ABSTRACT

Design, Synthesis, and Biological Evaluation of Tumor-Selective Vascular Disrupting Agents, Water-Soluble Amino Acid Prodrug Conjugates, and Bioreductively Activatable Prodrug Conjugates Targeting Tumor Hypoxia

Laxman Devkota, Ph.D.

Mentor: Kevin G. Pinney, Ph.D.

Selective targeting of tumor cells in comparison to healthy cells is vital in order to increase drug efficacy and reduce side effects to cancer patients. The underlying morphological and physiological differences between vasculature feeding healthy tissue versus tumor bearing tissue provides an opportunity to selectively target tumor vasculature as a promising therapeutic option for cancer treatment. Combretastatin A-1 (CA1) and combretastatin A-4 (CA4) are members of a family of inhibitors of tubulin polymerization which function as tumor-selective vascular disrupting agents (VDAs). The success of clinical trials involving the phosphate prodrugs of CA1 and CA4 (CA1P and CA4P respectively) inspired the design, synthesis, and biological evaluation of structurally related benzosuberene analogues as potent VDAs. A phenolic benzosuberene analogue (referred to as KGP18) and its amino congener (referred to as KGP156) emerged as potential preclinical candidates because of their robust cytotoxicity (sub-nM to pM) against selected human cancer cell lines *in vitro,* and excellent tubulin inhibitory

capabilities. A series of benzosuberene based analogues were synthesized in order to expand the relationship of structure to function in this class of anti-cancer agents. Also, a series of tri- and pentafluoro substituted amino-based combretastatin analogues were synthesized.

In an effort to discover new VDAs with improved water solubility and bioavailability, various amino-acid prodrug conjugates (AAPCs) of potent amino-based combretastatin analogues were synthesized. The corresponding water-soluble hydrochloride salts of these AAPCs were investigated for their ability to be cleaved by the leucine aminopeptidase (LAP) enzyme through a collaborative effort with the Trawick Research Group (Baylor University). The glycinamide hydrochloride salt 2' CA4-amine were cleaved quantitatively by LAP, however only partial cleavage was evident for their serinamide and *bis*-serinamide counterparts.

Since hypoxia is regarded as a hallmark of most solid tumors, a series of hypoxia-activated prodrugs [referred to as bioreductively activated prodrug conjugates (BAPCs)] of CA1 were synthesized regioselectively. These CA1-BAPCs are capable of being reduced by one or two-electron reductases, principally NAD(P)H cytochrome p450, cleaving the bioreductive trigger and releasing the cytotoxic drug in the hypoxic tumor microenvironment. A new and efficient synthetic methodology was explored to generate three nitrothiophene triggers from a common starting material.

Design, Synthesis, and Biological Evaluation of Tumor-Selective Vascular Disrupting Agents, Water-Soluble Amino Acid Prodrug Conjugates, and Bioreductively Activatable Prodrug Conjugates Targeting Tumor Hypoxia

by

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TABLE OF CONTENTS

LIST OF FIGURES

INTRODUCTION

LIST OF SCHEMES

LIST OF TABLES

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DEDICATION

To My Parents
Desh Nath Devkota and Sita Devi Devkota

In memory of
Shree Kanta Devkota and Dev Kumari Devkota

CHAPTER ONE

Introduction

In order to transport oxygen and nutrients throughout the body, a well organized vascular network is required.[1] Normal blood vasculature is arranged in a hierarchy of well-organized arteries, arterioles, capillaries, venules and veins.[2] The metabolic demand-driven balance automatically regulates the function of blood vessels, which evenly distributes oxygen and nutrients to all cells throughout the body.[1]

The tumor microenvironment exhibits distinct physiological characteristics in comparison to their healthy tissue counterpart.[3] The blood vasculature network feeding solid tumors is regarded as an attractive target for cancer therapy mainly because of its characteristics that are aberrant from normal tissue vasculature.[4–9] Tumor vasculature is devoid of a traditional classification layout of arteries-capillaries-veins.[1,3,6] Rapid and disorganized proliferation of the endothelial cells of tumor blood vessels cause them to exhibit variable shapes and diameters, irregular bulges and dead ends (Figure **1.1**).[10–12] The arteriolar-venous shunts due to the irregular branching add additional fragility to the blood vessels supplying nutrients and oxygen to the tumor cells. Tumor vasculature has an irregular flow of blood that at times, proceeds in alternating directions through the same vessels.[13] The discontinuous endothelial cell lining of blood vasculature with an abnormal basement membrane contributes to its immature and leaky nature. Such immature

and leaky nature consequently leads to the elevated interstitial fluid pressure of the tumor.[1,14–17]

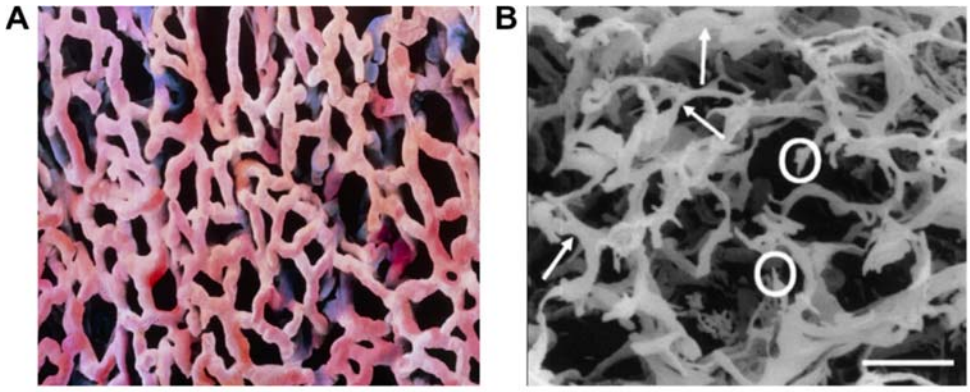


Figure **1.1**. Scanning Electron Microscopy (SEM) Image of a Microvascular Cast. A. From Normal Long Tissue. B. A SEM of Human Sigmoidal Adenocarcinoma (bar = 100 μm), Showing Blind Ends (Circled) and Abnormal Bulges (Arrowed) (Reprinted from Cancer Treatment Reviews 2011, with Permission from Elsevier, Ref 1)

In order to deliver necessary oxygen, blood, and nutrients to the tumor cells, a functional vascular network is essential.[3,18] Depriving tumors of these vital supplies restricts their growth beyond the size of 1-2 mm^3. Consequently, this deficiency is regarded as a contributing factor for their inability to metastasize to other regions of the host body.[4,19] However, once they establish their own vascular supply, the tumor cells exhibit a very high proliferation rate and enhanced metastatic spread..[20,21] In patients with the aggressive type of brain cancer, glioblastoma, it is reported that genetic markers of cancer cells are present in the blood vessel cells within the tumors. This suggests that the blood vessels within the tumor cells were developed by the tumor.

In contrast to normal vasculature, a tumor blood vascular network exhibits subtle differences in terms of its morphological and functional aspects, which offers a unique opportunity to target it selectively. Both small-molecules and biologics that specifically interact with tumor vasculature are referred to as vascular targeting agents (VTAs). The VTAs are further classified as angiogenesis-inhibiting agents (AIAs) and tumor-selective vascular disrupting agents (VDAs).[1,3,5,22]

Angiogenesis-Inhibiting Agents

Tumor angiogenesis is defined as the process of formation of new blood vessels as a result of unbalanced secretion of cytokines, particularly vascular endothelial growth factor-A(VEGF-A). The over-expression of VEGF and its receptor VEGFR in the normal tissue are indicative of an affected vascular network. Angiogenesis Inhibiting Agents (AIAs) are typically represented by compounds that target and inhibit VEGF-A and its receptor (VEGFR) to prevent the development of new tumor vasculature.[1,23–25] These agents, AIAs, are particularly active in the periphery of the tumor microenvironment, where nascent vessels are predominantly found. During the treatment of cancer patients using AIAs, tumor-vessels normalization was observed, which ultimately allows for the efficient delivery of oxygen and chemotherapeutic agents (Figure **1.2**).The efficient oxygenation of a tumor is required for the success of radiotherapy treatments. However, such types of VEGF inhibitory capability of AIAs potentially suffer from several side effects including hypertension, diabetic kidney disease,[26,27] and enhanced surgical wound healing complications.[28] A more selective strategy to

target tumor vasculature based on their fundamental structural differences over normal vasculature is imperative.

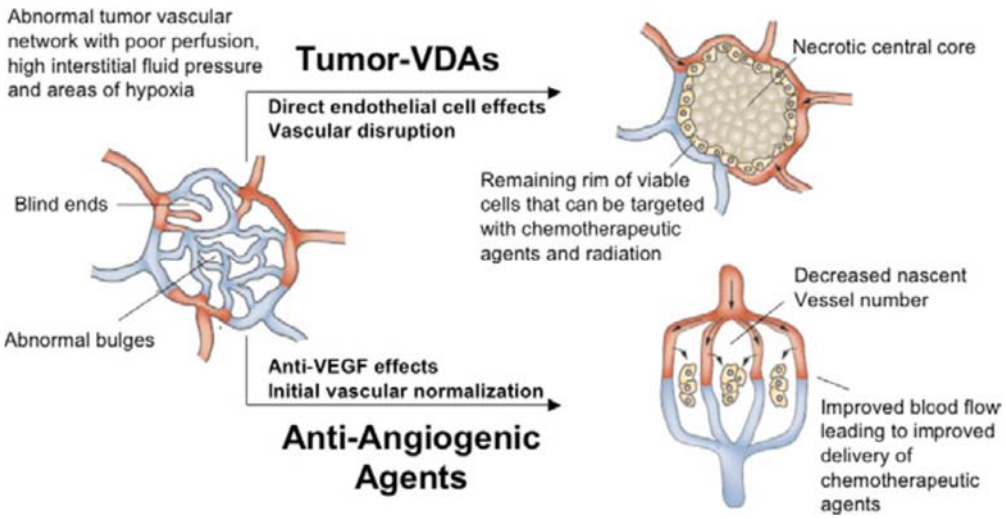


Figure **1.2**. Different Preclinical Effects of AIAs and Tumor-VDAs on Abnormal Tumor Blood Vessels. The treatment strategy of AIAs involve the tumor vessel normalization to help efficient delivery of chemotherapeutic agents and increased oxygenation to aid radiotherapy. In contrast, tumor-selective VDAs treatment lead to rapid vascular shutdown and extensive central necrosis leaving a thin viable rim which can be targeted with standard v therapies. (Reprinted from Cancer Treatment Reviews, with Permission from Elsevier, Refs. 1,29,30)

Tumor-Selective Vascular Disrupting Agents

Tumor-selective vascular disrupting agents (VDAs) are comprised of compounds that are capable of directly damaging established tumor vasculature by affecting rapidly growing endothelial cells which, in turn, suppress the tumor blood flow. The pioneering work from Dr. Juliana Denekamp and co-workers highlighted the importance of targeting these endothelial cells selectively over healthy tissue.[8,9,31] Tumor VDAs are known for their rapid damaging effect on existing tumor vasculature, requiring shorter periods of drug exposure.[32] They are potent

inhibitors of tubulin polymerization, as well as remarkably strong inhibitors of colchicine binding to tubulin. The pronounced endothelial cell apoptosis causes tumor blood flow to collapse, resulting in massive tumor necrosis around the central area of the tumor.[32–36]

It is believed that the VDAs potentially exhibit a synergistic effect with chemotherapy and radiotherapy, as they predominantly target the core region of the tumor, which is typically resistant to conventional chemotherapy.[1] Tumor-selective VDAs can be classified in two classes: tubulin depolymerizing tumor-VDAs and flavonoid tumor-VDAs. One class of tumor-VDAs, namely tubulin depolymerizing tumor-VDAs, interacts with the colchicine binding site located on the α,β-heterodimer of tubulin (Figure **1.3**). Binding of these agents to tubulin causes the inhibition of microtubule polymerization, cell retraction, rounding, and ultimately detachment from the polymeric chain.[3,5,8,22,34,37] The net result of such cell morphological changes are increased vessel permeability and inhibition of tumor blood flow.[5,7,37,38] However, the mechanism of action of flavonoid-based tumor-VDAs is independent of tubulin.[1]

The natural products combretastatin A-4 and combretastatin A-1 (CA4 and CA1 respectively, Figure. **1.4**), originally isolated by Pettit and co-workers (Arizona State University) from the South African bush-willow tree, *Combretum caffrum* Kuntze, are among the colchicine site class of tubulin binding tumor-selective VDAs.[39–42] The combretastatin family of natural products, along with a number of synthetic derivatives and analogues,[43–47] is capable of significantly inhibiting microtubule assembly in endothelial cell lining tumor-feeding vasculature, leading

to a series of cell signaling events, which ultimately result in endothelial cell morphology changes, blood flow reduction,[1,5,47] and rapid vascular collapse leading to tumor cell necrosis.[45,48,49]

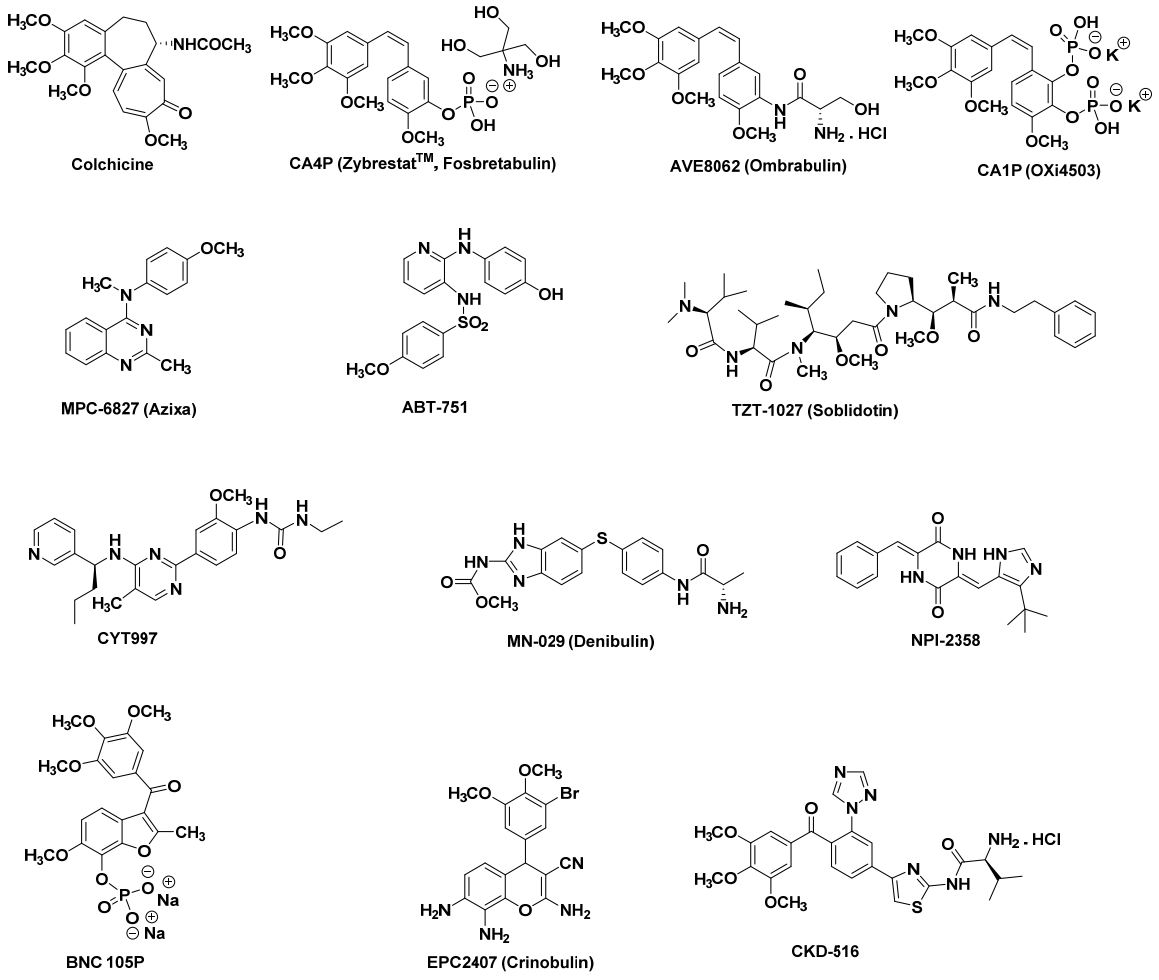


Figure **1.3**. List of Tubulin Binding Small-Molecule Vascular Disrupting Agents. (Reproduced from Ref 3 with permission of The Royal Society of Chemistry)

A compilation of VDAs currently in human clinical trials is shown in Figure **1.3,** which includes: Colchicine[50], CA4P[39], AVE8062[51], CA1P[52], MPC-6827[53], ABT-751[54], TZT-1027[55] , CYT997[56], MN-029[57], NPI-2358[58], BNC105P[59], EPC2407[60], CKD-516[61]. These VDAs function, in part, through a tubulin mechanism, and they are capable of

imparting tumor necrosis in affected laboratory mice in amounts much lower than their maximum tolerated doses.[7,42] The water-soluble phosphate prodrug conjugates of CA4 and CA1 (CA4P also known as Zybrestat™ and CA1P also known as OXi4503 respectively, Fig. **1.4**) are among a group of VDAs which have demonstrated promising efficacy in human clinical trials.[36,48,49,62–68]

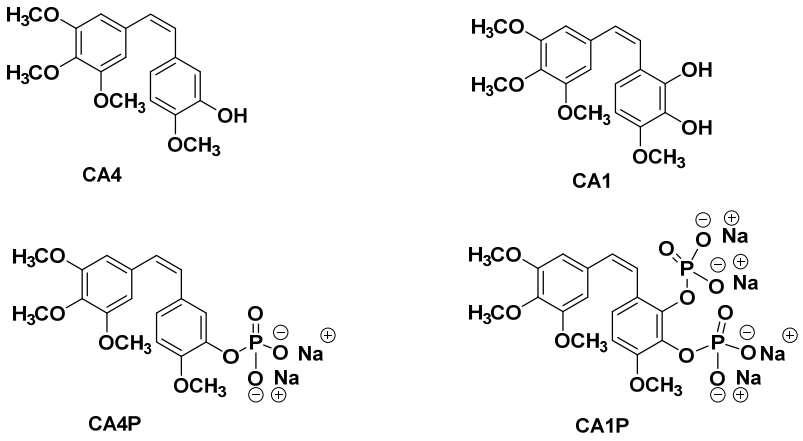


Figure **1.4**. Combretastatin Natural Products and their Water-Soluble Phosphate Prodrugs

The enzyme-mediated dephosphorylation of these prodrugs results in the release their respective parent compounds (CA4 and CA1). They are shown to bind and interfere with the tubulin-microtubule protein system, leading to pronounced morphological effects in the endothelial cell lining tumor vasculature.[36,37,64–69] Pre-clinical and pharmacokinetic studies of these combretastatin prodrug conjugates in patients bearing advanced solid tumors highlight their efficacy as VDAs.[52,64,70,71] It is noteworthy to mention that CA1P has shown dual mechanistic capability. It can function both as VDA and as a cytotoxic agent based on its *in vivo* mediated conversion to a highly reactive ortho-quinone intermediate.[48,52,70,71] The structural

simplicity of the combretastatins has motivated the Pinney Researh Group and other synthetic chemists to develop libraries of structurally-inspired analogues through alteration of the A–ring, the B–ring, and the ethylene bridge of the stilbene system.[45,46,72–75]

Amino Acid Prodrug Conjugates

A progressive research agenda in the Pinney Research Group led to the discovery, design, and development of highly cytotoxic amino-based CA4, CA1 and fused-ring systems.

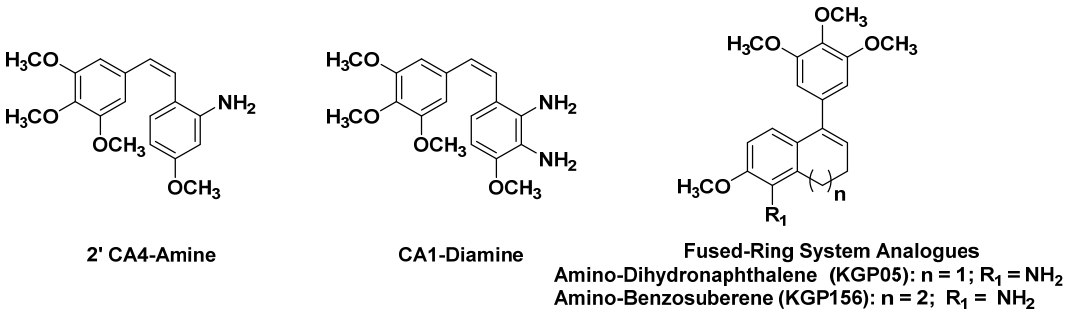


Figure **1.5**. Pinney Research Group Amino-Based Structural Modifications of Combretastatin and Fused-Ring System Analogues[45,46,76]

The synthesis guided by structural modifications of combretastatin family of natural products resulted in new amino-dihydronaphthalene and amino-benzosuberene analogues, that exhibited important biological activities.[45,46,76,77] Ohsumi and co-workers reported the first synthesis and biological analysis of an amino acid prodrug of the amino-based CA4 analogue [referred to as AVE8062 (Ombrabulin, Figure. **1.3**)], which showed significant promise in human clinical trials phase III as a VDA.[51,77,78] Enhanced antitumor activity and decreased toxicity (towards normal

cells) in both *in vitro* and *in vivo* models was the hallmark of AVE8062.[34,78–80] Inspired by this pioneering work, the Pinney group reported the first synthesis of the 2'-amino variant of CA4 (2' CA4-Amine, Figure **1.5**).[45,72] This compound exhibited potent inhibition of tubulin assembly and significant *in vivo* blood flow shutdown capability. Subsequently, our work showed that the di-amino-based-combretastatin A-1 (CA1-diamine, Figure **1.5**) was strongly cytotoxic against selected human cancer cell lines *in vitro* (average GI_{50} = 13.9 nM) and also demonstrated potent activity in regard to inhibition of tubulin assembly (IC_{50} = 2.8 μM).[46] Moreover, inspired by the SAR studies associated with the combretastatins and their close structural analogues, we were the first to report the synthesis of amino congeners of the dihydronaphthalene tubulin-binding agent (KGP05, Fig. **1.3**)[81–83] followed by its seven membered counterpart (KGP156, Figure **1.5**).[76,84–87] These compounds have emerged as potential pre-clinical candidates due to their robust in vitro cytotoxicity (sub-nanomolar GI_{50} values) against selected human cancer cell lines and also their strong tubulin inhibitory capabilities.[76,85,86] However, these amino-based anti-cancer agents have limited water solubility. This issue of water solubility associated with these agents can be addressed through prodrug strategies.[88,89] Amino acid prodrugs, like glycine and serine, with shorter hydrocarbon or polar side chains are readily water-soluble because of their simpler structure, and they are also quite likely to undergo quantitative cleavage by hydrolytic enzymes, likely an aminopeptidase.[89,90] The parent drug can be generated after an amide bond is hydrolyzed by the hydrolytic enzymes such as leucine aminopeptidase (LAP).[79,89] LAP is one of a widely distributed group of

metalloenzymes that exhibit broad specificity. [91–97] It catalyzes the hydrolysis of amino acids from the amino terminus of polypeptide chains. LAP is found mainly in human cytosol of liver cells, which makes the serum leucine aminopeptidase a marker of hepatic disorders.[98,99] The increase in concentration of LAP in human sera is diagnostically indicative of a number of cancers, such as head and neck cancer and pancreas carcinoma.[100–102]

Bioreductively Activatable Prodrug Conjugates

The tumor microenvironment is vastly different than that of healthy tissues.[1,3,14] Solid tumors, once they reach to a particular size, have to develop their own vascular supply in order to meet their insatiable demand of oxygen and nutrients. As a consequence, the rapidly expanding neoplastic cell forms its own vascular network, which is extremely abnormal in terms of both of its structure and function. The blood vessels feeding tumor cells, which are rapidly proliferating, are highly disorganized in nature as well. As discussed earlier, normal tissue vasculature and that of tumor cells are vastly different. In short, the extreme aberrant nature of the vascular supply network, elevated interstitial pressure due to premature and leaky vasculature, and the pH gradient with cells distant from blood vessels being acidic are some of the main traits of tumor microenvironment.[14,103] More importantly, a variety of measurement techniques have demonstrated the significant gradient in oxygen concentration in the region of solid animal tumor models, human tumor xenografts, and numerous human cancers.[104] Such a region of oxygen deficiency in the tumor microenvironment is known as tumor hypoxia. Such

hypoxic nature of tumor microenvironment should be explored in detail in order to find a better treatment strategy.

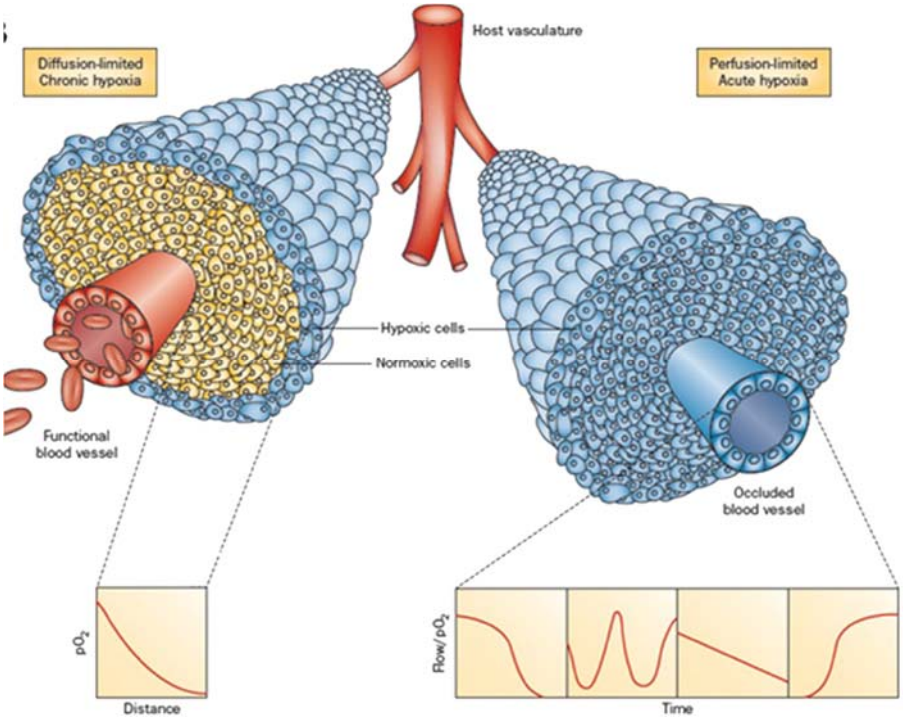


Figure. **1.6**. Illustration of Tumor Cells Growing as a Cord around Blood Vessels from which they Obtain Oxygen and Nutrients. The left side illustrates oxygen diffusion and utilization from the vessel resulting in the development of chronically hypoxic cells at the outer edge of the cord. The right side shows occluded blood vessel causing perfusion which results in the development of acute hypoxia. (Reprinted from Pharmacology and Therapeutics, 153, D. W. Siemann, M. R. Horsman, Modulation of the Tumor Vasculature and Oxygenation to Improve Therapy, Pg 107-124, Copyright 2015, with permission from Elsevier, Refs 105 and 14)

Over six decades ago, Thomlinson and Gray postulated the presence of regions of lower oxygen level in human tumors.[106] They found that radiation therapy was not uniformly successful because of the resistance developed by the hypoxic tumor cells.[105–107] The diffusion of oxygen to the neighboring tumor cells is limited, causing diffusion-controlled chronic hypoxia (Fig **1.6**).[106] However, the

clinical trials involving oxygenation of hypoxic tumor cells followed by radiation therapy were not particularly successful either.[106,107]

Later on, Chaplin and co-workers elegantly postulated the obstruction of blood flow in the tumor cells was another contributing factor to tumor hypoxia.[13,108,109] This type of hypoxia, known as perfusion-limited acute hypoxia, is believed to be one of the significant contributing factors of the failure of such clinical trials reported earlier (Figure **1.6**).[13,14] Hypoxia is one of the parameters in the tumor microenvironment that has been investigated extensively. There is a distinct gradient in the concentration of oxygen inside the solid tumor, varying from normoxic to hypoxic to anoxic. The tumor cells closer to the blood vessels are considered to be normoxic or slightly hypoxic, whereas those which are distant could be necrotic or anoxic in nature.[14]

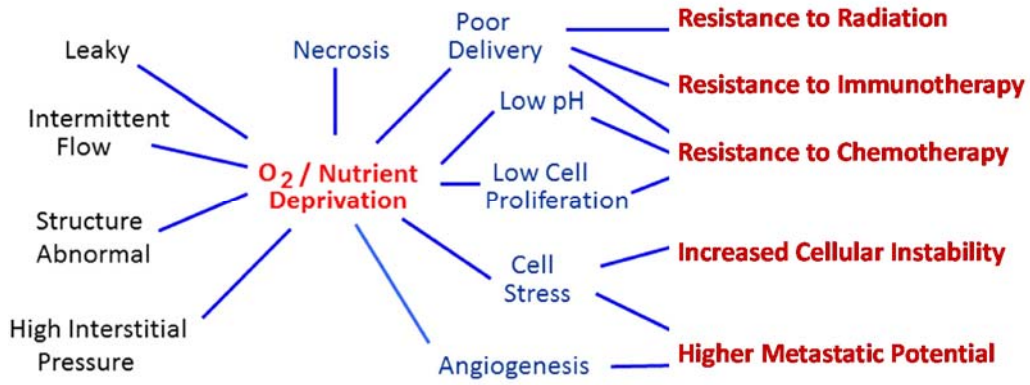


Figure **1.7**. The Aberrant Nature of Tumor Blood Vessel Network. This results in oxygen and nutrient deficiency. Such phenomenon promotes tumor metastasis resulting in enhanced failure of traditional anti-cancer therapies (Reprinted from Pharmacology and Therapeutics, 153, D. W. Siemann, M. R. Horsman, Modulation of the Tumor Vasculature and Oxygenation to Improve Therapy, Pg 107-124, Copyright 2015, with permission from Elsevier, Ref 14).

Tumor hypoxia is believed to be one of the significant contributing factors to the treatment failure and relapse among cancer patients bearing solid tumors, as the tumor cells in the hypoxic region are considered to be resistant to the most conventional anti-cancer therapies for several reasons (Figure **1.7**).[14] One of the reasons why tumor cells are resistant to conventional anti-cancer therapy relates to the distance of hypoxic cells from the blood vessels supplying anti-cancer drugs to them.[110–112] In addition, hypoxia is believed to be involved in the up-regulation of genes involved in drug resistance, including-glycoprotein.[113] Other notable effects of hypoxia are elevated mutation rates,[114] and the increase in VEGF associated with tumor angiogenesis,[115] tumor invasion[116] and metastatic phenotype of human cancers.[117,118] Also, significant over-expression of hypoxia inducible factor-1α (HIF-1α) has been observed in most solid tumors studied, which include the colon, breast, pancreas, kidneys, prostate, ovary, brain, and bladder.[119,120] HIF-1α increases the levels of survival factors related to VEGF, which can protect the tumor vasculature from being damaged during radiation therapy.[113,121] As hypoxia seems to be one of the main contributing factors for enhanced metastasis, it can easily compromise the curability of tumors by surgery.[112]

However, as the presence of low oxygen concentration and cell necrosis are unique features of solid tumors, they do not usually occur in normal tissues. So, this type of hypoxic nature of tumor microenvironment can be exploited in order to target them selectively in cancer therapy. Several strategies are currently being explored to target those hypoxic tumor cells at the clinical and preclinical levels, including the use of hypoxia-selective gene therapy, targeting the HIF-1α

transcriptional factor, use of recombinant obligate anaerobic bacteria, prodrugs activated by hypoxia.[14,111,112] Here, our main focus of discussion will be on the latter strategy, which deals with designing prodrugs of highly cytotoxic vascular disrupting agents that are capable of being cleaved once they reach the target. Such drugs are known as hypoxia-activated prodrugs,[14,105,112,122] or bioreductively activated prodrug conjugates (BAPCs). These prodrug conjugates are activated through either one- or two-electron reductase enzymes, principally NAD(P)H-dependent flavoproteins for one-electron reduction and cytochrome P450s for two-electron reductions.[14,122]

Tirapazamine is a type of hypoxia-activated bioreductive drug, which upon reduction of its triazine moiety to a free radical activates the compound to cause DNA damage and poisoning topoisomerase II (Figure **1.8**).[123,124]

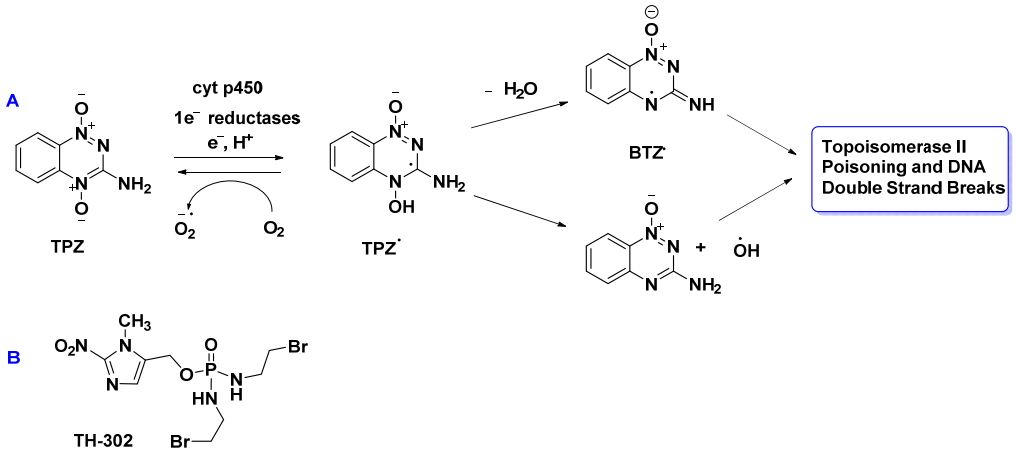


Figure **1.8**. A. The Mechanism by which Tirapazamine Selectively Kills Hypoxic Cells. B. Structure of TH-302

Combining tirapazamine with conventional anti-cancer agents like cisplatin was particularly effective in a phase III clinical trial with advanced non-small-cell

lung cancer. [14,125] Such a combination of tirapazamine doubled the response rate and significantly prolonged the survival of the patients. Due to its selectivity towards hypoxic conditions *in vitro* and its success in clinical trials, tirapazamine has become the positive control against which new bioreductive conjugates are tested.[122] TH-302,[126] a 2-nitroimidazole-based nitrogen mustard prodrug (Figure **1.8**. B) which is progressing in human Phase III clinical trials, releases its parent drug, bromoisophosphoramide mustard, in hypoxic conditions.[127,128]

Each conjugate is active over a different range of oxygen concentrations in the cell, as tirapazamine is activated in moderately hypoxic conditions whereas TH-302 is only activated in extremely hypoxic conditions.[127] Another type of BAPC, which has the reducing moiety simply as a masking agent, gets cleaved from the prodrug during reduction in hypoxic tumor microenvironment. Such masking agents are known as bioreductive triggers, which are usually bound to the drug through an ether linkage.[14,129] Once cleaved, the therapeutic agents are selectively released in the tumor microenvironment. The fact that the presence of lower oxygen concentration is unique to solid tumors provides an excellent opportunity to target them selectively over normal tissue, which will invariably drive the preclinical and clinical studies of this therapeutic approach.[14,122]

A recent surge in the exploration of alternative strategies to target solid tumors has highlighted hypoxia-activated prodrug conjugates as a promising avenue for current and future research.[14,122,124] Peter Davis and co-workers previously reported the synthesis of a series of nitrothienyl BAPCs of CA4, including *nor*-, *mono*-, and *gem*-dimethyl triggers. They demonstrated the ability of those CA4

BAPCs to efficiently release CA4 from A549 cells under tumor hypoxia. [129] These BAPCs tend to get activated mainly in the hypoxic tumor microenvironment, hence releasing a cytotoxic compound presumably through one or two-electron reductases. Mainly it is considered that NADPH-dependent flavoproteins are responsible for one-electron reductions and cytochrome P450 for two-electron reductions.

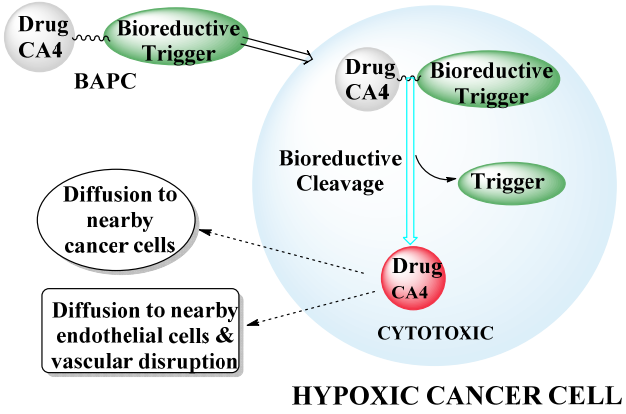
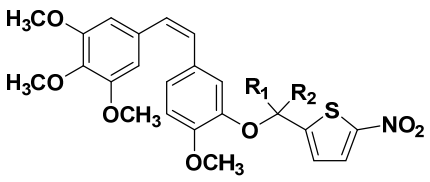


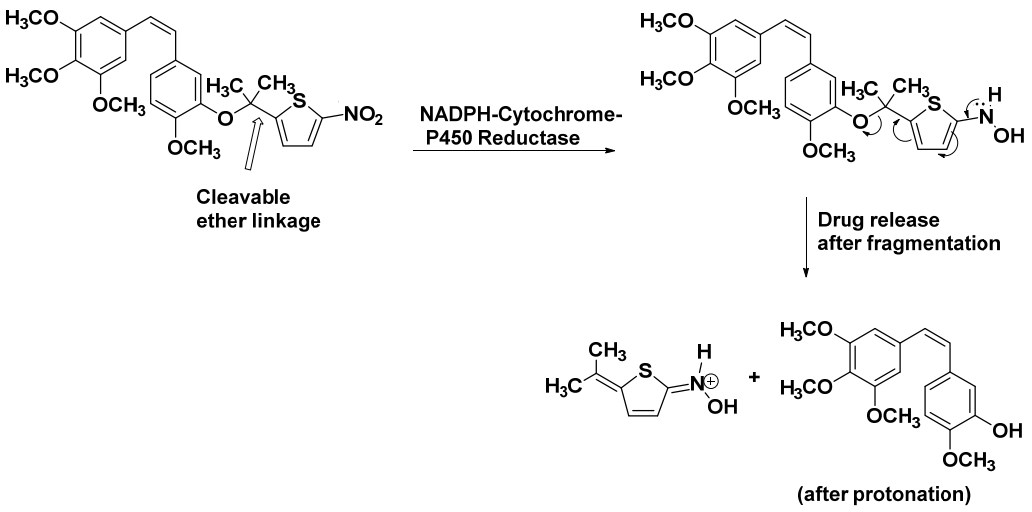
Figure **1.9**. Proposed Mechanism for Selective Release of Cytotoxic Agent (CA4) from Non-Toxic BAPC under Tumor Hypoxia. BAPCs tend to activate only in the hypoxic tumor micro environment thereby releasing a cytotoxic compound presumably through one or two-electron NADPH cytochrome P450 reductases (Reproduced with permission from Strecker, T. E.;Tanpure, R. P.; George, C. S.; Chaplin, D.j.; Trawick, M. L. and Pinney, K. G. Bioreductively Activatable Prodrug Conjugates Designed To Target Hypoxic Tumors.2012 CPRIT Annual Conference, Austin, Texas, October 24-26, 2012.)



Normethyl; $R_1=R_2=H$
Monomethyl; $R_1=H$, $R_2=CH_3$
Gem-dimethyl; $R_1=R_2=CH_3$

Figure **1.10**. Combretastatin A-4 with Nitrothiophene-Based Bioreductive Trigger

The efficacy of these BAPCs was evaluated by determining their differential cytotoxicity under normoxia versus hypoxia, measured as the hypoxic cytotoxic ratio (HCR), in selected human cancer cell lines.[129] The *gem*-dimethyl CA4 BAPC (Scheme. **1.1**) showed a higher susceptibility to cleavage by the cytochrome P450 reductase enzyme in the hypoxic tumor microenvironment in comparison to the *nor*-and *mono*-methyl substituted compounds. It was determined that inhibition of tubulin polymerization by CA4 was not observed since the prodrug was not cleaved in higher oxygen environments.[129]



Scheme. **1.1**. Biological Reduction and Cleavage of CA4 *gem*-Dimethyl Nitrothiophene Trigger Releasing CA4

While the *gem*- and *mono*-substituted CA4 BAPCs were effective across a range of oxygen concentrations, the unsubstituted (*nor*-methyl) was only effective in extreme hypoxic environments (<0.01% O_2).[129]

Benzosuberene-Based Vascular Disrupting Agents

The chaotic nature of tumor vasculature with abnormal bulges, blind ends, shunts, leaky and irregular networks collectively provide an attractive target for the selective disruption of tumor vasculature through small-molecule drug intervention.[1,5,14,47] Ongoing research progress highlights the evidence that VDAs preferentially bind to the colchicine binding site on the α,β-tubulin heterodimer,[47,130] affecting activated endothelial cells lining the vasculature supplying tumors rather than quiescent endothelial cells found in the vasculature feeding normal tissue.[59]

As discussed earlier, combretastatin A-1 (CA1) and combretastatin A-4 (CA4) are among the class of colchicine-binding VDAs. These natural products were originally isolated from the bush willow tree *Combretum caffrum* by George R. Pettit (Arizona State University).[40,131] This family of natural products, along with a number of synthetic derivatives and analogues, has demonstrated the ability to significantly inhibit endothelial cell microtubule assembly, ultimately resulting in morphology changes and blood flow reduction.[42]

In an effort to discover new classes of tubulin binding active compounds, the Pinney Research Group has designed and synthesized a variety of compounds that mimic the structure of the natural products colchicine and the combretastatins. This list includes (in part), dihydronaphthalene,[82,85] benzosuberene,[76,82,85,86] and indole analogues,[132] all of which incorporate the aryl ring linked in a pseudo-cis orientation to another aryl ring. The highly active parent benzosuberene phenol KGP18 and its amino-variant KGP156, have shown potential to be a pre-clinical candidates.[86,87] Our

previous studies in this area highlighted two separate synthetic strategy for the synthesis of KGP18[85] and KGP156.[76] The synthetic route utilized for the synthesis of KGP18 involved a ring expansion strategy in order to install the 6:7 fused ring system, ultimately leading to the highly active parent benzosuberene compound KGP18 (Figure **1.11**).

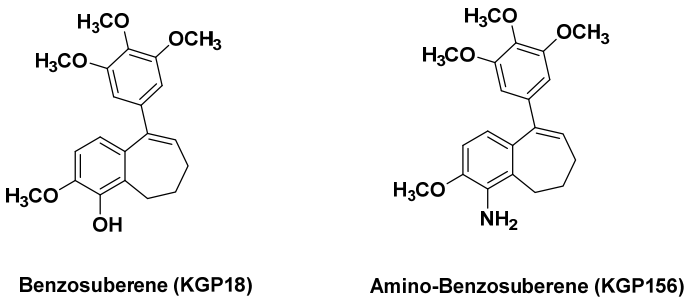


Figure **1.11.** Benzosuberene (KGP18) and its Amino-Variant Amino-Benzosuberene (KGP156)

Inspired by our original work, Maderna and co-workers later prepared and evaluated a series of structurally diversed benzosuberene analogues using a ring closing metathesis step, followed by the Suzuki coupling reaction. This synthetic modification allowed them to generate KGP18 efficiently.[133] Independently, and in the same time period, Pinney and coworkers reported a series of functionalized benzosuberene-based analogues employing very efficient Wittig olefination, catalytic hydrogenation, intramolecular Friedel-Crafts annulations with Eaton's reagent (7.7 weight percent P_2O_5 in CH_3SO_3H) followed by halogen metal exchange reactions to install the required structural moieties in the molecules.[76,86] Thus, utilizing this new strategy and functional group modifications designed to probe structure activity relationship considerations, we chemically prepared a small

library of benzosuberene analogues as potential inhibitors of tubulin polymerization.

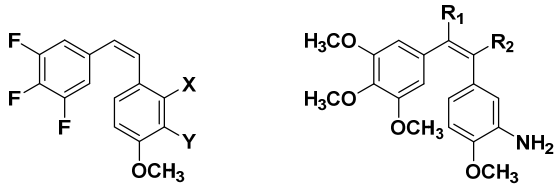
^{19}F Analogues of Amino-Based Combretastatins

Incorporation of fluorine atoms in drug candidates has been extensively studied.[134,135] Approximately 5-15% of the total number of launched drugs worldwide over the past 60 years are fluorinated.[135] As new methods to incorporate fluorine atoms or trifluoromethyl groups have become more readily available, medicinal chemists will have even more opportunity to explore these fluorinated drugs.[134–136] Since the fluorine atom is small and highly electronegative, it can change the overall physical and chemical properties of molecule. Due to its smaller size, fluorine can often be introduced into a compound without changing its steric layout.[137,138] The strong electronegativity of fluorine is regarded as a powerful tool for modulating the pK_a of the compound.[134] The change in pK_a of a drug can eventually change its pharmacokinetic properties including adsorption, distribution, metabolism and excretion.[134,139–141]

Introduction of fluorine influences the solubility as well as permeability of the compound of interest.[134] In general, fluorination of alkanes decreases lipophilicity, whereas the addition of fluorine or a trifluoromethyl moiety on an aryl ring or adjacent to a π-system or heteroatom-containing functional group increases lipophilicity.[135] In addition, fluorination can strongly polarize the parent molecule.[142] Replacing a hydrogen atom with fluorine increases the overall size of the molecule, in addition to its chemical reactivity. Whereas, due to its strong

electronegativity, replacing oxygen with fluorine in the drug retained comparable activities.[135] Additionally, replacing groups with fluorine could impart additional capacities for analytical techniques such as fluorine is useful for a number of imaging techniques.[143] Specifically, fluorine-19 magnetic resonance imaging (^{19}F MRI) has the capability to be used in conjunction with fluorinated CA1 and CA4 analogues to assess tumor oxygenation levels in certain cancer cell lines.[3] Such a method would allow deep tissue imaging in a non-invasive as well as non-radioactive fashion for assessment of the biochemical effectiveness of those combretastatin molecules.[144,145]

The amino variants of CA4 and CA1 (3' CA4- amine and CA1-diamine respectively) have shown significant promise as potential VDAs.[34,45,46,79] Furthermore, substitutions of fluorine atoms in place of methoxy groups on the A-ring of CA4 have been reported, and the resultant analogues have shown inhibition of tubulin polymerization.[146] Such fluorinated amino combretastatin analogues in initial studies have been effective at inhibiting tubulin polymerization, and one of these analogues (compound **2,** Figure. **1.12**), was moderately inhibitory in two notable cancer cell lines, NCI-H460 (lung) and DU-145 (prostate).[137]



1. X = NH_2, Y = H
2. X = H, Y = NH_2
3. X = H, Y = OH

4. R_1, R_2 = F
5. R_1 = F, R_2 = H
6. R_1 = H, R_2 = F
7. R_1 = Br, R_2 = F

Figure **1.12**. Representative Fluorine Derivatives of Combretastatins[137,138,146]

This lead compound of the CA4 A-ring fluorinated derivatives (compound **2**, Fig. 1.12) has led us to investigate the biological efficacy of numerous amino substituted combretastatin analogues that contain tri or penta fluorinated A-rings of amino-variants of the combretastatins. However, substituting vinyl protons with halogen atoms (compounds **4** and **7**, Figure **1.12**) reduced the activity.[138] Altogether, fluorine substitutions on the A-ring with amino-based combretastatins could lead to new VDAs with a myriad of useful biochemical properties. For example, if a smaller but equally (or more) hydrophobic, electron-dense A-ring is coupled with a hydrophilic B-ring containing polar, hydrogen-bonding amino or serinamide substituents, these new compounds may exhibit better water solubility and be more bioavailable as their configuration would allow for easier embedding in, or transport through, membranes.

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CHAPTER TWO

Design, Synthesis, and Biological Evaluation of Water-Soluble Amino Acid Prodrug Conjugates Derived from Combretastatin, Dihydronaphthalene, and Benzosuberene-Based Parent Vascular Disrupting Agents

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The author L. Devkota contributed to this manuscript with the synthesis of compounds **1** through **24**, in addition to their full characterization. Also, L. Devkota played a significant role in the preparation of manuscript including writing and making edits based on co-author's comments and suggestions.

Abstract

Targeting tumor vasculature represents an intriguing therapeutic strategy in the treatment of cancer. In an effort to discover new vascular disrupting agents (VDAs) with improved water solubility and potentially greater bioavailability, various amino acid prodrug conjugates (AAPCs) of potent amino combretastatin, amino dihydronaphthalene, and amino benzosuberene analogues were synthesized along with their corresponding water-soluble hydrochloride salts. These compounds were evaluated for their ability to inhibit tubulin polymerization and for their cytotoxicity (*in vitro*) against selected human cancer cell lines. The amino-based parent anti-cancer agents **7**, **8**, **32** (KGP05) and **33** (KGP156) demonstrated potent cytotoxicity (GI_{50} = 0.11 to 40 nM) across all evaluated cell lines, and they were strong inhibitors of tubulin polymerization (IC_{50} = 0.62 to 1.5 μM). The various

prodrug conjugates and their corresponding salts were investigated for their ability to be cleaved by the enzyme leucine aminopeptidase (LAP). Four of the glycine water-soluble AAPCs (**16**, **18**, **44** and **45**) showed quantitative cleavage by LAP, resulting in the release of the highly cytotoxic parent drug, whereas partial cleavage (<10-90%) was observed for other prodrugs (**15**, **17**, **24**, **38** and **39**). Eight of the nineteen AAPCs (**13-16**, **42-45**) showed significant cytotoxicity against selected human cancer cell lines. The previously reported CA1-diamine analogue and its corresponding hydrochloride salt (**8** and **10** respectively**)** caused extensive disruption (at a concentration of 1.0 μM) of human umbilical vein endothelial cells (HUVECs) growing in a two-dimensional tubular network on matrigel. In addition, compound **10** exhibited pronounced reduction in bioluminescence (greater than 95% compared to saline control) in a tumor bearing (MDA-MB-231-luc) SCID mouse model 2 h post treatment (80 mg/kg), with similar results observed upon treatment (15 mg/kg) with the glycine amino-dihydronaphthalene AAPC (compound **44**). Collectively, these results strongly support a mechanism involving vascular damage for the amino variants of combretastatin, dihydronaphthalene, and benzosuberene analogues.

Introduction

Tumor vasculature is as an attractive target for the treatment of cancer due, in part, to its distinct characteristics, such as rapid and disorganized proliferation of endothelial cells.[1–5] Both small-molecules and biologics that specifically interact with tumor vasculature are referred to as vascular targeting agents (VTAs), which are further sub-classified as angiogenesis-inhibiting agents (AIAs) and vascular

disrupting agents (VDAs).[6,7] AIAs are typically represented by compounds that target vascular endothelial growth factors to prevent the development of new tumor vasculature.[7] Conversely, VDAs are comprised of compounds that disrupt and directly damage established tumor vasculature, by affecting rapidly growing endothelial cells, to suppress tumor blood flow. One class of VDAs interacts with the colchicine (Fig. **2.1)** site located on the α,β- heterodimer of tubulin, causing cell retraction, rounding, and ultimately detachment from the aggregated sheet of cells.[1,4,8,9]

The natural products combretastatin A-4 (CA4) and combretastatin A-1 (CA1) (Fig. **2.1**), originally isolated by Pettit and co-workers (Arizona State University) from the South African bush-willow tree, *Combretum caffrum* Kuntze, are among the colchicine site class of tubulin binding VDAs.[10–13] This family of natural products, along with a number of synthetic derivatives and analogues[6,14–17], significantly inhibit microtubule assembly in endothelial cells lining tumor-feeding vasculature, leading to a series of cell signaling events that ultimately result in endothelial cell morphology changes and blood flow reduction.[1,7,17] The inhibition of tubulin polymerization results in activation of RhoA, an intracellular coordinator of the cytoskeletal rearrangement of microtubules and actin, and leads to rapid vascular collapse.[15,18,19] Administration of these VDAs in amounts significantly lower than their maximum tolerated doses has resulted in tumor necrosis in treated laboratory mice.[3,12]

The combretastatin water-soluble phosphate prodrugs, combretastatin A-4 phosphate (CA4P also known as Zybrestat™, Fig. **2.1**) and combretastatin A-1

diphosphate (CA1P also known as OXi4503, Fig. **2.1**) are among a group of VDAs which have demonstrated promising efficacy in human clinical trials.[18–27] They undergo enzyme-mediated dephosphorylation and, as their parent compounds (CA4 and CA1), they bind to tubulin and interfere with the tubulin-microtubule protein system. This causes pronounced morphological effects in the tumor vasculature.[8,22–28] Pre-clinical and pharmacokinetic evaluations of these combretastatin prodrugs in patients bearing advanced tumors indicate their efficacy as VDAs.[22,29–31] Interestingly, CA1P has dual mechanistic capability, functioning as both a VDA and as a cytotoxic agent based on its *in vivo* mediated conversion to a highly reactive ortho-quinone.[18,29–31] The relative structural simplicity of the combretastatins has motivated synthetic chemists to develop libraries of structurally inspired analogues through alteration of the A–ring, the B–ring, and the ethylene bridge.[6,15,16,32–35]

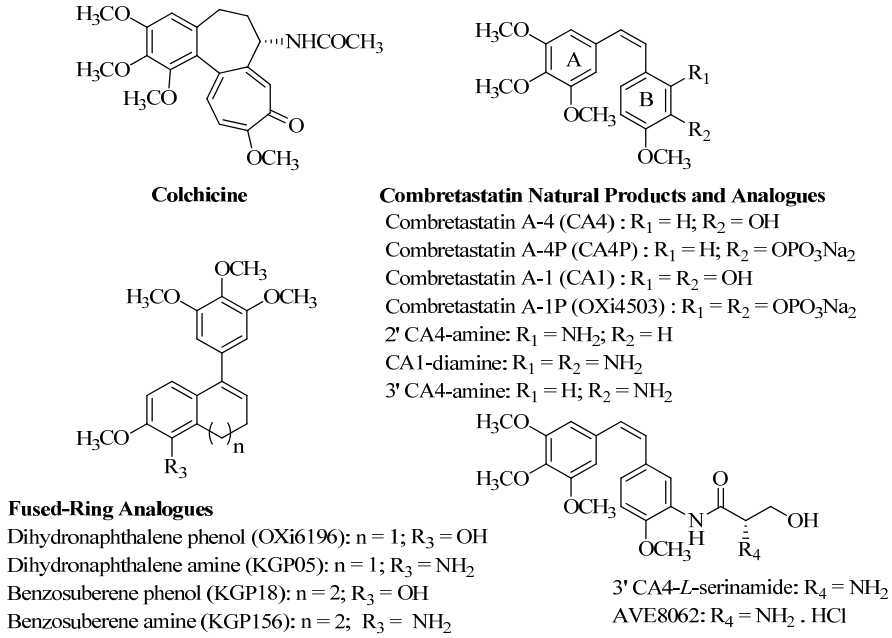


Figure **2.1**. Colchicine and natural and synthetic combretastatin, dihydronaphthalene, and benzosuberene analogues

Incorporation of the NH_2 substituent within either ring A or ring B in the combretastatin family resulted in new analogues that exhibited important biological activity.[15,36,37] In 2006, we reported the initial design and synthesis of the 2' CA4-amine (Fig. **2.1**) and described its potent inhibition of tubulin assembly and its activity as a VDA.[15] Later work by others confirmed the potency of this 2' CA4-amine analogue and related compounds.[34] Subsequently, our studies showed that the di-amino variant of combretastatin A-1 (CA1-diamine, Fig. **2.1**)[16] was strongly cytotoxic against human cancer cell lines (average GI_{50} = 13.9 nM) and also demonstrated potent activity in regard to inhibition of tubulin assembly (IC_{50} = 2.8 μM).[16] It is fairly common for compounds that interact with tubulin in the low μM range (cell free assay) to demonstrate nM cytotoxicity against human cancer cell lines; a variety of factors are postulated to influence this activity differential.[38] The trimethoxyphenyl moiety, the *p*-methoxyphenyl moiety, the *Z*-configuration of the two aryl rings, and the optimal 4-5 Å aryl-aryl distance all proved important for enhanced tubulin binding activity of the combretastatin analogues.[17,39–41] Inspired, in part, by the SAR studies associated with the combretastatins and their close structural analogues, we were the first to report the synthesis and biological activity of related dihydronaphthalene tubulin-binding agents [for example, OXi6196 (also referred to as KGP05), Fig. **2.1**),[42–44] followed by the discovery of a phenolic-based benzosuberene (KGP18, Fig. **2.1**) analogue and its corresponding amino congener (KGP156, Fig. **2.1**).[40,45–48] These compounds have emerged as potential pre-clinical candidates due to their robust in vitro cytotoxicity (sub-nanomolar to picomolar GI_{50} values) against selected human cancer cell lines and strong tubulin inhibitory

activities.[45–47] In our previous work,[46] we also demonstrated robust tubule disruption (in a human umbilical vein endothelial cell (HUVEC) tube disruption assay) and cell rounding capability of KGP156, which is one of the parent compounds for several of the prodrugs designed and synthesized in this study.

The issue of limited water solubility associated with these aniline based anti-cancer agents can be addressed through prodrug strategies.[49,50] Amino acid prodrugs, like glycine and serine, with shorter hydrocarbon or polar side chains, are reported to be more readily water soluble and more likely to be cleaved because of their structural simplicity.[49] Another advantage of using amino acids is that they are capable of undergoing quantitative cleavage by hydrolytic enzymes, likely an aminopeptidase.[50,51] The synthesis and biological evaluation of a series of water-soluble amino acid prodrugs of amino-combretastatin were previously reported.[15,35,52] A water-soluble serinamide prodrug of 3' CA4-amine (Fig. **2.1**) known as AVE8062 (Ombrabulin, synthesis[36] is described in supplementary data), showed significant promise as a VDA in phase III human clinical trials[53,54] and enhanced antitumor activity and decreased toxicity (for normal cells) in both *in vitro* and *in vivo* models.[36,52,54–56] The parent drug is generated after amide bond cleavage by a hydrolytic enzyme.[50,52]

Leucine aminopeptidase (LAP)[57–59] is one of a widely distributed group of aminopeptidases that exhibit broad specificity[60–62] and that catalyzes the hydrolysis of amino acids from the amino terminus of polypeptide chains.[63] There are also a number of literature reports of amino acid prodrugs cleaved by LAP.[49,52] In humans, LAP is found primarily in the cytosol of liver cells, which makes the serum leucine

aminopeptidase a marker of hepatic disorders.[64,65] The increase of LAP in human sera can also be diagnostically indicative of a number of cancers such as carcinoma of the pancreas and head and neck cancer.[66–68]

Inspired by these developments, herein we report the synthesis of eight glycine and serine AAPCs of highly potent amino-bearing structural variants incorporated within the combretastatin, dihydronaphthalene, and benzosuberene molecular scaffolds.[42,44,69,70] Furthermore, in order to enhance water solubility (and potentially bioavailability)[71] of these newly synthesized AAPCs, their eleven hydrochloride salts were synthesized.[50,52] The synthesis of compounds **13**, **15**, **37**, **39**, 3' CA4-*L*-serinamide and AVE8062 were previously reported,[15,36,47,52] and they were re-synthesized as a part of our ongoing biological studies. Each of the parent amino-based anti-cancer agents and their corresponding AAPCs were evaluated for their cytotoxicity against selected human cancer cell lines and for their ability to inhibit tubulin polymerization. In addition, these amino acid prodrug hydrochloride salts and two selected serinamide prodrugs (non-salts) were evaluated for their ability to undergo amide bond cleavage by LAP.[52]

Results and Discussion

2.1 Synthesis

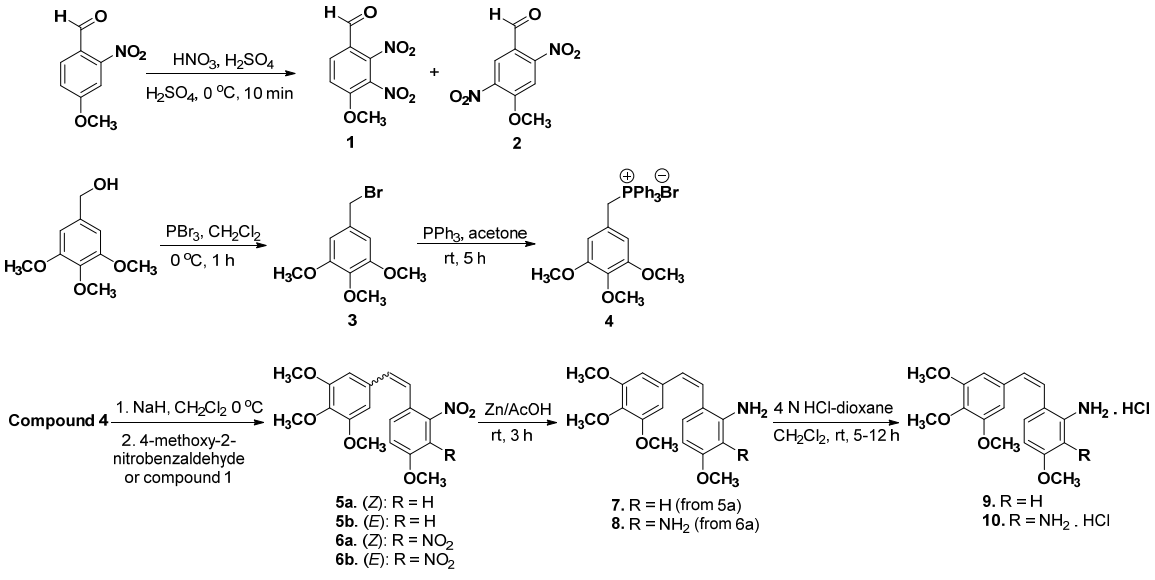
Twenty six compounds (including amino combretastatin, dihydronaphthalene and benzosuberene parent compounds, amino acid prodrugs, and their corresponding water soluble hydrochloride salts) were synthesized for this study. Among these, compounds **7-10**, **13**, **15**,[15,16] **32**,[42,43] **33**, **37**, **39**,[46,47] 3' CA4-

amine, 3' CA4-L-serinamide and AVE8062[36,52] were re-synthesized for the purpose of further biological evaluation.

2.1.1. Synthesis of Amino Acid Prodrug Conjugates of Amino Combretastatins

1. Synthesis of Combretastatin Amines

Our previous synthesis of 2' CA4-amine **7** involved a Wittig reaction between (4-methoxy-2-nitrobenzyl)triphenylphosphonium bromide and 3,4,5-trimethoxybenzaldehyde, followed by a reduction of the nitro group to form the corresponding amine using $Na_2S_2O_4$.[15,32] Later, this compound was synthesized and reported by another group as well.[34] Switching the Wittig reaction partners to 3,4,5-trimethoxybenzyltriphenylphosphoniumbromide **4** and 4-methoxy-2-dinitrobenzaldehyde (scheme **1**), followed by the separation of *Z* and *E*-isomers (1:0.4 ratio) and reduction of *Z*-isomer **5a** to amine **6a** using zinc[35] in acetic acid (AcOH) increased the overall yield for compound **7** about 4-fold over two steps.

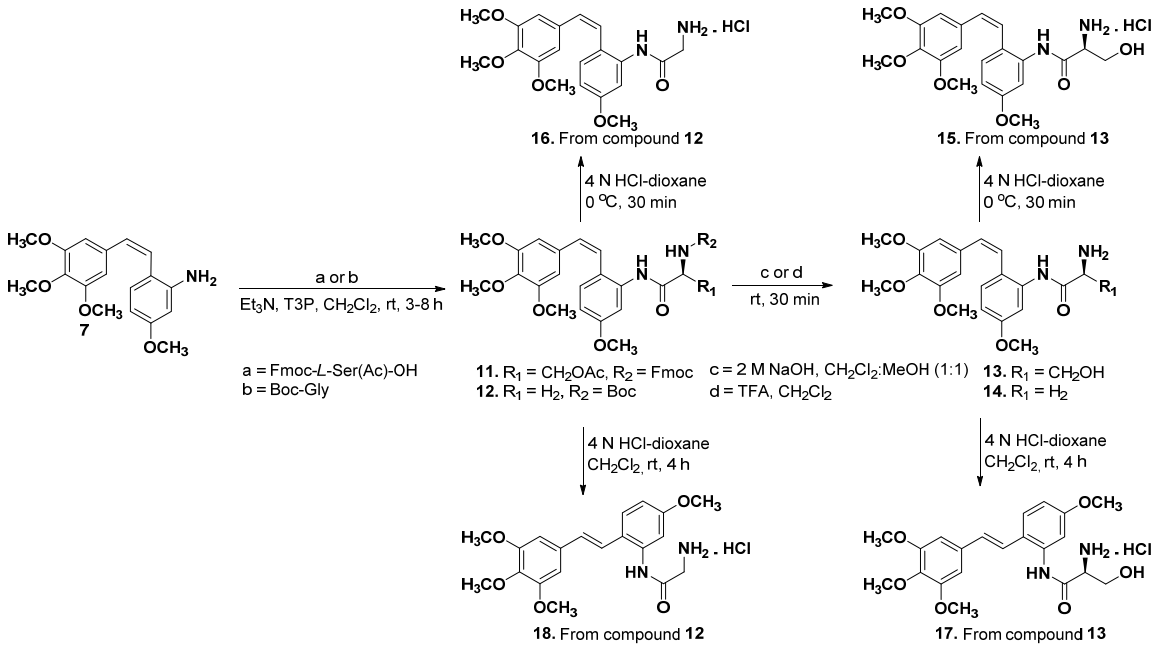


Scheme **2.1.** Synthesis of Combretastatin Amines[15,16]

The synthesis of CA1-diamine (**8,** scheme **1**) was achieved as reported earlier,[16] involving a key Wittig reaction between 3,4,5-trimethoxybenzyltriphenylphosphonium bromide **4** and 4-methoxy-2,3-dinitrobenzaldehyde **1**, followed by reduction of the nitro group of *Z*-isomer **6a** to achieve the desired target compound (CA1-diamine **8**). Upon treatment with HCl (4 N), these combretastatin-based amines (**7** and **8**) were converted to their corresponding hydrochloride salts (**9** and **10**).

2. Synthesis of Amino Acid Prodrug Conjugates

The serine and glycine amino acid prodrug conjugates (**13** and **14,** scheme **2**) of 2' CA4-amine **7** were prepared by coupling with Fmoc-*L*-ser(Ac)-OH and Boc-glycine-OH, respectively, in the presence of Et_3N and the peptide coupling reagent propylphosphonic anhydride (T3P), followed by deprotection.

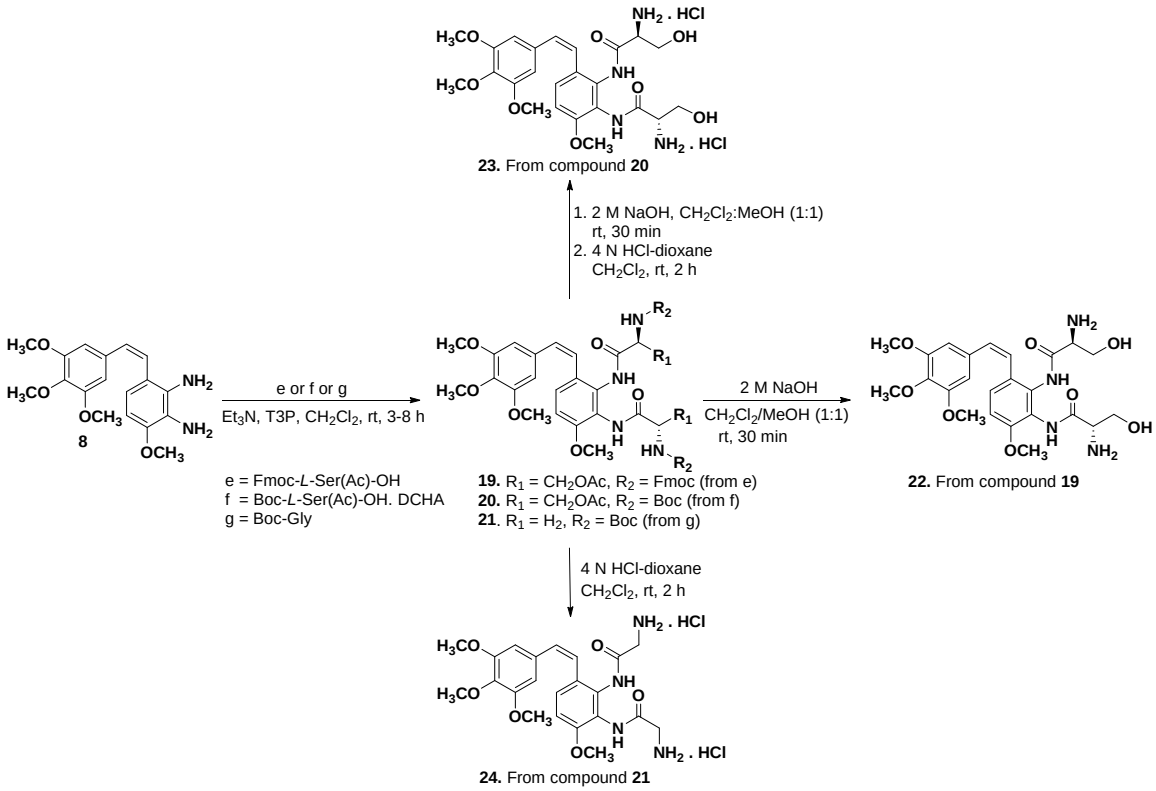


Scheme **2.2.** Synthesis of Amino Acid Prodrug Conjugates of 2' Combretastatin Amines[15,52]

This procedure is reminiscent of the synthetic strategy employed by Ohsumi and co-workers[52] for the synthesis of water-soluble amino acid prodrug salts of 3' CA4-amine[55] and our previous studies,[15,16] but this modified synthetic route has benefits in terms of higher yield and ease of purification from the use of T3P. Similarly, the serine and glycine amino acid prodrug conjugates (**19-21**, Scheme **3**) of CA1-diamine (**8**) were synthesized using a standard peptide coupling procedure (scheme **2**).

3. Synthesis of Hydrochloride Salts of Amino Acid Prodrug Conjugates

The synthesis of compound **15** is described in our earlier publication.[15] Our initial efforts to re-synthesize compound **15** and compound **16** (new compound) at room temperature in the presence of solvent resulted in the complete isomerization from *Z* to *E*, affording compounds **17** and **18**. These *E*-isomers were used as a model system to evaluate LAP mediated cleavage of their corresponding amino acid prodrugs. Utilizing solvent free reaction conditions,[72] the respective HCl salts of glycine and serine amino acid prodrug conjugates (**15** and **16**, scheme **2**) were synthesized from compounds **12** and **13**. The water-soluble hydrochloride salt of the glycine variant of CA1-diamine (**24**, scheme **2.3**) was obtained using a similar synthetic strategy with only about 10% isomerization to the *E*-isomer. Our initial attempts to prepare the hydrochloride salt of compound **22** were unsuccessful. However, treatment of compound **20** (scheme **2.3**) with NaOH (2 M) followed by HCl (4 N) yielded the desired CA1-diamine-based water-soluble amino acid prodrug conjugate (**23**) in a single step.



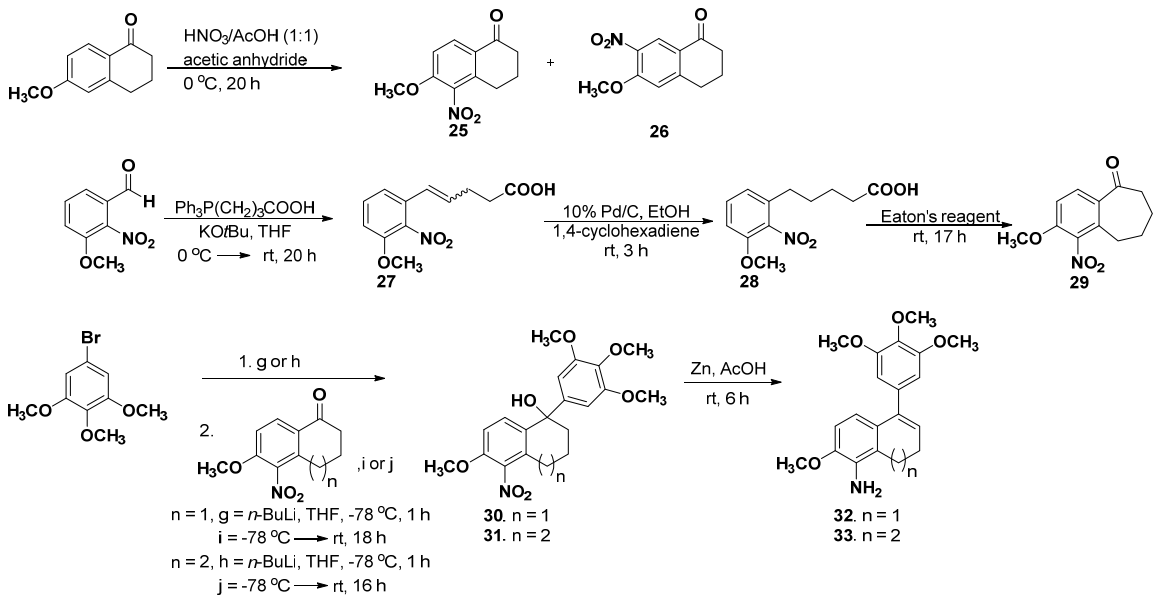
Scheme **2.3.** Synthesis of Amino Acid Prodrug Conjugates of Combretastatin Diamine[15,16,52]

1. Synthesis of Dihydronaphthalene and Benzosuberene Amines

The synthesis of amino dihydronaphthalene (**32**)[42,44] and amino benzosuberene (**33**),[45–47] as previously reported by our group, is illustrated in scheme **4**. Initially, 6-methoxy-1-tetralone was nitrated to form two constitutional isomers, with one isomer being the desired product, 5-nitro-6-methoxy-1-tetralone (**25**). Compound **32** was obtained by reaction of 5-bromo-1,2,3-trimethoxybenzene with *n*-butyllithium, followed by the addition of compound **25**.

2.1.2. Synthesis of Amino Acid Prodrug Conjugates of Amino Dihydronaphthalene and Amino Benzosuberene.

The reaction of compound **30** with Zn in the presence of AcOH resulted in reduction of the nitro group, and, following a subsequent condensation reaction, the desired product (**32**) was obtained in a 53% yield (over these two steps).

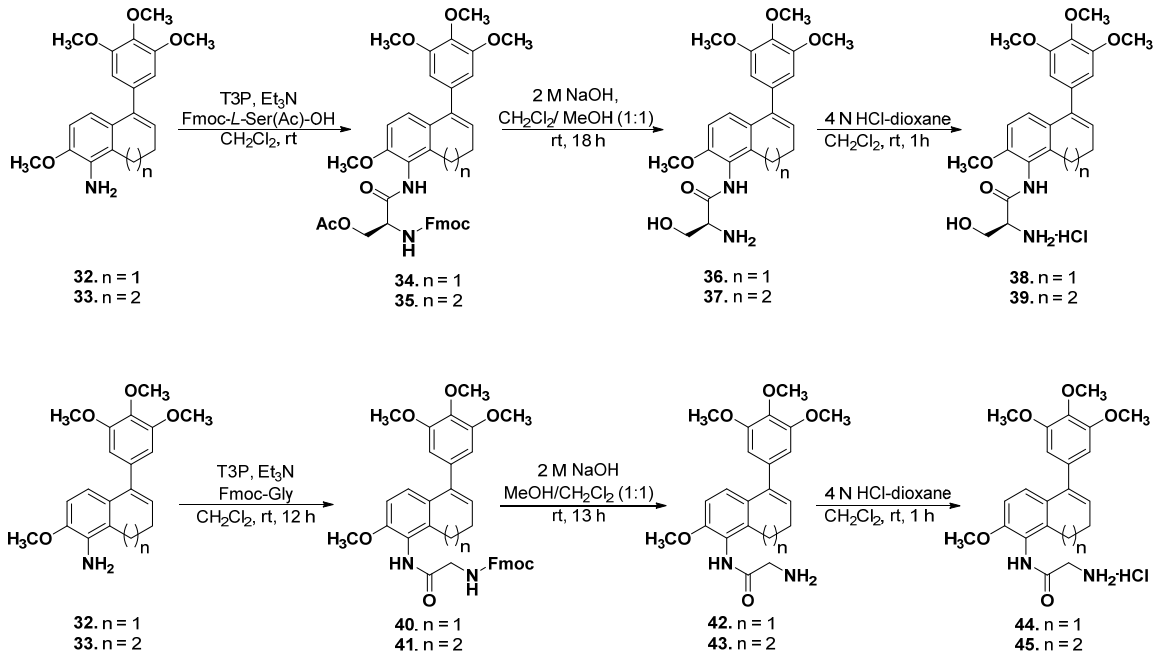


Scheme **2.4.** Synthesis of Dihydronaphthalene[42,44] and Benzosuberene Amines[42,45,46,73]

The synthesis of amino-benzosuberene KGP156 (**33**), initially reported by us in 2012,[46] utilized a sequential Wittig olefination, selective reduction with 1,4-cyclohexadiene, Eaton's reagent mediated cyclization, 1,2-addition of the appropriately functionalized aryl ring, followed by condensation and reduction.

2. Synthesis of Hydrochloride Salt of Amino Acid Prodrug Conjugates of Amino Dihydronaphthalene and Benzosuberene

To obtain the desired amino acid prodrugs of amino dihydronaphthalene (**32**), Fmoc-*L*-ser(Ac)-OH or Fmoc-gly-OH were reacted utilizing general peptide synthetic methodology. Compound **32** was treated with T3P, Et_3N, and the appropriate Fmoc-amino acid to obtain *N*-Fmoc protected amino acid amides (**34**) and (**40**), which upon deprotection resulted in the desired amino acid prodrugs (**36**) and (**42**). The AAPCs **37** and **39** (previously reported) were re-synthesized for this study.[73]



Scheme **2.5.** Synthesis of Amino Acid Prodrug Conjugates of Amino Dihydronaphthalene and Benzosuberene[45,47]

Similarly, the hydrochloride salts (**38**, **44, and 45**) of amino acids (**36**, **42,** and **43**, respectively), were obtained upon treatment with a 4 N HCl in dioxane solution as shown in scheme **5**.

2.2 Biological Evaluation

Each of the twenty six compounds synthesized for this study (including parent drugs, amino acid prodrugs, and their corresponding water soluble hydrochloride salts; compiled in Fig. **2.2**) were evaluated for their cytotoxicity against selected human cancer cell lines (Table **2.1**). Furthermore, these compounds were evaluated biologically for their ability to inhibit tubulin polymerization (cell free assay) and colchicine binding (Table **2.2)**. In addition, thirteen water soluble AAPCs (including two as their non-salt forms) were evaluated for enzymatic cleavage by LAP (Table **2.3** and Fig**. 2.3**).[37, 42–47]

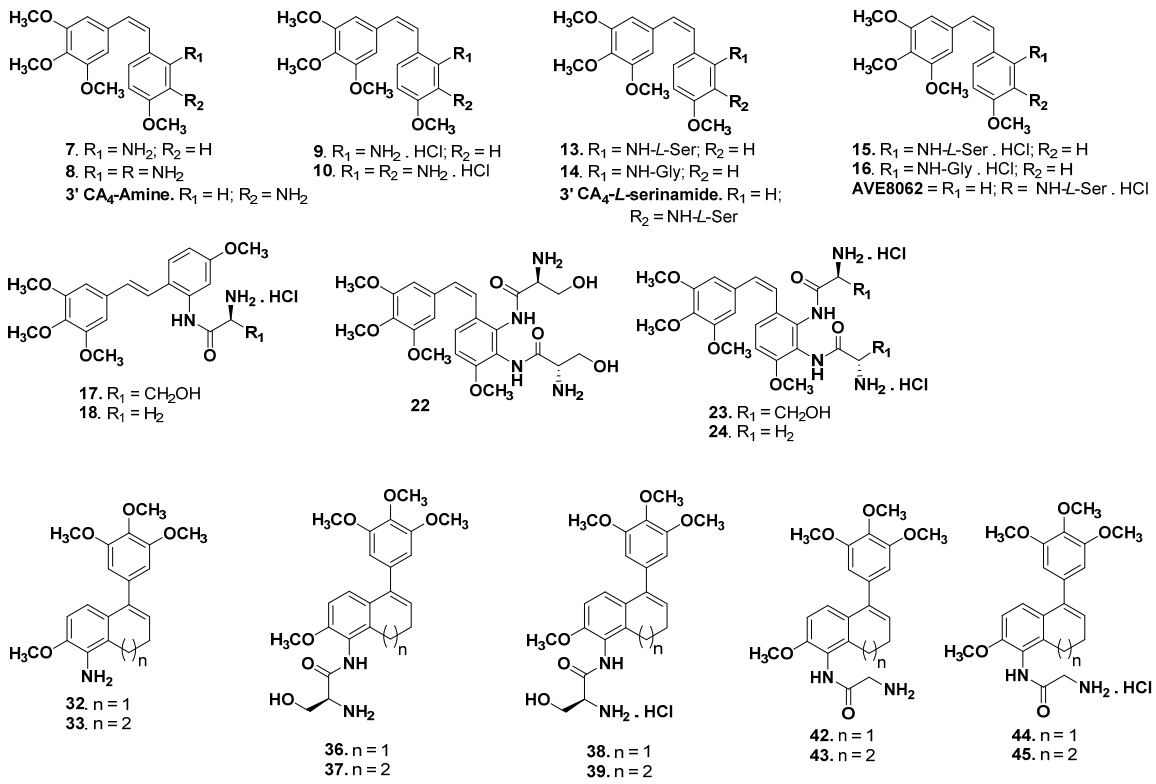


Figure **2.2.** Compilation of parent drugs and their amino-acid prodrug conjugates evaluated in this study

An endothelial tube disruption assay for CA1-diamine (compound **8,** Fig**. 2.2**) was carried out utilizing HUVECs (Fig. **2.4**). The hydrochloride salts of CA1-diamine (compound **10,** Fig**. 2.5**) and dihydronaphthalene glycinamide (compound **44**, Fig. 2.**2**) were initially evaluated for their *in vivo* ability to disrupt tumor blood flow utilizing dynamic bioluminescence imaging (BLI, Fig. **2.6** and **2.7**) studies.

2.2.1. Cytotoxicity, Inhibition of Tubulin Polymerization, and Percent Inhibition of Colchicine Binding

Cytotoxicity studies carried out with all twenty six compounds against selected human cancer cell lines (Table **2.1**) indicated that each of the parent drugs (compounds **7,**[15] **8,**[16] **32**[42,43] and **33**[46,73]) were highly potent with GI_{50} values ranging from sub-nanomolar to nanomolar.

The amino dihydronaphthalene **32** was found to be especially potent (GI_{50} = 1-3 nM across all three of the human cancer cell lines evaluated in this study) and was comparable to the clinically relevant agent CA4.[35] Both the serinamide and glycinamide AAPCs (compound **15** and **16**, respectively) of the 2' CA4-amine **7** demonstrated significant cytotoxicity against each of the cancer cell lines. When comparing the *E*-isomers **17** and **18**, the glycinamide AAPC **18** was more cytotoxic than the corresponding serinamide AAPC **17**. Negligible cytotoxicity was observed for the *bis*-substituted AAPCs (**22-24**). The serinamide AAPCs (**36-39**) of the parent compounds **32** (KGP05) and **33** (KGP156) demonstrated limited cytotoxicity against these cancer cell lines, while the glycinamide analogues (**42-45**) were more active.

Table **2. 1.** Cytotoxicity data for the amino-based combretastatin, dihydronaphthalene, and benzosuberene analogues against human cancer cell lines [SK-OV-3 (ovarian), NCI-H460 (lung), and DU-145 (prostate)]

Compound	GI_{50} (μM) SRB Assay[a]		
	SK-OV-3	NCI-H460	DU-145
CA1[b]	0.0384±0.0242	0.0153±0.0158	0.0326±0.0173
CA4[c]	0.00506±0.000145	0.005000±0.000359	0.00602±0.000661
3' CA4-amine[d]	0.00445±0.000870	0.00449±0.000894	0.00330±0.000281
3' CA4-*L*-serinamide[d]	0.00902±0.00481	0.0241±0.00200	0.0164±0.00729
AVE8062[d]	0.00396±0.000912	0.00387±0.000297	0.00334±0.000261
7[e]	0.0266±0.0226	0.0401±0.0171	0.0331±0.0214
8[f]	0.0235±0.0234	0.0359±0.0218	0.0391±0.0265
9	0.00735±0.00350	0.0263±0.0161	0.0285±0.0232
10[f]	0.0228±0.0152	0.124±0.116	0.205±0.241
13[e]	0.0395±0.0211	0.0333±0.0190	0.0347±0.00827
14	0.0323±0.00638	0.0532±0.00523	0.0436±0.00243
15[e]	0.0516±0.00688	0.0317±0.00487	0.0484±0.00336
16	0.0485±0.00853	0.0292±0.00567	0.0447±0.00353
17	2.29±1.17	2.24±1.45	4.55±2.70
18	0.427±0.111 (D) 0.426±0.0941 (W)	0.418±0.137 (D) 0.380±0.0602 (W)	0.630±0.306 (D) 0.674±0.255 (W)
22	25.9±2.55	24.4±4.42	33.6±2.60
23	>86.6	>86.6	>86.6
24	39.3±13.5 (D) >56.4 (W)	>68.5 (D) >96.6 (W)	>64.7 (D) >90.4 (W)
32[g]	0.000308±0.000402	0.000290±0.000126	0.000111±0.000114
33[h]	0.000137	0.00185	0.00226
36	13.7±9.70	31.1±9.94	40.6±32.3
37	44.9±4.10	45.9±1.79	>81.3
38	11.6±10.2	18.6±7.67	26.0±15.7
39	47.0±21.4	53.8±0.864	69.4±31.3
42[i]	0.0138±0.00754	0.0365±0.000899	0.0501±0.00480
43	0.195±0.0319	0.566±0.0382	0.507±0.188
44	0.0485±0.0325	0.0420±0.00372	0.0585±0.0120
45	0.203±0.0294	0.567±0.0507	0.773±0.302

[a] Average of n ≥ 3 independent determinations
[b] Data from ref. [74], see ref. [14] for additional data
[c] For additional data, see ref. [14]
[d] For additional data, see ref. [15,36,37,52]
[e] For additional data, see ref. [15]
[f] For additional data, see ref. [16]
[g] For additional data, see ref. [42,43]
[h] For additional data, see ref.[46,73]
[i] For additional data, see ref. [73]
D = compound dissolved in DMSO
W = compound dissolved in water

Pronounced inhibition of tubulin polymerization and inhibition of colchicine binding were observed for amino-dihydronaphthalene **32** (assembly IC_{50} = 0.62 μM and 92% inhibition of colchicine binding at 5 μM, respectively, Table **2.2**). Similarly, the other parent compounds **7**, **8** and **33** proved highly potent in these tubulin assays (ranging from 1.1 μM to 1.5 μM and 76% to 88% at 5 μM, respectively).

Table **2.2**. Inhibition of tubulin polymerization and percent inhibition of colchicine binding

Compound	Inhibition of Tubulin Polymerization IC_{50} (μM) ±SD	Inhibition of Colchicine Binding (% Inhibition±SD)	
		1 μM	5 μM
CA1	1.9[a]	ND	99.6±0.7[b]
CA4	1.2[a]	84±3[b]	98±0.007[b]
3' CA4-amine[c]	1.2±0.06[c]	84±2	97±2
3' CA4-*L*-serinamide[c]	14±1[d]	ND	ND
AVE8062[c]	13 ± 0.3[d]	ND	ND
7	1.1±0.06[d]	55±4	88±0.9
8	1.5±0.1[e]	55±0.1	87±0.2
9	2.5±0.2	ND	75±0.9
10	2.3±0.2[e]	46±2	86±4
32[f]	0.62±0.02	65±4	92±0.2
33	1.3±0.1[g]	ND	82±2

[a]Data from ref.[16] see ref.[14] for additional data
[b]Data from ref. [14]
[c]For additional data, see ref.[15]
[d]For additional data, see ref. [15]
[e]For additional data, see ref. [16]
[f]For additional data, see ref.[42,43]
[g]For additional data, see ref. [46,73]
ND = Not determined for this study

Uniformly, the AAPCs (compounds **13**-**18**, **22**-**24**, **36**-**39** and **42**-**45**) proved to be inactive (IC_{50} > 20 μM) as inhibitors of tubulin assembly, which was anticipated in this type of cell free assay.

2.2.2. Enzymatic Assay

Preliminary studies were carried out to determine the ability of LAP to cleave individual amino acid prodrug conjugates (Table **2.3**). The mono-glycinamide prodrugs **16**, **18**, **44**, **45** were quantitatively cleaved to their parent compounds. Treatment of the *bis*-glycinamide prodrug (**24**) with LAP (0.12 units) resulted in disappearance of greater than 95% of the prodrug by 25 h, but only 9% of the parent CA1-diamine (compound **8)** was formed.

Table **2.3.** Activity of LAP toward amino acid prodrug conjugates

Compound	Prodrug Type	% Cleavage[a]	Specific Activity of LAP (μM/min/enzyme unit)
15	Serinamide	62%	1.2
16	Glycinamide	100%	180
17	Serinamide	90%	C[e]
18	Glycinamide	100%	C[e]
22	Serinamide	NC	ND
23	Serinamide	NC	ND
24	Glycinamide	C[b]	ND
38	Serinamide	C[c], <10%	ND
39	Serinamide	C[c], <10%	ND
44	Glycinamide	100%	15.5
45	Glycinamide	100%	0.12
3' CA4-*L*-serinamide	Serinamide	C[d], 95%	ND
AVE8062	Serinamide	C[d], 95%	ND

[a]:Percentage of cleavage is calculated based on the area ratio of the parent drug and prodrug at a specific wavelength.
NC: No cleavage observed using 1.5 units LAP
C: Cleaved (a rate study was not carried out)
C[b], disappearance of the *bis*-glycinamide prodrug **24** was observed, but less than 50% of the expected final product **8** was detected
C[c], the serinamide prodrug was cleaved, but less than 10% of the expected product was obtained
C[d], used as positive controls (see ref 52 for additional info)
C[e], the prodrug was readily cleaved, but the absolute rate could not be determined because the parent compound (*E*-isomer of 2'-CA4-amine) was not available since isomerization (*Z* to *E* took place as a byproduct during salt formation after installation of the amino acid prodrug)
ND: Not determined

Two additional peaks, presumably the mono-glycinamide intermediates, were observed in the HPLC chromatogram. Using 1.5 and 3.7 units of LAP, the amount of CA1 diamine (compound **8)** detected increased to 19% and 24% respectively. The mono-serinamide prodrugs of both the *Z* and *E*-isomers (**15** and **17**, respectively) of 2' CA4-amine were effectively cleaved by LAP, as was the mono-serinamide derivative of 3' CA4-amine and its hydrochloride salt (AVE8062), used as positive controls.[52] Only a partial release (less than 10%) was achieved with excess LAP (3.6 units, 24 h) for the mono-serinamide analogues of the amino dihydronaphthalene **38** and amino benzosuberene **39** compounds. Treatment of the *bis*-serinamide prodrug **22** of CA1-diamine or its dihydrochloride salt **23** with a large amount (1.5 units) of LAP did not result in any observable cleavage. For AAPCs that produced more than 50% of the final product upon cleavage with LAP in preliminary experiments, rate studies were conducted. The glycinamide prodrug of the *Z*-isomer 2' CA4-amine **16** was cleaved at a rate of 180 μM/min/enzyme unit (Fig**. 2.3. A, B**), 150 times faster than its corresponding serinamide analogue **15 (**1.2 μM/min/enzyme unit). A similar result was observed for the glycinamide prodrug of the *E*-isomer 2' CA4-amine **18**, which was cleaved at a rate more than 100 times faster than its serinamide analogue **17**, as determined by the relative rates of cleavage of each prodrug. Differences were also found in the rate of cleavage of the glycinamide prodrugs of amino dihydronaphthalene **44 (**15.5 μM/min/enzyme unit, Fig**. 2.3. C, D**) and amino benzosuberene **45 (**0.12 μM/min/enzyme unit).

Despite a significantly faster cleavage of the glycinamide prodrug **16** versus the serinamide **15** by LAP, the cytotoxicity toward cancer cell lines over a 48 h

treatment was very similar for both prodrugs and the parent Z-isomer 2' CA4-amine **7** (Table **2.1**), indicating that release of compound **7** from both prodrugs in the presence of these cancer cell lines was efficient.

The slower rate of cleavage for the *E*-isomer series resulted in a differential cytotoxicity for the serinamide **17** and glycinamide **18** prodrugs. The lack of cleavage by LAP of the *bis*-serinamide prodrugs **22** and **23** and the very low production of parent CA1-diamine (compound **8)** from the *bis*-glycinamide **24** was reflected in the lack of cytotoxicity for all three compounds (**22**-**24**). This may indicate steric hindrance in the interaction of these compounds with LAP. There is a marked difference in the LAP cleavage rates between glycinamide prodrugs (compounds **44** and **45**) that was reflected in their differential cytotoxicity in cancer cell lines, and their cytotoxicity was significantly less than that of either of the corresponding parent compounds (KGP05, KGP156, respectively).

Overall, the cancer cell line cytotoxicity mirrored the cleavage by LAP of the amino acid prodrug conjugates. In amino acid amides and peptides, an *N*-terminal L-serine residue is preferred over glycine, but there is clearly a contribution of binding interactions, steric factors and stereospecificity (L vs. D) in the position adjacent to the *N*-terminal amino acid that is observed in peptide substrates and in some non-peptidic amino acid conjugates.[75,76] For example, Pettit et al. reported that the serinamide prodrug of 3' CA2-amine displayed significantly less activity compared to the glycine derivative in a cancer cell line panel.[35]

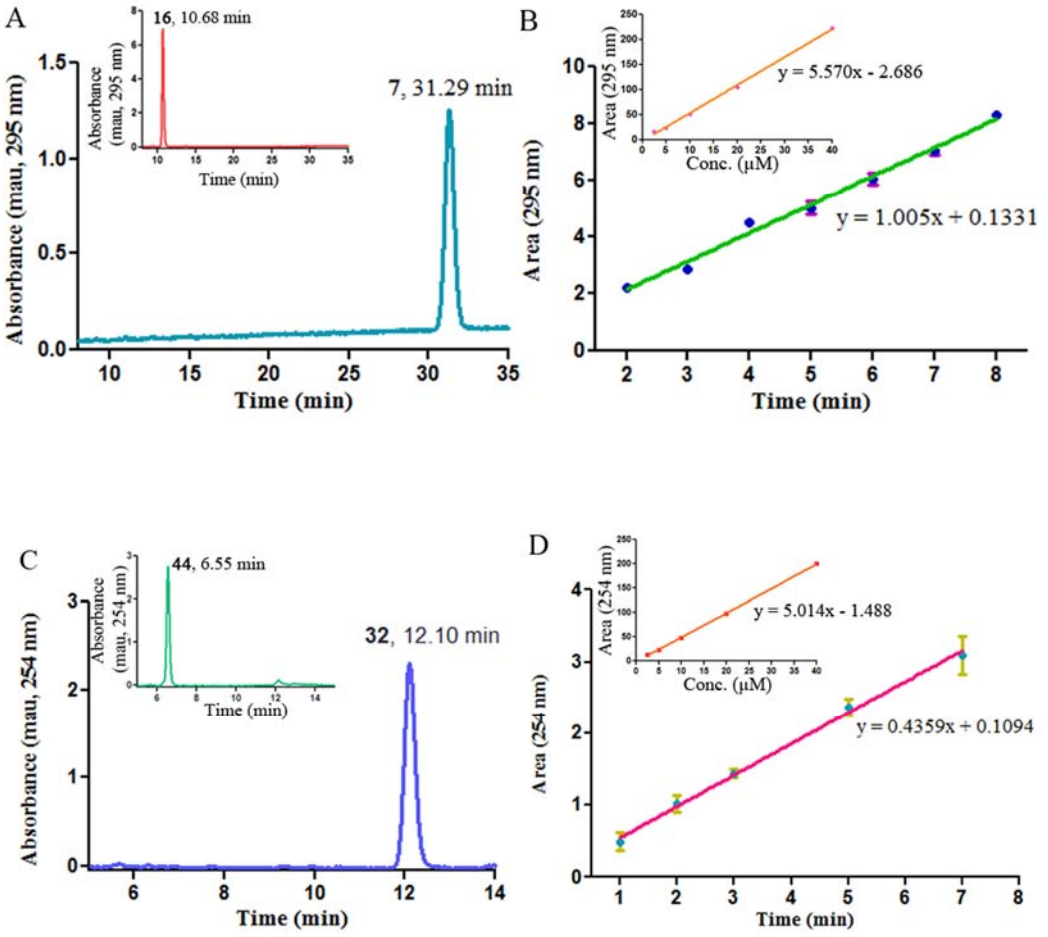


Figure 2.3. Cleavage of the glycine conjugates of 2' CA4-amine **16** and the amino dihydronaphthalene **44** by LAP. (A) Prodrug **16** was treated with 0.5 units of LAP for 40 h. A single peak corresponding to the product 2' CA4 amine **7** (t_R = 31.29 min) was observed (HPLC chromatogram mobile phase: 26% acetonitrile/74% water containing 0.05%TFA). Inset: Control, compound **16** was incubated for 40 h without LAP. (B) Rate study for the cleavage of prodrug **16** to form 2' CA4 amine **7**. Inset: Calibration curve for 2' CA4 monoamine **7**. (C) Prodrug **44** was treated with 0.05 units of LAP for 2 h. A single peak corresponding to the product amino dihydronaphthalene **32** (t_R = 12.10 min) was observed (HPLC chromatogram mobile phase: 28% acetonitrile/72% water containing 0.05%TFA). Inset: Control, compound **44** was incubated for 2 h without LAP. (D) Rate study for the cleavage of prodrug **44** to form amino dihydronaphthalene **32.** Inset: Calibration curve for amino dihydronaphthalene **32**

2.2.3. Endothelial Tube Disruption Assay[77]

Rapidly growing HUVECs can be induced to form a capillary-like network of tubules, which serves as a model for evaluating VDAs. CA1-diamine (compound **8**) and its hydrochloride salt (compound **10**) demonstrated significant disruption of a capillary-like network of tubules (from HUVECs) at a concentration of 0.1 μM. Our previous work shows a significant tubule disruption and cell rounding effect for compound **33** (KGP156) at 0.1 μM, and this effect was greatly enhanced at a 1 μM concentration.[46]

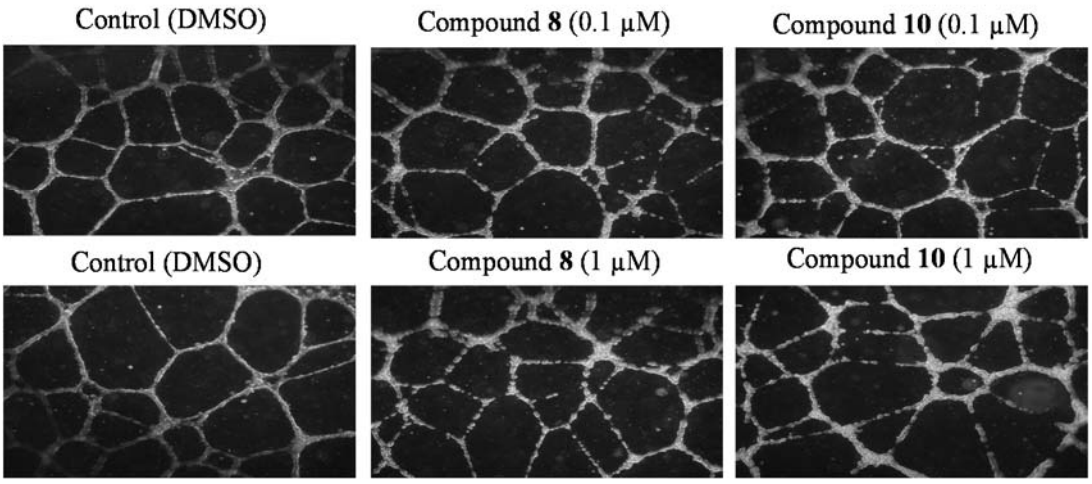


Figure 2.4. HUVEC Tubule Disruption at Various Inhibitor Concentrations

2.2.4. Dynamic Bioluminescence Imaging [77]

Bioluminescence Imaging (BLI) represents a valuable indirect *in vivo* technique for the assessment of tumor-specific vascular damage in response to treatment with VDAs.[9,78,79] A decrease in light flux emission at various time points post VDA administration presumably results from an inability of the injected

luciferin to reach the tumor (grown from luciferase expressing transfected cancer cells) due to VDA-induced damage of the vasculature feeding the tumor. Treatment (Fig. **2.5**) of an MDA-MB-231-luc human breast tumor induced in a SCID mouse model with the diamino-CA1 salt (compound **10**) at a dose of 80 mg/kg resulted in approximately 95% reduction in light emission [at 17 min time-point (midpoint)] at 2 h post VDA treatment relative to baseline. This reduction was still maintained (94% decrease) at the 4 h time point (fresh luciferin injection but no further VDA).

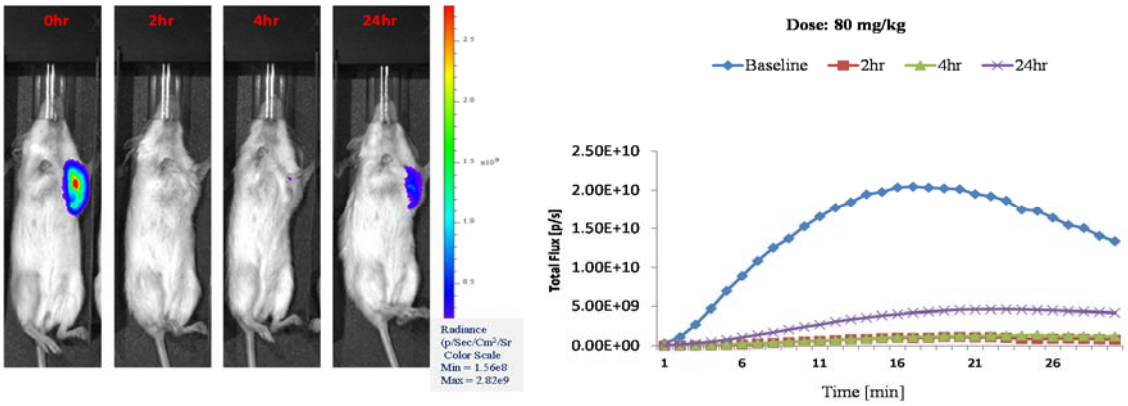


Figure 2. 5. Dynamic BLI of Tumor in Response to Treatment with Compound **10**.

There was some recovery (79% reduction, compared to baseline) at 24 h, which is typical due to the so-called viable rim.[9] The glycinamide dihydronaphthalene AAPC (**44**) demonstrated (Fig. **2.6**) similarly promising results (97% and 90% decrease in BLI signal at 4 and 24 h, respectively) when administered at a dose of 15 mg/kg, while the signal reduction at a dose of 10 mg/kg was 70% at 4 h and 55% at 24 h. In this experiment, the control mouse (saline administration) demonstrated a 22% decrease in signal at 4 h and 24% reduction at 24 h. These results, which are comparable to the clinically relevant VDA, CA4P (Fig.

2.7), provide further preliminary evidence suggesting that compounds **10** and **44** function as water-soluble VDAs and that glycinamide **44** is capable of being converted in vivo (presumably through the action of LAP) to its corresponding tubulin-active parent compound KGP05 (compound **32**), which then causes the tumor-specific vascular damage.

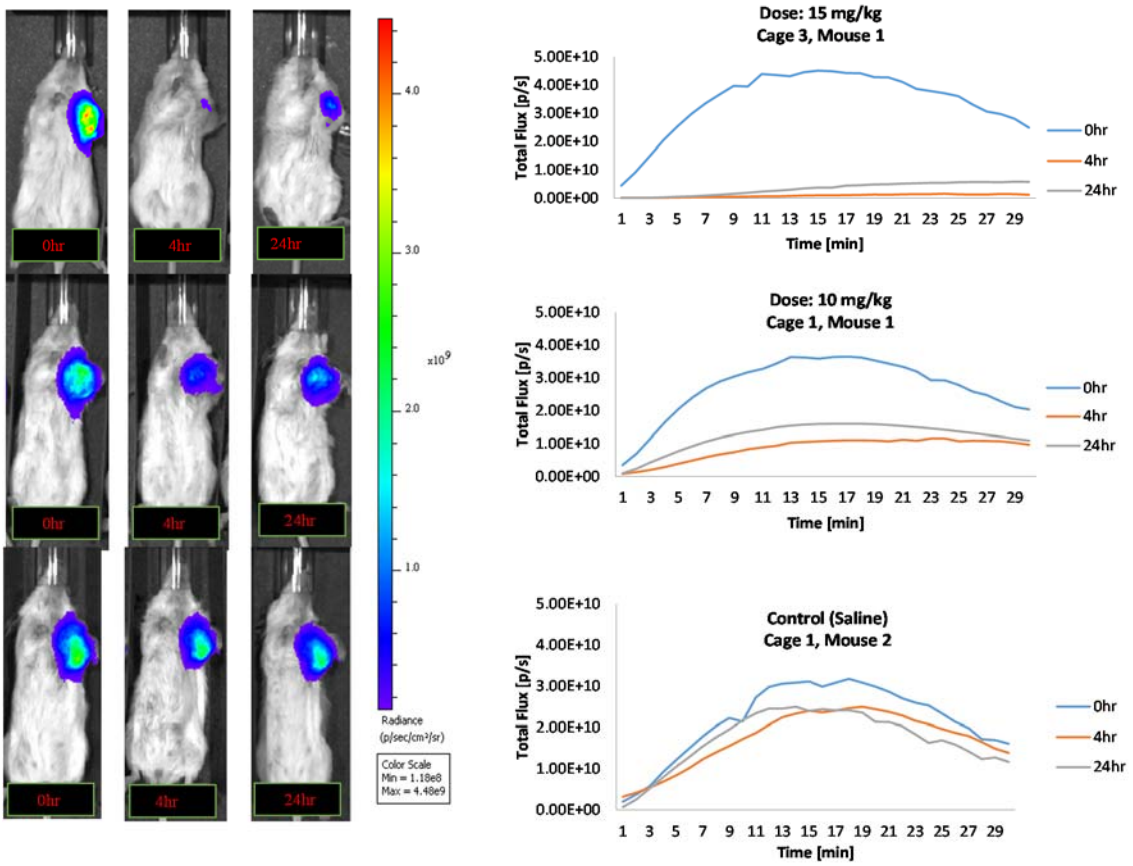


Figure 2.6. Dynamic BLI of Tumor in Response to Treatment with Compound **44**.

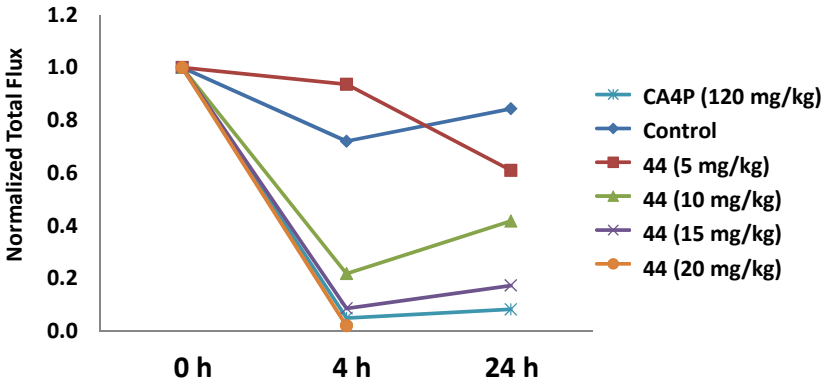


Figure 2.7. Dose Escalation Data for BLI Evaluation of Compound **44.** CA4P and compound **44** (at 10 or 15 mg/kg) each gave significantly less signal at both 4 h and 24 h compared with baseline. Signal from the saline group was significantly reduced at the 4 h time point. At the 4 h time point compound **44** (5 mg/kg) was not significantly different from saline control, but was significantly different ($p<0.05$) from compound **44** (10 mg/kg) and compound **44** (15 mg/kg). Similarly compound **44** (15 mg/kg) was significantly different from saline and compound **44** (5 mg/kg), but not compound **44** (10 mg/kg). In general it appeared that an increase of 10, but not 5 mg /kg generated significantly greater response, as judged by reduced light emission. CA4P was significantly different from saline, compound **44** (5 mg/kg), and compound **44** (10 mg/kg), but not compound **44** (15 mg/kg) or compound **44** (20 mg/kg)

Conclusions

Eleven water-soluble amino acid prodrug conjugates (AAPCs) derived from parent amino combretastatin, dihydronaphthalene, and benzosuberene derivatives (**7**, **8**, **32** and **33**) were designed and synthesized. Each of the parent compounds (**7**, **8**, **32** and **33**) were potent inhibitors of tubulin assembly binding to the colchicine site and were strongly cytotoxic against human cancer cell lines [SK-OV-3 (ovarian), NCI-H460 (lung), and DU-145 (prostate)]. Without exception, the glycinamide AAPCs based on the dihydronaphthalene and benzosuberene molecular scaffolds demonstrated efficient cleavage by LAP and were superior in this regard to their serinamide counterparts. Cytotoxicity mirrored the relative cleavage of these agents

by LAP. The story was slightly different for the AAPCs derived from the combretastatins in that both the glycinamide and serinamide AAPCs (**15**-**18**) were efficiently cleaved by LAP for both the 2' and 3' amino variants, but the diamine-CA1 derived glycinamide (**24**) and serinamide (**22** and **23**) were resistant to LAP mediated cleavage to regenerate the parent compound (**8**). CA1-diamine (**8**) and its corresponding hydrochloride salt (**10**) caused significant disruption to a network of HUVECs growing on matrigel, and both CA1-diamine salt **10** and dihydronaphthalene glycinamide **44** demonstrated a significant reduction in light emission in a BLI study in SCID mice bearing the luciferase expressing MDA-MB-231-luc human breast cancer cell line induced tumor. These results demonstrate that these compounds can function as water-soluble VDAs.

Experimental Section

4.1. Chemistry

General Materials and Methods

AcOH, acetic anhydride, acetonitrile, CH_2Cl_2, dimethylformamide (DMF), ethanol, methanol, HNO_3, H_2SO_4, and tetrahydrofuran (THF) were used in their anhydrous forms or as obtained from the chemical suppliers. Reactions were performed under N_2. Thin-layer chromatography (TLC) plates (precoated glass plates with silica gel 60 F254, 0.25 mm thickness) were used to monitor reactions. De-ionized (DI) water was used to quench and wash the reaction mixture as appropriate. Purification of intermediates and products was carried out with a Biotage Isolera flash purification system using silica gel (200−400 mesh, 60 Å) or RP-18 pre-packed columns or manually in glass columns. Intermediates and

products synthesized during this study 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. Spectral data were recorded in $CDCl_3$, D_2O, $(CD_3)_2CO$, DMSO-d6, or CD_3OD. All chemical shifts are expressed in ppm (δ), coupling constants (J) are presented in Hz, and peak patterns are reported as broad singlet (bs), singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), double doublet (dd), triplet of doublets (td), doublet of triplets (dt) 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 (λ = 190−400 nm), a Zorbax XDB-C18 HPLC column (4.6 mm Å~ 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; method A: solvent A, acetonitrile, solvent B, 0.1% TFA in H_2O; or method B: solvent A, acetonitrile, solvent B, H_2O; gradient, 10% A/90% B to 100% A/0% B over 0 to 40 min; post-time 10 min; or method C: solvent A, acetonitrile, solvent B, 0.1% TFA in H_2O; isocratic, 10% A/90% B over 0 to 5 min, 10% A/90% B to 100% A/0% B over 5 to 25 min; post-time 5 min, flow rate 1.0 mL/min; injection volume 20 μL; monitored at wavelengths of 210, 254, 230, 280, and 360 nm. Mass spectrometry was carried out under positive or negative electrospray ionization (ESI) using a Thermo Scientific LTQ Orbitrap Discovery instrument. Compound numbering for the combretastatin molecular scaffold followed this protocol: the trimethoxy A ring was numbered 1-6, and the B ring was numbered 1'-6'. The ethylene bridging atoms were numbered as 1a and 1a' respectively for the carbons connected to the A ring and B ring, respectively.

Synthesis of Amino Acid Prodrug Conjugates of Amino Combretastatins

4.1.1. Nitration of aldehyde[16]

4-Methoxy-2-nitrobenzaldehyde (1.53 g, 8.45 mmol) was dissolved in concentrated H_2SO_4 (25 mL). To this solution, a pre-cooled mixture of HNO_3 and H_2SO_4 (3.85 mL/2.85mL) was added drop wise. The reaction mixture was stirred for 10 min, and then the resulting solution was added drop wise into ice-water (100 mL). After stirring for 2 h at 0 ^oC, the solution was filtered, and the solid containing the desired product was rinsed with ice-water (10 mL). Purification by flash column chromatography (40% EtOAc/hexanes) yielded regio-isomers **1** and **2.**

4-methoxy-2,3-dinitrobenzaldehyde (1). This compound was isolated as a yellow solid (0.879 g, 3.89 mmol, 46%). ^1H NMR (500 MHz, $CDCl_3$): δ 9.95 (1H, s), 8.15 (1H, d, *J* = 8.9 Hz), 7.40 (1H, d, *J* = 8.9 Hz), 4.09 (3H, s), ^{13}C NMR (125 MHz, $CDCl_3$): δ 184.05, 155.69, 133.11, 128.96, 121.04, 115.92, 114.53, 57.88.

4-methoxy-2,5-dinitrobenzaldehyde (2). This compound was isolated as a colorless solid (0.725 g, 3.20 mmol, 38%). ^1H NMR (500 MHz, $CDCl_3$): δ 10.30 (1H, s) 8.43 (1H, s), 7.74 (1H, s), 4.15 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 184.63, 155.99, 127.12, 123.78, 123.04, 109.63, 57.89.

4.1.2. Synthesis of Wittig salt[80]

3,4,5-Trimethoxybenzyl bromide (3). The mixture of 3,4,5-trimethoxybenzyl alcohol (20.1 g, 101 mmol) and PBr_3 (4.80 mL, 50.7 mmol) in anhydrous CH_2Cl_2 (100 mL) was stirred for 1 h at 0 ^oC. Water (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and removed by evaporation under reduced

pressure. After the recrystallization of the crude solid with EtOAc/hexanes, the bromide **3** (23.6 g, 90.3 mmol, 89%) was obtained as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 6.62 (2H, s) 4.47 (2H, s), 3.87 (6H, s), 3.85 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$): *δ* 153.3, 138.2, 133.2, 106.1, 60.9, 56.1, 34.3.

3,4,5-Trimethoxybenzyltriphenylphosphonium bromide (4).[80] A mixture of bromide **3** (11.0 g, 42.1 mmol) and PPh_3 (12.1 g, 46.3 mmol) in anhydrous acetone (100 mL) was stirred for 5 h. The resulting suspension was filtered through a Buchner funnel, and the solid was washed with acetone (100 mL) followed by hexanes (50 mL) to afford an off-white solid. The solid was dried in vacuum to obtain the phosphonium salt **4** (20.3 g, 38.2 mmol, 92%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 – 7.64 (9H, m), 7.58 – 7.50 (6H, m), 6.43 (2H, d, *J*=2.6 Hz), 5.29 (2H, d, *J*=14.1 Hz), 3.70 (3H, s), 3.43 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$): ^{13}C NMR (151 MHz, $CDCl_3$): δ 152.91 (d, *J*=3.6 Hz), 137.49 d, *J*=3.7 Hz), 134.84 (d, *J*=2.9 Hz), 134.58 (d, *J*=9.8 Hz), 129.99 (d, *J*=12.5 Hz), 122.44 (d, *J*=8.9 Hz), 117.76 (d, *J*=85.7 Hz), 108.76 (d, *J*=5.2 Hz), 60.83 (d, *J*=2.3 Hz), 56.16 (s), 30.72 (d, *J*=46.7 Hz) ^{31}P NMR (240 MHz, $CDCl_3$): δ 23.2.

4.1.3. Procedure for the synthesis of *Z*- and *E*-stilbenes (5a and 5b).[15,16]

At 0 ^{o}C, a NaH suspension (0.478 g, 19.9 mmol) in dry CH_2Cl_2 (50 mL) was stirred for 10 min. The previously prepared solution of 3,4,5-trimethoxybenzylphosphonium bromide (1.73 g, 3.30 mmol) was added drop wise. On addition, the color of solution changed to bright orange. After stirring for 20 min, 4-methoxy-2-nitrobenzaldehyde (0.501 g, 2.76 mmol) was added. The resulting mixture was stirred for 5 h. At this point, ice-water was added carefully until H_2 evolution stopped. Workup of the

reaction mixture was carried out by extraction with CH_2Cl_2 (2 × 25 mL), followed by washing the combined organic layers with water (twice) and brine and finally drying over Na_2SO_4. The *Z*-and *E*-isomers were isolated after flash column chromatography (40% EtOAc/hexanes) and re-crystallization of the *Z* and *E*-isomer mixture from EtOAc and hexanes.

(*Z*)-2-(4'-Methoxy-2'-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (5a). This compound was isolated as a yellow solid (0.690 g, 1.99 mmol, 72%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.59 (1H, d, *J*=2.7 Hz), 7.24 (1H, d, *J*=8.6 Hz), 7.01 (1H, dd, *J*=8.6, 2.6 Hz), 6.80 (1H, d, *J*=11.9 Hz), 6.62 (1H, d, *J*=11.9 Hz), 6.29 (2H, s), 3.87 (3H, s), 3.81 (3H, s), 3.62 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.0, 152.9, 148.6, 137.4, 133.4, 131.5, 131.2, 125.9, 125.7, 120.0, 108.7, 106.3, 60.9, 55.9, 55.8.

(*E*)-1,2,3-Trimethoxy-5-(4-methoxy-2-nitrostyryl)benzene (5b). This compound was isolated as an orange solid (0.239 g, 0.692 mmol, 25%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.67 (1H, d, *J*=8.8 Hz), 7.47 (1H, d, *J*=2.7 Hz), 7.44 (1H, d, *J*=16.0 Hz), 7.16 (1H, dd, *J*=8.7, 2.7 Hz), 6.92 (1H, d, *J*=16.0 Hz), 6.74 (2H, s), 3.91 (6H, s), 3.90 (3H, s), 3.87 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$): δ 159.1, 153.4, 148.3, 138.4, 132.5, 132.2, 129.1, 125.5, 122.8, 120.4, 108.9, 103.9, 61.0, 56.2, 55.9.

4.1.4. Procedure for the synthesis of *Z*- and *E*-stilbenes (6a and 6b). These compounds were synthesized using the procedure as described for compounds **5a** and **5b.** Starting from NaH (1.57g, 65.4 mmol), 3,4,5-trimethoxybenzylphosphonium bromide (5.71 g, 17.6 mmol), and 4-methoxy-2,3-dinitrobenzaldehyde (2.24 g, 9.90 mmol) in CH_2Cl_2 (150 mL), the desired *Z* and *E*-isomers were isolated after flash column chromatography (50% EtOAc/hexanes).

(*Z*)-2-(4'-Methoxy-2',3'-dinitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (6a). This compound was isolated as a yellow solid (0.818 g, 2.09 mmol, 52%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35 (1H, d, *J* = 8.9 Hz), 7.08 (1H, d, *J* = 8.9 Hz), 6.77 (1H, d, *J* = 11.8 Hz), 6.50 (1H, d, *J* = 11.8 Hz), 6.30 (2H, s), 3.95 (3H, s), 3.83 (3H, s), 3.69 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$): δ 153.1, 150.9, 143.0, 137.9, 135.2, 134.5, 134.3, 130.7, 124.5, 121.6, 115.9, 106.0, 60.9, 57.4, 56.0.

(*E*)-2-(4'-Methoxy-2',3'-dinitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (6b). This compound was isolated as an orange solid (0.630 g, 1.61 mmol, 40%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.84 (1H, d, *J* = 9.0 Hz), 7.25 (1H, d, *J* = 9 Hz), 6.96 (2H, dd, *J* = 38.2, 16.0 Hz), 6.69 (2H, s), 4.01 (3H, s), 3.91 (6H, s), 3.88 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 153.7, 151.0, 142.3, 139.3, 135.0, 134.6, 131.5, 130.0, 124.3, 118.8, 116.3, 104.4, 61.1, 57.5, 56.4.

4.1.5. General procedure for the reduction of nitro groups to amines[15,16]

(*Z*)-2-(2′-amino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (7).[15] Zinc (8.36 g, 128 mmol) was added slowly to a well stirred solution of *Z*-stilbene (0.884 g, 2.56 mmol) in glacial AcOH (75 mL). The resulting suspension was stirred for 3 h at room temperature. At this point, the reaction mixture was filtered through Celite, and the Celite was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. The desired amine was purified by flash column chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20%A / 80%B (1 CV), 20%A / 80%B → 75%A / 25%B (10 CV), 75%A / 25%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford the amine **7** (0.615 g, 1.95 mmol, 76%) as a white solid. ^{1}H NMR (500 MHz,

$CDCl_3$): δ 7.04 (1H, d, *J* = 8.4 Hz), 6.52 (2H, s), 6.51 (1H, d, *J* = 12 Hz), 6.43 (1H, d, *J* = 11.9 Hz), 6.30 (1H, dd, *J* = 8.4, 2.5 Hz), 6.26 (1H, d, *J* = 2.5 Hz), 3.81 (3H, s), 3.76 (3H, s), 3.74 (2H, b), 3.65 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.1, 152.7, 144.9, 137.3, 132.3, 131.0, 130.6, 125.7, 116.1, 105.8, 104.3, 100.7, 60.8, 55.8, 55.2. HRMS: *m*/*z*: obsd 316.1544 $[M+H]^+$, calcd for $C_{18}H_{22}NO_4^+$, 316.1543. HPLC (Method B): 13.153 min.

(*Z*)-2-(2′,3′-Diamino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (8).[16] This compound was synthesized using the procedure as described for compound **7.** Starting from zinc (13.4 g, 205 mmol) and *Z*-stilbene (0.797 g, 2.04 mmol) in glacial AcOH (72 mL), the desired product **8** (0.403 g, 1.22 mmol, 60%) was obtained as a brown oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.66 (1H, d, *J*=8.4 Hz), 6.52 (1H, d, *J*=12 Hz), 6.50 (1H, d, *J*=12 Hz), 6.48 (2H, s), 6.39 (1H, d, *J*=8.4 Hz), 3.83 (3H, s), 3.81 (3H, s), 3.62 (6H, s), 3.49 (4H, bs). ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.68, 147.72, 137.31, 133.00, 132.22, 131.26, 126.14, 123.31, 119.53, 117.99, 105.90, 102.23, 60.84, 55.82, 55.73. HRMS: *m*/*z*: obsd 331.1656 $[M+H]^+$, calcd for $C_{18}H_{23}N_2O_4^+$, 331.1658. HPLC (Method B): 11.603 min.

4.1.6. Preparation of hydrochloride salts of amino combretastatin analogues[15,16]

(*Z*)-2-(2'-Methoxy-4'-aminophenyl)-1-(3,4,5-trimethoxyphenyl)ethene hydrochloride (9).[15] To a solution of 2' CA4-amine **7** (0.0550 g, 0.175 mmol) in anhydrous CH_2Cl_2 (8 mL) was added a HCl solution (4 N in dioxane, 3 equiv.) at 0 ^{o}C. The reaction was monitored by TLC and stirred for 12 h. After evaporating the solvents under reduced pressure, the final hydrochloride salt **9** (0.0440 g, 0.125

mmol, 72%) was obtained as a green solid after recrystallization with ethanol/diethyl ether solution. ^{1}H NMR (500 MHz, CD_3OD): δ 7.27 (1H, d, *J*=8.3 Hz), 6.97 – 6.91 (2H, m), 6.78 (1H, d, *J*=11.9 Hz), 6.55 (1H, d, *J*=11.8 Hz), 6.46 (2H, s), 3.83 (3H, s), 3.71 (3H, s), 3.60 (6H, s). ^{13}C NMR (125 MHz, CD_3OD): δ 160.11, 152.83, 152.82, 133.86, 132.04, 131.52, 130.51, 123.86, 122.08, 113.51, 108.42, 106.21, 59.66, 54.89, 54.83. HRMS: *m*/*z*: obsd 316.1544 $[M\text{-}Cl]^+$, calcd for $C_{18}H_{22}NO_4^+$, 316.1543. HPLC (Method B): 13.02 min.

(*Z*)-2-(2′,3′-Diamine hydrochloride-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (10). To a solution of CA1-diamine **8** (0.320 g, 0.970 mmol) in anhydrous CH_2Cl_2 (50 mL) was added a HCl solution (4 N in dioxane, 5 equiv.), and the reaction mixture was stirred for 5 h. The resulting crystals were filtered and re-crystallized from anhydrous methanol at -20 °C. The brown crystals thus obtained were washed with dry CH_2Cl_2 to obtain the salt **10** (0.130 g, 0.320 mmol, 30%). ^{1}H NMR (600 MHz, CD_3OD): δ 6.98 (1H, d, *J*=8.5 Hz), 6.60 (1H, d, *J*=11.9 Hz), 6.47 (1H, d, *J*=8.6 Hz), 6.42 (2H, s), 6.37 (1H, d, *J*=11.8 Hz), 3.83 (3H, s), 3.62 (3H, s), 3.52 (6H, s). ^{13}C NMR (150 MHz, CD_3OD): δ 152.7, 152.3, 137.1, 137.0, 132.6, 132.2, 129.2, 124.0, 119.0, 107.4, 106.0, 101.4, 59.7, 55.4, 54.9. HRMS: *m*/*z*: obsd 353.1476 $[M\text{ -}2HCl+\text{ }Na]^+$, calcd for $C_{18}H_{22}N_2O_4Na^+$, 353.1472. HPLC (Method C): 13.06 min.

4.1.7. Preparation of amino acid prodrug conjugates of 2' combretastatin A-4 amines[15,52]

(*S*,*Z*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((5-methoxy-2-(3,4,5-trimethoxystyryl)phenyl)amino)-3-oxopropyl acetate (11). To a solution of 2' CA4-amine **7** (0.220 g, 0.700 mmol) in anhydrous CH_2Cl_2 (30 mL) was added Et_3N (0.110 mL, 0.760 mmol), peptide coupling reagent T3P (50% in EtOAc, 0.830 mL, 1.40 mmol) and Fmoc-*L*-ser(Ac)-OH (0.284 g, 0.760 mmol). The reaction mixture was stirred for 8 h at room temperature. H_2O (20 mL) was added, and the reaction mixture was extracted with CH_2Cl_2. The organic extract was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure, and the residue was purified using flash column chromatography (50% EtOAc/hexanes) to afford the desired Fmoc-*L*-serinamide acetate **11** (0.388 g, 0.580 mmol, 83%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.23 (1H, b), 7.93 (2H, d, *J* = 7.5), 7.60 (2H, d, *J* = 7.5), 7.60 (2H, d, *J* = 7.5), 7.40 (2H, dd, *J* = 7.5, 7.0), 7.33 (2H, dd, *J* = 7.5, 7.0), 6.98 (1H, d, *J* = 8.5), 6.65 (1H, d, *J* = 9.0), 6.56 (1H, s), 6.00 (1H, t, *J* = 4.5), 5.75 (1H, d, *J* = 6.5), 4.47 (4H, m), 4.23 (1H, d, *J* = 11.5), 3.89 (3H, s), 3.84 (6H, s), 3.79 (3H, s), 2.67 (2H, t, *J* = 7.5), 2.30 (2H, td, *J* = 7.5, 4.5), 2.11 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.2, 166.6, 159.8, 153.2, 143.9, 141.7, 138.1, 135.3, 133.2, 131.5, 130.2, 128.2, 127.5, 127.5, 125.2, 125.1, 124.2, 120.4, 120.4, 111.7, 105.8, 105.8, 67.2, 63.8, 61.1, 56.43, 56.0, 55.8, 47.4, 20.8.

(*S*,*Z*)-2-amino-3-hydroxy-*N*-(5-methoxy-2-(3,4,5-trimethoxystyryl)phenyl)propanamide (13). Fmoc-*L*-serinamide acetate **11** (0.290 g, 0.435 mmol) was dissolved in a CH_2Cl_2/MeOH mixture (10 mL, 1:1 ratio), a

NaOH solution (2 M, 0.650 mL, 1.30 mmol) was added, and the mixture was stirred for 30 min. After the evaporation of solvent, the resulting oil was purified by normal phase preparative TLC (95% CH_2Cl_2/MeOH) to obtain 2' CA4-*L*-serinamide **13** (0.125 g, 0.311 mmol, 71%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.65 (1H, s), 8.04 (1H, d, *J*=2.6 Hz), 7.16 (1H, dd, *J*=8.5, 0.9 Hz), 6.69 (1H, dd, *J*=8.5, 2.6 Hz), 6.61 (1H, d, *J*=12.0 Hz), 6.50 (1H, d, *J*=12.0 Hz), 6.42 (2H, s), 3.82 (3H, s), 3.80 (3H, s), 3.76 (1H, dd, *J*=10.8, 5.2 Hz), 3.61 (7H, s), 3.37 (1H, t, *J*=5.4 Hz). ^{13}C NMR (90 MHz, $CDCl_3$): δ 171.7, 159.5, 152.8, 137.9, 135.8, 132.6, 131.8, 130.1, 124.3, 120.1, 110.8, 106.1, 105.6, 64.8, 60.8, 56.8, 55.9, 55.4. HRMS: *m*/*z*: obsd 403.1866 $[M+H]^+$, calcd for $C_{21}H_{27}N_2O_6^+$, 403.1864. HPLC (Method A): 12.97 min.

(*S*,*E*)-2-amino-3-hydroxy-*N*-(5-methoxy-2-(3,4,5-trimethoxystyryl)phenyl)propanamide hydrochloride (17).[52] To a well stirred solution of 2' CA4-*L*-serinamide **13** (0.0950 g, 0.236 mmol) in CH_2Cl_2 (10 mL) was added a HCl solution (4 N in dioxane, 0.8 mmol) at room temperature. After completion of the reaction (monitored by TLC), diethyl ether (20 mL) was added to the reaction mixture, producing a white solid that was recovered by filtration through a membrane filter. After the solid was washed with diethyl ether (10 mL), the water soluble 2' CA4-*L*-serinamide **17** (0.0780 g, 0.178 mmol, 75%) was obtained as a white solid. ^{1}H NMR (600 MHz, CD_3OD): δ 7.72 (1H, d, *J*=8.8 Hz), 7.23 (1H, d, *J*=16.2 Hz), 7.09 (1H, d, *J*=2.6 Hz), 7.01 (1H, d, *J*=16.1 Hz), 6.92 (1H, dd, *J*=8.7, 2.6 Hz), 6.89 (2H, s), 4.26 (1H, t, *J*=5.2 Hz), 4.12 (2H, d, *J*=5.2 Hz), 3.91 (6H, s), 3.84 (3H, s), 3.79 (3H, s). ^{13}C NMR (150 MHz, CD_3OD): δ 166.2, 159.5, 153.2, 137.4, 134.5, 133.9, 128.6, 126.5, 124.9, 122.4, 112.8, 111.0, 103.5, 60.5, 59.8, 55.3, 55.1, 54.5.

HRMS: *m*/*z*: obsd 403.1873 [M-Cl]$^+$, calcd for $C_{21}H_{27}N_2O_6^+$ 403.1864. HPLC (Method C): 13.23 min.

(*S*,*Z*)-2-amino-3-hydroxy-*N*-(5-methoxy-2-(3,4,5-trimethoxystyryl)phenyl)propanamide hydrochloride (15). To 2' CA4-*L*-serinamide **13** (0.0500 g, 0.124 mmol) was added a HCl solution (4 N in dioxane, 0.36 mmol) at 0 ^oC. The resulting reaction mixture was stirred for 3 h, followed by evaporation of the solvent under reduced pressure at 30 ^oC. The resultant green oil was washed with anhydrous diethyl ether to furnish the water soluble HCl salt of 2' CA4-*L*-serinamide **15** (0.0360 g, 0.0820 mmol, 66%) as a dark green solid. ^1H NMR (600 MHz, CD_3OD): δ 7.37 (1H, d, *J*=2.4 Hz), 7.12 (1H, dd, *J*=8.5, 2.5 Hz), 6.75 (1H, d, *J*=7.8 Hz), 6.56 (1H, d, *J*=11.8 Hz), 6.47 (1H, d, *J*=11.9 Hz), 6.42 (2H, s), 3.98 (1H, t, *J*=5.9 Hz), 3.75 (3H, s), 3.72 (1H, d, *J*=4.5 Hz), 3.67 (3H, s), 3.61 (1H, m), 3.55 (6H, s). ^{13}C NMR (150 MHz, CD_3OD): δ 165.35, 159.38, 152.70, 137.08, 135.26, 132.31, 131.57, 130.38, 124.80, 123.57, 111.27, 109.52, 105.93, 60.09, 59.70, 55.08, 54.87, 54.52. HRMS: *m*/*z*: obsd 403.1885 [M+H]$^+$, calcd for $C_{21}H_{27}N_2O_6^+$, 403.1884. HPLC (Method C): 13.05 min.

***Tert*-butyl (*Z*)-(2-((5-methoxy-2-(3,4,5-trimethoxystyryl)phenyl)amino)-2-oxoethyl) carbamate (12).** To a solution of 2' CA4-amine **7** (0.363 g, 0.700 mmol) in anhydrous CH_2Cl_2 (75 mL) was added Et_3N (0.300 mL, 1.72 mmol), peptide coupling reagent T3P (50% in EtOAc, 1.70 mL, 2.30 mmol) and Boc-glycine-OH (0.311 g, 1.44 mmol). The reaction mixture was stirred for 3 h at room temperature. Water (25 mL) was added, and the organic solvent was removed by evaporation under reduced pressure. The resultant crude mixture was extracted with EtOAc, and

the organic extract was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified using flash column chromatography (50% EtOAc/hexanes) to afford the desired Boc-protected glycinamide **12** (0.490 g, 1.04 mmol, 90%) as a white solid.^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95 (1H, s), 7.93 (1H, bs), 7.15 (1H, d, *J* = 8.5 Hz), 6.68 (1H, d, *J* = 7.7 Hz), 6.61 (1H, d, *J* = 12.0 Hz), 6.46 (1H, d, *J* = 12.0 Hz), 6.41 (2H, s), 4.80 (1H, s), 3.81 (3H, s), 3.80 (3H, s), 3.67 (1H, s), 3.61 (6H, d, *J* = 1.4 Hz), 1.46 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.26, 159.51, 155.67, 152.86, 137.83, 135.32, 132.51, 131.43, 130.06, 124.34, 119.50, 110.91, 109.99, 105.67, 105.64, 60.91, 55.80, 55.46, 45.10, 28.29.

(*Z*)-2-amino-*N*-(5-methoxy-2-(3,4,5-trimethoxystyryl)phenyl)acetamide (14).[81] The Boc- protected glycinamide **12** (0.128 g, 0.270 mmol) in anhydrous CH_2Cl_2 (2 mL) was reacted with TFA (0.650 mL, 1.30 mmol) and stirred for 30 min.[81] After evaporation of the solvent, the resulting oily residue was diluted with water (2 mL) and washed with ether (2 × 2 mL). The aqueous phase was treated with $NaHCO_3$ (1 N) until pH 7-8 and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified using flash column chromatography (50% EtOAc/hexanes) to obtain 2' CA4 glycinamide prodrug **14** (0.0680 g, 0.183 mmol, 71%) as a brown oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.52 (1H, s), 8.08 (1H, d, *J*=2.6 Hz), 7.14 (1H, d, *J*=8.5 Hz), 6.65 (1H, dd, *J*=8.6, 2.5 Hz), 6.60 (1H, d, *J*=12.0 Hz), 6.50 (1H, d, *J*=12.1 Hz), 6.41 (2H, s), 3.82 (3H, s), 3.80 (3H, s), 3.60 (6H, s), 3.28 (2H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.62, 159.52, 152.73, 137.63, 136.05, 132.44, 131.77, 130.01, 124.43, 119.59, 110.51,

105.85, 105.21, 60.89, 55.82, 55.45, 45.42. HRMS: *m*/*z*: obsd 373.1759 $[M+H]^+$, calcd for $C_{20}H_{25}N_2O_5^+$, 373.1758. HPLC (Method A): 8.14 min.

(*E*)-2-amino-*N*-(5-methoxy-2-(3,4,5-trimethoxystyryl)phenyl)acetamide hydrochloride (18).[52] To a well stirred solution of Boc-protected 2' CA4 glycinamide **12** (0.146 g, 0.310 mmol) in CH_2Cl_2 (10 mL) was added a HCl solution (4 N in dioxane, 1.20 mmol) at room temperature. After completion of the reaction (monitored by TLC), diethyl ether (25 mL) was added to the reaction mixture, producing a white solid that was filtered through a membrane filter. After washing the product on the membrane with diethyl ether (10 mL), the HCl salt of the *E*-isomer of 2' CA4 glycinamide **18** (0.109 g, 0.226 mmol, 79%) was obtained as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.66 (1H, d, *J*=8.8 Hz), 7.20 (1H, d, *J*=15 Hz), 7.19 (1H, d, *J*=2.7 Hz), 6.98 (1H, d, *J*=16.1 Hz), 6.88 (1H, d, *J*=2.5 Hz), 6.87 (2H, s), 3.98 (2H, s), 3.88 (6H, s), 3.81 (3H, s), 3.77 (3H, s). ^{13}C NMR (125 MHz, CD_3OD): δ 165.04, 159.47, 153.25, 137.58, 134.59, 133.83, 129.04, 126.75, 124.24, 122.22, 112.35, 110.48, 103.75, 59.76, 55.31, 54.46, 40.66. HRMS: *m*/*z*: obsd 373.1769 $[M-Cl]^+$, calcd for $C_{20}H_{25}N_2O5^+$ 373.1758. HPLC (Method B): 6.661 min.

(*Z*)-2-amino-*N*-(5-methoxy-2-(3,4,5-trimethoxystyryl)phenyl)acetamide hydrochloride (16).[72] To *boc*-protected 2' CA4 glycinamide **12** (0.0898 g, 0.190 mmol) was added a HCl solution (4 N in dioxane, 0.270 mmol) at 0 ^{o}C. The resulting reaction mixture was stirred for 30 min., followed by evaporation of the solvent under reduced pressure at 30 ^{o}C. The resulting brownish oil, on washing with anhydrous diethyl ether, furnished the water soluble HCl salt of 2' CA4 glycinamide **16** (0.0691 g, 0.171 mmol, 90%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD): δ 7.37

(1H, d, *J*=2.6 Hz), 7.12 (1H, d, *J*=8.4 Hz), 6.73 (1H, dd, *J*=8.3, 2.4 Hz), 6.57 (1H, d, *J*=11.8 Hz), 6.48 (1H, d, *J*=11.9 Hz), 6.43 (2H, s), 3.75 (3H, s), 3.67 (3H, d, *J*=1.6 Hz), 3.63 (2H, s), 3.56 (6H, s). ^{13}C NMR (150 MHz, CD_3OD): δ 164.38, 159.37, 152.65, 136.96, 135.29, 132.57, 131.37, 130.58, 124.93, 123.22, 111.06, 109.45, 105.84, 59.75, 54.89, 54.51, 40.59. HRMS: *m*/*z*: obsd 373.1759 $[M\text{-}Cl]^+$, calcd for $C_{20}H_{25}N_2O_5^+$, 373.1759. HPLC (Method C): 12.94 min.

4.1.8. Preparation of amino acid prodrug conjugates of combretastatin A-1 diamines[15,16,52]

(*Z*)-2-(2',3′-Diamino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene-Fmoc-*L*-serinamide (19). To a solution of CA1-diamine **8** (0.780 g, 2.37 mmol) in dry CH_2Cl_2 (150 mL) was added Fmoc-*L*-serine (Ac)-OH (2.40 g, 7.11 mmol), Et_3N (1.00 mL, 7.11 mmol), and T3P (50% in EtOAc, 5.62 mL, 9.48 mmol). The resulting solution was stirred for 8 h at room temperature. At this point, the reaction mixture was washed with water (3 × 50 mL) and brine and dried over Na_2SO_4. The organic solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography (50% EtOAc/hexanes) to afford the Fmoc-*L*-diserinamide acetate **19** (1.31 g, 1.30 mmol, 55%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.35 (1H, s), 8.05 (1H, s), 7.71 (2H, d, *J* = 6.6 Hz), 7.66 (2H, s), 7.49 (4H, d, *J* = 6.0 Hz), 7.33 (4H, m), 7.23 – 7.15 (4H, m), 7.18 (1H, d, *J* = 9.0 Hz), 6.78 (1H, d, *J* = 8.0 Hz), 6.46 (1H, d, *J* = 12.0 Hz), 6.43 (2H, s), 6.39 (1H, d, *J* = 12.0 Hz), 6.09 (1H, s), 5.84 (1H, s), 4.68 – 4.02 (12H, m), 3.79 (6H, s), 3.62 (6H, s), 2.04 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.0, 171.0, 168.1, 167.5, 156.6, 156.4, 152.9, 152.5, 143.6, 143.6, 143.5, 143.5, 141.3, 141.2, 137.3, 132.1, 131.0, 130.4, 128.8, 127.8, 127.8,

127.1, 127.1, 125.9, 125.1, 125.1, 125.0, 120.0, 109.6, 105.9, 67.6, 67.5, 63.7, 63.6, 60.9, 56.2, 55.9, 54.8, 46.9, 34.7, 29.1, 25.3, 20.8.

(2*S*, 2'*S*)-*N, N'*-(3-methoxy-6-((*Z*)-3,4,5-trimethoxystyryl)-1,2-phenylene)*bis*(2-amino-3-hydroxypropanamide) (22). Fmoc-*L*-diserinamide acetate **19** (0.501 g, 0.485 mmol) was dissolved in a CH_2Cl_2/MeOH mixture (20 mL, 1:1 ratio) to which a NaOH solution (2 M, 0.970 mL, 1.98 mmol) was added, followed by stirring (30 min). After the evaporation of solvent, the resulting oil was purified by normal phase preparative TLC (90% CH_2Cl_2/MeOH. 0.2% Et_3N) to obtain CA1-*L*-diserinamide prodrug **22** (0.0650 g, 0.128 mmol, 27%) as a white solid. ^{1}H NMR (600 MHz, CD_3OD): δ 7.17 (1H, d, *J*=8.6 Hz), 6.98 (1H, d, *J*=8.7 Hz), 6.54 (1H, d, *J*=12 Hz), 6.49 (2H, s), 6.48 (1H, d, *J*=13.3 Hz), 3.90 (2H, d, *J*=5.7 Hz), 3.89 – 3.87 (1H, m), 3.86 (3H, s), 3.85 – 3.80 (2H, m), 3.76 (1H, m), 3.71 (3H, s), 3.61 (6H, s), 1.95 (4H, s). ^{13}C NMR (150 MHz, CD_3OD): δ 180.70, 173.77, 155.83, 154.95, 139.05, 134.89, 133.67, 132.82, 130.62, 130.45, 128.38, 124.07, 111.92, 108.28, 65.55, 65.50, 61.94, 58.85, 58.54, 57.51, 57.20. HRMS: *m*/*z*: obsd 505.2289 $[M+H]^+$, calcd for $C_{24}H_{33}N_4O_8^+$, 505.2293. HPLC (Method C): 10.94 min.

(2*S*, 2'*S*)-((3-methoxy-6-((*Z*)-3,4,5-trimethoxystyryl)-1,2-phenylene)*bis*(azanediyl))*bis*(2-((*tert*-butoxycarbonyl)amino)-3-oxopropane-3,1-diyl) diacetate (20). To a solution of CA1 diamine **8** (0.270 g, 0.817 mmol) in anhydrous CH_2Cl_2 (50 mL) was added Boc-serine (Ac)-OH·DCHA (0.875 g, 2.04 mmol), Et_3N (0.290 mL, 2.04 mmol), and T3P (50% in EtOAc, 2.10 mL, 3.27 mmol). The resulting solution was stirred for 8 h at room temperature. At this point, the reaction mixture was washed with water (3 × 20 mL) and brine and dried over

Na_2SO_4. The organic solvent was removed under reduced pressure, and the crude reaction mixture was purified by flash column chromatography (50% EtOAc/hexanes) to afford the Boc-protected-*L*-serinamide acetate **20** (0.251 g, 0.318 mmol, 39%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (1H, s), 8.21 (1H, s), 7.14 (1H, d, *J*=8.6 Hz), 6.75 (1H, d, *J*=8.6 Hz), 6.45 (1H, d, *J*=12.0 Hz), 6.41 (2H, s), 6.38 (1H, d, *J*=11.9 Hz), 5.66 (1H, s), 5.48 – 5.41 (1H, m), 4.62 (1H, s), 4.48 (2H, d, *J*=5.2 Hz), 4.40 (2H, d, *J*=7.5 Hz), 4.30 (1H, dd, *J*=7.0 Hz), 3.80 (3H, s, 1 Hz), 3.79 (3H, s), 3.62 (6H, s), 2.05 (3H, s), 2.02 (3H, s), 1.46 (9H, s), 1.43 (9H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.97, 170.80, 168.40, 167.67, 155.56, 155.33, 152.82, 152.40, 137.27, 132.00, 130.91, 130.42, 128.50, 128.36, 125.95, 121.24, 109.53, 105.98, 64.00, 63.92, 60.80, 60.38, 56.13, 55.83, 54.05, 28.31, 28.28, 20.66, 20.65.

(2*S*, 2'*S*)-*N, N'*-(3-methoxy-6-((*Z*)-3,4,5-trimethoxystyryl)-1,2-phenylene)bis(2-amino-3-hydroxypropanamide) dihydrochloride (23). Boc-*L*-serinamide acetate **20** (0.203 g, 0.258 mmol) was dissolved in a CH_2Cl_2/MeOH mixture (20 mL, 1:1 ratio) to which a NaOH solution (2 M, 0.650 mL, 1.30 mmol) was added. The reaction mixture was stirred for 30 min. After the evaporation of solvent, the resulting white solid (0.130 g, 0.184 mmol) in CH_2Cl_2 (15 mL) was reacted with a HCl solution (4 N in dioxane, 2.27 mmol) at room temperature for 2 h. Following evaporation of solvent under reduced pressure, the solid was purified by reversed phase column chromatography (RP-18 silica column, acetonitrile/water) to afford compound **20** (0.0590 g, 0.102 mmol, 40%) as an off-white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.16 (1H, d, *J* = 8.7 Hz), 6.99 (1H, d, *J* = 8.7 Hz), 6.55 (1H, d, *J* = 12.0 Hz), 6.49 (2H, s), 6.48 (1H, d, *J* = 12.0 Hz), 4.32 (1H, dd, *J* = 5.5, 4.3 Hz), 4.27 (1H, dd, *J* =

6.1, 4.8 Hz), 4.12 (1H, dd, *J* = 11.5, 4.5 Hz), 4.03 (1H, dd, *J* = 11.5, 4.5 Hz), 4.00 (1H, dd, *J* = 12.0, 8.5 Hz), 3.89 (1H, dd, *J* = 12.0, 8.5 Hz), 3.85 (3H, s), 3.71 (3H, s), 3.61 (6H, s). ^{13}C NMR (150 MHz, CD_3OD): δ 166.1, 165.6, 153.9, 152.6, 136.8, 132.4, 131.4, 131.0, 129.0, 128.7, 125.9, 121.7, 110.3, 106.2, 60.4, 59.7, 55.4, 55.1, 55.1, 55.0, 55.0. HRMS: *m*/*z*: obsd 527.2102 $[M - HCl + Na]^+$, calcd for $C_{24}H_{32}N_4O_8Na^+$, 527.2112. HPLC (Method C): 11.17 min.

Di-*tert*-butyl (((3-methoxy-6-(3,4,5-trimethoxystyryl)-1,2-phenylene)*bis*(azanediyl))*bis*(2-oxoethane-2,1-diyl))(*Z*)-dicarbamate (21). To a solution of CA1-diamine **8** (0.300 g, 0.908 mmol) in anhydrous CH_2Cl_2 (50 mL) was added Boc-glycine-OH (0.398 g, 2.27 mmol), Et_3N (0.270 mL, 1.91 mmol), and T3P (50% in EtOAc, 2.25 mL, 3.63 mmol). The resulting solution was stirred for 3 h at room temperature. At this point, the reaction mixture was washed with water (3 × 20 mL) and brine and dried over Na_2SO_4. The organic solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography (50% EtOAc/hexanes) to afford the Boc-protected CA1-diglycinamide **21** (0.503 g, 0.780 mmol, 86%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.21 (1H, bs), 7.96 (1H, bs), 7.14 (1H, d, *J*=8.6 Hz), 6.74 (1H, d, *J*=8.6 Hz), 6.49 (1H, d, *J*=12.0 Hz), 6.44 (2H, s), 6.43 (1H, d, *J*=11.6 Hz), 5.54 (1H, bs), 5.38 (1H, bs), 3.96 (2H, d, *J*=5.6 Hz), 3.83 (2H, d, *J*=5.8 Hz), 3.80 (6H, s), 3.63 (6H, s), 1.47 (9H, s), 1.45 (9H, s). ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.2, 168.8, 156.6, 156.4, 153.1, 152.8, 137.4, 132.6, 131.2, 128.9, 128.7, 126.5, 121.7, 109.8, 106.2, 61.2, 56.4, 56.2, 45.2, 44.8, 28.7, 28.7.

(*Z*)-*N,N′*-(3-methoxy-6-(3,4,5-trimethoxystyryl)-1,2-phenylene)*bis*(2-aminoacetamide) (24). To a solution of Boc-protected CA1-diglycinamide **21** (0.355 g, 0.550 mmol) in CH_2Cl_2 (50 mL) was added a HCl solution (4 N in dioxane, 2.75 mmol) at room temperature, and the mixture was stirred for 3 h. On adding diethyl ether (25 mL) to the reaction mixture, a white solid formed, and it was filtered through a membrane filter. After washing the solid with diethyl ether (20 mL), CA1-diglycinamide **24** (0.142 g, 0.275 mmol, 50%) was obtained as a white solid. ^{1}H NMR (600 MHz, CD_3OD): δ 7.18 (1H, d, *J*=8.3 Hz), 7.01 (1H, d, *J*=8.4 Hz), 6.59 (1H, d, *J*=11.8 Hz), 6.53 (1H, d, *J*=12 Hz), 6.51 (2H, s), 4.02 (4H, bs, NH_2, CH_2), 3.93 (2H, bs, NH_2), 3.92 (2H, bs, CH_2), 3.87 (3H, s), 3.73 (3H, s), 3.63 (6H, s). ^{13}C NMR (150 MHz, CD_3OD): δ 164.9, 164.7, 153.9, 152.6, 136.8, 132.5, 131.4, 131.0, 128.9, 128.7, 126.0, 121.7, 110.2, 106.1, 59.7, 55.3, 55.1, 40.7, 40.6. HRMS: m/z: obsd 467.1897 [M -HCl+ Na]$^+$, calcd for $C_{22}H_{28}N_4O_6Na^+$, 467.1901. HPLC (Method A): 6.06 min.

4.1.9. Synthesis of Amino Acid Prodrug Conjugates of Amino Dihydronaphthalene and Amino Benzosuberene

6-Methoxy-5-nitro-1-tetralone (25). To a well-stirred solution of 6-methoxy-1-tetralone (20.0 g, 114 mmol) in acetic anhydride (200 mL), a solution of HNO_3 (10 mL) and AcOH (10 mL) was slowly added over the course of 1 h at 0 ºC. The reaction was stirred for another 20 h and was allowed to reach room temperature. Water (20 mL) was added to the reaction mixture, and the crude product was extracted with CH_2Cl_2 (3 × 50 mL). The organic phase was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography

(20% EtOAc/hexanes) to give the desired 6-methoxy-5-nitro-tetralone **25** (8.28 g, 37.5 mmol, 33%) as a light yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.18 (1H, d, *J* = 8.8 Hz), 7.02 (1H, d, *J* = 8.8 Hz), 3.97 (3H, s), 2.86 (2H, t, *J* = 6.5 Hz), 2.65 (2H, t, *J* = 6.5 Hz), 2.15 (2H, p, *J* = 6.5 Hz).^{13}C NMR (150 MHz, $CDCl_3$): δ 195.5, 154.4, 140.3, 137.3, 131.2, 126.1, 110.7, 56.8, 38.2, 24.7, 22.3.

2-Methoxy-1-nitro-5-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (30). To a solution of 5-bromo-1,2,3-trimethoxybenzene (1.11 g, 4.49 mmol) in THF (20 mL) at -78 °C, *n*-BuLi (1.80 mL, 2.50 M) was added, and the reaction mixture was stirred for 1 h. Compound **25 (**0.500 g, 2.30 mmol) in THF (3 mL) was added to the reaction mixture dropwise. The reaction mixture was stirred for 18 h and was allowed to warm to room temperature. The reaction mixture was quenched with H_2O (20 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The organic extract was dried over Na_2SO_4, filtered, and concentrated under reduced pressure, and the residue was purified by flash column chromatography (40% EtOAc/hexanes) to yield alcohol **30** (0.521 g, 1.34 mmol, 59% yield) as a light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.18 (1H, d, *J*=8.8 Hz), 6.85 (1H, d, *J*=8.8 Hz), 6.54 (2H, s), 3.87 (3H, s), 3.85 (3H, s), 3.81 (6H, s), 2.79 (2H, t, *J*=7.4 Hz), 2.12 (1H, s), 2.10 (2H, m), 2.04 (1H, m), 1.86 (1H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 152.9, 150.0, 143.7, 137.0, 135.3, 132.1, 130.6, 110.8, 103.8, 74.9, 61.0, 56.5, 56.4, 40.6, 24.6, 18.6.

2-Methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalen-1-amine (32). Alcohol **30** (0.521 g, 1.34 mmol) was dissolved in AcOH (18 mL) in a round-bottom reaction flask, followed by the addition of Zn (1.75 g, 26.8 mmol). The reaction

mixture was stirred for 6 h at room temperature and then filtered through Celite®, which was washed with CH_2Cl_2. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (30% EtOAc/hexanes) to give the desired amino-dihydronaphthalene (**32**) (0.410 g, 1.27 mmol, 89% yield) as a dark brown solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.59 (1H, d, *J* = 8.5 Hz), δ 6.56 (2H, s), δ 6.51 (1H, d, *J* = 8.5 Hz), δ 5.92 (1H, t, *J* = 4.5 Hz), δ 3.89 (3H, s), δ 3.85 (3H, s), δ 3.83 (6H, s), δ 2.67 (2H, t, *J* = 7.5 Hz), δ 2.41 (2H, td, *J* = 7.5, 4.5 Hz). ^{13}C NMR (125 MHz, DMSO-d6): δ 152.9, 146.7, 140.0, 137.1, 136.9, 134.0, 127.8, 124.2, 120.3, 115.1, 107.6, 106.2, 60.4, 56.2, 55.7, 23.0, 21.7. HRMS: m/z: obsd 342.1698 $[M+H]^+$, calcd for $C_{20}H_{24}NO_4^+$, 342.1705. HPLC (Method B): 14.18 min.

5-(3'-Methoxy-2'-nitrophenyl)pent-4-enoic acid (27). To a well-stirred solution of 3-(carboxypropyl) triphenylphosphonium bromide (2.66 g, 6.21 mmol) in THF (60 mL), potassium *tert*-butoxide (1.68 g, 14.9 mmol) was added, and the reaction mixture was stirred for 1 h at ambient temperature. The reaction mixture was cooled to 0 °C, and 3-methoxy-2-nitrobenzaldehyde (1.07 g, 5.91 mmol) in THF (5 mL) was added dropwise. After the reaction mixture was stirred for 16 h, warming from 0 °C to room temperature, the reaction was quenched with HCl (2 M, 20 mL). The organic solvent was removed by evaporation under reduced pressure, and the residue was extracted with EtOAc (4 × 30 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting organic crude product was purified by flash column chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (1 CV), 12%A / 88%B → 100%A / 0%B (15 CV),

100%A / 0%B (4 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] affording a mixture of *E* and *Z*-isomers **27** (0.750 g, 2.98 mmol, 50%) as a red solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (1H, t, *J*=8.1 Hz), 7.33 (1H, t, *J*=8.2 Hz), 7.12 (1H, d, *J*=7.8 Hz), 6.95 (1H, d, *J*=8.3 Hz), 6.89 (2H, d, *J*=7.7 Hz), 6.37 (1H, d, *J*=11.3 Hz), 6.31 (2H, m), 5.83 (1H, dt, *J*=11.5, 7.0 Hz), 3.89 (3H, s), 3.87 (3H, s), 2.53 (4H, m), 2.45 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.5, 178.4, 150.9, 150.9, 134.9, 134.5, 130.8, 130.7, 123.7, 123.6, 121.7, 118.2, 111.4, 111.0, 56.5, 56.5, 33.6, 33.3, 28.1, 23.9.

5-(3'-Methoxy-2'-nitrophenyl)pentanoic acid (28). Anhydrous ethanol (18 mL) was added to a flask containing pentenoic acid **27** (0.750 g, 2.98 mmol) and Pd/C (10%, 0.830 g), and the solution was stirred for 10 min at ambient temperature. 1,4-Cyclohexadiene (8.90 mL, 93.8 mmol) was added to the solution, which was then stirred for another 3 h. The reaction mixture was filtered through Celite, and the Celite was washed with EtOAc (3 × 20 mL). The combined filtrate was removed by evaporation under reduced pressure, and the residue was purified by flash column chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (1 CV), 12%A / 88%B → 100%A / 0%B (12 CV), 100%A / 0%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford pentanoic acid analogue **28** (0.490 g, 1.93 mmol, 65%) as a tan solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33 (1H, t, *J*=8.1 z), 6.87 (2H, dd, *J*=8.1, 3.7 Hz), 3.87 (3H, s), 2.58 (2H, m), 2.36 (2H, m), 1.67 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.3, 150.8, 142.0, 134.8, 130.8, 121.7, 110.3, 56.5, 33.7, 30.8, 29.9, 24.4.

2-Methoxy-1-nitro-benzosuber-5-one (29). Pentanoic acid analogue **28** (0.490 g, 1.93 mmol) was dissolved in Eaton's reagent under N_2, and the reaction mixture was

stirred for 16 h at ambient temperature. To the solution was added ice, which allowed to melt, and the reaction mixture was neutralized slowly with $NaHCO_3$ (aq.) and extracted with EtOAc (3 × 20 mL). The combined organic phase was dried over Na_2SO_4, filtered, and removed by evaporation under reduced pressure, and the residue 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 (1 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford benzosuberone analogue **29** (0.340 g, 1.45 mmol, 75%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.84 (1H, d, *J*=8.8 Hz), 6.98 (1H, d, *J*=8.8 Hz), 3.95 (3H, s), 2.78 (2H, m), 2.71 (2H, m), 1.91 (2H, m), 1.81 (2H, m).^{13}C NMR (125 MHz, $CDCl_3$): δ 203.2, 153.4, 141.5, 134.1, 132.3, 131.9, 110.4, 56.7, 40.4, 26.3, 24.5, 20.3.

2-Methoxy-1-nitro-5-(3',4',5'-trimethoxyphenyl)-benzosuber-5-ol (31). To a well-stirred solution of 3,4,5-trimethoxyphenyl bromide (0.680 g, 2.75 mmol) in THF (25 mL) at -78 °C, *n*-BuLi (1.00 mL, 2.50 M in hexanes) was added, and the reaction mixture was stirred for 1 h. Benzosuberone analogue **29** (0.390 g, 1.66 mmol) in THF (5 mL) was added dropwise into the reaction mixture, which was then stirred for 18 h and allowed to warm from -78 °C to room temperature. The reaction was quenched with H_2O (20 mL), and the solvent was removed under reduced pressure. The mixture was extracted with EtOAc (3 × 25 mL), and the combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue 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 → 100%A / 0%B (13 CV), 100%A / 0%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford alcohol **31** (0.468 g, 1.16 mmol, 70%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (1H, d, *J*=8.7 Hz), 6.88 (1H, d, *J*=8.7 Hz), 6.46 (2H, s), 3.90 (3H, s), 3.84 (3H, s), 3.76 (6H, s), 2.60 (2H, m), 2.41 (1H, m), 2.37 (1H, s), 2.11 (1H, m), 1.95 (1H, m), 1.79 (2H, m), 1.53 (1H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 153.3, 149.4, 142.4, 140.5, 138.6, 137.6, 133.6, 129.4, 109.3, 104.1, 79.7, 60.9, 56.3, 56.2, 40.9, 28.7, 26.4, 26.0.

1-Amino-2-methoxy-5-(3',4',5'-trimethoxyphenyl)-benzosuber-5-ene (33). To a solution of alcohol **31** (0.498 g, 1.16 mmol) in AcOH (15 mL) was added Zn dust (1.52 g, 23.2 mmol), and the reaction was stirred for 6 h at ambient temperature. The reaction mixture was filtered through Celite®, which was washed with CH_2Cl_2, and the filtrate was concentrated under reduced pressure. The concentrated residue was neutralized with $NaHCO_3$ and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and removed by evaporation 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 → 60%A / 40%B (13 CV), 60%A / 40%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **33** (KGP156, 0.350 g, 0.980 mmol, 85%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.67 (1H, d, *J*=8.4 Hz), 6.52 (2H, s), 6.49 (1H, d, *J*=8.4 Hz), 6.30 (1H, t, *J*=7.3 Hz), 3.88 (3H, s), 3.86 (3H, s), 3.80 (6H, s), 2.59 (2H, t, *J*=6.9 Hz), 2.12 (2H, p, *J*=7.0 Hz), 1.95 (2H, q, *J*=7.2 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ

152.9, 146.5, 143.7, 138.7, 137.4, 133.7, 132.6, 126.9, 126.4, 120.0, 107.7, 105.4, 61.1, 56.3, 55.7, 33.4, 25.8, 25.4. HRMS: *m*/*z*: obsd 356.1863 $[M+H]^+$, calcd for $C_{21}H_{26}NO_4^+$, 356.1862. HPLC (Method B): 15.34 min.

(*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-((2-methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalen-1-yl)amino)-3-oxopropyl acetate (34). To a well-stirred solution of amino compound **32** (0.500 g, 1.45 mmol) in CH_2Cl_2 (30 mL), Fmoc-*L*-ser(Ac)-OH (0.648 g, 2.18 mmol), T3P (50% in EtOAc, 2.60 mL, 4.35 mmol), and Et_3N (0.31 mL, 2.18 mmol) were added, and the reaction mixture was stirred for 17 h at room temperature. Water (30 mL) was added, and the reaction mixture was extracted with EtOAc (3 × 30 mL). The organic phase was rinsed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography (35% EtOAc/hexanes) to afford the desired Fmoc-*L*-serinamide acetate **34** (0.695 g, 1.12 mmol, 77%) as a yellow solid. ^1H NMR (500 MHz, $CDCl_3$): δ 7.77 (2H, d, *J* = 7.5 Hz), 7.60 (2H, d, *J* = 7.5 Hz), 7.60 (1H, s), 7.40 (2H, dd, *J* = 7.5, 7.0 Hz), 7.33 (2H, dd, *J* = 7.5, 7.0 Hz), 6.98 (1H, d, *J* = 8.5 Hz), 6.65 (1H, d, *J* = 9.0 Hz), 6.56 (2H, s), 6.00 (1H, t, *J* = 4.5 Hz), 5.75 (1H, d, *J* = 6.5 Hz), 4.75 (1H, t, *J* = 11.5 Hz), 4.47 (4H, m), 4.23 (1H, t, *J* = 11.5 Hz), 3.89 (3H, s), 3.84 (6H, s), 3.79 (3H, s), 2.67 (2H, t, *J* = 7.5 Hz), 2.30 (2H, td, *J* = 7.5, 4.5 Hz), 2.11 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.77, 167.42, 152.96, 143.62, 143.55, 141.32, 139.47, 137.11, 136.55, 135.32, 128.81, 127.80, 127.11, 125.30, 125.16, 124.98, 121.79, 120.03, 120.01, 107.62, 105.78, 67.27, 64.47, 60.92, 56.15, 55.67, 54.36, 47.15, 23.99, 22.92, 20.78. HRMS: *m*/*z*: obsd 715.2625 $[M+Na]^+$, calcd for $C_{40}H_{40}N_2O_9Na^+$, 715.2630.

(*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-((3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)amino)-3-oxopropyl acetate (35). This compound was synthesized using the procedure as described for compound **34.** Starting from amino compound **33** (0.355 g, 1.00 mmol), Fmoc-*L*-ser(Ac)-OH (0.553 g, 1.50 mmol), T3P (50% in EtOAc, 1.50 mL, 2.50 mmol), and Et_3N (0.210 mL, 1.50 mmol) in CH_2Cl_2 (25 mL), the desired product **35** (0.393 g, 0.556 mmol, 56%) was obtained as a white solid. ^1H NMR (500 MHz, $CDCl_3$): δ 7.74 (2H, d, *J* = 7 Hz), 7.65 (1H, s), 7.57 (2H, d, *J* = 7.5 Hz), 7.38 (2H, dd, *J* = 7.5, 7.0 Hz), 7.27 (2H, dd, *J* = 7.5, 7.0 Hz), 6.97 (1H, d, *J* = 8.5 Hz), 6.73 (1H, d, *J* = 8.5 Hz), 6.49 (2H, s), 6.36 (1H, t, *J* = 7.5 Hz), 5.94 (1H, d, *J* = 6.5 Hz), 4.82 (1H, bs), 4.49 (4H, m), 4.23 (1H, t, *J* = 6.5 Hz), 3.86 (3H, s), 3.79 (6H, s), 3.74 (3H, s), 2.59 (2H, t, *J* = 7.5 Hz), 2.14 (2H, m,), 2.10 (3H, s). 1.95 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.90, 168.51, 156.24, 153.31, 152.94, 143.69, 143.60, 142.46, 141.32, 140.74, 138.38, 137.42, 133.53, 129.45, 127.84, 127.15, 125.04, 122.19, 120.04, 108.42, 105.36, 67.32, 64.61, 60.95, 56.20, 55.71, 54.26, 47.14, 33.91, 27.09, 25.63, 20.82.

(*S*)-2-Amino-3-hydroxy-*N*-(2-methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalen-1-yl)propanamide (36). To a stirred solution of CH_2Cl_2/MeOH (6 mL, 1:1 ratio) were added Fmoc-*L*-serinamide acetate **34** (0.30 g, 0.43 mmol) and a NaOH solution (2 M, 0.48 mL, 0.97 mmol). After stirring for 18 h at room temperature, the solvent was evaporated under reduced pressure, and water (6 mL) was added. The solution was extracted with EtOAc (3 × 10 mL), and then the combined organic phase were rinsed with brine, dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column

chromatography (5% MeOH/ CH_2Cl_2) to afford the desired serinamide **36** (0.12 g, 0.28 mmol, 65%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.91 (1H, s), 6.98 (1H, d, *J* = 8.5 Hz), 6.65 (1H, d, *J* = 9.0 Hz), 6.56 (2H, s), 6.00 (1H, t, *J* = 4.5 Hz), 4.00 (1H, dd, J = 11, 4.5 Hz), 3.88 (3H, s), 3.84 (6H, s), 3.81 (3H, s), 3.81 (1H, dd, *J* = 11, 4.5 Hz), 3.69 (1H, t, *J* = 4.5 Hz), 2.67 (2H, t, *J* = 7.5 Hz), 2.30 (2H, td, *J* = 7.5, 4.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.6, 152.9, 152.8, 139.4, 137.0, 136.6, 135.2, 128.7, 125.1, 125.0, 122.3, 107.7, 105.7, 65.3, 60.8, 56.6, 56.1, 55.7, 23.9, 22.9. HRMS: *m*/*z*: obsd 429.2041 $[M+H]^+$, calcd for $C_{23}H_{29}N_2O_6^+$, 429.2026. HPLC (Method A): 7.80 min.

(*S*)-2-Amino-3-hydroxy-*N*-(3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)propanamide (37). This compound was synthesized using the procedure as described for compound **36.** Starting from Fmoc-*L*-serinamide acetate **35** (0.393 g, 0.556 mmol) and a NaOH solution (2 N, 0.630 mL, 1.26 mmol) in CH_2Cl_2/MeOH (6 mL, 1:1 ratio), the desired product **37** (0.185 g, 0.418 mmol, 75%) was obtained as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.73 (1H, s), 6.97 (1H, d, *J* = 8.5 Hz), 6.78 (1H, d, *J* = 8.5 Hz), 6.49 (2H, s), 6.36 (1H, t, *J* = 7.5 Hz), 4.04 (1H, dd, *J* = 11, 4.5 Hz), 3.86 (3H, s), 3.84 (6H, s), 3.80 (3H, s), 3.80 (1H, dd, *J* = 11, 4.5 Hz), 3.71 (1H, t, *J* = 4.5 Hz), 2.62 (2H, t, *J* = 6.5 Hz), 2.16 (2H, m), 1.98 (2H, q, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.5, 153.1, 152.8, 142.5, 140.6, 138.3, 133.5, 129.1, 127.6, 122.5, 109.9, 108.4, 105.3, 65.9, 60.9, 56.3, 56.1, 55.8, 34.0, 27.0, 25.5. HRMS: *m*/*z*: obsd 443.2190 $[M+H]^+$, calcd for $C_{24}H_{31}N_2O_6^+$, 443.2177. HPLC (Method A): 8.23 min.

(*S*)-2-Amino-3-hydroxy-*N*-(2-methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalen-1-yl)propanamide hydrochloride (38). Serinamide **36** (0.055 g, 0.13 mmol) was dissolved in CH_2Cl_2 (0.8 mL), and HCl (4 N in dioxane, 0.080 mL, 0.32 mmol) was added to the solution. After stirring for 1 h, the mixture was removed by evaporation to dryness, and the crude solid was re-crystallized using EtOAc/MeOH to afford desired serinamide salt **38** (0.015 g, 0.032 mmol, 25%) as a light yellow solid ^{1}H NMR (500 MHz, CD_3OD): δ 6.98 (1H, d, *J* = 8.5 Hz), 6.83 (1H, d, *J* = 8.5 Hz), 6.58 (2H, s), 6.01 (1H, t, *J* = 4.5 Hz), 4.20 (1H, dd, *J* = 7.0, 4.0 Hz), 4.15 (1H, dd, *J* = 11, 4.0 Hz), 3.97 (1H, dd, *J* = 11, 4.0 Hz), 3.82 (3H, s), 3.80 (6H, s), 3.79 (3H, s), 2.67 (2H, m), 2.30 (2H, m). ^{13}C NMR (125 MHz, CD_3OD): δ 166.1, 153.7, 152.9, 139.4, 136.9, 136.8, 135.6, 128.3, 125.3, 124.6, 121.6, 107.8, 105.7, 60.6, 59.7, 55.1, 55.1, 54.8, 23.2, 22.4. HRMS: *m/z*: obsd 429.2063 $[M\text{-}Cl]^+$, calcd for $C_{23}H_{29}N_2O_6^+$, 429.2026. HPLC (Method A): 7.94 min.

(*S*)-2-Amino-3-hydroxy-*N*-(3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)propanamide hydrochloride (39). This compound was synthesized using the procedure as described for compound **38.** Starting from serinamide **37** (0.10 g, 0.23 mmol) and HCl (4 N in dioxane, 0.28 mL, 1.1 mmol) in CH_2Cl_2/MeOH (6 mL, 1:1 ratio), the desired product **39** (0.074 g, 0.15 mmol, 67%) was obtained as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 6.96 (1H, d, *J* = 8.5 Hz), 6.92 (1H, d, *J* = 8.5 Hz), 6.53 (2H, s), 6.39 (1H, t, *J* = 7.5 Hz), 4.26 (1H, dd, *J* = 7.5, 4.5 Hz), 4.19 (1H, dd, *J* = 11, 4.0 Hz), 3.99 (1H, dd, *J* = 11, 7.5 Hz), 3.82 (3H, s), 3.76 (6H, s), 3.75 (3H, s), 2.62 (2H, m), 2.16 (2H, m), 1.92 (2H, m). ^{13}C NMR (125 MHz, CD_3OD): δ 166.9, 153.9, 152.8, 142.5, 140.7, 138.4, 137.1, 133.3, 129.3, 126.9,

121.7, 108.5, 105.1, 60.7, 59.7, 55.1, 55.1, 54.8, 33.5, 26.2, 24.9. HRMS: *m*/*z*: obsd 443.2214 $[M-Cl]^+$, calcd for $C_{24}H_{31}N_2O_6^+$, 443.2177. HPLC (Method A): 8.32 min.

(9*H*-fluoren-9-yl)methyl (2-((2-methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalen-1-yl)amino)-2-oxoethyl)carbamate (40). This compound was synthesized using the procedure as described for compound **34.** Starting from amino compound **32** (0.500 g, 1.45 mmol), Fmoc-glycine-OH (0.648 g, 2.18 mmol), T3P (50% in EtOAc, 2.60 mL, 4.35 mmol), and Et_3N (0.310 mL, 2.18 mmol) in CH_2Cl_2 (30 mL) with 12 h stirring, the desired product **40** (0.678 g, 1.10 mmol, 75%) was obtained as a yellow solid. ^1H NMR (500 MHz, $CDCl_3$): δ 7.75 (2H, d, *J* = 7.5 Hz), 7.60 (2H, d, *J* = 7.5 Hz), 7.46 (1H, s), 7.38 (2H, t, *J* = 7.5 Hz), 7.29 (2H, t, *J* = 7.5 Hz), 6.97 (1H, d, *J* = 8.5 Hz), 6.65 (1H, d, *J* = 9.0 Hz), 6.57 (2H, s), 5.99 (1H, t, *J* = 4.5 Hz), 5.61 (1H, s), 4.48 (2H, d, *J* = 7 Hz), 4.24 (1H, t, *J* = 7 Hz), 4.12 (2H, s), 3.88 (3H, s), 3.84 (6H, s), 3.76 (3H, s), 2.67 (2H, t, *J* = 7.5 Hz), 2.30 (2H, td, *J* = 7.5, 4.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.71, 156.57, 152.94, 152.63, 143.84, 143.66, 141.31, 139.47, 137.09, 136.61, 135.38, 128.80, 127.76, 127.07, 125.19, 125.00, 121.92, 120.00, 107.57, 105.80, 67.19, 60.92, 56.16, 55.65, 47.17, 44.86, 24.05, 22.94.

(9*H*-fluoren-9-yl)methyl (2-((3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)amino)-2-oxoethyl)carbamate (41). This compound was synthesized using the procedure as described for compound **34.** Starting from amino compound **33** (0.200 g, 0.563 mmol), Fmoc-glycine-OH (0.250 g, 0.841 mmol), T3P (50% in EtOAc, 1.07 mL, 1.68 mmol), and Et_3N (0.118 mL, 0.841 mmol) in CH_2Cl_2 (10 mL) with 12 h stirring, the desired product **41** (0.350 g, 0.552 mmol, 98%) was obtained as a white solid. ^1H NMR (500 MHz, $CDCl_3$): δ 7.73

(2H, d, *J* = 7.04 Hz), 7.58 (2H, d, *J* = 7.04 Hz), 7.42 (1H, s), 7.35 (2H, t, *J* = 6.80 Hz), 7.27 (2H, t, *J* = 6.80), 6.96 (1H, d, *J* = 8.20 Hz), 6.73 (1H, d, *J* = 7.87 Hz), 6.49 (2H, s), 6.35 (1H, t, *J* = 6.45 Hz), 5.80 (1H, s), 4.45 (2H, m), 4.22 (1H, t, *J* = 6.93 Hz), 4.13 (2H, s), 3.86 (3H, s), 3.79 (6H, s), 3.76 (3H, s), 2.58 (2H, t, *J* = 6.93 Hz), 2.14 (2H, td, *J* = 6.93, 7.32 Hz), 1.95 (2H, q, *J* = 7.32 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.93, 156.84, 153.43, 152.97, 143.76, 142.50, 141.36, 140.86, 138.45, 137.46, 133.53, 129.40, 127.89, 127.82, 127.14, 125.10, 122.34, 120.05, 108.44, 105.41, 67.26, 60.99, 56.26, 55.75, 47.23, 44.93, 33.97, 27.18, 25.69.

2-Amino-*N*-(2-methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalen-1-yl)acetamide (42). This compound was synthesized using the procedure as described for compound **36.** Starting from Fmoc-glycinamide **40** (0.477 g, 0.768 mmol) and a NaOH solution (2 M, 0.770 mL, 1.54 mmol) in CH_2Cl_2/MeOH (9 mL/ 1:1 ratio) with 13 h stirring, the desired product **42** (0.285 g, 0.715 mmol, 93%) was obtained as a tan solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (1H, s), 6.93 (1H, d, *J* = 8.5 Hz), 6.63 (1H, d, *J* = 9.0 Hz), 6.55 (2H, s Hz), 5.95 (1H, t, *J* = 4.5 Hz), 3.85 (3H, s), 3.80 (6H, s), 3.77 (3H, s), 3.55 (2H, s), 2.67 (2H, t, *J* = 7.5 Hz), 2.26 (2H, td, *J* = 7.5, 4.5 Hz), 2.02 (2H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.5, 152.8, 152.8, 139.5, 136.9, 136.7, 135.1, 128.6, 125.0, 124.7, 122.5, 107.6, 105.7, 60.8, 56.1, 55.6, 45.0, 24.1, 22.9. HRMS: *m*/*z*: obsd 399.1930 $[M+H]^+$, calcd for $C_{22}H_{27}N_2O_5^+$, 399.1914. HPLC (Method A): 8.10 min.

2-Amino-*N*-(3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)acetamide (43). This compound was synthesized using the procedure as described for compound **36.** Starting from Fmoc-glycinamide **41**

(0.350 g, 0.552 mmol) and a NaOH solution (2 M, 0.560 mL, 1.12 mmol) in CH_2Cl_2/MeOH (6 mL, 1:1 ratio) with 13 h stirring, the desired product **43** (0.193 g, 0.468 mmol, 83%) was obtained as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 6.92 (1H, d, *J* = 8.58 Hz), 6.88 (1H, d, *J* = 8.61 Hz), 6.53 (2H, s), 6.37 (1H, t, *J* = 7.31 Hz), 3.80 (3H, s), 3.76 (3H, s), 3.74 (6H, s), 3.53 (2H, s), 2.60 (2H, t, *J* = 6.81 Hz), 2.13 (2H, p, *J* = 6.88 Hz), 1.93 (2H, q, *J* = 7.11 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 175.20, 155.54, 154.20, 144.12, 142.12, 139.97, 138.54, 134.61, 130.37, 128.23, 123.91, 109.79, 106.61, 61.15, 56.57, 56.19, 45.06, 34.96, 27.69, 26.38. HRMS: *m*/*z*: obsd 413.2074 $[M+H]^+$, calcd for $C_{23}H_{29}N_2O_5^+$, 413.2071. HPLC (Method A): 8.45 min.

2-Amino-*N*-(2-methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalen-1-yl)acetamide hydrochloride (44). This compound was synthesized using the procedure as described for compound **38.** Starting from glycinamide **42** (0.274 g, 0.688 mmol) and HCl (4 N in dioxane, 0.510 mL, 2.04 mmol) in MeOH (5 mL), the desired product **44** (0.171 g, 0.393 mmol, 57%) was obtained as a light yellow solid. ^{1}H NMR (500 MHz, CD_3OD): δ 6.97 (1H, d, *J* = 8.5 Hz), 6.82 (1H, d, *J* = 8.5 Hz), 6.58 (2H, s), 6.01 (1H, t, *J* = 4.5 Hz), 3.97 (2H, s), 3.81 (3H, s), 3.80 (6H, s), 3.79 (3H, s), 2.69 (2H, m), 2.31 (2H, m). ^{13}C NMR (125 MHz, CD_3OD): δ 165.1, 153.7, 152.9, 139.5, 136.9, 136.8, 135.6, 128.3, 125.3, 124.6, 121.6, 107.8, 105.7, 59.7, 55.1, 54.7, 40.2, 33.2, 22.47. HRMS: *m*/*z*: obsd 399.1919 $[M-Cl]^+$, calcd for $C_{22}H_{27}N_2O_5^+$, 399.1914. HPLC (Method A): 8.03 min.

2-Amino-*N*-(3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)acetamide hydrochloride (45). This compound was synthesized using the procedure as described for compound **38.** Starting from

glycinamide **43** (0.150 g, 0.364 mmol) and HCl (4 N in dioxane, 0.450 mL, 1.80 mmol) in MeOH/CH_2Cl_2 (4 mL, 1:1 ratio), the desired product **45** (0.0847 g, 0.189 mmol, 53%) was obtained as a light yellow solid. ^{1}H NMR (500 MHz, CD_3OD): δ 6.98 (1H, d, *J* = 8.59 Hz), 6.94 (1H, d, *J* = 8.62 Hz), 6.54 (2H, s), 6.41 (1H, t, *J* = 7.31 Hz), 3.99 (2H, s), 3.84 (3H, s), 3.77 (3H, s), 3.77 (6H, s), 2.64 (2H, t, *J* = 6.81 Hz), 2.16 (2H, p, *J* = 6.72 Hz), 1.95 (2H, q, *J* = 7.13 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 167.39, 155.48, 154.25, 144.05, 142.07, 139.85, 138.63, 134.75, 130.80, 128.30, 123.13, 109.93, 106.63, 61.17, 56.59, 56.24, 41.63, 35.01, 27.70, 26.35. HRMS: *m*/*z*: obsd 413.2074 $[M\text{-}Cl]^+$, calcd for $C_{23}H_{29}N_2O_5^+$, 413.2071. HPLC (Method A): 8.47 min.

4.2. Biological Evaluations

4.2.1. Cell Culture and SRB Cytotoxicity Assay[16,82–84]

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% CO_2, 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.[16,82–84] 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.

4.2.2. Inhibition of Tubulin Polymerization[85]

Tubulin assembly experiments were performed using 0.25 mL reaction mixtures (final volume),[85] which contained 1.0 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 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.

4.2.3. Colchicine Binding Assay[86]

Inhibition of [^{3}H]colchicine binding to tubulin was determined using 100 μL reaction mixtures, each containing 1.0 μM tubulin, 5.0 μM [^{3}H]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[86] (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.

4.2.4. *In Vitro* LAP Cleavage Assay

LAP (from porcine kidney microsomes) was purchased from Sigma-Aldrich or Calzyme Laboratories Inc, and its activity (enzyme units) was determined with 24 mM *L*-leucine *p*-nitroanilide as substrate in 50 mM sodium phosphate buffer (pH 7.2) at 37 °C.[5] In preliminary experiments, the extent of cleavage of individual amino acid prodrug conjugates were determined. In a general procedure, LAP (appropriate amount of units) was pre-incubated in 50 mM sodium phosphate buffer (pH 7.2) at 37 °C. The enzymatic reaction was initiated by the addition of substrate. After varying periods of incubation, a small portion of the reaction mixture was quenched by the addition of acetonitrile on ice and centrifuged. The resulting supernatant was diluted by HPLC solvent (acetonitrile/water) and filtered through a 0.2 μm nylon syringe filter and analyzed by HPLC (Agilent Technologies 1200 series) with an

Agilent ZORBAX Eclipse XDB-18 column (4.6 × 150 mm, 5 μm). HPLC operating conditions were: mobile phase, acetonitrile/water containing 0.05% TFA, flow rate, 1.0 mL/min; column temperature, 22 °C; diode array UV detector. For rate studies, aliquots were withdrawn from incubation mixtures at different time points (1-15 min), and they were immediately quenched with acetonitrile and analyzed by HPLC (see supplemental data for more information). Standard calibration curves were used to determine the concentration of individual amino acid prodrug conjugates and their parent compounds.[87]

4.2.5. *In Vitro* HUVEC Tubule Disruption Assay

A layer of Matrigel™ (9.5 mg/mL) was plated manually into each well of a 24-well plate, which was then placed into an incubator at 37 °C for 60 min prior to cell plating. HUVECs (124,000 cells suspended in 300 μL of M200 growth factors) were plated into 24 wells and allowed to incubate at 37 °C for 16 h. The incubated HUVECs were treated with varying concentrations of inhibitors (0.01 μM, 0.1 μM, 1.0 μM), and a photographic record was obtained (9 fields/well, 40x objective).

4.2.5. *In Vivo* Tumor Model[78]

Human breast cancer cells, MDA-MB-231(ATCC), were transfected with a lentivirus containing a firefly luciferase reporter. Induction of tumors was carried out by injecting 10^6 cells mixed with 30% Matrigel™ (BD Biosciences, San Jose, CA) into the mammary fat pads of female SCID mice (UTSW breeding colony). Tumors were allowed to grow to about 5 mm in diameter, determined by calipers, before BLI and assessment of VDAs. All animal procedures were approved by the University of Texas Southwestern Medical Center Animal Care and Use Committee.

4.2.6. *In Vivo* Bioluminescence Imaging (BLI)[79]

BLI was performed as described previously.[79] Briefly, anesthetized, tumor bearing mice (O_2, 2% isoflurane, Henry Schein Inc., Melville, NY) were injected subcutaneously in the fore-back neck region with 80 μL of a solution of luciferase substrate, *D*-luciferin (sodium salt, 120 mg/kg, in saline, Gold Biotechnology, St. Louis, MO). Mice were maintained under anesthesia (2% isoflurane in oxygen, 1 dm^3/min,), while baseline BLI was performed using a Xenogen IVIS® Spectrum (Perkin-Elmer, Alameda, CA). A series of BLI images was collected over 35 min using the following settings: auto exposure time, f-stop = 2, Field of view = D, binning = 4 (medium). Light intensity-time curves obtained from these images were analyzed using Living Image® software. Mice were injected intraperitoneally with either 120 μL of saline (vehicle), CA4P (provided by OXiGENE at 120 mg/kg in saline as used previously[9]) or compound **44** (5, 10, 15, or 20 mg/kg) in saline immediately after baseline BLI. Bioluminescence imaging was repeated, with new luciferin injections 4 and 24 h later. Dosing with 20 and 30 mg/kg was repeated in a separate cohort of mice. Analysis of variance was used to compare the maximum BLI light intensity observed on each occasion with respect to drug administration. Time-point and significance assessed using Fisher's PLSD (protected least squares difference) using Statview software with $p<0.05$ considered significant.

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Supplementary Data

Supplementary data for the synthesis of AVE8062, and characterization data (^{1}H NMR, ^{13}C NMR, ^{31}P NMR, HPLC, HRMS) for final compounds and intermediates (^{1}H NMR, ^{13}C NMR), HPLC chromatograms, calibration curves and rate studies related to LAP enzymatic assays associated with this article is available online.

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CHAPTER THREE

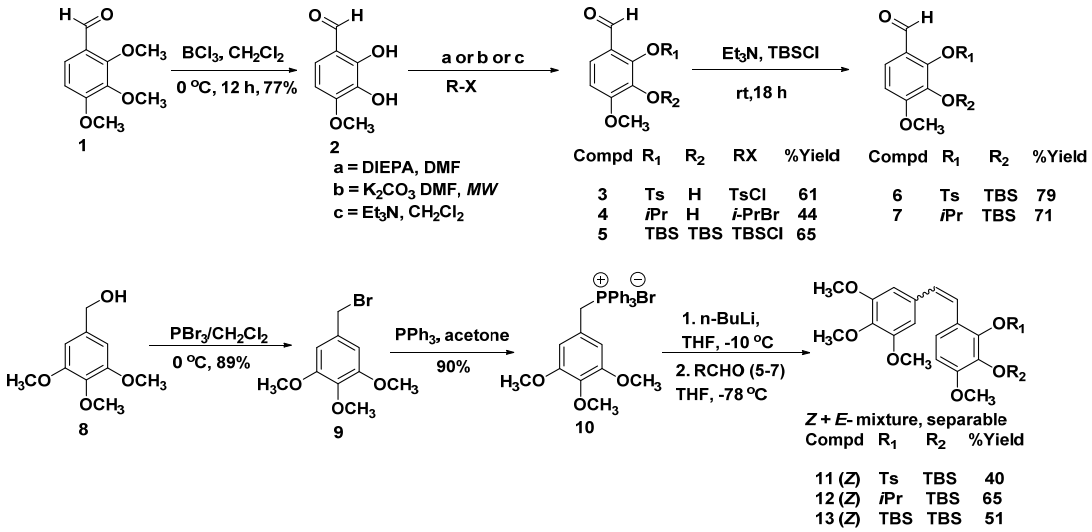
Design, Synthesis and Biological Evaluation of Bioreductively Activatable Prodrug Conjugates of Combretastatin A-1 as anti-cancer Agents - Targeted towards Tumor Hypoxia

Compounds **23**, **31**, **34**, **36**, **38** and **40** were synthesized by Mr. Blake Winn. For compound **41**, Mr. Winn contributed equally

Synthesis

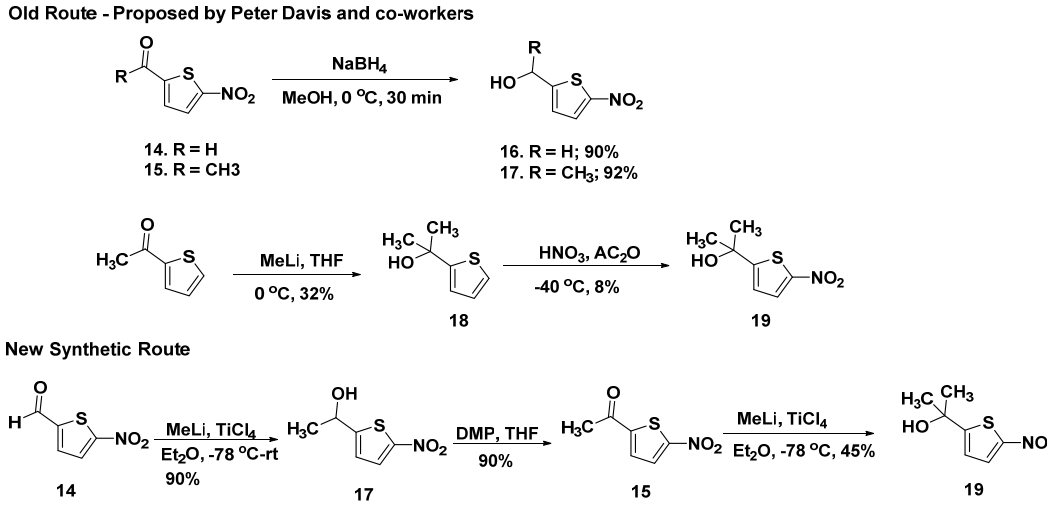
The CA1-BAPCs were obtained by utilizing two key reactions- a Wittig olifination to generate regio-selectively protected CA1, and a Mitsunobu reaction between protected CA1 (**20**, **21**, **27**, and **28**, Schemes **3.3** and **3.5**) and nitrothionyl triggers (**16**, **17** and **19**, Scheme **3.2**).[1,2] The synthesis of regioselectively protected *Z*-CA1 analogs (**11-13**, Scheme **3.1**) has been successfully executed utilizing the Wittig olifination reaction between aldehydes (Scheme **3.1**) and triphenyl phosphonium salt **10**.[1] The Wittig reaction produced both the *Z*- and *E*- isomers of the stilbene, but favored the *Z*- over *E*-isomer (Scheme **3.1**).[1,3-5]

Selective demethoxylation of aldehyde **1** using boron trichloride yielded catechol **2**, which generated selectively protected aldehydes **5**-**7** using a previously reported synthetic strategy.[2–4] Phosphonium salt **10** is 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 the *Z*- and *E*- isomers of stilbenes (**11**-**13**), which were separated using flash column chromatography, favoring the *Z*- isomer.



Scheme **3.1**. Synthesis of Regio-Selectively Protected CA1 **11-13**[3,4]

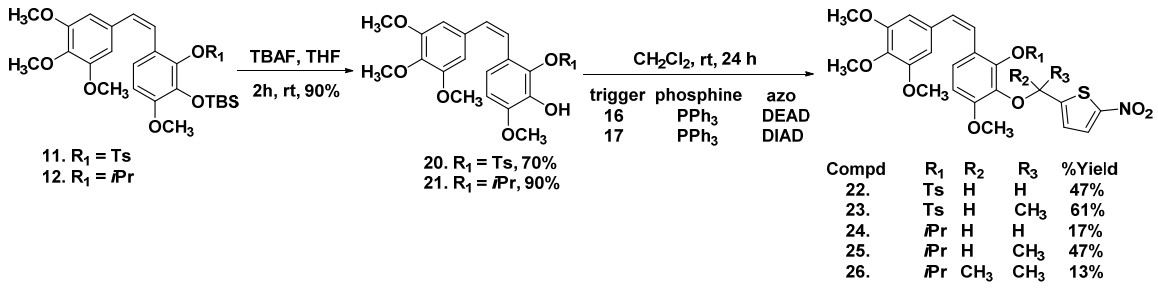
Scheme **3.2** details the synthesis of the three nitrothiophene triggers used in the Mitsunobu reactions. The synthetic route reported by Peter Davis and co-workers highlights the synthesis of *nor*- and *mono*-methyl nitrothiophene triggers **16** and **17** in very good yields through the reduction of their respective aldehyde and ketone **14** and **15**.[6]



Scheme **3.2**. Synthesis of Nitrothiophene Triggers Using Peter Davis and Co-Workers Route and New Route[5,11]

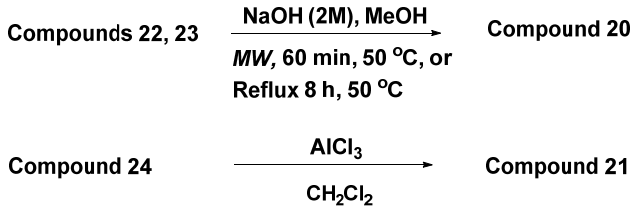
However, in our hands (Scheme **3.2**), synthesis of gem-dimethyl nitrothiophene trigger **19** suffered with two consecutive low yielding steps, methylation followed by nitration. In order to scale up the production of compound **19**, it was imperative for us to develop a new and improved synthetic route. The new synthetic route provided all triggers (*nor*-, *mono*- and *gem*-) from a single starting material **14**. The methylation of aldehyde **14** furnished *mono*-methyl trigger **17**, which on subsequent oxidation and methylation yielded *gem*-dimethyl trigger **19** in a good yield (scheme **3.2**).

The deprotection of selectively protected CA1 **11** and **12** using TBAF yielded their respective phenols **20** and **21**, which were further reacted under Mitsunobu conditions with nitrothiophene triggers (**16**, **17** and **19**), phosphines (PPh_3 or PBu_3) and azo compounds (DEAD, DIAD or ADDP) to generate their respective products **22-26** (Scheme **3.3**).



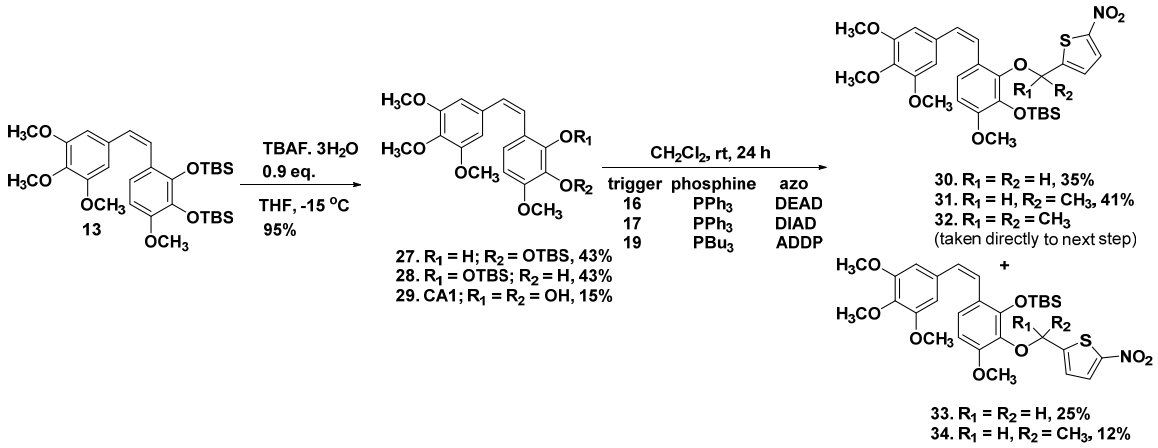
Scheme **3.3**. Synthesis of Regio-Selectively Protected CA1-BAPCs **22-26**

The attempted deprotection of compounds **22** and **23** using either microwave or reflux conditions did not yield the desired product, instead the trigger component was cleaved from the starting material, regenerating compound **20 (**scheme **3.4).**



Scheme **3.4**. Attempted Deprotection of Compounds **22**, **23** and **24**

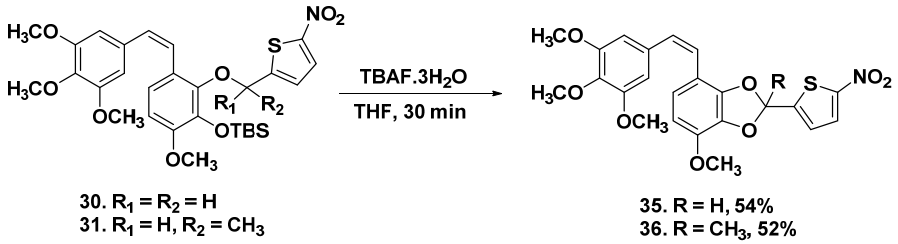
Similarly, compound **24** regenerated compound **21** during its attempted deprotection using $AlCl_3$ (Scheme **3.4**). In order to solve this problem, we employed a method to partially cleave the di-TBS protected CA1 **13** using less than 1 molar equivalent of Tetra-n-butylammonium fluoride (TBAF, Scheme **3.5**) resulting in regio-isomers **27** and **28**, which were inseparable in flash column chromatography.



Scheme **3.5**. Synthesis of TBS-Protected CA1-BAPCs **30**-**34**

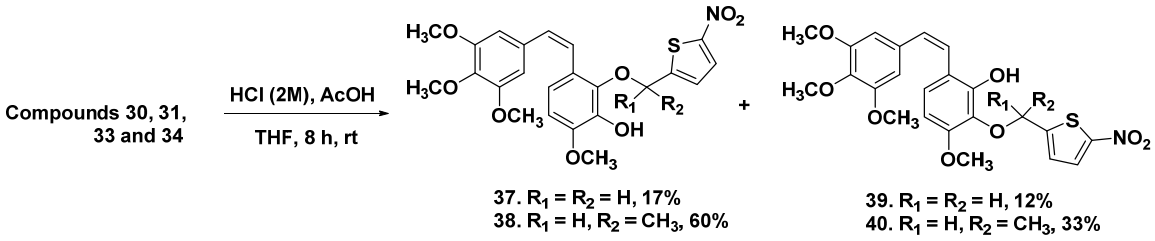
While the mixture of regio-isomers **27** and **28** produced their respective Mitsunobu products **30**-**34**, CA1 **29** did not react at all under these conditions. Interestingly, the conventional TBS-deprotection of compounds **30** and **31** using

TBAF yielded ring-cyclized products **35** and **36** (proposed structures based on the NMRs and HRMS) without producing any other side products (Scheme **3.6**).



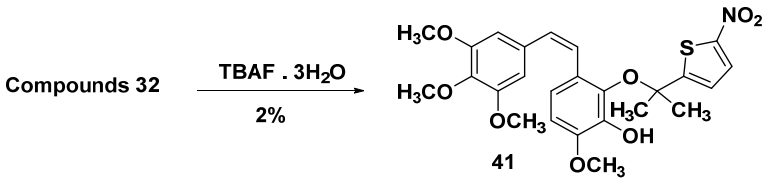
Scheme **3.6**. TBS-Deprotection Generating Ring-Cyclized Products **35** and **36**

However, on switching the deprotecting reagent from TBAF to 2M HCl/AcOH, the desired CA1 BAPCs **37**-**40** were obtained (Scheme **3.7**).



Scheme **3.7**. Synthesis of *Nor*- and *Mono*- Methyl CA1 BAPCs **37**-**40**

As the purification of TBS-protected *gem*-dimethyl CA1 BAPC **32** by column chromatography proved to be unsuccessful, it is taken directly to the deprotection step as detailed in scheme **3.8** to generate *gem*-dimethyl CA1 BAPC **41**. As opposed to earlier (Scheme **3.6**), in this case of deprotection reaction using TBAF as a reagent, we did not observe any ring-cyclized product.



Scheme **3.8**. Synthesis of Gem-Dimethyl CA1 BAPC **41**

Biological Evaluation

The BAPCs and their parent drugs were also evaluated for their ability to inhibit tubulin polymerization and colchicine binding, and the results are shown in Table **3.1**. The inhibition of tubulin polymerization results obtained with the synthesized CA1, CA4 BAPCs are shown in comparison to the standards CA1 and CA4. The inhibition of tubulin polymerization of the synthesized CA1 BAPCs are shown with the standards CA1, CA4 (Table **3.1**). The tosyl and TBS-protected CA1 parent drugs showed excellent inhibition of tubulin polymerization (0.84 and 0.82 μM respectively), and were found to be more active than CA1 (1.9 μM). Similarly, they exhibited excellent percent inhibition to the colchicine binding as well. Seven of the newly synthesized prodrug conjugates showed significant activity for inhibition of tubulin assembly. Among them, compounds **35**, **37**, **38** and **41** shown the inhibition of tubulin polymerization IC_{50} value (ranging from 0.82 to 1.7 μM) which were comparable to CA1 and CA4. Similarly, five of the BAPCs showed inhibition of colchicine binding, 92% being the highest inhibition shown by compound **37**.

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

Compd	Inhibition of Tubulin Polymerizat-ion IC_{50}	Inhibition of Colchicine Binding % Inhibition μM ±SD	
	(μM)±SD	1 μM	5 μM
29 KGP439	1.9[c]	ND	99.6±0.7
CA1[b]			
CA4[d]	0.64	84±2	97±0.7
20 KGP400	0.84±0.1	50±5	84±1
21 KGP440	0.82±0.04	72±4	94±0.7
22 KGP441	>20	ND	ND
23 KGP442	>20	ND	ND
25 KGP443	12±1	ND	ND
26 KGP444	>20	ND	ND
27 KGP445	9.5±0.9	ND	ND
35 KGP446	1.7±0.2	ND	25±3
36 KGP455	>20	ND	ND
37 KGP457	1.7±0.01	53±3	92±0.5
38 KGP454	0.84±0.1	34±3	90±0.7
39 KGP456	4.3±0.4	ND	58±4
40 KGP461	ND	ND	ND
41	1.3±0.08	ND	43±4

[a] Average of n ≥ 3 independent determinations
[b] Data from ref. 6, see ref. 9 for additional data
[c] Data from ref. 10, see ref. 9 for additional data
[d] For additionl data, see ref. 9
ND= Data not available

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 (λ = 190–400 nm), a Zorbax XDB-C18 HPLC column (4.6 mm Å~ 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; solvent A acetonitrile, solvent B H_2O; Method A: H_2O; gradient, 10% A/90% B to 100% A/0% B over 0 to 40 min; post-time 10 min, Method B: H_2O; gradient,

50% A/50% B to 90% A/10% B over 0 to 30 min; post-time 10 min; flow rate 1.0 mL/min; injection volume 20 μL; monitored at wavelengths of 210, 230, 254, 280, and 320 nm. Mass spectrometry was carried out under positive or negative ESI (electrospray ionization) using a Thermo Scientific LTQ OrbitrapDiscovery instrument.

Experimental Section

*2,3-Dihydroxy-4-methoxybenzaldehyde (**2**)*[10,11]

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.

^{1}H NMR (500 MHz, $CDCl_3$): δ 11.12 (1H, s), 9.76 (1H, s), 7.15 (1H, d, *J* = 8.5 Hz), 6.63 (1H, d, *J* = 8.5 Hz), 5.46 (1H, s), 3.99 (3H, s).

^{13}C NMR (125 MHz, $CDCl_3$): δ 195.2, 153.0, 149.0, 133.0, 126.1, 116.1, 103.6, 56.4.

*6-formyl-2-hydroxy-3-methoxyphenyl 4-methylbenzenesulfonate (**3**)*

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 H_2O (20 mL), and extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, 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.

^{1}H NMR (600 MHz, $CDCl_3$): 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).

^{13}C NMR (151 MHz, $CDCl_3$): δ 187.0, 153.2, 146.2, 139.2, 138.2, 132.0, 130.0, 128.7, 124.1, 120.6, 109.2, 77.3, 77.0, 76.8, 56.7, 21.8.

*3-Hydroxy-2-isopropoxy-4-methoxybenzaldehyde (**4**)*[10,11]

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 (13) (0.220 g, 1.05 mmol, 44%) as a tan solid.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 10.24 (1H, s), 7.41 (1H, d, *J*=8.7 Hz), 6.71 (1H, d, *J*=8.7 Hz), 5.77 (1H, d, *J*=4.8 Hz), 4.67 (1H, hept, *J*=6.1 Hz), 3.94 (3H, s), 1.34 (7H, d, *J*=6.2 Hz)

^{13}C NMR (151 MHz, $CDCl_3$): δ 189.68, 152.75, 147.90, 138.62, 124.34, 120.36, 106.22, 77.00, 56.44, 22.43.

*2,3-bis((tert-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (**5**)*

To a solution of 2,3-Dihydroxy-4-methoxybenzaldehyde (1.00 g, 5.95 mmol), Et_3N (2.00 mL, 14.3 mmol), and DMAP (0.025 g, 0.200 mmol) in CH_2Cl_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_2O was used 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 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 189.64, 157.88, 151.32, 137.10, 123.64, 121.69, 105.73, 77.57, 77.36, 77.15, 55.53, 26.51, 26.36, 19.07, 18.89, -3.51.

*2-((tert-butyldimethylsilyl)oxy)-6-formyl-3-methoxyphenyl 4-methylbenzenesulfonate (**6**)*

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**)*

Aldehyde **4** (1.39 g, 6.61 mmol), Et_3N (1.40 mL, 9.91 mmol), and DMAP (0.050 g, 0.40 mmol) were dissolved in dry CH_2Cl_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**)*

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 ^oC 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 **3** (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, O*C*H_3-4), 56.1 (CH_3, O*C*H_3-3, -5), 34.3 (CH_2, -*C*H_2Br).

*3,4,5-Trimethoxybenzyltriphenylphosphonium Bromide (**10**)*

A mixture of bromide **3** (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 **4** (20.3 g, 38.2 mmol, 92% yield) as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 – 7.64 (9H, m, Ar*H*), 7.58 – 7.50 (6H, m, Ar*H*), 6.43 (2H, d, *J*=2.6 Hz), 5.29 (2H, d, *J*=14.1 Hz, benzylic CH_2), 3.70 (3H, d, *J*=3.4 Hz), 3.43 (6H, d, *J*=3.7 Hz) **^{13}C NMR** (125 MHz, $CDCl_3$): δ 153.0 (C, C-3, C-5), 137.6 (C, C-4), 134.8 [CH, Ph(C-4)], 134.6 [CH, Ph(C-3, C-5)], 130.0 [CH, Ph(C-2, C-6)], 122.4 (C, C-1), 117.8 [C, Ph(C-1)], 108.8 (CH, C-2, C-6), 60.8 (CH_3, O*C*H_3-4), 56.2 (CH_3, O*C*H_3-3, -5), 30.8 (CH_2, -*C*H_2P). ^{31}P NMR (243 MHz, $CDCl_3$) δ 23.2.

*(Z)-2-((tert-butyldimethylsilyl)oxy)-3-methoxy-6-(3,4,5-trimethoxystyryl)phenyl 4-methylbenzenesulfonate (**11**)*

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).

^{13}C 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**)*

Triphenyl(3,4,5-trimethoxybenzyl)phosphonium bromide (1.94 g, 3.70 mmol) was dissolved in dry THF (50 mL) and cooled to -15 °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. Reaction mixture was cooled to -78 °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.

^{1}H 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).

^{13}C 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**)*

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, 137.1, 133.1, 128.0, 127.7, 123.5, 122.5, 106.2, 104.5, 77.6, 77.4, 77.1, 61.2, 56.1, 55.3, 26.7, 26.4, 19.1, 18.9, -2.9, -3.6.

*Synthesis of Compounds **16**, **17** and **19** using Old Route*[6]

*(5-nitrothiophen-2-yl)methanol (**16**)*[6]

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 **7** (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**)*[6]

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 **8** (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**)*

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 (**36**) (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**)*

The tertiary alcohol **18** (6.22 g, 4.37 mmol) was dissolved in Ac_2O (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 repeatedly with brine, water and saturated sodium bicarbonate, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified using flash column chromatography affording the alcohol product **19** (0.655 g, 0.35 mmol, 8%) as an orange wax.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.79 (1H, d, *J*=4.2 Hz), 6.87 (1H, d, *J*=4.2 Hz), 2.13 (1H, s), 1.67 (3H, s).

^{13}C NMR (151 MHz, $CDCl_3$): δ 163.6, 133.9, 128.9, 121.4, 77.4, 77.2, 76.9, 72.0, 32.2.

Synthesis of Compounds **16**, **17** and **19** using New Route

*1-(5-nitrothiophen-2-yl)ethan-1-ol (**17**)*[11]

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.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.81 (1H, d, *J* = 4 Hz), 6.90 (1H, d, *J* = 4 Hz), 5.15 (1H, dq, *J* = 6 Hz, *J* = 5 Hz), 2.23 (1H, d, *J* = 5 Hz), 1.63 (3H, d, *J* = 6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.0, 149.9, 129.1, 122.2, 66.3, 25.1.

*1-(5-nitrothiophen-2-yl)ethan-1-one (**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.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.89 (1H, d, *J*=4.3 Hz), 7.58 (1H, d, *J*=4.3 Hz), 2.60 (3H, s)

^{13}C NMR (151 MHz, $CDCl_3$): δ 190.5, 156.5, 148.2, 130.2, 128.4, 26.6.

*2-(5-nitrothiophen-2-yl)propan-2-ol (**19**)*[11]

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.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.79 (1H, d, *J*=4.2 Hz), 6.87 (1H, d, *J*=4.2 Hz), 2.13 (1H, s), 1.67 (3H, s).

^{13}C NMR (151 MHz, $CDCl_3$): δ 163.6, 133.9, 128.9, 121.4, 77.4, 77.2, 76.9, 72.0, 32.2.

*(Z)-2-hydroxy-3-methoxy-6-(3,4,5-trimethoxystyryl)phenyl 4-methylbenzene-sulfonate (**20**)*

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.

^{1}H NMR (500 MHz, $CDCl_3$): 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).

^{13}C NMR (126 MHz, $CDCl_3$): 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, 76.4, 76.1, 76.1, 75.9, 60.0, 55.6, 55.0, 20.9.

*(Z)-2-isopropoxy-6-methoxy-3-(3,4,5-trimethoxystyryl)phenol (**21**)*

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.

^{1}H NMR (500 MHz, $CDCl_3$): 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).

^{13}C NMR (126 MHz, $CDCl_3$): 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, 77.2, 77.0, 76.7, 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 CH_2Cl_2 (10 mL), PPh_3 (0.610 g, 2.33 mmol) was dissolved in CH_2Cl_2 and added drop-wise. The reaction was allowed to stir for 12 h at room temperature. H_2O was used to quench the reaction and the residue was extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude 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.

^{1}H NMR (600 MHz, $CDCl_3$): 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_2Cl_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 7.84 (2H, d, *J* = 8.5 Hz), 7.72 (1H, d, *J* = 4 Hz), 7.25 (2H, d, *J* = 8 Hz), 6.94 (1H, d, *J* = 8.5 Hz), 6.75 (1H, d, *J* = 4.5 Hz), 6.66 (1H, d, *J* = 8.5 Hz), 6.48 (2H, s), 6.48 (1H, d, *J* = 10.5 Hz), 6.42 (1H, d, *J* = 12 Hz), 5.32 (1H, q, *J* = 6 Hz), 3.83 (3H, s), 3.80 (3H, s), 3.67 (6H, s), 1.42 (3H, d, *J* = 6.5 Hz).

^{13}C NMR (125 MHz, $CDCl_3$): δ 154.0, 152.8, 152.5, 145.1, 138.8, 134.5, 132.1, 131.7, 129.5, 128.4, 127.8, 126.1, 126.0, 124.2, 123.8, 110.3, 106.2, 75.4, 60.9, 56.1, 55.9, 21.9, 21.6, 21.5.

*(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 CH_2Cl_2 (10 mL), PPh_3 (0.430 g, 1.64 mmol) was dissolved in CH_2Cl_2 and added drop-wise. The reaction was allowed to stir for 24 h at room temperature. H_2O (40 mL) was used 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 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 **25** (0.0600 g, 0.116 mmol, 17%) as a yellow oil.

^{1}H 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, 77.3, 77.0, 76.8, 76.0, 69.2, 60.9, 55.9, 55.8, 22.6.

HRMS: *m*/*z*: obsd 538.1506 $[M+23]^+$, calcd for $C_{26}H_{29}NO_8S^+$ 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_2Cl_2 (10 mL), PPh_3 (0.364 g, 1.39 mmol) was dissolved in CH_2Cl_2 and added drop-wise. The reaction was allowed to stir for 12 h at room temperature. H_2O was used 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: 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 **26** (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). **^{13}C NMR** (151 MHz, $CDCl_3$): 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 $C_{27}H_{31}NO_8S^+$, 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 (0.091 g, 0.486 mmol), and ADDP (0.137 g, 0.543 mmol) in CH_2Cl_2 (10 mL), PBu_3 (0.199 mL) was added drop-wise. The reaction was allowed to stir for 24 h at room temperature. H_2O was used 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 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 **27** (0.020 g, 0.037 mmol, 13%) as an orange oil.

^{1}H NMR (600 MHz, $CDCl_3$): 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).

^{13}C 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 $C_{28}H_{33}NO_8S^+$, 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**)*

To a solution of di-TBS CA1 **13** (2.00 g, 3.57 mmol) in THF (150 mL) at -15 °C, TBAF·$3H_2O$ (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_2O 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_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 70%A / 30%B (13 CV), 70%A / 30%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm]

affording a mixture of compounds **27** and **28** (0.860 g, 2.59 mmol, 43%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.80 (1H, d, *J*=8.7 Hz), 6.71 (1H, d, *J*=8.7 Hz), 6.58 (2H, d, *J*=12.0 Hz), 6.52 (4H, s), 6.47 (1H, d, *J*=12.1 Hz), 6.41 (1H, d, *J*=12.2 Hz), 6.36 (1H, d, *J*=8.5 Hz), 6.30 (1H, d, *J*=8.6 Hz), 5.66 (1H, s), 5.45 (1H, s), 3.81 (6H, s), 3.78 (3H, s), 3.74 (3H, s), 3.64 (12H, d, *J*=2.2 Hz), 1.01 (9H, d, *J*=5.2 Hz), 1.00 (9H, s), 0.22 (6H, s), 0.19 (6H, s).

^{13}C NMR (126 MHz, $CDCl_3$): δ 152.7, 152.7, 149.3, 146.9, 145.9, 141.2, 137.0, 137.0, 136.8, 132.9, 132.8, 131.6, 129.6, 129.0, 126.8, 124.5, 123.2, 122.0, 120.1, 117.1, 106.1, 106.0, 103.8, 103.0, 60.9, 60.8, 56.1, 55.8, 55.7, 55.2, 26.0, 26.0, 18.6, 18.6, -3.9, -4.4.

*(Z)-3-methoxy-6-(3,4,5-trimethoxystyryl)benzene-1,2-diol (**29**)*

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

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.76 (1H, d, *J*=8.6 Hz), 6.59 (1H, d, *J*=12.1 Hz), 6.54 (1H, d, *J*=11.9 Hz), 6.52 (2H, s), 6.39 (1H, d, *J*=8.6 Hz), 5.39 (2H, s), 3.86 (3H, s), 3.83 (3H, s), 3.67 (6H, s).

^{13}C NMR (126 MHz, $CDCl_3$): δ 152.9, 146.5, 141.7, 137.4, 132.7, 132.6, 130.5, 124.2, 120.5, 118.0, 106.1, 103.1, 77.4, 77.2, 77.2, 76.9, 61.0, 56.3, 56.0.

HRMS: *m*/*z*: obsd 355.154 $[M+Na]^+$, calcd for $C_{18}H_{20}O_6^+$, 332.1260. **HPLC** (Method A): 11.3 min.

*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 CH_2Cl_2 (50 mL), PPh_3 (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.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.81 (1H, d, *J*=4.1 Hz), 7.00 (1H, d, *J*=8.7 Hz), 6.96 (1H, d, *J*=4.1 Hz), 6.56 (1H, d, *J*=12.2 Hz), 6.52 (2H, s), 6.44 (1H, d, *J*=11.6 Hz), 6.42 (1H, d, *J*=8.5 Hz), 5.30 (2H, s), 3.83 (3H, s), 3.80 (3H, s), 3.67 (6H, s), 1.01 (9H, s), 0.18 (6H, s).

^{13}C NMR (151 MHz, $CDCl_3$): δ 153.4, 152.8, 152.7, 148.8, 147.8, 138.4, 137.0, 132.6, 129.1, 128.2, 126.3, 125.9, 125.4, 123.3, 105.9, 104.9, 68.8, 60.9, 55.9, 55.8, 26.1, 18.6, -3.9.

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

Mono TBS CA1 (0.680 g, 1.52 mmol), diisopropyl azodicarboxylate (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.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.73 (1H, d, *J*=4.2 Hz), 6.86 (1H, d, *J*=8.5 Hz), 6.85 (1H, d, *J*=4.1 Hz), 6.53 (1H, d, *J*=8.4 Hz), 6.51 (1H, d, *J*=11.3 Hz), 6.44 (2H, s), 6.41 (1H, d, *J*=12.2 Hz), 5.71 (1H, q, *J*=6.4 Hz), 3.82 (3H, s), 3.78 (3H, s), 3.64 (6H, s), 1.61 (3H, d, *J*=6.4 Hz), 0.98 (9H, s), 0.12 (3H, s), 0.11 (3H, s).

^{13}C NMR (151 MHz, $CDCl_3$): δ 154.9, 152.7, 151.4, 150.8, 146.0, 138.4, 137.2, 132.4, 129.9, 128.1, 125.5, 124.9, 123.5, 122.4, 107.1, 106.0, 74.4, 60.7, 55.7, 55.2, 25.8, 22.2, 18.6, -4.4, -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.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77 (1H, d, *J*=4.2 Hz), 7.00 (1H, d, *J*=8.7 Hz), 6.88 (1H, d, *J*=4.2 Hz), 6.54 (1H, d, *J*=11.9 Hz), 6.53 (1H, s), 6.42 (1H, d, *J*=7.5 Hz), 6.40 (1H, d, *J*=4.0 Hz), 5.42 (1H, q, *J*=6.5 Hz), 3.83 (1H, s), 3.78 (1H, s), 3.66 (2H, s), 1.64 (1H, d, *J*=6.5 Hz), 1.00 (3H, s), 0.19 (1H, s), 0.16 (1H, s).

^{13}C NMR (151 MHz, $CDCl_3$): δ 155.2, 153.0, 152.8, 151.0, 148.0, 137.4, 137.1, 132.6, 128.8, 128.1, 126.5, 125.6, 123.4, 123.3, 106.0, 104.9, 75.0, 60.9, 55.8, 55.8, 26.1, 21.7, 18.5, -3.3, -3.8.

*(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· $3H_2O$ (0.0672 g, 0.213 mmol) was dissolved in THF (10 mL) and added drop-wise. The reaction was stirred (30 min) and H_2O (5 mL) was added. 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 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.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.83 (1H, d, *J*=4.2 Hz), 7.15 (1H, d, *J*=4.2 Hz), 7.07 (1H, s), 6.86 (1H, d, *J*=8.8 Hz), 6.56 (1H, d, *J*=12.0 Hz), 6.50 (2H, s), 6.48 (1H, d, *J*=8.8 Hz), 6.44 (1H, d, *J*=12.0 Hz), 3.90 (3H, s), 3.83 (3H, s), 3.69 (6H, s).

^{13}C NMR (151 MHz, $CDCl_3$): δ 152.9, 146.0, 145.2, 143.2, 137.3, 133.8, 132.6, 131.2, 128.1, 126.0, 123.4, 121.7, 113.5, 107.8, 105.7, 105.6, 105.2, 60.9, 56.6, 55.9.

^{13}C NMR DEPT ($CDCl_3$, 151 MHz): δ 131.2, 128.1, 126.0, 123.4, 121.7, 107.8, 105.6, 105.2, 60.9, 56.6, 55.9.

HRMS: *m/z*: obsd 494.0881 $[M+23]^+$, calcd for $C_{23}H_{21}NO_8S^+$, 471.0988. **HPLC** (Method A): 17.2 min.

*(Z)-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 °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 7.90 (1H, d, *J*=4.3 Hz), 7.25 (1H, d, *J*=4.3 Hz), 6.82 (1H, d, *J*=8.7 Hz), 6.63 (1H, d, *J*=8.8 Hz), 6.58 (1H, d, *J*=12.0 Hz), 6.53 (2H, s), 6.44 (1H, d, *J*=12.0 Hz), 3.88 (3H, s), 3.68 (3H, s), 3.61 6H, s), 2.06 (3H, s).

^{13}C NMR (126 MHz, acetone): δ 153.0, 151.3, 145.1, 143.4, 137.8, 134.0, 132.5, 131.4, 129.0, 124.9, 122.9, 121.7, 113.8, 113.5, 108.1, 106.1, 67.2, 59.6, 56.1, 55.2, 25.5.

*(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.

^{1}H NMR (600 MHz, $CDCl_3$): 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).

^{1}H NMR (600 MHz, Acetone): δ 8.15 (1H, s), 7.93 (1H, d, *J*=4.2 Hz), 7.21 (1H, d, *J*=4.1 Hz), 6.95 (1H, d, *J*=8.7 Hz), 6.58 (2H, s), 6.54 (1H, d, *J*=12.2 Hz), 6.49 (1H, d, *J*=8.7 Hz), 6.44 (1H, d, *J*=12.2 Hz), 5.29 (2H, s), 3.86 (3H, s), 3.68 (3H, s), 3.62 (6H, s).

^{13}C NMR (151 MHz, Acetone): δ 153.1, 152.3, 149.2, 148.6, 137.4, 134.1, 132.7, 129.0, 128.6, 126.5, 125.1, 124.5, 124.5, 117.9, 106.2, 103.0, 68.5, 59.6, 55.4, 55.2.

^{13}C NMR DEPT (151 MHz, Acetone): δ 129.0, 128.6, 126.5, 125.1, 124.5, 106.2, 103.0, 68.5, 59.6, 55.4, 55.2.

HRMS: *m/z*: obsd 496.1034 $[M+Na]^+$, calcd for $C_{23}H_{23}NO_8S^+$, 473.1144. **HPLC** (Method B): 10.0 min.

*(Z)-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*=8.2 Hz), 6.55 (1H, d, *J*=8.6 Hz), 6.54 (1H, d, *J*=12.4 Hz), 6.47 (2H, s), 6.45 (1H, d, *J*=12.3 Hz), 5.71 (1H, q, *J*=6.4 Hz), 5.65 (1H, s), 3.87 (3H, s), 3.84 (3H, s), 3.66 (6H, s), 1.71 (3H, d, *J*=6.5 Hz).

^{13}C NMR (126 MHz, $CDCl_3$): δ 154.5, 152.7, 146.9, 141.6, 138.5, 137.2, 132.3, 130.2, 128.1, 125.0, 124.5, 123.7, 120.6, 110.0, 106.4, 106.0, 74.9, 60.9, 56.3, 55.8, 22.2.

*(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_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 **33** (0.030 g, 0.0634 mmol, 12%) as a brown oil.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.77 (1H, d, *J*=4.1 Hz), 6.97 (1H, d, *J*=4.1 Hz), 6.79 (1H, d, *J*= 8.4 Hz), 6.56 (1H, d, *J*= 8.4 Hz), 6.55 (1H, d, *J*=12.2 Hz), 6.50 (1H, d, *J*=12.2 Hz), 6.45 (2H, s), 5.59 (1H, s), 5.24 (2H, s), 3.88 (3H, s), 3.82 (3H, s), 3.65 (6H, s).

^{13}C NMR ($CDCl_3$, 151 MHz): δ 152.8, 151.8, 148.5, 147.0, 142.6, 138.4, 137.2, 132.4, 130.7, 128.2, 125.5, 124.6, 124.3, 120.5, 106.6, 106.0, 68.7, 60.9, 56.4, 55.8.

HRMS: *m/z*: obsd 496.1033 $[M+Na]^+$, calcd for $C_{23}H_{23}NO_8S^+$, 473.1144. **HPLC** (Method B): 12.5 min.

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

Compound **38** (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). H_2O (20 mL) was used to quench the reaction and it was concentrated under reduced pressure. The residue was extracted with CH_2Cl_2 (3 x 20 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 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 **38** (0.026 g, 0.055 mmol, 33%) as a yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.78 (1H, d, *J*=4.2 Hz), 6.95 (1H, d, *J*=8.7 Hz), 6.92 (1H, d, *J*=4.2 Hz), 6.52 (2H, s), 6.48 (2H, s), 6.36 (1H, d, *J*=8.8 Hz), 5.71 (1H, s), 5.56 (1H, q, *J*=6.5 Hz), 3.83 (3H, s), 3.82 (3H, s), 3.67 (6H, s), 1.72 (3H, d, *J*=6.5 Hz).

^{13}C NMR (126 MHz, $CDCl_3$): 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, 110.0, 105.8, 103.4, 103.3, 75.2, 60.9, 55.9, 55.8, 21.7. **HPLC** (Method B): 11.5 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·$3H_2O$ (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. H_2O (40 mL) was used to quench the reaction, THF was evaporated off completely, and the residue was extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (1 CV), 7%A / 93%B → 60%A / 40%B (13 CV), 60%A / 40%B (2 CV); flow rate: 20 mL/min; monitored at 254 and 280 nm] affording compound **32** (0.050 g, 0.980 mmol, 2%) as a brownish-yellow solid.

^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.77 (1H, d, *J*=4.2 Hz), 6.93 (1H, d, *J*=4.2 Hz), 6.84 (1H, d, *J*=8.6 Hz), 6.56 (1H, d, *J*=8.6 Hz), 6.52 (2H, s), 6.48 (1H, d, *J*=12.2 Hz), 6.30 (1H, d, *J*=12.2 Hz), 5.47 (1H, s), 3.86 (3H, s), 3.84 (3H, s), 3.67 (6H, s), 1.79 (6H, s).

^{13}C NMR ($CDCl_3$, 151 MHz): δ 161.5, 152.9, 150.6, 147.1, 140.6, 140.2, 137.3, 132.5, 129.3, 128.4, 127.2, 126.6, 122.2, 120.6, 106.9, 106.0, 81.9, 77.4, 77.2, 76.9, 61.1, 56.4, 56.0, 29.5.

HRMS: *m*/*z*: obsd 524.1352 $[M+Na]^+$, calcd for $C_{25}H_{27}NO_8S^+$, 501.1457. **HPLC** (Method B): 12.3 min.

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CHAPTER FOUR

Benzosuberene-Based Tumor-Vascular Disrupting Agents

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The author L. Devkota contributed in this manuscript by synthesizing a final compound **32** and all of its intermediates. Also, the author L. Devkota characterized and verified the compounds synthesized by R. P. Tanpure. In addition, L. Devkota contributed significantly to the preparation of the manuscript.

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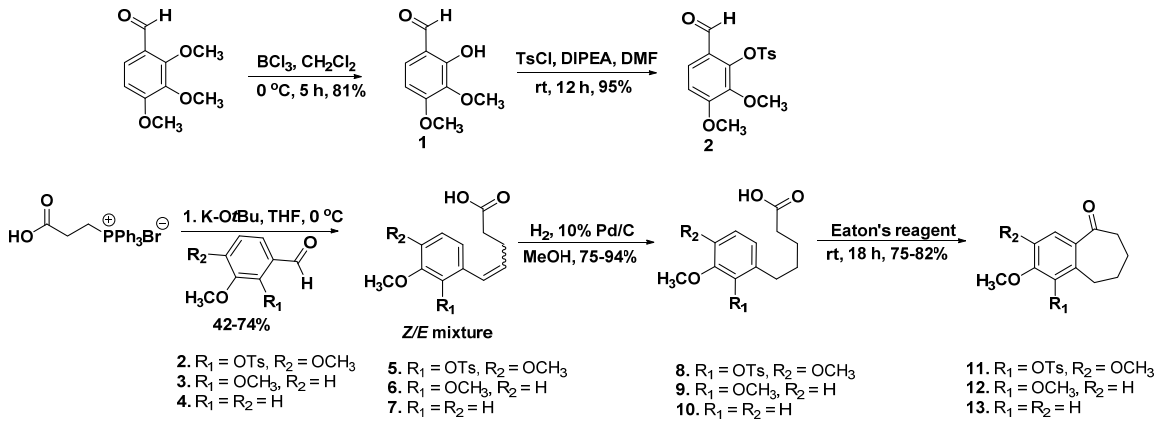
The author L. Devkota contributed in this manuscript by synthesizing five final compounds **27**, **30**, and **33-36** and all of their intermediates. Also, the author L. Devkota contributed significantly to the preparation of the manuscript.

Synthesis

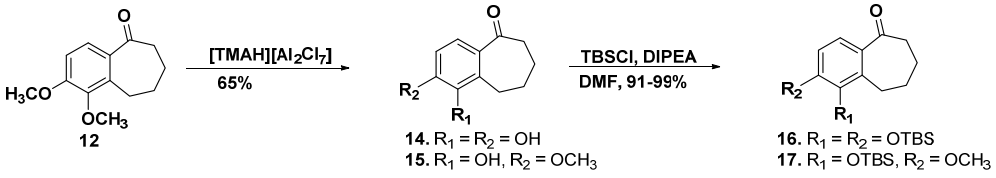
Seven benzosuberene analogues, including KGP18, were synthesized and evaluated for their cytotoxicity (*in vitro*) against selected human cancer cell lines (SK-OV-3, NCI-H460 and DU-145). Furthermore, the inhibition of tubulin polymerization ability of these compounds were also evaluated. The synthesis of each benzosuberene analogues involved the Wittig olefination reaction between the suitably designed aldehydes **2-4** and Wittig salt followed by a catalytic hydrogenation reaction to afford carboxylic acid intermediates **8-10**. Protecting group strategies were included where necessary. The use of Eaton's reagent[1,2]

facilitated an intramolecular Friedel-Crafts annulation of the carboxylic acid intermediates yielding the desired benzosuberone intermediates **11-13** (Scheme **4.1**).



Scheme **4.1**. Synthesis of Benzosuberone Intermediates **11-13**

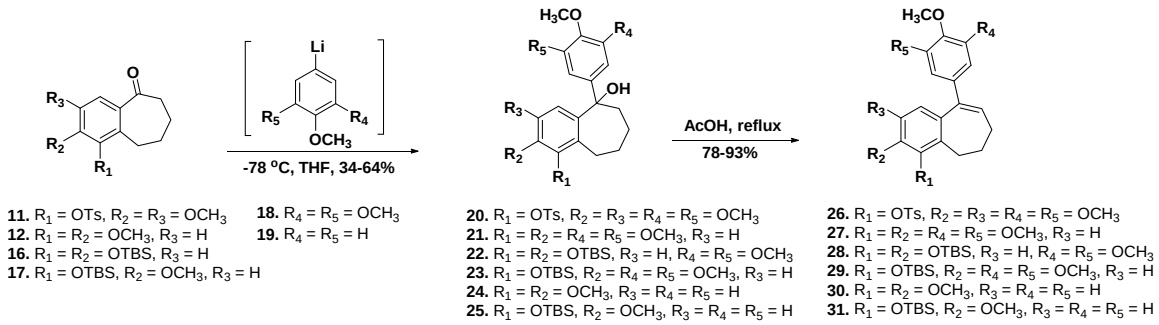
Additional modifications of benzosuberone **12** were achieved through its selective demethylation with the ionic liquid [TMAH][Al_2Cl_7], resulting in phenolic benzosuberones **14** and **15,** which were then subsequently converted to their respective silyl ethers **16** and **17** through reaction with TBSCl (Scheme **4.2).**[3,4]



Scheme **4.2**. Synthetic Modifications Affording Protected Benzosuberone Intermediates **16** and **17**

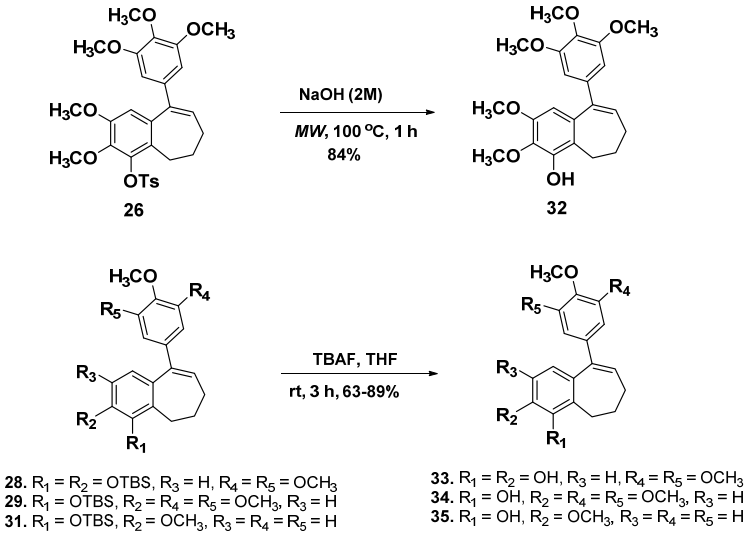
The benzosuberone intermediates **11**, **12**, **16** and **17** were subsequently treated with their requisite aryl-lithium reagents generated *in situ* after halogen-metal exchange reaction between suitable bromo benzenes and n-BuLi. The tertiary

alcohol intermediates **20-25** then underwent an elimination reaction to form the benzosuberene core compounds **26-31** (Scheme **4.3**).



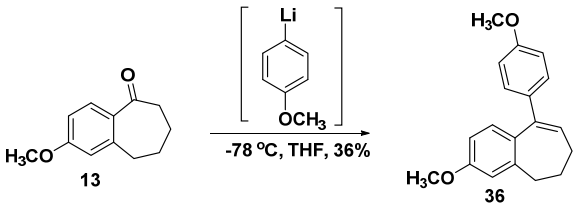
Scheme **4.3**. Synthetic Route to Benzosuberenes **26-31**

The pentamethoxy benzosuberene analogue **32** was obtained after reacting the tosyl protected intermediate **26** with NaOH (2M) solution. Similarly, deprotection of the silyl protecting groups of **28, 29** and **31** using TBAF.3H_2O furnished the final benzosuberene analogs **33-35** in high overall yields (Scheme **4.4**).



Scheme **4.4.** Synthetic route to Benzosuberene Analogues **33-35**

Interestingly, a concomitant elimination accompanied the addition of 4-methoxyphenyl lithium, generating the analogue **36** (Scheme **4.5**) in a single step.



Scheme **4.5**. Synthetic Route to Benzosuberene Analogue **36**

Biological Evaluation

Table 4.1. Inhibition of Tubulin Polymerization, Percent Inhibition of Colchicine Binding, and Cytotoxicity of the Target Benzosuberene Analogues

Compound	Inhibition of tubulin polymerization IC_{50} (μM) ± SD	% Inhibition of colchicine binding ± SD	GI_{50} (μM) SRB assay[a]		
			SK-OV-3	NCI-H460	DU-145
CA4	1.0[b]	84 ± 3 (1 μM), 98 ± 0.007 (5 μM)	0.00455	0.00223[b]	0.00327[b]
27	3.1 ± 0.03	30 ± 4 (5 μM), 56 ± 4 (50 μM)	0.277	0.593	0.708
30	>20	nr	20.5	33.4	48.3
32	>40	nr	18.1	25.9	38.4
33	7.7 ± 0.2	nr	0.346	0.691	1.53
34	1.4[c]	nr	0.0000543[c]	0.0000418[c]	0.0000249[c]
35[c]	7.4 ± 0.06	nr	18.4	10.6	8.59
36[c]	>20	nr	40.7	57.7	68.7

[a] Average of n ≥ 3 independent determinations,
[b] Data from ref. 5
[c] For additional data, see ref. 3, 6 and 7. nr = not reported

Three of the newly synthesized compounds (**27**, **33**, and **35**) inhibited tubulin polymerization, with the IC_{50} values 3.1 μM, 7.7 μM, and 7.4 μM respectively, which were less than that of parent benzosuberene **34** (Table **4.1**). This result suggested the importance of trimethoxy aryl unit and functionalization at the 5-position as key structural modification in benzosuberene analogues. Similarly, the compound **27** exhibited highest cytotoxicity of the compounds synthesized for this study followed by compounds **33** and **35** respectively correlating with the IC_{50} values obtained for the inhibition of tubulin polymerization as discussed earlier.

Materials and Methods

*2-Hydroxy-3-4-dimethoxybenzaldehyde (**1**).*[3,8,9,]

2,3,4-Trimethoxybenzaldehyde (5.00 g, 25.5 mmol) was dissolved in dry CH_2Cl_2 (15 mL) at 0 ºC under nitrogen. Anhydrous BCl_3 (28.0 mL, 1.0 M soln in CH_2Cl_2) was added dropwise, and the reaction mixture stirred for 5 h. The reaction was quenched with H_2O (10 mL), and the aqueous phase was extracted with CH_2Cl_2 (2 × 25 mL). The organic extract was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was subjected to flash column chromatography (silica gel, 30:70 EtOAc-hexanes) to yield aldehyde **1** (3.75 g, 20.6 mmol, 81% yield) as a white powder.

1H NMR (500 MHz, $CDCl_3$): δ 11.2 (1H, s, O*H*-2), 9.75 (1H, s, C*H*O-1a), 7.29 (1H, d, J = 9.0 Hz, H-6), 6.61 (1H, d, J = 9.0 Hz, H-5), 3.95 (3H, s, OCH3-4), 3.91 (3H, s, OCH3- 3).

13C NMR (126 MHz, $CDCl_3$): δ 194.9 (CH, C-1a), 159.4 (C, C-4), 155.7 (C, C-2), 136.2 (C, C-3), 130.2 (CH, C-6), 116.6 (C, C-1), 104.0 (CH, C-5), 60.8 (CH3, O*C*H3-3), 56.3 (CH3, O*C*H3-4).

*2-Tosyloxy-3,4-dimethoxybenzyldehyde (**2**).*[3]

To a stirred solution of aldehyde **1** (2.0 g,11.0 mmol), DIPEA (4.0 mL, 23.0 mmol) in anhydrous DMF (10 mL) at rt, p-TsCl (4.18 g, 22.0 mmol) was added in portions. The reaction mixture was stirred 12 h and quenched with H_2O (10 mL). The solution was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and evaporated S95 under reduced pressure. The crude product was subjected to flash column chromatography (silica gel, 10:90 EtOAc-hexanes) to afford aldehyde **2** (3.50 g, 10.4 mmol, 95% yield) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.86 (1H, s, CHO-1a), 7.86 (2H, d, J = 8.35 Hz, H-2′, -6′), 7.68 (1H, d, J = 8.8 Hz, H-6), 7.37 (2H, d, J = 8.35 Hz, H-3′, -5′), 6.94 (1H, d, J = 8.8 Hz, H-5), 3.94 (3H, s, OCH3-4), 3.74 (3H, s, OCH3-3), 2.48 (3H, s, CH3-4′).

^{13}C NMR (126 MHz, $CDCl_3$): δ 187.0 (CH, C-1a), 158.9 (C, C-4), 145.8 (C, C-2), 145.1 (C, C-3), 142.3 (C, C-3), 132.8 (C, C-1′), 129.9 (CH, C-3′,-5′), 128.4 (C, C-2′,-6′), 124.0 (C, C-1), 123.9 (CH, C-6), 110.6 (CH, C-5), 61.0 (CH3, OCH3- 3), 56.4 (CH3, OCH3-4), 21.8 (CH3, CH3-4′).

*(Z)/(E)- 5-(3′,4′-dimethoxy-2′-(tosyloxy)phenyl)pent-4-enoic acid (**5**).*[3]

To a well-stirred solution of (3-carboxypropyl) triphenylphosphonium bromide (3.82 g, 8.90 mmol) in THF (200 mL) at -50 °C was added *n*-BuLi (5.4 mL, 2.5 M in hexanes). The reaction mixture was allowed to warm to room temperature and stirred for 15 min and then cooled to -78 ºC. Aldehyde **2** (2.01 g, 5.97 mmol)

dissolved in THF (15 mL) was added dropwise and the reaction mixture was allowed to reach room temperature. H_2O (50 mL) was added and the aqueous phase was extracted with EtOAc (3 x 200 mL). The organic extract was washed with brine, dried with $MgSO_4$, concentrated under reduced pressure, and subjected to flash chromatography [silica gel, 40% EtOAc, 60% Hexanes] to obtain a mixture of *E/Z*-isomers **5** (1.03 g, 2.53 mmol, 42%) as an off-white solid.

^{1}H NMR (Mixture of *E* and *Z*) ($CDCl_3$, 500 MHz): δ 7.89 (2H, d, *J* = 8.2 Hz, H-2′, -6′), 7.87 (2H, d, *J* = 8.2 Hz, H-2′′, -6′′), 7.35 (2H, d, *J* = 8.2 Hz, H-3′′, -5′′), 7.32 (2H, d, *J* = 8.2 Hz, H-3′, -5′), 7.16 (1H, d, *J* = 8.8 Hz, H-6′), 6.96 (1H, d, *J* = 8.6 Hz, H-6′), 6.81 (1H, d, *J* = 8.6 Hz, H-5′), 6.79 (1H, d, *J* = 8.8 Hz, H-5′), 6.35 (1H, d, *J* = 16.0 Hz, H-5), 6.35 (1H, dt, *J* = 11.5 Hz, H-5), 6.04 (1H, m, *J* = 16.0 Hz, H-4), 5.55 (1H, m, *J* = 11.5 Hz, H-4), 3.88-3.83 (2 × 3H, s, OC*H*$_3$-2′, -3′), 3.88-3.83 (2 × 3H, s, OC*H*$_3$-2′, -3′), 2.59 (2H, m, H-2/3), 2.56-2.54 (4H, m, H-2, -3), 2.47 (2H, m, H-2/3).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 152.7, 152.7, 144.9, 144.8, 142.5, 141.1, 134.7, 134.6, 131.1, 129.5, 129.4, 128.9, 128.4, 128.3, 128.3, 128.3, 125.3, 125.1, 124.7, 124.4, 124.2, 120.3, 111.1, 110.4, 110.4, 105.0, 60.7, 56.2, 56.1, 34.7, 33.6, 33.3, 27.9, 23.8, 21.7, 21.7.**HRMS**: *m/z*: observed 429.0977 $[M+Na]^+$, calculated for $C_{20}H_{22}O_7NaS^+$, 429.0978. **HPLC**: 13.53 min.

*5-(3′,4′-dimethoxy-2′-(tosyloxy)phenyl)pentanoic acid (**8**).*[3,9]

To a solution of pentanoic acid **5** (1.25 g, 20.2 mmol) in MeOH (40 mL) and EtOH (15 mL) was added 10% Pd-C (400 mg). The flask was evacuated and H_2 gas was introduced via balloons. The reaction mixture was stirred for 12 h and was checked for completion by filtering a small amount of the reaction mixture through Celite®,

concentrating under reduced pressure, and recording the ^{1}H NMR spectrum. Upon completion, the reaction mixture was filtered through Celite® and concentrated under reduced pressure to obtain **8** (0.94 g, 2.3 mmol, 75%) as an off-white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.93 (2H, d, *J* = 8.2 Hz, H-2′′, -6′′), 7.35 (2H, d, *J* = 8.2 Hz, H-3′′, -5′′), 6.89 (1H, d, *J* = 8.6 Hz, H-6′), 6.77 (1H, d, *J* = 8.6 Hz, H-5′), 3.82 (3H, s, OC*H*$_3$-4′), 3.51 (3H, s, OC*H*$_3$-3′), 2.58 (2H, t, H-5), 2.46 (3H, s, C*H*$_3$-4′′), 2.34 (2H, m, H-2), 1.61 (4H, m, H-3,-4).

^{13}C NMR ($CDCl_3$,125 MHz): δ 179.0 (C, C-1), 151.8 (C, C-4′), 144.7 (C, C-4′′), 142.3 (C, C-2′), 142.1 (C, C-3′), 134.9 (C, C-1′′), 129.5 (CH, C-3′′,-5′′), 129.0 (C, C-1′), 128.1 (C, C-2′′,-6′′), 123.9 (CH, C-6′), 110.8 (CH, C-5′), 60.5 (CH_3, O*C*H$_3$-3′), 56.1 (CH_3, O*C*H$_3$-4′), 33.7 (CH_2, C-2), 29.6 (CH_2, C-5), 29.5 (CH_2, C-4), 24.4 (CH_2, C-3), 21.7 (CH_3, *C*H$_3$-4′′).

5-(2′,3′-Dimethoxyphenyl)pentanoic acid **(9).**

To dissolved carboxylic acid **6** (6.40 g, 25.5 mmol) in methanol (60 mL) was added 10% palladium on carbon (0.222 g) and hydrogen gas. The reaction mixture was stirred at room temperature for 12 h and filtered through Celite®, and the Celite® was washed with EtOAc (3 x 75 mL). The combined organic phase (MeOH and EtOAc) was evaporated under reduced pressure. The resulting organic material 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 (10 CV), 40%A / 60%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **9** (6.05 g, 23.9 mmol, 94%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 11.67 (1H, s), 6.99 7(1H, t, *J* = 8 Hz), 6.78 (2H, m), 3.86

(3H, s), 3.84 (3H, s), 2.68 (2H, t, *J* = 8 Hz), 2.41 (2H, t, *J* = 7.5 Hz), 1.70 (4H, m).

^{13}C NMR (125 MHz, $CDCl_3$): δ 180.2, 152.7, 147.1, 135.8, 123.8, 121.9, 110.2, 60.6, 55.6, 34.0, 30.8, 29.4, 24.5.

*1-Tosyloxy-2,3-dimethoxy-benzocycloheptan-5-one (**11**).*

Pentanoic acid **8** (0.90 g, 2.2 mmol) was dissolved in Eaton's reagent (14 mL) and the solution was stirred for 12 h. The reaction mixture was poured over ice and the ice was allowed to melt. The aqueous phase was extracted with CH_2Cl_2 (3 × 100 mL) and $NaHCO_3$ powder was added in small amounts until neutralized. The organic extract was washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, and subjected to flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 25%A / 75%B (1 CV), 25%A / 75%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] affording **11,** (0.70 g, 1.8 mmol, 81%) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.93 (2H, d, *J* = 8.0 Hz, H-2′, -6′), 7.37 (2H, d, *J* = 8.0 Hz, H-3′, -5′), 7.29 (1H, s, H-4), 3.87 (3H, s, OC*H*$_3$-2), 3.58 (3H, s, OCH_3-3), 2.95 (2H, dd, *J* = 4.9, 6.9 Hz, H-9), 2.73 (3H, m, H-6), 2.48 (3H, s, C*H*$_3$-4′′), 1.84 (2H, m, H-8), 1.81 (2H, m, H-7).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 204.0 (C, C-5), 151.3 (C, C-3), 145.4 (C, C-2), 145.0 (C, C-4′), 141.2 (C, C-1), 134.32 (C, C-10/11), 134.28 (CH, C-1′), 129.9 (C, C-10/11), 129.5 (CH, C-3′,-5′), 128.2 (CH, C-2′,-6′), 110.9 (CH, C-4), 60.5 (CH_3, O*C*H_3-3), 56.0 (CH_3, O*C*H_3-2), 40.7 (CH_2, C-6), 24.7 (CH_2, C-8), 24.5 (CH_2, C-9), 21.7 (CH_3, *C*H_3-4′), 20.8 (CH_2, C-7).

*1-Tosyloxy-2,3-dimethoxy-5-(3′,4′,5′-trimethoxyphenyl-benzocycloheptan-5-ol (**20**).*[9]

To a solution of 3,4,5-triemethoxyphenyl bromide (0.85 g, 3.4 mmol) in THF (100 mL) at -78 ºC was added *n*-BuLi (1.4 mL, 2.5 M in hexanes) and the reaction mixture was stirred for 30 min. Benzosuberone **11** (0.67 g, 1.7 mmol) in THF (15 mL) was added using an addition funnel over a period of 15 min. The reaction mixture was allowed to reach room temperature over 12 h. Upon completion, the reaction mixture was extracted with Et_2O (150 mL) and EtOAc (15 mL). The organic extract was washed with brine, dried over $MgSO_4$, filtered, concentrated under reduced pressure, and subjected to flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc, solvent B: hexanes; gradient: 25%A / 75%B (1 CV), 25%A / 75%B → 80%A / 20%B (10 CV), 80%A / 20%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] afforded alcohol **20** (0.61 g, 1.1 mmol, 64%) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.91 (2H, d, *J* = 8.3 Hz, H-2′′, -6′′), 7.35 (2H, d, *J* = 8.3 Hz, H-3′′, -5′′), 7.37 (1H, s, H-4), 6.46 (2H, s, H-2′, H-6′), 3.844 (3H, s, OC*H*$_3$-4′), 3.840 (3H, s, OC*H*$_3$-3), 3.78 (6H, s, OC*H*$_3$-3′, -5′), 3.54 (3H, s, OC*H*$_3$-2), 3.12 (1H, m, C*H*$_2$-9), 2.62 (1H, m, C*H*$_2$-6), 2.46 (3H, s, C*H*$_3$-4′′), 2.13 (1H, m, C*H*$_2$-6), 1.88 (1H, s, C*H*$_2$-7/8), 1.72 (3H, m, C*H*$_2$-7/8), 1.36 (1H, m) .

^{13}C NMR ($CDCl_3$, 125 MHz): δ 153.2 (C, C-3′, C-5′), 150.5 (C, C-3), 144.7 (C, C-4′′), 141.7 (C, C-1), 141.4 (C, C-10/11), 140.7 (C, C-2), 139.9 (C, C-1′), 137.5 (CH, C-4′), 134.7 (C, C-1′′), 129.4 (C, C-3′′, C-5′′), 128.4 (C, C-10/11), 128.1 (C, C-2′′, C-6′′), 110.2 (CH, C-4), 104.2 (CH, C-2′, C-6′), 80.2 (C, C-5), 60.8 (CH_3, O*C*H_3-4′), 60.5 (CH_3, O*C*H_3-

2), 56.2 (CH_3, O*C*H_3-3′, -5′), 56.0 (CH_3, O*C*H_3-3), 41.1 (CH_2, C-6), 26.7 (CH_2, C-7/8), 26.6 (CH_2, C-9), 26.3 (CH_2, C-7/8), 21.7 (CH_3, *C*H_3-4′′).

*1-Tosyloxy-2,3-dimethoxy-5-(3′,4′,5′-trimethoxyphenyl)-benzocycloheptan-5-ene (**26**).* Alcohol **20** (0.54 g, 0.97 mmol) was dissolved in AcOH (20 mL) and H_2O (30 mL) and was heated to reflux at 180 °C for 12 h. The reaction mixture was cooled, concentrated under reduced pressure, subjected to flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 15%A / 85%B (1 CV), 15%A / 85%B → 50%A / 50%B (10 CV), 50%A / 50%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] affording benzosuberene **26** (0.41 g, 0.75 mmol, 78%) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.99 (2H, d, *J* = 8.3 Hz, H-2′′, -6′′), 7.37 (2H, d, *J* = 8.3 Hz, H-3′′, -5′′), 6.54 (1H, s, H-4), 6.48 (2H, s, H-2′, H-6′), 6.44 (1H, t, *J* = 7.4 Hz, H-6), 3.87 (3H, s, OC*H*$_3$-4′), 3.82 (6H, s, OC*H*$_3$-3′, -5′), 3.69 (3H, s, OC*H*$_3$-3), 3.54 (3H, s, OC*H*$_3$-2), 2.71 (2H, t, *J* = 6.5 Hz, H-9), 2.48 (3H, s, C*H*$_3$-4′′), 2.21 (2H, p, *J* = 7.0 Hz, H-8), 1.99 (2H, q, *J* = 7.1 Hz, H-7).

^{13}C NMR ($CDCl_3$, 125 MHz): δ 153.0 (C, C-3′, C-5′), 151.0 (C, C-3), 144.7 (C, C-4′′), 141.9 (C, C-5), 141.6 (C, C-1), 140.8 (C, C-2), 137.6 (C, C-1′), 137.5 (C, C-4′), 136.1 (C, C-10/11), 134.8 (C, C-1′′), 129.6 (C, C-10/11), 129.5 (CH, C-3′′, C-5′′), 129.4 (CH, C-6), 128.2 (CH, C-2′′, C-6′′), 112.0 (CH, C-4), 105.2 (CH, C-2′, C-6′), 60.94 (CH_3, O*C*H_3-4′), 60.93 (CH_3, O*C*H_3-2), 60.5 (CH_3, O*C*H_3-3′, -5′), 56.2 (CH_3, O*C*H_3-3) 34.6 (CH_2, C-8), 25.5 (CH_2, C-7), 25.1 (CH_2, C-9), 21.7 (CH_3, *C*H_3-4′′).

*1-Hydroxy-2,3-dimethoxy-5-(3′,4′,5′-trimethoxyphenyl)-benzocycloheptan-5-ene (**32**).*[4]

A solution of sulfonate ester **26** (0.250 g, 0.462 mmol) dissolved in NaOH (1 mL, 2 M) and methanol (4 mL) in a 5 mL microwave safe sealed vial was subjected to microwaved irradiation at 100 °C for 1h. Upon completion, the reaction mixture was neutralized (1 mL, 2 M HCl), concentrated under reduced pressure, and subjected to flash chromatography using a pre-packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 0%A / 100%B (1 CV), 0%A / 100% B → 40%A / 60%B (10 CV), 40%A / 60%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] affording phenol **43** (0.15 g, 0.388 mmol, 84%) as an off-white solid.

^{1}H NMR (CDCl3, 500 MHz): δ 6.50 (2H, s, H-2′, H-6′), 6.39 (1H, t, *J* = 7.4 Hz, H-6), 6.18 (1H, s, H-4), 5.94 (1H, s, O*H*-1), 3.95 (3H, s, OC*H*3-2), 3.86 (3H, s, OC*H*3-4′), 3.81 (6H, s, OC*H*3-3′, -5′), 3.70 (3H, s, OC*H*3-3), 2.67 (2H, t, *J* = 6.9 Hz, H-9), 2.13 (2H, p, *J* = 6.9 Hz, H-8), 1.96 (2H, q, *J* = 7.1 Hz, H-7).

^{13}C NMR (CDCl3, 125 MHz): δ 153.0 (C, C-3′, C-5′), 149.8 (C, C-3), 146.3 (C, C-1), 142.8 (C, C-5), 138.1(C, C-1′), 137.5 (C, C-4′), 136.3 (C, C-10/11), 134.3 (C, C-2), 128.5 (CH, C-6), 121.4 (C, C-10/11), 105.3 (CH, C-2′, C-6′), 105.1 (CH, C-4), 61.08 (CH3, O*C*H3-2), 61.06 (CH3, O*C*H3-4′), 56.3 (CH3, O*C*H3-3′, -5′), 56.1 (CH3, O*C*H3-2), 34.3 (CH2, C-8), 25.8 (CH2, C-7), 23.4 (CH2, C-9).

HRMS: *m/z:* observed 387.1807 [M+H]+, calculated for $C_{22}H_{27}O_6$+, 387.1802. **HPLC** (Method A): 15.16 min.

*5-(2′,3′-Dimethoxyphenyl)pent-4-enoic acid (**6**).*[10]

To dissolved 3-(carboxypropyl)triphenyl phosphonium bromide (13.04 g, 30.39 mmol) in THF (500 mL) was added potassium *tert*-butoxide (7.43 g, 66.2 mmol),

and the reaction mixture was stirred at room temperature for 1 h. 2,3-Dimethoxybenzaldehyde **3** (5.02 g, 30.1 mmol) dissolved in THF (100 mL) was added, and the mixture was stirred at room temperature for 12 h. The THF was removed under reduced pressure, and the resulting material was quenched with 2 M HCl (75 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were evaporated under reduced pressure, and the crude reaction 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 (10 CV), 50%A / 50%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford compound **6** (5.39 g, 21.5 mmol, 72%) as a yellow oil. NMR characterization was conducted after the next step.

*5-(2´,3´-Dimethoxyphenyl)pentanoic acid (**9**).*[10,11]

To a solution of carboxylic acid **6** (6.40 g, 25.6 mmol) in methanol (60 mL) was added 10% palladium on carbon (0.223 g) and hydrogen gas. The reaction mixture was stirred at room temperature for 12 h and then filtered through Celite®, and the Celite® was washed with EtOAc (3 x 50 mL). The combined organic phase (MeOH and EtOAc) was evaporated under reduced pressure. The resulting organic material 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 (10 CV), 40%A / 60%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **9** (6.05 g, 23.9 mmol, 82%) as a pale yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.98 (1H, t, *J*=8 Hz), 6.80 – 6.71 (2H, m), 3.85 (3H, s),

3.81 (3H, s), 2.65 (2H, t, *J*=7.3 Hz), 2.38 (2H, t, *J*=7.0 Hz), 1.79 – 1.56 (4H, m).

^{13}C NMR (126 MHz, $CDCl_3$): δ 179.4, 152.7, 147.1, 135.9, 123.8, 121.8, 110.2, 60.6, 55.7, 33.9, 30.1, 29.4, 24.5.

*1,2-Dimethoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (**12**).*[10,11,12]

To carboxylic acid **9** (5.80 g, 23.0 mmol) was added Eaton's reagent (72.2 mL), and he reaction mixture was stirred at room temperature for 12 h. It was then poured over ice and neutralized with sodium bicarbonate. The reaction mixture was extracted with EtOAc (3 x 50 mL), and the combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The reaction 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 (10 CV), 40%A / 60%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford benzosuberone **12** (3.917 g, 17.8 mmol, 77%) as a light yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53 (dt, *J* = 1.39, 8.67 Hz, 1H), 6.83 (dt, *J* = 1.05, 8.78 Hz, 1H), 3.90 (s, 1H), 3.79 (s, 1H), 3.03 – 2.96 (m, 2H), 2.72 – 2.65 (m, 2H), 1.89 – 1.81 (m, 2H), 1.81 – 1.72 (m, 2H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 204.82, 156.05, 145.92, 135.70, 132.80, 125.44, 109.66, 61.06, 55.74, 40.61, 24.86, 23.24, 20.86.

*5-(3´-Methoxyphenyl)pent-4-enoic acid (**7**).*[3,10,11,12]

To dissolved 3-(carboxypropyl)triphenyl phosphonium bromide (15.92 g, 37.09 mmol) in THF (500 mL) was added potassium *tert*-butoxide (8.20 g, 73.4 mmol), and the reaction mixture was stirred at room temperature for 1 h. 3-

Methoxybenzaldehyde **4** (4.5 mL, 37 mmol) was added, and the reaction mixture was stirred at room temperature for 12 h. The THF was evaporated, and the resulting material was quenched with 2 M HCl (75 mL) and extracted with EtOAc (3 x 50 mL). The combined organic phase was dried over sodium sulfate, filtered, and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (1 CV), 12%A / 88%B → 70%A / 30%B (10 CV), 70%A / 30%B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **7** (5.63 g, 27.3 mmol, 74%) as a yellow solid. NMR characterization was performed after the next step.

*5-(3´-Methoxyphenyl)pentanoic acid (**10**).*[3,10,11,12]

To dissolved carboxylic acid **7** (5.63 g, 27.3 mmol) in MeOH (100 mL) was added 10% palladium on carbon (0.44 g) and hydrogen gas. The reaction was stirred at room temperature for 12 h. The reaction mixture was then filtered through Celite®, and the Celite® was washed with EtOAc (3 x 50 mL). The organic phase (MeOH and EtOAc) was evaporated under reduced pressure to afford carboxylic acid **8** (4.27 g, 20.5 mmol, 75%) as a yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 11.2 (1H, s), 7.03 (1H, d, *J* = 8 Hz), 6.62 (2H, d, *J* = 5 Hz), 6.59 (1H, d, *J* = 8.5 Hz), 3.59 (3H, s), 2.44 (2H, t, *J* = 7.5 Hz), 2.21 (2H, d, *J* = 7.5 Hz), 1.52 (4H, m).

^{13}C NMR (125 MHz, $CDCl_3$): δ 180.2, 159.7, 143.7, 129.4, 120.9.

*2-Methoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (**13**).*

To carboxylic acid **10** (4.43 g, 21.3 mmol) was added Eaton's reagent (43 mL, 3 g per

mmol of compound **10**), and the mixture was stirred at room temperature for 12 h. The mixture was then poured over ice and neutralized with sodium bicarbonate. The aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic phase was dried over sodium sulfate, evaporated under reduced pressure, and 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 → 30%A / 70%B (10 CV), 30%A / 70%B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford benzosuberone **13** (2.80 g, 14.7 mmol, 70%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.78 (1H, d, *J*=8.6 Hz), 6.81 (1H, dd, *J*=8.6, 2.6 Hz), 6.70 (1H, d, *J*=2.5 Hz), 2.91 (1H, t, *J*=6.0 Hz), 2.74 – 2.68 (1H, m), 1.91 – 1.84 (1H, m), 1.83 – 1.75 (1H, m)

^{13}C NMR (125 MHz, $CDCl_3$): δ 204.2, 162.7, 144.2, 131.6, 131.2, 114.9, 111.7, 55.3, 40.7, 32.9, 25.1, 20.7.

*1,2-Dihydroxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (**14**)*[3]

Ketone **12** (0.88 g, 4.0 mmol) was added to ionic liquid $[Al_2Cl_7][TMAH]$ (20.0 mL, 0.497 M). The reaction mixture was allowed to microwave at 80 °C and 1 atm for 1 h. H_2O (20 mL) was added to the mixture and the resulting brown liquid was extracted with dichloromethane (3 x 20 mL). The organic extracts were dried over Na_2SO_4, filtered, evaporated under reduced pressure, and purified by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 60%A / 40%B (13 CV), 60%A / 40%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] affording benzosuberone **14** (0.50 g, 2.60 mmol, 65% yield) as a brown solid.

^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ 7.14 (1H, d, *J*=8.3 Hz), 6.78 (1H, d, *J*=8.8 Hz), 3.07 – 2.99 (2H, m), 2.67 – 2.59 (2H, m), 1.84 – 1.77 (2H, m), 1.77 – 1.71 (2H, m).

^{13}C NMR (126 MHz, $(CD_3)_2CO$): δ 205.37, 148.11, 142.01, 132.32, 128.93, 120.55, 112.34, 40.25, 24.40, 22.76, 21.02.

*1,2-Bis((tert-butyldimethylsilyl)oxy)-benzocycloheptan-5-one (**16**)*

To a solution of catechol **14** (0.68 g, 3.5 mmol) and DIPEA (2.7 mL, 16.3 mmol) in DMF (5 mL) at 0 °C was added TBSCl (1.60 g, 10.6 mmol) in portions. The reaction mixture was stirred for 18 h, diluted with H_2O (5 mL), and extracted with Et_2O (2 × 20 mL). The organic extract was dried over Na_2SO_4, filtered, concentrated under reduced pressure, and purified by flash chromatography using a pre packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 0%A / 100%B (1 CV), 0%A / 100%B → 30%A / 70%B (10 CV), 30%A / 70%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] affording ketone **16** (1.51 g, 3.59 mmol, 99 %) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.29 (1H, d, *J*=8.5 Hz), 6.75 (1H, d, *J*=8.5 Hz), 2.97 – 2.94 (2H, m), 2.70 (2H, t, *J*=6.2 Hz), 1.89 – 1.70 (4H, m), 1.02 (9H, s), 0.96 (9H, s), 0.24 (6H, s), 0.15 (6H, s).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 205.25, 151.15, 143.94, 135.48, 134.03, 122.64, 118.61, 41.03, 26.51, 26.45, 25.33, 25.09, 21.92, 19.18, 18.89, -3.15, -3.19.

*1-Hydroxy-2-dimethoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (**15**).*[13]

To benzosuberone **12** (1.03 g, 4.68 mmol) in a 20 mL microwave vial was added [TMAH][Al_2Cl_7] (18.4 mL, 9.02 mmol), and the mixture was subjected to microwave irradiation for 1 h at 80 °C on high absorbance. The solution was then poured into

water (50 mL) and extracted with EtOAc (3 x 25 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction 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 (10 CV), 60%A / 40%B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford benzosuberone **15** (0.61 g, 3.0 mmol, 65%) as a yellow oil.

^{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), 3.93 (3H, d, *J*=0.9 Hz), 3.01 (2H, dd, *J*=7.1, 5.0 Hz), 2.76 – 2.65 (2H, m), 1.90 – 1.72 (4H, m).

^{13}C NMR (126 MHz, $CDCl_3$): δ 205.01, 149.20, 142.41, 133.27, 127.67, 120.83, 107.90, 56.05, 40.75, 24.49, 23.05, 21.31.

*1-((tert-Butyldimethylsilyl)oxy)-2-dimethoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (**17**).*[3]

Benzosuberone **15** (1.28 g, 6.22 mmol) was dissolved in dimethylformamide (15 mL). TBSCl (1.40 g, 9.33 mmol) and DIPEA (1.63 mL, 9.33 mmol) were added, and the solution was stirred for 6 h at room temperature. The reaction mixture was washed with water (3 x 25 mL) and extracted with Et_2O (2 x 20 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 0%A / 100%B (1 CV), 0%A / 100%B → 30%A / 70%B (10 CV), 30%A / 70%B (2 CV);

flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford TBS protected ketone **17** (1.81 g, 7.38 mmol, 91%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37 (1H, d, *J*=8.5 Hz), 6.76 (1H, d, *J*=8.5 Hz), 3.82 (3H, s), 3.00 (2H, dd, *J*=7.0, 4.9 Hz), 2.69 (2H, dd, *J*=7.3, 4.4 Hz), 1.79 (5H, dddd, *J*=13.3, 10.5, 7.1, 3.9 Hz), 1.01 (11H, s), 0.18 (7H, s).

^{13}C NMR (126 MHz, $CDCl_3$): δ 205.26, 153.16, 141.72, 133.12, 133.08, 122.29, 108.74, 54.82, 40.69, 26.05, 24.65, 23.94, 21.20, -3.91.

*1,2-dimethoxy-5-(3,4,5-trimethoxyphenyl)-benzocycloheptan-5-ol (**21**)*

To a solution of 3,4,5-triemethoxyphenyl bromide (0.673 g, 2.7 mmol) in THF (100 mL) at -78 ºC was added *n*-BuLi (1.1 mL, 2.5 M in hexanes) and the reaction mixture was stirred for 30 min. Benzosuberone **12** (0.60 g, 2.72 mmol) in THF (15 mL) was added drop wise over a period of 15 min. The reaction mixture was allowed to reach room temperature over 12 h. Upon completion, the water was added and mixture was extracted with EtOAc (100 mL). The organic extract was washed with brine, dried over Na_2SO4, filtered, concentrated under reduced pressure, and subjected to 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 → 56%A / 44%B (9.2 CV), 56%A / 44%B (1 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] afforded alcohol **21** (0.385 g, 0.99 mmol, 34%) as light yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.26 (1H, d, *J*=8.7 Hz), 6.75 (1H, d, *J*=8.8 Hz), 6.49 (2H, s), 3.87 (3H, s), 3.83 (3H, s), 3.74 (6H, s), 3.26 – 3.20 (1H, m), 2.56 (1H, ddd, *J*=14.1, 6.9, 3.0 Hz), 2.38 – 2.30 (1H, m), 2.21 – 2.21 (2H, m), 2.11 (1H, ddd, *J*=14.0, 10.7, 3.1 Hz), 1.93 (1H, ddd, *J*=15.3, 7.4, 3.8 Hz), 1.81-1.71 (2H, m), 1.50-1.42 (1H, m).

^{13}C NMR (126 MHz, $CDCl_3$): δ 152.98, 151.80, 146.23, 141.60, 138.59, 137.19, 135.51, 122.79, 108.77, 104.23, 79.91, 60.97, 60.81, 56.06, 55.53, 41.28, 27.13, 26.28, 25.06.

*1,2-dimethoxy-5-(3,4,5-trimethoxyphenyl)-benzocyclohept-5-ene (**27**)*

Tertiary alcohol analogue **21** (0.5 g, 1.29 mmol) was dissolved in acetic acid (10 mL) and refluxed for 2 hours. Deionized water (30 mL) was added and refluxed for 2 more hours. The white precipitate thus obtained was filtered and washed with hexane. On drying, it afforded benzosuberene analogue **27** (0.444 g, 1.2 mmol, 93%) as colorless solid without further purification.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.79 (1H, d, *J*=8.5 Hz), 6.77 (1H, d, *J*=8.5 Hz), 6.51 (2H, s), 6.35 (1H, t, *J*=7.3 Hz), 3.90 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.82 (6H, s), 2.77 (2H, t, *J*=7.0 Hz), 2.17 (2H, p, *J*=7.1 Hz), 1.98 (2H, q, *J*=7.2 Hz).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 152.86, 151.48, 146.06, 142.85, 138.38, 137.36, 135.89, 133.78, 127.07, 125.28, 109.26, 105.28, 61.25, 60.92, 56.17, 55.63, 34.56, 25.61, 24.15. **HRMS**: Obsvd 393.1682 [M + Na^+], Calcd for $C_{22}H_{25}O_5Na$: 393.1672.

HPLC (Method A): 17.52 min.

*1,2-bis((tert-butyldimethylsilyl)oxy)-5-(3,4,5-trimethoxyphenyl)-benzocycloheptan-5-ol (**22**)*

To a solution of 3,4,5-triemethoxyphenyl bromide (0.458 g, 1.85 mmol) in THF (20 mL) at -78 ºC was added *n*-BuLi (0.96 mL, 2.5 M in hexanes) and the reaction mixture was stirred for 60 min. Benzosuberone **16** (0.639 g, 1.51 mmol) in THF (20 mL) was added drop wise over a period of 15 min. The reaction mixture was allowed to reach room temperature over 12 h. Upon completion, water was added

and the mixture was extracted with EtOAc (4 x 20 mL). The organic extract was washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, and subjected to flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc, solvent B: hexanes; gradient: 10%A / 90%B (1 CV), 10%A / 90%B → 42%A / 58%B (6 CV), 42%A / 58%B→70%A / 30%B (1 CV), 70%A / 30%B → 100%A/0%B, 100%A/0% B (1.1 CV) ; flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded tertiary alcohol **22** (0.480 g, 0.81 mmol, 54%) as a clear oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.10 (1H, d, *J*=8.7 Hz), 6.72 (1H, d, *J*=8.6 Hz), 6.46 (2H, s), 3.83 (3H, s), 3.74 (6H, s), 3.23 – 3.15 (1H, m), 2.57 (1H, ddd, *J*=14.0, 6.2, 3.0 Hz), 2.23 – 2.07 (3H, m), 1.94 – 1.84 (1H, m), 1.81 – 1.66 (2H, m), 1.46 – 1.33 (1H, m), 1.00 (9H, s), 0.95 (9H, s), 0.24 (3H, s), 0.23 (3H, s), 0.15 (3H, s), 0.10 (3H, s).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 152.92, 146.37, 143.67, 141.76, 139.13, 137.07, 133.91, 120.00, 117.46, 104.12, 79.97, 60.81, 55.96, 40.87, 26.84, 26.30, 26.23, 26.12, 25.86, 18.91, 18.59, -3.38, -3.61.

*1,2-bis((tert-butyldimethylsilyl)oxy)-5-(3,4,5-trimethoxyphenyl)-benzocyclohept-5-ene (**28**)*

Tertiary alcohol **22** (0.44 g, 0.75) mmol was dissolved in acetic acid (5 mL) and stirred for 12 hours at room temperature. The reaction was quenched by adding deionized water (10 mL). The reaction mixture was then extracted with Et_2O (3 x 10 mL). The combined organic extracts were washed with sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. Thus obtained pure

clear oil of TBS-protected benzosuberene analogue **28** (0.66 mmol, 89%) was taken to the next step of the reaction without further purification.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.69 (1H, d, *J*=8.4 Hz), 6.52 (1H, d, *J*=8.4 Hz), 6.45 (2H, s), 6.33 (1H, t, *J*=7.3 Hz), 3.85 (3H, s), 3.78 (6H, s), 2.70 (2H, t, *J*=6.9 Hz), 2.10 (2H, q, J=7.0 Hz), 1.95 (2H, q, *J*=7.0 Hz), 1.04 (9H, s), 0.95 (9H, s), 0.24 (6H, s), 0.20 (6H, s).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 152.73, 145.95, 143.38, 142.98, 138.55, 137.10, 134.38, 134.24, 126.76, 122.69, 117.86, 105.01, 60.90, 55.96, 33.91, 26.27, 26.25, 25.57, 24.55, 18.90, 18.70, -3.30, -3.37.

*1,2-dihydroxy-5-(3,4,5-trimethoxyphenyl)-benzocyclohept-5-ene (**33**)*

The di-TBS-protected analogue **28** (0.32 g, 0.56 mmol) was dissolved in THF (5 mL). To the solution, TBAF.$3H_2O$ (1.4 mmol) was added and stirred for 3h at room temperature. The reaction was quenched with the addition of deionized water (15 mL) followed by the evaporation of organic solvent under reduced pressure. The resultant aqueous phase was then extracted with EtOAc (3 x 20 mL). The combined organic extract were washed with brine solution, dried over Na_2SO_4, filtered, evaporated under reduced pressure and purified using flash chromatography using a pre-packed 25 g silica gel column [eluent; 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 (5.2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] affording the catechol analogue **33** (0.169 g, 0.49 mmol, 88%) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.68 (1H, d, *J*=8.2 Hz), 6.52 (1H, d, *J*=8.2 Hz), 6.33 (1H, t, *J*=7.4 Hz), 3.86 (3H, s), 3.79 (6H, s), 2.71 (2H, t, *J*=7.0 Hz), 2.15 (2H, p, *J*=7.1 Hz), 1.96 (2H, q, *J*=7.2 Hz).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 152.80, 142.90, 141.82, 140.81, 138.32, 137.27, 134.10, 128.51, 127.00, 121.70, 112.30, 105.27, 60.91, 56.10, 33.78, 25.58, 23.80. **HRMS**: Obsvd 365.1444 [M + H^+], Calcd for C_{12} $H_{22}O_5$ H: 365.1359. **HPLC** (Method A): 16.18 min.

*1-((tert-Butyldimethylsilyl)oxy)-2-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol (**23**).*[3,6]

To an oven dried flask, under inert gas condition, THF (50 mL) and 3,4,5-trimethoxyphenyl bromide (0.315 g, 1.28 mmol) were added, and the solution was cooled to -78 °C. *n*-BuLi (0.65 mL, 1.49 mmol) was slowly added to the reaction mixture, which was then stirred at -78 °C for 1 h. Benzosuberone **17** (0.340 g, 1.06 mmol) was then added dropwise to the flask, and the reaction mixture was stirred while warming from -78° C to room temperature over 12 h. The reaction mixture was washed with water and extracted with EtOAc (3 x 50 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction 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 → 65%A / 35%B (10 CV), 65%A / 35%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford compound **23** (0.231 g, 0.473 mmol, 45%) 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 (**29**).*

Acetic acid (5 mL) was added to the solution of tertiary alcohol **23** (0.210 g, 0.429

mmol), and the reaction mixture was stirred for 12 h at room temperature. The mixture was washed with water and extracted with EtOAc (3 x 30 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography to afford TBS-protected benzosuberene **29** (0.172 mg, 0.365 mmol, 85%) as a clear oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.69 (1H, d, *J* = 8.5 Hz), 6.62 (1H, d, *J* = 8.4 Hz), 6.49 (2H, s), 6.32 (1H, t, *J* = 7.3 Hz), 3.85 (3H s,), 3.80 (s, 3H), 3.79 (s, 6H), 2.77 (t, *J* = 6.9 Hz, 2H), 2.11 (p, *J* = 7.0 Hz, 2H), 1.95 (q, *J* = 7.1 Hz, 2H), 1.05 (s, 9H), 0.24 (s, 6H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 152.89, 148.75, 143.15, 141.57, 138.70, 137.36, 133.88, 133.33, 126.89, 122.46, 108.46, 105.33, 60.94, 56.16, 54.70, 34.02, 26.26, 25.69, 24.32, 19.11, -3.74.

*3-Methoxy-9-(3´,4´,5´-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-ol (**34**).*[14]

Silyl protected benzosuberene **29** (0.130 g, 0.276 mmol) was dissolved in THF (5 mL), TBAF (0.105 mg, 0.331 mmol) was added, and the reaction mixture was stirred at room temperature for 12 h. The solution was washed with water and extracted with EtOAc (3 x 30 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure affording benzosuberene **34** (0.062 g, 0.191 mmol, 63%) as an orange solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ 6.71 (1H, d, J=8.4 Hz), 6.57 (1H, d, J=8.4 Hz), 6.50 (2H, s), 6.34 (1H, t, J=7.4 Hz), 5.76 (1H, s), 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 (151 MHz, CDCl3): δ 153.11, 145.36, 143.08, 142.61, 138.80, 137.50, 134.51, 128.05, 127.55, 121.14, 107.94, 105.48, 77.57, 77.36, 77.15, 61.24, 56.41, 56.25, 33.91, 26.01, 23.85.

HRMS: Obsvd 379.1516 [M + Na^+], Calcd for $C_{21}H_{24}O_5$ 356.1618. **HPLC** (Method B): 9.45 min.

*1,2-dimethoxy-5-(4-methoxyphenyl)-benzocycloheptan-5-ol (**24**)*

To a solution of 4-methoxyphenyl bromide (0.719 g, 3.84 mmol) in THF (50 mL) at -78 ºC was added *n*-BuLi (2.6 mL, 2.5 M in hexanes) and the reaction mixture was stirred for 30 min. Benzosuberone **12** (0.58 g, 2.56 mmol) in THF (15 mL) was added drop wise over a period of 15 min. The reaction mixture was allowed to reach room temperature over 12 h. Upon completion, the water was added and mixture was extracted with EtOAc (3 x 20 mL). The organic extract was washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, and subjected to flash chromatography using a pre packed silica 80g gold column [eluent; solvent A, EtOAc, solvent B, hexanes; gradient 0%A/100%B →100%A/0%B over 20.3 min; flow rate: 50 mL/min; monitored at 254 and 280 nm] furnishing alcohol **24** (0.45 g, 1.37 mmol, 54%) as clear oil which solidifies as white.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39 (1H, d, *J*=8.7 Hz), 7.15 (2H, d, *J*=8.8 Hz), 6.83 (2H, d, *J*=8.8 Hz), 6.79 (1H, d, *J*=8.7 Hz), 3.89 (3H, s), 3.79 (3H, s), 3.75 (3H, s), 3.25 – 3.18 (1H, m), 2.67 – 2.59 (1H, m), 2.15 – 2.08 (2H, m), 1.93 – 1.86 (1H, m), 1.80 – 1.69 (2H, m), 1.43 – 1.35 (1H, m).

^{13}C NMR (126 MHz, $CDCl_3$): δ 158.78, 151.74, 146.28, 139.08, 137.53, 135.39, 128.20, 122.31, 113.69, 108.75, 79.35, 60.98, 55.56, 55.22, 41.23, 27.27, 26.60, 25.16.

*1,2-dimethoxy-5-(4-methoxyphenyl)-benzocyclohept-5-ene (**30**)*

Tertiary alcohol analogue **24** (0.401 g, 1.22 mmol) was dissolved in acetic acid (10 mL) and stirred for 12 hours in ambient temperature. Deionized water (30 mL) was added and stirred for 2 more hours. Upon completion, the water was added and mixture was extracted with EtOAc (3 x 20 mL). The organic solvent was evaporated under reduced pressure and extracted with EtOAc (3 x 20 mL). The combined organic solvent was washed with brine, dried over Na_2SO4, filtered, concentrated under reduced pressure, and subjected to flash chromatography using a pre packed 25 g silica column [eluent; solvent A: EtOAc, solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 50%A / 50%B (10 CV), 50%A / 50%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] afforded clear oil to afford benzosuberene analogue **30** (0.31 g, 0.998 mmol, 82%).

^{1}H NMR (500 MHz, CD_3OD): δ 7.17 (2H, d, *J*=8.8 Hz), 6.87 (1H, d, *J*=8.5 Hz), 6.86 (2H, d, *J*=8.8 Hz), 6.71 (1H, d, *J*=8.5 Hz), 6.30 (1H, t, *J*=7.4 Hz), 3.89 (3H, s), 3.85 (3H, s), 3.81 (3H, s), 2.74 (2H, t, *J*=7.0 Hz), 2.15 (2H, p, *J*=7.1 Hz), 1.94 (2H, q, *J*=7.2 Hz).

^{13}C NMR (126 MHz, $CDCl_3$): δ 158.78, 151.34, 146.08, 142.19, 135.88, 135.23, 134.34, 128.99, 125.90, 125.01, 113.46, 109.23, 61.18, 55.62, 55.28, 34.56, 25.49, 24.04.

HRMS: Obsvd 281.1597 [M + H^+], Calcd for C_{19} $H_{20}O_2$ H: 281.1536. **HPLC** (Method A): 19.08 min.

*1-((tert-butyldimethylsilyl)oxy)-2-methoxy-5-(4-methoxyphenyl)- benzocycloheptan-5-ol (**25**)*

To a solution of 4-methoxyphenyl bromide (0.52 g, 2.78 mmol) in THF (40 mL) at -78 ºC was added *n*-BuLi (0.34 mL, 2.5 M in hexanes) and the reaction mixture was stirred for 30 min. Benzosuberone **17** (0.61 g, 1.9 mmol) in THF (20 mL) was added drop wise over a period of 15 min. The reaction mixture was allowed to reach room temperature over 12 h. Upon completion, water was added and the mixture was extracted with EtOAc (3 x 20 mL). The organic extract was washed with brine, dried over Na_2SO4, filtered, concentrated under reduced pressure, and subjected to flash chromatography using a prepacked 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 (10 CV), 60%A / 40%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded alcohol **25** (0.32 g, 0.75 mmol, 39%) as a clear oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.26 (1H, d, *J*=8.6 Hz), 7.15 (2H, d, *J*=8.8 Hz), 6.81 (2H, d, *J*=8.8 Hz), 6.73 (1H, d, *J*=8.7 Hz), 3.81 (3H, s), 3.79 (3H, s), 3.30 (1H, dd, *J*=14.6, 7.5 Hz), 2.67 – 2.59 (1H, m), 2.14 – 2.04 (2H, m), 1.77 – 1.65 (2H, m), 1.35 – 1.25 (2H, m), 0.99 (9H, s), 0.20 (3H, s), 0.14 (3H, s).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 158.72, 149.16, 141.84, 139.03, 137.63, 132.68, 128.29, 119.13, 113.61, 107.83, 79.36, 55.21, 54.63, 41.15, 27.13, 26.68, 26.06, 25.46, -3.86, -4.22.

*1-((tert-butyldimethylsilyl)oxy)-2-methoxy-5-(4-methoxyphenyl)- benzocyclohept-5-ene (**31**)*

Tertiary alcohol **25** (0.53 g, 1.23) mmol was dissolved in acetic acid (5 mL) and refluxed for 5h. No apparent change in TLC is noticed, so deionized water (15 mL) is added in the reaction mixture and refluxed for 2 more hours. The solvents evaporated and extracted with EtOAc (3 x 15 mL). The combined organic extracts were washed with sat. $NaHCO_3$, brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure, and subjected to flash chromatography using a pre packed 25 g silica column [eluent; solvent A: EtOAc, solvent B: hexanes; gradient: 5%A / 95%B (1 CV), 5%A / 95%B → 50%A / 50%B (10 CV), 50%A / 50%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] afforded clear oil which solidified as colorless solid of TBS-protected benzosuberene analogue **31** (1.05 mmol, 85%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.20 (1H, d, *J*=8.7 Hz), 6.83 (1H, d, *J*=8.8 Hz), 6.68 (1H, d, *J*=8.4 Hz), 6.58 (1H, d, *J*=8.4 Hz), 6.26 (1H, t, *J*=7.3 Hz), 3.81 (2H, s), 3.79 (2H, s), 2.75 (1H, t, *J*=6.9 Hz), 2.09 (1H, p, *J*=7.1 Hz), 1.93 (1H, q, *J*=7.2 Hz).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 158.70, 148.54, 142.39, 141.53, 135.48, 134.31, 133.28, 128.98, 125.69, 122.02, 113.40, 108.31, 55.27, 54.65, 33.94, 26.17, 25.51, 24.19, 19.00, -3.85.

*1-hydroxy-2-methoxy-5-(4-methoxyphenyl)- benzocyclohept-5-ene (**35**)*

The TBS-protected analogue **31** (0.33 g, 0.8 mmol) was dissolved in THF (5 mL). To the solution, TBAF.$3H_2O$ (0.96 mmol) was added and stirred for 3h at room temperature. The reaction was quenched with the addition of deionized water (15 mL) followed by the evaporation of organic solvent under reduced pressure. The

resultant aqueous phase was then extracted with EtOAc (3 x 20 mL). The combined organic extract were washed with brine solution, dried over Na_2SO_4, filtered, evaporated under reduced pressure and subjected to flash chromatography using a pre-packed silica 40g gold column [eluent; solvent A, EtOAc, solvent B, hexanes; gradient 0%A/100%B →100%A/0%B over 9.0 min; flow rate: 50 mL/min; monitored at 254 and 280 nm] affording the benzosuberene analogue **35** (0.21 g, 0.71 mmol, 89%) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.21 (2H, d, *J*=8.7 Hz), 6.83 (2H, d, *J*=8.7 Hz), 6.70 (1H, d, *J*=8.4 Hz), 6.54 (1H, d, *J*=8.4 Hz), 6.28 (1H, t, *J*=7.4 Hz), 5.72 (1H, s), 3.90 (3H, s), 3.81 (3H, s), 2.75 (2H, t, *J*=7.0 Hz), 2.13 (2H, p, *J*=7.0 Hz), 1.95 (2H, q, *J*=7.2 Hz).

^{13}C NMR ($CDCl_3$, 126 MHz): δ 158.76, 144.94, 142.35, 142.15, 135.34, 134.80, 129.03, 127.80, 126.05, 120.56, 113.44, 107.63, 55.94, 55.28, 33.68, 25.63, 23.48.

HRMS: Obsvd 297.1492 [M + H^+], Calcd for C_{19} $H_{20}O_3$ H: 297.1485. **HPLC** (Method A): 17.42 min.

*2-methoxy-5-(4-methoxyphenyl)- benzocyclohept-5-ene (**36**)*

To a solution of 4-methoxyphenyl bromide (0.886 g, 4.73 mmol) in THF (50 mL) at -78 ºC was added *n*-BuLi (3.4 mL, 2.5 M in hexanes) and the reaction mixture was stirred for 30 min. Benzosuberone **13** (0.601 g, 3.15 mmol) in THF (25 mL) was added drop wise over a period of 15 min. The reaction mixture was allowed to reach room temperature over 12 h. Upon completion, the water was added and mixture was extracted with EtOAc (100 mL). The organic extract was washed with brine, dried over Na2SO4, filtered, concentrated under reduced pressure, and subjected to flash chromatography using a pre-packed silica 40g gold column [eluent; solvent A,

EtOAc, solvent B, hexanes; gradient 0%A/100%B →100%A/0%B over 22.9 min; flow rate: 50 mL/min; monitored at 254 and 280 nm], which afforded benzosuberene analogue **36** (0.32 g, 1.14 mmol, 36%) as clear oil which solidified as white.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20 (2H, d, *J*=8.7 Hz), 6.94 (1H, d, *J*=8.4 Hz), 6.83 (2H, d, *J*=8.5 Hz), 6.82 (1H, d, J=2.5 Hz), 6.73 (1H, dd, *J*=8.5, 2.7 Hz), 6.29 (1H, t, J=7.5 Hz) 3.83 (3H, s), 3.81 (3H, s), 2.63 (2H, t, *J*=7.0 Hz), 2.16 (2H, p, *J*=7.1 Hz), 1.96 (2H, q, *J*=7.2 Hz).

^{13}C NMR (126 MHz, $CDCl_3$): δ 158.82, 158.34, 143.82, 142.13, 135.27, 133.00, 130.37, 125.87, 113.91, 113.50, 113.50, 111.10, 55.28, 55.17, 35.16, 32.77, 25.40. **HRMS**: Obsvd 311.1713 [M + H^+], Calcd for $C_{20}H_{22}O_3$ H: 311.1642. **HPLC** (Method A): 17.65 min.

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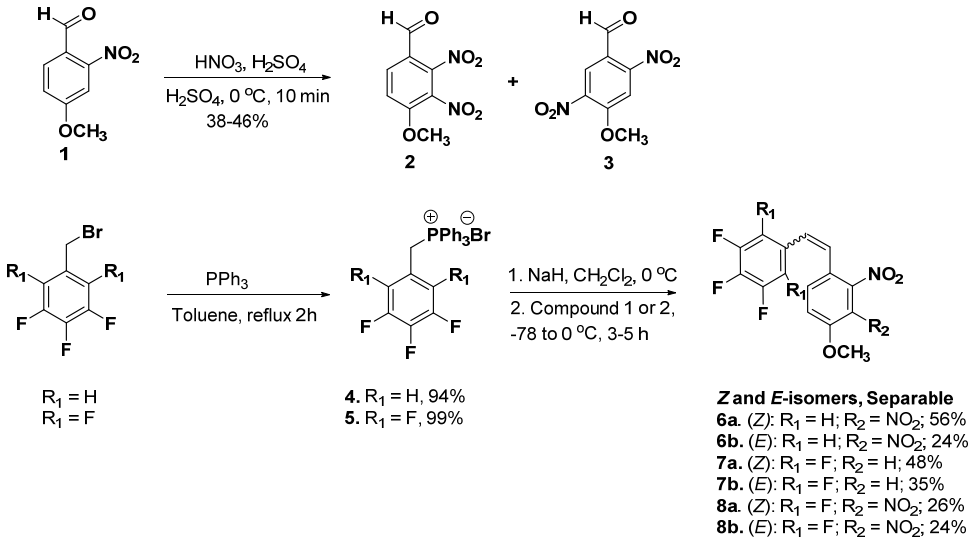
CHAPTER FIVE

Synthesis of ^{19}F Analogues of Amino-Based Combretastatins

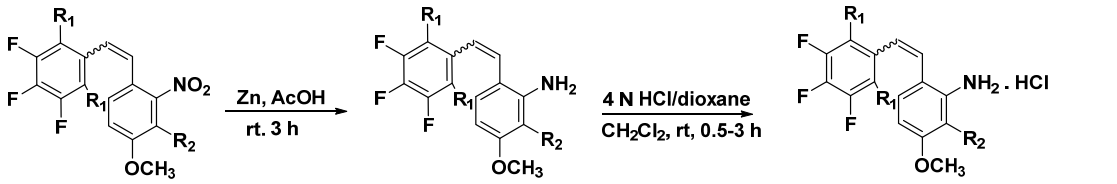
Synthesis

Eight fluorinated amino-based combretastatin derivatives, including free amines and their corresponding hydrochloride salts were synthesized. The synthetic strategy that allowed for preparation of these fluorinated amino-based combretastatins utilized a Wittig reaction as a key step to synthesize the desired Z-stilbenes. The commercially available 4-methoxy-2-nitrobenzaldehyde **1** or its nitrated derivative 4-methoxy-2,3-dinitrobenzaldehyde **2** served as the aldehyde for the Wittig reaction (Scheme **5.1**).[1,2]



Scheme **5.1**. Synthesis of Fluorinated Combretastatin Stilbenes **6a-8b**

The tri- and penta-fluorinated benzylbromide were refluxed with PPh_3 in toluene to generate their respective Wittig salts **4** and **5** in excellent yields.[3] Reduction of the nitro group, of selected *Z* and *E* stilbenes, afforded their corresponding amines, which on treatment with 4 N HCl/dioxane generated their respective hydrochloride salts (Scheme **5.2**).[2,4,5]



6a. (*Z*): R_1 = H; R_2 = NO_2
6b. (*E*): R_1 = H; R_2 = NO_2
7a. (*Z*): R_1 = F; R_2 = H
7b. (*E*): R_1 = F; R_2 = H
8a. (*Z*): R_1 = F; R_2 = NO_2
8b. (*E*): R_1 = F; R_2 = NO_2

9. (*Z*): R_1 = H; R_2 = NH_2; 61%
10. (*Z*): R_1 = F; R_2 = H; 74%
11. (*E*): R_1 = F; R_2 = H; 80%
12. (*Z*): R_1 = F; R_2 = NH_2; 85%

13. (*Z*): R_1 = H; R_2 = NH_2 . HCl; 88%
14. (*Z*): R_1 = F; R_2 = H; 93%
15. (*Z*): R_1 = F; R_2 = NH_2 . HCl; 66%

Scheme **5.2**. Synthesis of Hydrochloride Salts of Combretastatin-Amines **13-15**

Biological Evaluation

Table **5.1**. Inhibition of Tubulin Polymerization, Percent Inhibition of Colchicine Binding, and Cytotoxicity of the Fluorinated Amino-Based Combretastatins

Compound	Inhibition of tubulin polymerization IC_{50} (μM) ± SD	% Inhibition of colchicine binding ± SD	GI_{50} (μM) SRB assay[a]		
			SK-OV-3	NCI-H460	DU-145
CA1[b]	1.9[c]	99.6±0.7[d]	0.0384	0.0153	0.0326
CA4[e]	1.2[e]	84 ± 3[d] (1 μM), 98 ± 0.007[d] (5 μM)	0.00455	0.00223	0.00327
9	7.5±0.1	77±0.3 (50 μM)	1.38	2.04	5.37
10	Nd	Nd	4.57	Nd	9.36(Avg.) 6.15
11	Nd	Nd	Nd	Nd	Nd
12	8.4±0.6	67±0.2	2.41	0.650	5.18

Compound	Inhibition of tubulin polymerization IC_{50} (μM) ± SD	% Inhibition of colchicine binding ± SD	GI_{50} (μM) SRB assay[a]	Compound	Inhibition of tubulin polymerization IC_{50} (μM) ± SD
13	6.2±0.3	80±0.7 (50 μM)	1.39	2.80	4.86
14	Nd	Nd	3.74	7.27	4.97
15	Nd	Nd	Nd	Nd	Nd
16	6.2±0.2	72±2	2.55	0.521	2.61

[a] Average of n ≥ 3 independent determinations
[b] Data from ref. 6, for additional data, see ref. 7
[c] Data from ref. 2, for additional data, see ref. 7
[d] Data from ref. 7
[e] For additional data, see ref. 7
Nd = not detected

Four of the newly synthesized compounds were biologically evaluated for their ability to inhibit tubulin polymerization and colchicine binding. Each of the compounds demonstrated inhibition of tubulin polymerization in the lower μM range (Table **5.1**). In addition, they showed good percent inhibition of colchicine binding (Table **5.1**) as well as good cytotoxicity against selected human cancer cell lines varying from sub μM to lower μM range.

Materials and Methods

Nitration of aldehyde[2]

The appropriate aldehyde (1.53 g, 8.45 mmol) was dissolved in concentrated H_2SO_4 (25 mL). To this solution, a pre-cooled mixture (1:1 by volume) of HNO_3 and H_2SO_4 was added dropwise. The reaction mixture was stirred for 10 minutes. The resulting solution was added dropwise into ice-water (approximately 100 mL). After stirring for 2 h at 0 ^oC, the solution was filtered and the solid containing the desired product

was rinsed with ice-water (approximately 10 mL). Purification by flash column chromatography (EtOAc/hexane, 20:80) yielded region-isomers **1** and **2.**

*4-methoxy-2,3-dinitrobenzaldehyde (**2**).*

This compound was isolated as a yellow solid (0.879 g, 3.89 mmol, 46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.95 (1H, s), 8.15 (1H, d, *J* = 8.9 Hz), 7.40 (1H, d, *J* = 8.9 Hz), 4.09 (3H, s), ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.05, 155.69, 133.11, 128.96, 121.04, 115.92, 114.53, 57.88.

*4-methoxy-2,5-dinitrobenzaldehyde (**3**).*

This compound was isolated as a colorless solid (0.725 g, 3.20 mmol, 38%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.30 (1H, s) 8.43 (1H, s), 7.74 (1H, s), 4.15 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.63, 155.99, 127.12, 123.78, 123.04, 109.63, 57.89.

Synthesis of Wittig Salt[2,3,5]

To a solution of suitably fluorinated benzyl bromide in toluene (0.2 M solution), PPh_3 (1.2 equivalence) was added and refluxed for 2 h (at 110 ^{O}C). The reaction mixture was cooled down to room temperature, filtered and washed with plenty of diethyl ether and dried.

*(3,4,5-trifluorobenzyl)phosphonium bromide (**4**)*

This compound was isolated as a white solid (0.408 g, 0.838 mmol, 94%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 – 7.72 (9H, m), 7.66 (6H, td, *J*=7.8, 3.5 Hz), 6.91 (2H, t, *J*=7.1 Hz), 5.77 (2H, dd, *J*=14.7, 4.5 Hz).

^{19}F NMR (470 MHz, $CDCl_3$,) δ -131.52 – -134.15 (m), -159.86 (d, *J*=6.6 Hz)

*((perfluorophenyl)methyl)phosphonium bromide (**5**)*

This compound was isolated as a white solid (0.840 g, 1.60 mmol, 99%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 – 7.77 (m, 4H), 7.74 – 7.66 (m, 3H), 5.72 (d, *J* = 14.0 Hz, 1H).

^{19}F NMR (470 MHz, cdcl$_3$) δ -135.94 (d, *J* = 16.9 Hz), -150.98 (td, *J* = 21.0, 6.8 Hz), -159.66 – -160.12 (m).

General procedure for the synthesis of *Z*- and *E*-stilbenes.[2,4]

A NaH suspension (at 0 ^{o}C for **6a** and **6b,** 6.0 equiv) or n-BuLi (at -10 ^{o}C for **7a**, **7b** and -78 ^{o}C for **8a**, **8b**, 1.5 equiv) in dry CH_2Cl_2 was stirred for about 10 minutes under an inert (N_2) atmosphere. The previously prepared solution of Wittig salt (**4** or **5,** 1.1 equiv, approximately 0.1 M in CH_2Cl_2) was added dropwise. After stirring for 20 min, 1.0 equiv of 4-methoxy-2-nitrobenzaldehyde or 4-methoxy-2,3-dinitrobenzaldehyde (0.1 M in CH_2Cl_2) was added. The resulting mixture was stirred for 2-5 h. At this point, ice water was added carefully until the hydrogen gas evolution stopped. Workup was carried out by extracting with CH_2Cl_2, washing twice with water followed by brine, and drying over Na_2SO_4. The *Z*-and *E*-isomers were isolated (in 24-56% yield) after flash column chromatography or by re-crystallization of the mixture in ethyl acetate and hexane.

*(Z)-1-methoxy-2,3-dinitro-4-(3,4,5-trifluorostyryl)benzene (**6a**)*

This isomer was isolated as a yellow solid (0.92 g, 2.58 mmol, 56%)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (1H, d, *J*=8.9 Hz), 7.11 (1H, d, *J*=8.9 Hz), 6.75 (1H, d, *J*=7.2 Hz), 6.74 (1H, d, *J*=7.4 Hz), 6.69 (1H, d, *J*=12.0 Hz), 6.57 (1H, d, *J*=11.9 Hz), 3.98 (4H, s).

^{13}C NMR (126 MHz, $CDCl_3$,) δ 151.45, 151.18 (ddd, *J*=251.0, 10.1, 4.2 Hz), 142.84 (d, *J*=3.6 Hz), 139.38 (dt, *J*=254.2, 15.3 Hz), 134.47, 133.81, 131.78, 131.19 (td, *J*=7.9, 5.0 Hz), 124.37, 122.80, 116.14, 113.00 (dd, *J*=16.4, 5.1 Hz), 57.32.

*(Z)-1,2,3,4,5-pentafluoro-6-(4-methoxy-2-nitrostyryl)benzene (**7a**)*

This isomer was isolated as a pale yellow solid (0.460 g, 1.33 mmol, 48%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (1H, d, *J*=2.1 Hz), 7.32 (1H, d, *J*=11.7 Hz), 6.98 – 6.92 (2H, m), 6.42 (1H, dd, *J*=11.7, 1.1 Hz), 3.87 (4H, s).

^{13}C NMR (151 MHz, $CDCl_3$) δ 159.9, 147.9, 144.1 (dddd, *J*=249.1, 15.7, 7.9, 3.7 Hz), 141.70 – 139.63 (m), 138.70 – 136.56 (m), 135.33, 131.17, 125.03, 120.36, 114.52 (H, d, *J*=2.2 Hz), 111.36 (H, td, *J*=18.2, 4.4 Hz), 109.68.

*(E)-1,2,3,4,5-pentafluoro-6-(4-methoxy-2-nitrostyryl)benzene (**7b**)*

This isomer was isolated as a pale yellow solid (0.332 g, 0.961 mmol, 35%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (1H, d, *J*=16.5 Hz), 7.65 (1H, d, *J*=8.7 Hz), 7.52 (1H, d, *J*=2.5 Hz), 7.20 (1H, dd, *J*=8.7, 2.6 Hz), 6.82 (1H, d, *J*=16.5 Hz), 3.91 (3H, s).

^{13}C NMR (151 MHz, $CDCl_3$) δ 160.07, 148.64, 144.90 (dddd, *J*=251.4, 12.0, 7.9, 3.7 Hz), 140.19 (dddd, *J*=249.7, 25.0, 14.2, 5.5 Hz), 138.86 – 136.62 (m), 132.38 (td, *J*=9.2, 2.7 Hz), 129.46, 124.90, 120.38, 115.86 (d, *J*=3.1 Hz), 111.92 (td, *J*=14.1, 4.5 Hz), 109.25, 56.02.

*(Z)-1,2,3,4,5-pentafluoro-6-(4-methoxy-2,3-dinitrostyryl)benzene (**8a**)*

This isomer was isolated as a yellow solid (0.550 g, 1.40 mmol, 26%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (1H, d, *J*=8.9 Hz), 7.08 (1H, d, *J*=8.9 Hz), 7.01 (1H, d, *J*=11.8 Hz), 6.55 (1H, d, *J*=11.8 Hz), 3.97 (3H, s).

^{13}C NMR ($CDCl_3$, 151 MHz) δ 144.05 (H, dddd, *J*=251.3, 16.3, 8.3, 4.3 Hz), 142.10 (H, s), 142.29 – 140.24 (H, m), 137.84 (H, dddd, *J*=252.3, 18.5, 12.5, 2.9 Hz), 134.83 (H, s), 132.07 (H, s), 130.77 (H, s), 123.51 (H, s), 118.40 (H, d, *J*=2.3 Hz), 116.27 (H, s), 110.28 (H, td, *J*=18.0, 4.5 Hz), 57.43 (H, s).

*(E)-1,2,3,4,5-pentafluoro-6-(4-methoxy-2,3-dinitrostyryl)benzene (**8b**)*

This isomer was isolated as a pale yellow solid (0.500 g, 1.28 mmol, 24%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (1H, d, *J*=9.0 Hz), 7.42 (1H, d, *J*=16.5 Hz), 7.33 (1H, d, *J*=9.0 Hz), 7.28 (1H, s), 6.96 (1H, d, *J*=16.5 Hz), 4.05 (3H, s).

General procedure for the reduction of nitro to amines[2]

Zinc powder (50-80 equiv.) was added slowly to a well stirred solution of *Z*-stilbenes **6a, 7a, 7b** and **8a** (1.0 equiv, 0.03 M) in glacial AcOH. The resulting suspension was stirred for approximately 3 hours at room temperature. At this point, the reaction mixture was filtered through Celite and washed with ethyl acetate, and the filtrate was concentrated at reduced pressure. The desired amine was purified by flash chromatography using flash chromatography to afford the amines **9**-**12** in 61-85% yield.

*(Z)-3-methoxy-6-(3,4,5-trifluorostyryl)benzene-1,2-diamine (**9**)*

This isomer was isolated as a white solid (0.464 g, 1.58 mmol, 61%).

^{1}H NMR (500 MHz, $CDCl_3$,) δ 6.83 (1H, d, *J*=6.9 Hz), 6.82 (1H, d, *J*=6.8 Hz), 6.59 (1H, d, *J*=11.9 Hz), 6.55 (1H, d, *J*=8.5 Hz), 6.43 (1H, d, *J*=11.9 Hz), 6.36 (1H, d, *J*=8.4 Hz), 3.86 (3H, s), 3.49 (4H, s).

^{13}C NMR (151 MHz, $CDCl_3$) δ 150.82 (ddd, *J*=248.4, 10.3, 4.5 Hz), 148.28, 138.69 (dt, *J*=252.2, 15.8 Hz), 133.21, 132.95 (td, *J*=8.4, 5.1 Hz), 129.00 (d, *J*=2.3 Hz), 128.30 (dd, *J*=5.0, 2.4 Hz), 123.20, 119.54, 116.15, 112.61 (dd, *J*=17.4, 4.9 Hz), 102.32, 55.69.

HPLC (Method A) 14.67 min.

*(Z)-5-methoxy-2-(2-(perfluorophenyl)vinyl)aniline (**10**)*

This isomer was isolated as a white solid (0.305 g, 0.967 mmol, 74%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.86 (1H, d, *J*=11.5 Hz), 6.66 (1H, d, *J*=8.5 Hz), 6.26 (1H, dd, *J*=11.5, 1.3 Hz), 6.23 (1H, d, *J*=2.4 Hz), 6.14 (1H, dd, *J*=8.5, 2.4 Hz), 3.74 (3H, s), 3.71 (2H, s).

^{13}C NMR (151 MHz, $CDCl_3$) δ 160.81, 145.45, 144.02 (dddd, *J*=248.6, 15.8, 8.1, 3.8 Hz), 141.24 – 139.19 (m), 138.59 – 136.43 (m), 133.98, 129.16, 115.58, 113.27 (H, d, *J*=2.1 Hz), 112.45 (H, td, *J*=18.6, 4.5 Hz), 104.60, 100.96, 55.09.

*(E)-5-methoxy-2-(2-(perfluorophenyl)vinyl)aniline (**11**)*

This isomer was isolated as a white solid (0.510 g, 1.62 mmol, 80%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (1H, d, *J*=16.4 Hz), 7.36 (1H, d, *J*=8.6 Hz), 6.74 (1H, d, *J*=16.4 Hz), 6.41 (1H, dd, *J*=8.7, 2.4 Hz), 6.25 (1H, d, *J*=2.4 Hz), 3.86 (2H, s).

^{13}C NMR (151 MHz, $CDCl_3$) δ 159.54, 146.78 – 143.90 (m), 145.09, 140.67-138.77 (m), 138.33 – 135.64 (m), 128.25, 127.19, 115.30, 113.69 (t, *J*=18.5 Hz), 105.68, 104.08, 101.41.

*(Z)-3-methoxy-6-(2-(perfluorophenyl)vinyl)benzene-1,2-diamine (**12**)*

This isomer was isolated as a white solid (0.201 g, 0.608 mmol, 85%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (1H, d, *J*=11.5 Hz), 6.34 (1H, d, *J*=8.6 Hz), 6.31 (1H, d, *J*=11.6 Hz), 6.23 (1H, d, *J*=8.5 Hz), 3.83 (3H, s), 3.50 (4H, s).

^{13}C NMR (151 MHz, $CDCl_3$) δ 148.69 (H, s), 144.06 (H, dddd, *J*=248.8, 15.8, 8.1, 3.9 Hz), 141.25 – 139.09 (H, m), 138.49 – 136.14 (H, m), 134.58 (H, s), 133.91 (H, s), 122.73 (H, s), 118.97 (H, s), 116.96 (H, s), 113.67 (H, d, *J*=2.1 Hz), 112.52 (H, td, *J*=18.6, 4.4 Hz), 101.83 (H, s), 55.57 (H, s).

^{19}F NMR (470 MHz, $CDCl_3$) δ -139.08 (H, dd, *J*=22.7, 7.8 Hz), -156.54 (H, t, *J*=20.9 Hz), -162.77 (H, td, *J*=22.8, 8.1 Hz). **HPLC** (Method A): 14.60 min

General Procedure for the Preparation of hydrochloride salts of amino combretastatin analogues[2]

To a solution of combretastatin-amines **9-12** (1.0 equiv, approximately 0.2 M in anhydrous CH_2Cl_2 was added HCl solution (4N/dioxane, 3 equiv.) at room temperature. The reaction is monitored by TLC and stirred for 30 min to 3 h. The solvents were either evaporated or solid is filtered. Thus obtained product was washed with plenty of dry diethyl ether to furnish the hydrochloride salts **13**-**16** in 66-93% as white solid.

*(Z)-3-methoxy-6-(3,4,5-trifluorostyryl)benzene-1,2-diamine dihydrochloride (**13**)*

This isomer was isolated as a white solid (0.082 g, 0.223 mmol, 88%).

^{1}H NMR (500 MHz, MeOD) δ 6.97 (1H, d, *J*=8.6 Hz), 6.90 (2H, dd, *J*=9.2, 6.8 Hz), 6.64 (1H, d, *J*=11.9 Hz), 6.61 (1H, d, *J*=11.9 Hz), 6.51 (1H, d, *J*=8.6 Hz), 3.93 (3H, s).

^{13}C NMR (151 MHz, MeOD) δ 152.93, 150.70 (ddd, *J*=247.5, 10.4, 4.4 Hz), 138.47 (dt, *J*=250.7, 15.9 Hz), 136.63, 133.29 (td, *J*=8.5, 5.1 Hz), 129.55, 129.45, 127.12 (d, *J*=1.6 Hz), 117.90, 112.60 (dd, *J*=17.6, 4.9 Hz), 107.67, 102.33, 55.61. **HPLC** (Method A): 9.86 min.

*(Z)-5-methoxy-2-(2-(perfluorophenyl)vinyl)aniline hydrochloride LD-III-91 (**14**)*

This isomer was isolated as a white solid (0.083 g, 0.236 mmol, 93%).

^{1}H NMR (600 MHz, MeOD) δ 7.05 (1H, d, *J*=11.7 Hz), 7.00 – 6.96 (2H, m), 6.78 (1H, dd, *J*=8.7, 2.1 Hz), 6.54 (1H, d, *J*=11.7 Hz), 3.77 (3H, s).

^{13}C NMR (151 MHz, MeOD) δ 160.77 (t, *J*=6.8 Hz), 145.12 – 142.89 (m), 140.73 (ddd, *J*=252.6, 16.9, 11.6 Hz), 138.84 – 136.55 (m), 130.67, 130.17 – 129.48 (m), 123.22, 116.43, 114.01, 111.47 (td, *J*=18.5, 4.3 Hz), 109.46d, *J*=7.6 Hz), 54.94.

*(Z)-3-methoxy-6-(2-(perfluorophenyl)vinyl)benzene-1,2-diamine dihydrochloride LD-II-121-1A (**15**)*

This isomer was isolated as a white solid (0.032 g, 0.080 mmol, 66%).

^{1}H NMR (500 MHz, MeOD) δ 6.93 (1H, d, *J*=11.4 Hz), 6.70 (1H, d, *J*=8.5 Hz), 6.37 (1H, d, *J*=11.4 Hz), 6.29 (1H, d, *J*=8.5 Hz), 3.82 (3H, s).

^{13}C NMR (151 MHz, MeOD) δ 153.08, 143.99 (dddd, *J*=247.4, 15.4, 7.9, 3.7 Hz), 141.33 – 139.20 (m), 139.14, 138.56 – 136.25 (m), 133.24, 127.85, 117.40, 113.81, 112.33 (td, *J*=18.8, 4.4 Hz), 106.16 (d, *J*=13.9 Hz), 100.20, 55.31.

HPLC (Method A): 10.05 min.

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CHAPTER SIX

Conclusion and Future Direction

Several amino-based analogues of the combretastatin family of natural products were synthesized in addition to their hydrochloride salts. As parent drugs, 2' CA4-amine, 3' CA4-amine, and CA1-diamine, which were previously reported by us and others, all showed excellent cytotoxicity (in lower nano molar range) against selected human cancer cell lines. The CA1-Diamine and its hydrochloride salt (compounds **8** and **10** respectively, chapter two) demonstrated significant disruption of a HUVEC capillary-like network of tubules at a concentration of 0.1 μM. The tubule disruption and cell rounding effects were greatly enhanced at the concentration of 1 μM. In addition, compound **10** (dose of 80 mg/kg) resulted in approximately a 95% reduction in light emission in a Bioluminescence Imaging (BLI) study in SCID mice bearing the luciferase expressing MDA-MB-231-luc human breast cancer cell line induced tumor. In an effort to increase potential bioavailability along with water-solubility, the glycine- and serine-based amino acid prodrug conjugates (AAPCs) were synthesized. LAP cleavage study demonstrated the ability of AAPCs to be cleaved efficiently releasing their parent amino-based compounds, which suggests their capability to function as water soluble VDAs.

In an effort to develop drugs that can particularly target the tumor microenvironment, a series of bioreductively activatable prodrug conjugates (BAPCs) of CA1 were designed and synthesized regioselectively utilizing the Wittig

and Mitsunobu reactions as the main coupling methods. The idea was to mask the activity of CA1 by converting CA1 to its corresponding nitrothionyl prodrug conjugates. However, these BAPCs are insoluble or partially soluble in water. So, in order to increase the water solubility and potentially bioavailability, the synthesis of their respective phosphate salts is warranted in the near future. The synthesis of the BAPCs phosphate salts will also potentially solve the issue of regio-isomers via X-ray crystallography.

A series of VDAs based on benzosuberene analogues (**27**, **30**, and **32-36**) were synthesized utilizing a Wittig olefination reaction followed by an intramolecular Friedel-Crafts annulation facilitated by Eaton's reagent (chapter four). Also, a series of fluorinated amino-based combretastatin derivatives and their respective hydrochloride salts (compounds **9-16**, chapter five) were synthesized and biologically evaluated for their cytotoxicity against selected human cancer cell lines, inhibition of tubulin polymerization as well as percent inhibition of colchicine binding.

APPENDICES

APPENDIX A

Supplimentary Data: Design, Synthesis, and Biological Evaluation of Water-Soluble Amino Acid Prodrug Conjugates Derived from Combretastatin, Dihydronaphthalene, and Benzosuberene-Based Parent Vascular Disrupting Agents

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The author L. Devkota contributed to this manuscript with the synthesis of compounds **1** through **24**, in addition to their full characterization. Also, L. Devkota played a significant role in the preparation of manuscript including writing and making edits based on co-author's comments and suggestions.

Supplimentary Data

Design, Synthesis, and Biological Evaluation of Water-Soluble Amino Acid Prodrug Conjugates Derived from Combretastatin, Dihydronaphthalene, and Benzosuberene-Based Parent Vascular Disrupting Agents

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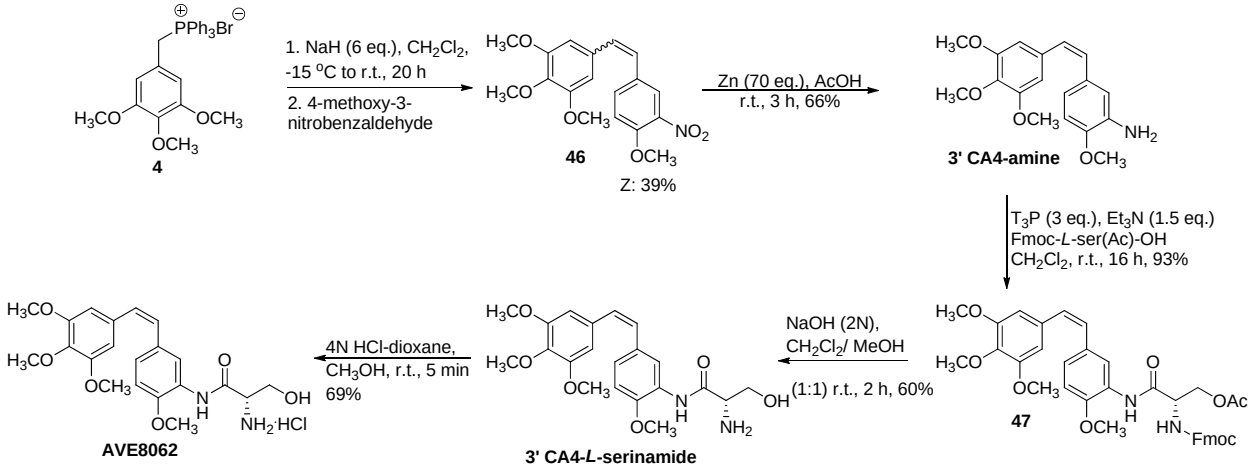
Table of Contents

Synthesis of AVE8062



Scheme. Synthesis of AVE8062

Experimental Section for the Synthesis of AVE8062

*(Z)-1,2,3-trimethoxy-5-(4-methoxy-3-nitrostyryl)benzene (**46**)*

NaH (1.54 g, 64.2 mmol) was added into an oven-dried round-bottom reaction flask. Anhydrous dichloromethane (100 mL) and 3,4,5-trimethoxybenzyl-triphenylphosphonium bromide (6.94 g, 13.3 mmol) were added to the reaction flask and the flask was stirred for an hour. The reaction mixture was cooled to -15 ºC, and 4-methoxy-3-nitrobenzaldehyde (2.01 g 11.1 mmol) was added to the reaction flask. The reaction mixture was allowed to stir for 20 h warming to ambient temperature under a nitrogen atmosphere. DI water (100 mL) was slowly added to the reaction and the product was extracted with dichloromethane (100 mL × 3). The organic phase was rinsed with brine, dried with Na_2SO_4, concentrated under reduced pressure, and 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 (10 CV), 70%A / 30%B (5 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] affording 3' CA4-nitro **46** (1.51 g, 4.34 mmol, 39% yield) as a yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (1H, d, *J*=2.0 Hz), 7.44 (1H, dd, *J*=8.7, 2.1 Hz), 6.95 (1H, d, *J*=8.7 Hz), 6.58 (1H, d, *J*=12.1 Hz), 6.47 (2H, s), 6.45 (1H, d, *J*=12.1 Hz), 3.94 (3H, s), 3.85 (3H, s), 3.72 (6H, s). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.50, 152.00, 139.67, 137.89, 134.99, 132.12, 131.59, 129.97, 127.15, 126.29, 113.36, 106.03, 61.29, 56.88, 56.31.

(Z)-2-methoxy-5-(3,4,5-trimethoxystyryl)aniline (3' CA4-amine)

To a well-stirred solution of 3' CA4-nitro **46** (0.560 g, 1.78 mmol) in acetic acid (40 mL), Zn (8.20 g, 125 mmol) was added and the reaction was allowed to stir for 3 h at room temperature. The reaction was filtered using Celite®, and washed with EtOAc. The filtrate was concentrated under reduced pressure and purified by flash column 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 (15 CV), 60%A / 40%B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] affording 3' CA4-amine (0.370 g, 1.17 mmol, 66% yield) as a brown solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.71 – 6.65 (2H, m), 6.67 (1H, d, *J*=1.8 Hz), 6.55 (2H, s), 6.45 (1H, d, *J*=12.2 Hz), 6.37 (1H, d, *J*=12.2 Hz), 3.84 (3H, s), 3.82 (3H, s), 3.70 (6H, s). ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.09, 146.91, 137.18, 136.02, 133.30, 130.32, 130.29, 128.64, 119.82, 115.53, 110.29, 106.20, 61.23, 56.20, 55.80. HRMS (ESI) calculated for $C_{18}H_{21}NO_4$ 315.1471, $(M+H)^+$ 316.1549, found 316.1543.

(S,Z)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-((2-methoxy-5-(3,4,5-trimethoxystyryl)phenyl)amino)-3-oxopropyl acetate (47)

3' CA4-amine (0.11 g, 0.35 mmol) was dissolved in CH_2Cl_2 (10 mL), and then Fmoc-serine acetate (0.19 g, 0.52 mmol), T3P (0.62 mL, 1.0 mmol), and Et_3N (0.073 mL, 0.52 mmol) were added. After stirring for 16 h at room temperature, DI water (10 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (10 mL × 3). The combined organic phase was rinsed with brine, dried with Na_2SO_4, concentrated under reduced pressure, and 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 (10 CV), 100%A / 0%B (2 CV); flow rate: 36 mL/min; monitored at 254 and 280 nm] affording the desired Fmoc-*L*-serinamide acetate **47** (0.22 g, 0.32 mmol, 93%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.42 (1H, s), 8.31 (1H, d, *J*=1.8 Hz), 7.77 (2H, d, *J*=7.0 Hz), 7.60 (2H, d, *J*=6.7 Hz), 7.40 (2H, t, *J*=7.0 Hz), 7.31 (2H, d, *J*=5.6 Hz), 7.03 (1H, d, *J*=8.2 Hz), 6.70 (1H, d, *J*=8.5 Hz), 6.51 (2H, s), 6.50 (2H, d, *J*=12.9 Hz), 6.45 (1H, d, *J*=12.1 Hz), 5.79 (1H, d, *J*=7.0 Hz), 4.65 (1H, d, *J*=4.8 Hz), 4.53 – 4.48 (2H, m), 4.35 – 4.30 (1H, m), 4.24 (1H, t, *J*=7.0 Hz), 4.02 (1H, dd, *J*=9.4, 2.7 Hz), 3.84 (3H, s), 3.79 (3H, s), 3.68 (6H, s), 2.09 (3H, s). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.99, 166.74, 153.17, 147.41, 143.93, 143.84, 141.59, 137.32, 133.05, 130.45, 129.75, 129.61, 128.13, 127.42, 125.35, 125.28, 121.14, 120.37, 120.35, 109.87, 106.19, 67.78, 64.23, 61.21, 56.19, 56.16, 55.01, 47.34, 21.05.

(S,Z)-2-amino-3-hydroxy-N-(2-methoxy-5-(3,4,5-trimethoxystyryl)phenyl)propanamide (3' CA4-L-serinamide)

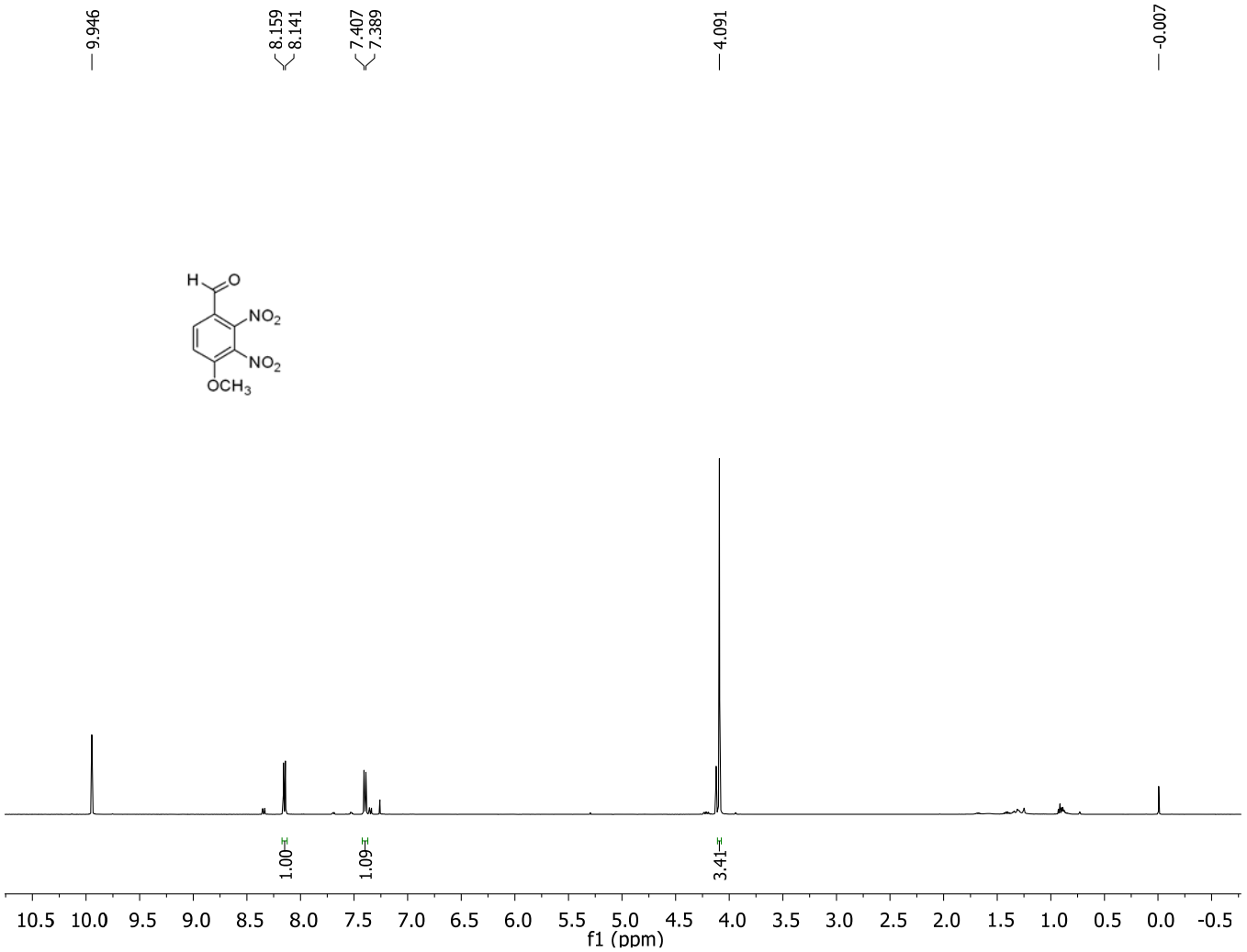
To a well-stirred solution of Fmoc-*L*-serinamide acetate **47** (0.22 g, 0.32 mmol) in dichloromethane/ methanol (2 mL/ 2 mL), 2N NaOH (2.00 eq.) was added to the reaction flask, and reaction was stirred for 2 h at room temperature under a nitrogen atmosphere. The solvent was evaporated under reduced pressure, and sat. $NaHCO_3$ (10 mL) was added. The solution was extracted by CH_2Cl_2 (10 mL × 3), and then the combined organic phase was rinsed with brine, dried with Na_2SO_4, concentrated under reduced pressure, and purified by preparative TLC (10% methanol/ 90% dichloromethane) to give the desired 3' CA4-*L*-serinamide prodrug (0.077 g, 0.19 mmol, 60%) as a yellow oil. ^{1}H NMR (600 MHz, CD_3OD) δ 8.21 (1H, d, *J*=1.9 Hz), 6.97 (1H, d, *J*=8.4 Hz), 6.88 (1H, d, *J*=8.3 Hz), 6.53 (2H, s), 6.49 (1H, d, *J*=12.1 Hz), 6.43 (1H, d, *J*=12.1 Hz), 3.84 (3H, s), 3.72 (2H, d, *J*=6 Hz), 3.71 (3H, s), 3.61 (6H, s), 3.49 (1H, t, *J*=5.3 Hz). ^{13}C NMR (CD_3OD, 151 MHz) δ 172.37, 152.72, 148.32, 136.81, 132.96, 129.88, 129.24, 128.95, 126.76, 124.82, 120.56, 109.89, 106.02, 63.84, 59.74, 57.03, 55.04, 54.93. HRMS (ESI) calculated for $C_{21}H_{26}N_2O_6$ 402.1791, $(M+H)^+$ 403.1864, found 403.1869.

(S,Z)-2-amino-3-hydroxy-N-(2-methoxy-5-(3,4,5-trimethoxystyryl)phenyl)propanamide hydrochloride (AVE8062)

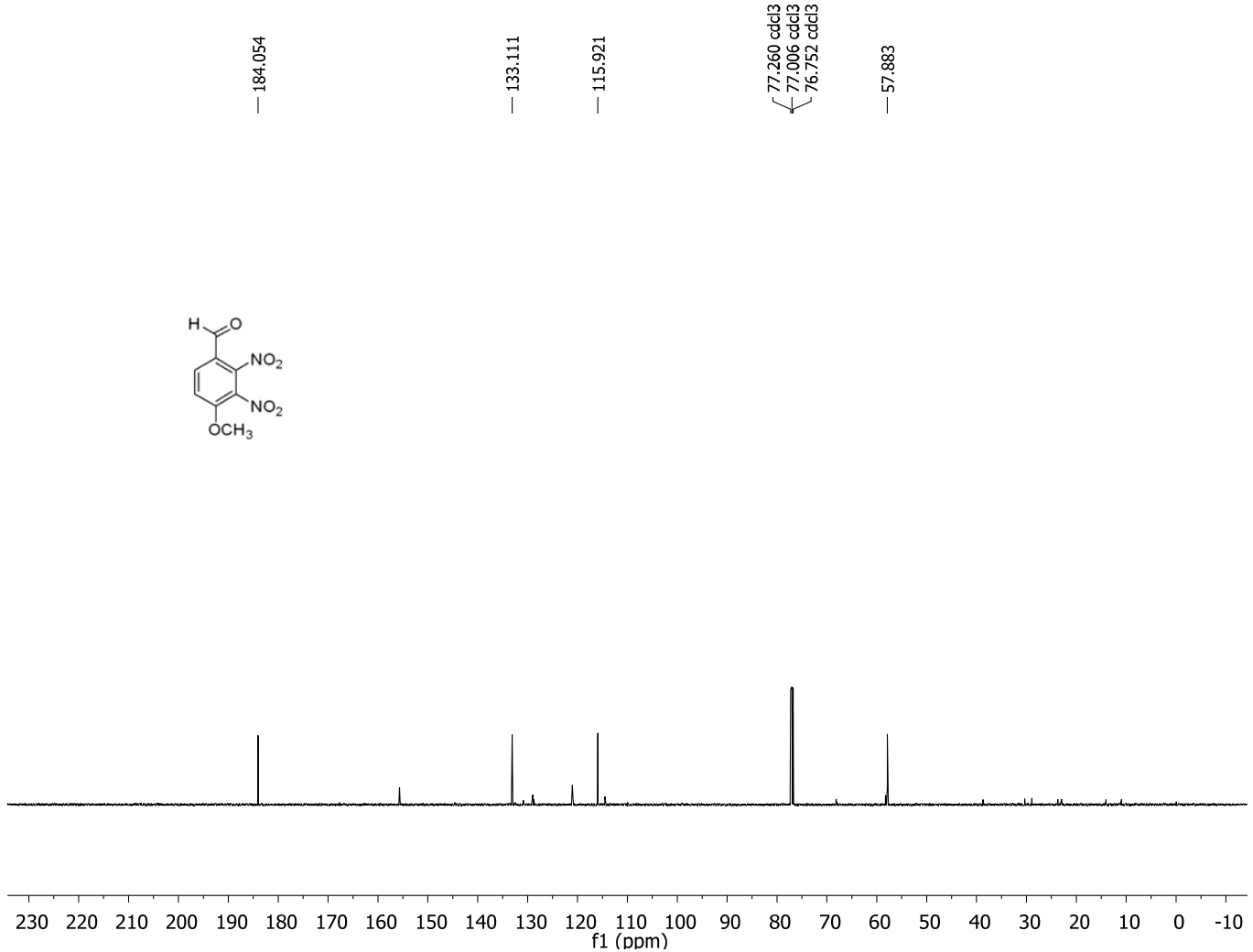
3' CA4-*L*-serinamide prodrug (0.027 g, 0.067 mmol) was dissolved in CH_3OH (0.50 mL), and 4N HCl-dioxane (0.08 mL, 0.32 mmol) was added into the solution. After stirring for 5 min, the solvent was evaporated to dryness and the resultant was washed by diethyl ether (2 mL × 3) to give desired 3' CA4-*L*-serinamide prodrug salt

AVE8062 (0.020 g, 0.046 mmol, 69%) as a colorless solid. ^{1}H NMR (600 MHz, CD_3OD) δ 7.97 (1H, d, *J*=2.0 Hz), 7.04 (1H, dd, *J*=8.5, 2.0 Hz), 6.93 (1H, d, *J*=8.5 Hz), 6.52 (2H, s), 6.49 (1H, d, *J*=12.2 Hz), 6.45 (1H, d, *J*=12.2 Hz), 4.14 (1H, dd, *J*=6.3, 5.0 Hz), 3.94 (1H, dd, *J*=11.2, 4.7 Hz), 3.87 – 3.83 (4H, m), 3.71 (3H, s), 3.62 (6H, s). ^{13}C NMR (151 MHz, CD_3OD) δ 165.22, 152.77, 149.21, 136.74, 132.96, 129.80, 129.07, 128.93, 126.15, 125.87, 122.42, 110.28, 105.90, 60.38, 59.78, 55.04, 55.02, 54.93. HRMS (ESI) calculated for $C_{21}H_{27}ClN_2O_6$ 438.1558, $(M\text{-}Cl)^+$ 403.1864, found 403.1865.

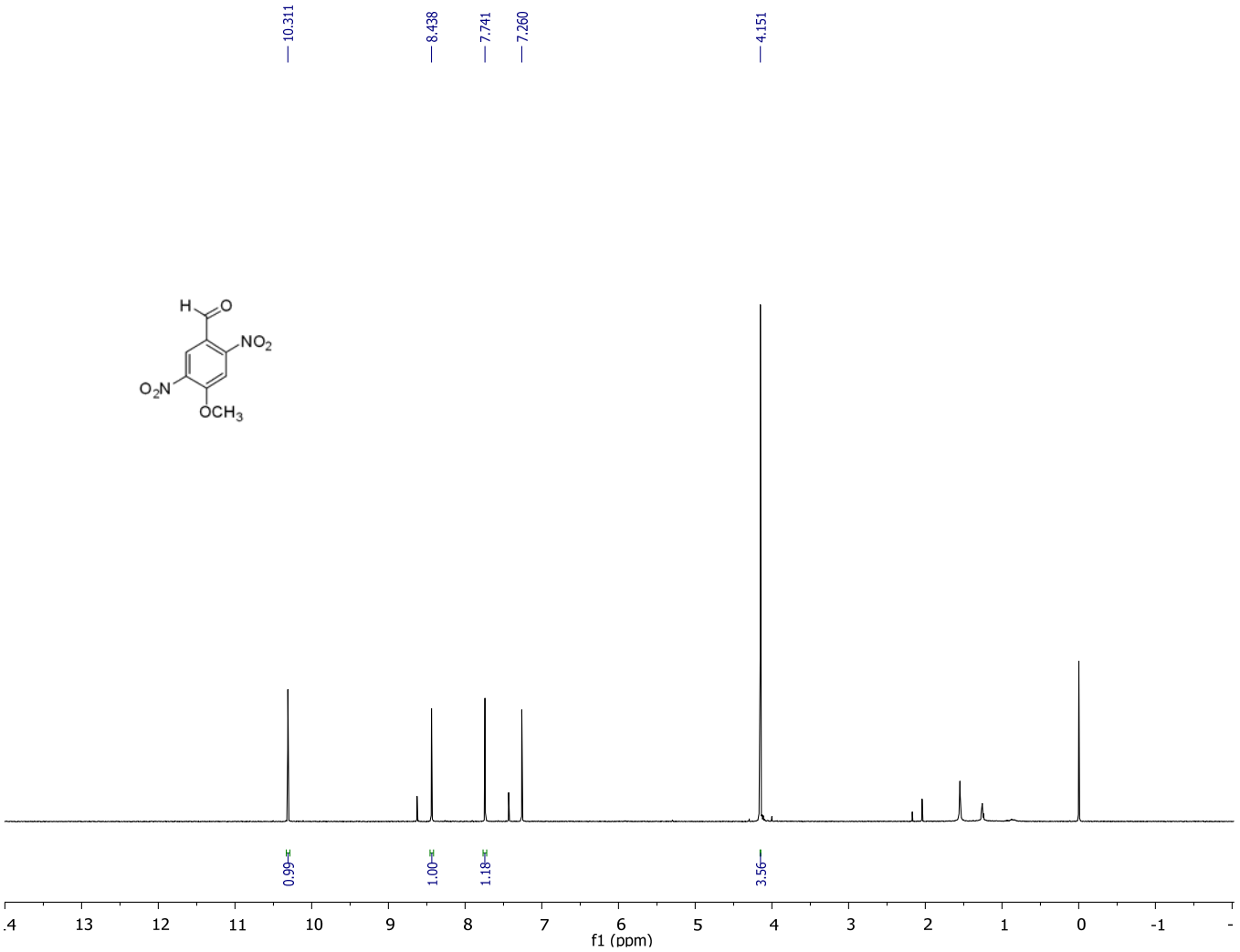
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **1**



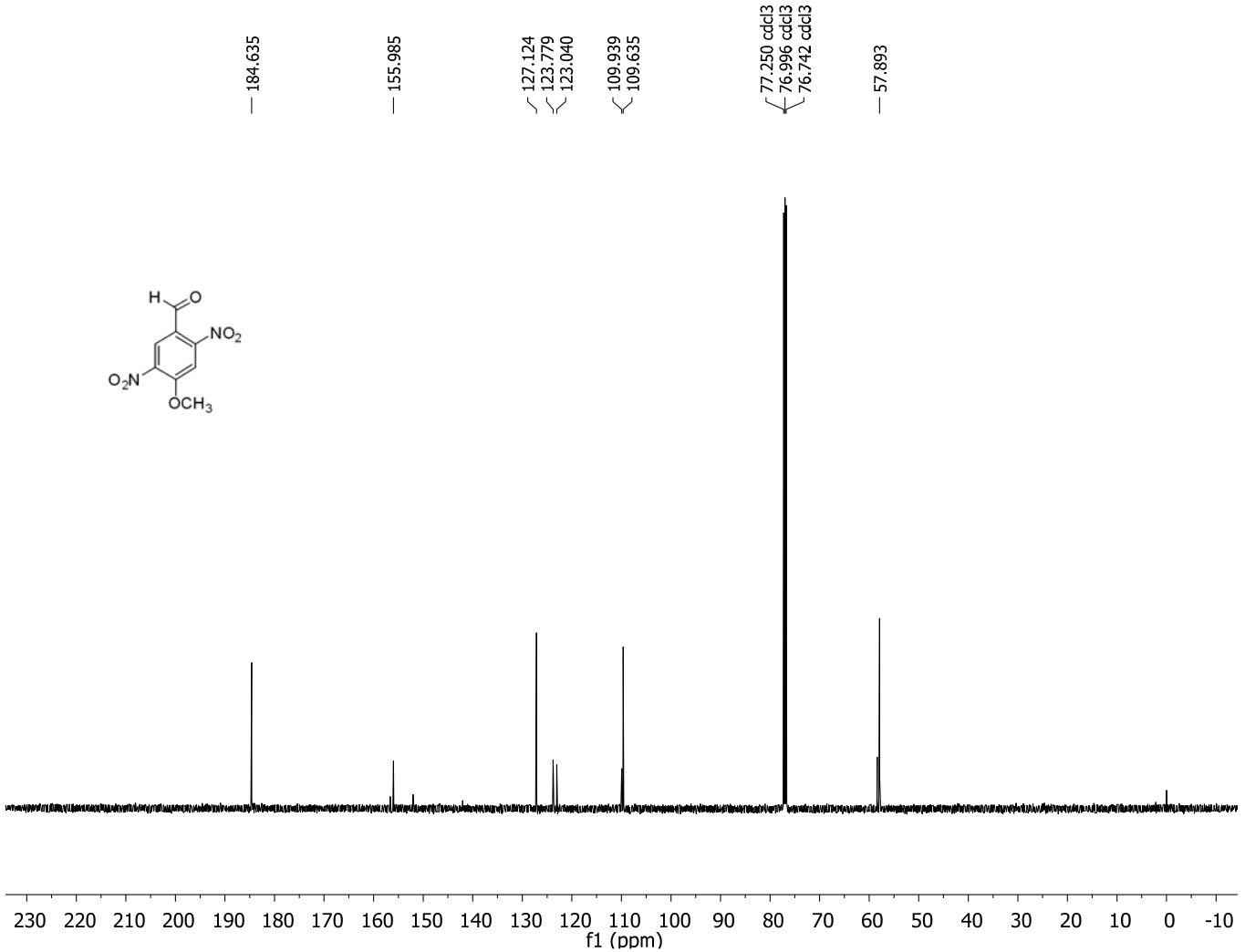
^{13}C NMR ($CDCl_3$, 125 MHz) Compound **1**



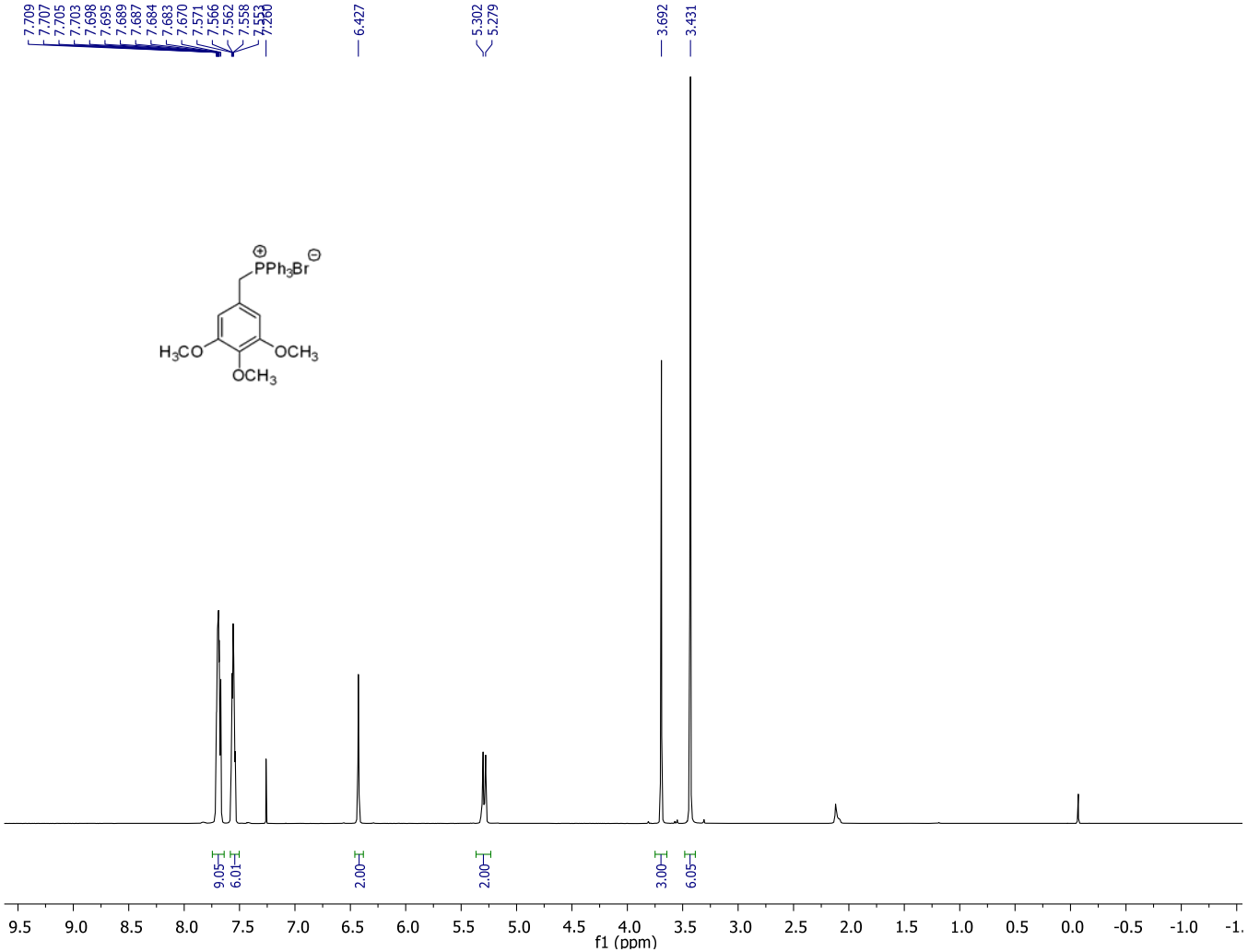
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **2**



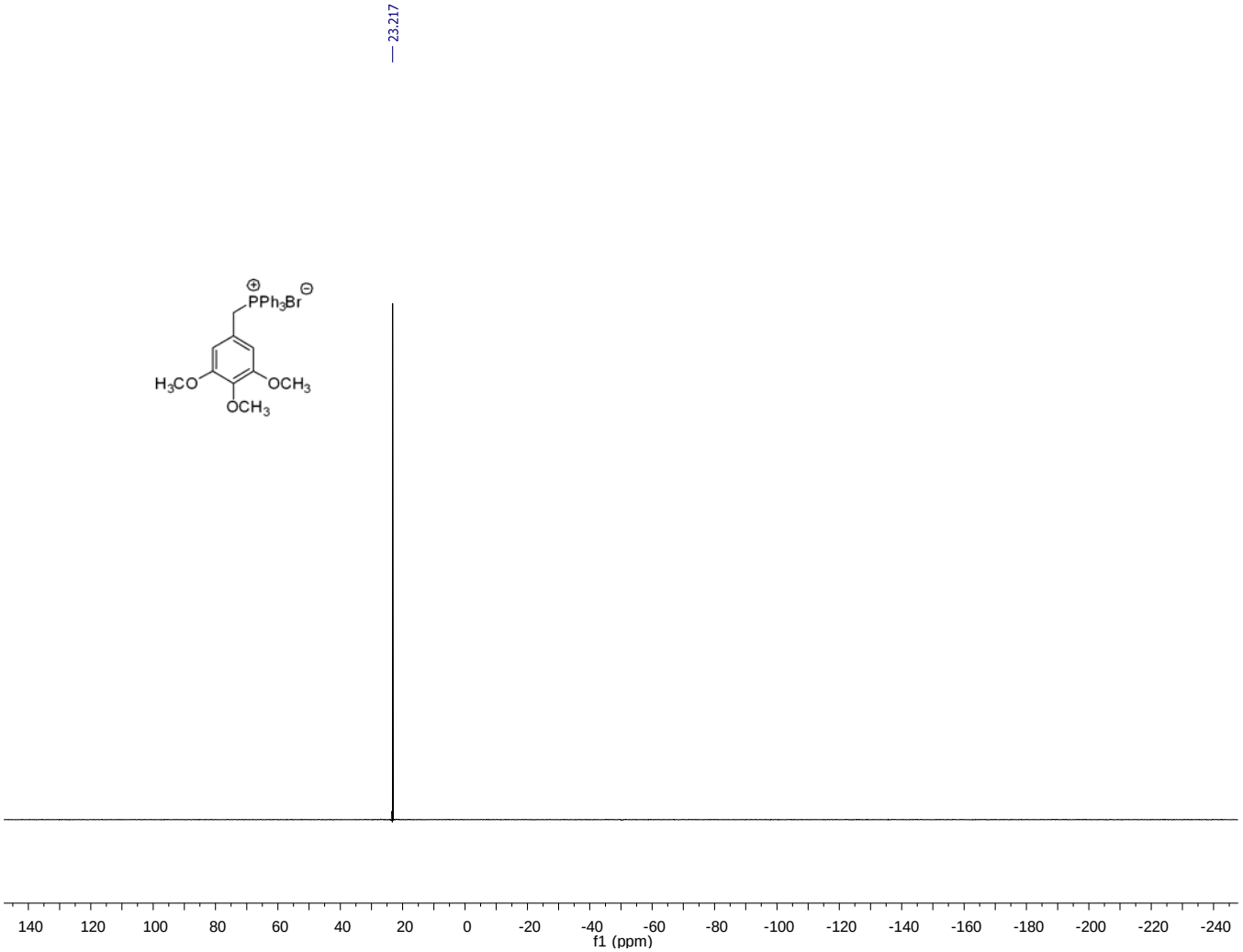
^{13}C NMR ($CDCl_3$, 125 MHz) Compound **2**



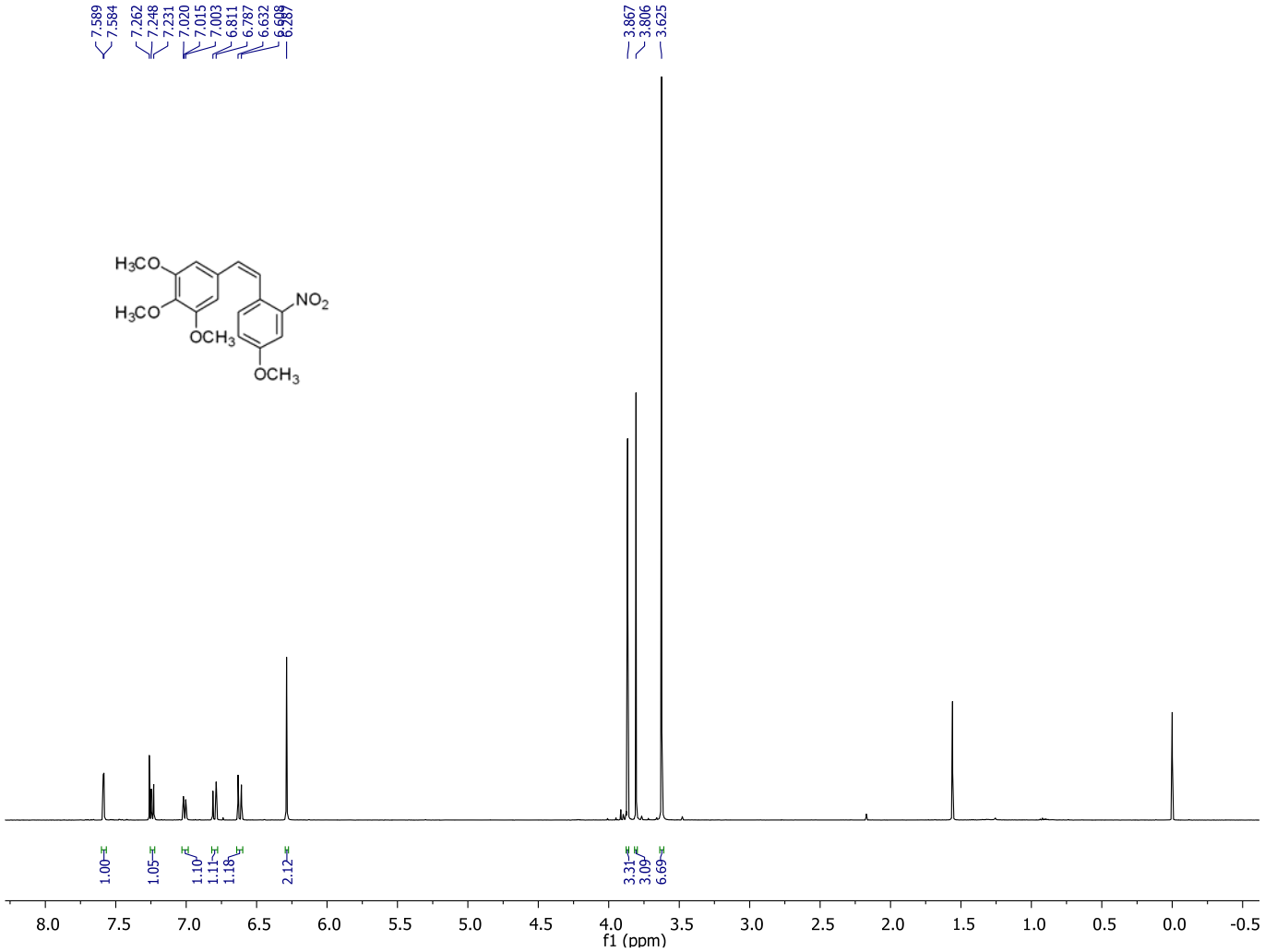
^{1}H NMR ($CDCl_3$, 600 MHz) Compound **4**



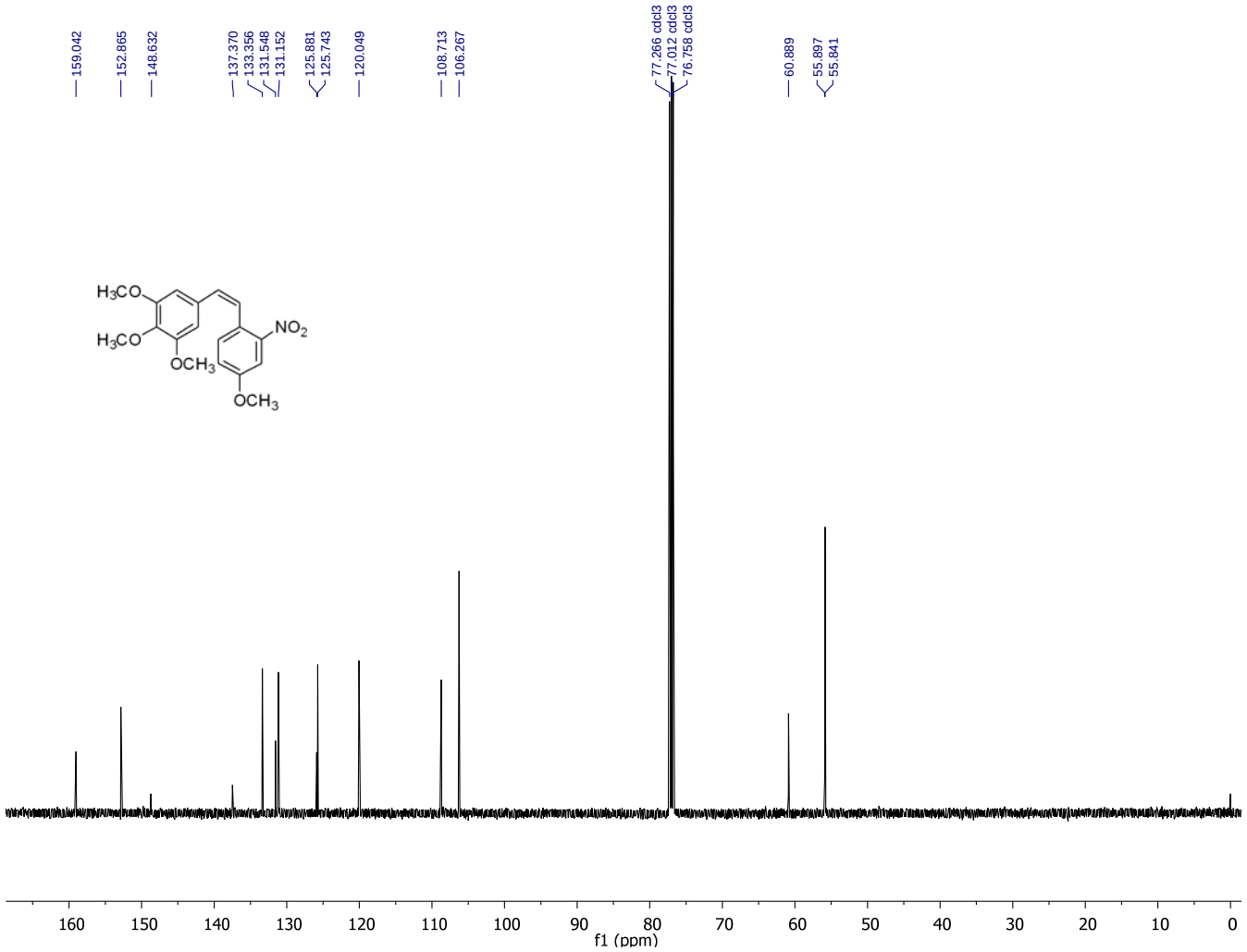
^{31}P NMR ($CDCl_3$, 240 MHz) Compound **4**



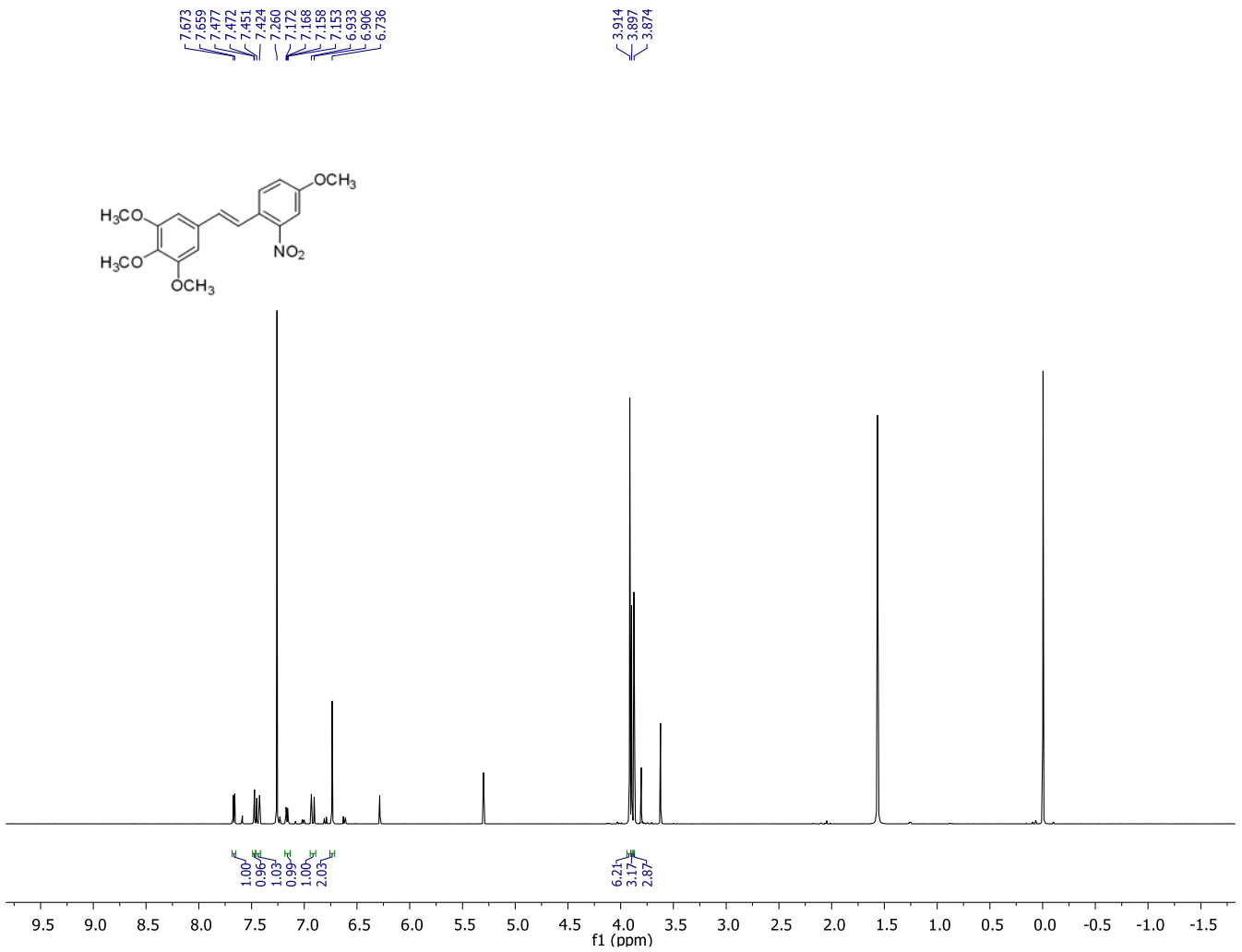
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **5a**



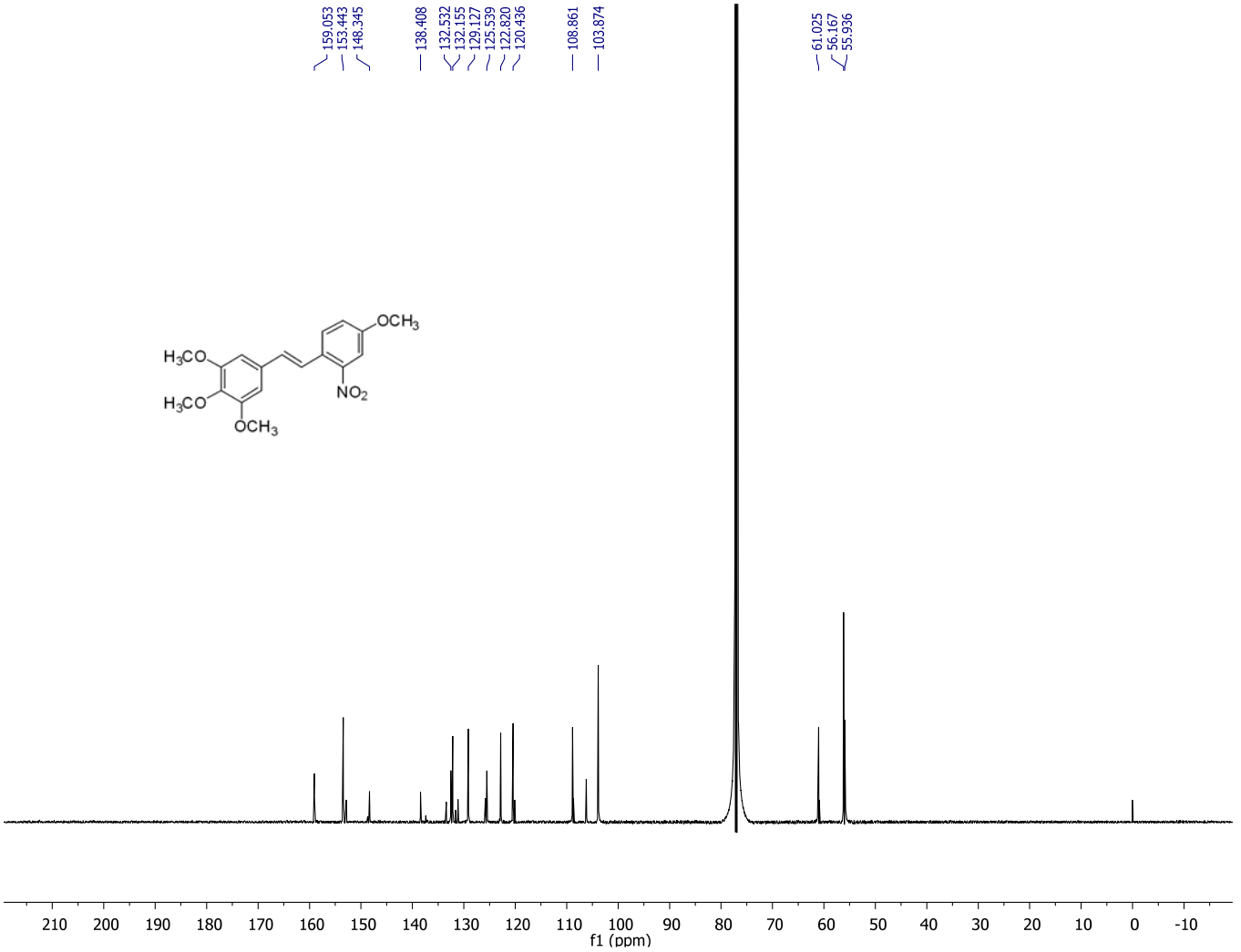
^{13}C NMR ($CDCl_3$, 125 MHz) Compound **5a**



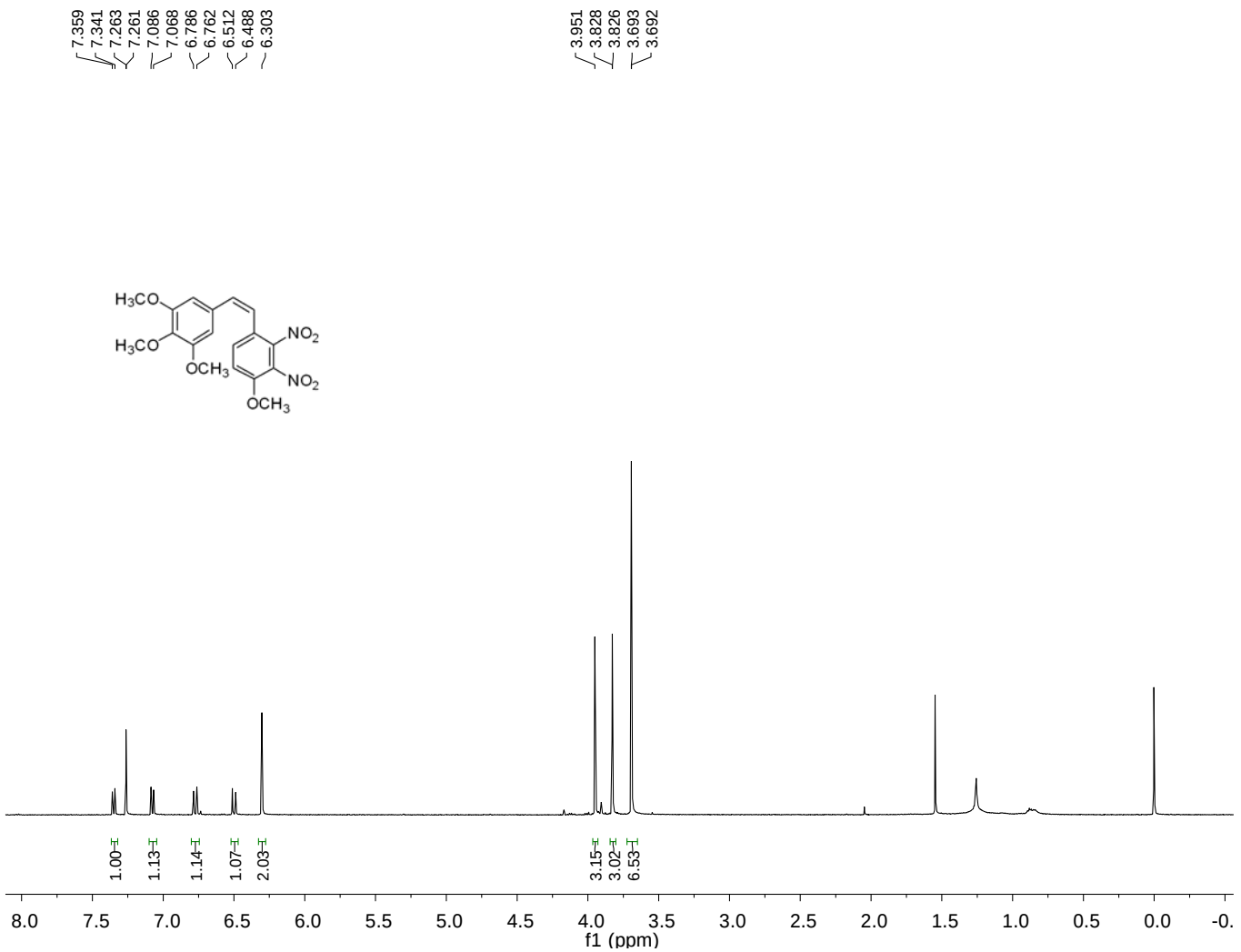
^{1}H NMR ($CDCl_3$, 600 MHz) Compound **5b**



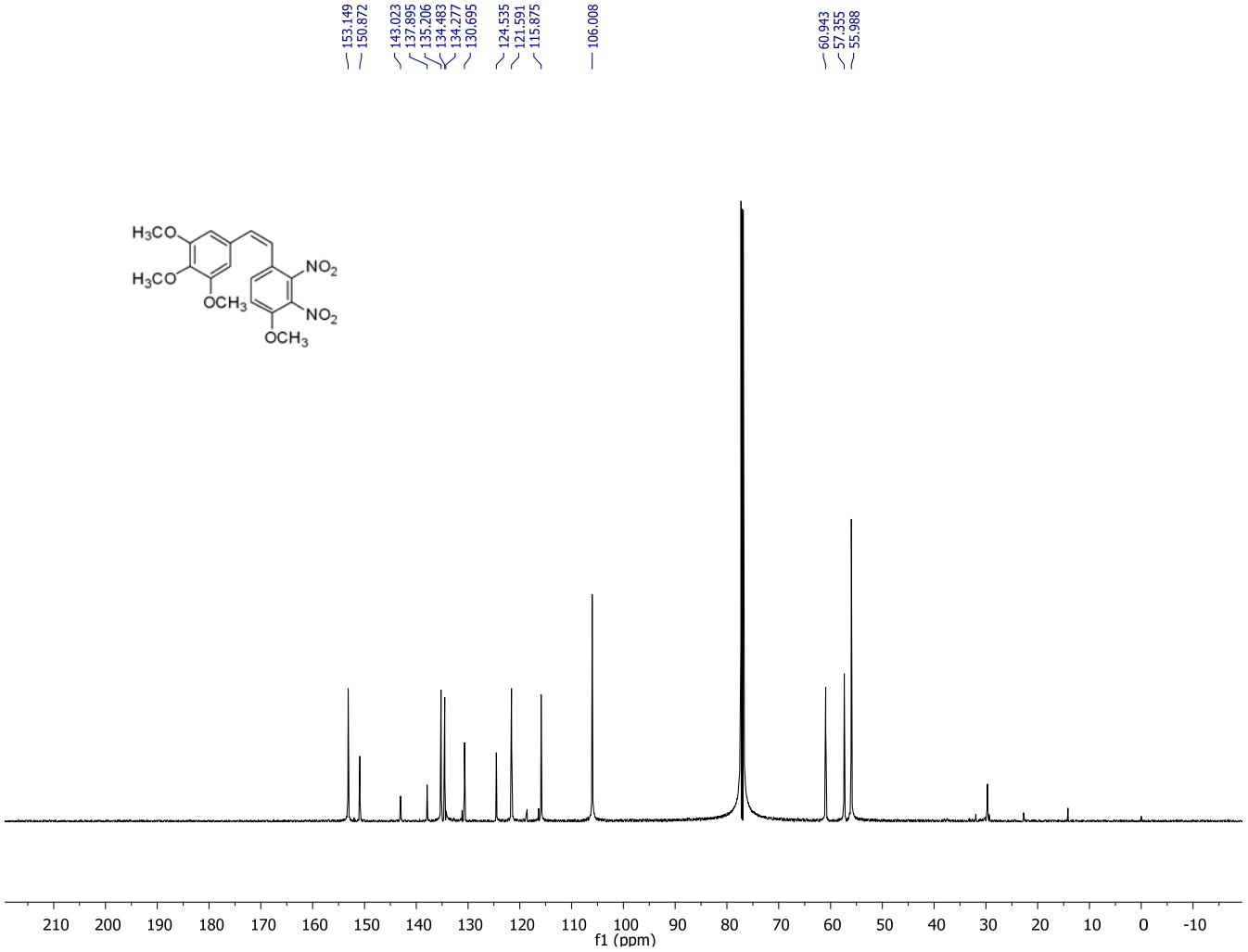
^{13}C NMR ($CDCl_3$, 150 MHz) Compound **5b**



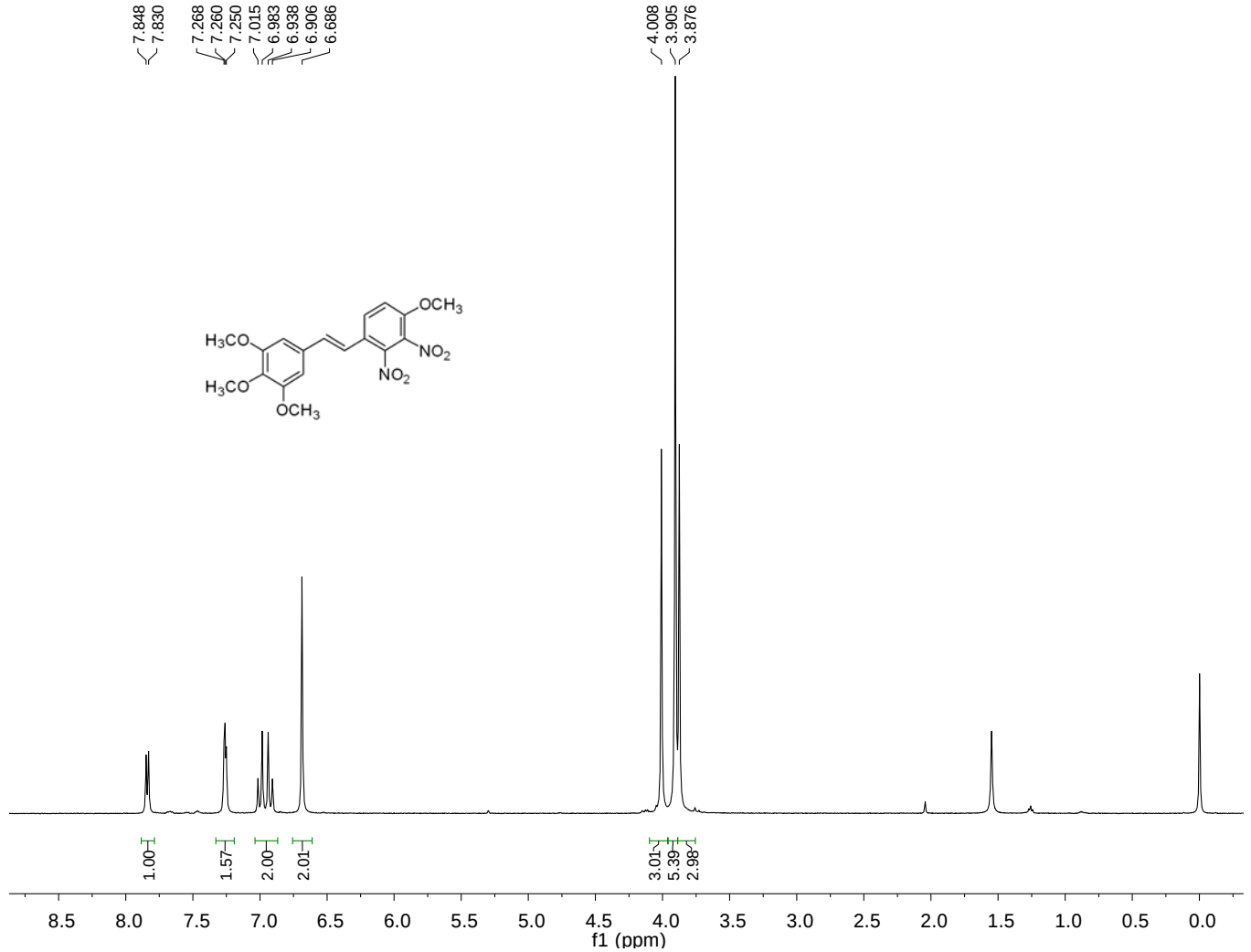
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **6a**



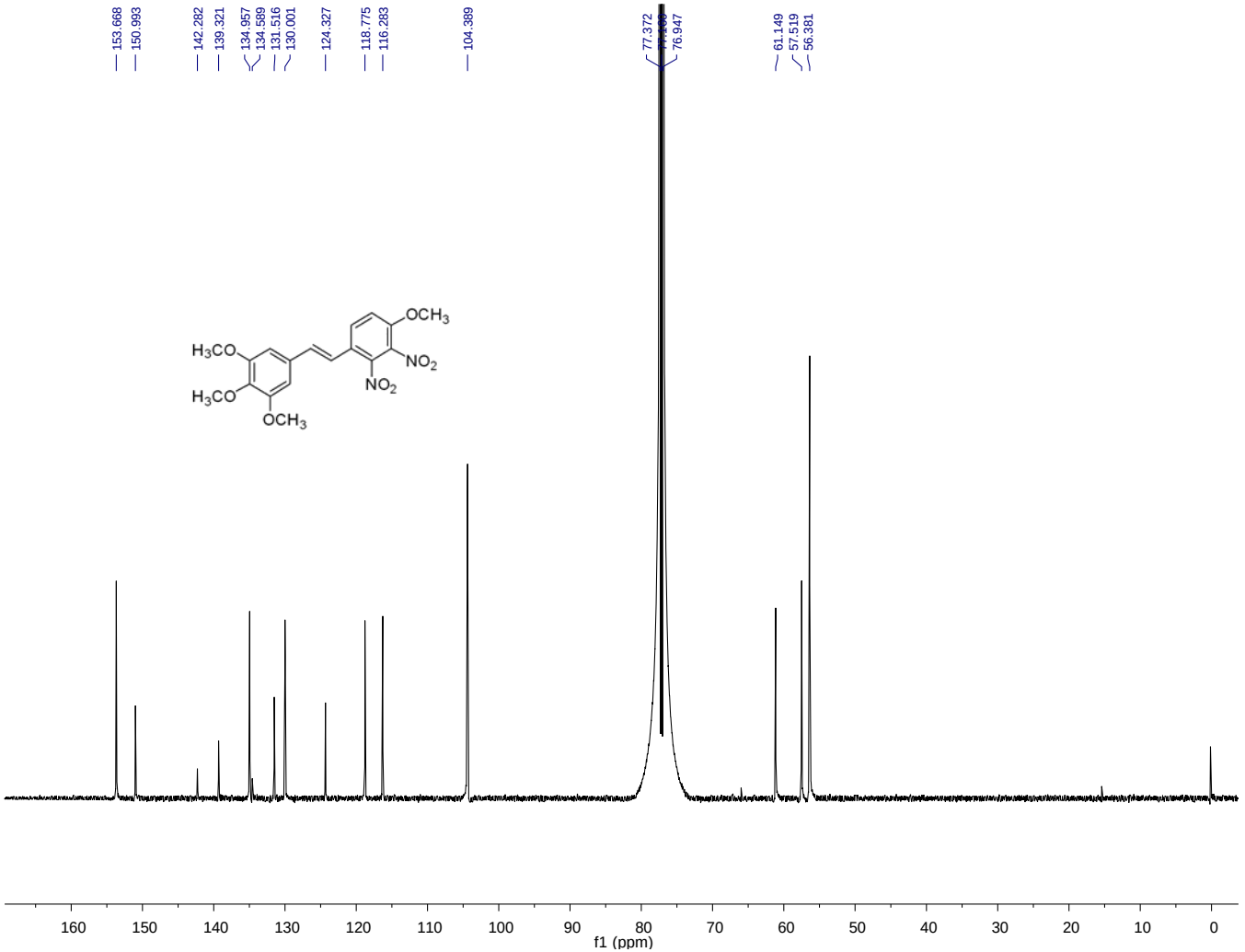
^{13}C NMR ($CDCl_3$, 150 MHz) Compound **6a**



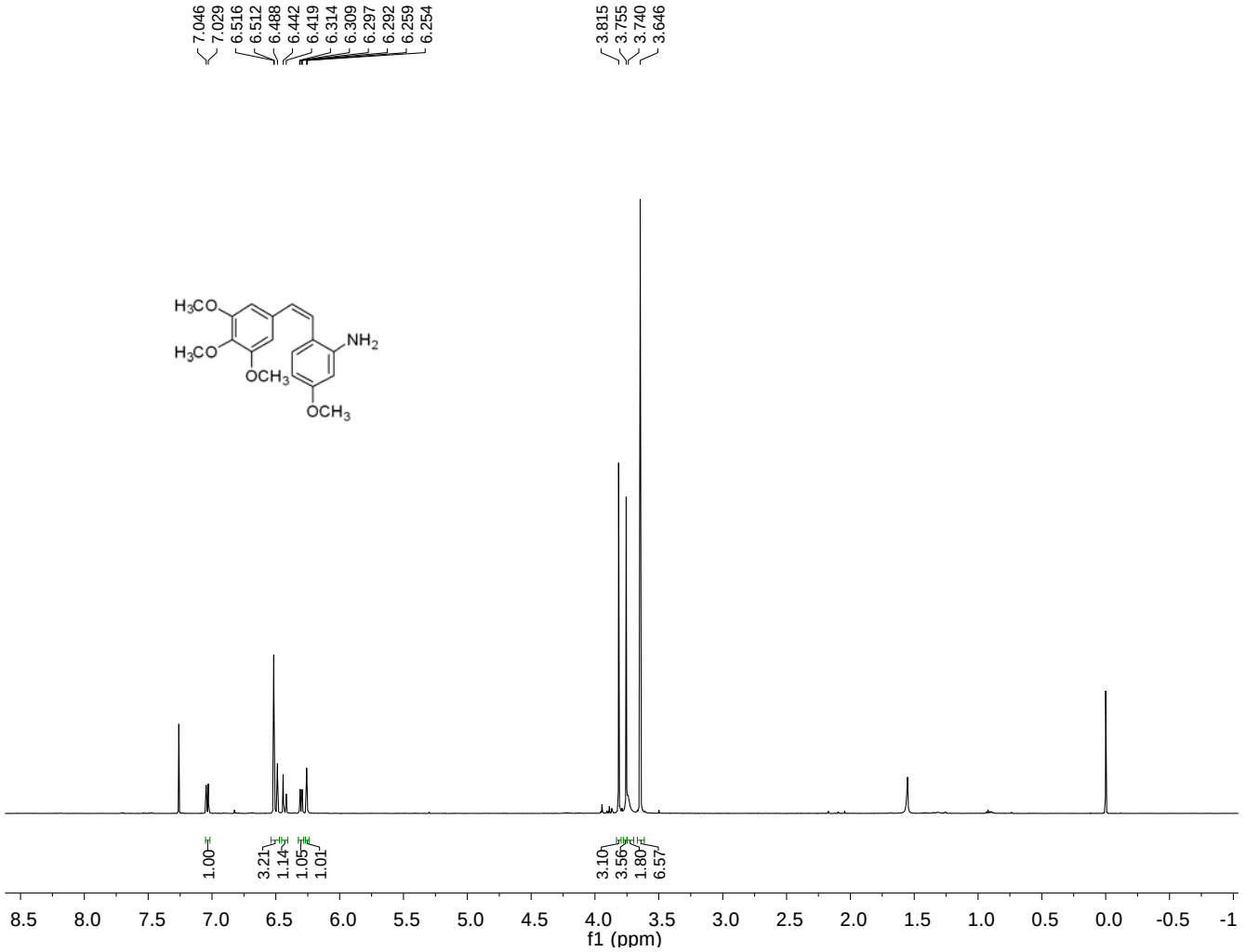
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **6b**



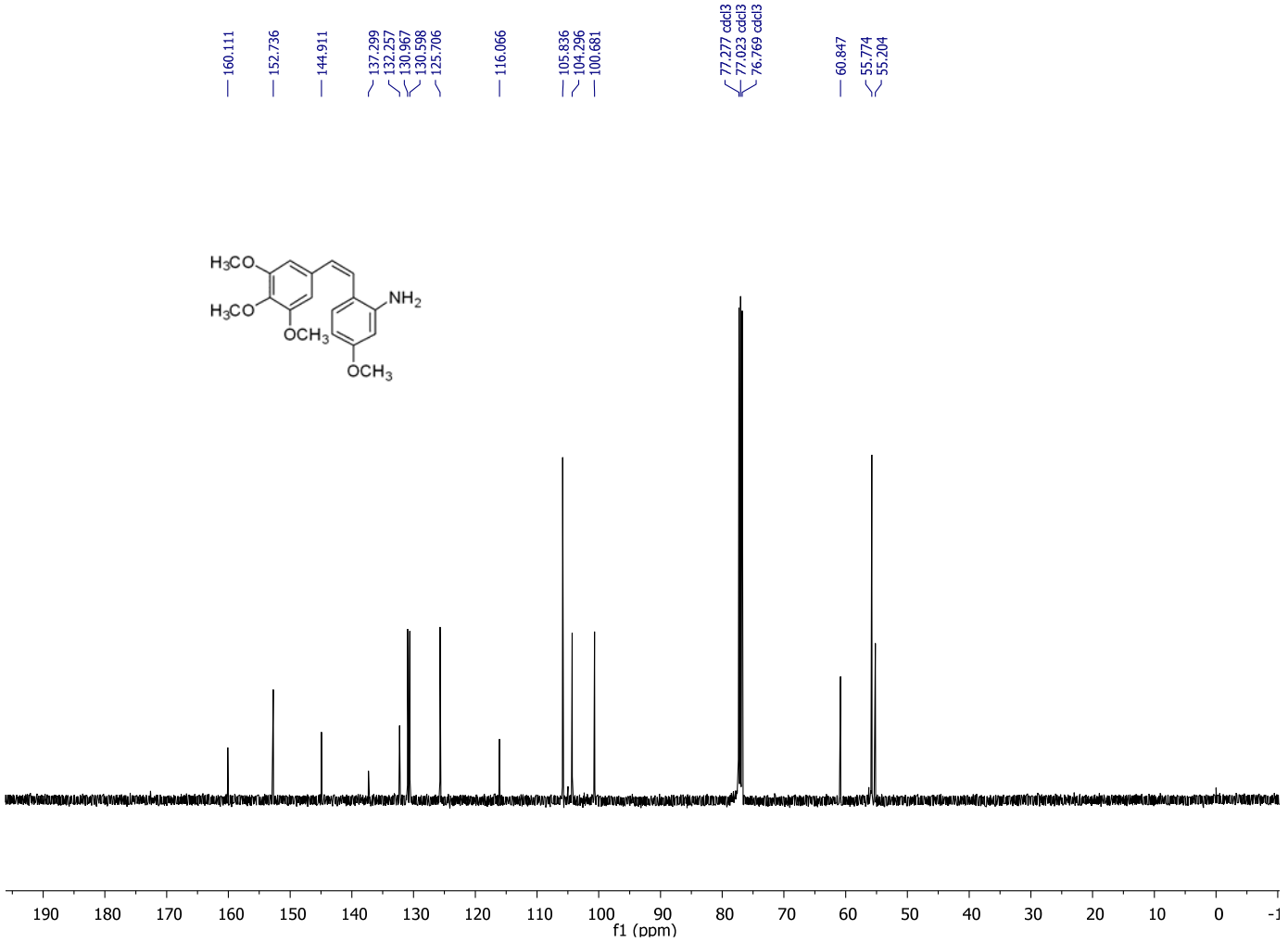
^{13}C NMR ($CDCl_3$, 125 MHz) Compound **6b**



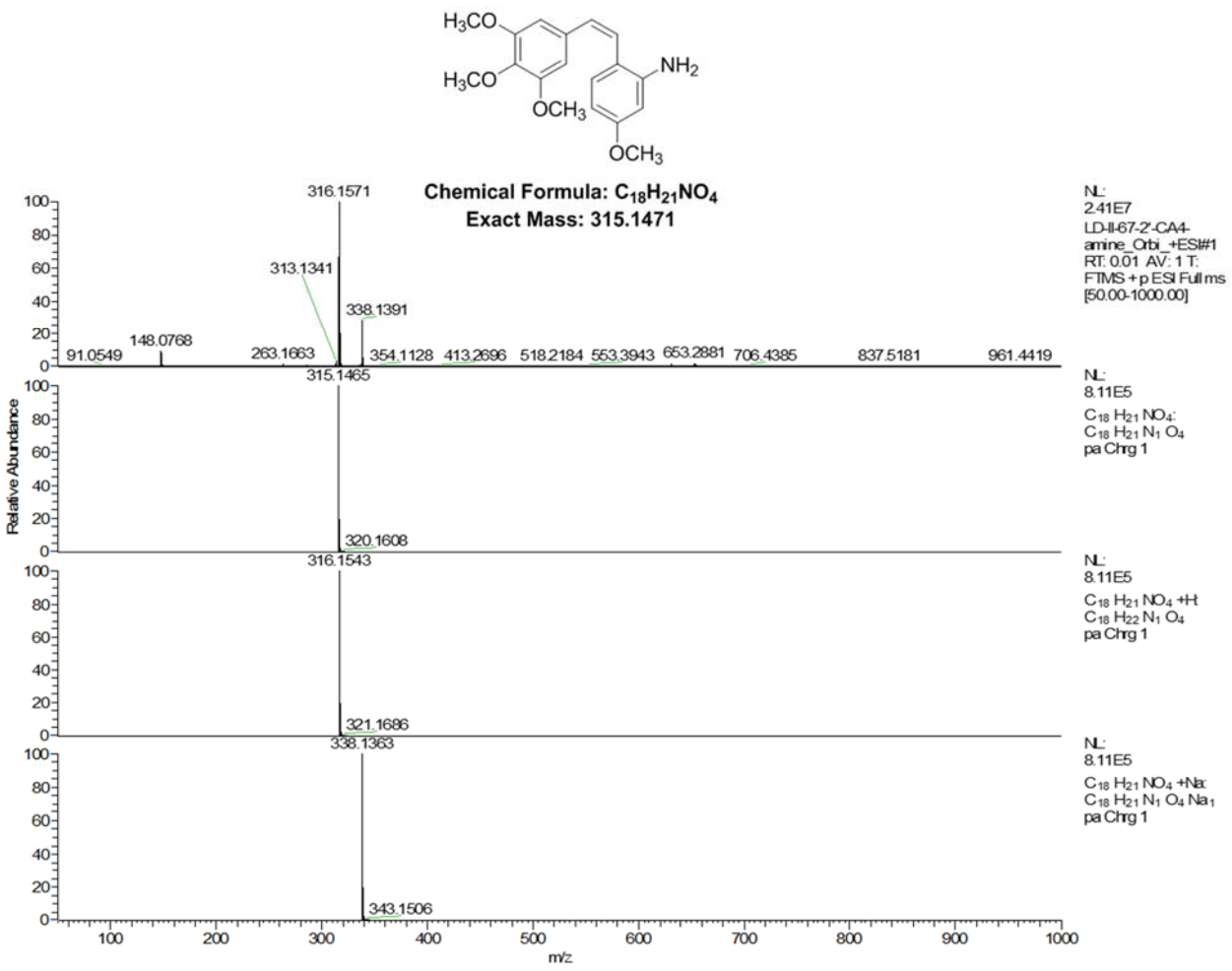
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **7**



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



HRMS Compound **7**



HPLC for Compound **7**

Data File C:\CHEM32\1\DATA\LAXMAN\LDII-67\RUN2\LDII67-RUN00001.D
Sample Name: LD-II-67-1-run2

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Acq. Instrument : Instrument 1                    Location :   -
Injection Date  : 7/3/2013 10:53:44 AM
Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
Last changed    : 7/3/2013 10:38:50 AM by Laxman
Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LDII-67\RUN2\LDII67-RUN00001.D\DA.M (MASTERMETHOD.M)
Last changed    : 7/3/2013 11:43:58 AM by Laxman
Sample Info     :
                  mastermethod
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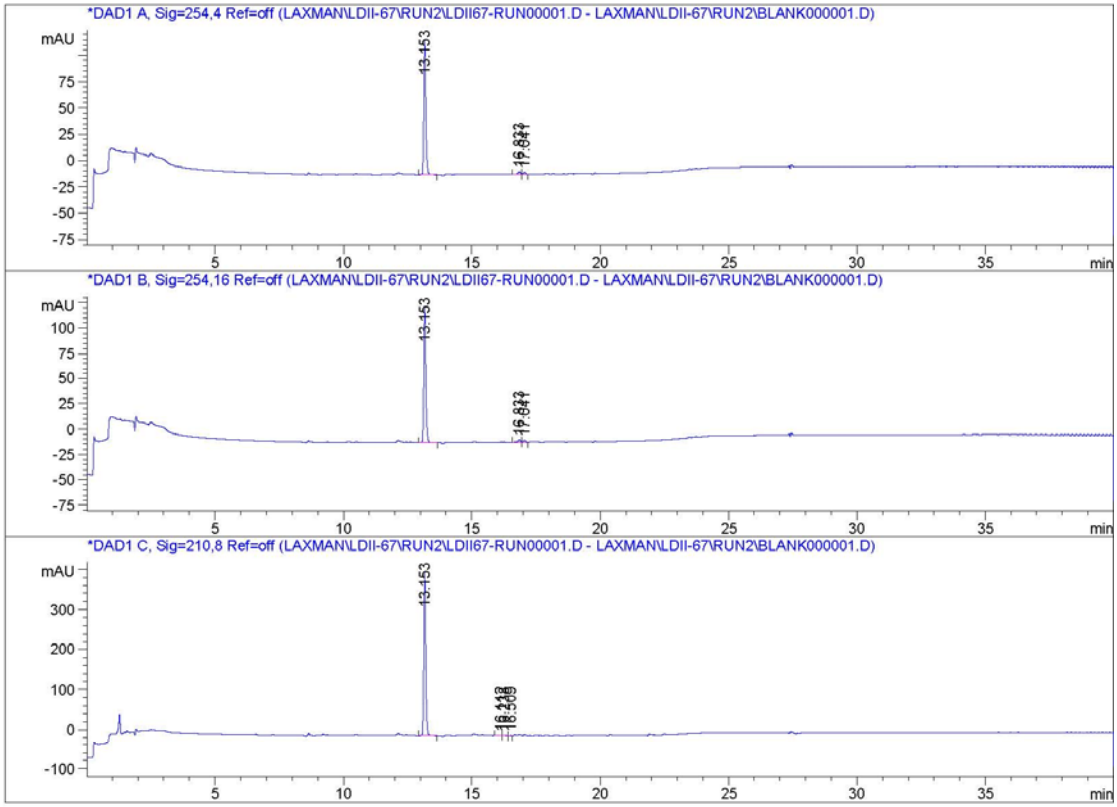
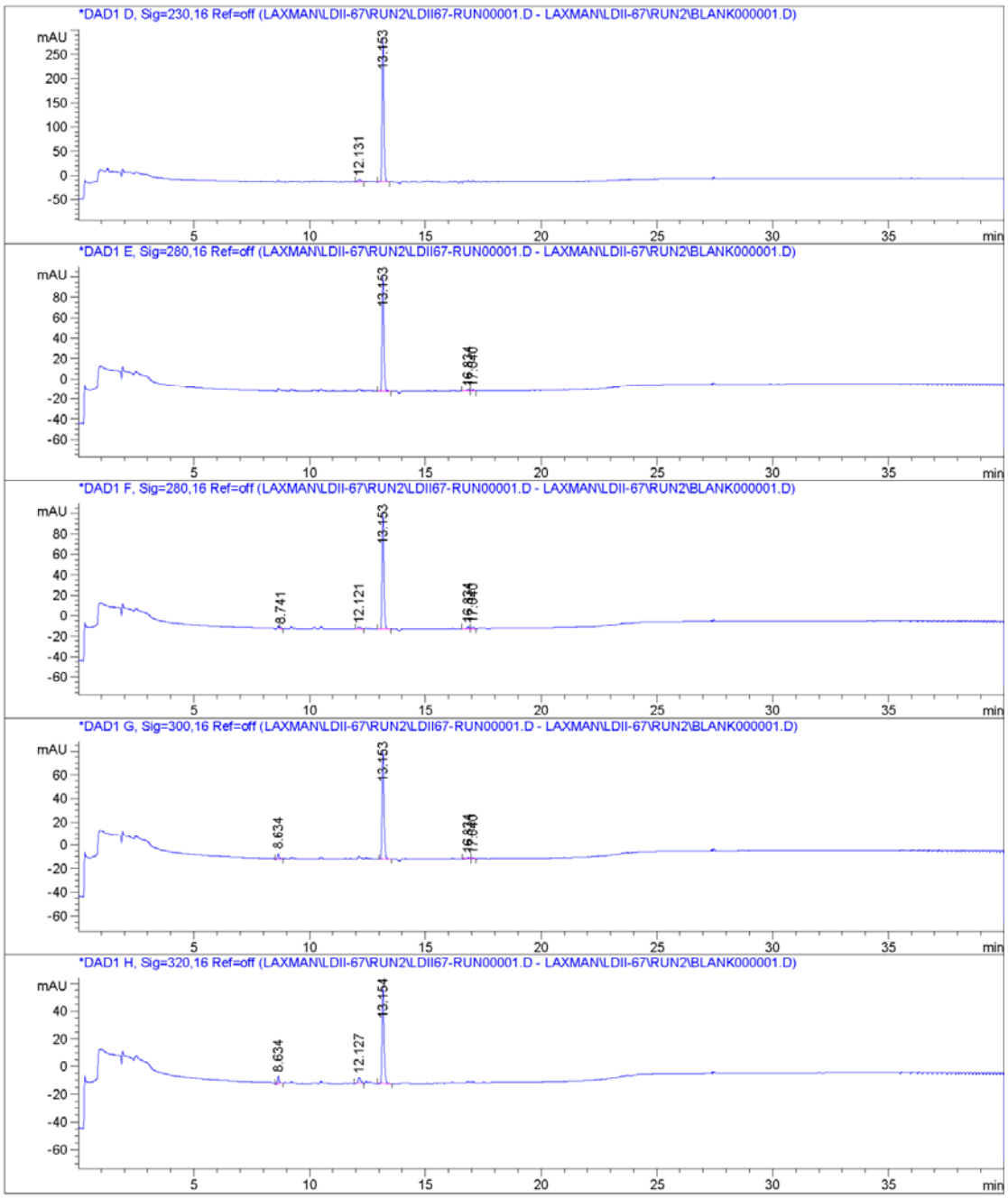


Data File C:\CHEM32\1\DATA\LAXMAN\LDII-67\RUN2\LDII67-RUN00001.D
Sample Name: LD-II-67-1-run2



Instrument 1 7/3/2013 3:03:49 PM Laxman Page 2 of 5

Data File C:\CHEM32\1\DATA\LAXMAN\LDII-67\RUN2\LDII67-RUN00001.D
Sample Name: LD-II-67-1-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
    Signal has been modified after loading from rawdata file!

   Peak RetTime Type  Width     Area      Height     Area
     #   [min]        [min]   [mAU*s]     [mAU]        %
   ----|-------|----|-------|----------|----------|--------|
      1  13.153 BB    0.0801  684.21429  128.44377  95.8285
      2  16.833 BV    0.1042   18.81558    2.54608   2.6352
      3  17.041 VB    0.0833   10.96856    2.02302   1.5362

   Totals :                   713.99843  133.01288



   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  13.153 BB    0.0801  715.88000  134.46823  95.9599
      2  16.833 BV    0.1076   19.28228    2.51098   2.5847
      3  17.041 VB    0.0835   10.85781    1.99546   1.4554

   Totals :                   746.02010  138.97467



   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  13.153 BB    0.0786 2124.96387  408.88397  98.1593
      2  16.112 BV    0.1162   16.52923    1.96462   0.7635
      3  16.238 VB    0.1149   15.90247    1.95409   0.7346
      4  16.509 BB    0.0770    7.41531    1.63260   0.3425

   Totals :                  2164.81088  414.43528
```

Data File C:\CHEM32\1\DATA\LAXMAN\LDII-67\RUN2\LDII67-RUN00001.D
Sample Name: LD-II-67-1-run2

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.131	VV	0.1468	52.76670	4.93102	3.2789
2	13.153	BB	0.0790	1556.49365	297.68036	96.7211
Totals :				1609.26036	302.61138	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.153	BB	0.0796	603.33063	114.21896	97.2505
2	16.834	BV	0.0993	10.60114	1.56055	1.7088
3	17.040	VB	0.0835	6.45647	1.18643	1.0407
Totals :				620.38824	116.96593	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.741	VB	0.0797	5.59504	1.05788	0.8730
2	12.121	BB	0.1405	14.87934	1.48978	2.3218
3	13.153	BB	0.0796	603.33063	114.21896	94.1435
4	16.834	BV	0.0993	10.60114	1.56055	1.6542
5	17.040	VB	0.0835	6.45647	1.18643	1.0075
Totals :				640.86262	119.51359	

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	8.634	BB	0.0769	27.28100	5.22612	4.8981
2	13.153	BB	0.0824	514.18933	93.18947	92.3180
3	16.834	BV	0.1026	9.83546	1.35601	1.7659

Data File C:\CHEM32\1\DATA\LAXMAN\LDII-67\RUN2\LDII67-RUN00001.D
Sample Name: LD-II-67-1-run2

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
4	17.040	VB	0.0835	5.67047	1.04138	1.0181
Totals :				556.97626	100.81297	

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	8.634	BB	0.0773	29.33656	5.58494	6.4560
2	12.127	BB	0.1151	33.01547	4.22876	7.2656
3	13.154	BB	0.0836	392.05457	69.73837	86.2784
Totals :				454.40660	79.55207	

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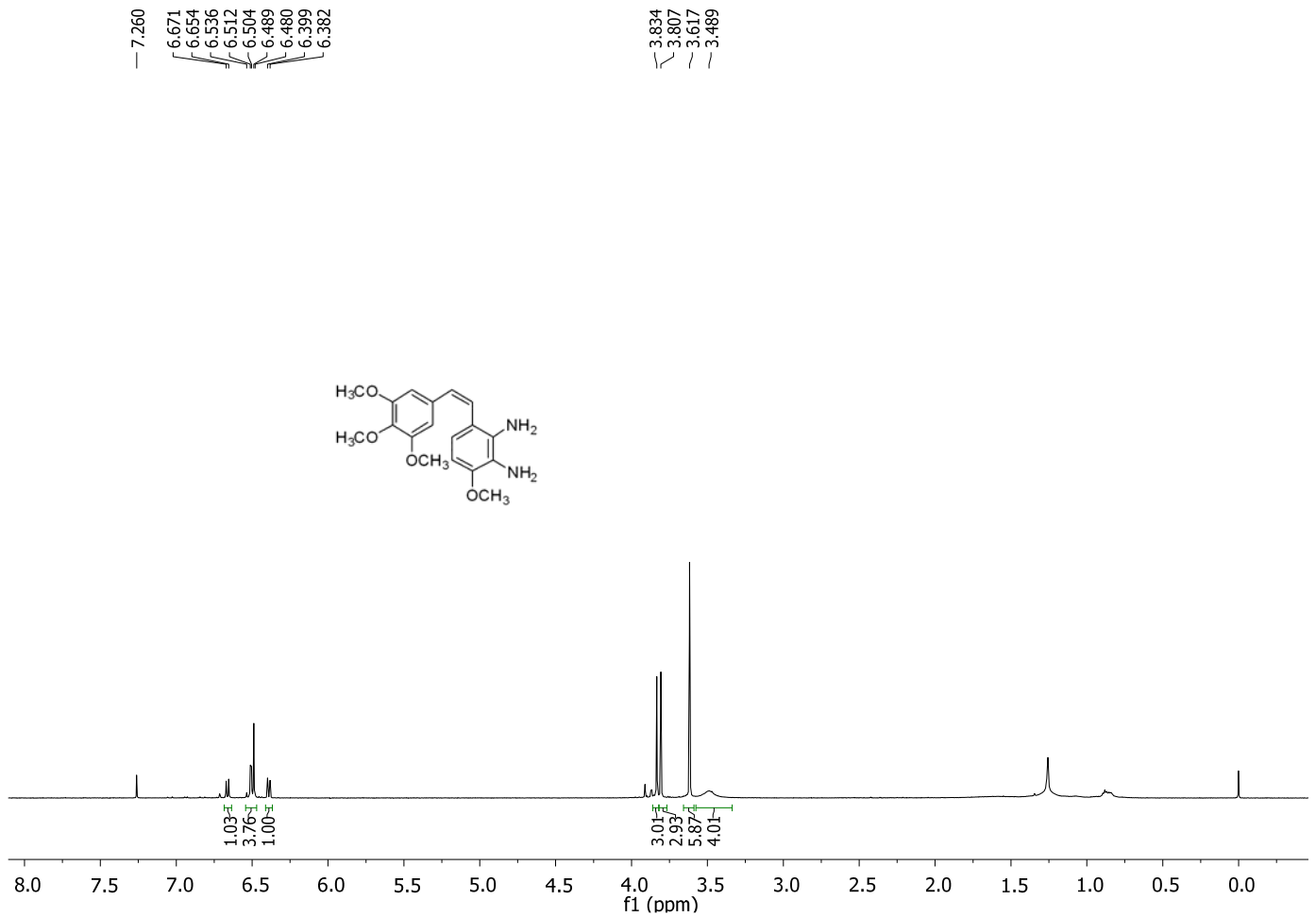
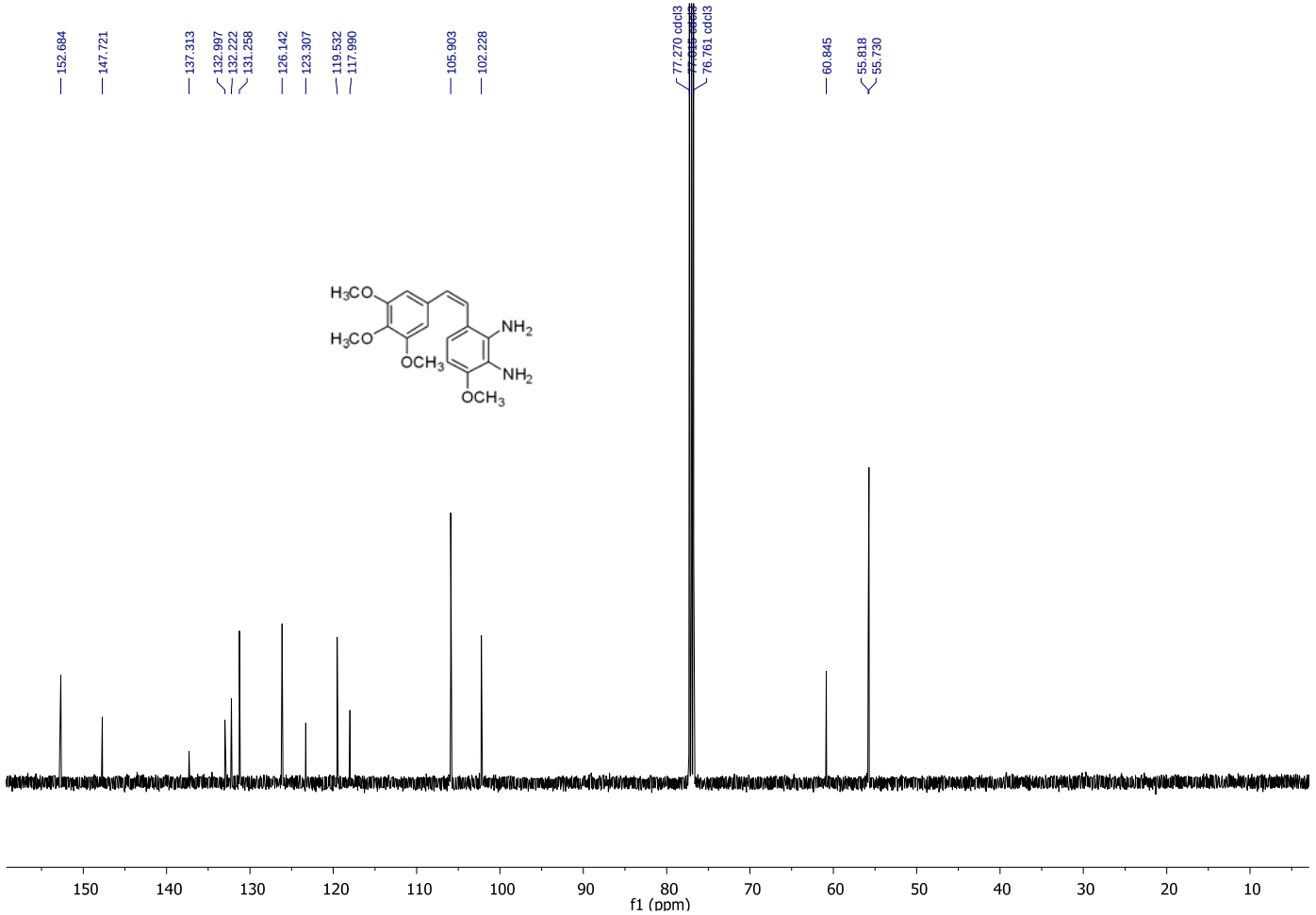
*** End of Report ***

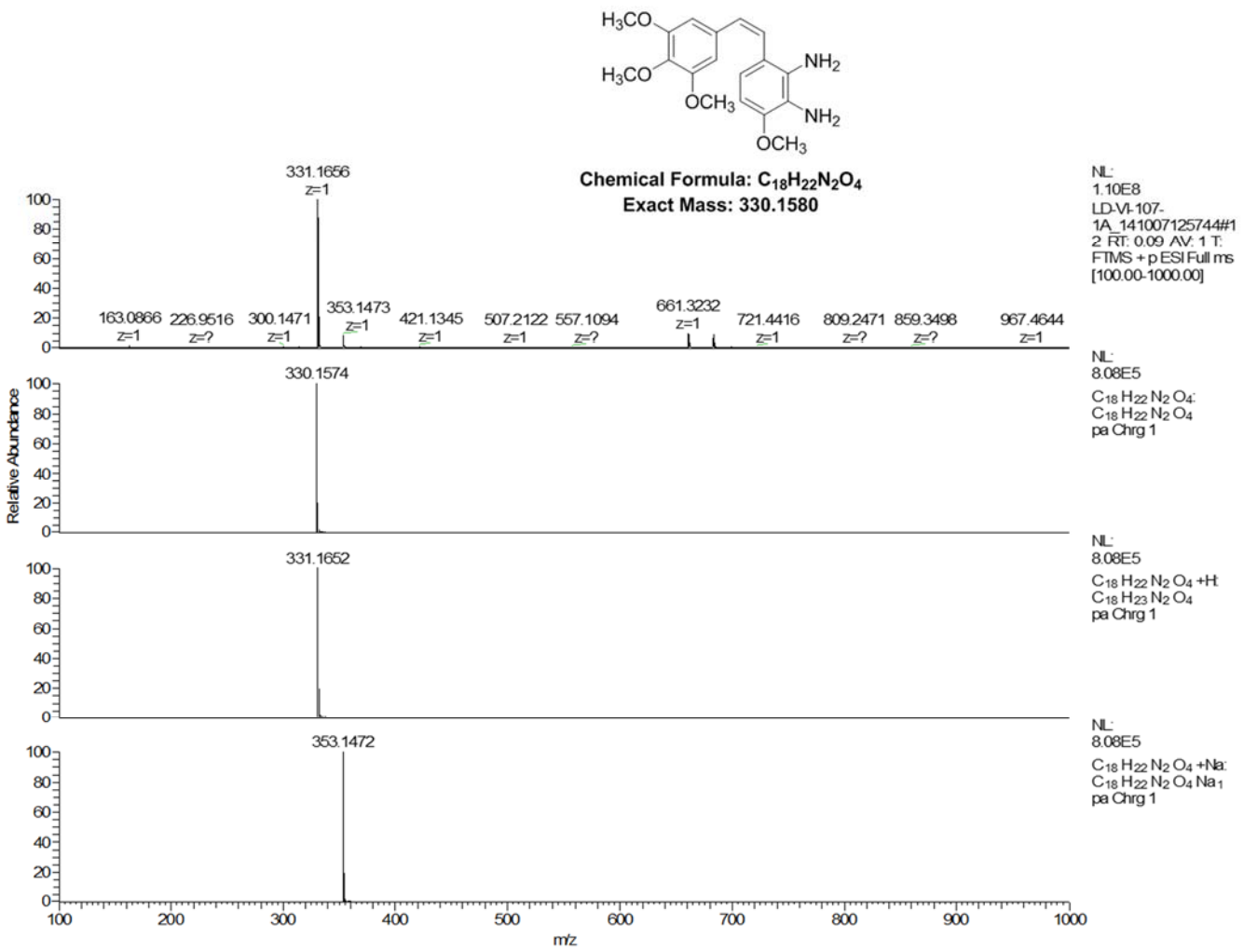
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **8**



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



HRMS Compound **8**



HPLC for Compound **8**

```
Data File C:\CHEM32\1\DATA\LAXMAN\JPT1-24-RUN1001.D
Sample Name: JPT-I-24-2- run1

    ======================================================================
    Acq. Operator   : Laxman
    Acq. Instrument : Instrument 1                    Location :    -
    Injection Date  : 3/8/2013 11:34:37 AM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 3/8/2013 10:58:56 AM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\JPT1-24-RUN1001.D\DA.M (MASTERMETHOD.M)
    Last changed    : 3/8/2013 12:22:23 PM by Laxman
                      (modified after loading)
    Sample Info     : CA1 Diamine
                      Run 1 - 10% ACN/Water
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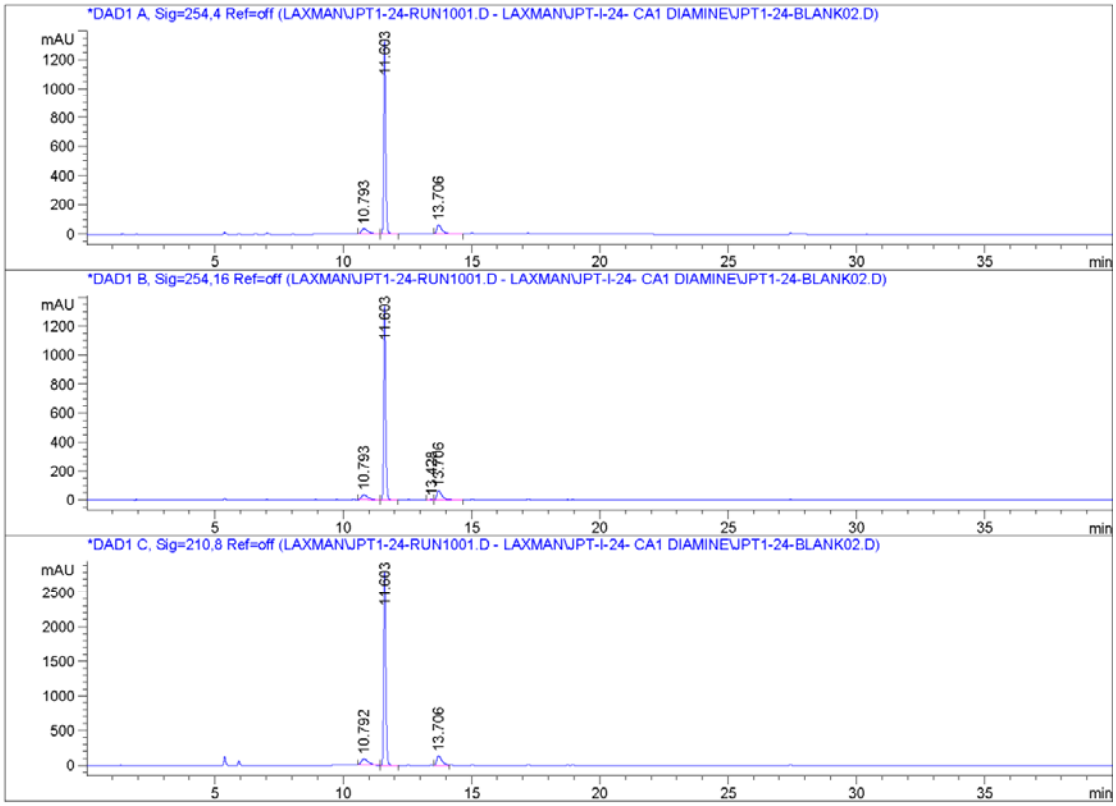
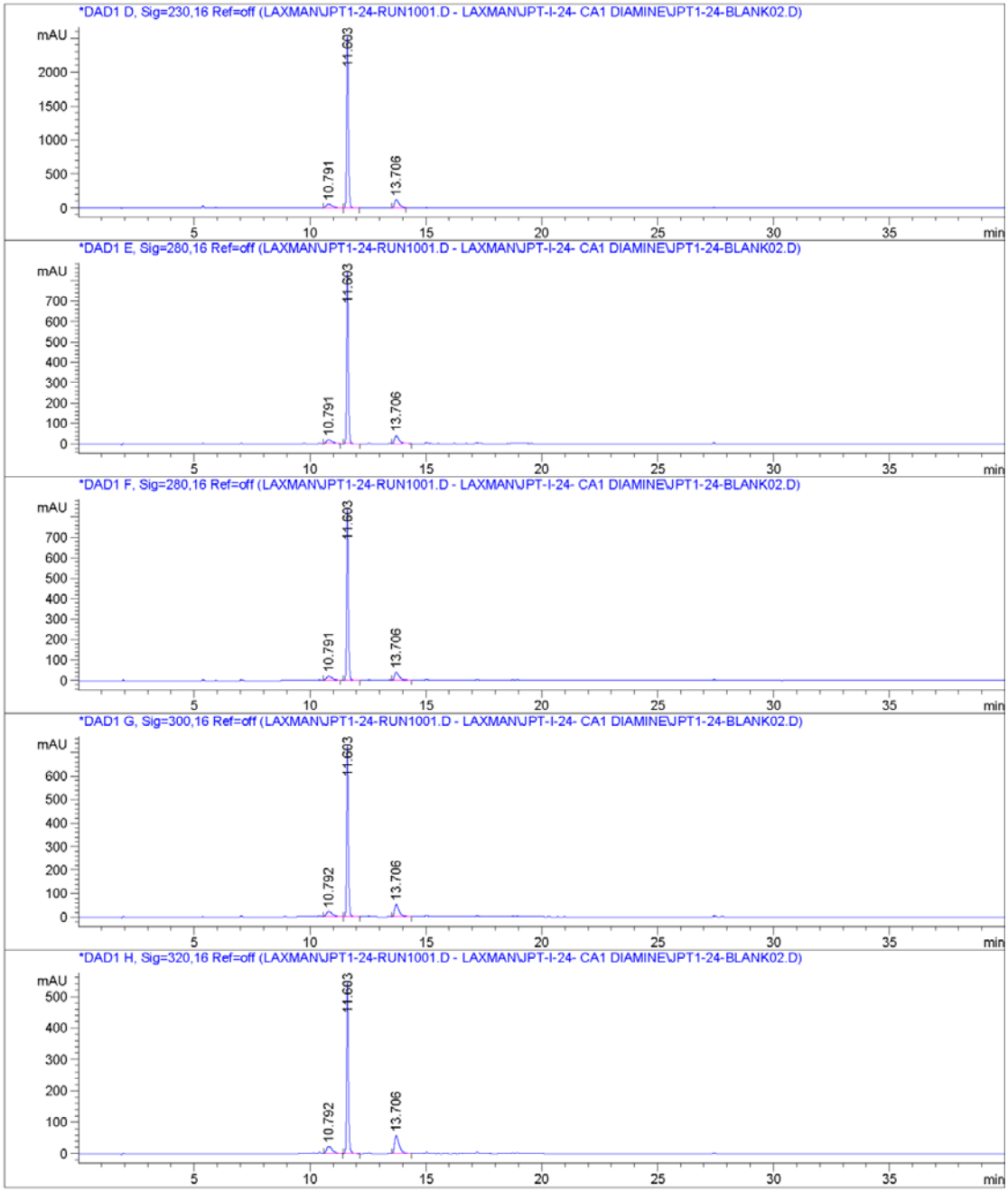


Data File C:\CHEM32\1\DATA\LAXMAN\JPT1-24-RUN1001.D
Sample Name: JPT-I-24-2- run1



Data File C:\CHEM32\1\DATA\LAXMAN\JPT1-24-RUN1001.D
Sample Name: JPT-I-24-2- run1

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.793	VB	0.2658	636.82605	36.46028	7.4422
2	11.603	BB	0.0813	7032.83594	1338.65552	82.1882
3	13.706	VB	0.2133	887.33014	61.89165	10.3697
Totals :				8556.99213	1437.00745	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.793	VB	0.2683	634.71381	36.23970	7.3312
2	11.603	BB	0.0813	7088.87402	1349.12854	81.8791
3	13.428	BV	0.1080	28.47644	3.95348	0.3289
4	13.706	VB	0.2133	905.67200	63.14117	10.4608
Totals :				8657.73626	1452.46289	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.792	BB	0.2721	1686.11475	93.64143	8.4590
2	11.603	BB	0.0918	1.63806e4	2819.08179	82.1797
3	13.706	VV	0.2051	1865.96326	136.85098	9.3613
Totals :				1.99327e4	3049.57420	

Data File C:\CHEM32\1\DATA\LAXMAN\JPT1-24-RUN1001.D
Sample Name: JPT-I-24-2- run1

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.791	VB	0.2794	1063.52295	56.54724	6.3234
2	11.603	BB	0.0863	1.40428e4	2549.48462	83.4944
3	13.706	VV	0.2068	1712.51416	124.25601	10.1821
Totals :				1.68188e4	2730.28787	

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	10.791	VB	0.2803	362.46127	19.91856	6.7384
2	11.603	BB	0.0812	4438.77637	845.72125	82.5199
3	13.706	VB	0.2105	577.80243	40.99035	10.7417
Totals :				5379.04007	906.63016	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.791	VB	0.2803	362.46127	19.91856	6.7384
2	11.603	BB	0.0812	4438.77637	845.72125	82.5199
3	13.706	VB	0.2105	577.80243	40.99035	10.7417
Totals :				5379.04007	906.63016	

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	10.792	VB	0.2793	422.46881	23.11047	8.3678
2	11.603	BB	0.0815	3879.14600	736.06525	76.8335
3	13.706	VB	0.2095	747.15320	53.30036	14.7987

Data File C:\CHEM32\1\DATA\LAXMAN\JPT1-24-RUN1001.D
Sample Name: JPT-I-24-2- run1

Totals : 5048.76801 812.47607

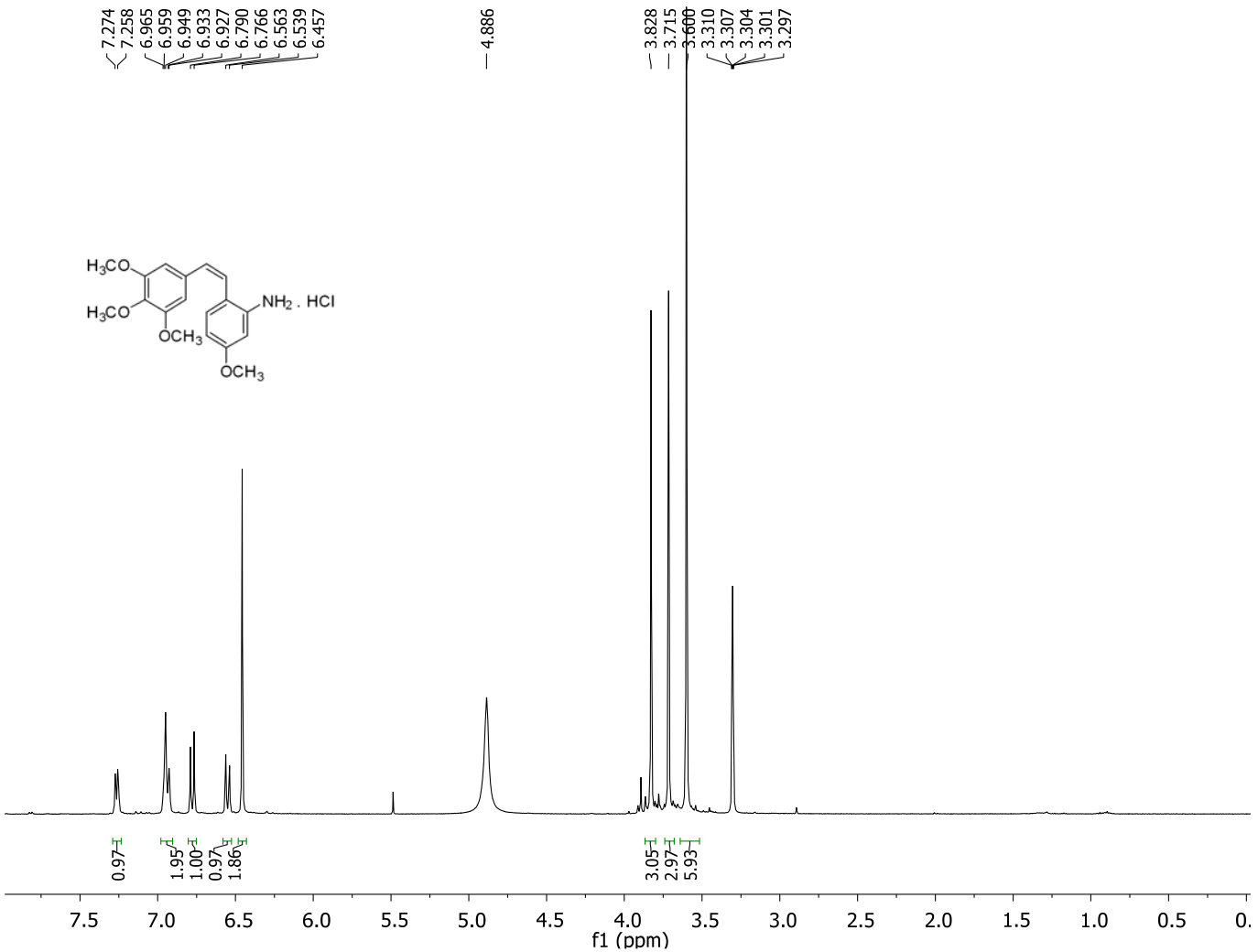
Signal 8: DAD1 H, Sig=320,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.792	VB	0.2743	400.08136	22.62302	9.6661
2	11.603	BB	0.0822	2933.52075	549.82037	70.8749
3	13.706	VB	0.2086	805.40729	57.80400	19.4589

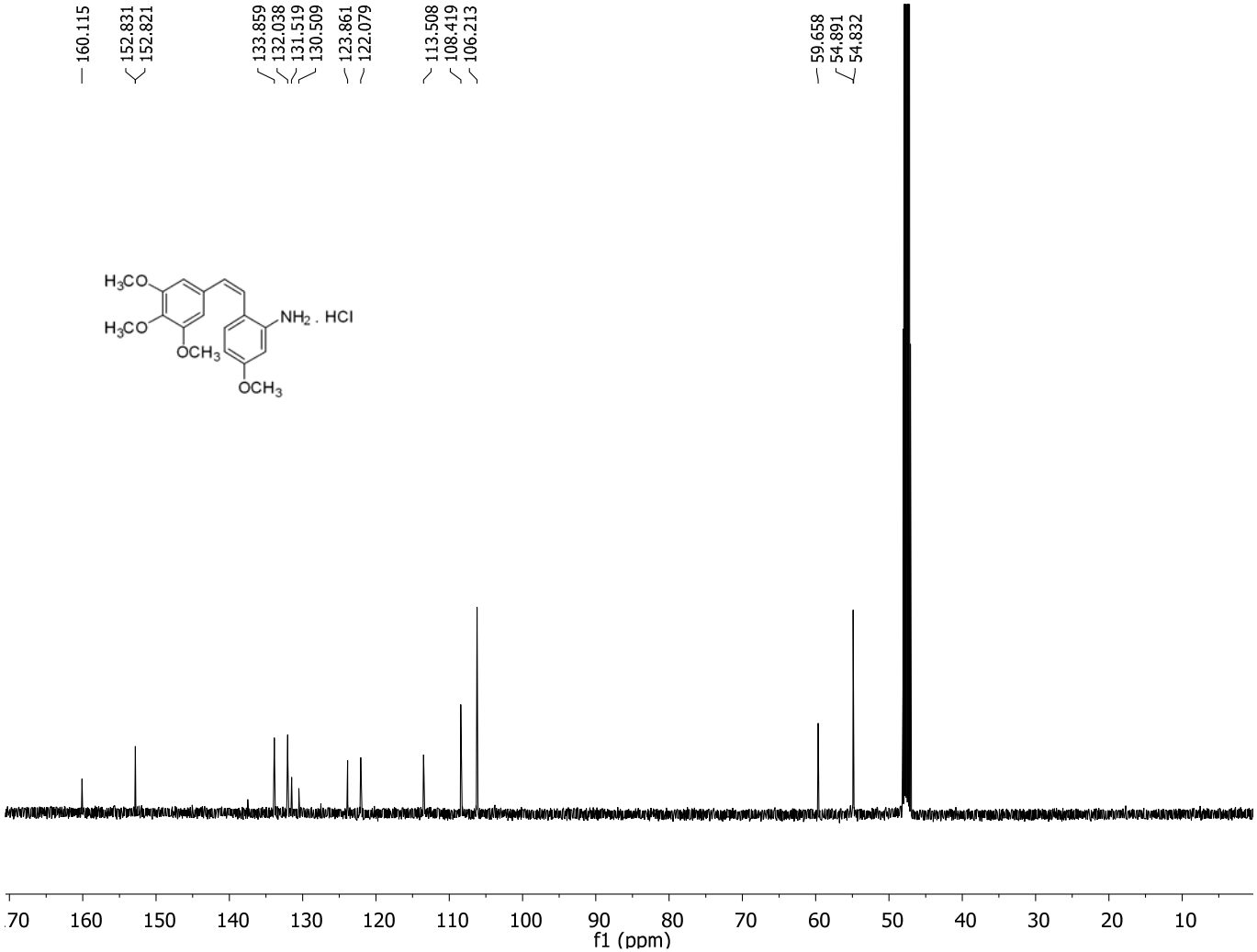
Totals : 4139.00940 630.24739

*** End of Report ***

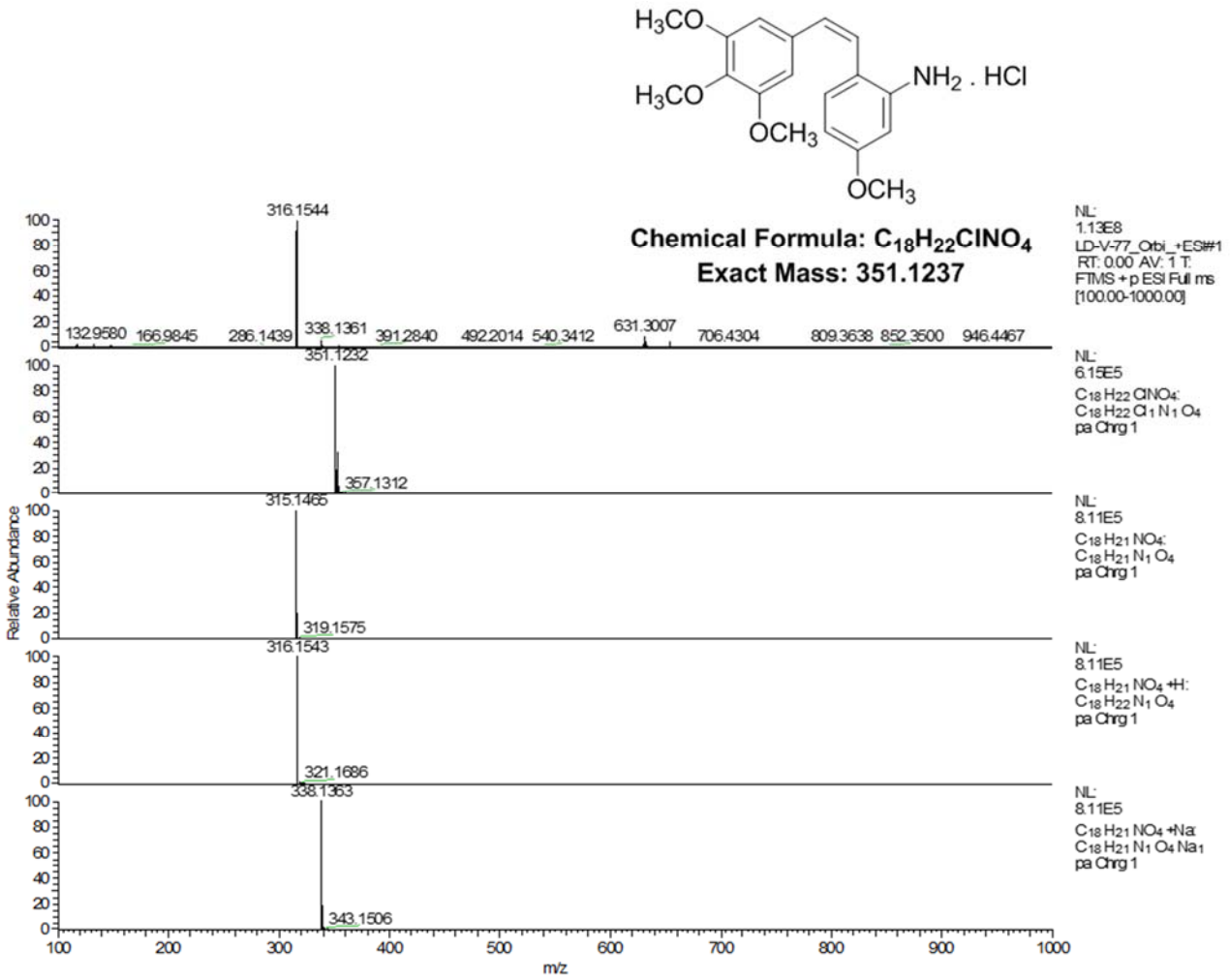
^{1}H NMR (CD_3OD, 500 MHz) Compound **9**



^{13}C NMR (CD_3OD, 125 MHz) Compound **9**



HRMS Compound **9**



HPLC for Compound **9**

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Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-77-RUN0001.D
Sample Name: LD-V-77-1A-run1

    ========================================================================
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    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 7/23/2014 10:58:30 AM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 7/23/2014 10:57:14 AM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-V-77-RUN0001.D\DA.M (MASTERMETHOD.M)
    Last changed    : 7/23/2014 11:48:44 AM by Laxman
    Sample Info     : Method- Mastermethod
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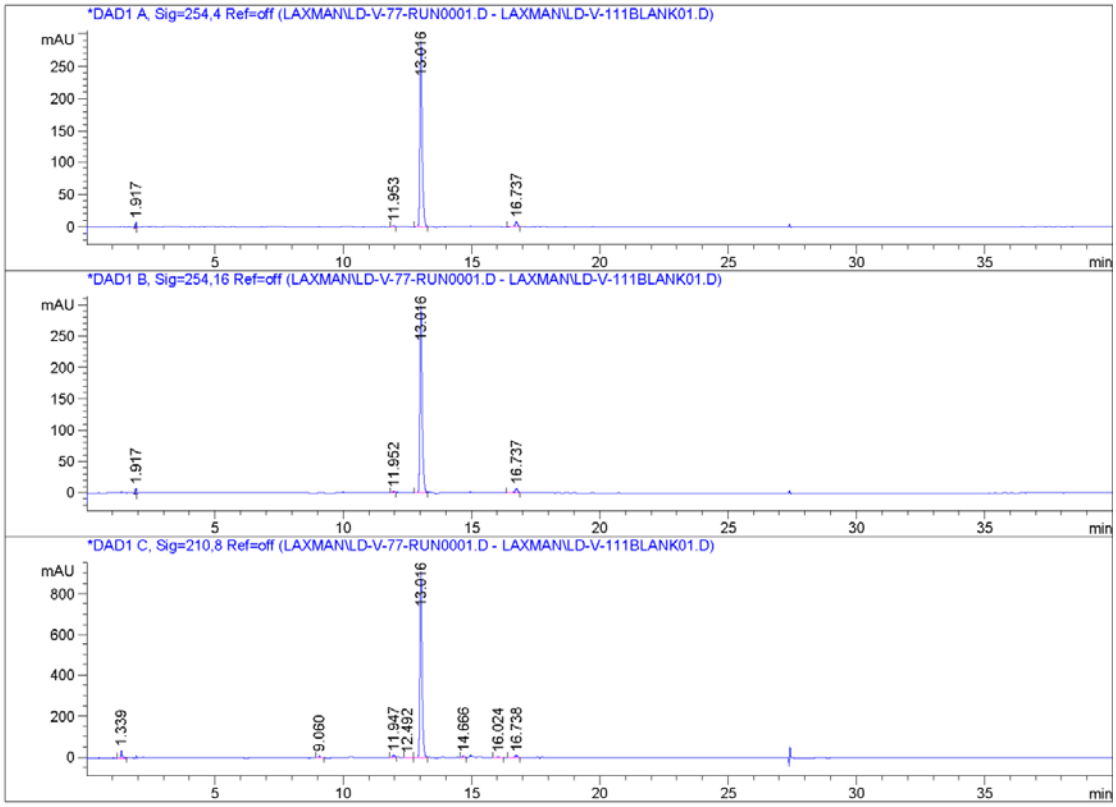
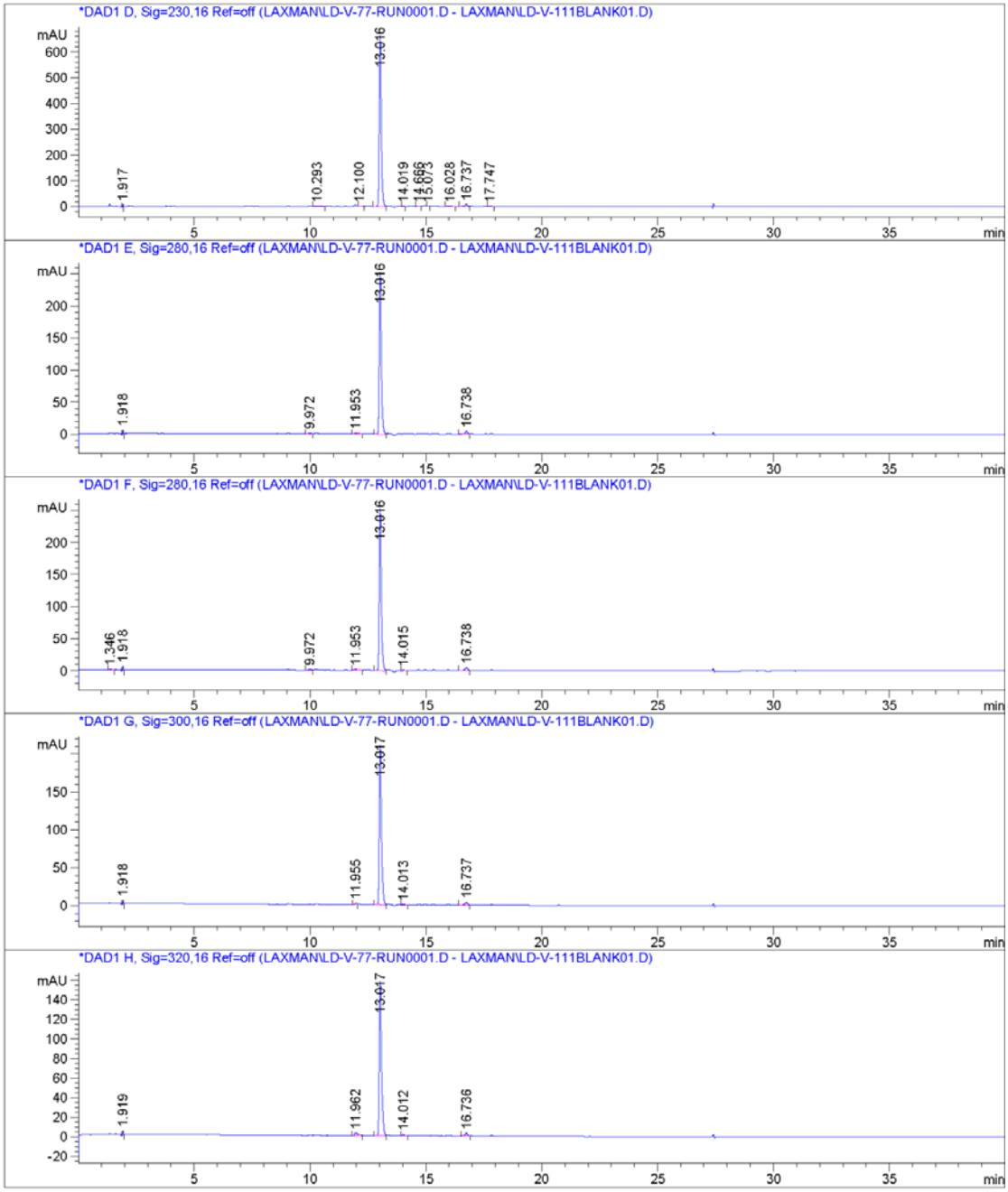


Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-77-RUN0001.D
Sample Name: LD-V-77-1A-run1



Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-77-RUN0001.D
Sample Name: LD-V-77-1A-run1

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.917	BB	0.0398	21.88001	9.19392	1.2022
2	11.953	BB	0.0959	9.57182	1.59724	0.5259
3	13.016	BV	0.0896	1734.00476	290.73328	95.2719
4	16.737	BV	0.1027	54.60278	7.70488	3.0001
Totals :				1820.05937	309.22931	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.917	BB	0.0398	21.54620	9.05059	1.1550
2	11.952	BB	0.0936	10.00687	1.67807	0.5364
3	13.016	BV	0.0894	1780.96240	299.31604	95.4728
4	16.737	BV	0.1033	52.89812	7.40820	2.8357
Totals :				1865.41360	317.45291	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.339	BV	0.0678	178.51530	37.33842	3.1609
2	9.060	VB	0.0861	49.31191	8.45259	0.8731
3	11.947	BV	0.1015	70.69033	10.36870	1.2517
4	12.492	VB	0.1447	22.89972	2.17788	0.4055
5	13.016	BV	0.0865	5184.50049	909.86755	91.8002
6	14.666	BB	0.0940	28.75089	4.79432	0.5091
7	16.024	VB	0.1287	33.65904	3.61362	0.5960

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-77-RUN0001.D
Sample Name: LD-V-77-1A-run1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
8	16.738	BV	0.1192	79.26709	9.51872	1.4036
Totals :				5647.59478	986.13178	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.917	BV	0.0412	27.87641	11.13194	0.6840
2	10.293	VB	0.2294	37.74120	2.37437	0.9261
3	12.100	VB	0.0996	12.79910	1.92545	0.3141
4	13.016	BV	0.0878	3866.90088	665.83099	94.8834
5	14.019	VB	0.0972	7.92666	1.22969	0.1945
6	14.666	BB	0.0922	13.23263	2.19939	0.3247
7	15.073	VB	0.0824	7.13054	1.37812	0.1750
8	16.028	BB	0.1512	16.67805	1.46031	0.4092
9	16.737	BV	0.1155	71.10069	8.87124	1.7446
10	17.747	BB	0.1336	14.03684	1.44144	0.3444
Totals :				4075.42300	697.84294	

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	1.918	BB	0.0398	16.67188	6.99573	1.0558
2	9.972	BV	0.1246	10.85515	1.31168	0.6874
3	11.953	BB	0.1431	16.43839	1.61048	1.0410
4	13.016	BV	0.0890	1503.59937	254.13380	95.2219
5	16.738	BB	0.1006	31.48283	4.67115	1.9938
Totals :				1579.04762	268.72284	

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

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-77-RUN0001.D
Sample Name: LD-V-77-1A-run1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.346	BV	0.1634	13.47963	1.03886	0.8410
2	1.918	BB	0.0398	16.67188	6.99573	1.0401
3	9.972	BV	0.1246	10.85515	1.31168	0.6772
4	11.953	BB	0.1431	16.43839	1.61048	1.0256
5	13.016	BV	0.0890	1503.59937	254.13380	93.8061
6	14.015	VB	0.1280	10.35211	1.13919	0.6458
7	16.738	BB	0.1006	31.48283	4.67115	1.9641
Totals :				1602.87935	270.90089	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.918	BB	0.0399	13.10246	5.47127	0.9396
2	11.955	BB	0.0952	8.87040	1.49495	0.6361
3	13.017	BV	0.0933	1331.21448	211.96465	95.4686
4	14.013	VB	0.1117	12.31849	1.60284	0.8834
5	16.737	BB	0.1013	28.89387	4.14858	2.0721
Totals :				1394.39970	224.68228	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.919	BB	0.0399	11.75484	4.92294	1.0894
2	11.962	BB	0.1461	25.13461	2.52625	2.3294
3	13.017	BV	0.0946	1012.13507	158.33987	93.8010
4	14.012	BB	0.0878	7.24201	1.28391	0.6712
5	16.736	BB	0.1061	22.75686	3.08307	2.1090
Totals :				1079.02340	170.15604	

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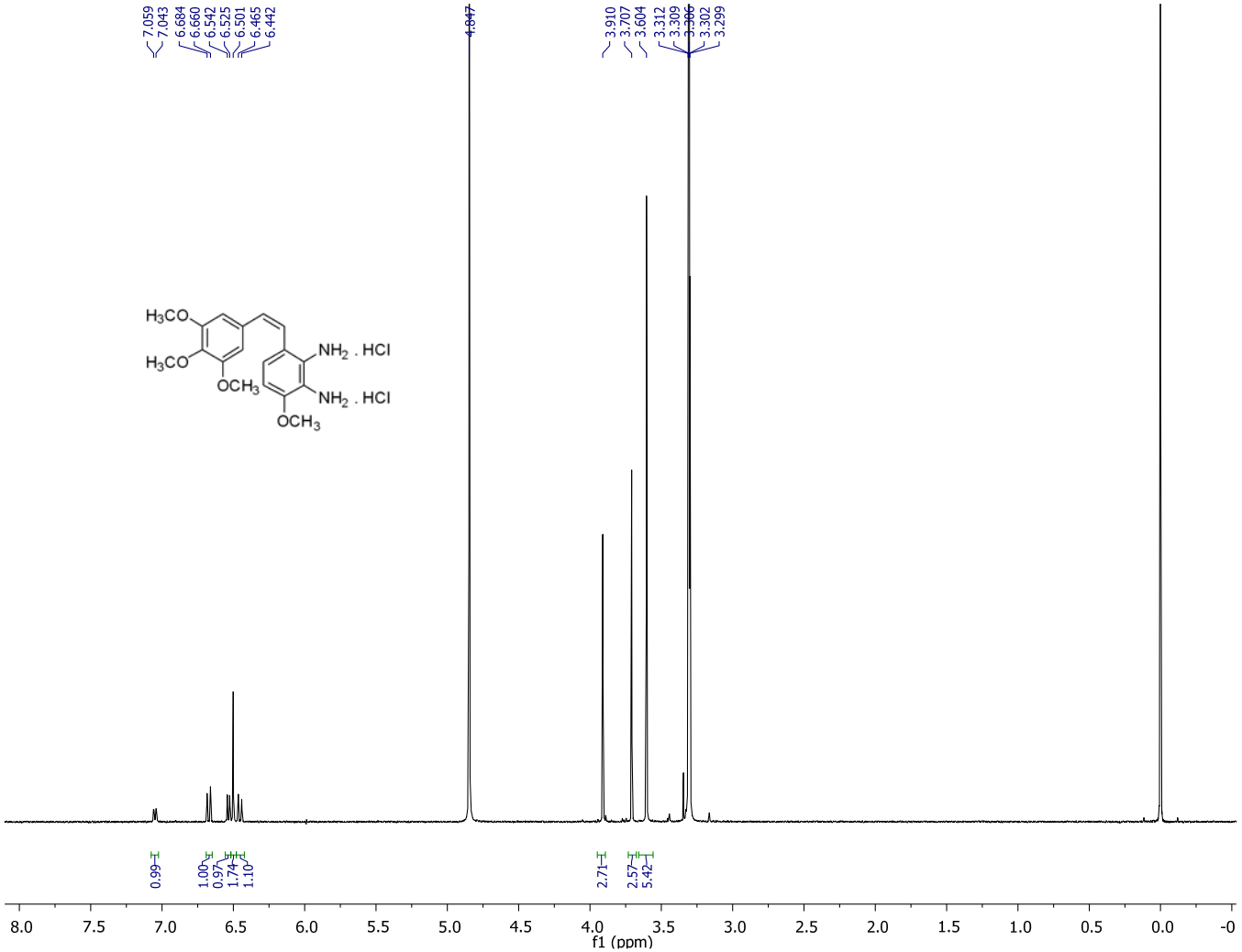
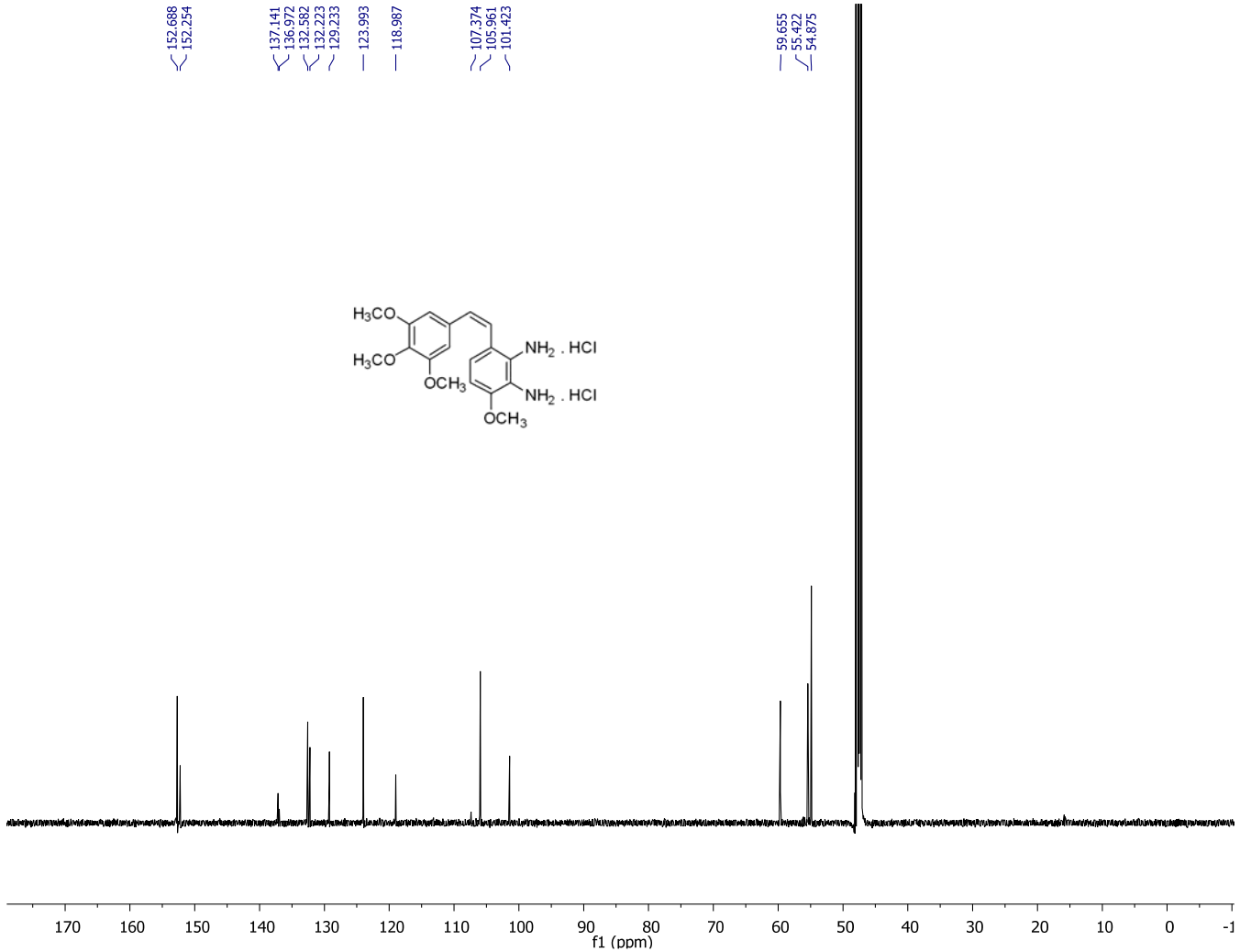
*** End of Report ***

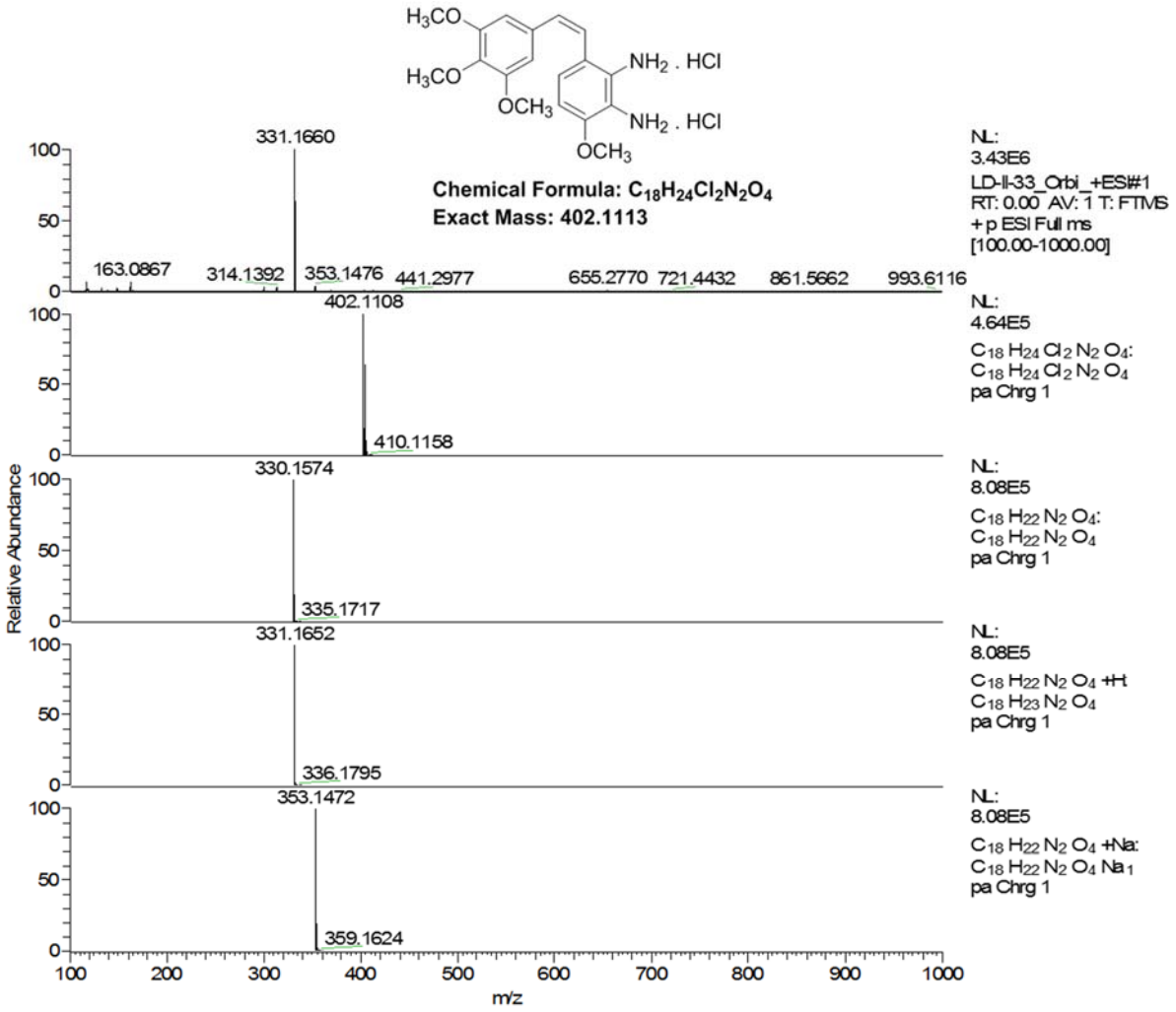
^{1}H NMR (CD_3OD, 600 MHz) Compound **10**



^{13}C NMR (CD_3OD, 150 MHz) Compound **10**



HRMS Compound **10**



HPLC for Compound **10**

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Data File C:\CHEM32\1\DATA\LAXMAN\LD-II-32000001.D
Sample Name: LD-II-32-run1

    ======================================================================
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    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 6/28/2015 4:33:05 PM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD2.M
    Last changed    : 6/28/2015 4:06:36 PM by Eric Lin
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-II-32000001.D\DA.M (MASTERMETHOD2.M)
    Last changed    : 6/28/2015 5:21:03 PM by Laxman
    Sample Info     : Method-Mastermethod2
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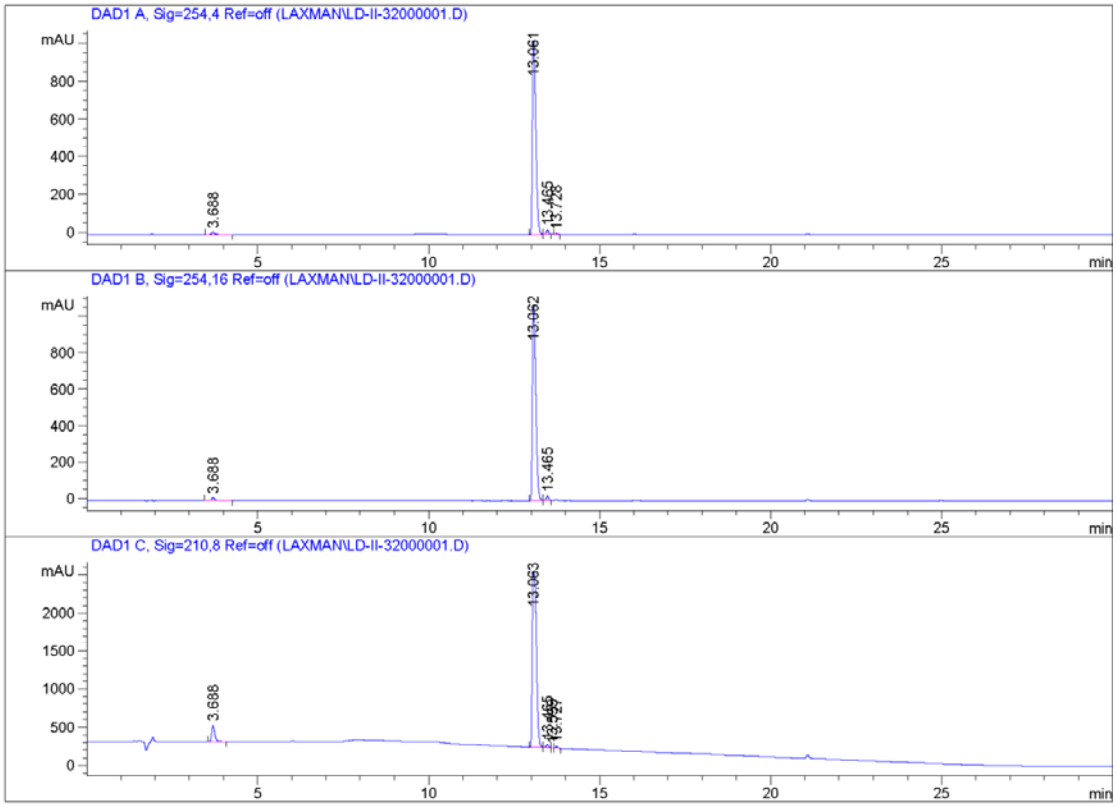
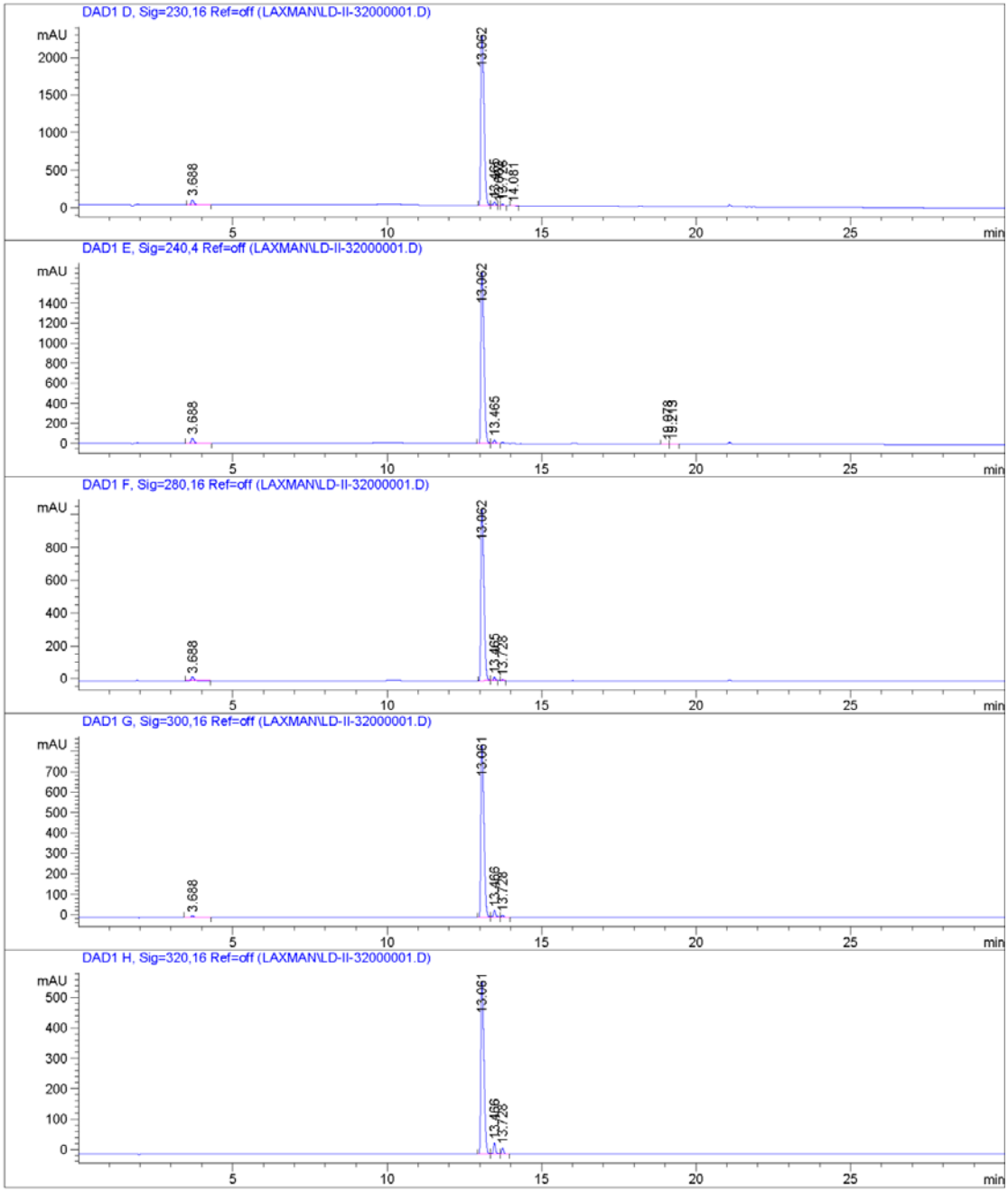


Data File C:\CHEM32\1\DATA\LAXMAN\LD-II-32000001.D
Sample Name: LD-II-32-run1



Data File C:\CHEM32\1\DATA\LAXMAN\LD-II-32000001.D
Sample Name: LD-II-32-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	3.688	BB	0.0941	70.04690	11.33046	0.9650
2	13.061	BV	0.1080	7049.17139	1027.60767	97.1129
3	13.465	VV	0.0763	120.15994	24.05628	1.6554
4	13.728	BB	0.0639	19.36081	4.71832	0.2667
Totals :				7258.73903	1067.71272	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.688	BB	0.0939	90.60506	14.70064	1.1994
2	13.062	BV	0.1080	7342.37061	1070.50842	97.1965
3	13.465	VV	0.0764	121.17745	24.21496	1.6041
Totals :				7554.15312	1109.42402	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.688	BB	0.0958	1287.63904	209.26445	6.1710
2	13.063	BV	0.1355	1.92646e4	2304.68799	92.3255
3	13.465	VV	0.0819	230.33527	42.03444	1.1039
4	13.599	VB	0.0544	11.39503	3.29091	0.0546
5	13.727	BB	0.0648	71.98167	17.20097	0.3450
Totals :				2.08659e4	2576.47876	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-II-32000001.D
Sample Name: LD-II-32-run1

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.688	BB	0.0936	411.70078	67.10372	2.3965
2	13.062	BV	0.1164	1.64493e4	2272.70361	95.7515
3	13.465	VV	0.0778	231.34738	45.13335	1.3467
4	13.602	VB	0.0543	8.60591	2.49010	0.0501
5	13.728	BB	0.0650	70.94254	16.90570	0.4130
6	14.081	BV	0.1019	7.25227	1.00877	0.0422
Totals :				1.71791e4	2405.34525	

Signal 5: DAD1 E, Sig=240,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.688	BB	0.0935	340.03543	55.47417	2.7247
2	13.062	BV	0.1086	1.18929e4	1721.22375	95.2988
3	13.465	VV	0.0842	224.57724	39.56317	1.7996
4	19.078	BV	0.1209	10.11429	1.24328	0.0810
5	19.213	VB	0.1184	11.95831	1.41727	0.0958
Totals :				1.24796e4	1818.92165	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.688	BB	0.0936	149.57484	24.38544	1.9959
2	13.062	BV	0.1079	7213.50439	1053.68127	96.2542
3	13.465	VV	0.0766	108.89276	21.67045	1.4530
4	13.728	BB	0.0643	22.24905	5.37009	0.2969
Totals :				7494.22105	1105.10725	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.688	BB	0.0965	37.61830	6.05143	0.6159
2	13.061	BV	0.1083	5836.52344	848.33795	95.5620

Data File C:\CHEM32\1\DATA\LAXMAN\LD-II-32000001.D
Sample Name: LD-II-32-run1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
3	13.466	VV	0.0789	182.86238	35.00428	2.9940
4	13.728	VB	0.0714	50.57355	10.64560	0.8280
Totals :				6107.57767	900.03926	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.061	BV	0.1086	3937.61230	569.77728	93.4290
2	13.466	VV	0.0762	185.44067	37.14345	4.4000
3	13.728	VB	0.0697	91.49677	19.89258	2.1710
Totals :				4214.54975	626.81331	

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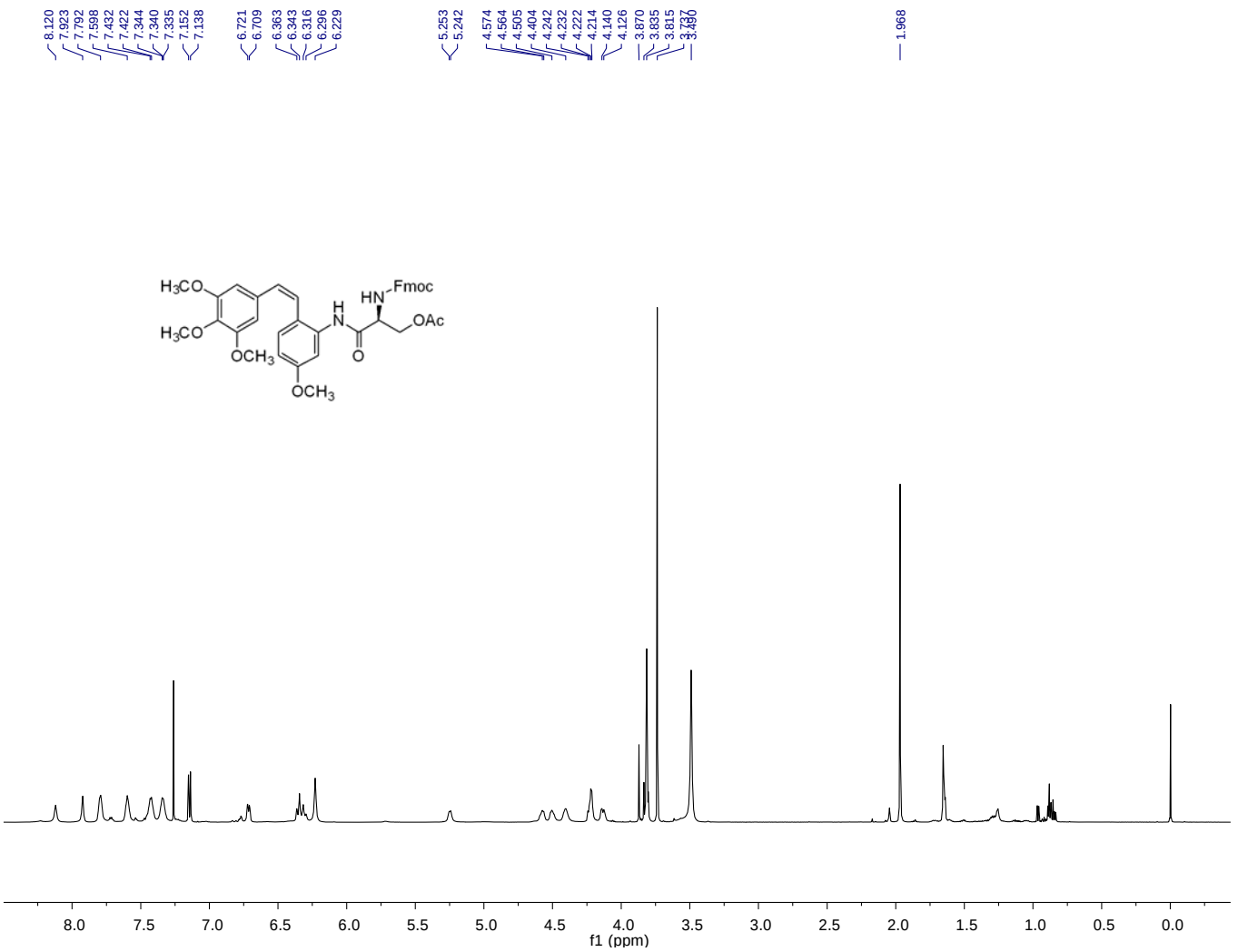
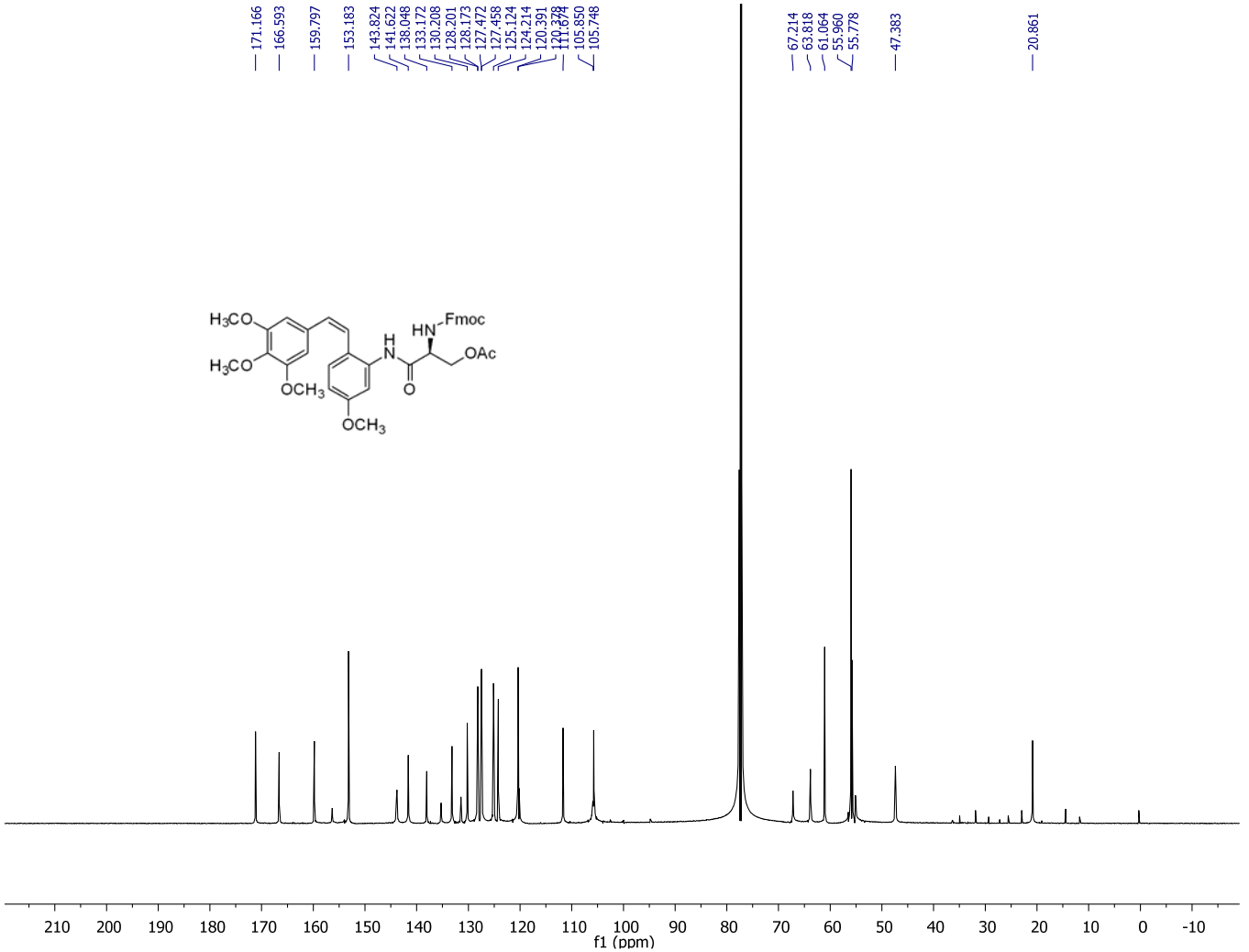
*** End of Report ***

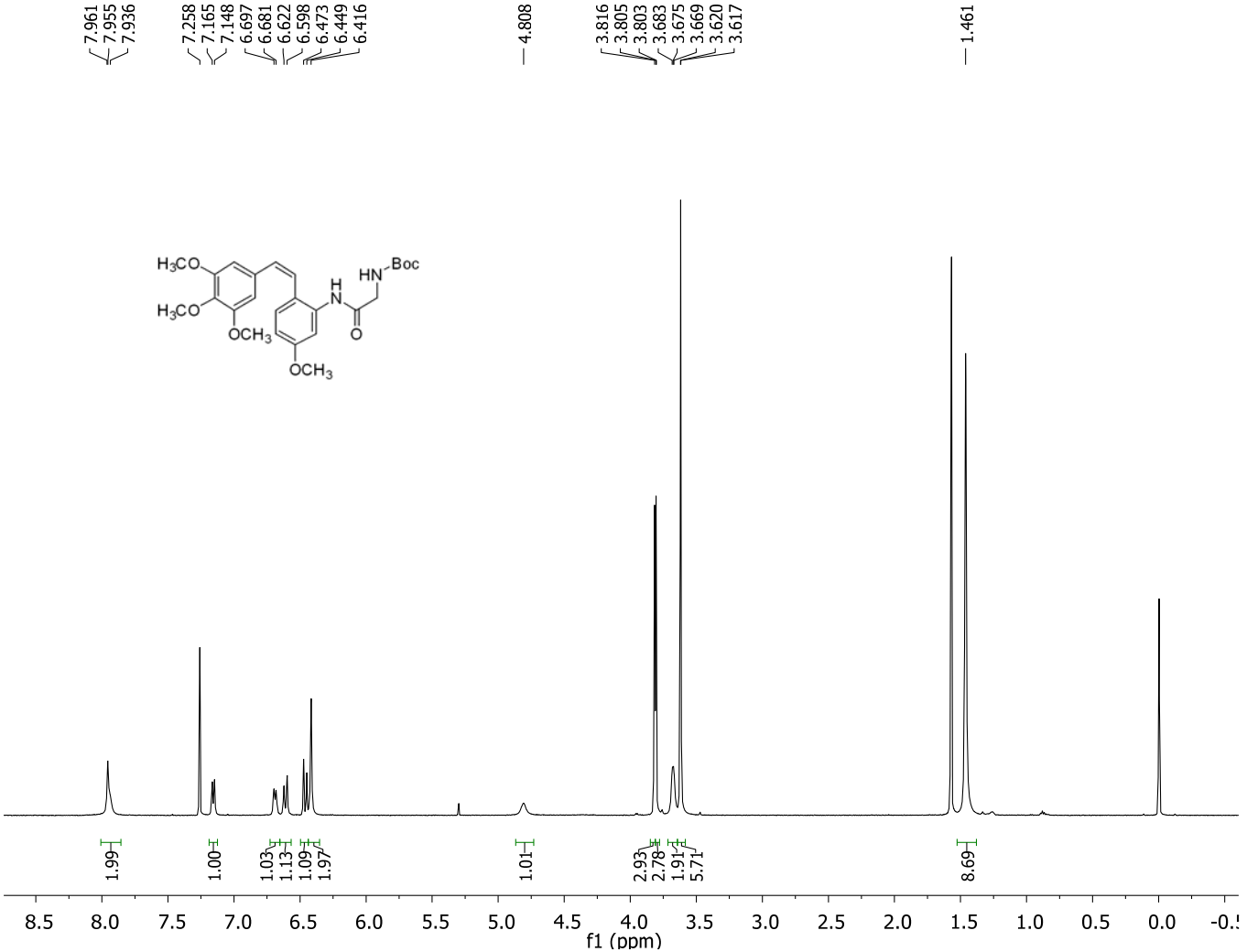
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **11**



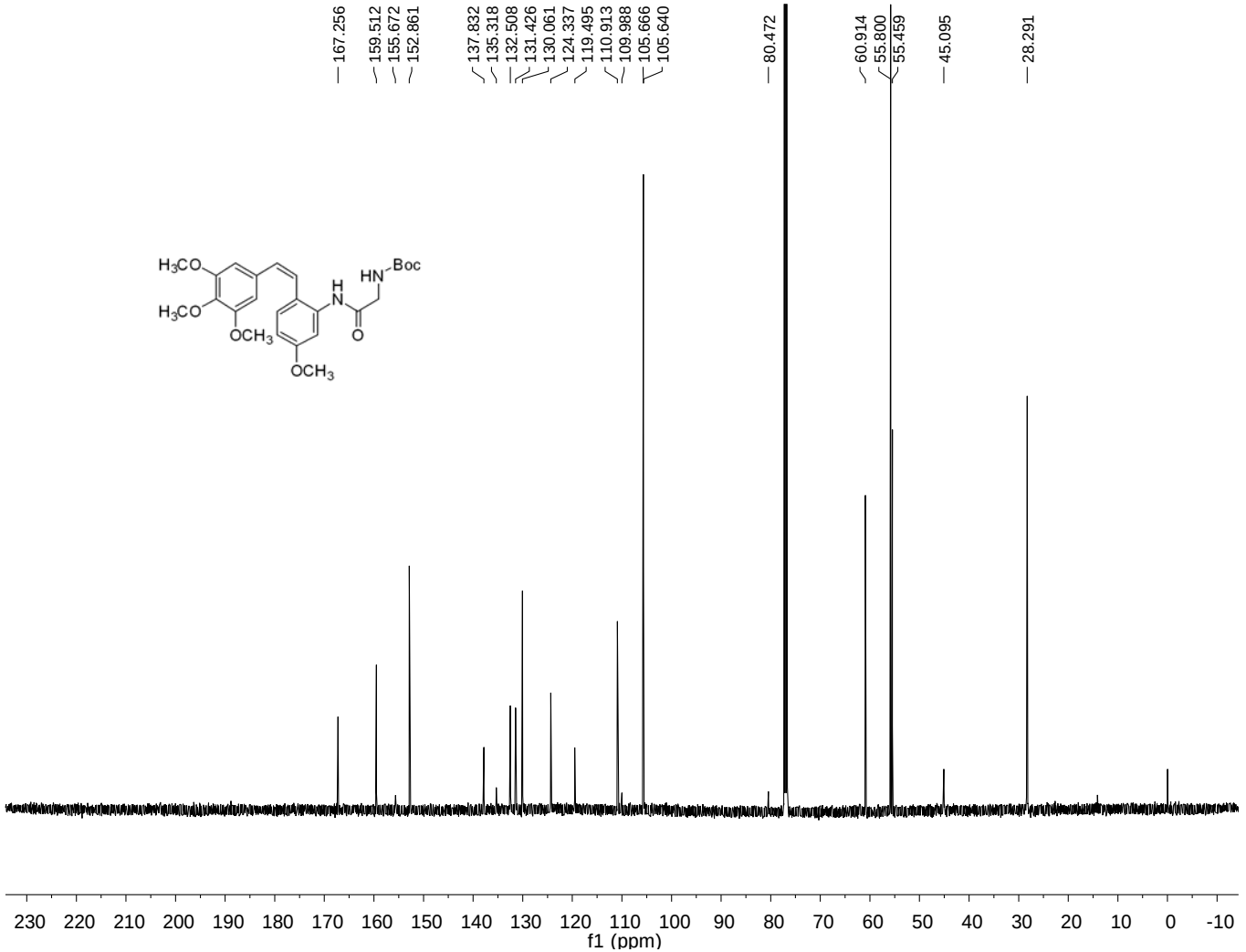
^{13}C NMR ($CDCl_3$, 150 MHz) Compound **11**



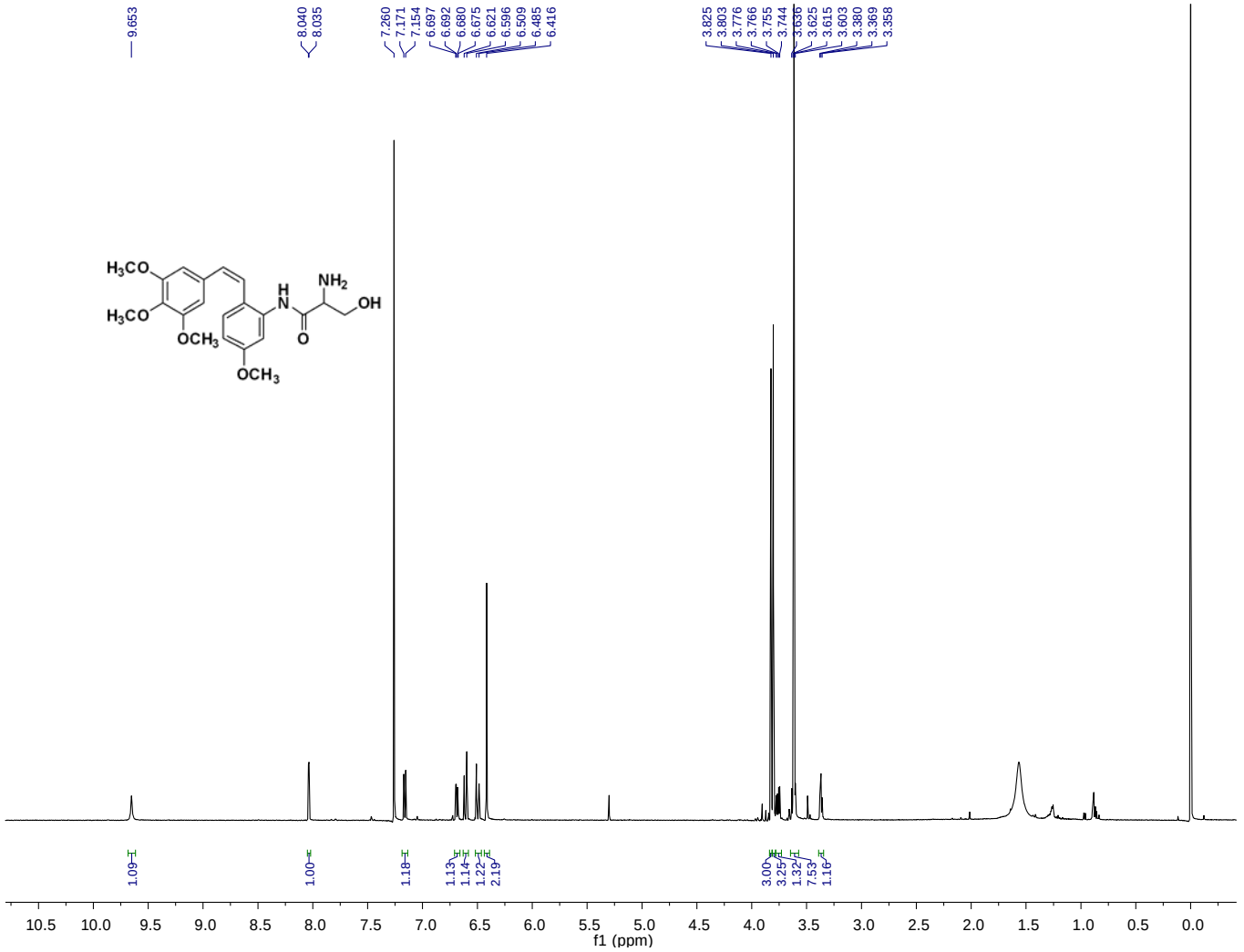
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **12**



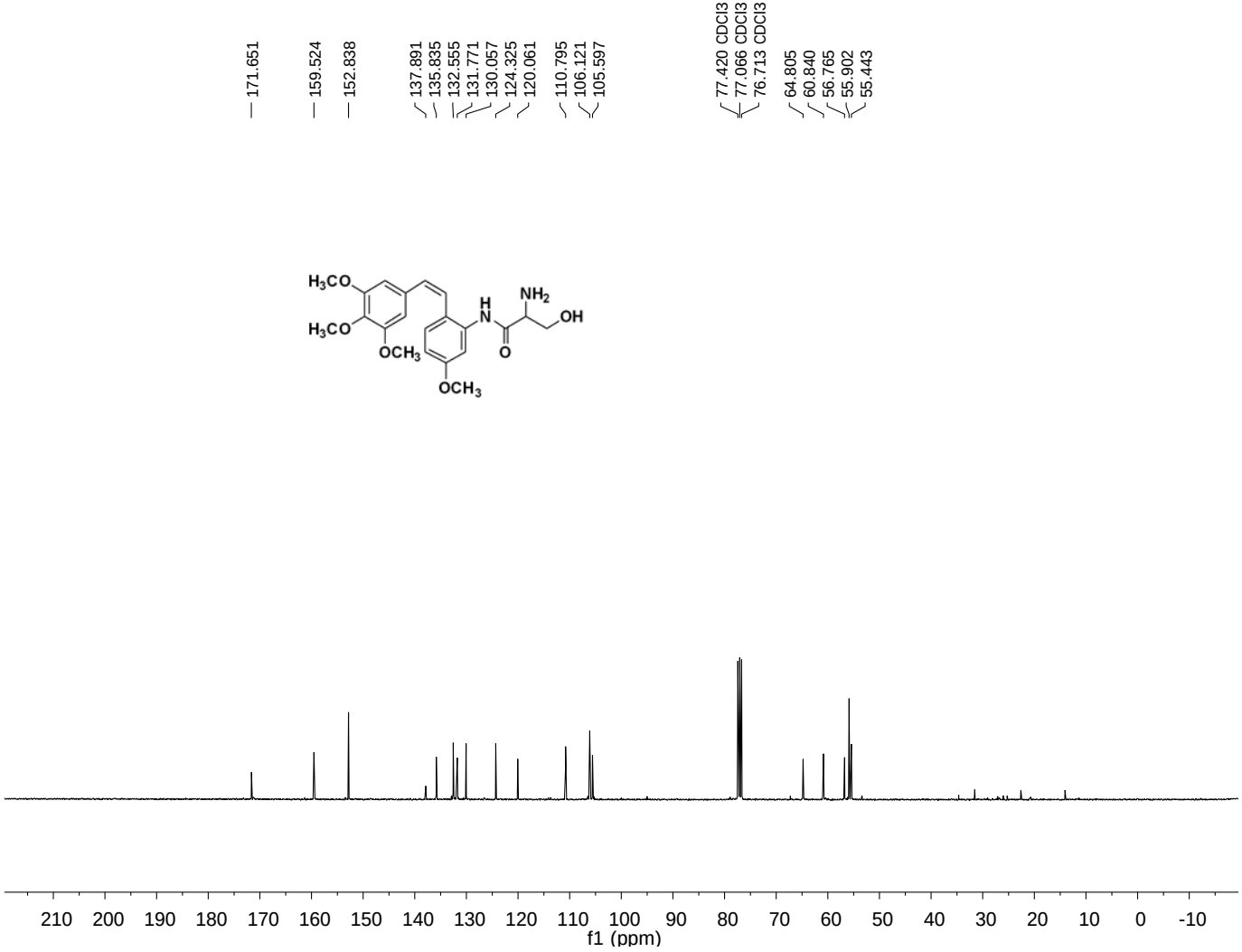
^{13}C NMR ($CDCl_3$, 125 MHz) Compound **12**



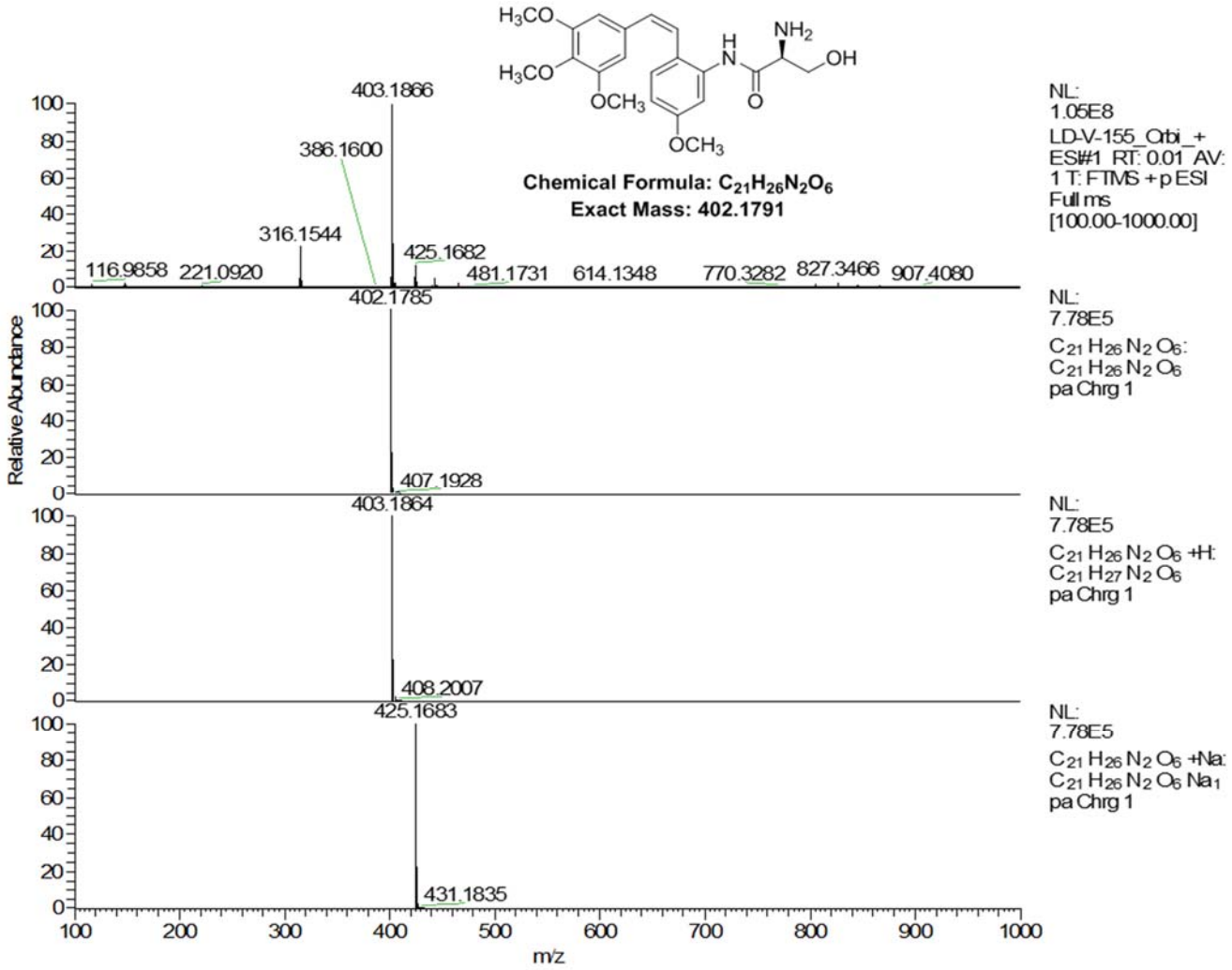
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **13**



^{13}C NMR ($CDCl_3$, 90 MHz) Compound **13**



HRMS Compound **13**



HPLC for Compound **13**

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    Last changed    : 7/31/2014 1:12:57 PM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-V-155-1A0001.D\DA.M (MASTERMETHOD2.M)
    Last changed    : 7/31/2014 2:17:20 PM by Laxman
    Sample Info     : Method- Mastermethod2
                      0-5 min 10:90 ACN: 0.1% TFA in Water
                      5-25 min 10:90 to 100:0 ACN: 0.1% TFA in Water
                      25-30 min 100:0 ACN: 0.1% TFA in Water
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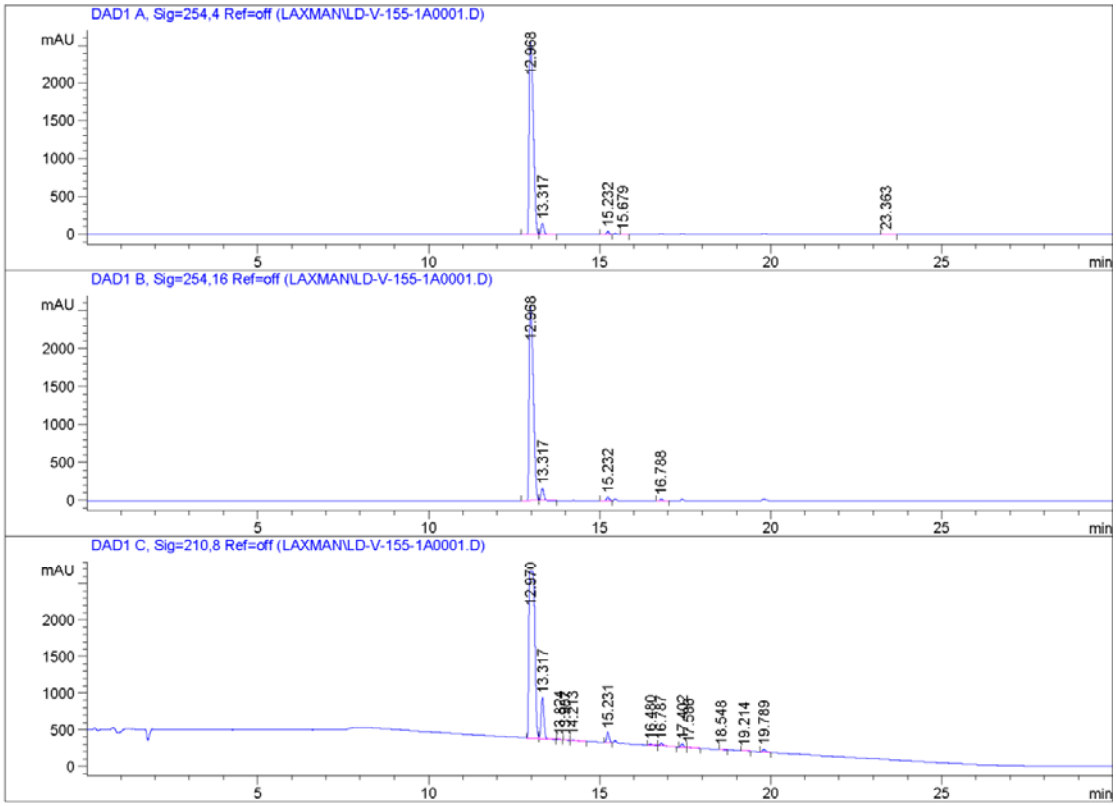
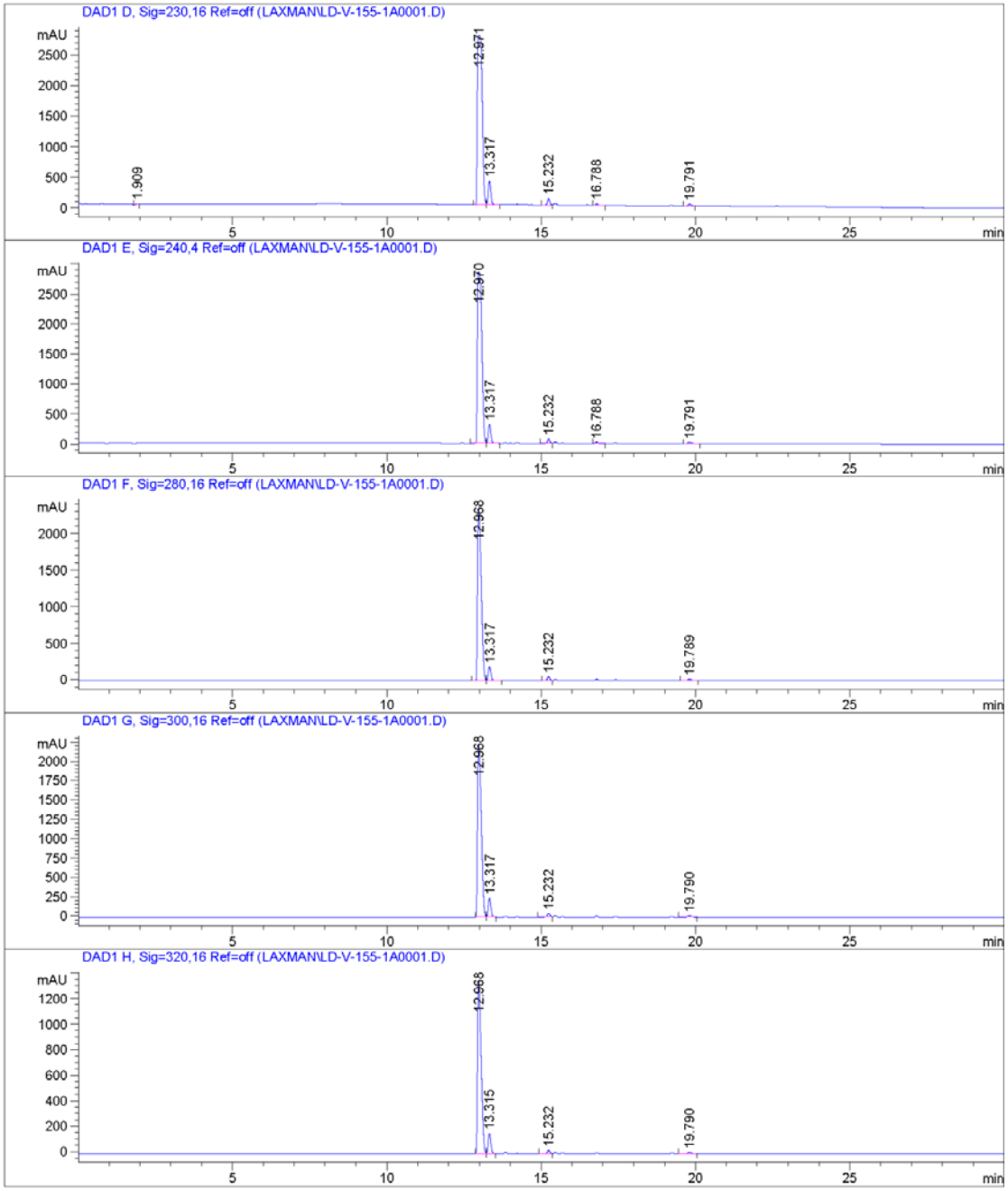


Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-155-1A0001.D
Sample Name: LD-V-155-1A-run1



Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-155-1A0001.D
Sample Name: LD-V-155-1A-run1

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.968	BV	0.1362	2.16384e4	2571.50366	95.2518
2	13.317	VB	0.0888	820.29114	143.31441	3.6109
3	15.232	BV	0.0812	234.08575	43.19005	1.0304
4	15.679	VB	0.0841	9.86208	1.79634	0.0434
5	23.363	VB	0.1591	14.41530	1.20862	0.0635

Totals : 2.27171e4 2761.01308

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.968	BV	0.1368	2.17823e4	2571.05396	94.5653
2	13.317	VB	0.0885	910.27527	159.73578	3.9519
3	15.232	BV	0.0812	252.29407	46.55761	1.0953
4	16.788	BB	0.0815	89.26048	16.92814	0.3875

Totals : 2.30341e4 2794.27549

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.970	VV	0.1930	2.69550e4	2294.62939	84.3049
2	13.317	VB	0.0884	3234.33423	568.48431	10.1158
3	13.824	BB	0.0694	35.76576	7.82016	0.1119
4	13.987	BV	0.1578	19.65191	1.68774	0.0615
5	14.213	VB	0.1124	88.28443	10.91145	0.2761
6	15.231	VV	0.0804	750.52655	140.31758	2.3474
7	16.480	BV	0.0813	87.05302	16.56549	0.2723
8	16.787	VB	0.0843	200.55069	36.40348	0.6272
9	17.402	BV	0.0832	244.03952	45.06736	0.7633

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-155-1A0001.D
Sample Name: LD-V-155-1A-run1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
10	17.586	VB	0.1047	37.94374	5.22775	0.1187
11	18.548	BB	0.0999	15.19789	2.27553	0.0475
12	19.214	BB	0.0957	43.13522	6.65227	0.1349
13	19.789	BB	0.1042	261.74756	36.26916	0.8186
Totals :				3.19732e4	3172.31167	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.909	BB	0.1103	64.22646	9.81579	0.1960
2	12.971	BV	0.1748	2.95450e4	2762.10352	90.1549
3	13.317	VB	0.0877	2179.68066	387.20401	6.6512
4	15.232	BV	0.0813	588.52106	108.53487	1.7958
5	16.788	VB	0.0830	164.31732	30.45298	0.5014
6	19.791	VV	0.1089	229.63806	30.12724	0.7007
Totals :				3.27713e4	3328.23841	

Signal 5: DAD1 E, Sig=240,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.970	BV	0.1595	2.78349e4	2861.06689	91.6270
2	13.317	VB	0.0877	1778.19116	315.66486	5.8535
3	15.232	BV	0.0815	446.13184	81.93338	1.4686
4	16.788	BV	0.0820	134.76234	25.35521	0.4436
5	19.791	VB	0.1138	184.50830	22.95249	0.6074
Totals :				3.03785e4	3306.97284	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.968	BV	0.1313	1.93204e4	2365.42163	92.6736
2	13.317	VB	0.0878	1085.78162	192.58340	5.2082
3	15.232	BV	0.0810	302.56723	56.02794	1.4513
4	19.789	BB	0.1053	139.03084	19.01400	0.6669

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-155-1A0001.D
Sample Name: LD-V-155-1A-run1

Totals : 2.08477e4 2633.04697

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.968	BV	0.1321	1.83907e4	2233.27051	91.3035
2	13.317	VB	0.0875	1356.34009	241.75505	6.7338
3	15.232	BV	0.0823	263.98782	47.88708	1.3106
4	19.790	BB	0.1094	131.35910	17.14645	0.6522

Totals : 2.01423e4 2540.05910

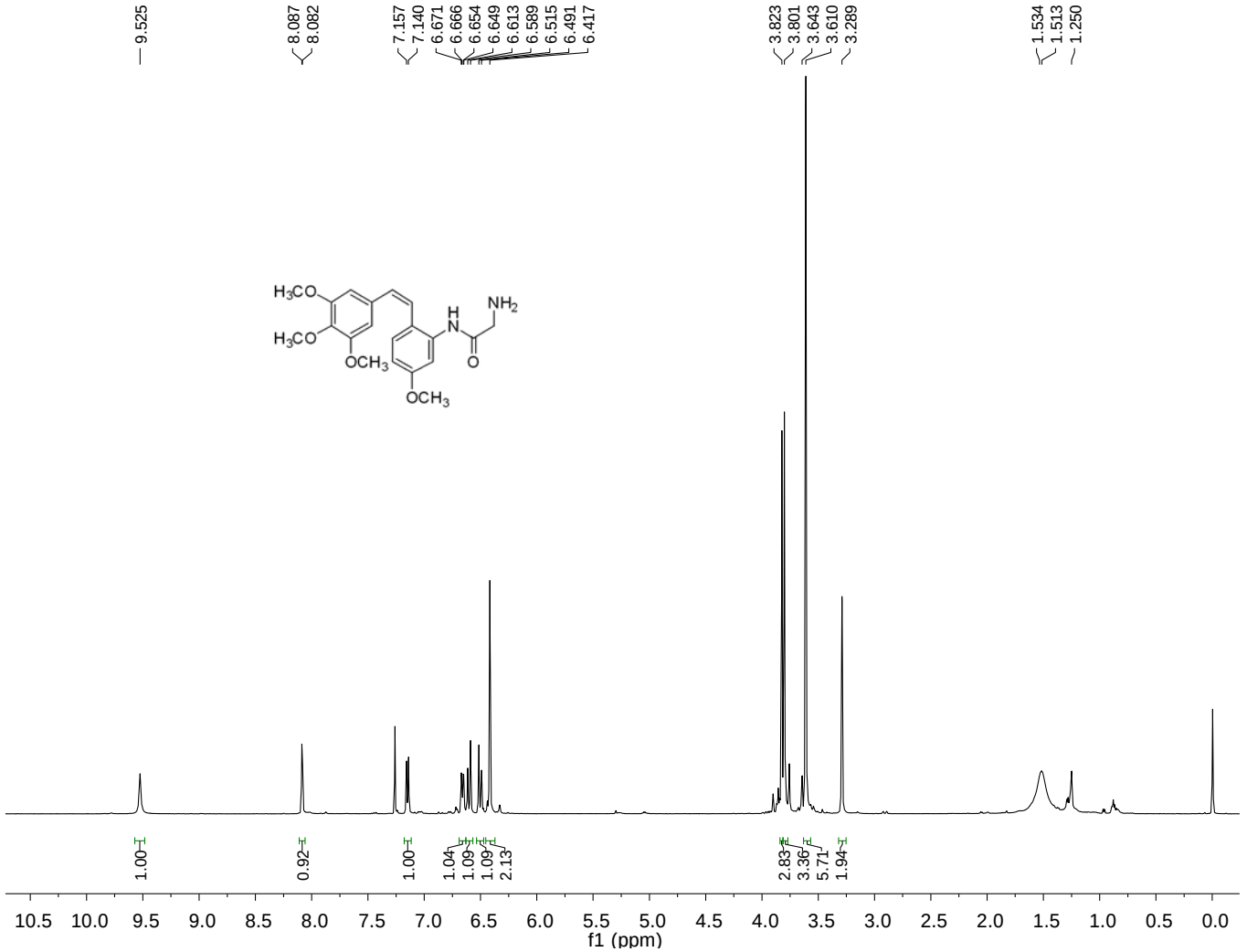
Signal 8: DAD1 H, Sig=320,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.968	BV	0.1217	1.06545e4	1356.26221	90.4901
2	13.315	VB	0.0905	890.75409	156.25275	7.5653
3	15.232	BV	0.0826	148.63684	26.85264	1.2624
4	19.790	BB	0.1090	80.32684	10.53331	0.6822

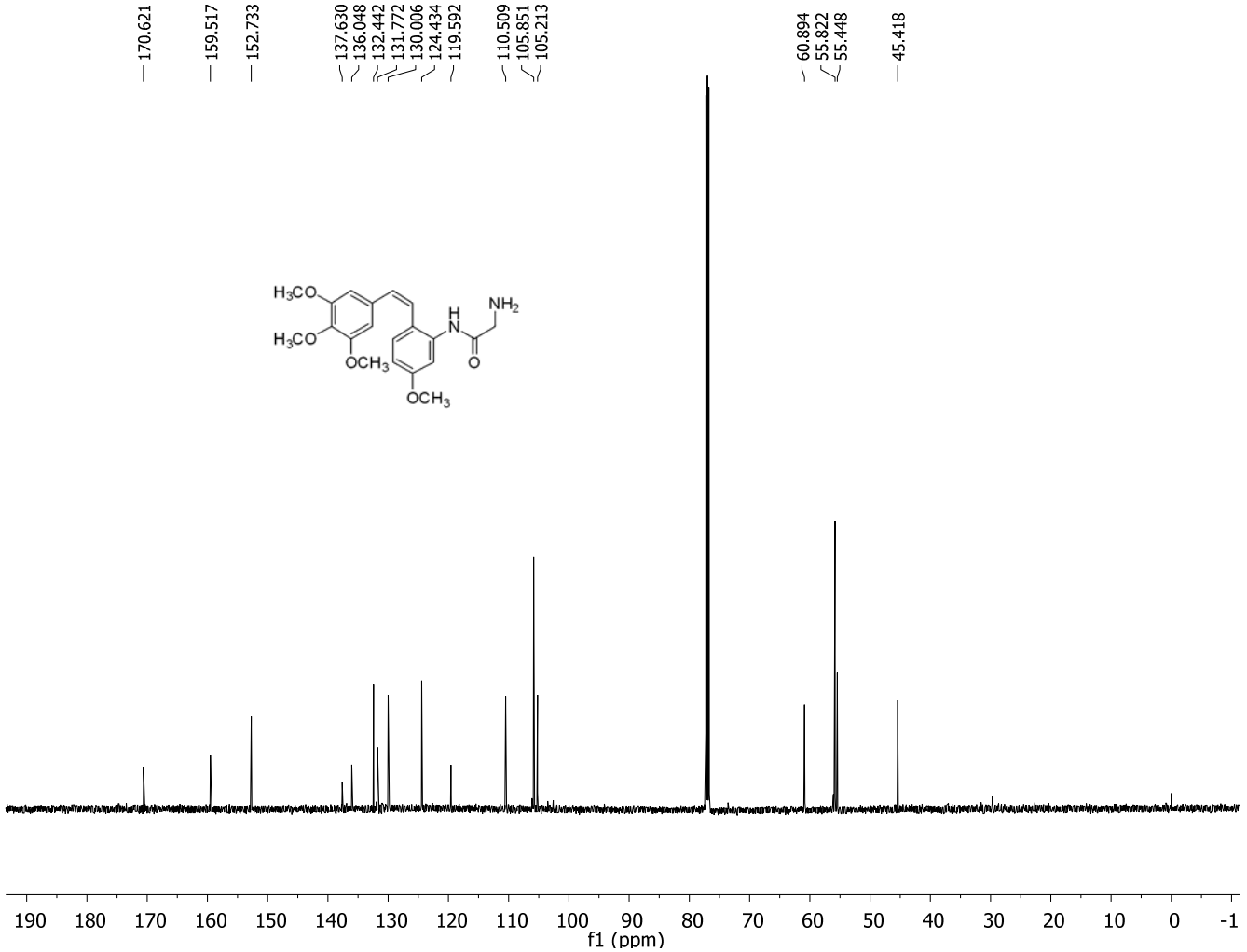
Totals : 1.17742e4 1549.90090

*** End of Report ***

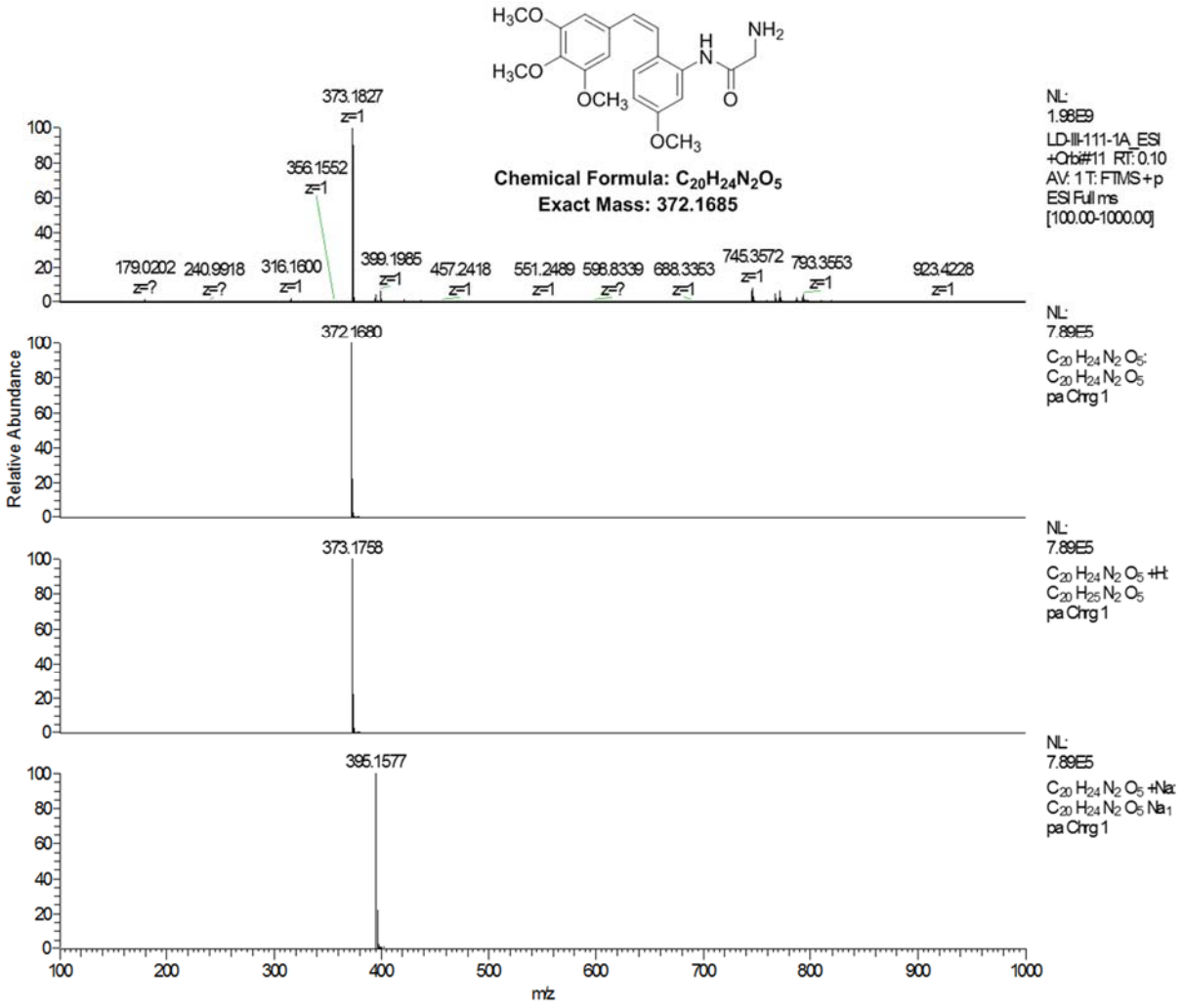
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **14**



^{13}C NMR ($CDCl_3$, 125 MHz) Compound **14**



HRMS Compound **14**



HPLC for Compound **14**

Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-111\RUN6-GOOD-0.1TFA\LDIII111RUN0006.D
Sample Name: LD-III-111-run6

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Acq. Operator   : Laxman
Acq. Instrument : Instrument 1                    Location :   -
Injection Date  : 7/9/2013 9:00:02 PM
Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
Last changed    : 7/9/2013 6:17:01 PM by Laxman
Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-III-111\RUN6-GOOD-0.1TFA\LDIII111RUN0006.D\DA.M (
                  MASTERMETHOD.M)
Last changed    : 4/1/2014 10:55:34 AM by ZHE
Sample Info     : Method- Mastermethod
                  0.1% TFA in Water
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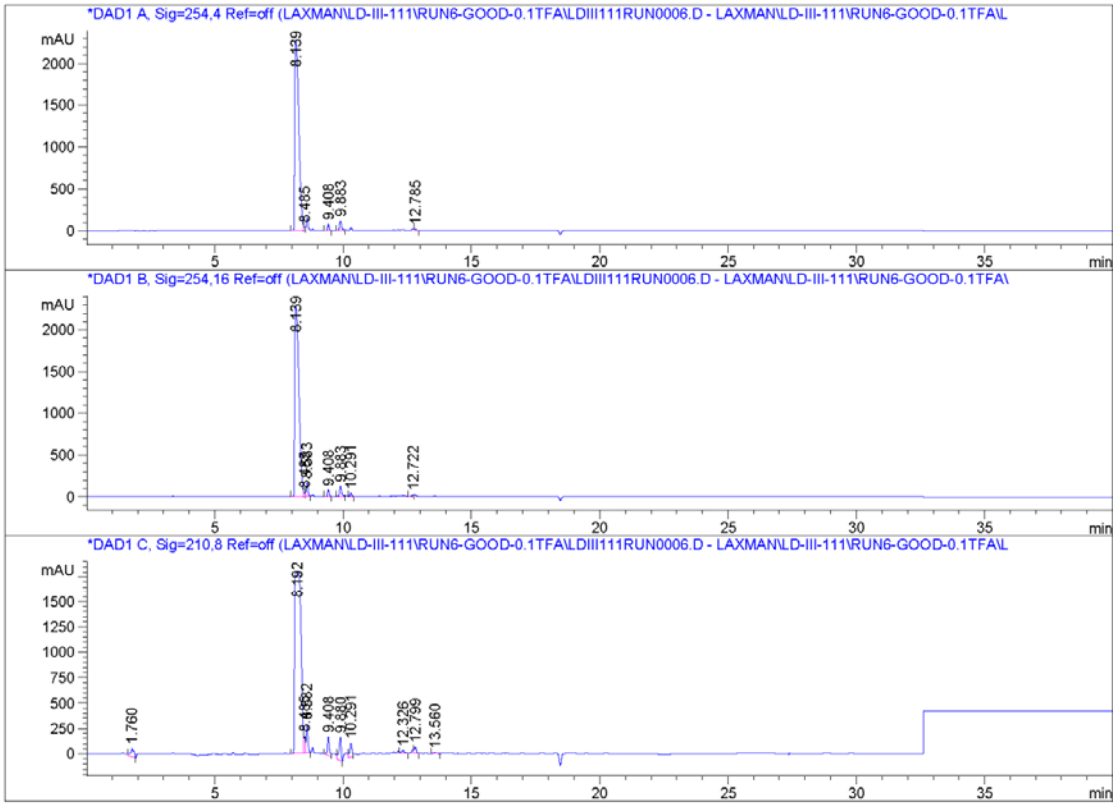
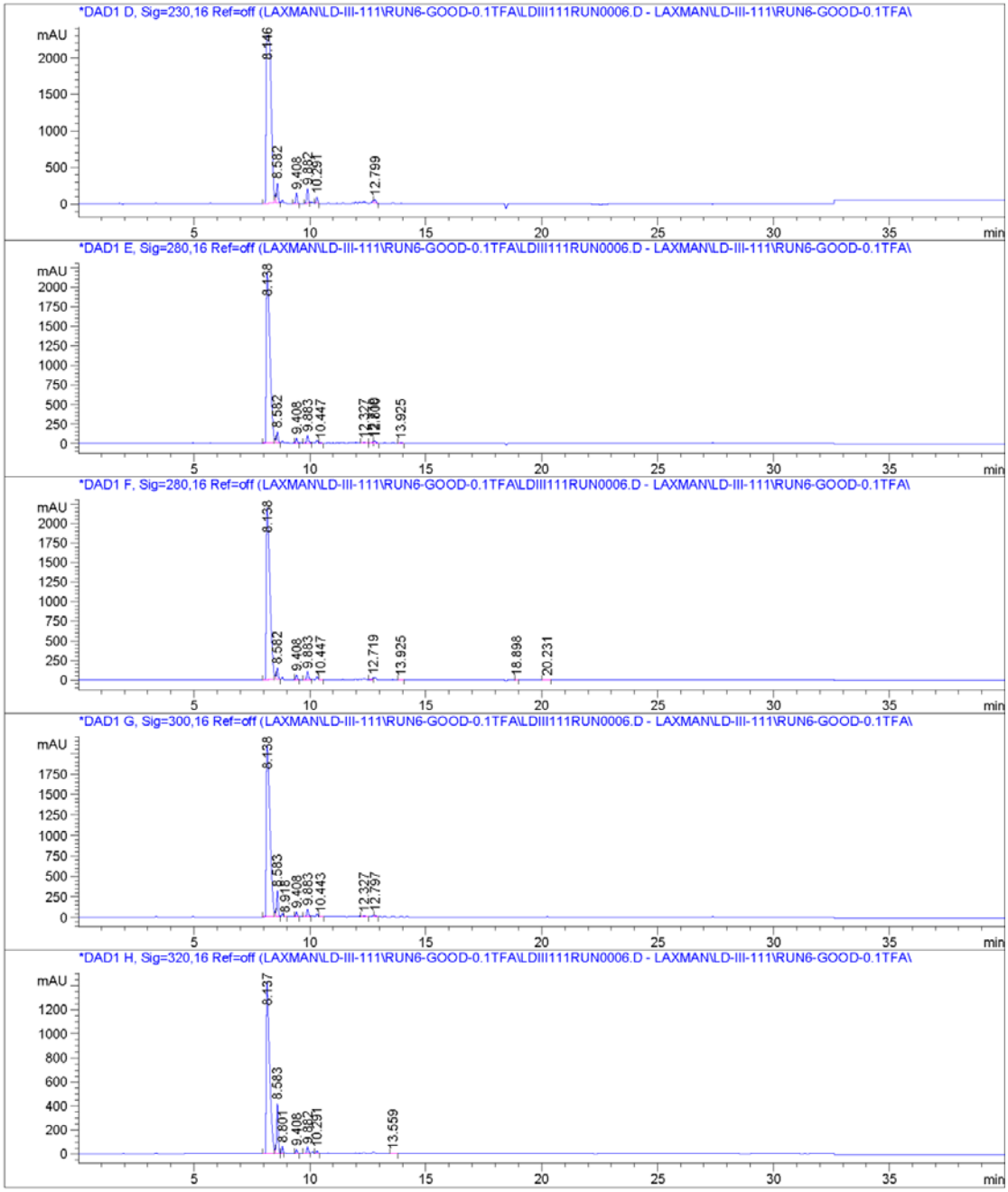


Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-111\RUN6-GOOD-0.1TFA\LDIII111RUN0006.D
Sample Name: LD-III-111-run6



Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-111\RUN6-GOOD-0.1TFA\LDIII111RUN0006.D
Sample Name: LD-III-111-run6

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.139	BV	0.1754	2.51894e4	2271.24341	95.4505
2	8.485	VV	0.0515	142.75304	42.16708	0.5409
3	9.408	BV	0.0674	326.38943	74.15012	1.2368
4	9.883	BV	0.0796	640.42310	117.46875	2.4268
5	12.785	VB	0.0730	91.04413	19.30842	0.3450
Totals :				2.63900e4	2524.33778	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.139	BV	0.1799	2.58701e4	2288.13721	92.2516
2	8.485	VV	0.0526	167.77173	48.26380	0.5983
3	8.583	VB	0.0693	721.26178	158.01703	2.5720
4	9.408	BV	0.0674	337.57236	76.65775	1.2038
5	9.883	BV	0.0794	656.34247	120.76386	2.3405
6	10.291	VB	0.0718	171.31871	37.16681	0.6109
7	12.722	BV	0.0809	118.60592	21.33296	0.4229
Totals :				2.80430e4	2750.33941	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.760	VB	0.1147	713.67432	81.08369	1.9280
2	8.192	BV	0.2641	3.05965e4	1801.82019	82.6552
3	8.485	VV	0.0556	607.23651	162.55066	1.6404

Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-111\RUN6-GOOD-0.1TFA\LDIII111RUN0006.D
Sample Name: LD-III-111-run6

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
4	8.582	VB	0.0698	1298.44763	281.44962	3.5077
5	9.408	BV	0.0794	991.03918	182.45256	2.6772
6	9.880	VB	0.0838	1236.96521	226.01939	3.3416
7	10.291	VV	0.0889	863.00427	141.99573	2.3314
8	12.326	VB	0.1201	283.79422	32.43545	0.7667
9	12.799	VV	0.0854	353.40680	63.03465	0.9547
10	13.560	BV	0.0934	72.98273	11.92350	0.1972
Totals :				3.70171e4	2984.76544	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.146	BV	0.2404	3.43759e4	2300.19604	90.7117
2	8.582	VB	0.0695	1254.74963	273.39069	3.3111
3	9.408	BV	0.0685	651.16071	144.70052	1.7183
4	9.882	BV	0.0693	926.08765	202.89774	2.4438
5	10.291	BV	0.0704	385.79929	85.91025	1.0181
6	12.799	VB	0.0832	302.09579	54.06322	0.7972
Totals :				3.78958e4	3061.15845	

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	8.138	BV	0.1715	2.42665e4	2186.85913	93.0409
2	8.582	VB	0.0692	656.98676	143.97168	2.5190
3	9.408	VB	0.0680	282.83658	63.43553	1.0844
4	9.883	BV	0.0788	524.90851	97.49480	2.0126
5	10.447	VB	0.0706	8.61436	1.84189	0.0330
6	12.327	VB	0.1122	90.34814	11.42952	0.3464
7	12.719	BV	0.0755	94.46789	19.16719	0.3622
8	12.800	VB	0.0819	142.46277	26.00443	0.5462
9	13.925	BB	0.1102	14.42253	2.09712	0.0553
Totals :				2.60816e4	2552.30129	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-111\RUN6-GOOD-0.1TFA\LDIII111RUN0006.D
Sample Name: LD-III-111-run6

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.138	BV	0.1715	2.42665e4	2186.85913	93.7362
2	8.582	VB	0.0692	656.98676	143.97168	2.5378
3	9.408	VB	0.0680	282.83658	63.43553	1.0925
4	9.883	BV	0.0788	524.90851	97.49480	2.0276
5	10.447	VB	0.0706	8.61436	1.84189	0.0333
6	12.719	BV	0.0755	94.46789	19.16719	0.3649
7	13.925	BB	0.1102	14.42253	2.09712	0.0557
8	18.898	VB	0.1124	30.22587	3.81837	0.1168
9	20.231	BB	0.0976	9.12180	1.40804	0.0352
Totals :				2.58881e4	2520.09375	

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	8.138	BV	0.1723	2.34731e4	2101.46777	90.7065
2	8.583	VB	0.0679	1408.04065	316.57404	5.4410
3	8.918	VB	0.0565	13.70495	3.76272	0.0530
4	9.408	VB	0.0681	275.41730	61.68370	1.0643
5	9.883	BV	0.0795	513.82751	94.44556	1.9856
6	10.443	VB	0.0686	8.27514	1.83509	0.0320
7	12.327	VB	0.1099	91.84422	11.91732	0.3549
8	12.797	VB	0.0747	93.86009	18.64861	0.3627
Totals :				2.58781e4	2610.33480	

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	8.137	BV	0.1477	1.41791e4	1430.12402	84.7866
2	8.583	VB	0.0668	1786.22986	410.09262	10.6811
3	8.801	BV	0.0635	214.57774	52.71780	1.2831
4	9.408	VB	0.0681	151.69165	33.99868	0.9071
5	9.882	BV	0.0780	268.97385	50.61902	1.6084
6	10.291	VV	0.0737	110.94556	23.25360	0.6634
7	13.559	BB	0.0920	11.75747	1.90529	0.0703

Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-111\RUN6-GOOD-0.1TFA\LDIII111RUN0006.D
Sample Name: LD-III-111-run6

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				1.67233e4	2002.71104	

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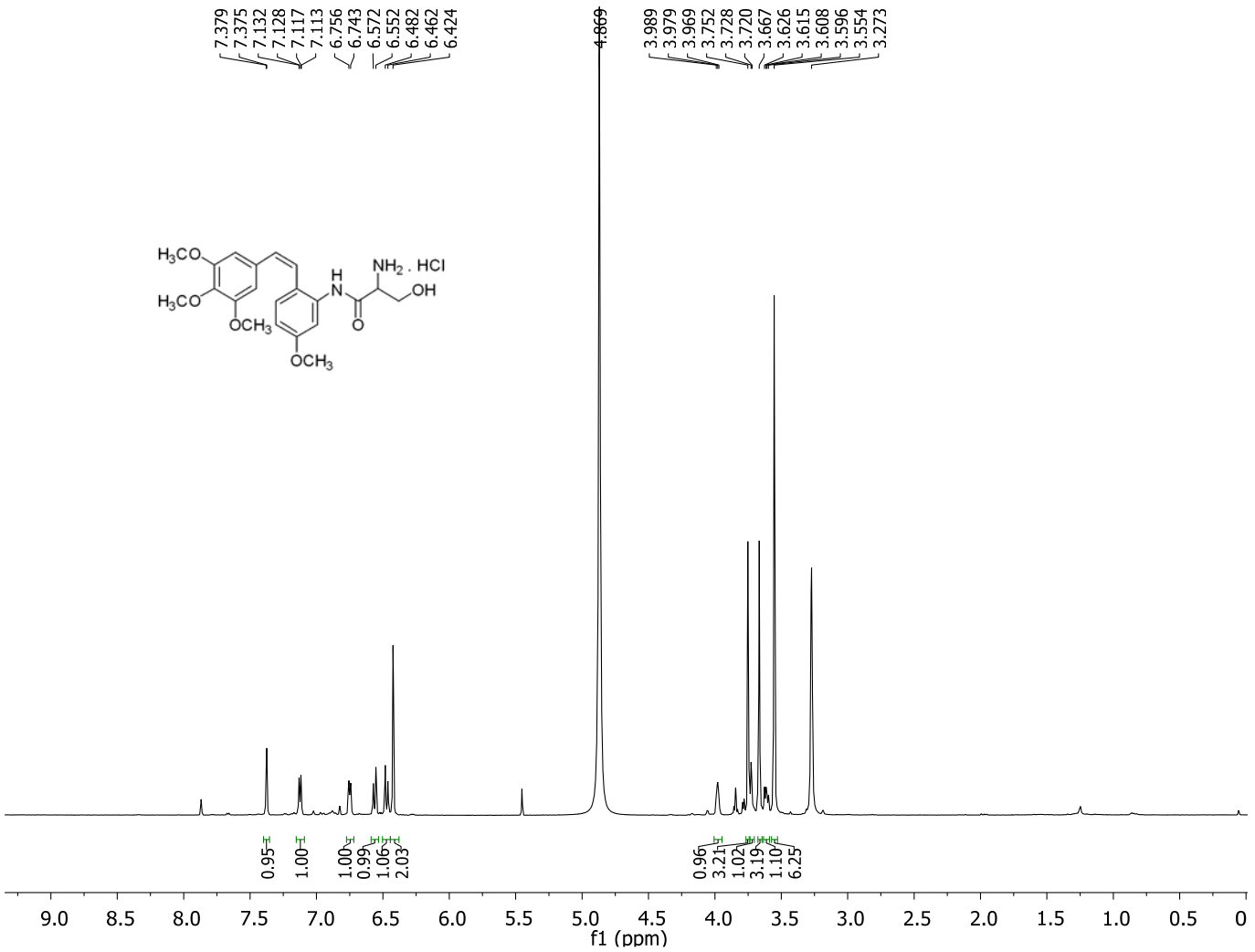
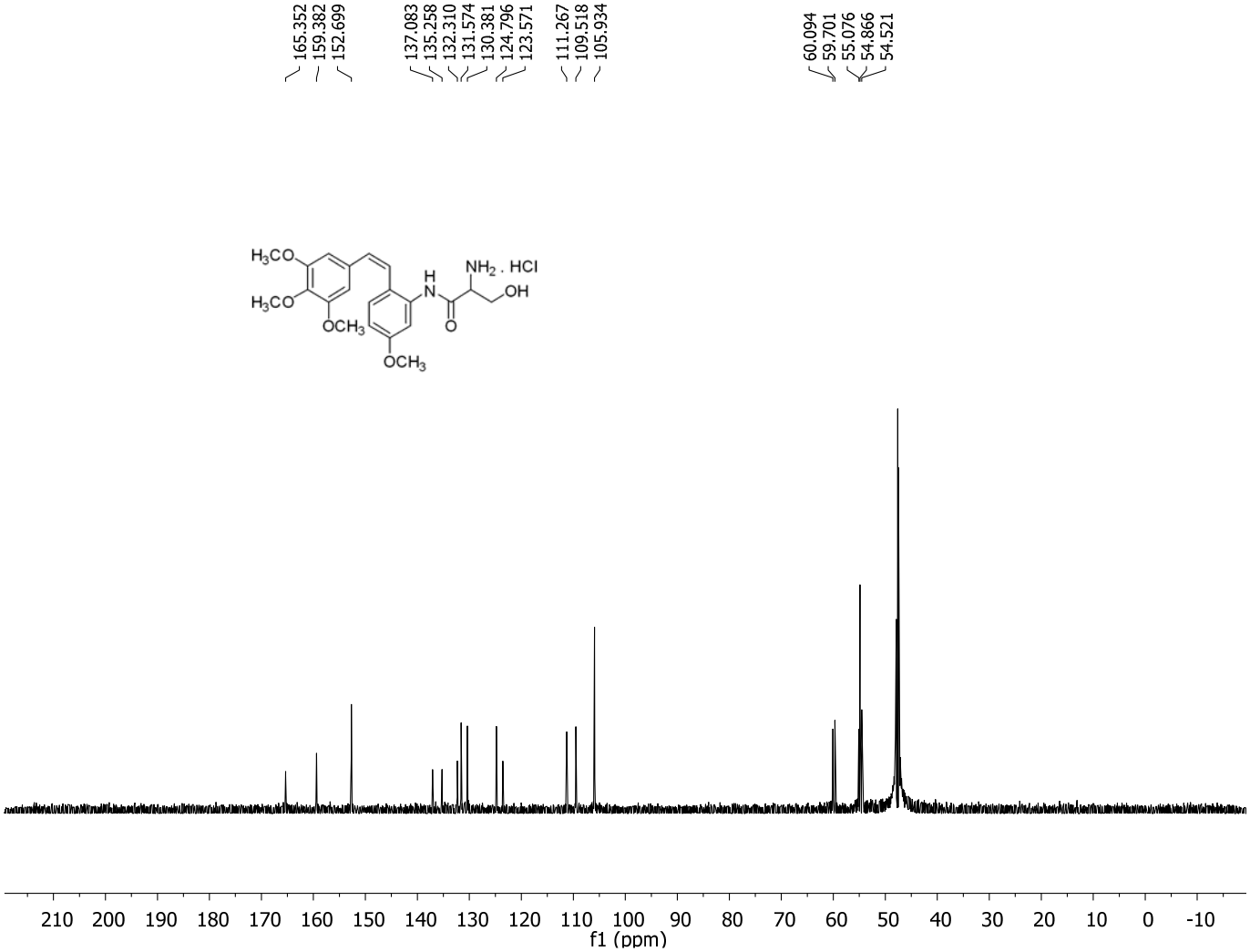
*** End of Report ***

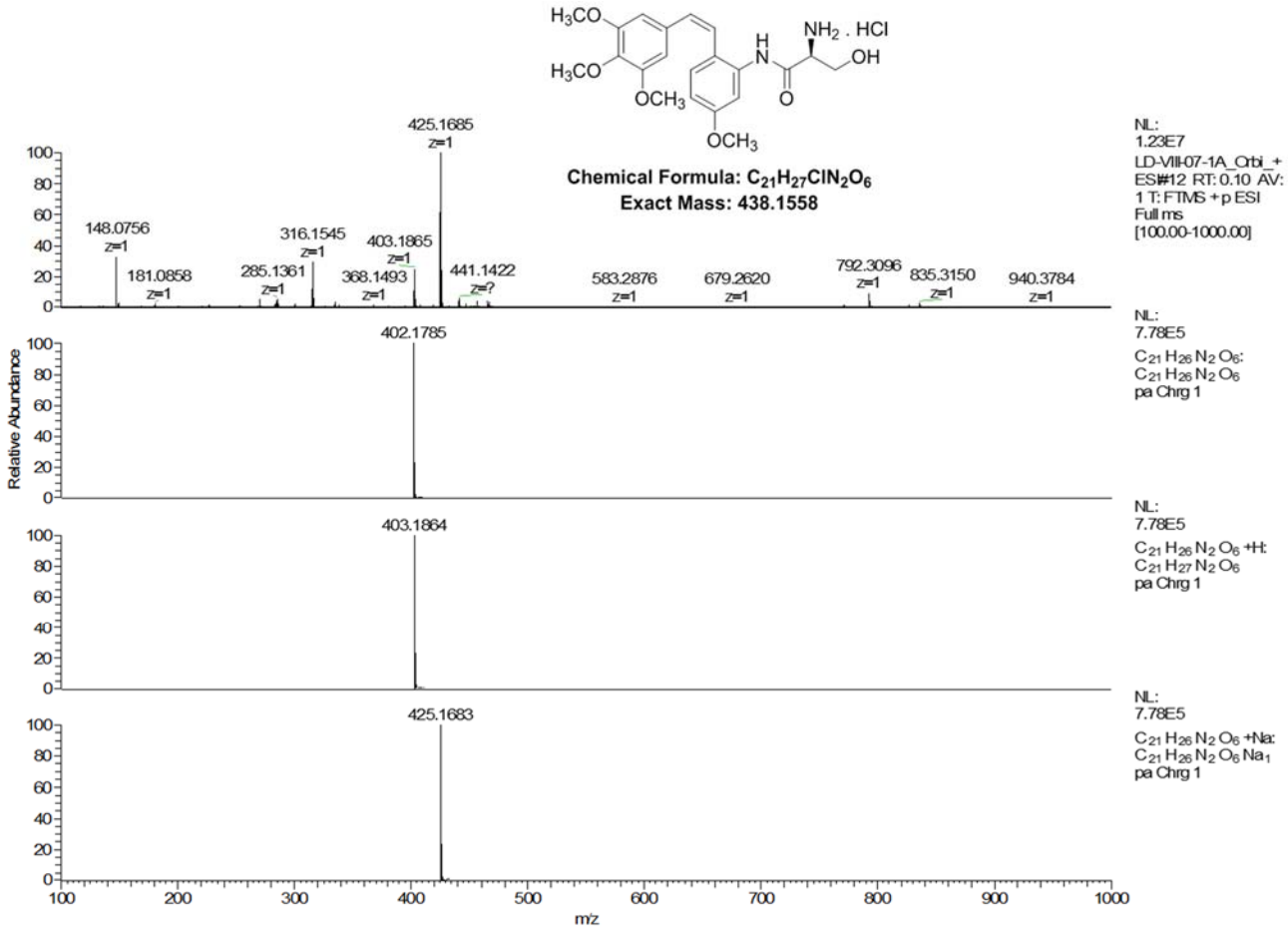
^{1}H NMR (CD_3OD, 600 MHz) Compound **15**



^{13}C NMR (CD_3OD, 150 MHz) Compound **15**



HRMS Compound **15**



HPLC for Compound **15**

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Data File C:\CHEM32\1\DATA\LAXMAN\LDVI-07-RUN0001.D
Sample Name: LD-VII-07-1A-run1

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    Acq. Instrument : Instrument 1                    Location :   -
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    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD2.M
    Last changed    : 1/30/2015 11:00:11 AM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LDVI-07-RUN0001.D\DA.M (MASTERMETHOD2.M)
    Last changed    : 1/30/2015 11:44:15 AM by Laxman
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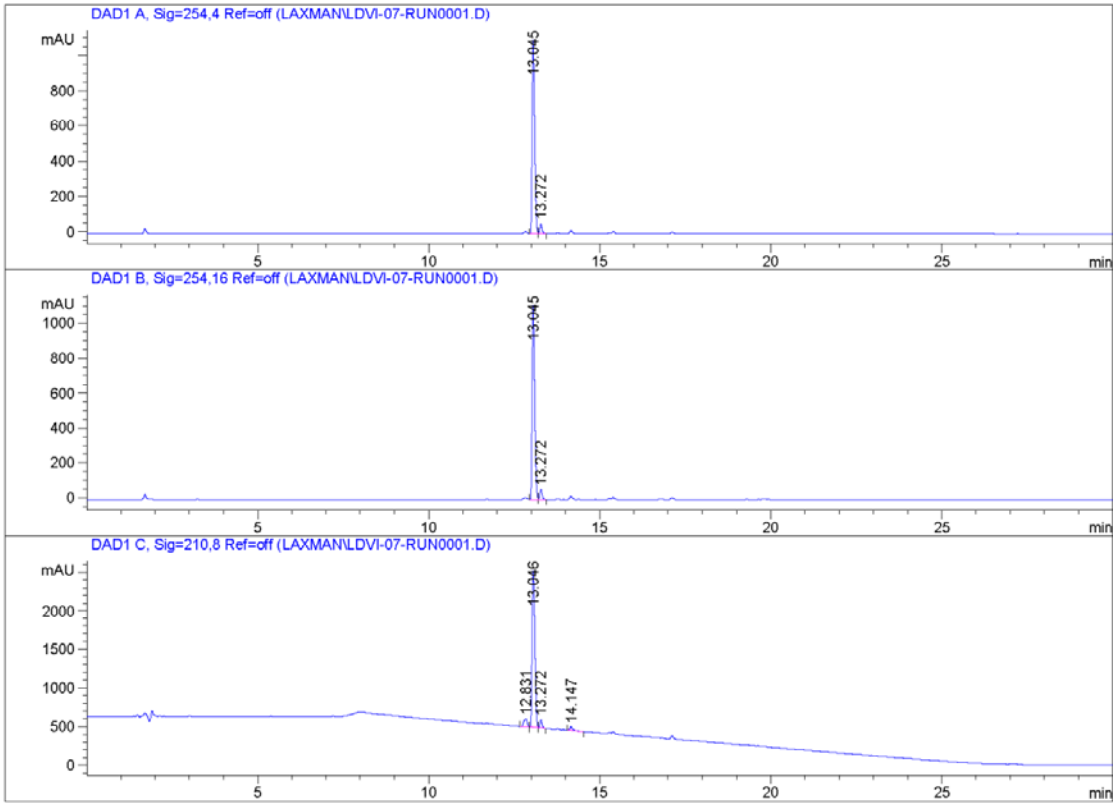
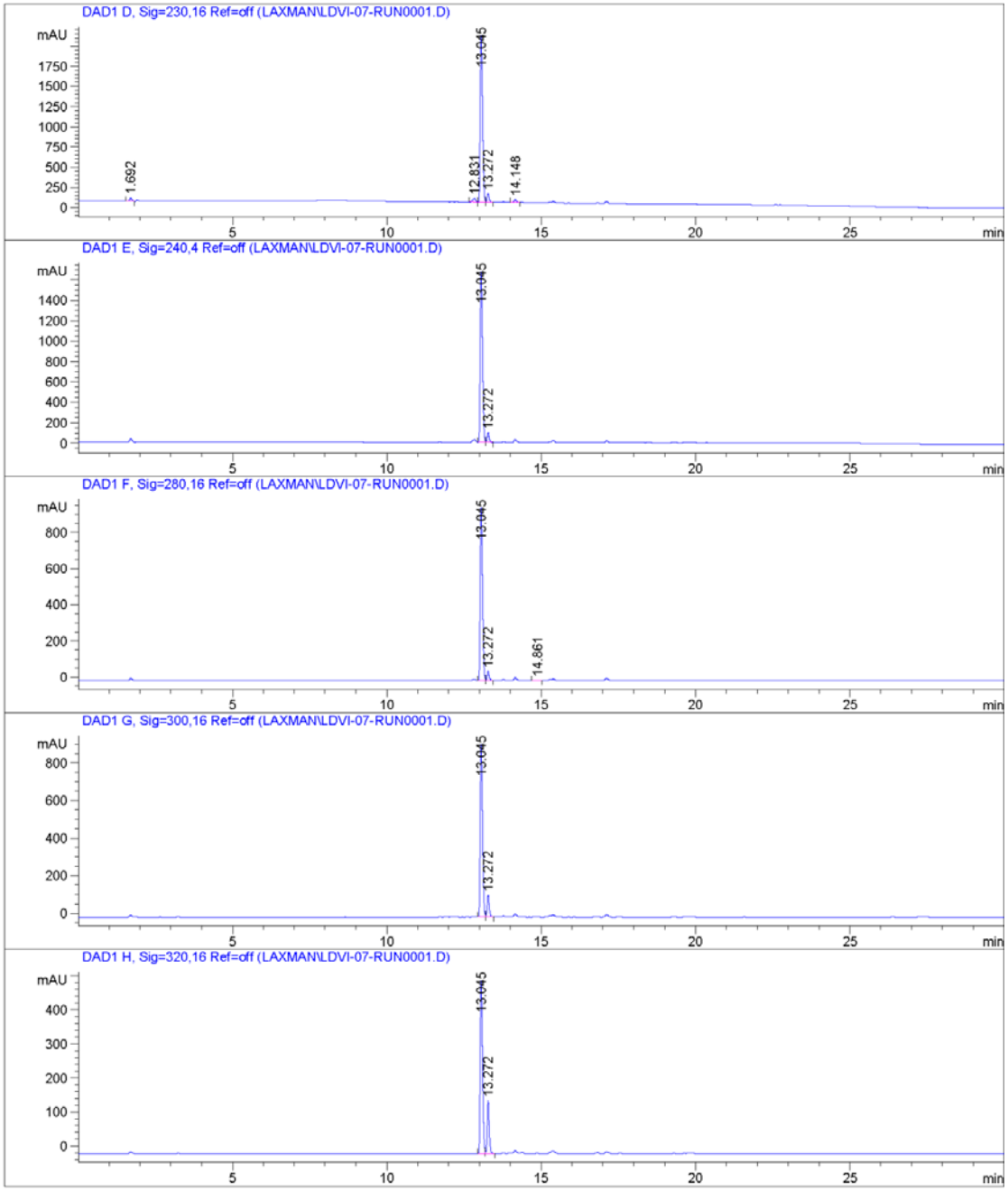


Data File C:\CHEM32\1\DATA\LAXMAN\LDVI-07-RUN0001.D
Sample Name: LD-VII-07-1A-run1



Data File C:\CHEM32\1\DATA\LAXMAN\LDVI-07-RUN0001.D
Sample Name: LD-VII-07-1A-run1

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Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.045	VV	0.0798	5641.81982	1100.64673	95.6171
2	13.272	VV	0.0679	258.60953	58.10617	4.3829
Totals :				5900.42935	1158.75290	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.045	VV	0.0798	5725.09717	1116.79260	95.6048
2	13.272	VV	0.0679	263.19711	59.14703	4.3952
Totals :				5988.29428	1175.93963	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.831	BB	0.1014	707.34418	101.35224	5.4677
2	13.046	BV	0.0917	1.14252e4	2030.26953	88.3164
3	13.272	VB	0.0674	483.14075	109.64965	3.7347
4	14.147	VB	0.0927	320.98795	48.82853	2.4812
Totals :				1.29367e4	2290.09996	

Data File C:\CHEM32\1\DATA\LAXMAN\LDVI-07-RUN0001.D
Sample Name: LD-VII-07-1A-run1

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.692	VB	0.0868	273.36813	45.01778	2.2595
2	12.831	VV	0.1009	308.57629	43.41265	2.5505
3	13.045	VV	0.0831	1.08210e4	2067.49194	89.4404
4	13.272	VB	0.0677	469.66138	105.94901	3.8819
5	14.148	VV	0.0830	225.95723	39.32711	1.8676
Totals :				1.20986e4	2301.19848	

Signal 5: DAD1 E, Sig=240,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.045	VV	0.0800	8622.23145	1675.22180	95.2748
2	13.272	VV	0.0678	427.61954	96.27460	4.7252
Totals :				9049.85098	1771.49640	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.045	BV	0.0797	4888.63330	955.13538	95.3672
2	13.272	VB	0.0677	227.55379	51.38308	4.4391
3	14.861	BB	0.0931	9.93156	1.54229	0.1937
Totals :				5126.11865	1008.06074	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.045	BV	0.0799	4717.08740	919.34308	90.2095
2	13.272	VB	0.0672	511.94711	116.60598	9.7905
Totals :				5229.03452	1035.94906	

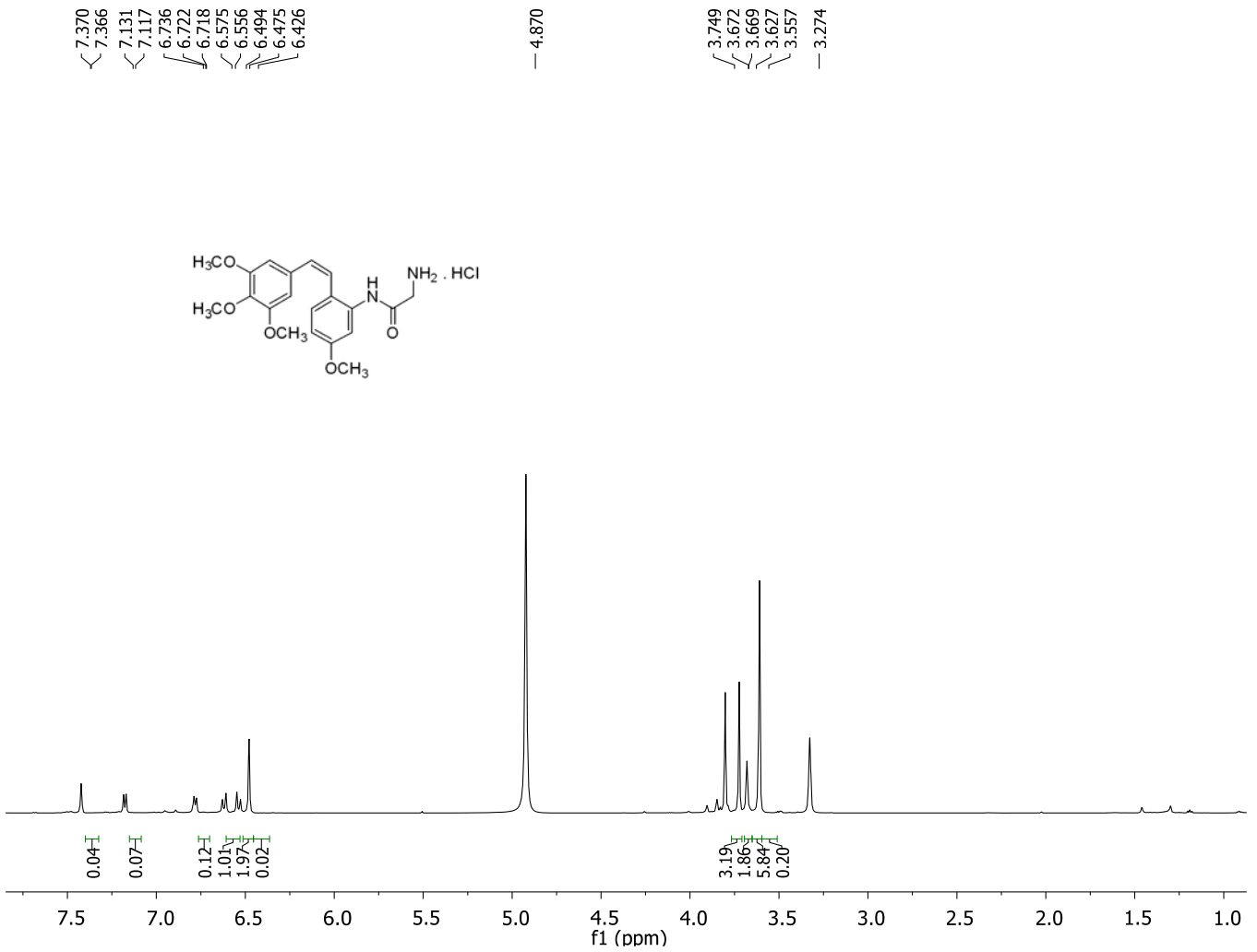
Data File C:\CHEM32\1\DATA\LAXMAN\LDVI-07-RUN0001.D
Sample Name: LD-VII-07-1A-run1

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

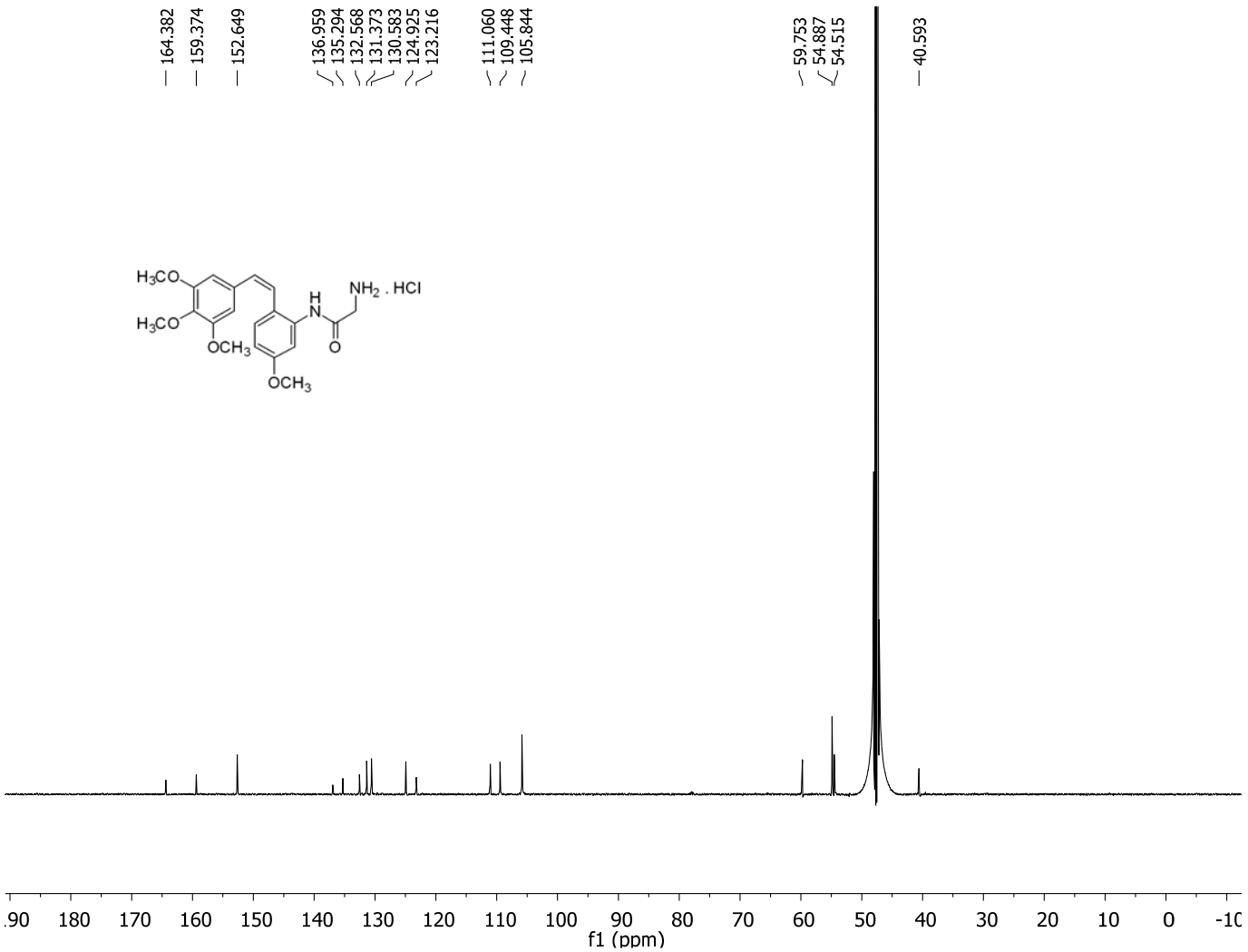
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.045	BV	0.0801	2620.03906	508.46661	79.2579
2	13.272	VB	0.0670	685.67603	156.98068	20.7421
Totals :				3305.71509	665.44730	

*** End of Report ***

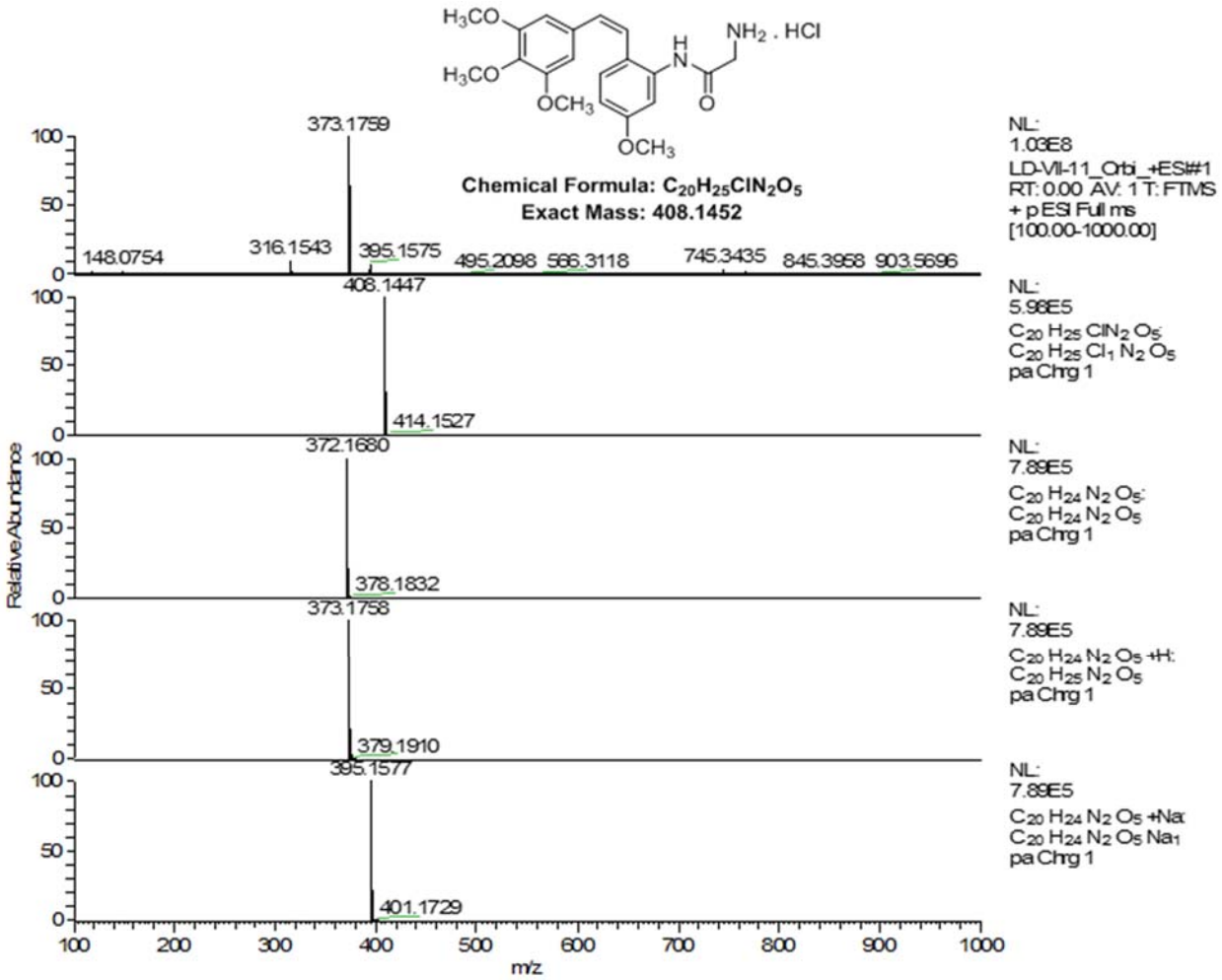
^{1}H NMR (CD_3OD, 600 MHz) Compound **16**



^{13}C NMR (CD_3OD, 150 MHz) Compound **16**



HRMS Compound **16**



HPLC for Compound **16**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-13000001.D
Sample Name: LD-VII-13-1A-run1

    ================================================================
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    Acq. Instrument : Instrument 1                  Location :   -
    Injection Date  : 1/30/2015 2:24:28 PM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD2.M
    Last changed    : 1/30/2015 2:22:10 PM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-VII-13000001.D\DA.M (MASTERMETHOD2.M)
    Last changed    : 2/3/2015 10:01:31 AM by Laxman
    Sample Info     : Method-Mastermethod2.M
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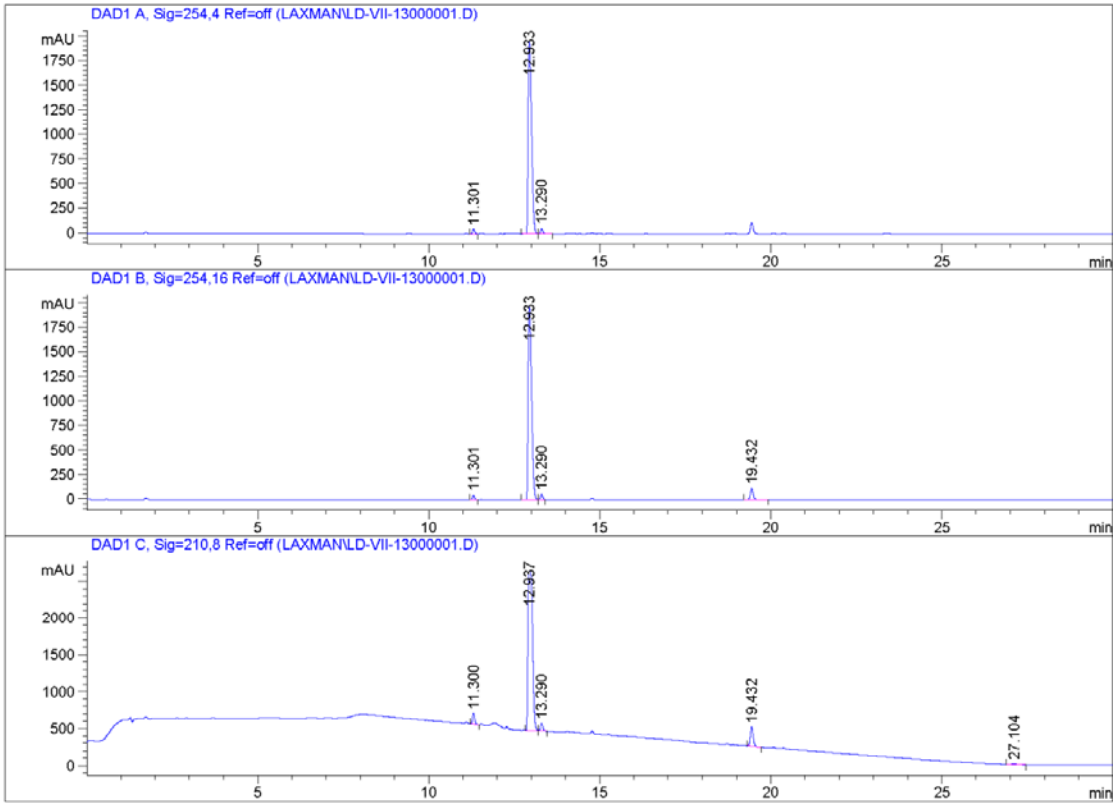
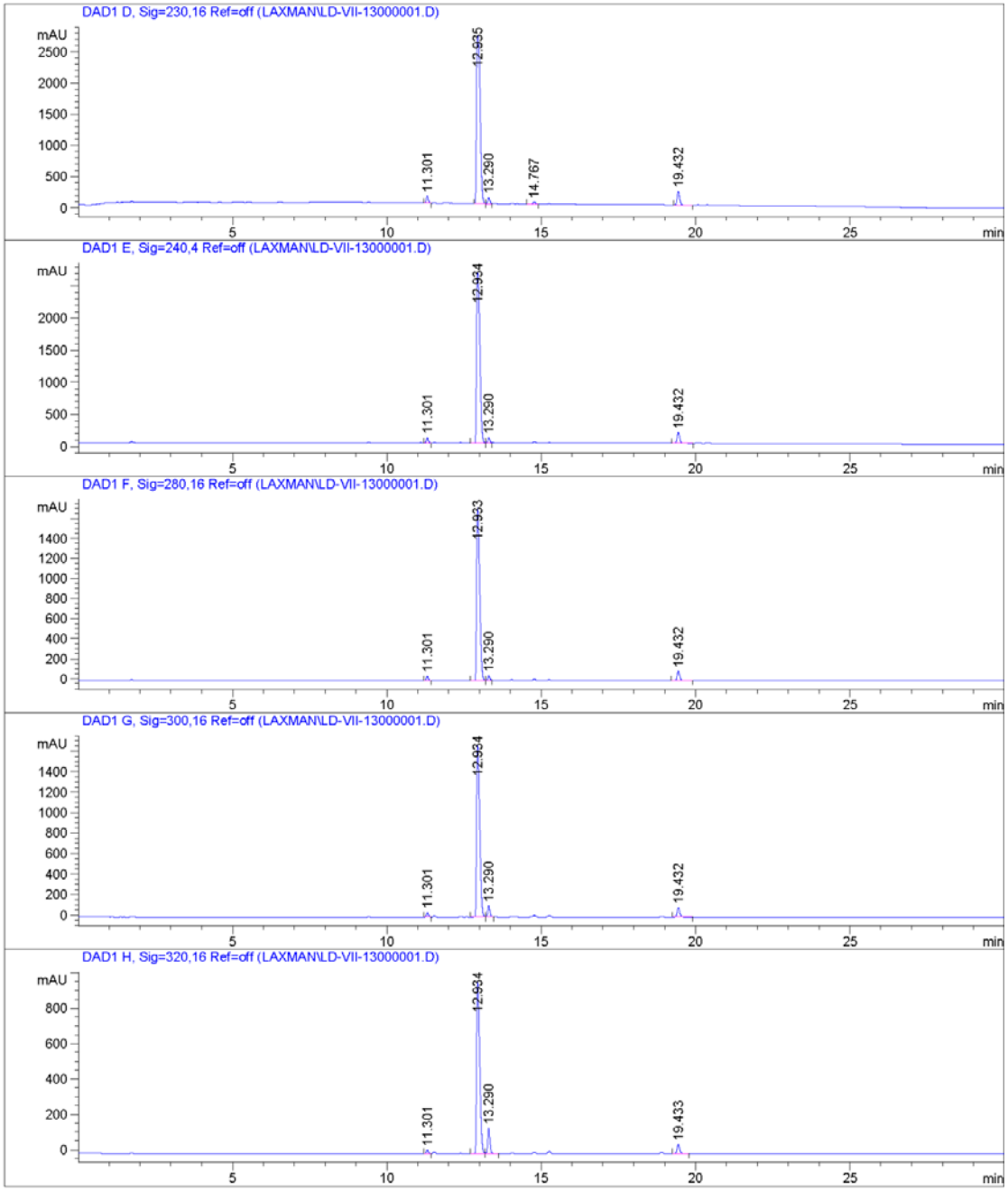


Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-13000001.D
Sample Name: LD-VII-13-1A-run1



Instrument 1 2/3/2015 10:04:46 AM Laxman Page 2 of 5

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-13000001.D
Sample Name: LD-VII-13-1A-run1

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.301	VV	0.0688	216.40208	49.69061	1.5422
2	12.933	BV	0.1082	1.35325e4	1968.75415	96.4400
3	13.290	VB	0.0767	283.14041	56.29315	2.0178
Totals :				1.40320e4	2074.73792	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.301	VV	0.0688	220.70474	50.70074	1.4896
2	12.933	BB	0.1083	1.36721e4	1986.53516	92.2783
3	13.290	BV	0.0707	249.83946	55.35923	1.6863
4	19.432	BB	0.0870	673.50708	117.34465	4.5458
Totals :				1.48161e4	2209.93979	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.300	BV	0.0709	674.63080	148.83742	2.9450
2	12.937	VV	0.1521	2.01127e4	2169.80444	87.7994
3	13.290	VB	0.0728	479.12888	102.04993	2.0916
4	19.432	BB	0.0865	1503.00769	263.54004	6.5612
5	27.104	BBA	0.2629	138.09706	8.43884	0.6028
Totals :				2.29076e4	2692.67067	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-13000001.D
Sample Name: LD-VII-13-1A-run1

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.301	VB	0.0686	494.89111	114.18317	2.0390
2	12.935	VV	0.1305	2.18511e4	2696.80225	90.0305
3	13.290	VV	0.0733	470.43481	99.17249	1.9383
4	14.767	BB	0.0844	173.55266	31.42022	0.7151
5	19.432	BB	0.0869	1280.80042	223.38777	5.2771
Totals :				2.42708e4	3164.96590	

Signal 5: DAD1 E, Sig=240,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.301	VV	0.0687	374.85321	86.20531	1.7492
2	12.934	BB	0.1178	1.96632e4	2673.26465	91.7548
3	13.290	BV	0.0707	396.78284	87.91900	1.8515
4	19.432	BB	0.0869	995.31000	173.60373	4.6444
Totals :				2.14301e4	3020.99268	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.301	VV	0.0689	198.91370	45.63385	1.5660
2	12.933	BB	0.1075	1.17454e4	1722.68103	92.4673
3	13.290	BV	0.0707	217.02390	48.02873	1.7085
4	19.432	BB	0.0868	540.88361	94.40138	4.2582
Totals :				1.27023e4	1910.74500	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.301	VV	0.0693	190.55530	43.33076	1.4858
2	12.934	BV	0.1082	1.15952e4	1687.50012	90.4100
3	13.290	VV	0.0725	517.15173	110.68778	4.0323
4	19.432	BB	0.0868	522.22614	91.22451	4.0719

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-13000001.D
Sample Name: LD-VII-13-1A-run1

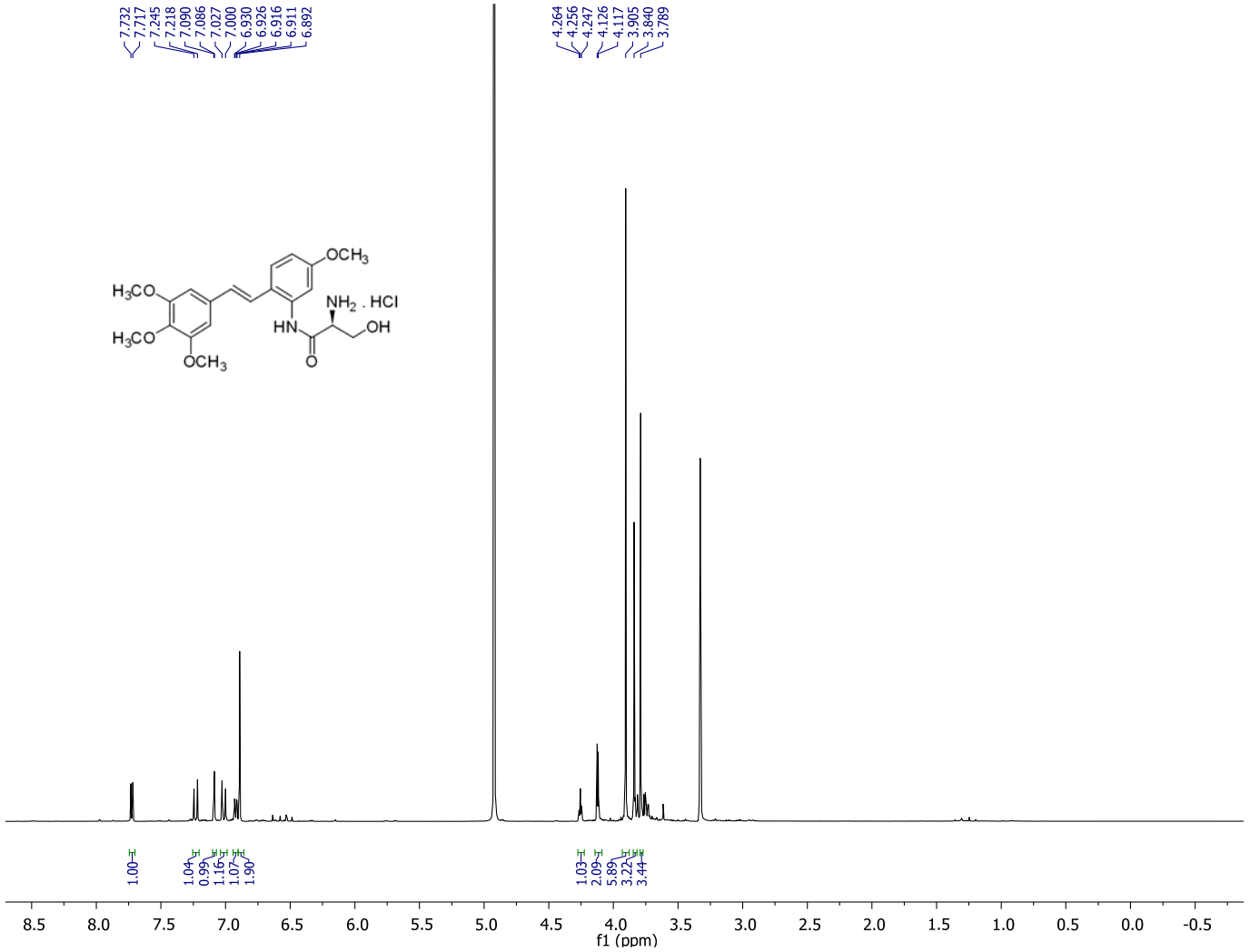
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				1.28252e4	1932.74317	

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

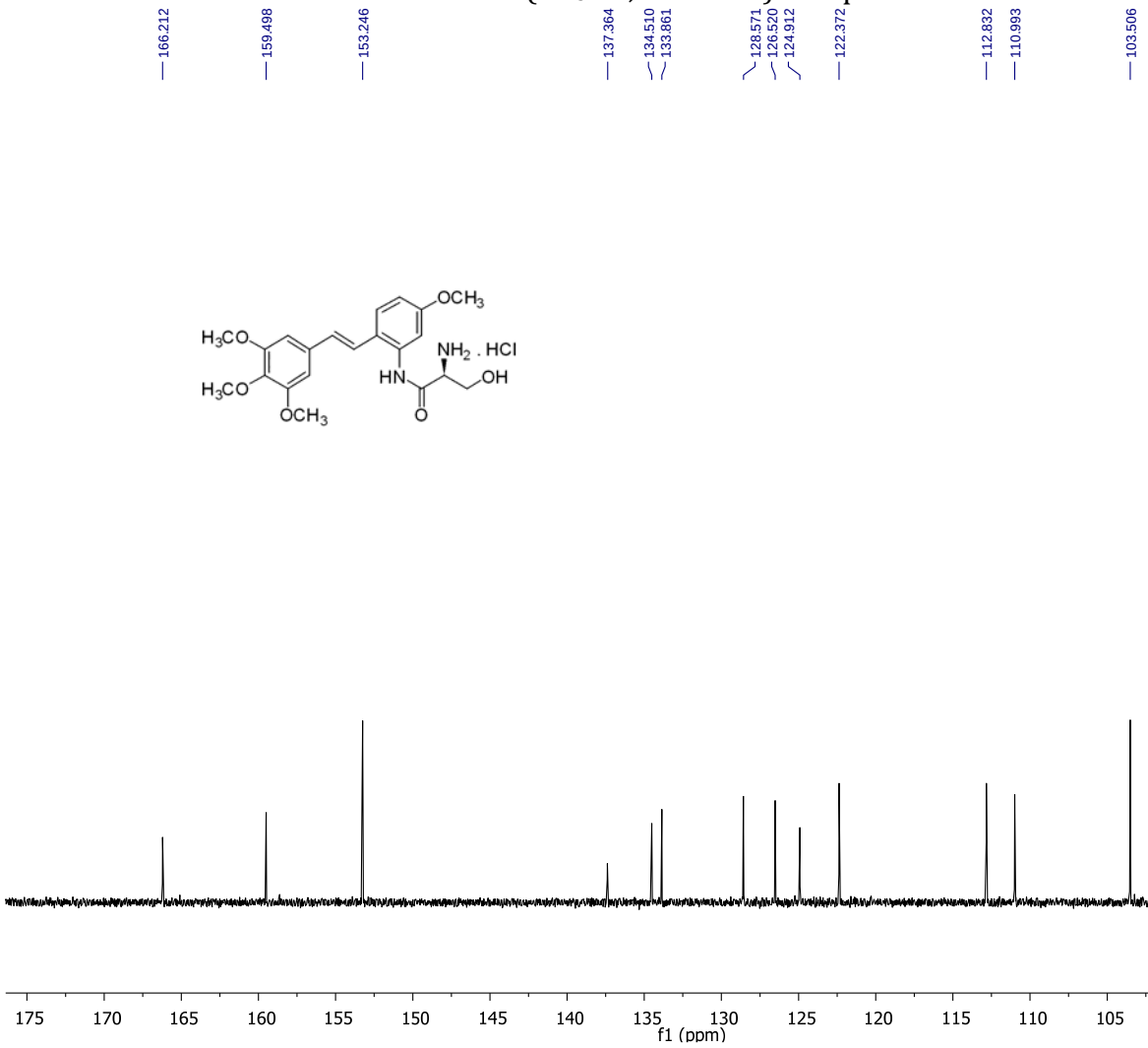
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.301	BV	0.0690	96.04256	21.98625	1.2361
2	12.934	BV	0.1078	6687.89258	978.16895	86.0774
3	13.290	VB	0.0727	684.77283	146.05211	8.8135
4	19.433	BB	0.0867	300.91791	52.62127	3.8730
Totals :				7769.62588	1198.82858	

*** End of Report ***

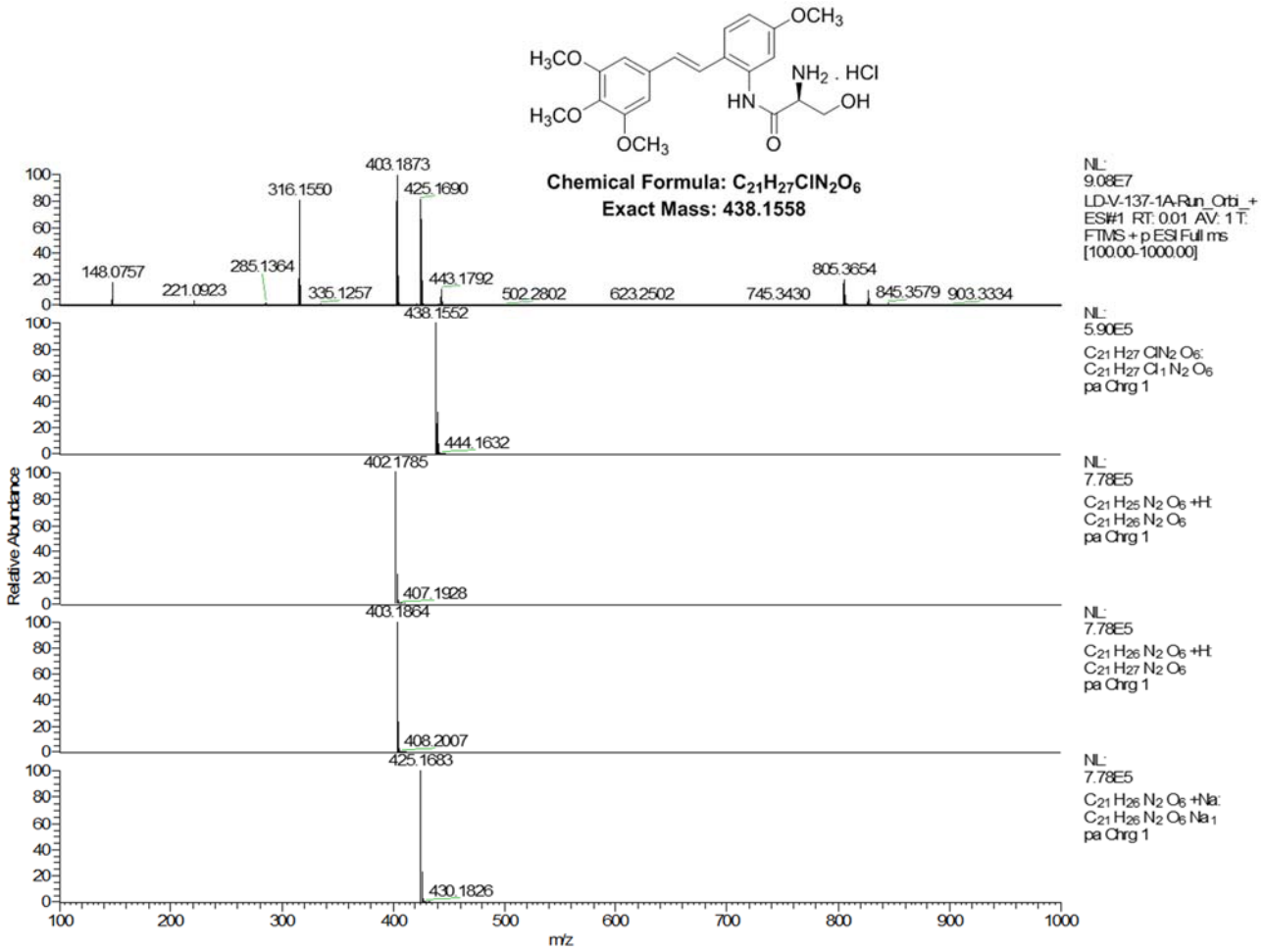
^{1}H NMR (CD_3OD, 600 MHz) Compound **17**



^{13}C NMR (CD_3OD, 150 MHz) Compound **17**



HRMS Compound **17**



HPLC for Compound **17**

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Data File C:\CHEM32\1\DATA\LAXMAN\LD-V137-2RUN001.D
Sample Name: LD-V-137-2-run1

    ======================================================================
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    Acq. Instrument : Instrument 1                  Location :   -
    Injection Date  : 7/14/2014 10:03:32 PM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD2.M
    Last changed    : 7/14/2014 9:54:41 PM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-V137-2RUN001.D\DA.M (MASTERMETHOD2.M)
    Last changed    : 7/14/2014 10:43:46 PM by Laxman
    Sample Info     : Mastermethod2
                      0-5 min 10:90  ACN: 0.1% TFA in Water
                      5-25 min 10:90 to 100:0 ACN: 0.1% TFA in Water
                      25-30 min 100:0 ACN: 0.1% TFA in Water
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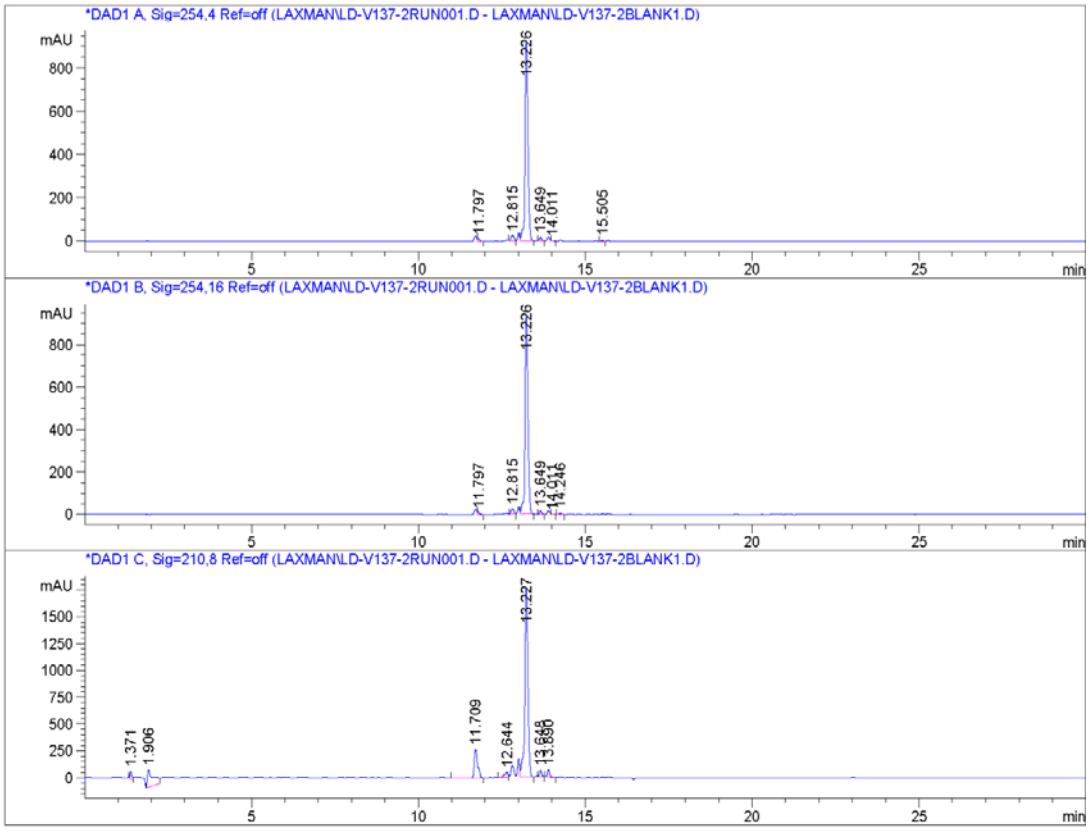
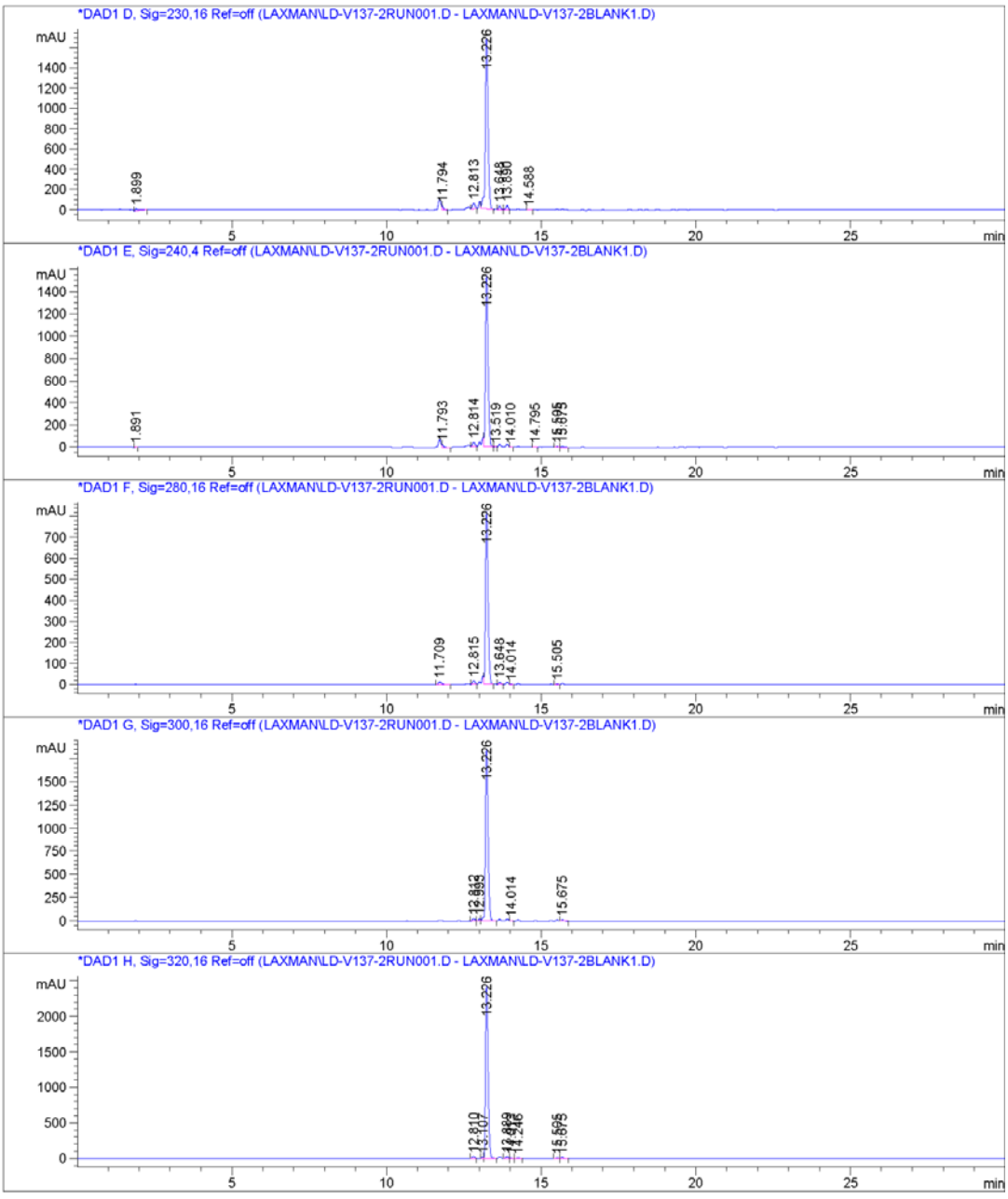


Data File C:\CHEM32\1\DATA\LAXMAN\LD-V137-2RUN001.D
Sample Name: LD-V-137-2-run1



Data File C:\CHEM32\1\DATA\LAXMAN\LD-V137-2RUN001.D
Sample Name: LD-V-137-2-run1

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.797	VB	0.0571	37.39809	10.12354	0.6524
2	12.815	BB	0.0943	141.96701	24.23329	2.4765
3	13.226	VB	0.0908	5449.38037	924.33972	95.0589
4	13.649	BB	0.0717	75.91022	16.47810	1.3242
5	14.011	VB	0.0582	5.78434	1.52517	0.1009
6	15.505	BV	0.0677	22.19742	5.01273	0.3872
Totals :				5732.63745	981.71255	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.797	VB	0.0543	37.51292	10.34681	0.6424
2	12.815	BB	0.0946	141.00084	23.99567	2.4146
3	13.226	VB	0.0907	5548.84961	942.08569	95.0223
4	13.649	BB	0.0717	74.49856	16.18236	1.2758
5	14.011	VB	0.0583	5.93264	1.56384	0.1016
6	14.246	BB	0.0727	31.73079	6.76175	0.5434
Totals :				5839.52536	1000.93613	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.371	BV	0.0676	340.71149	74.14693	2.0426
2	1.906	BV	0.1686	2097.38916	164.71938	12.5738
3	11.709	BB	0.1048	1859.09814	268.37698	11.1453

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V137-2RUN001.D
Sample Name: LD-V-137-2-run1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
4	12.644	BV	0.1103	423.80072	53.58047	2.5407
5	13.227	VB	0.0960	1.12451e4	1772.58142	67.4141
6	13.648	VV	0.0770	315.91071	62.49385	1.8939
7	13.890	VB	0.0834	398.60931	73.31020	2.3897
Totals :				1.66806e4	2469.20923	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.899	BB	0.1205	206.66499	23.52119	1.8437
2	11.794	VB	0.0507	132.52130	39.94218	1.1822
3	12.813	BB	0.0956	323.16006	54.13917	2.8830
4	13.226	VB	0.0918	1.01466e4	1696.98718	90.5198
5	13.648	VV	0.0736	178.25475	37.39213	1.5902
6	13.890	VV	0.0767	213.33083	42.40094	1.9032
7	14.588	VV	0.1051	8.73081	1.28800	0.0779
Totals :				1.12093e4	1895.67081	

Signal 5: DAD1 E, Sig=240,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.891	BB	0.0504	19.58302	6.28240	0.2135
2	11.793	VB	0.0534	76.00732	23.73110	0.8287
3	12.814	BB	0.0957	236.46078	39.57375	2.5782
4	13.226	VB	0.0880	8730.77148	1542.62524	95.1954
5	13.519	BV	0.0590	6.40798	1.82516	0.0699
6	14.010	VB	0.0571	10.10926	2.73744	0.1102
7	14.795	VB	0.0677	9.97771	2.34226	0.1088
8	15.505	VV	0.0689	35.71164	7.87916	0.3894
9	15.675	VB	0.0800	46.39381	8.72317	0.5059
Totals :				9171.42301	1635.71969	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V137-2RUN001.D
Sample Name: LD-V-137-2-run1

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.709	BB	0.1042	97.78605	14.22911	2.0067
2	12.815	BB	0.0938	97.29163	16.73797	1.9965
3	13.226	VB	0.0873	4604.29199	823.04236	94.4843
4	13.648	BB	0.0710	46.71126	10.28558	0.9586
5	14.014	VB	0.0629	6.04078	1.50296	0.1240
6	15.505	VV	0.0699	20.95428	4.53886	0.4300
Totals :				4873.07599	870.33683	

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

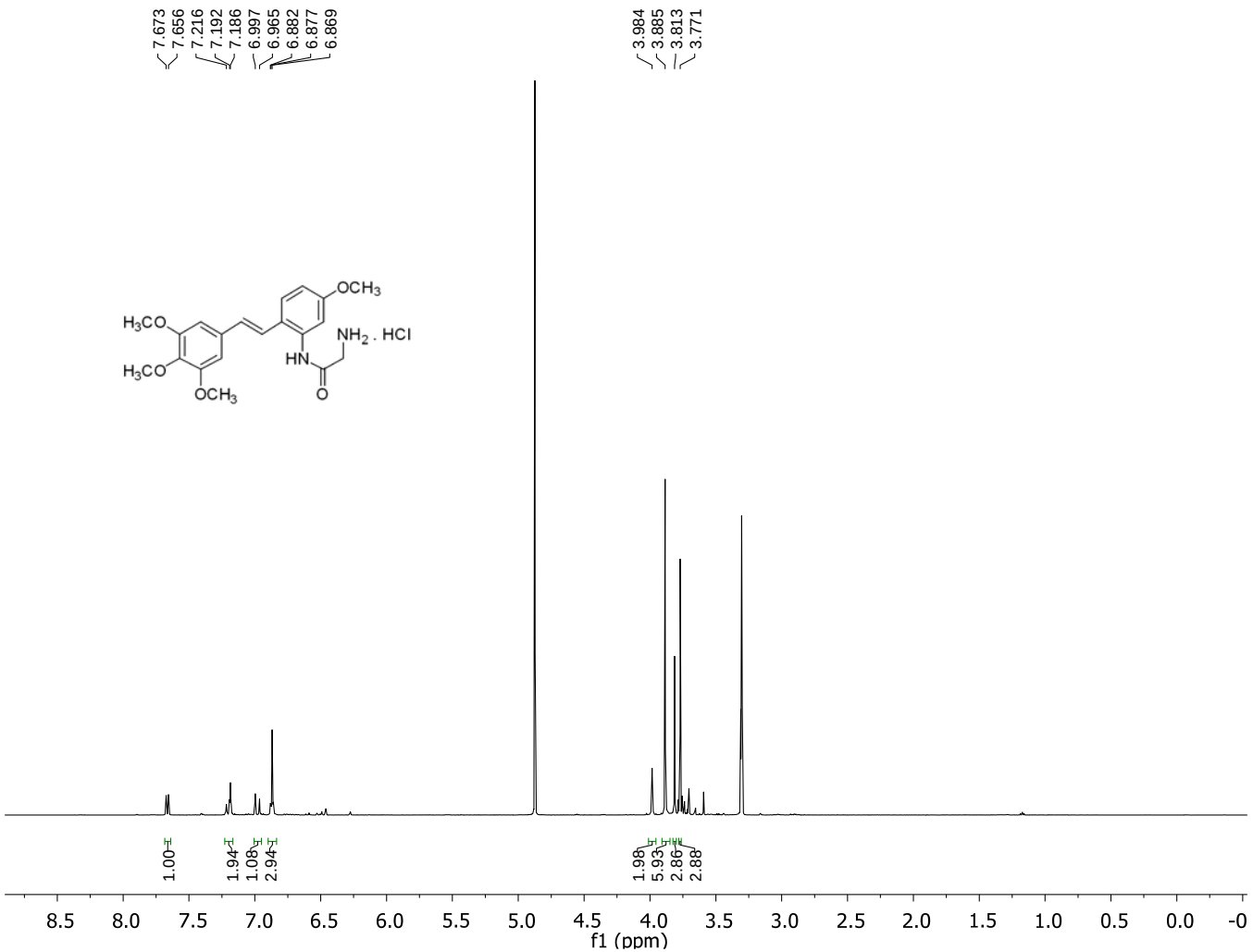
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.812	BB	0.0888	138.41168	25.71216	1.2652
2	12.993	BV	0.0620	88.38454	22.39664	0.8079
3	13.226	VB	0.0887	1.06324e4	1859.13770	97.1902
4	14.014	VB	0.0646	15.00118	3.60392	0.1371
5	15.675	VB	0.0780	65.59080	12.75470	0.5996
Totals :				1.09398e4	1923.60512	

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

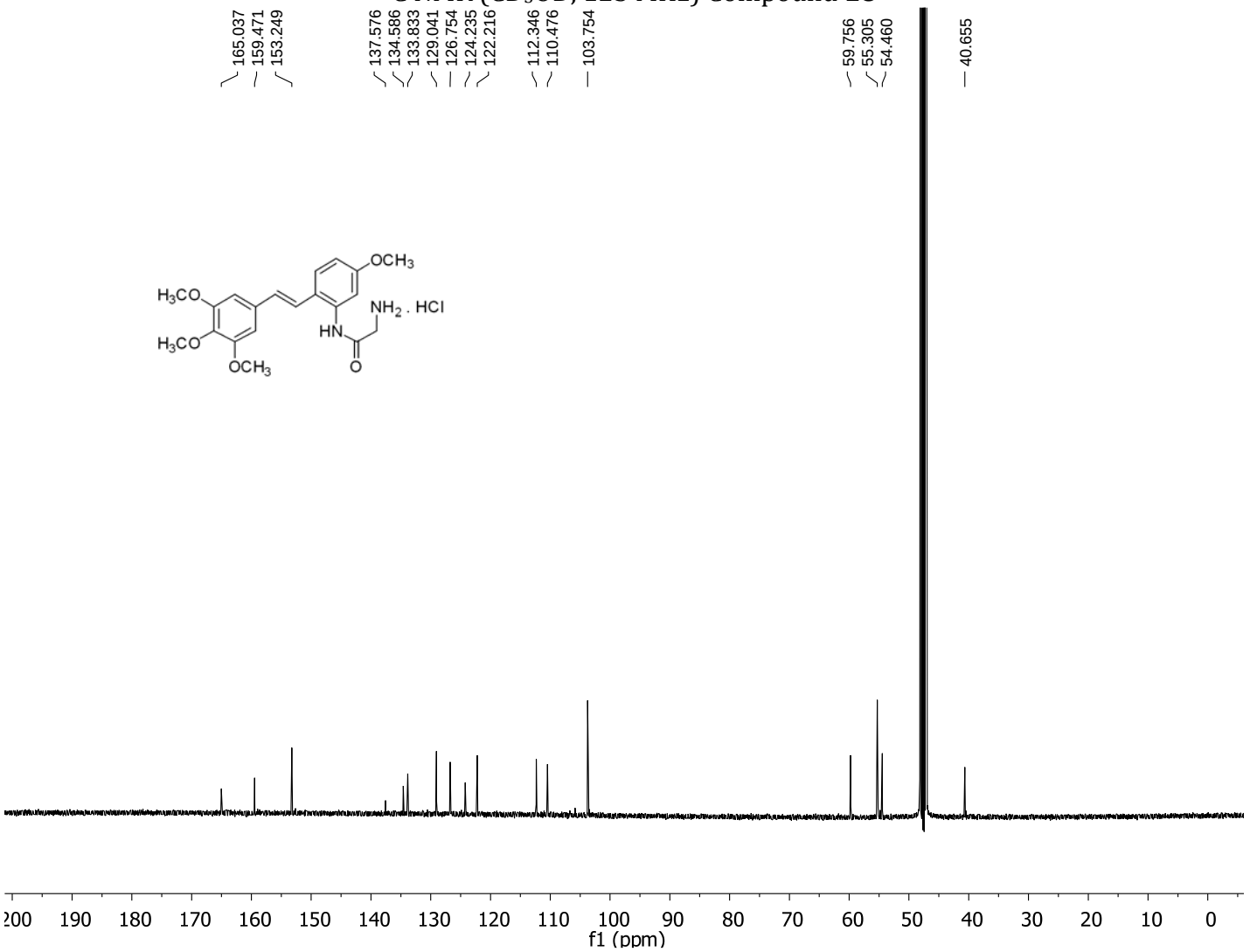
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.810	BB	0.0877	154.40775	29.18997	1.0733
2	13.107	BV	0.0546	66.88392	19.22609	0.4649
3	13.226	VB	0.0901	1.38478e4	2443.69946	96.2531
4	13.889	BV	0.0742	109.93297	22.84040	0.7641
5	14.013	VB	0.0647	19.88695	4.76055	0.1382
6	14.246	BB	0.0723	54.22525	11.65682	0.3769
7	15.505	VV	0.0698	57.71371	12.52080	0.4012
8	15.675	VB	0.0779	76.01604	14.81508	0.5284
Totals :				1.43869e4	2558.70918	

==========

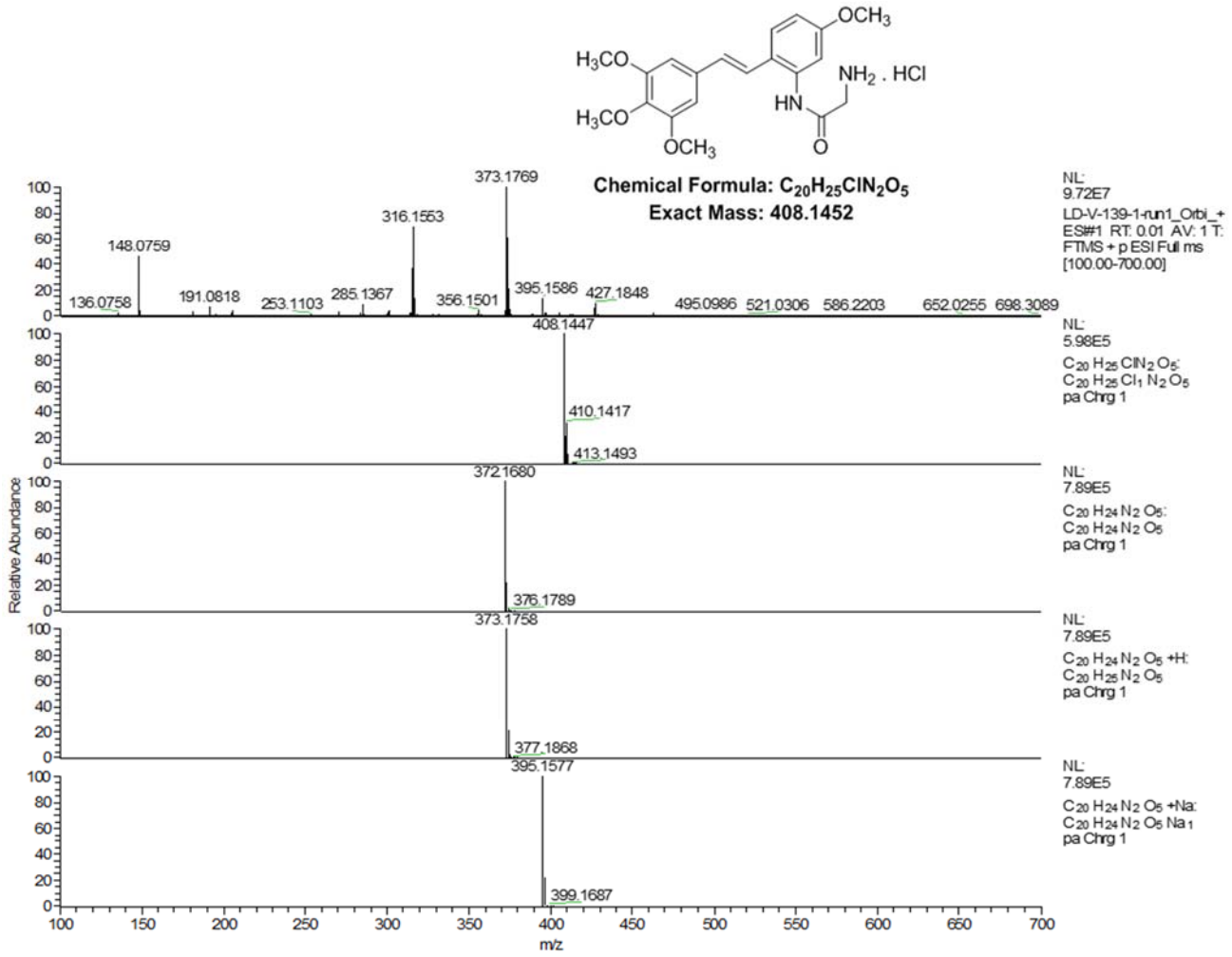
^{1}H NMR (CD_3OD, 500 MHz) Compound **18**



^{13}C NMR (CD_3OD, 125 MHz) Compound **18**



HRMS Compound **18**



HPLC for Compound **18**

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-139-2RUN01.D
Sample Name: LD-V-139-2run1

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Acq. Instrument : Instrument 1                    Location :   -
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Last changed    : 7/13/2014 12:04:39 PM by Laxman
Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-V-139-2RUN01.D\DA.M (MASTERMETHOD.M)
Last changed    : 7/13/2014 1:05:46 PM by Laxman
Sample Info     : Method
                  Mastermethod.M
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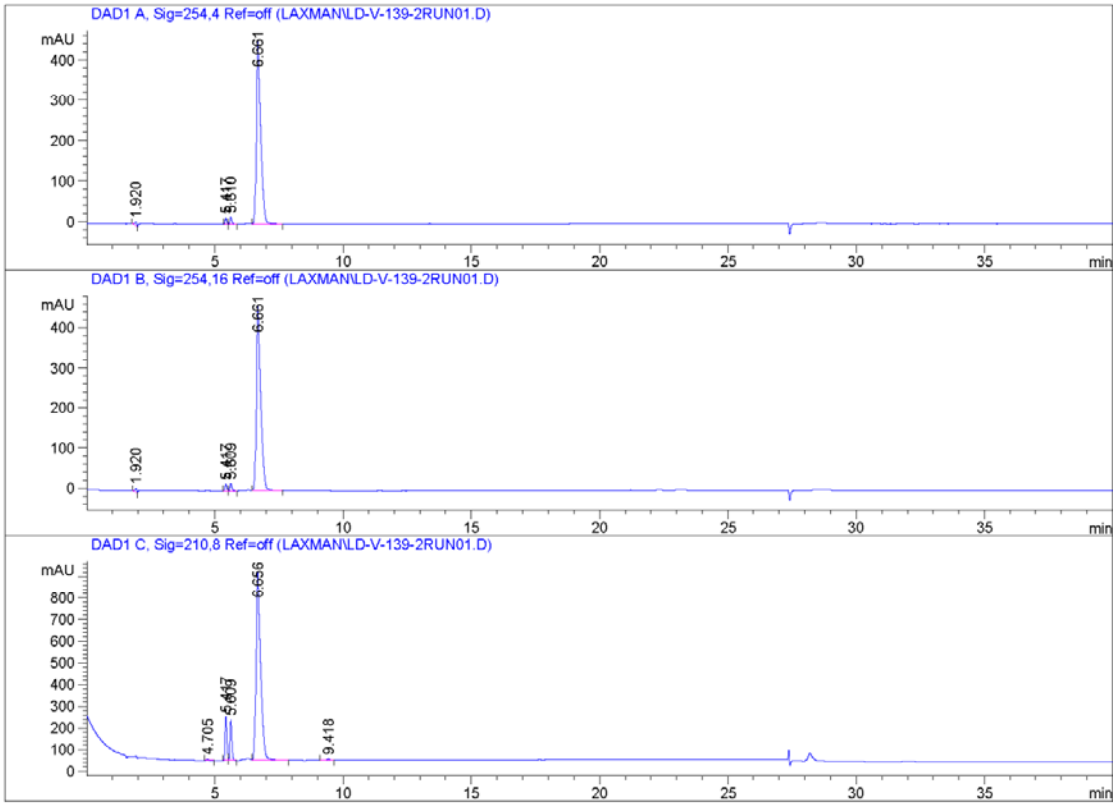
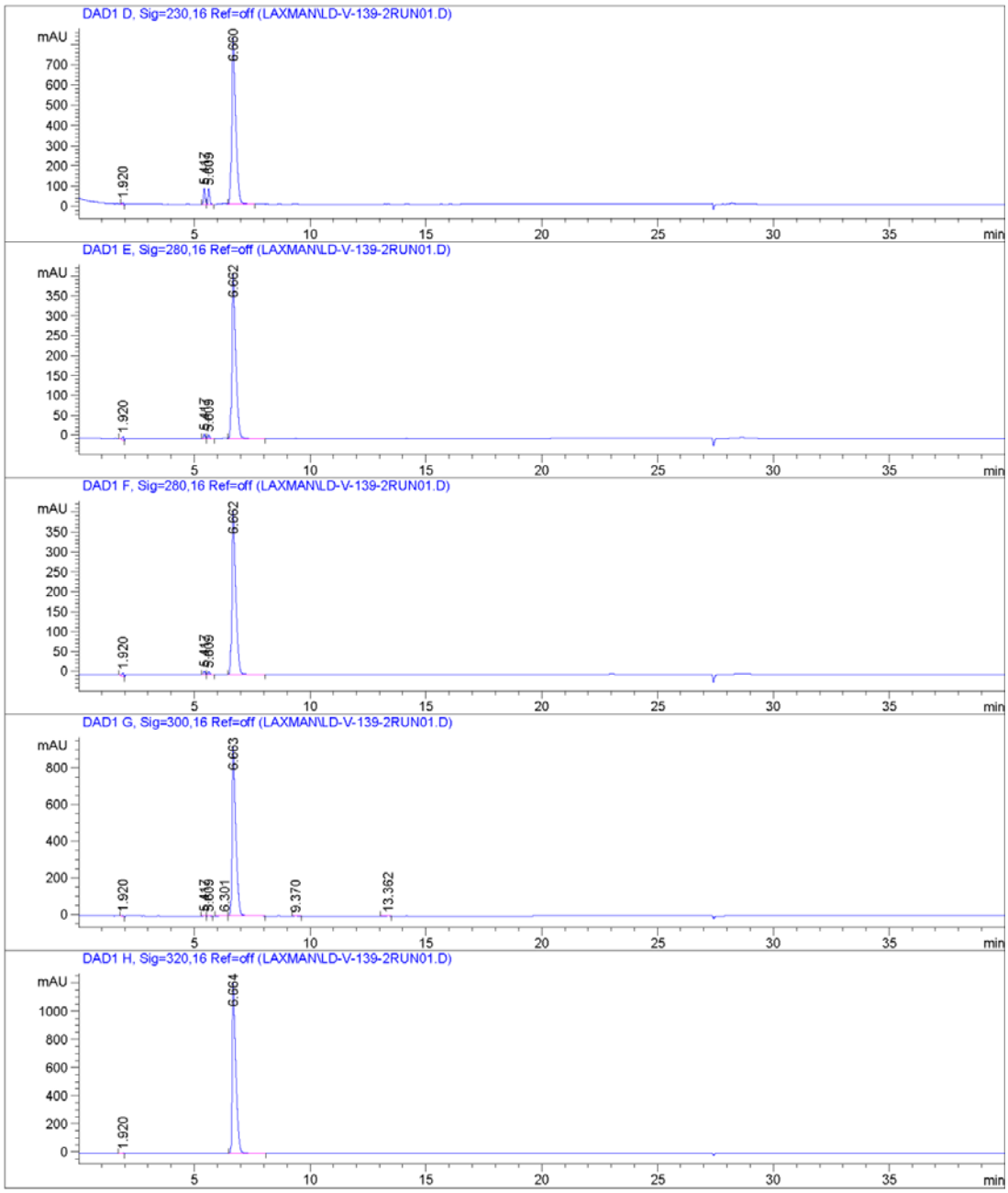


Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-139-2RUN01.D
Sample Name: LD-V-139-2run1



Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-139-2RUN01.D
Sample Name: LD-V-139-2run1

==========
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	1.920	BB	0.0775	46.97380	9.52554	0.8652
2	5.417	BV	0.0785	73.83537	14.72783	1.3600
3	5.610	VB	0.0783	87.99896	17.59993	1.6209
4	6.661	VB	0.1616	5220.14502	455.76682	96.1538
Totals :				5428.95316	497.62012	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.920	BB	0.0764	44.75594	9.26610	0.8119
2	5.417	BV	0.0785	80.30719	16.00931	1.4569
3	5.609	VB	0.0784	90.60959	18.10985	1.6438
4	6.661	VB	0.1615	5296.55029	462.70700	96.0874
Totals :				5512.22301	506.09225	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.705	BB	0.0736	31.72051	6.42426	0.2502
2	5.417	BV	0.0786	1011.34918	201.44394	7.9766
3	5.609	VB	0.0784	932.56183	186.28250	7.3552
4	6.656	VB	0.1680	1.06542e4	875.27972	84.0301
5	9.418	BB	0.1088	49.18766	6.46056	0.3879
Totals :				1.26790e4	1275.89098	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-139-2RUN01.D
Sample Name: LD-V-139-2run1

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.920	BB	0.0702	44.30437	10.30551	0.4233
2	5.417	BV	0.0786	388.58575	77.37257	3.7130
3	5.609	VB	0.0784	381.26849	76.18825	3.6430
4	6.660	VB	0.1644	9651.52832	825.70642	92.2207
Totals :				1.04657e4	989.57275	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.920	BB	0.0868	42.78221	7.47017	0.8821
2	5.417	BV	0.0786	51.21740	10.19095	1.0560
3	5.609	VB	0.0786	44.75695	8.91765	0.9228
4	6.662	VB	0.1583	4711.51318	415.42648	97.1392
Totals :				4850.26974	442.00525	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.920	BB	0.0868	42.78221	7.47017	0.8821
2	5.417	BV	0.0786	51.21740	10.19095	1.0560
3	5.609	VB	0.0786	44.75695	8.91765	0.9228
4	6.662	VB	0.1583	4711.51318	415.42648	97.1392
Totals :				4850.26974	442.00525	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.920	BB	0.0774	26.29604	5.34806	0.2568
2	5.417	BV	0.0789	11.63756	2.30688	0.1137
3	5.609	VB	0.0787	10.18231	2.02301	0.0995
4	6.301	BV	0.2271	25.72116	1.55393	0.2512
5	6.663	VB	0.1552	1.01509e4	930.72180	99.1451

Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-139-2RUN01.D
Sample Name: LD-V-139-2run1

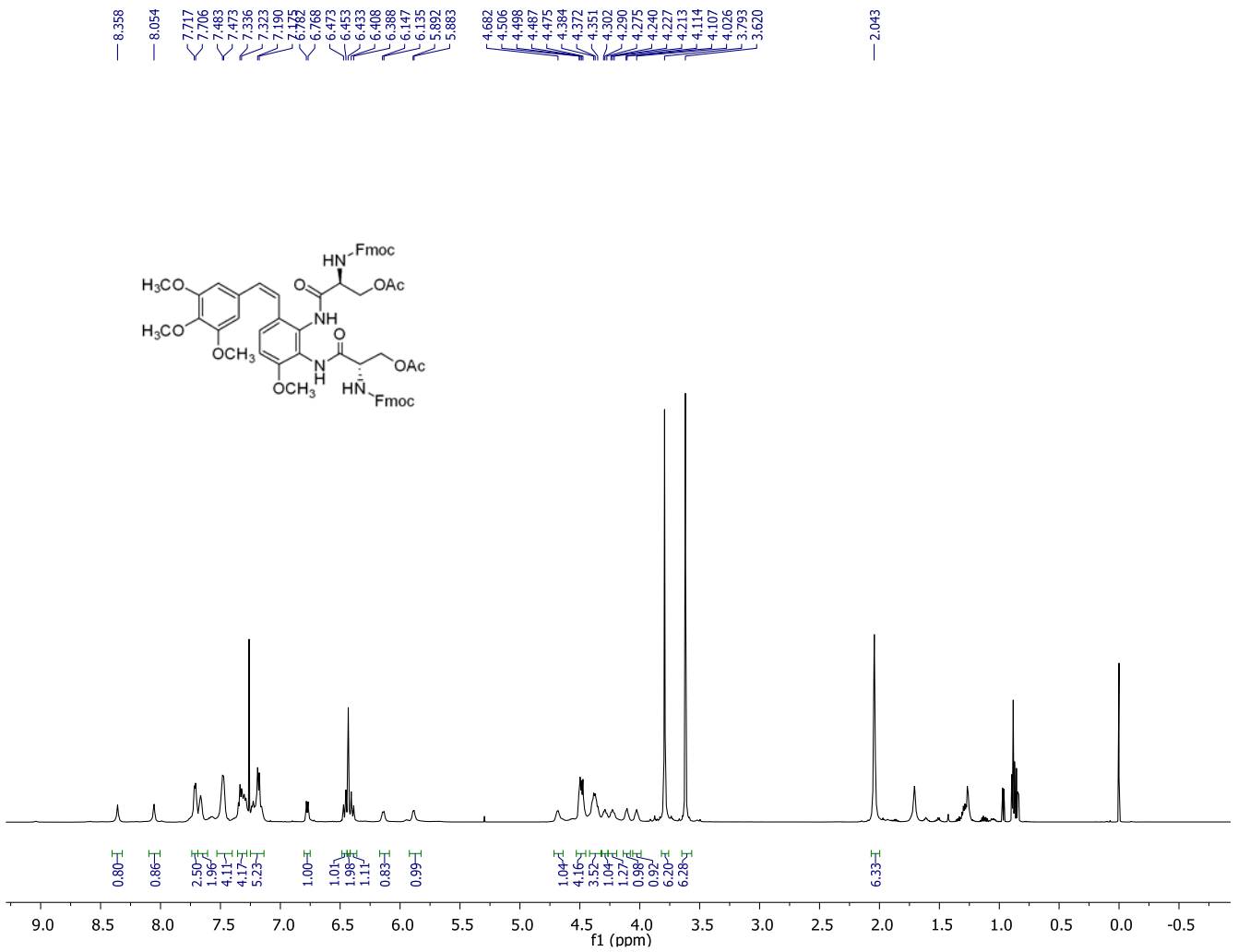
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
6	9.370	BB	0.0844	5.83878	1.05772	0.0570
7	13.362	BB	0.1049	7.84864	1.07818	0.0767
Totals :				1.02384e4	944.08958	

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

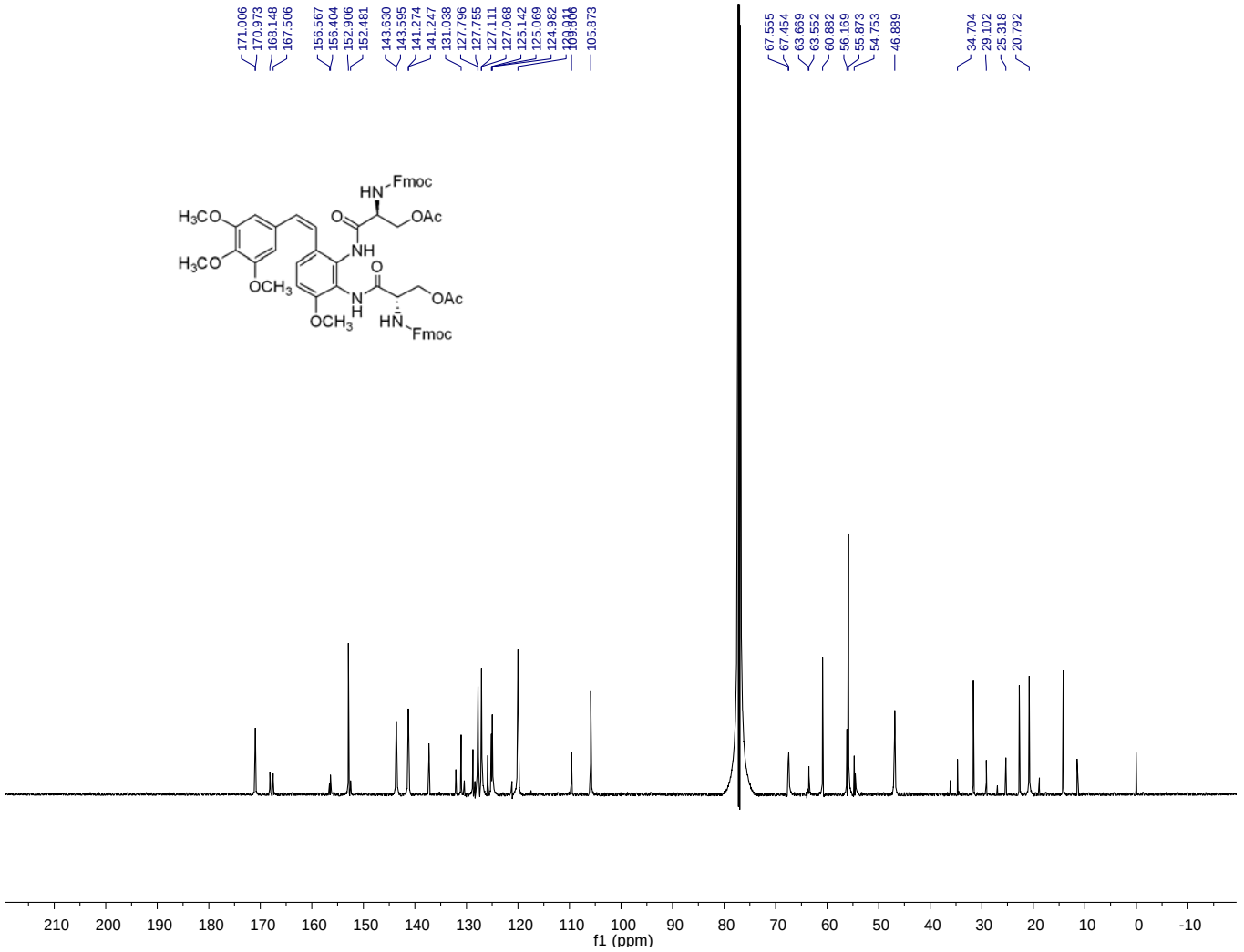
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.920	BB	0.0894	29.69990	4.99136	0.2281
2	6.664	VB	0.1527	1.29935e4	1215.47620	99.7719
Totals :				1.30232e4	1220.46756	

*** End of Report ***

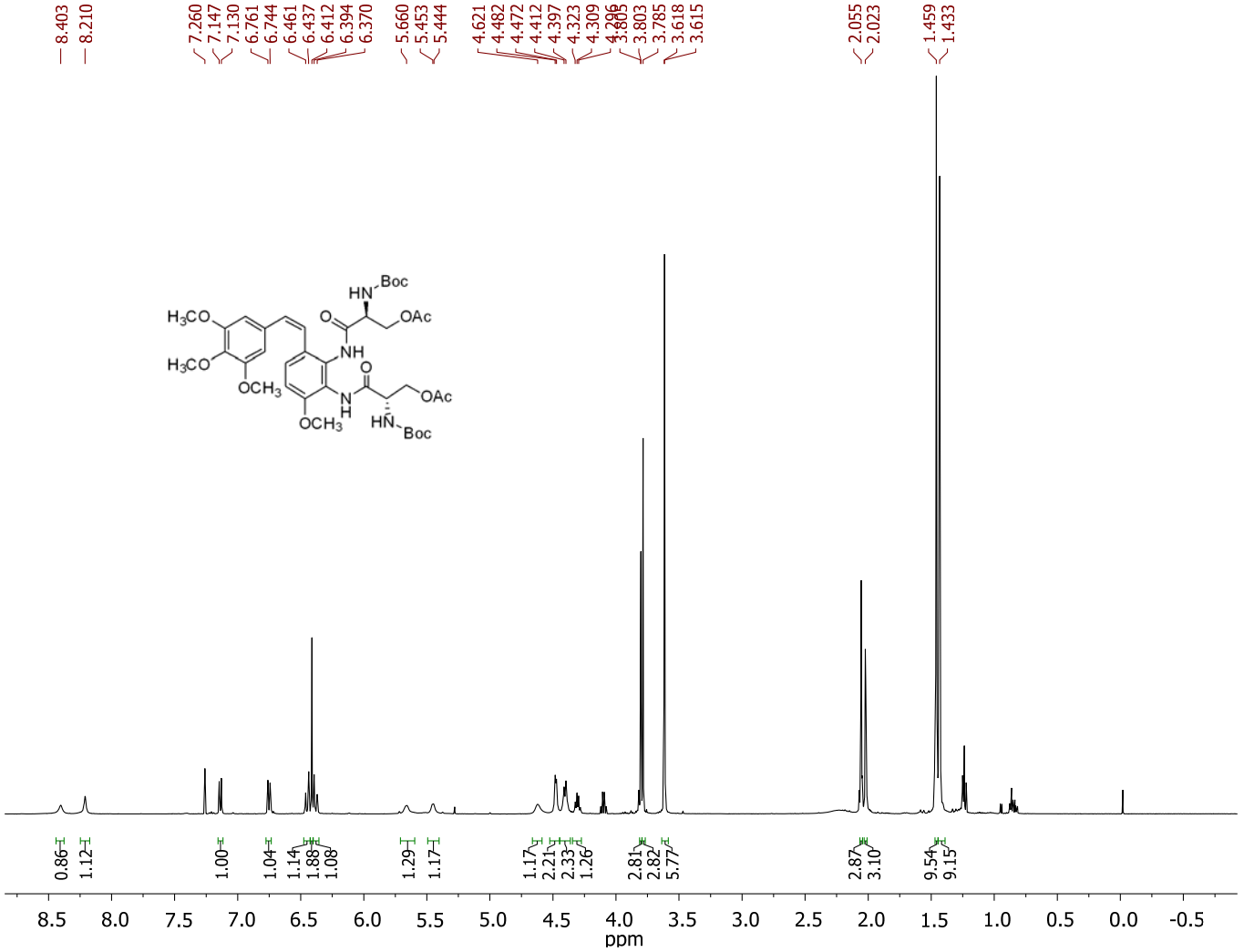
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **19**



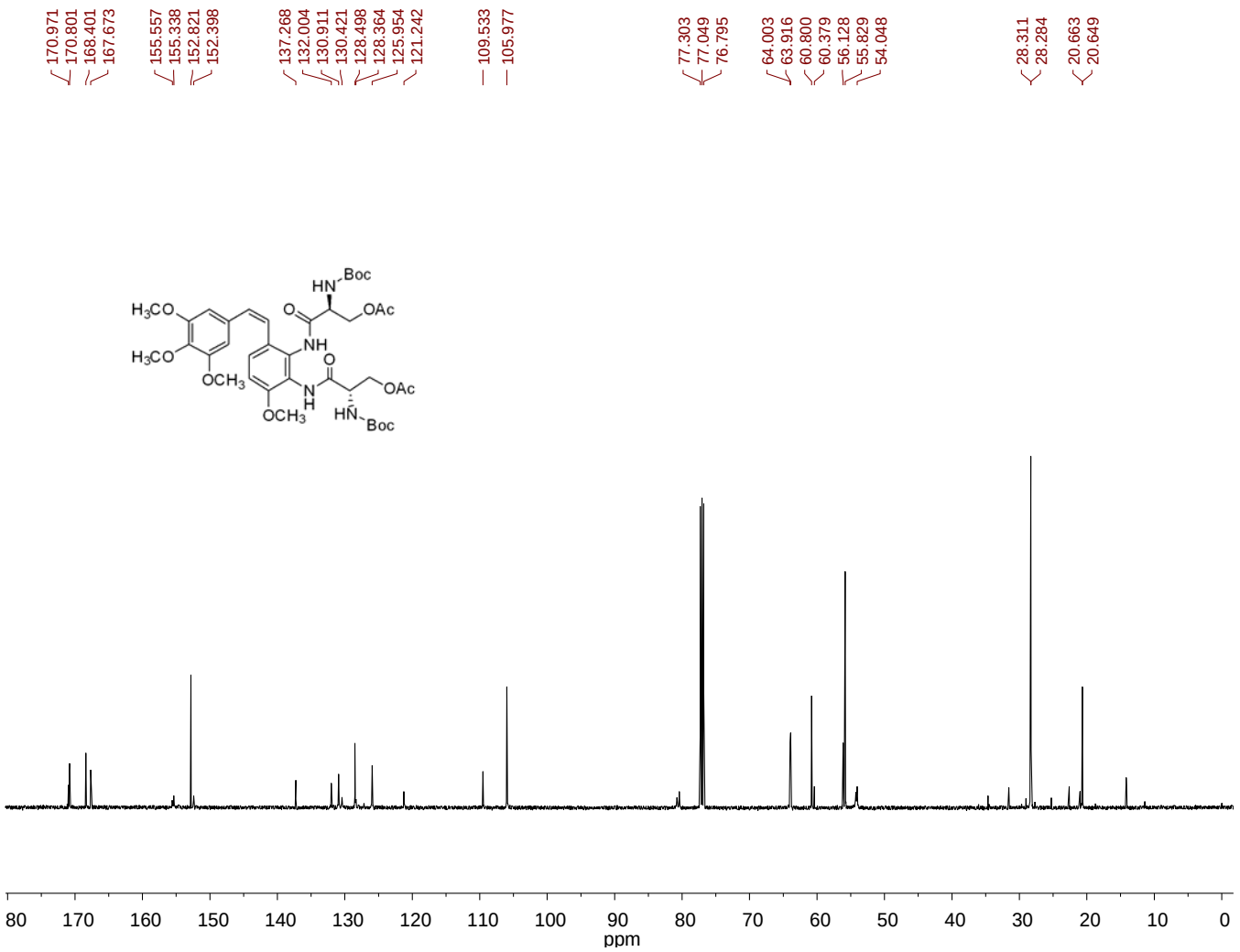
^{13}C NMR ($CDCl_3$, 150 MHz) Compound **19**



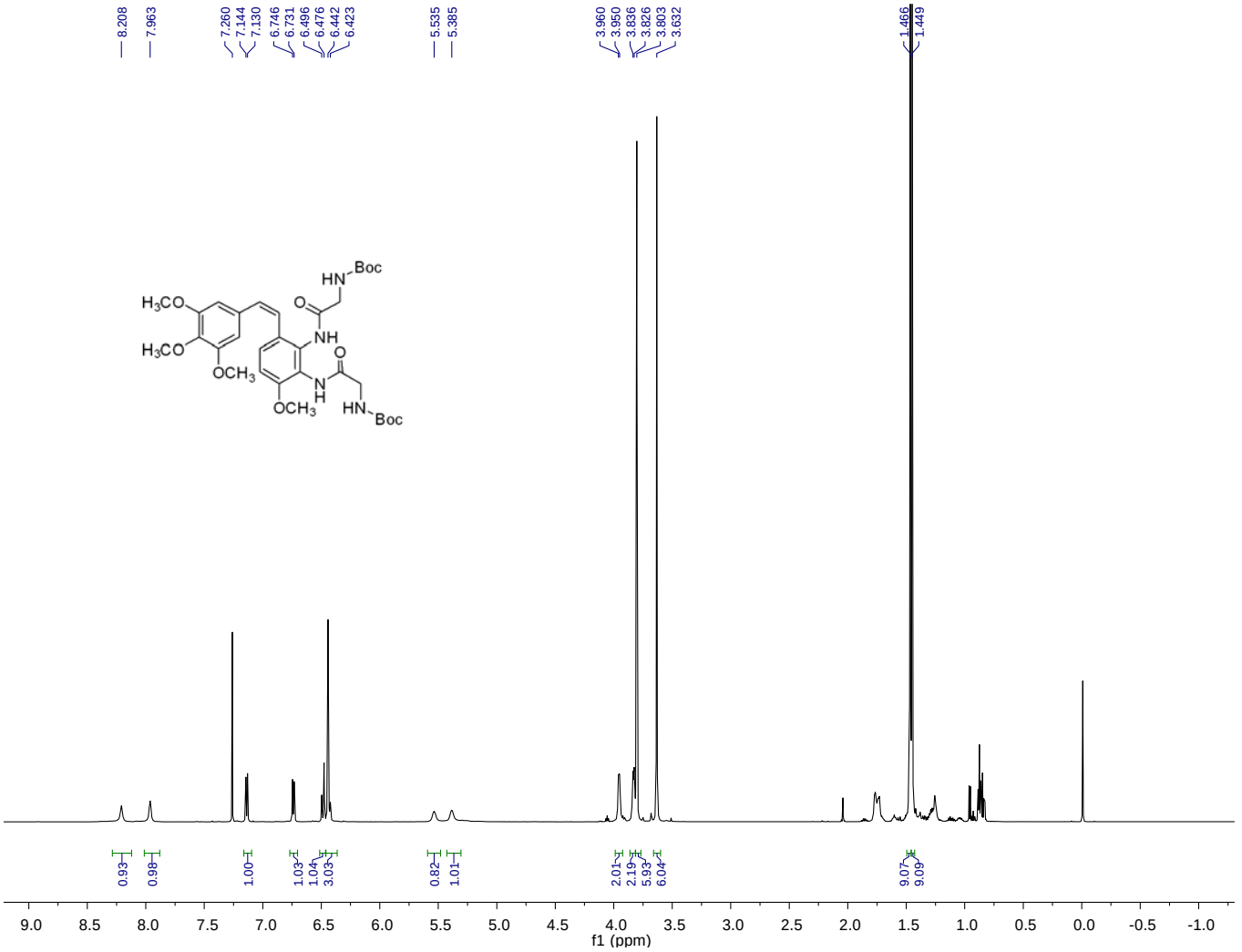
^{1}H NMR ($CDCl_3$, 500 MHz) Compound **20**



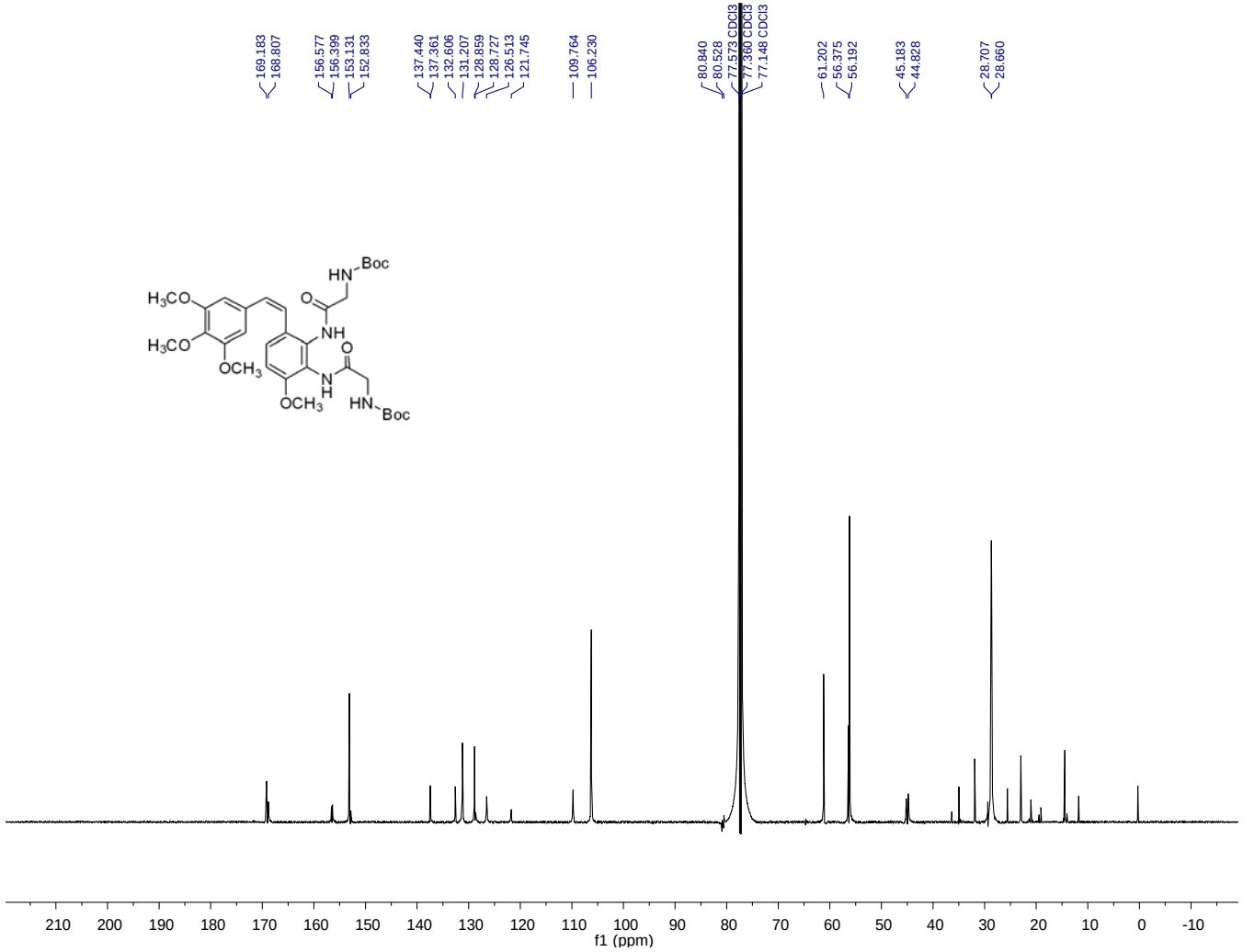
^{13}C NMR ($CDCl_3$, 125 MHz) Compound **20**



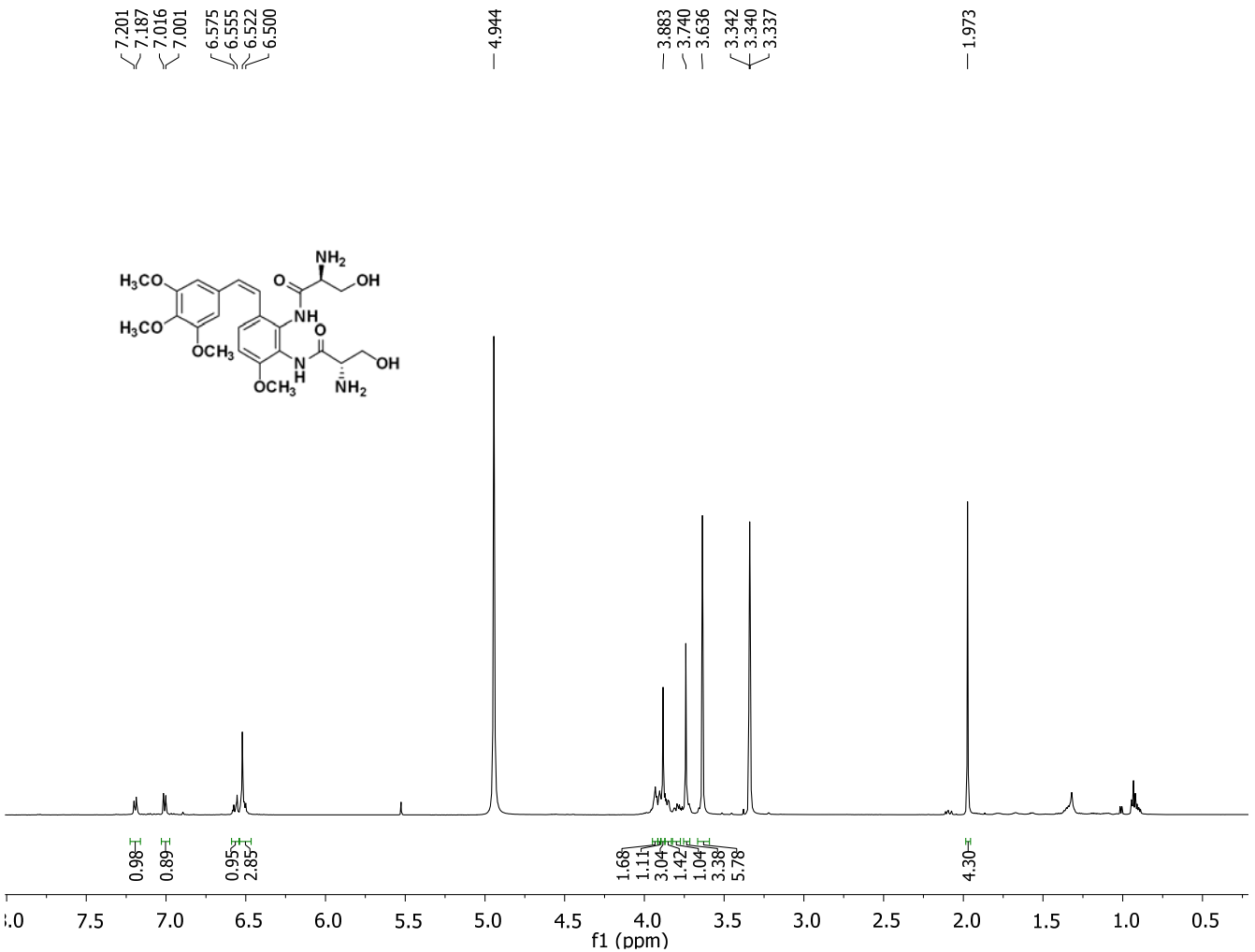
^{1}H NMR ($CDCl_3$, 600 MHz) Compound **21**



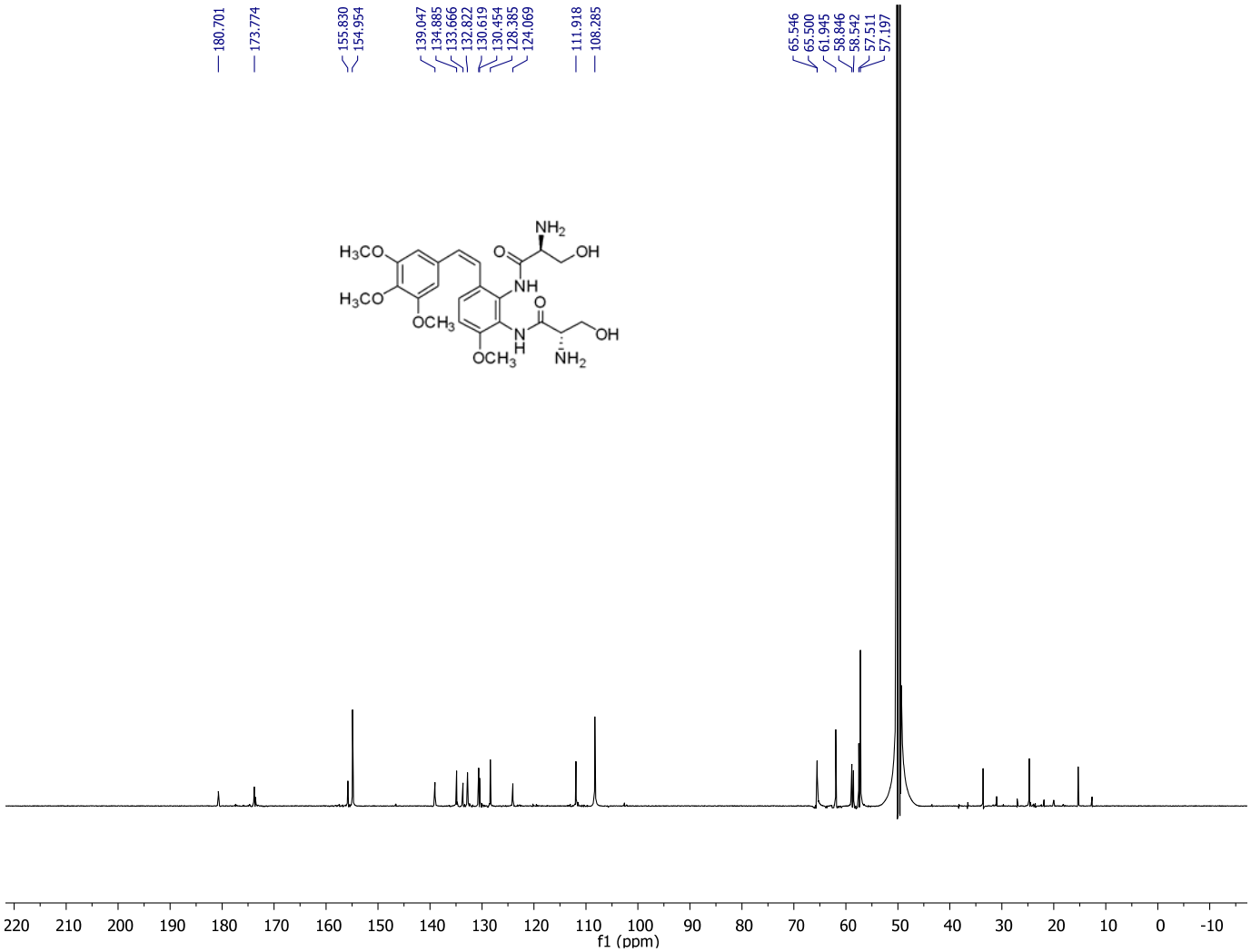
^{13}C NMR ($CDCl_3$, 150 MHz) Compound **21**



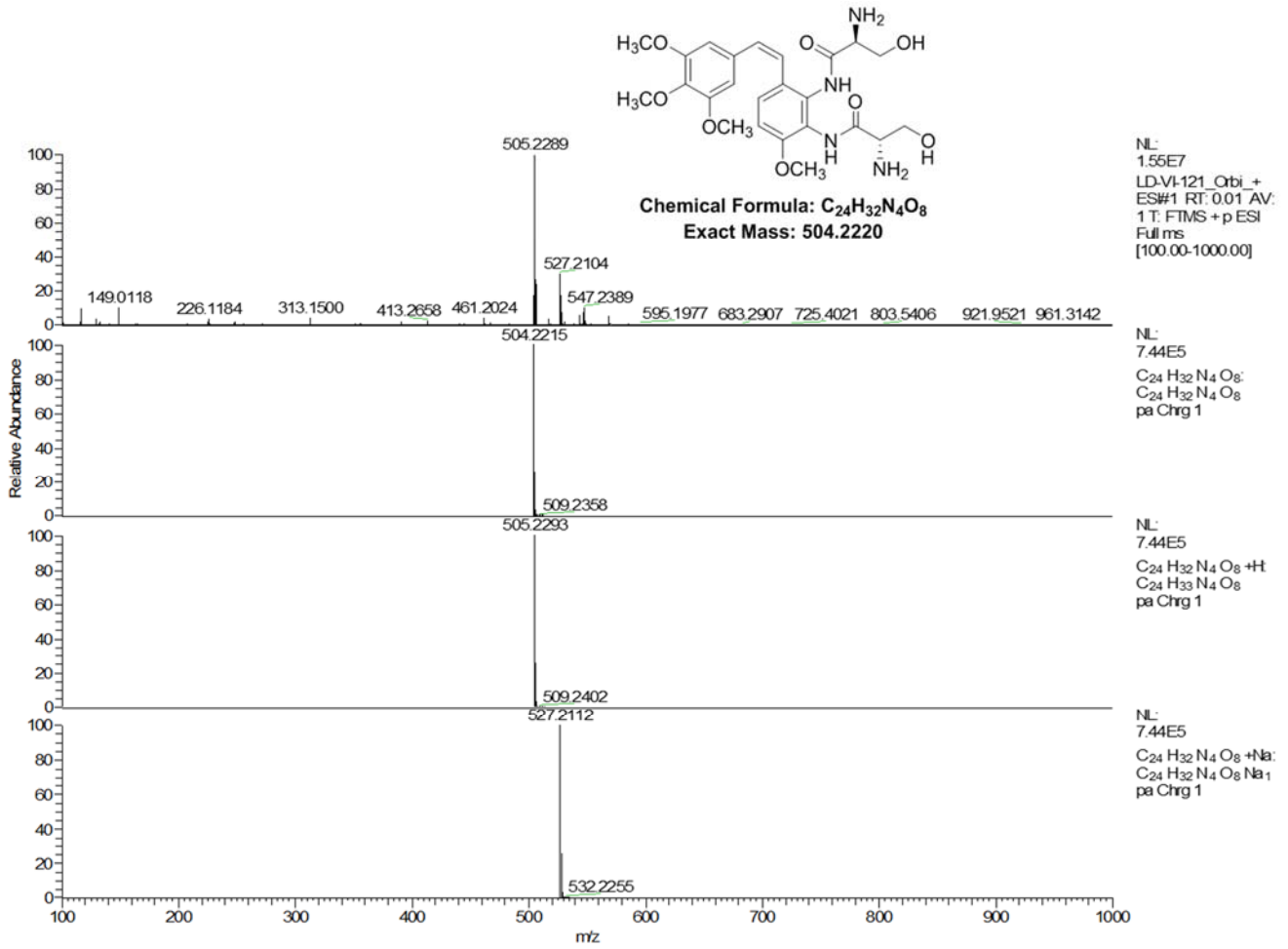
^{1}H NMR (CD_3OD, 600 MHz) Compound **22**



^{13}C NMR (CD_3OD, 150 MHz) Compound **22**



HRMS Compound **22**



HPLC for Compound **22**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-121-RUN05.D
Sample Name: LD-VI-121-run5

    ======================================================================
    Acq. Operator   : Laxman
    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 2/4/2015 9:18:24 AM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD2.M
    Last changed    : 2/4/2015 9:15:35 AM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-VI-121-RUN05.D\DA.M (MASTERMETHOD2.M)
    Last changed    : 2/4/2015 10:03:37 AM by Laxman
    Sample Info     : Method-Mastermethod2
                      0.05% TFA/H2O
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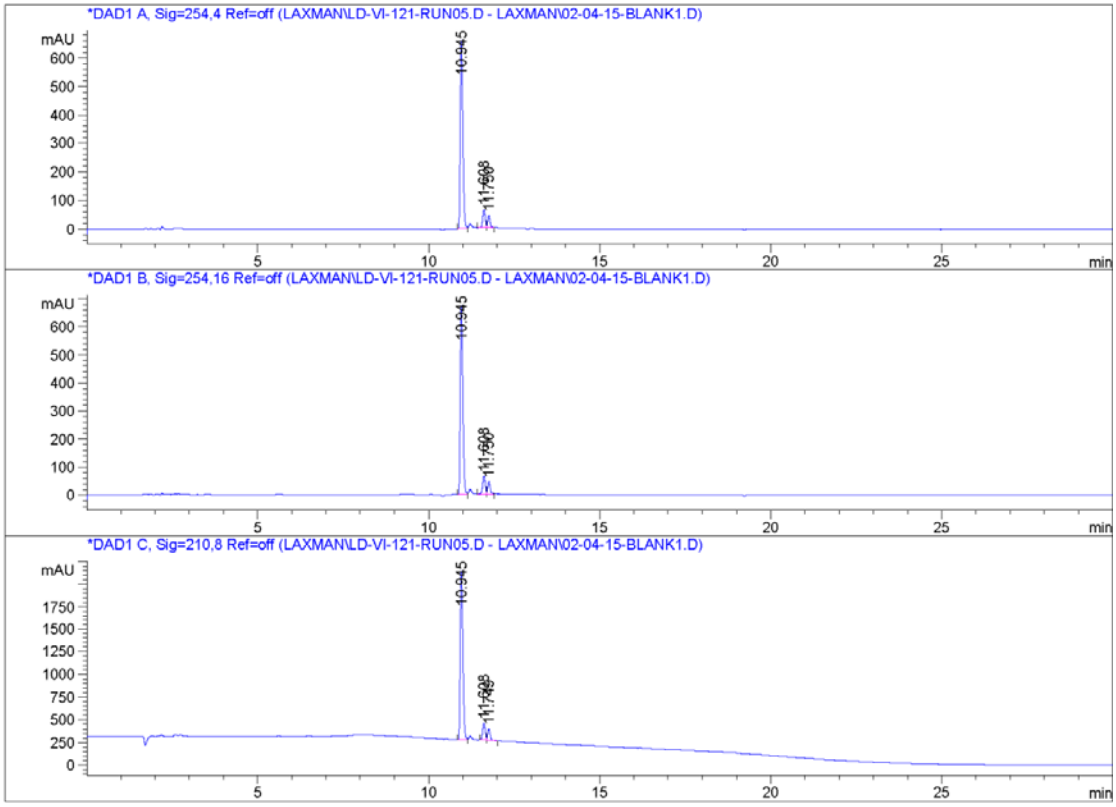
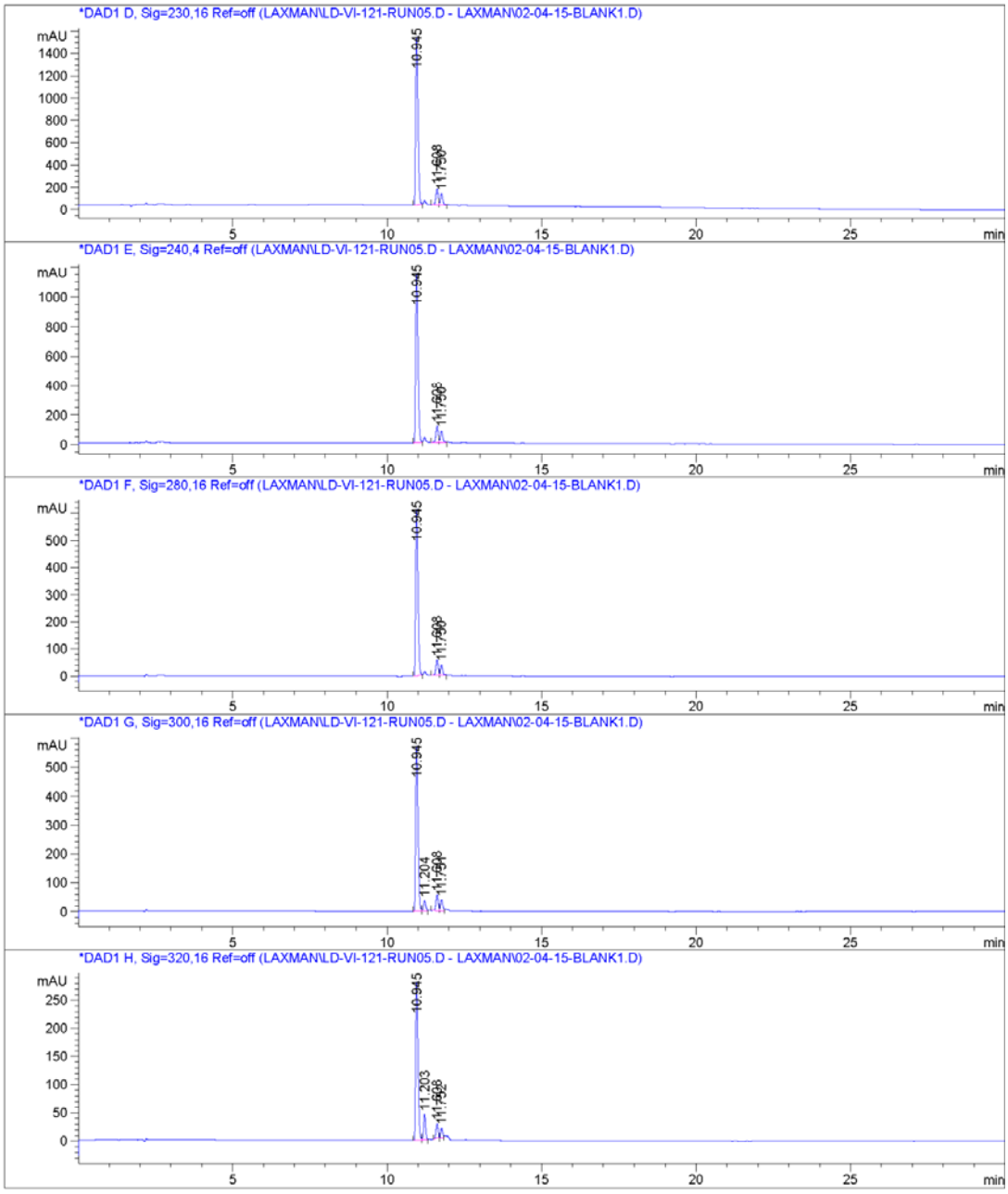


Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-121-RUN05.D
Sample Name: LD-VI-121-run5



Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-121-RUN05.D
Sample Name: LD-VI-121-run5

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.945	BV	0.0806	3429.38184	660.40332	85.7238
2	11.608	BV	0.0818	342.65540	64.67617	8.5653
3	11.750	VV	0.0771	228.46587	45.06903	5.7109
Totals :				4000.50310	770.14853	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.945	BV	0.0806	3503.13452	674.69476	85.7581
2	11.608	BV	0.0818	348.86224	65.84724	8.5403
3	11.750	VV	0.0771	232.90524	45.94286	5.7016
Totals :				4084.90201	786.48487	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.945	BV	0.0842	9926.38086	1861.91199	86.6126
2	11.608	BV	0.0789	927.72119	183.65503	8.0948
3	11.749	VB	0.0748	606.56696	124.58265	5.2926
Totals :				1.14607e4	2170.14967	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-121-RUN05.D
Sample Name: LD-VI-121-run5

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.945	BV	0.0808	7864.29395	1509.11328	85.7517
2	11.608	BV	0.0822	780.64148	146.49777	8.5121
3	11.750	VV	0.0783	526.07617	101.73031	5.7363
Totals :				9171.01160	1757.34136	

Signal 5: DAD1 E, Sig=240,4 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.945	BV	0.0806	5953.35156	1145.60352	85.4289
2	11.608	BV	0.0823	600.10669	112.39833	8.6114
3	11.750	VV	0.0793	415.31732	79.05017	5.9597
Totals :				6968.77557	1337.05202	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.945	BV	0.0805	3185.41992	613.96997	86.4240
2	11.608	BV	0.0817	305.75058	57.77947	8.2954
3	11.750	VV	0.0770	194.63496	38.46372	5.2807
Totals :				3685.80547	710.21316	

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	10.945	BV	0.0809	2988.00757	572.34558	81.8322
2	11.204	VV	0.0725	169.99767	35.08196	4.6557
3	11.608	BV	0.0823	296.85989	55.58070	8.1301
4	11.751	VV	0.0785	196.51675	37.88121	5.3820

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-121-RUN05.D
Sample Name: LD-VI-121-run5

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				3651.38188	700.88945	

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	10.945	BV	0.0814	1481.12476	281.65887	78.3325
2	11.203	VV	0.0685	204.32208	45.42173	10.8060
3	11.608	BB	0.0749	121.84870	25.89132	6.4442
4	11.752	BV	0.0733	83.52104	17.62517	4.4172
Totals :				1890.81658	370.59708	

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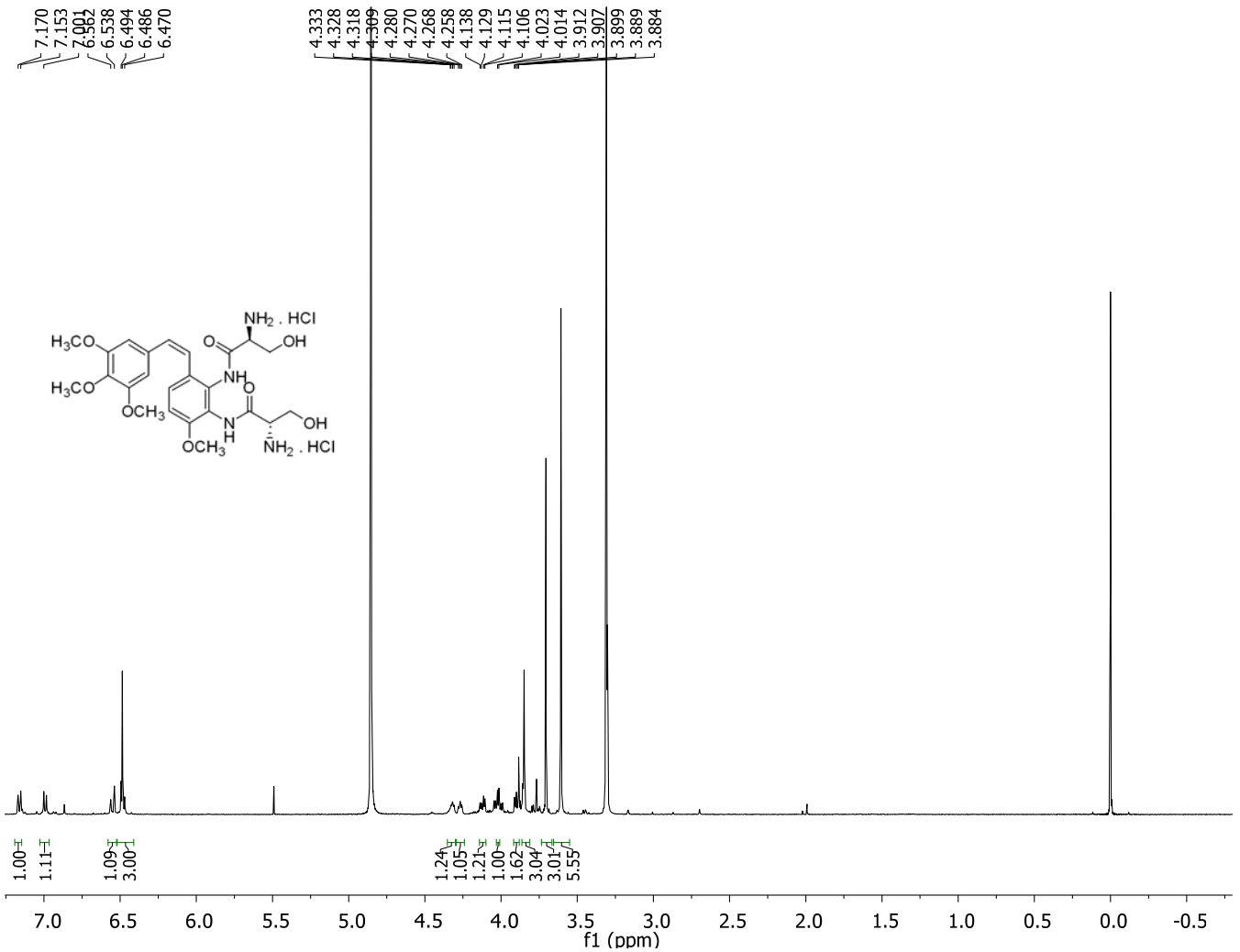
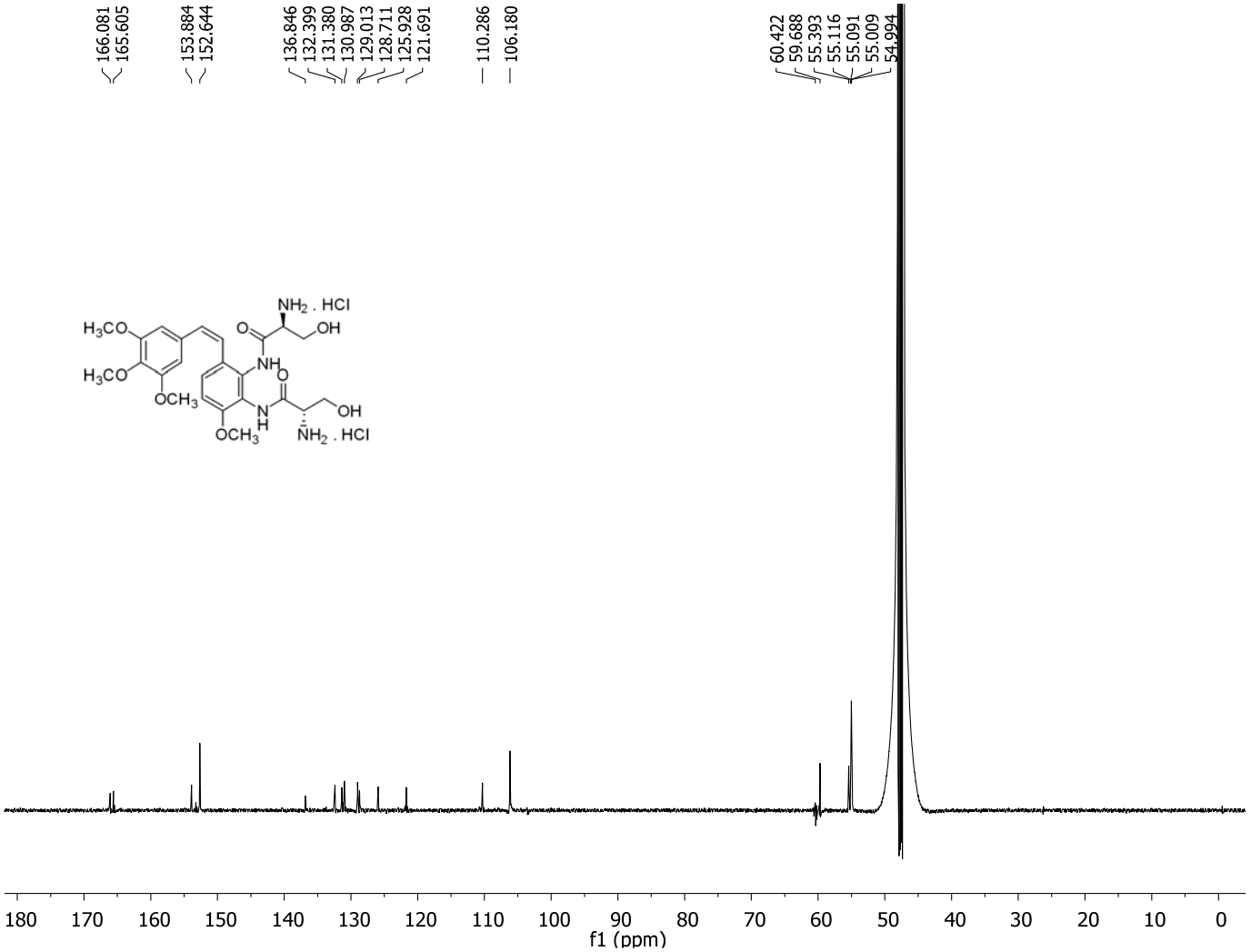
*** End of Report ***

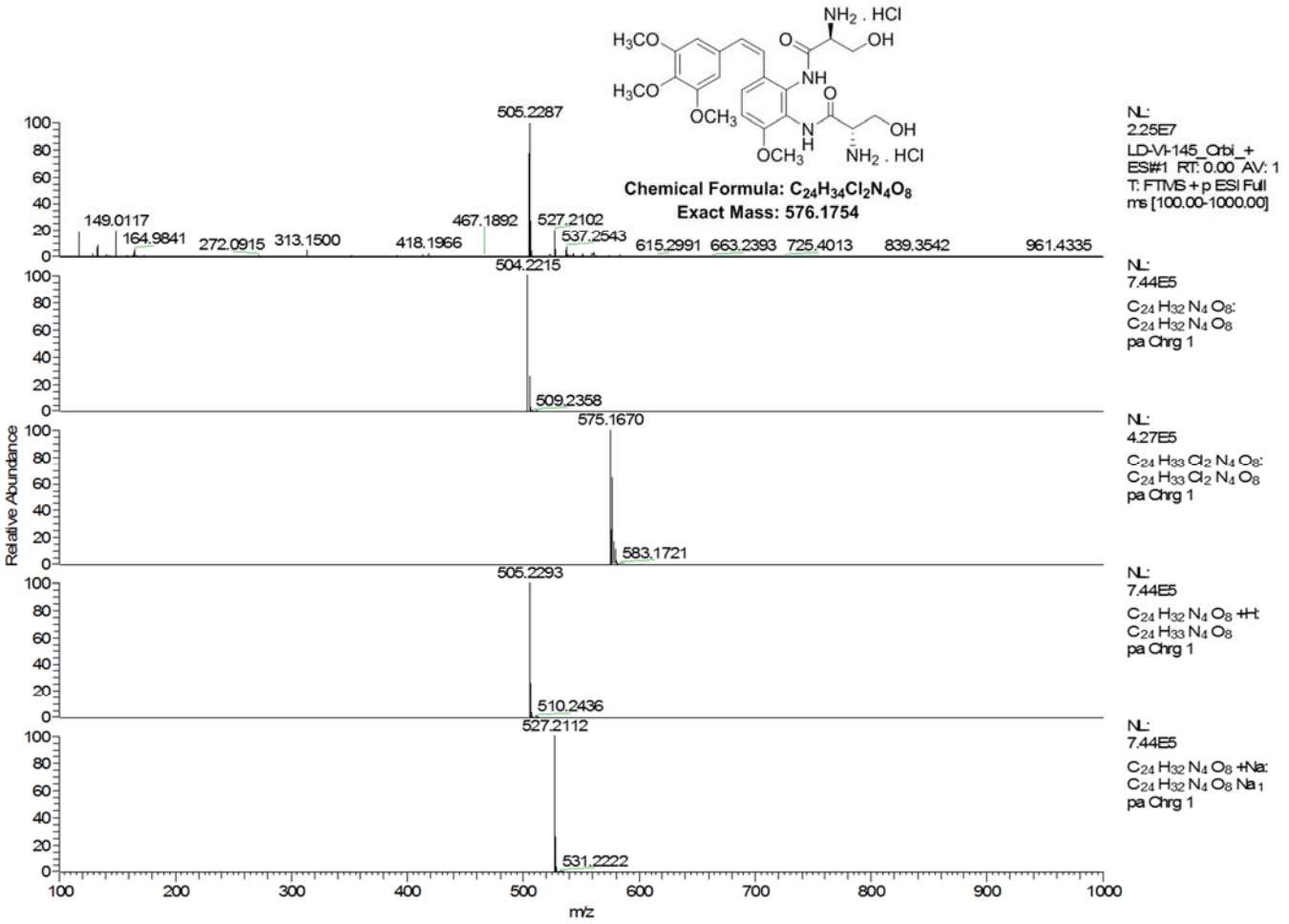
^{1}H NMR (CD_3OD, 500 MHz) Compound **23**



^{13}C NMR (CD_3OD, 150 MHz) Compound **23**



HRMS Compound **23**



HPLC for Compound **23**

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Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-145-00002.D
Sample Name: LD-VI-145-run2

    ======================================================================
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    Acq. Instrument : Instrument 1                  Location :   -
    Injection Date  : 2/3/2015 10:54:00 AM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD2.M
    Last changed    : 2/3/2015 10:52:45 AM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-VI-145-00002.D\DA.M (MASTERMETHOD2.M)
    Last changed    : 2/4/2015 9:20:40 AM by Laxman
    Sample Info     : Method-Mastermethod2
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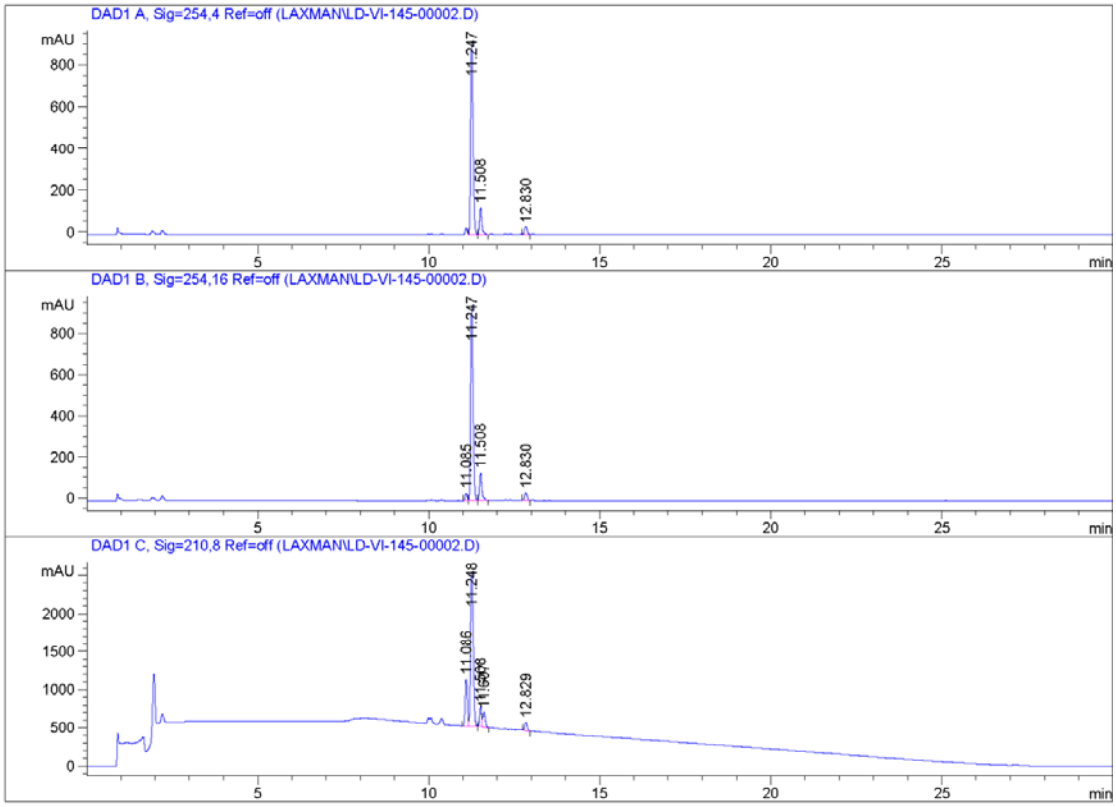
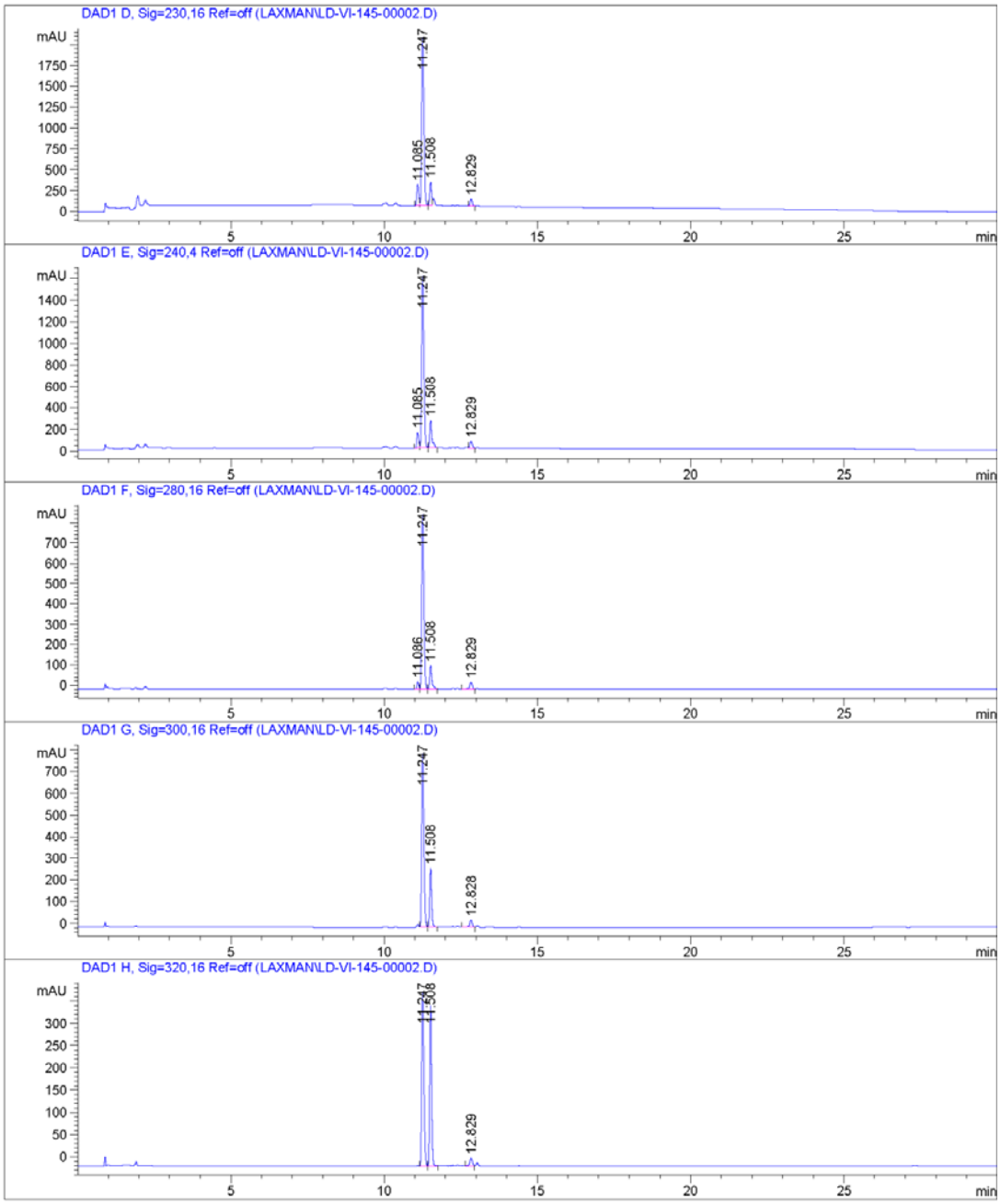


Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-145-00002.D
Sample Name: LD-VI-145-run2



Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-145-00002.D
Sample Name: LD-VI-145-run2

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

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.247	VV	0.0750	4529.85498	927.61292	84.7722
2	11.508	VB	0.0703	604.70068	129.86781	11.3164
3	12.830	VV	0.0862	209.00600	38.00933	3.9114
Totals :				5343.56166	1095.49006	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.085	BV	0.0666	158.56816	36.54854	2.8155
2	11.247	VV	0.0750	4626.72656	947.20282	82.1503
3	11.508	VB	0.0711	634.57526	134.26546	11.2673
4	12.830	VV	0.0861	212.15869	38.62234	3.7670
Totals :				5632.02867	1156.63916	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.086	BV	0.0678	2736.85107	615.96875	16.5802
2	11.248	VB	0.0883	1.11715e4	2027.12781	67.6783
3	11.508	BV	0.0638	1073.51233	262.24918	6.5035
4	11.607	VB	0.0726	937.48230	193.35208	5.6794
5	12.829	VB	0.0846	587.43060	109.54142	3.5587
Totals :				1.65068e4	3208.23923	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-145-00002.D
Sample Name: LD-VI-145-run2

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.085	BV	0.0671	1128.58777	257.43103	8.7839
2	11.247	VB	0.0801	1.00606e4	2019.74780	78.3026
3	11.508	BV	0.0658	1199.73914	281.16333	9.3377
4	12.829	VB	0.0847	459.43491	85.48837	3.5758
Totals :				1.28483e4	2643.83054	

Signal 5: DAD1 E, Sig=240,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.085	BV	0.0672	634.01740	144.39835	6.2688
2	11.247	VV	0.0754	7837.16797	1593.89563	77.4891
3	11.508	VB	0.0754	1302.56641	255.79105	12.8790
4	12.829	VB	0.0847	340.14511	63.32522	3.3631
Totals :				1.01139e4	2057.41024	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.086	BV	0.0671	158.43652	36.17655	3.0899
2	11.247	VV	0.0748	4190.94482	860.92053	81.7330
3	11.508	VV	0.0736	576.60266	116.70569	11.2451
4	12.829	BV	0.0917	201.62273	33.76098	3.9321
Totals :				5127.60674	1047.56375	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.247	VV	0.0750	3908.86646	800.75116	74.1305
2	11.508	VB	0.0668	1175.77905	270.14182	22.2983
3	12.828	BV	0.0913	188.30544	31.69815	3.5712
Totals :				5272.95094	1102.59113	

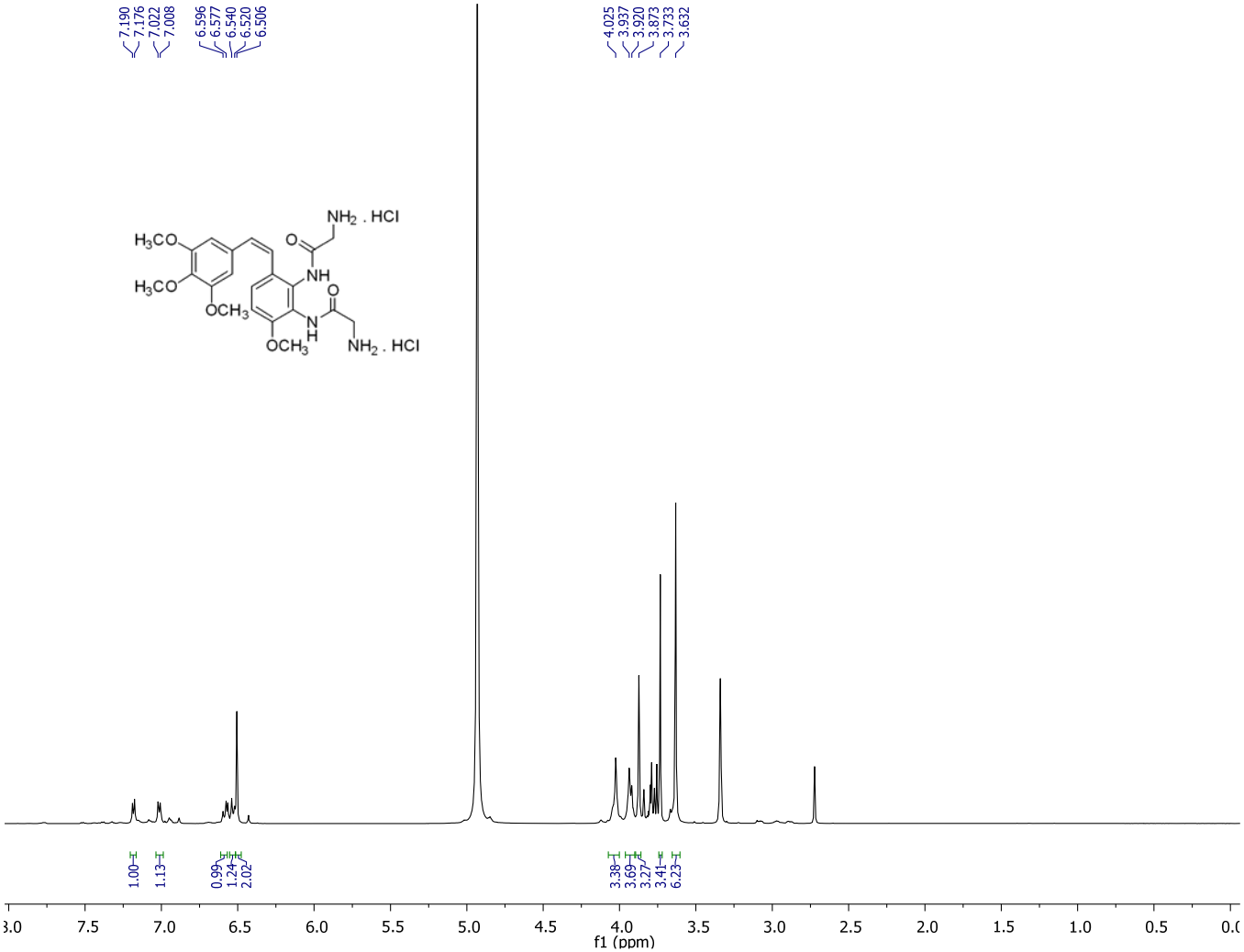
Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-145-00002.D
Sample Name: LD-VI-145-run2

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

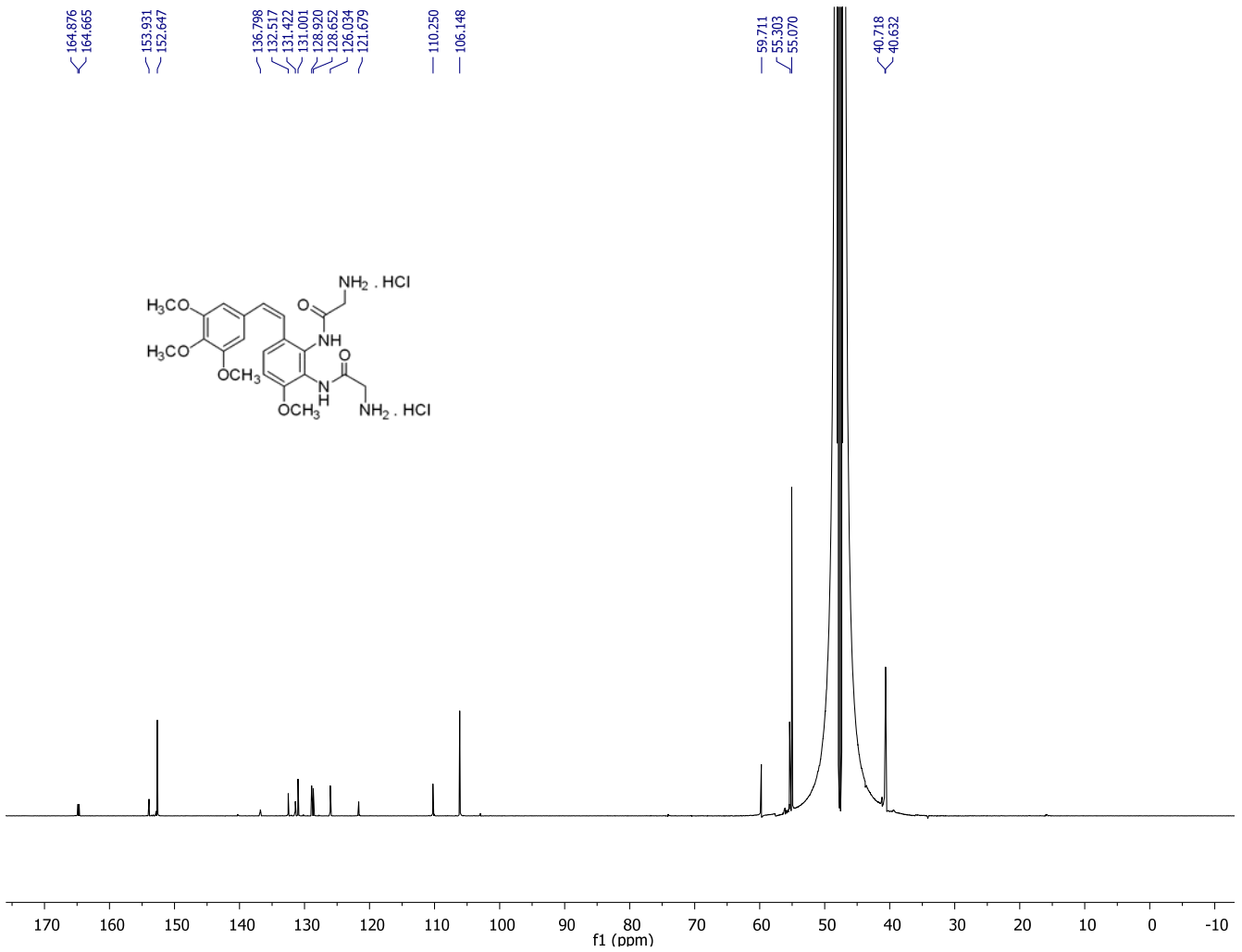
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.247	VV	0.0753	1927.66699	392.75601	53.8382
2	11.508	VV	0.0661	1547.21765	360.35504	43.2126
3	12.829	VV	0.0940	105.59404	17.09910	2.9492
Totals :				3580.47868	770.21016	

*** End of Report ***

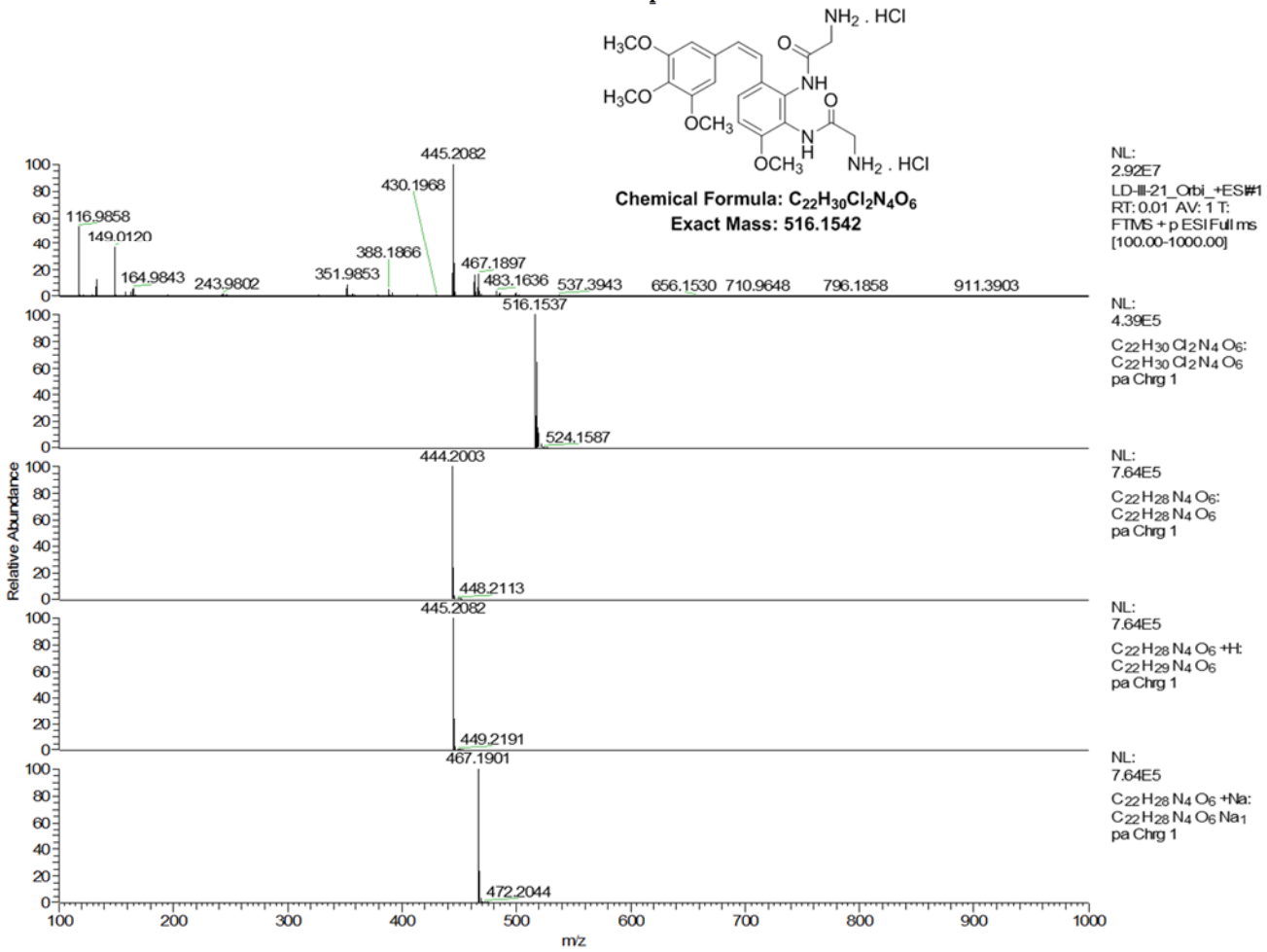
^{1}H NMR (CD_3OD, 600 MHz) Compound **24**



^{13}C NMR (CD_3OD, 150 MHz) Compound **24**



HRMS Compound **24**



HPLC for Compound **24**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-21\RUN1-III-21-0001.D
Sample Name: run1-LD-III-21-1A

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    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 12/21/2012 11:37:50 AM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 12/21/2012 11:34:21 AM by Laxman
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    Last changed    : 1/18/2013 10:56:37 AM by song
                      (modified after loading)
    Sample Info     : run1
                      10%ACN in 0.1%TFA water solution
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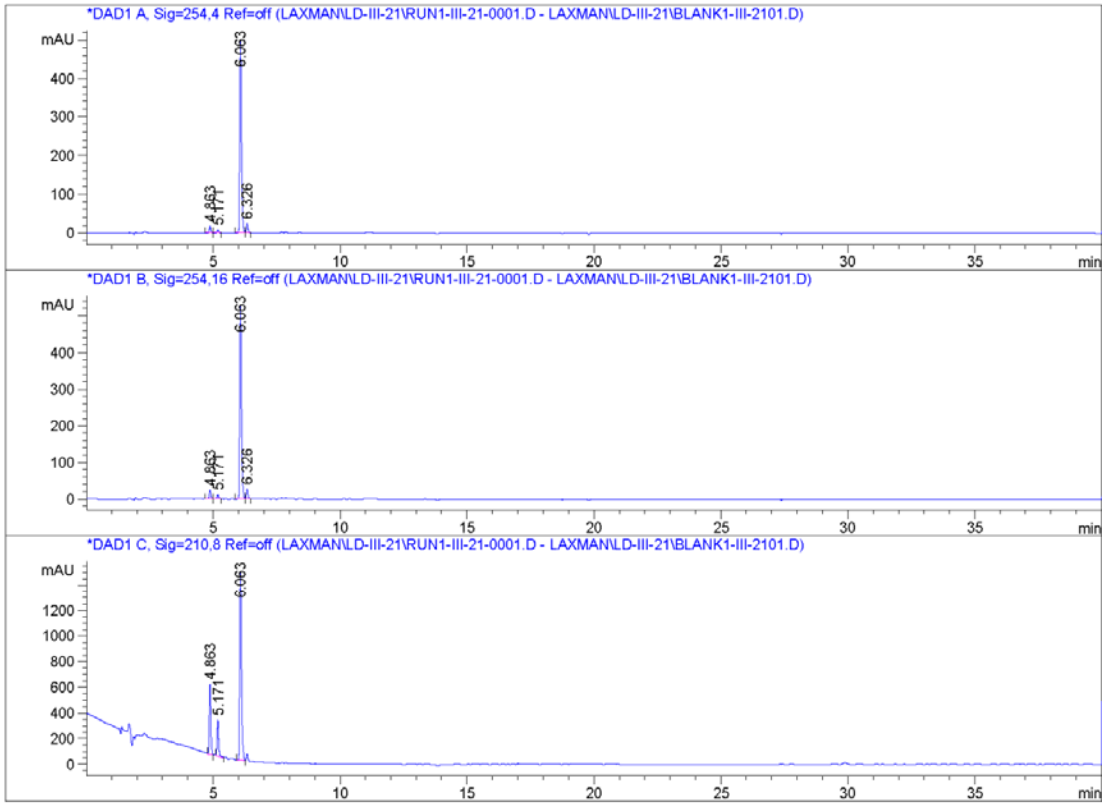
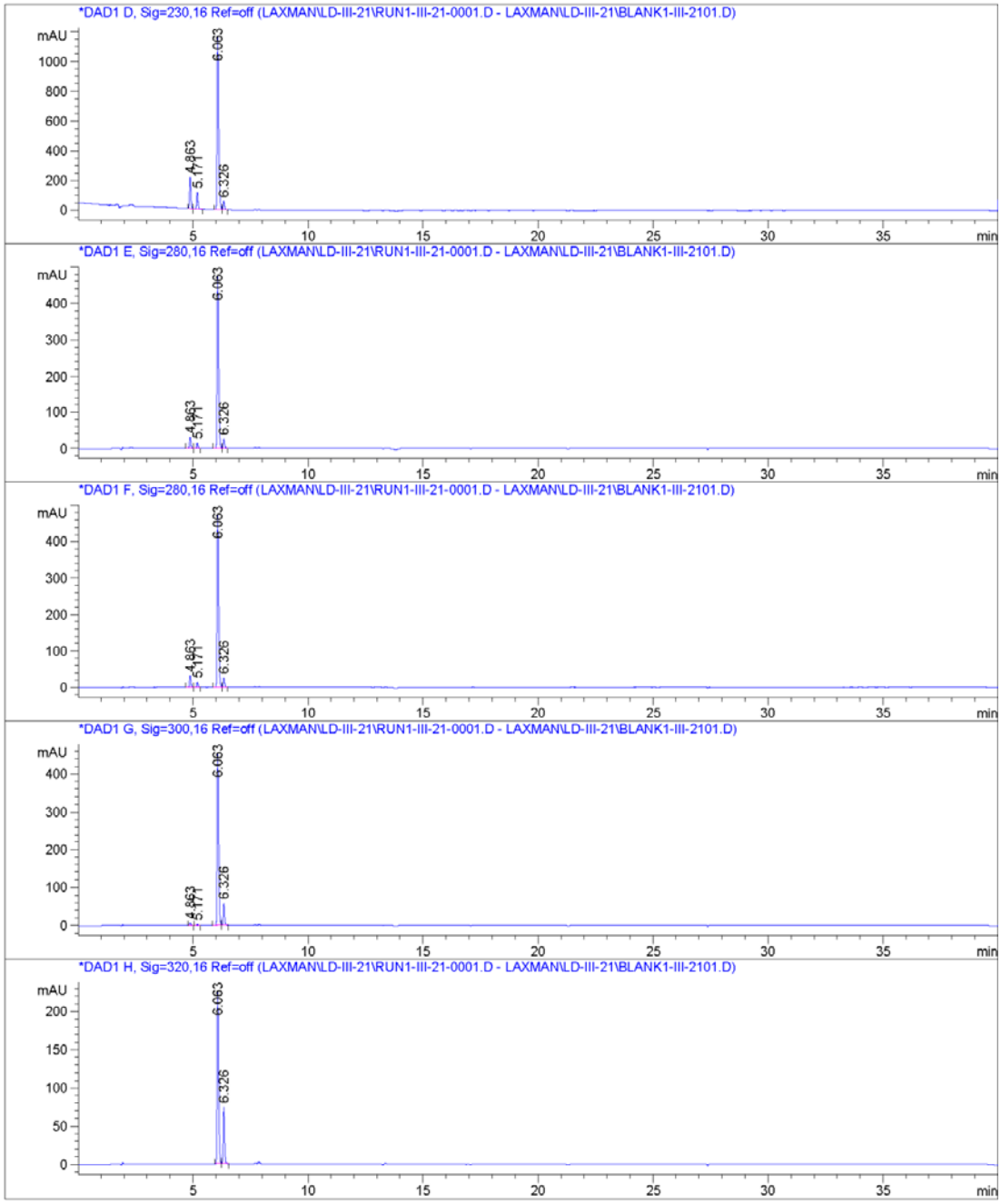


Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-21\RUN1-III-21-0001.D
Sample Name: run1-LD-III-21-1A



Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-21\RUN1-III-21-0001.D
Sample Name: run1-LD-III-21-1A

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
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.863	BB	0.0643	71.16022	17.17359	2.8439
2	5.171	BB	0.0645	37.81452	9.08890	1.5113
3	6.063	BV	0.0693	2286.42432	500.08197	91.3767
4	6.326	VB	0.0649	106.79793	24.49240	4.2682
Totals :				2502.19698	550.83686	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.863	BB	0.0642	98.68317	23.90006	3.6702
2	5.171	BB	0.0639	50.66319	12.33097	1.8843
3	6.063	BV	0.0694	2422.45068	529.47382	90.0960
4	6.326	VB	0.0648	116.94734	26.84951	4.3495
Totals :				2688.74438	592.55435	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.863	VB	0.0650	2312.86865	551.00055	21.8635
2	5.171	VV	0.0671	1246.27808	284.80121	11.7810
3	6.063	BV	0.0732	7019.53174	1483.43799	66.3555
Totals :				1.05787e4	2319.23975	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-21\RUN1-III-21-0001.D
Sample Name: run1-LD-III-21-1A

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.863	VV	0.0648	884.47058	211.53062	12.5062
2	5.171	VV	0.0674	505.99442	114.94447	7.1546
3	6.063	BV	0.0721	5426.37646	1169.56604	76.7275
4	6.326	VB	0.0645	255.42552	59.02868	3.6116
Totals :				7072.26698	1555.06981	

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	4.863	BB	0.0643	129.42253	31.24761	5.2490
2	5.171	BB	0.0635	60.69393	14.92229	2.4616
3	6.063	BV	0.0688	2160.37939	477.03757	87.6182
4	6.326	VB	0.0654	115.17860	26.14397	4.6713
Totals :				2465.67446	549.35143	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.863	BB	0.0643	129.42253	31.24761	5.2490
2	5.171	BB	0.0635	60.69393	14.92229	2.4616
3	6.063	BV	0.0688	2160.37939	477.03757	87.6182
4	6.326	VB	0.0654	115.17860	26.14397	4.6713
Totals :				2465.67446	549.35143	

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

Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-21\RUN1-III-21-0001.D
Sample Name: run1-LD-III-21-1A

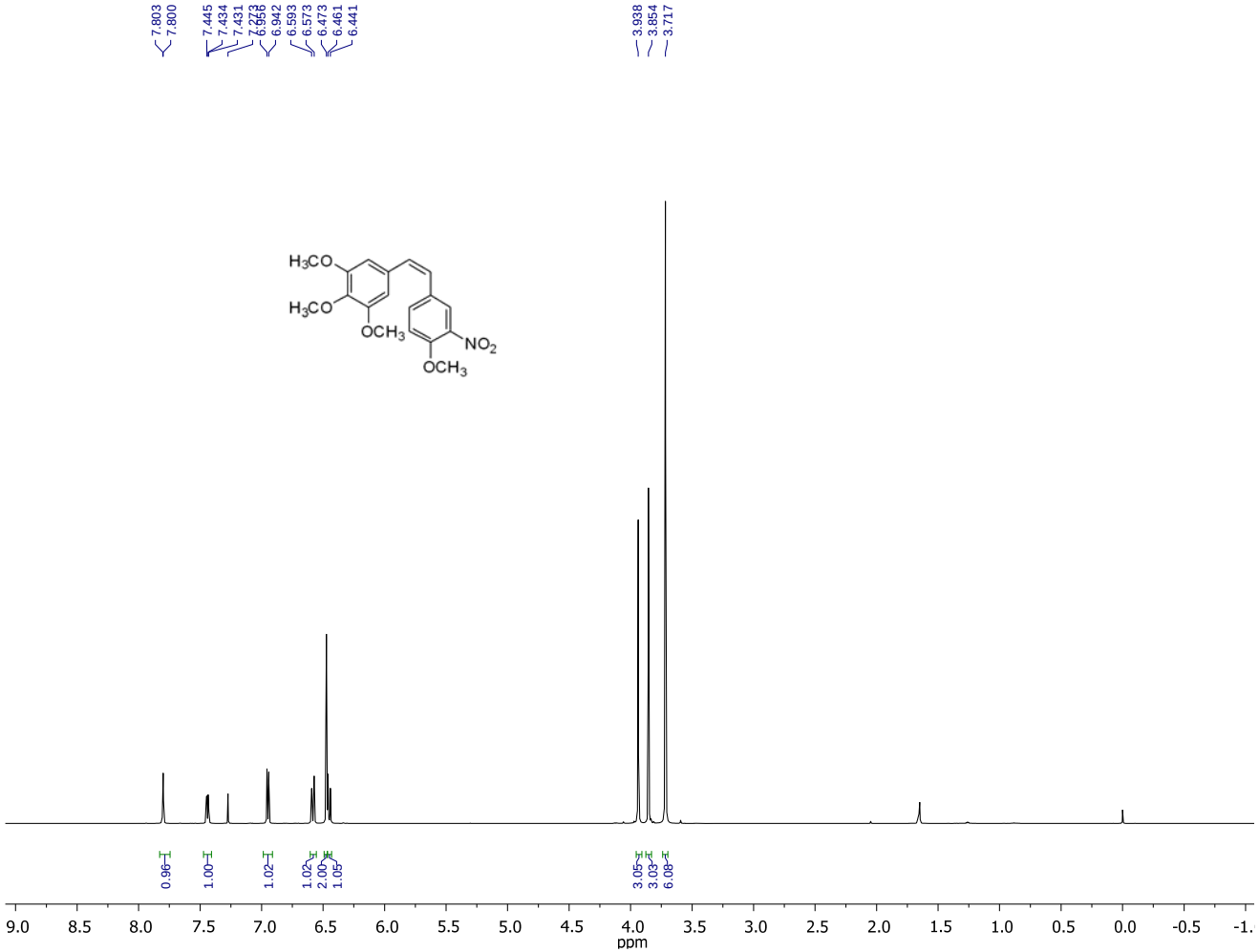
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.863	BB	0.0639	28.77723	7.00664	1.2205
2	5.171	BB	0.0640	11.33881	2.75776	0.4809
3	6.063	BV	0.0709	2067.91895	455.82397	87.7011
4	6.326	VB	0.0640	249.88266	58.33168	10.5976
Totals :				2357.91764	523.92005	

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

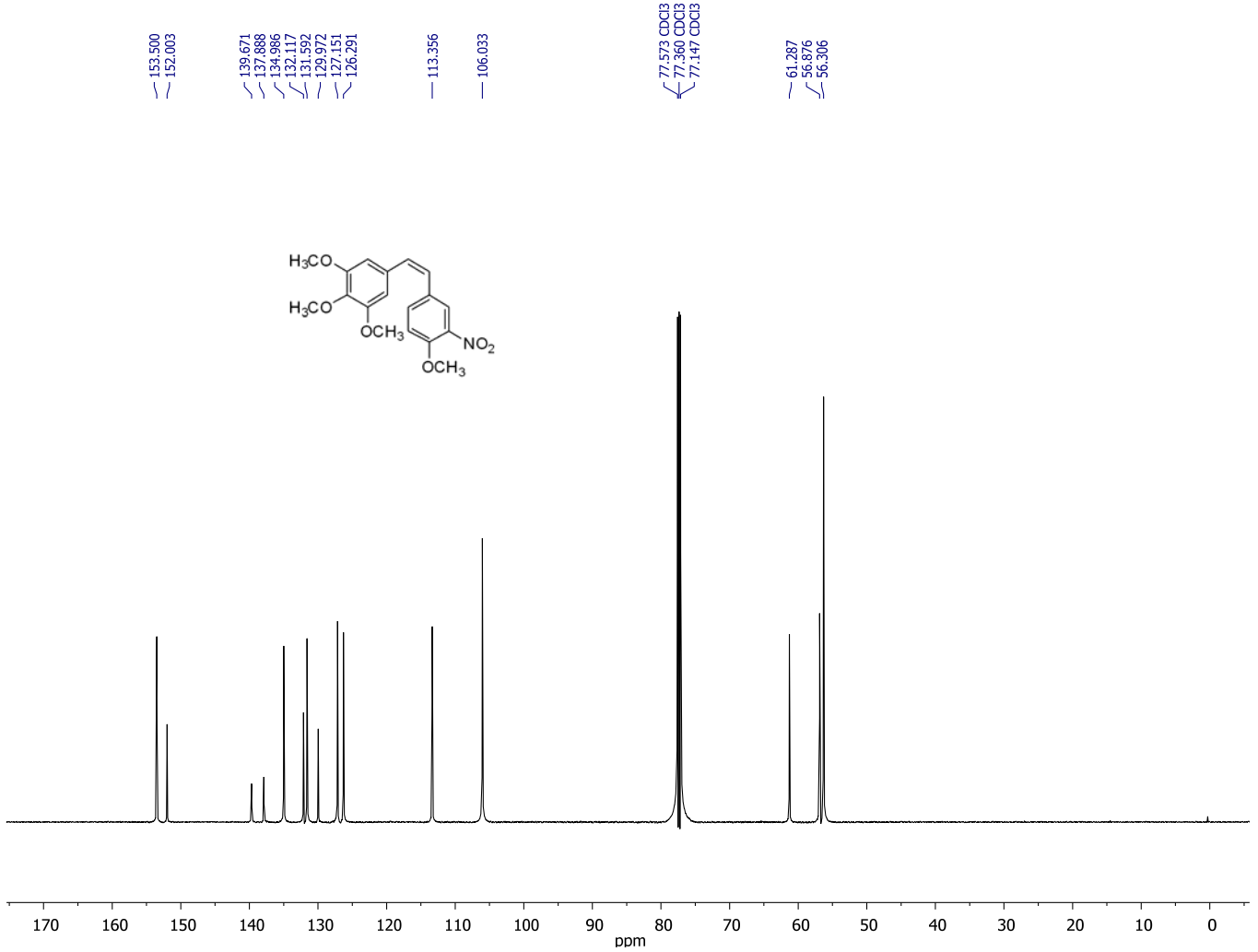
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.063	BV	0.0706	1020.73212	226.40169	76.5450
2	6.326	VB	0.0635	312.77420	73.71690	23.4550
Totals :				1333.50632	300.11858	

*** End of Report ***

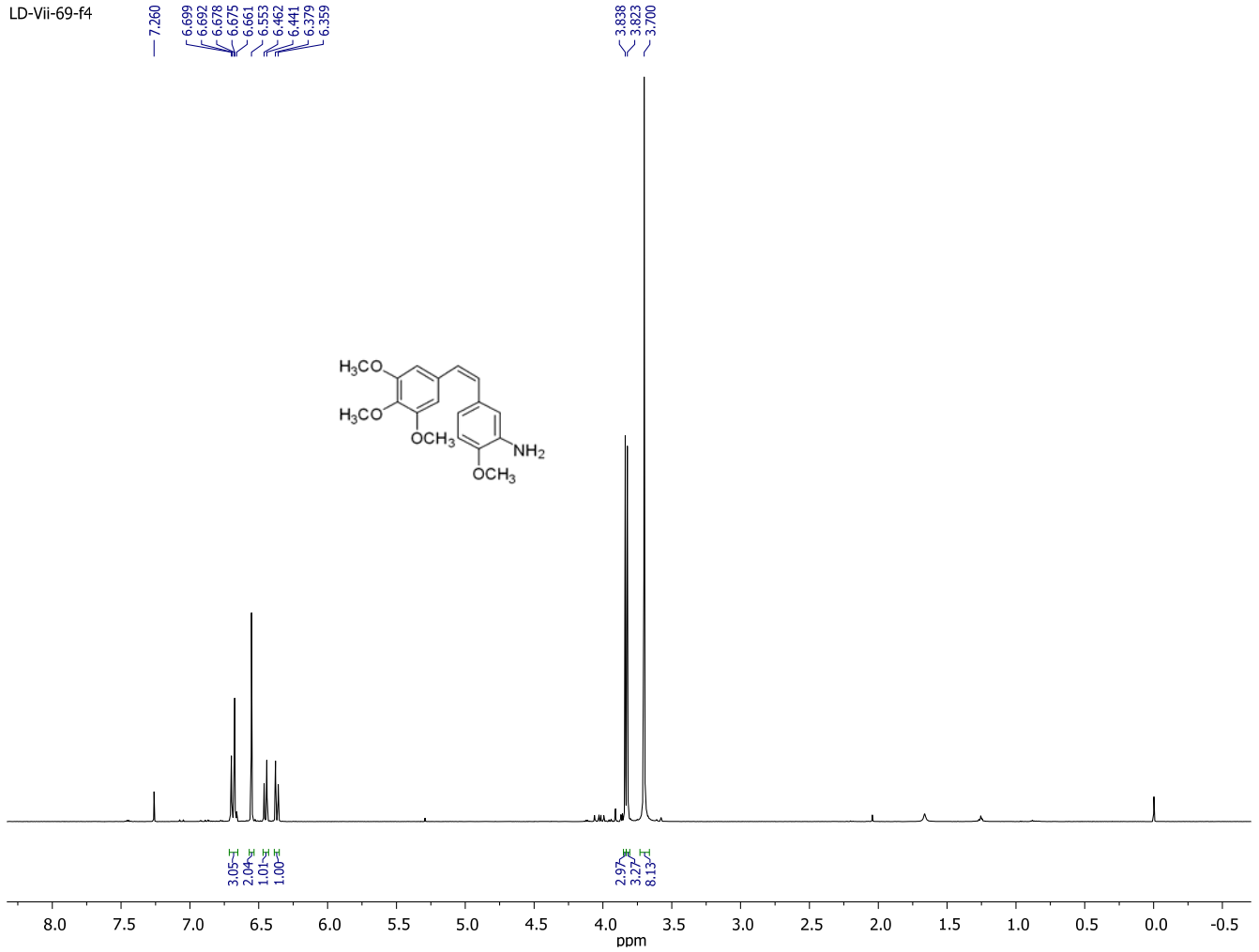
^{1}H NMR (CDCl3, 600 MHz) for Compound **46**



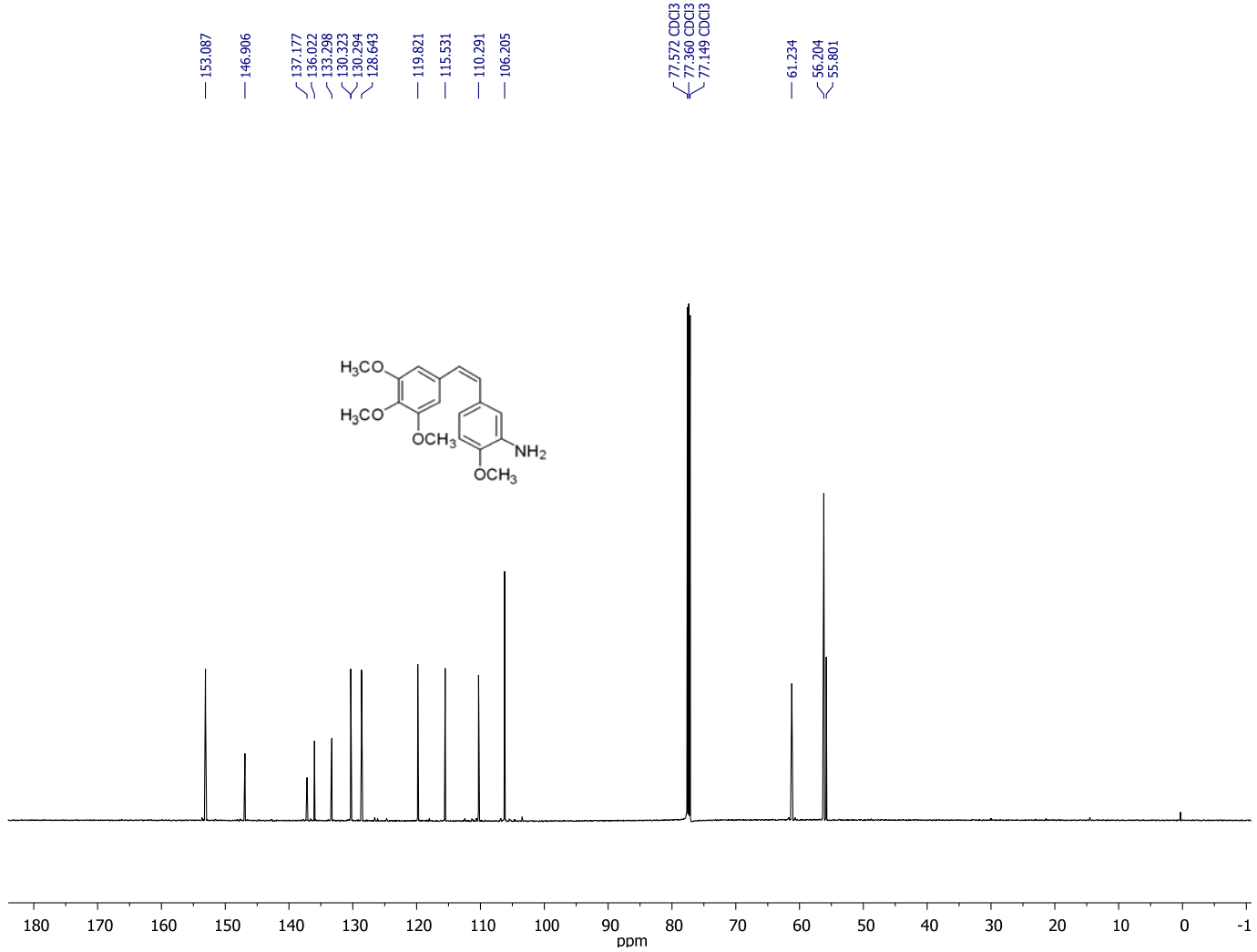
^{13}C NMR ($CDCl_3$, 150 MHz) Compound **46**



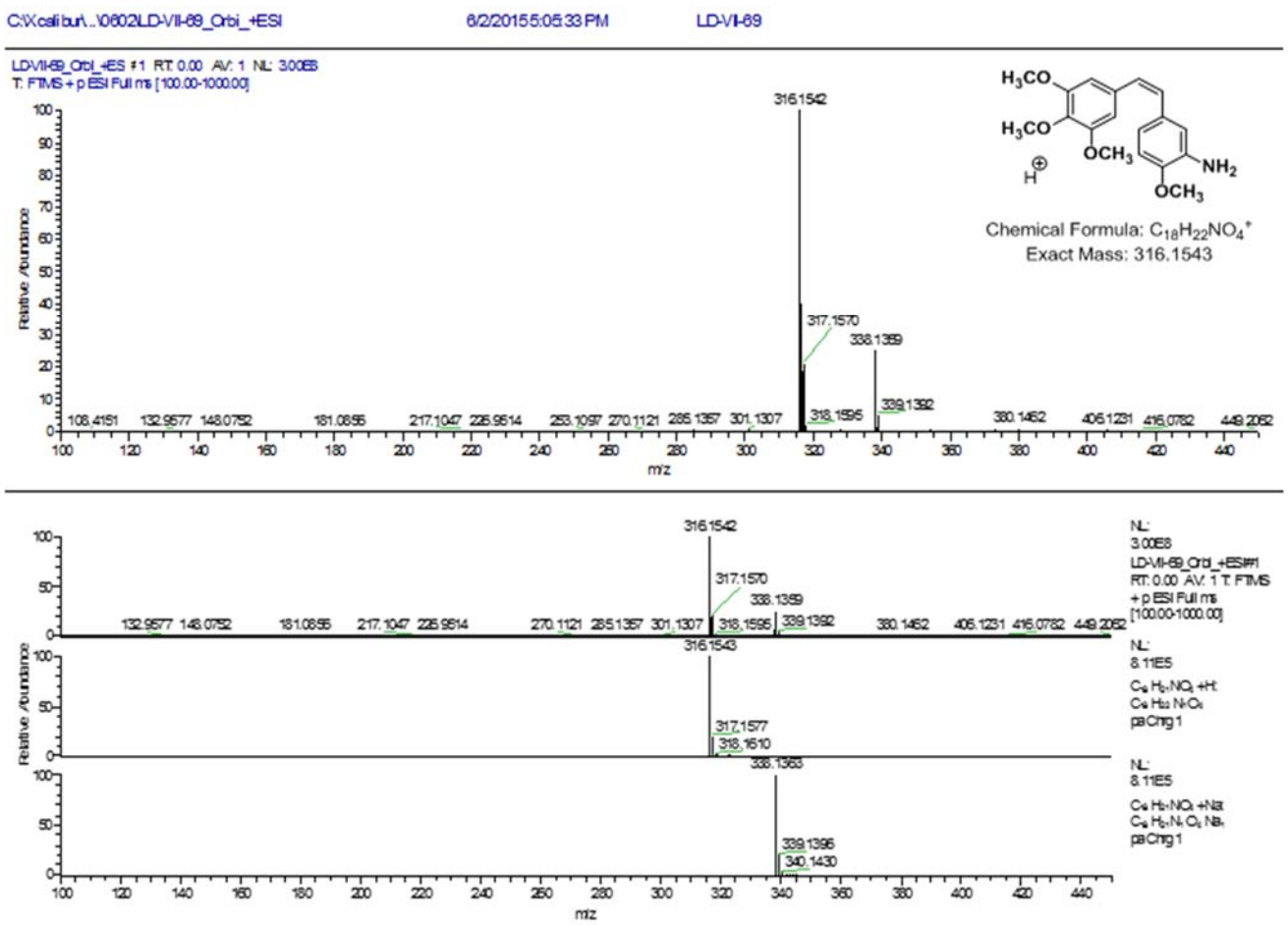
^{1}H NMR ($CDCl_3$, 600 MHz) for 3' CA4-amine



^{13}C NMR ($CDCl_3$, 150 MHz) for 3' CA4-amine



HRMS for 3' CA4-amine



HPLC for 3' CA4-amine

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-69-00001.D
Sample Name: LD-VII-69-1 3'CA4-Amine

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Acq. Operator   : Laxman
Acq. Instrument : Instrument 1                  Location :   -
Injection Date  : 6/1/2015 2:33:28 PM
Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD2.M
Last changed    : 6/1/2015 2:13:25 PM by Eric Lin
Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-VII-69-00001.D\DA.M (MASTERMETHOD2.M)
Last changed    : 6/1/2015 3:22:30 PM by Eric Lin
Sample Info     : Method-Mastermethod2
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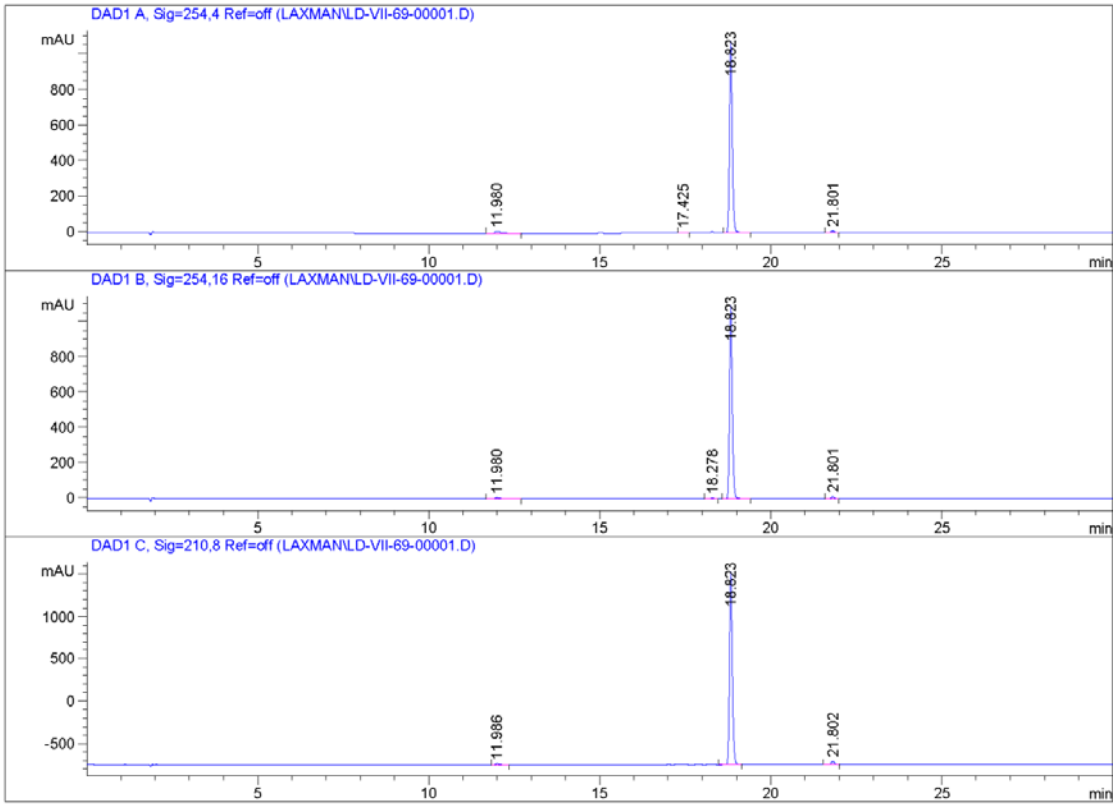
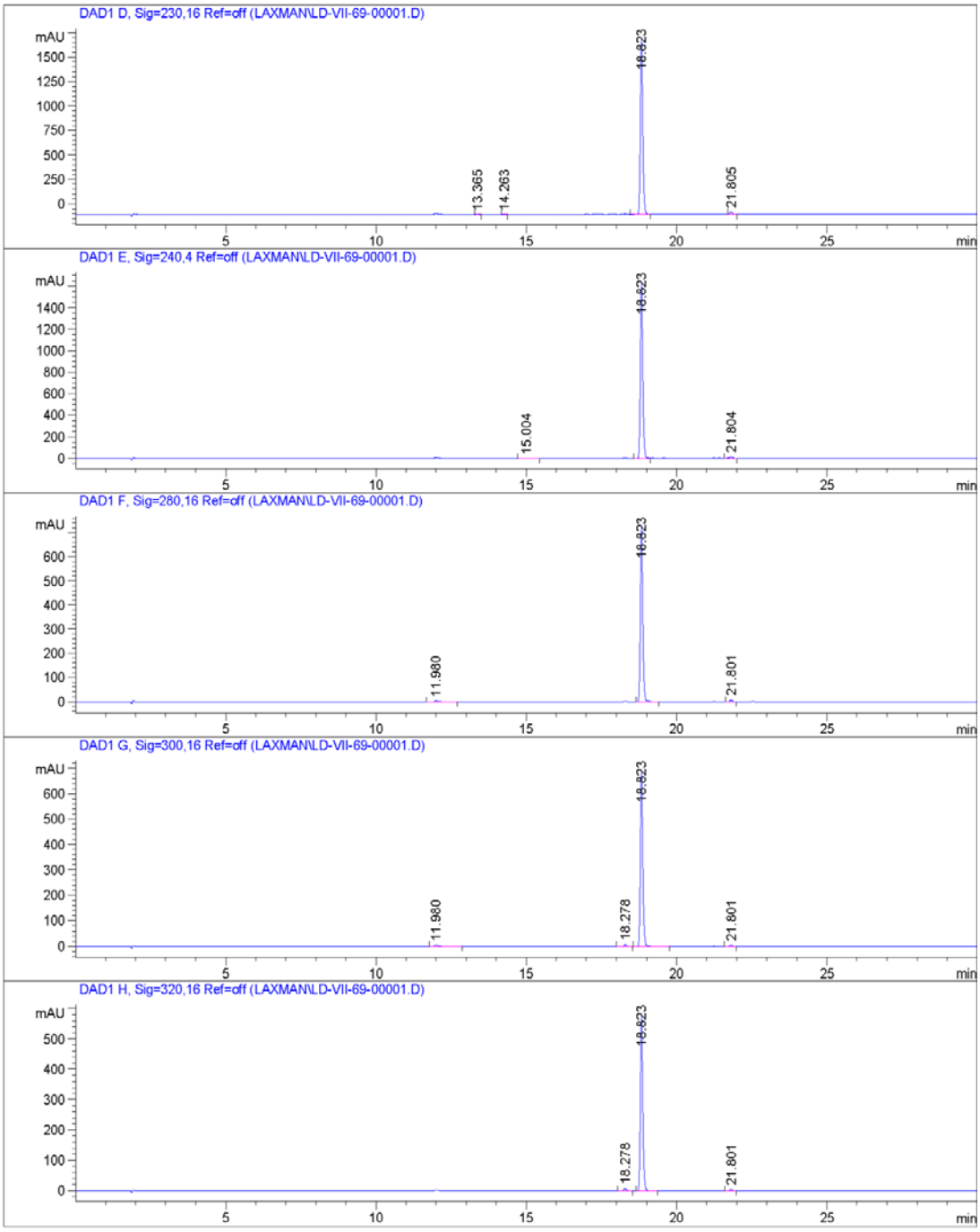


Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-69-00001.D
Sample Name: LD-VII-69-1 3'CA4-Amine



Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-69-00001.D
Sample Name: LD-VII-69-1 3'CA4-Amine

==========

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.980	BB	0.1751	89.59236	7.62506	1.4503
2	17.425	BB	0.0961	10.77919	1.65243	0.1745
3	18.823	BB	0.0851	6008.21289	1077.08765	97.2588
4	21.801	BB	0.0943	68.96559	11.13570	1.1164
Totals :				6177.55003	1097.50084	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.980	BB	0.1756	89.20059	7.56309	1.4138
2	18.278	BB	0.0868	26.60785	4.64551	0.4217
3	18.823	BB	0.0851	6123.01221	1097.30920	97.0493
4	21.801	BB	0.0947	70.35337	11.29626	1.1151
Totals :				6309.17402	1120.81407	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.986	BB	0.1510	134.73405	13.20541	1.0140
2	18.823	BV	0.0883	1.29139e4	2273.72705	97.1937
3	21.802	VB	0.0974	238.12749	36.85693	1.7922
Totals :				1.32868e4	2323.78940	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-69-00001.D
Sample Name: LD-VII-69-1 3'CA4-Amine

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.365	BB	0.0656	9.52854	2.24285	0.0928
2	14.263	BB	0.0820	5.22144	1.01602	0.0508
3	18.823	BV	0.0872	1.01168e4	1810.49146	98.5021
4	21.805	VB	0.0987	139.09056	21.72524	1.3543
Totals :				1.02707e4	1835.47557	

Signal 5: DAD1 E, Sig=240,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.004	BB	0.1330	14.18810	1.51891	0.1524
2	18.823	BV	0.0870	9179.72461	1646.90674	98.5840
3	21.804	VB	0.1042	117.66814	17.11329	1.2637
Totals :				9311.58084	1665.53893	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.980	BB	0.1822	64.04824	4.97383	1.5266
2	18.823	BB	0.0852	4088.56470	731.74805	97.4517
3	21.801	BB	0.0907	42.86423	7.27667	1.0217
Totals :				4195.47717	743.99854	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.980	BB	0.1797	62.46052	5.14319	1.5541
2	18.278	BB	0.0856	34.96275	6.22234	0.8699
3	18.823	BB	0.0852	3891.12744	695.78998	96.8142
4	21.801	BB	0.0907	30.62026	5.19646	0.7619
Totals :				4019.17097	712.35197	

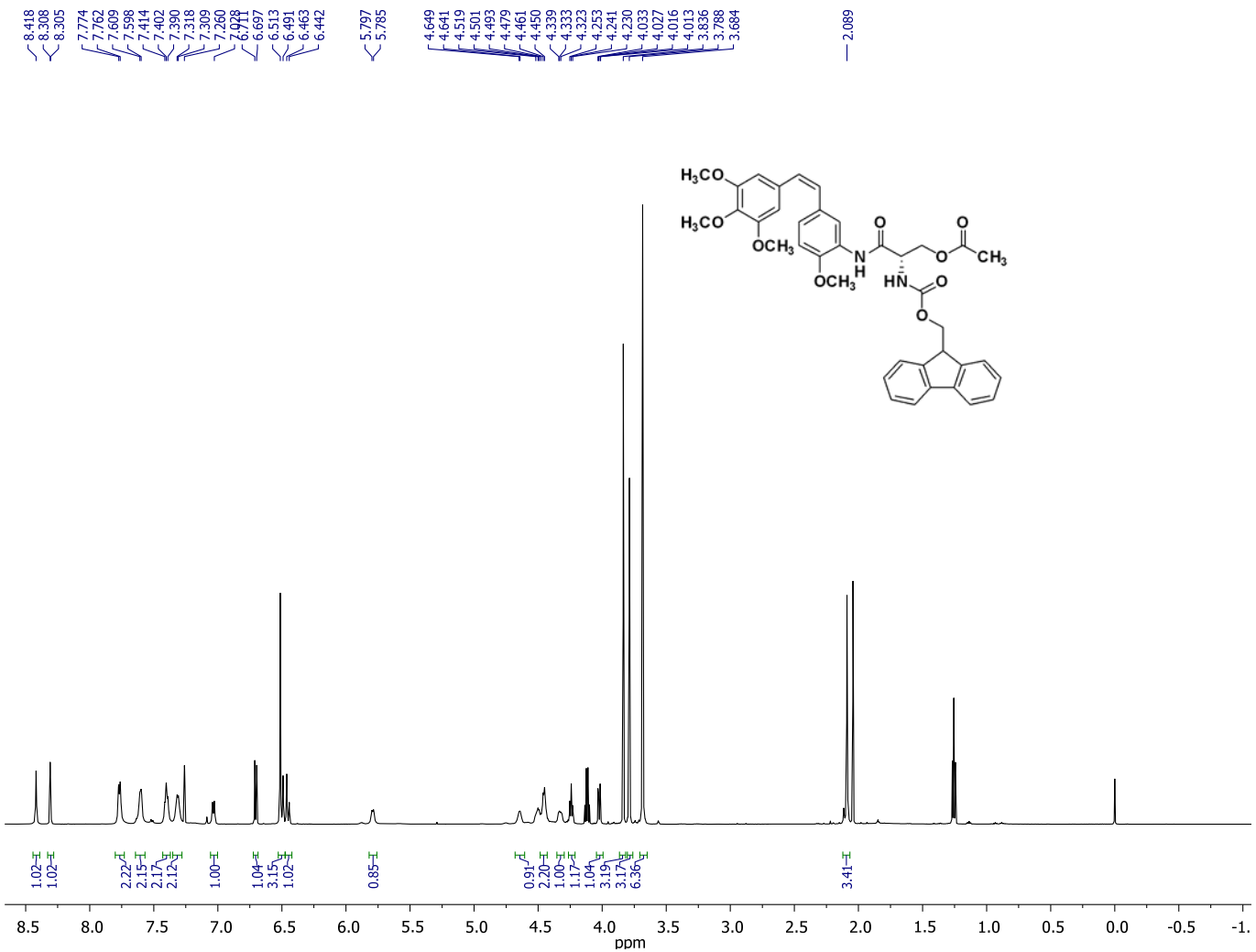
Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-69-00001.D
Sample Name: LD-VII-69-1 3'CA4-Amine

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

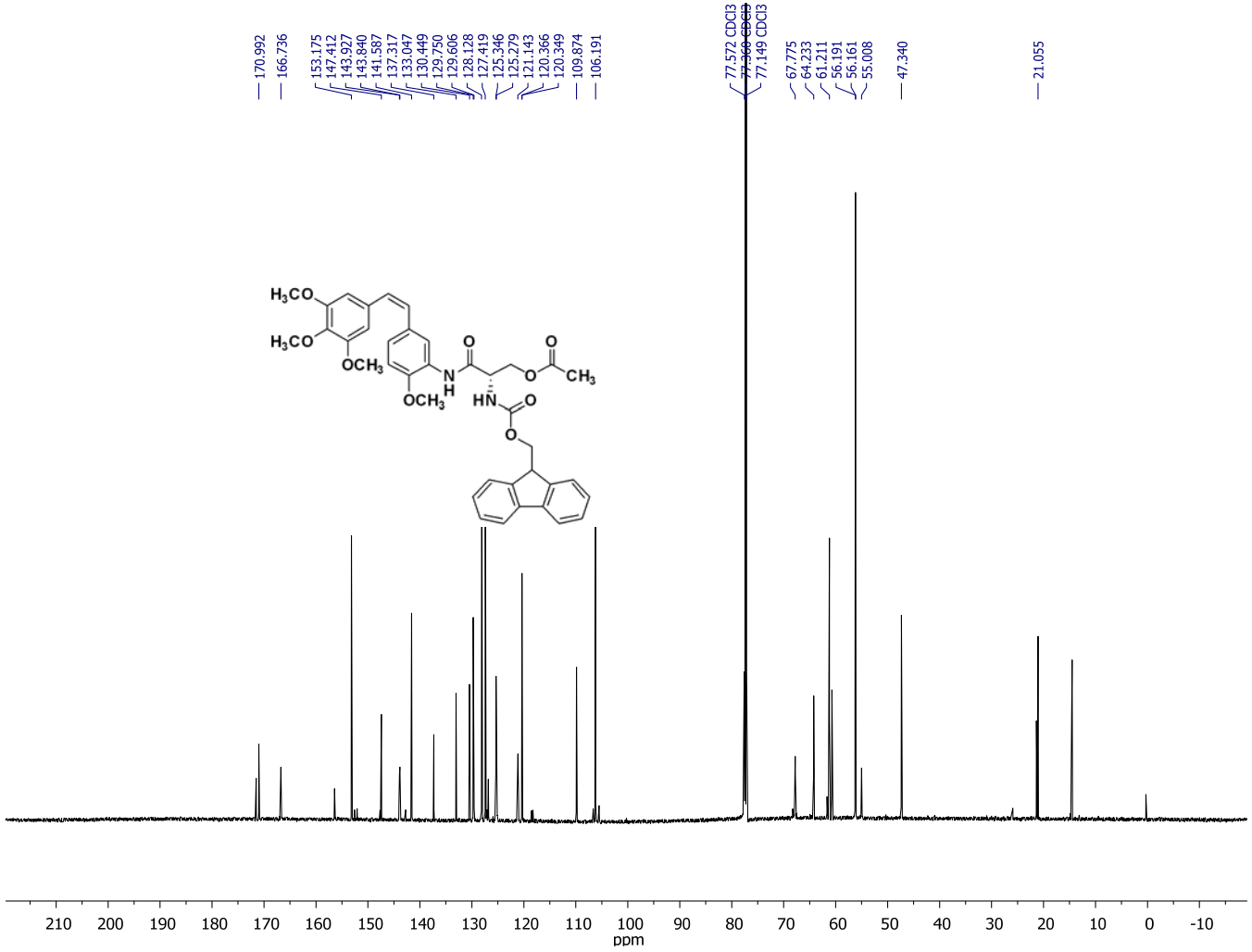
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	18.278	BB	0.0849	42.04350	7.55673	1.2647
2	18.823	BB	0.0851	3256.91406	583.29913	97.9669
3	21.801	BB	0.0909	25.54603	4.32569	0.7684
Totals :				3324.50358	595.18155	

*** End of Report ***

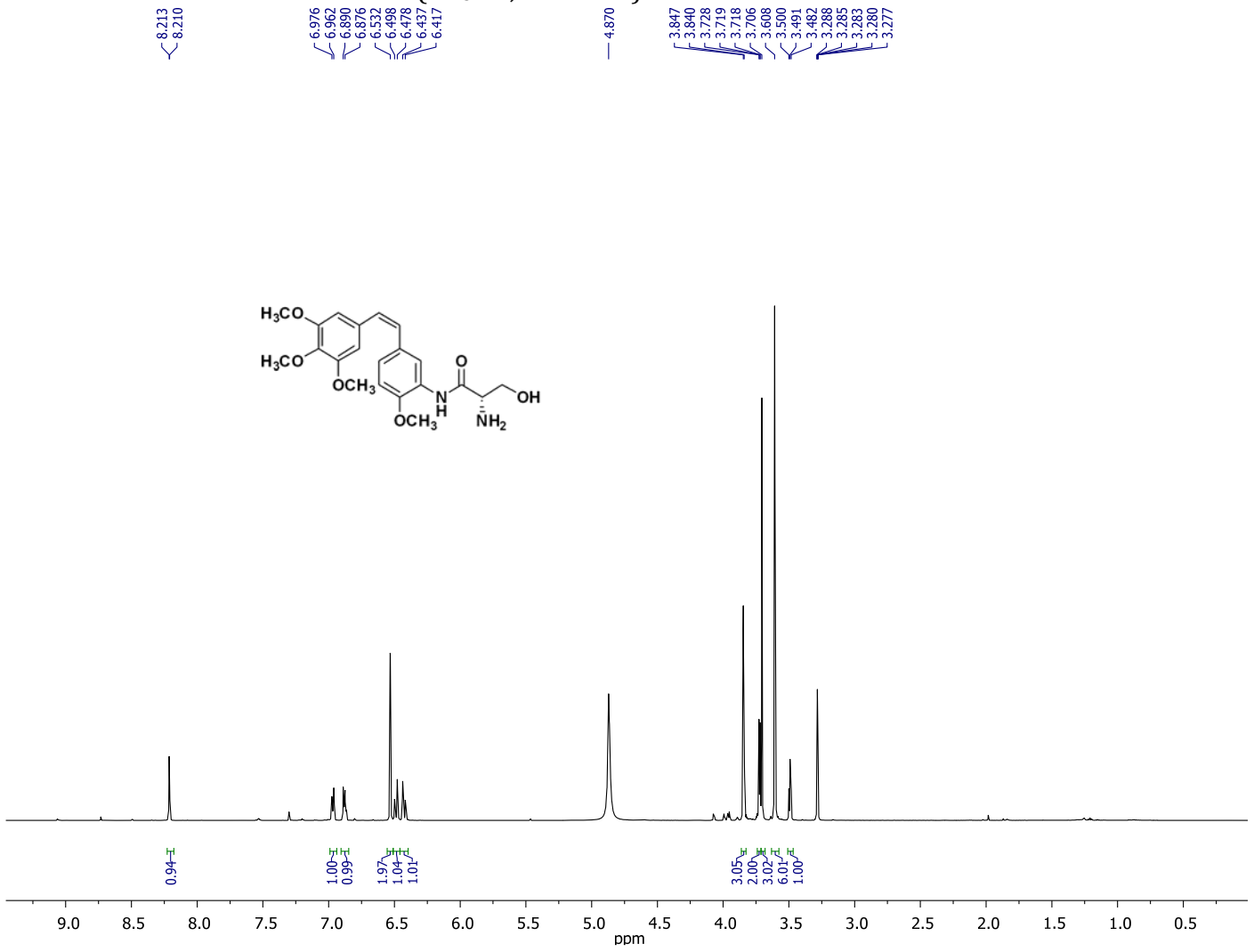
^{1}H NMR ($CDCl_3$, 600 MHz) Compound **47**



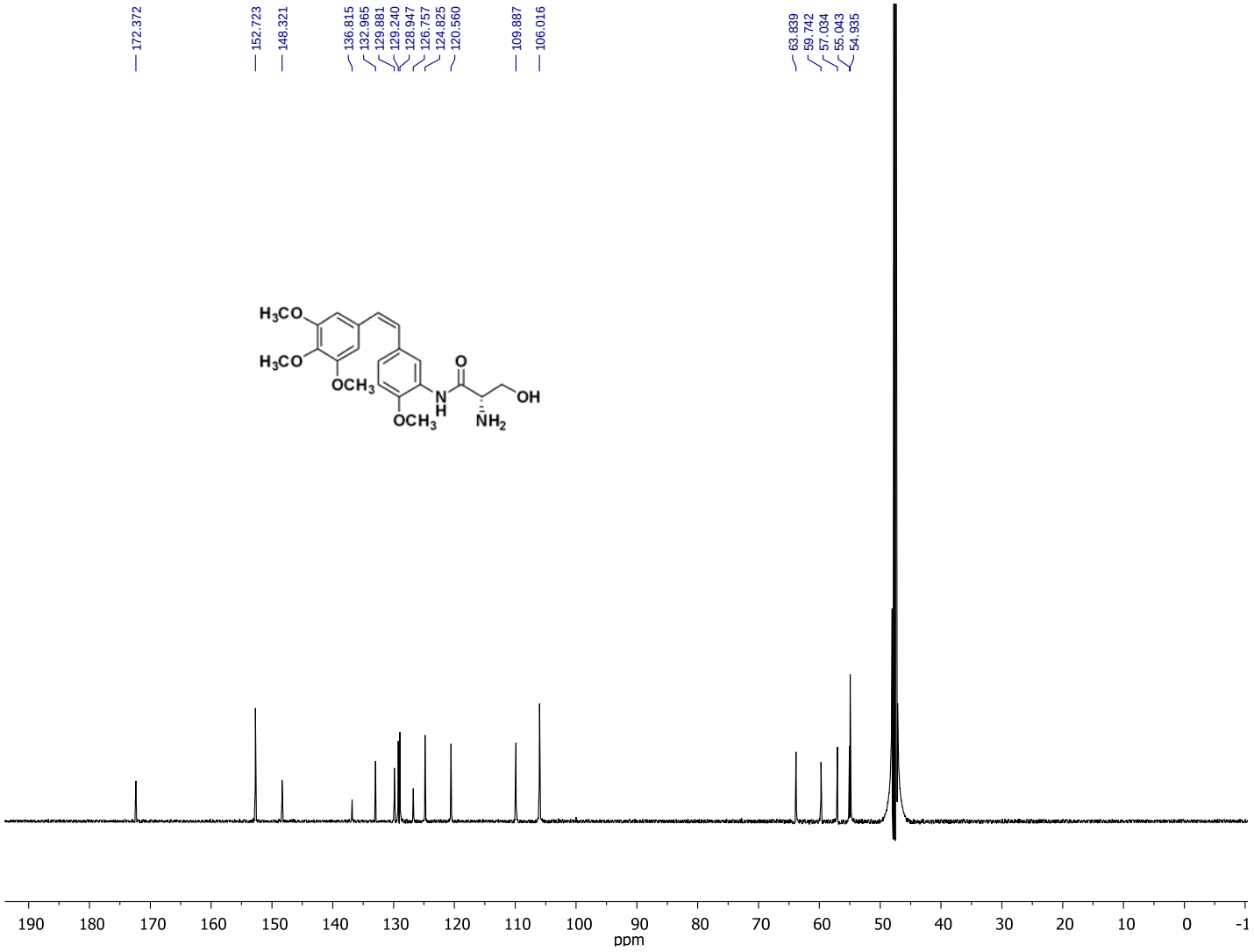
^{13}C NMR ($CDCl_3$, 150 MHz) Compound **47**



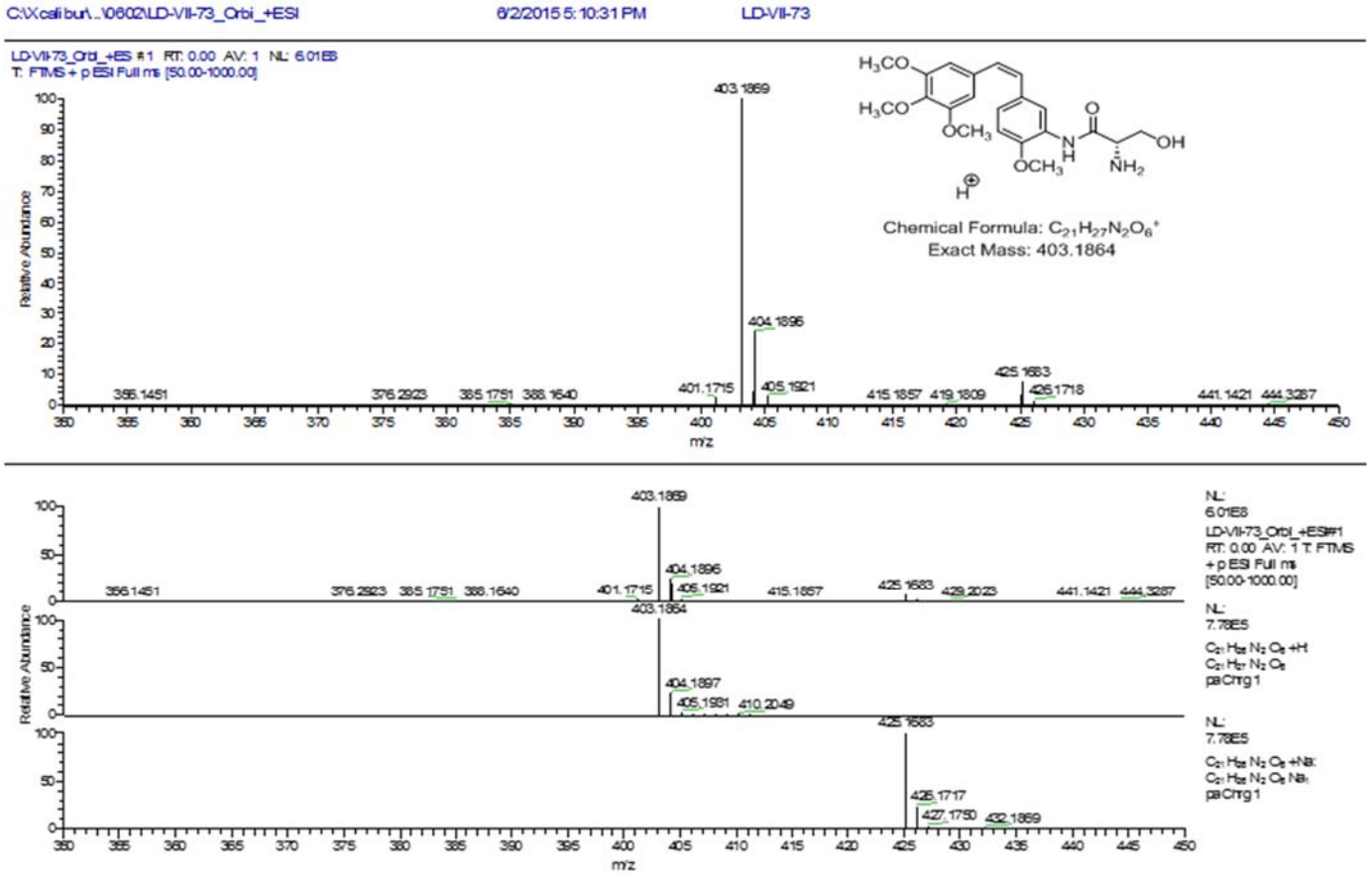
^{1}H NMR (CD_3OD, 600 MHz) for 3' CA4-*L*-serinamide



^{13}C NMR (CD_3OD, 150 MHz) for 3' CA4-*L*-serinamide



HRMS for 3' CA4-*L*-serinamide



HPLC for 3' CA4-*L*-serinamide

Data File C:\CHEM32\1\DATA\ERIC LIN\LD-VII-73A-R209.D
Sample Name: LD-VII-73A-R2

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Acq. Operator   : Eric Lin
Acq. Instrument : Instrument 1                    Location :   -
Injection Date  : 6/1/2015 12:29:03 PM
Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
Last changed    : 6/1/2015 11:52:37 AM by Eric Lin
Analysis Method : C:\CHEM32\1\DATA\ERIC LIN\LD-VII-73A-R209.D\DA.M (MASTERMETHOD.M)
Last changed    : 6/1/2015 1:19:18 PM by Eric Lin
Sample Info     : wash

                  Method:

                  0-5 min, 10:90 ACN/Water
                  5-25 min, gradient, 10:90 to 100:00 ACN/Water
                  25-30 min, 100:00 ACN/Water
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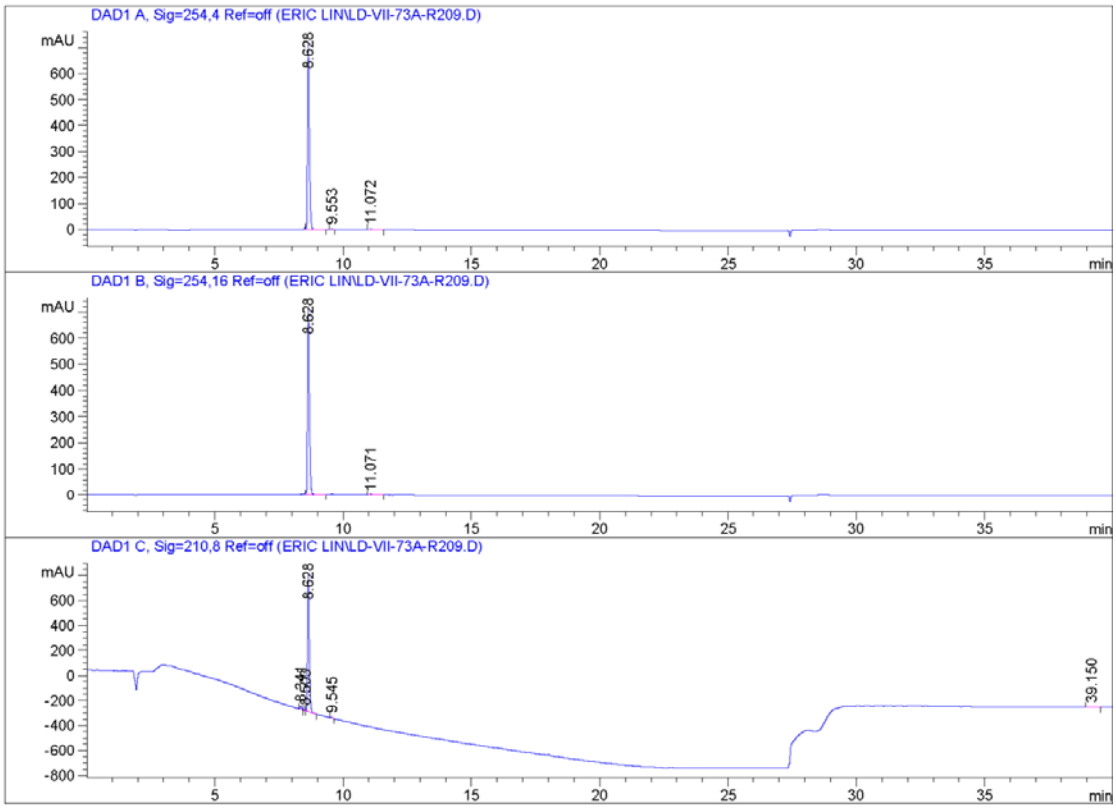
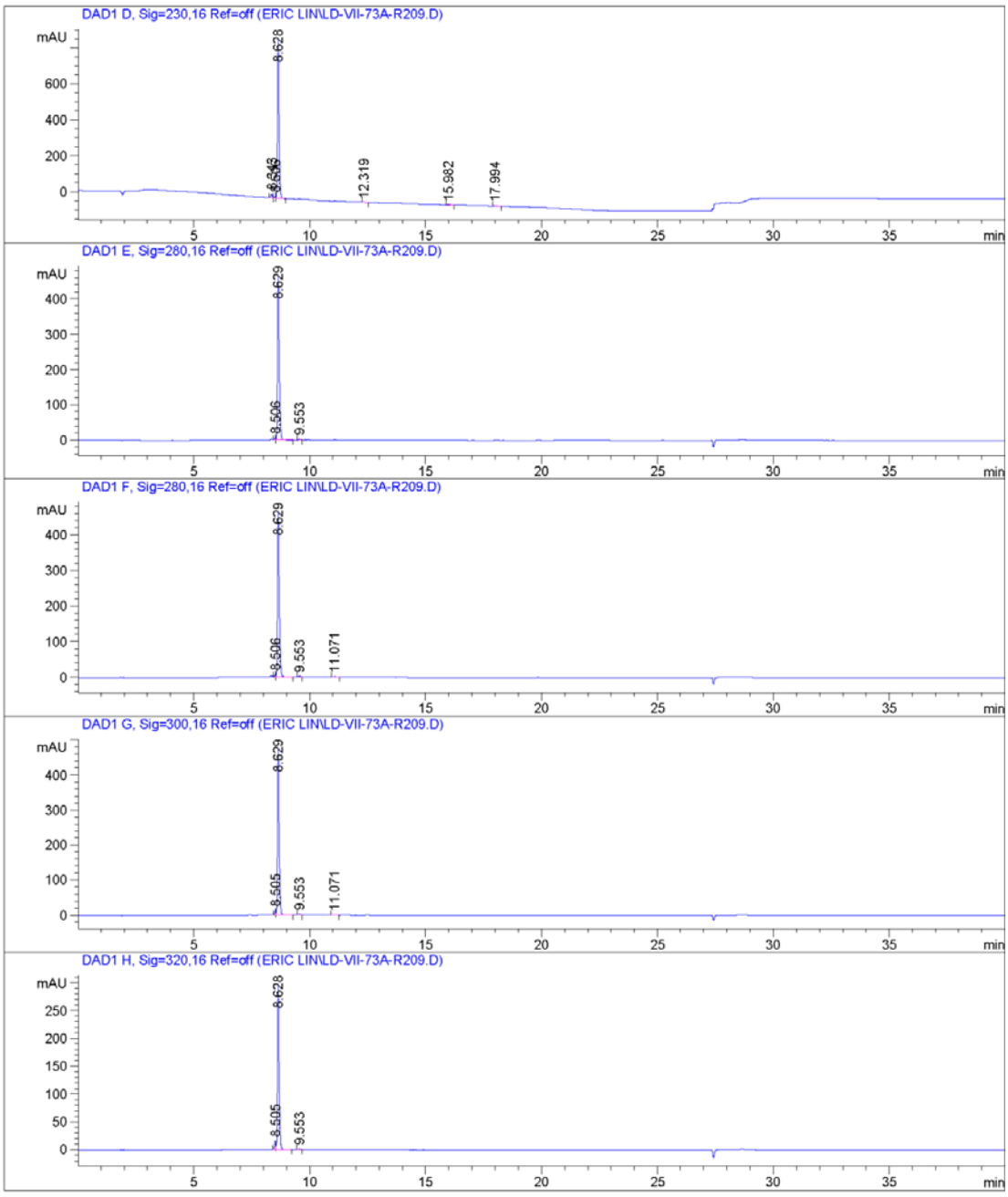


Data File C:\CHEM32\1\DATA\ERIC LIN\LD-VII-73A-R209.D
Sample Name: LD-VII-73A-R2



Data File C:\CHEM32\1\DATA\ERIC LIN\LD-VII-73A-R209.D
Sample Name: LD-VII-73A-R2

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

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.628	VB	0.0761	3615.06738	725.35986	99.2925
2	9.553	BB	0.0725	8.57860	1.83688	0.2356
3	11.072	BB	0.1303	17.18025	1.78545	0.4719
Totals :				3640.82624	728.98219	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.628	VB	0.0764	3597.92407	718.34595	99.5364
2	11.071	BB	0.1264	16.75720	1.77018	0.4636
Totals :				3614.68127	720.11613	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.341	BB	0.0702	125.12968	27.94563	2.2352
2	8.503	BV	0.0582	32.47391	8.98720	0.5801
3	8.628	VB	0.0744	5386.43848	1113.73535	96.2202
4	9.545	BB	0.0701	25.45053	5.69919	0.4546
5	39.150	BB	0.2871	28.53901	1.39256	0.5098
Totals :				5598.03160	1157.75993	

Data File C:\CHEM32\1\DATA\ERIC LIN\LD-VII-73A-R209.D
Sample Name: LD-VII-73A-R2

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.343	BB	0.0704	97.43347	21.70703	2.1572
2	8.506	BV	0.0582	33.81914	9.34663	0.7487
3	8.628	VB	0.0748	4350.40234	893.63580	96.3168
4	12.319	VB	0.1081	10.71834	1.41883	0.2373
5	15.982	BB	0.1050	6.85785	1.01244	0.1518
6	17.994	VB	0.0977	17.53352	2.63260	0.3882
Totals :				4516.76467	929.75333	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.506	VV	0.0609	26.17398	6.80126	1.0126
2	8.629	VB	0.0813	2547.08740	469.12259	98.5353
3	9.553	BB	0.0732	11.68830	2.47169	0.4522
Totals :				2584.94968	478.39554	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.506	VV	0.0609	26.17398	6.80126	1.0099
2	8.629	VB	0.0813	2547.08740	469.12259	98.2801
3	9.553	BB	0.0732	11.68830	2.47169	0.4510
4	11.071	BB	0.0906	6.71148	1.07787	0.2590
Totals :				2591.66116	479.47341	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.505	BV	0.0613	50.99851	13.13386	2.0432
2	8.629	VB	0.0773	2427.22803	477.66873	97.2444
3	9.553	BB	0.0727	11.26238	2.40055	0.4512
4	11.071	BB	0.0867	6.51913	1.10683	0.2612

Data File C:\CHEM32\1\DATA\ERIC LIN\LD-VII-73A-R209.D
Sample Name: LD-VII-73A-R2

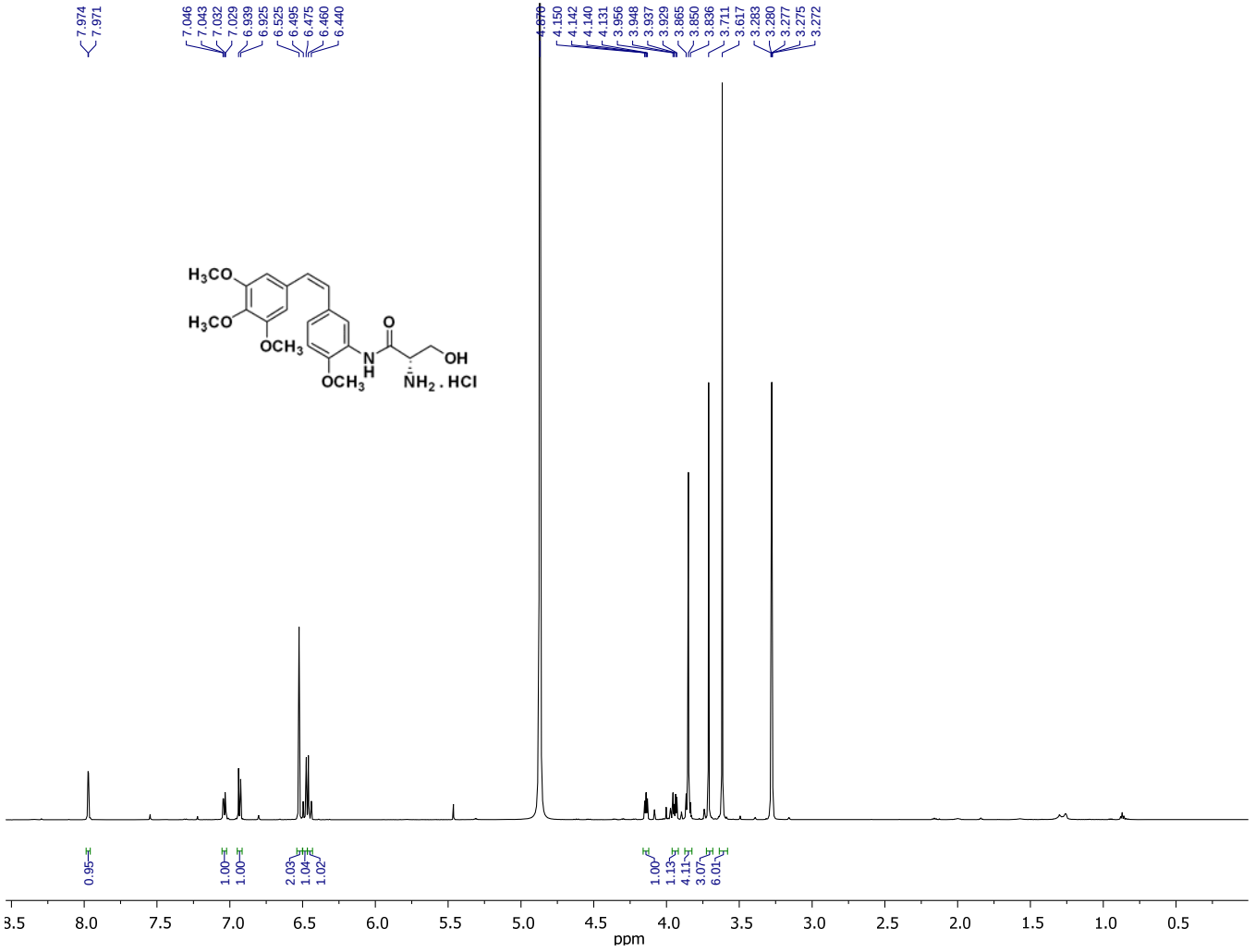
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				2496.00805	494.30997	

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

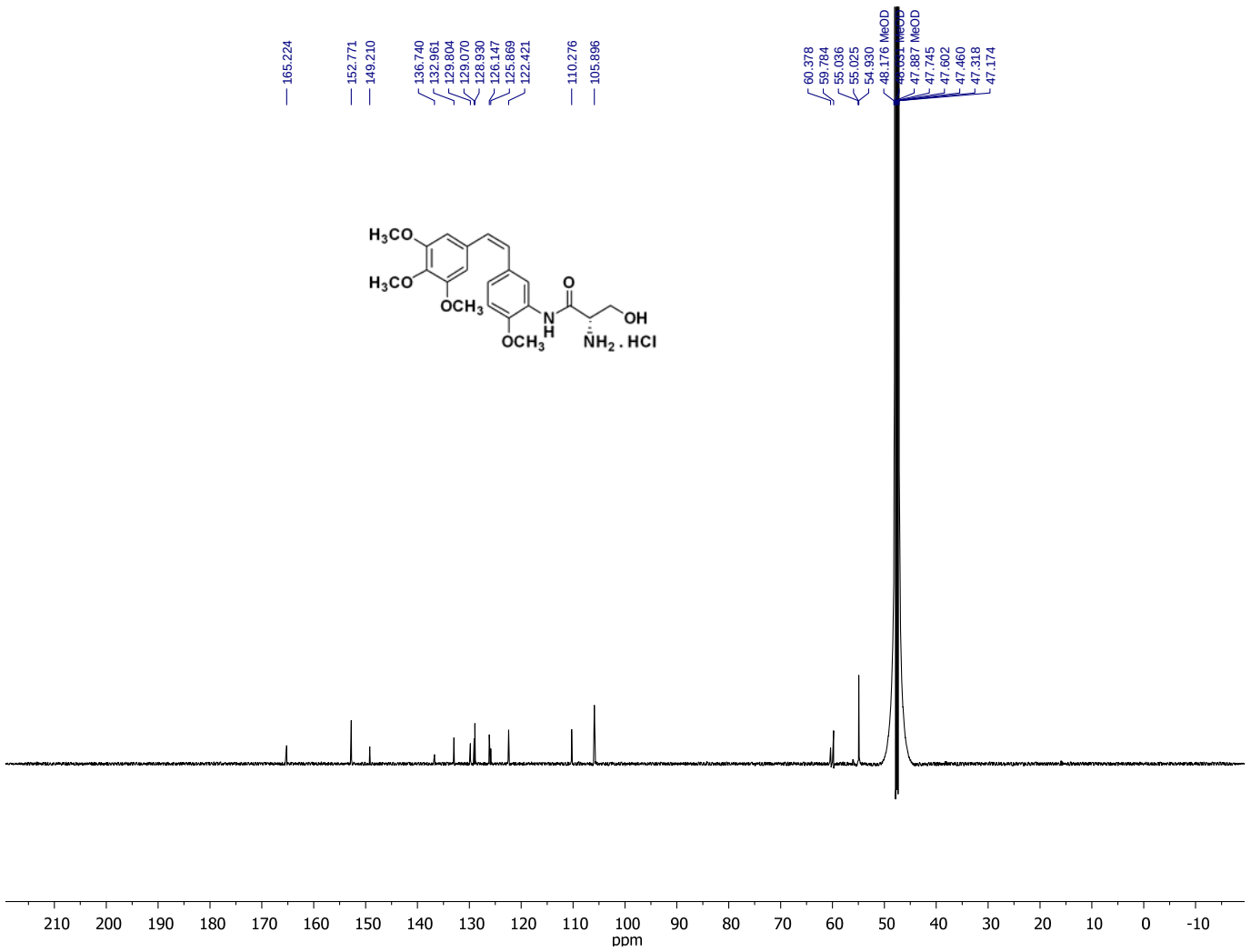
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.505	BV	0.0623	62.80982	15.83393	4.0939
2	8.628	VB	0.0754	1464.79163	297.60944	95.4747
3	9.553	BB	0.0730	6.61850	1.40323	0.4314
Totals :				1534.21994	314.84660	

*** End of Report ***

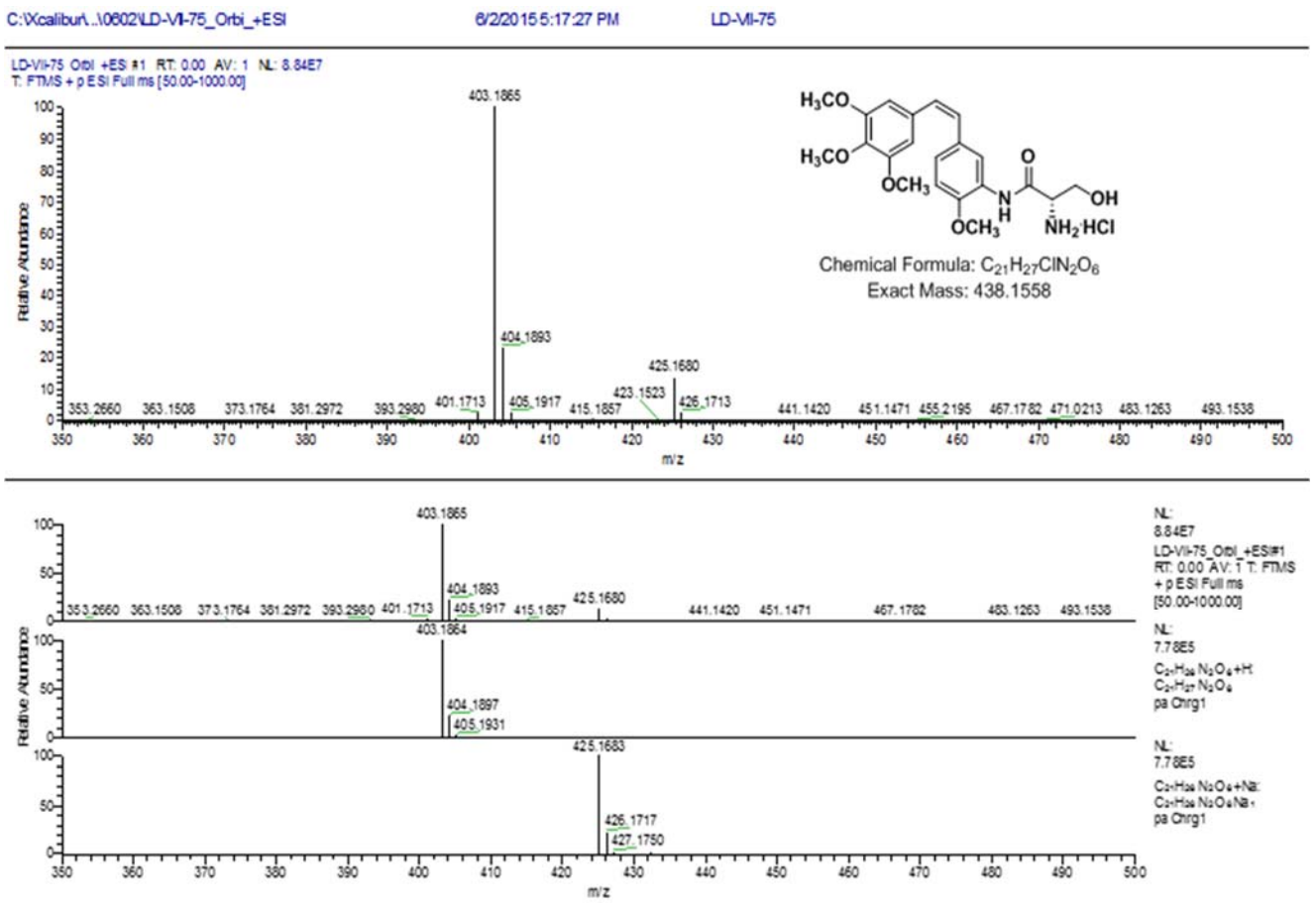
^{1}H NMR (CD_3OD, 600 MHz) for **AVE8062**



^{13}C NMR (CD_3OD, 150 MHz) for **AVE8062**



HRMS for **AVE8062**



HPLC for **AVE8062**

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Sample Name: AVE-8062-R1

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                      (modified after loading)
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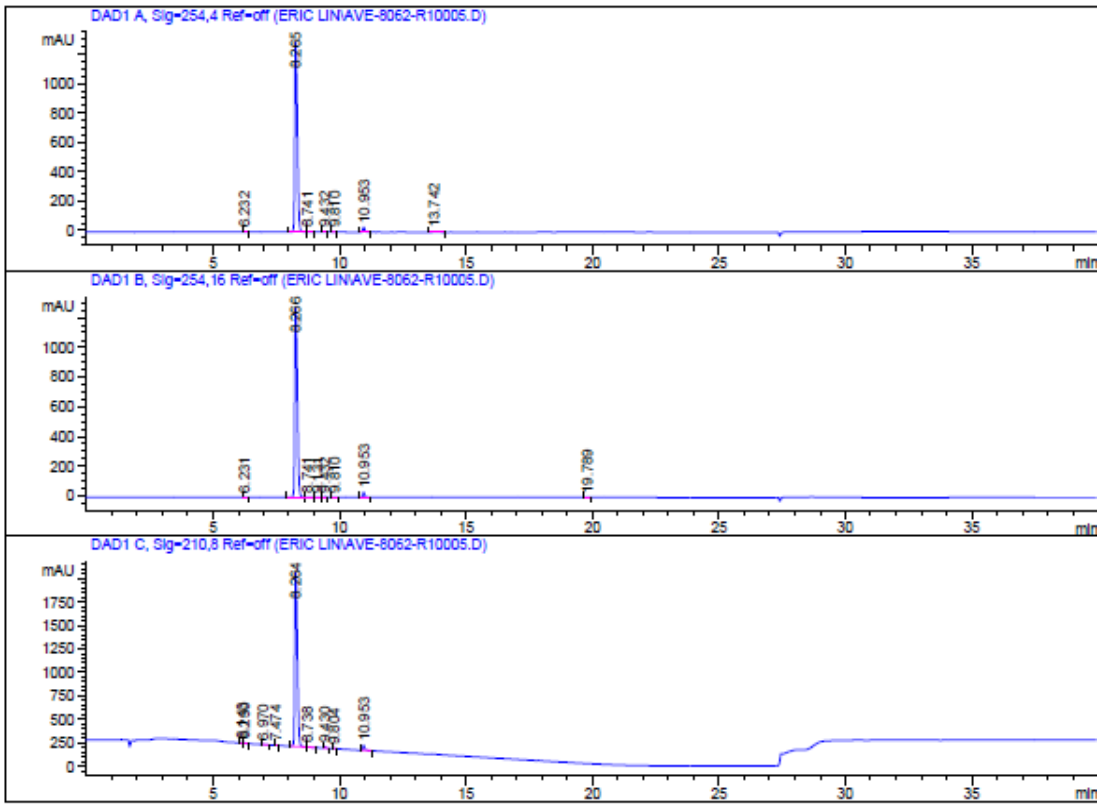

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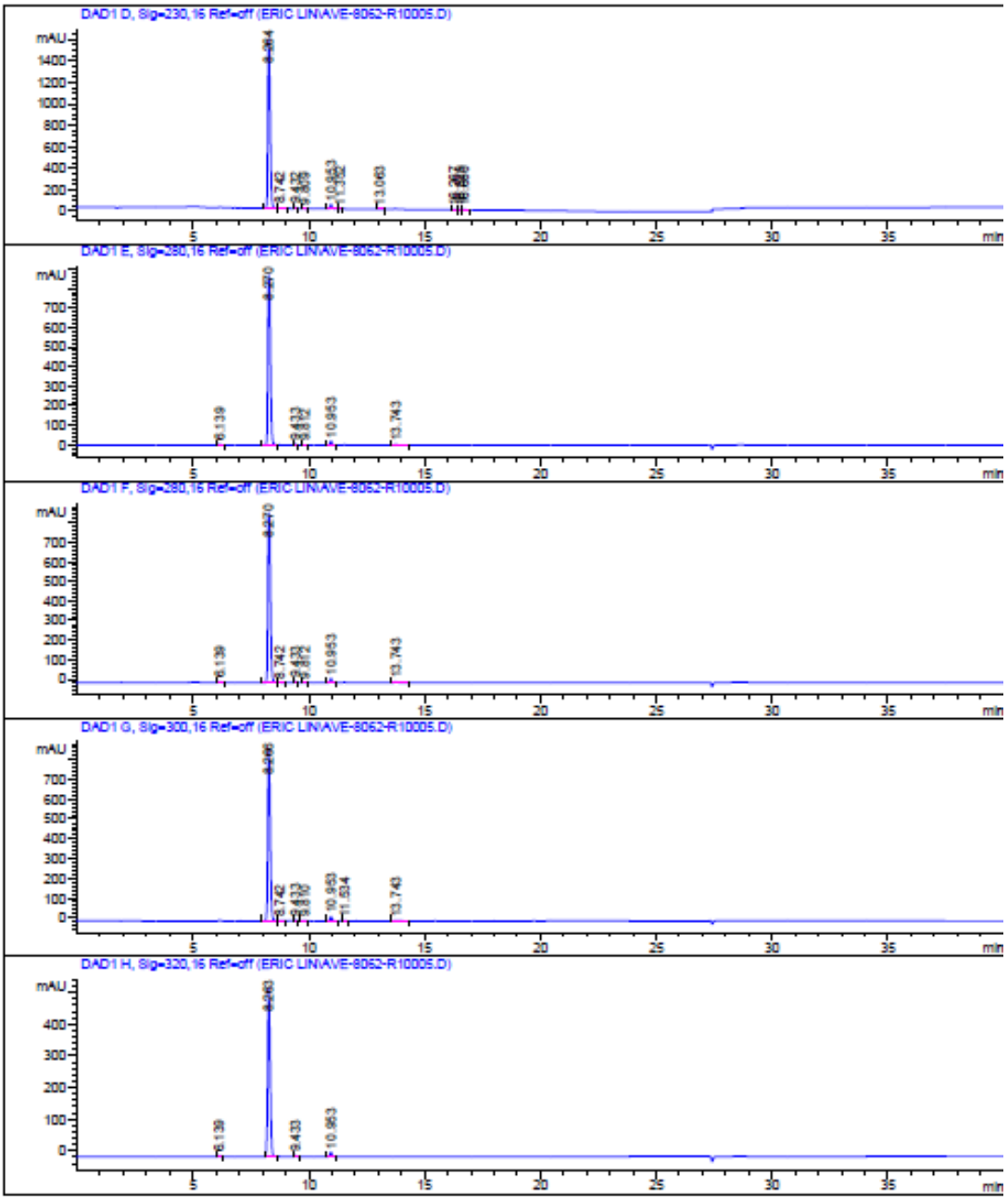

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Sample Name: AVE-8062-R1

Area Percent Report

Sorted By : Signal
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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	6.232	VB	0.0727	7.52764	1.54801	0.0804
2	8.265	BV	0.1118	9095.58008	1296.55225	97.1534
3	8.741	VV	0.1199	15.38805	1.72812	0.1644
4	9.432	VB	0.0763	19.83443	3.97215	0.2119
5	9.810	BB	0.0796	11.97414	2.26929	0.1279
6	10.953	BB	0.0813	187.22200	34.52772	1.9998
7	13.742	BB	0.1673	24.55193	2.11709	0.2622
Totals :				9362.07827	1342.71463	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.231	VB	0.0696	7.30522	1.53343	0.0782
2	8.266	BV	0.1122	9068.53711	1286.86707	97.0999
3	8.741	VV	0.1311	19.50147	1.97604	0.2088
4	9.131	VV	0.1193	8.92628	1.00854	0.0956
5	9.432	VV	0.0819	25.36259	4.63422	0.2716
6	9.810	VB	0.0887	14.70501	2.42779	0.1575
7	10.953	BB	0.0812	185.16338	34.15549	1.9826
8	19.789	BB	0.0890	9.88684	1.72187	0.1059
Totals :				9339.38790	1334.32444	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.143	BV	0.0688	29.88397	6.60556	0.2195
2	6.230	VB	0.0716	43.34299	9.09865	0.3183
3	6.970	VB	0.1280	22.19154	2.31232	0.1630

Data File C:\CHEM32\1\DATA\ERIC LIN\AVE-8062-R10005.D
Sample Name: AVE-8062-R1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
4	7.474	BB	0.0867	7.42790	1.19249	0.0546
5	8.264	BV	0.1121	1.31104e4	1863.22046	96.2920
6	8.738	VB	0.1087	29.73862	3.74297	0.2184
7	9.430	BB	0.0793	74.17680	14.59327	0.5448
8	9.804	BB	0.0708	12.46281	2.75495	0.0915
9	10.953	BV	0.0808	285.62799	53.03270	2.0979
Totals :				1.36152e4	1956.55338	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.264	BV	0.1086	1.09526e4	1585.03564	96.7945
2	8.742	VV	0.1154	24.17192	2.83805	0.2136
3	9.432	VB	0.0771	47.90776	9.44978	0.4234
4	9.809	BB	0.0822	14.80064	2.69172	0.1308
5	10.953	BV	0.0824	231.90778	42.03893	2.0495
6	11.352	VV	0.0903	10.09067	1.72249	0.0892
7	13.063	BB	0.1017	8.28207	1.24403	0.0732
8	16.267	BB	0.0863	7.61213	1.38036	0.0673
9	16.529	BV	0.0940	7.70709	1.28438	0.0681
10	16.680	VV	0.1121	10.23456	1.29656	0.0904
Totals :				1.13153e4	1648.98195	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.139	BB	0.0786	27.36969	5.27338	0.4053
2	8.270	BV	0.1215	6523.29443	869.58826	96.6053
3	9.433	BB	0.0751	25.17395	5.13926	0.3728
4	9.812	BB	0.0839	7.86839	1.39310	0.1165
5	10.953	BB	0.0813	117.99647	21.75365	1.7474
6	13.743	BB	0.1650	50.81811	4.39317	0.7526
Totals :				6752.52103	907.54081	

Data File C:\CHEM32\1\DATA\ERIC LIN\AVE-8062-R10005.D
Sample Name: AVE-8062-R1

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.139	BB	0.0786	27.37036	5.27350	0.4045
2	8.270	BV	0.1215	6523.28369	869.58813	96.4126
3	8.742	VB	0.1035	13.48111	1.84035	0.1992
4	9.433	BB	0.0751	25.17586	5.13947	0.3721
5	9.812	BB	0.0839	7.87010	1.39290	0.1163
6	10.953	BB	0.0813	118.00455	21.75415	1.7441
7	13.743	BB	0.1650	50.82064	4.39342	0.7511
Totals :				6766.00630	909.38193	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.266	BV	0.1147	6275.62891	864.02368	96.8008
2	8.742	VB	0.1013	11.85244	1.66092	0.1828
3	9.433	BB	0.0753	24.58060	5.00164	0.3792
4	9.810	BB	0.0814	7.66697	1.41149	0.1183
5	10.953	BB	0.0824	125.00182	22.64232	1.9281
6	11.534	VB	0.0826	7.59221	1.37027	0.1171
7	13.743	BB	0.1655	30.70807	2.64326	0.4737
Totals :				6483.03103	898.75359	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.139	BB	0.0714	17.48327	3.81790	0.4340
2	8.263	BV	0.1134	3913.47534	534.81958	97.1548
3	9.433	BB	0.0760	14.63547	2.94218	0.3633
4	10.953	BB	0.0840	82.49001	14.57937	2.0479
Totals :				4028.08409	556.15903	

*** End of Report ***

APPENDIX B

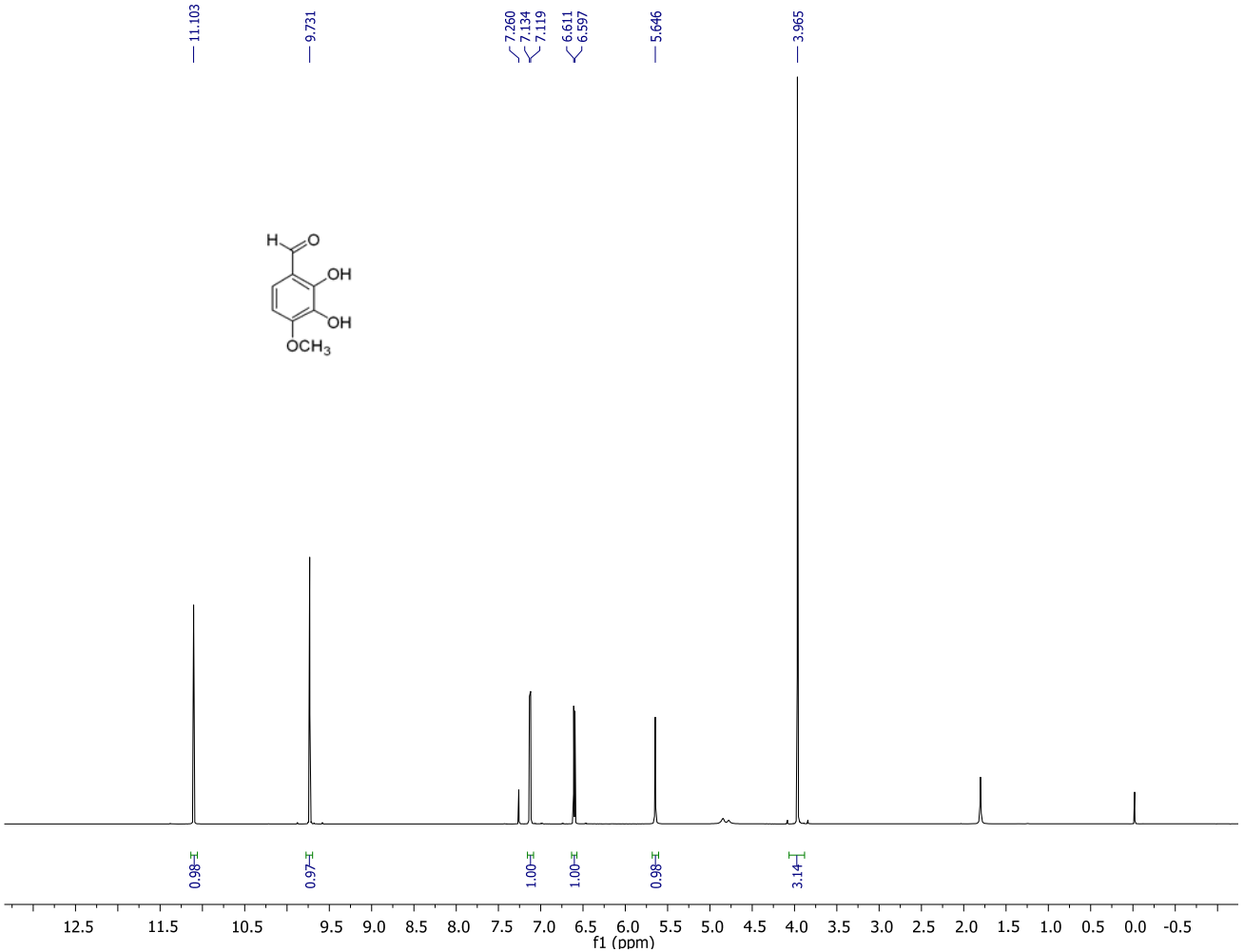
Design, Synthesis and Biological Evaluation of Bioreductively Activatable Prodrug Conjugates (BAPCs) of Combretastatin A-1 as anti-cancer Agents - Targeted towards Tumor Hypoxia

Compounds **23**, **31**, **34**, **36**, **38** and **40** were synthesized by Mr. Blake Winn. For compound **41**, Mr. Winn contributed equally

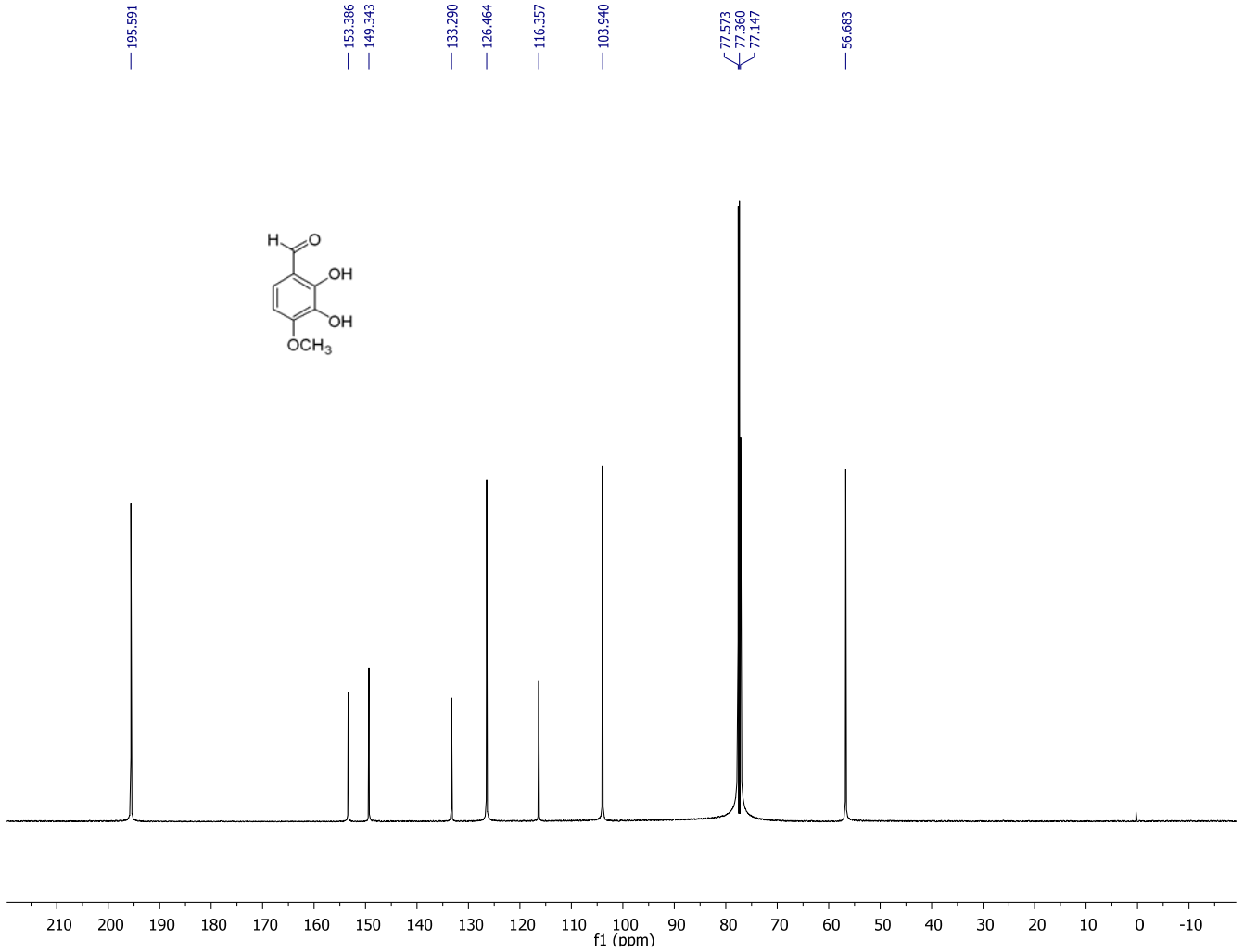
Table of Contents

NMRs, HRMS, and HPLC data:

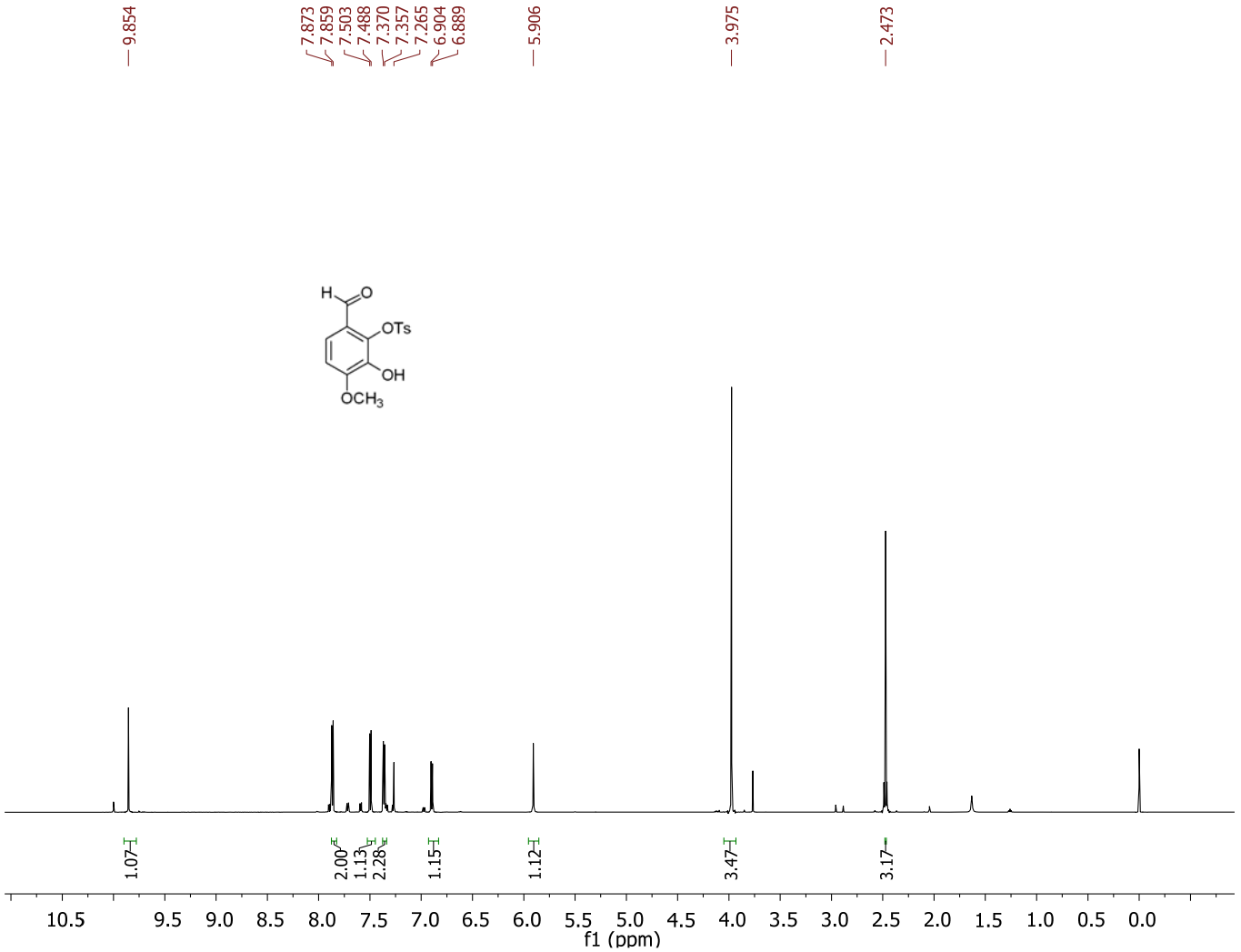
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **2**



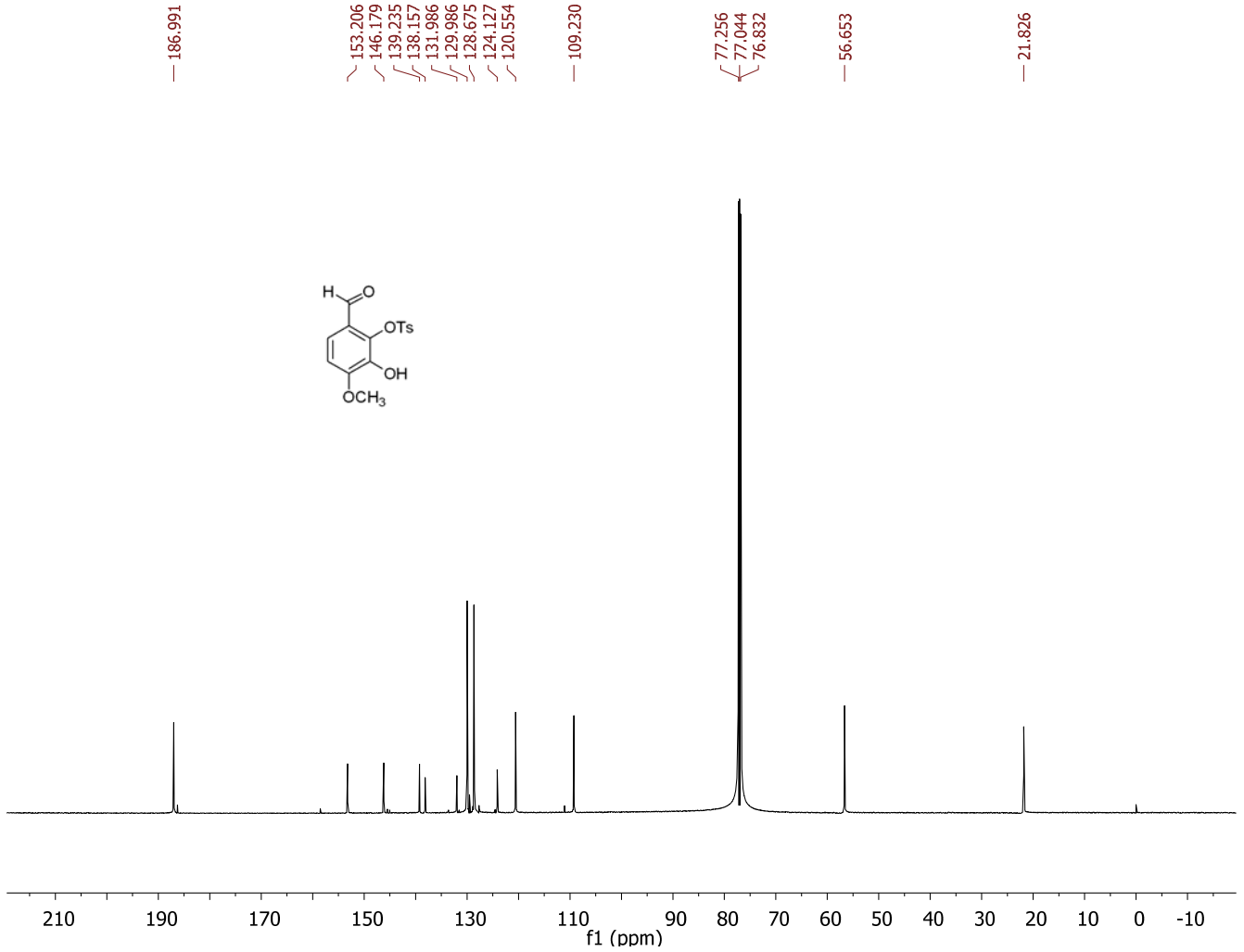
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **2**



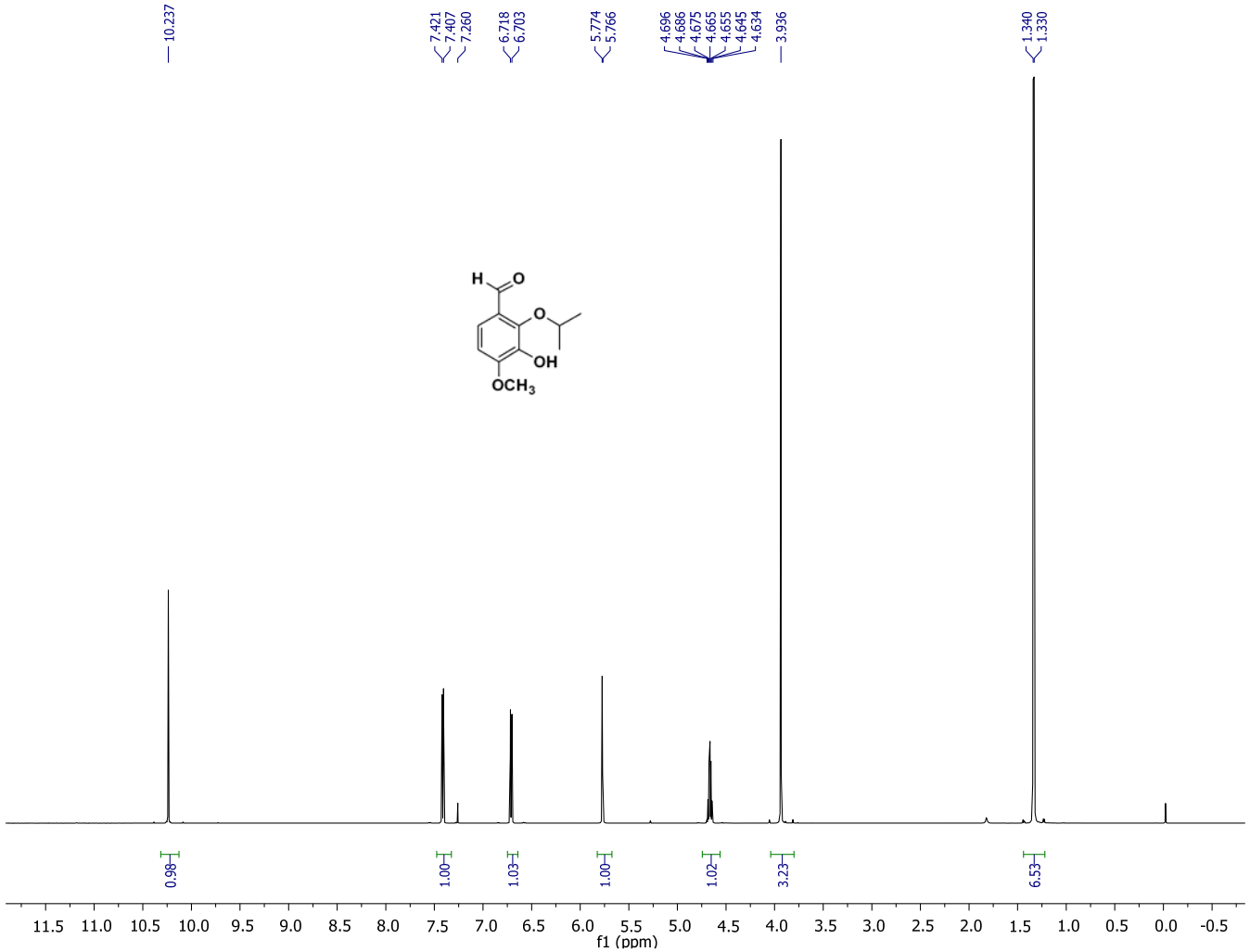
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **3**



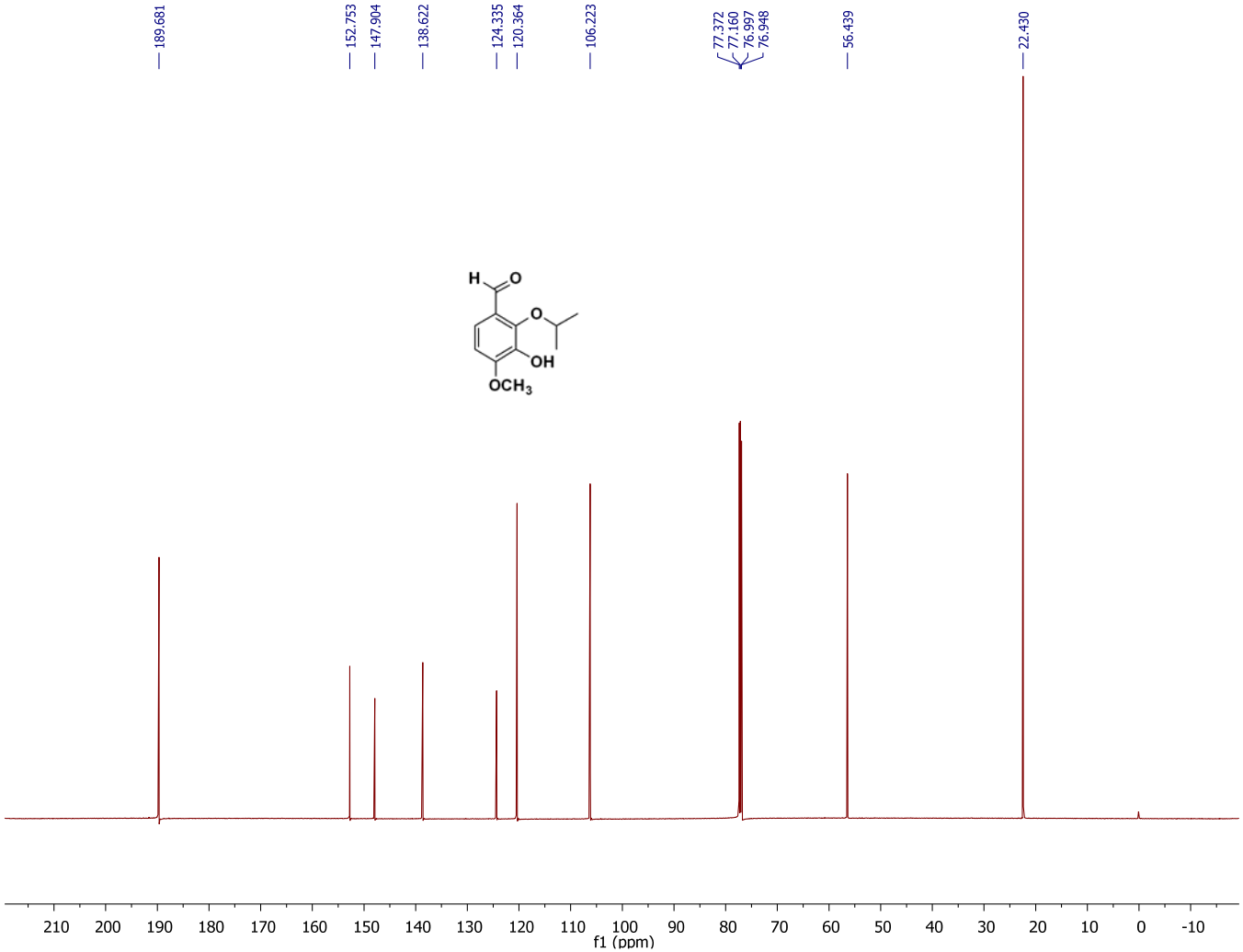
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **3**



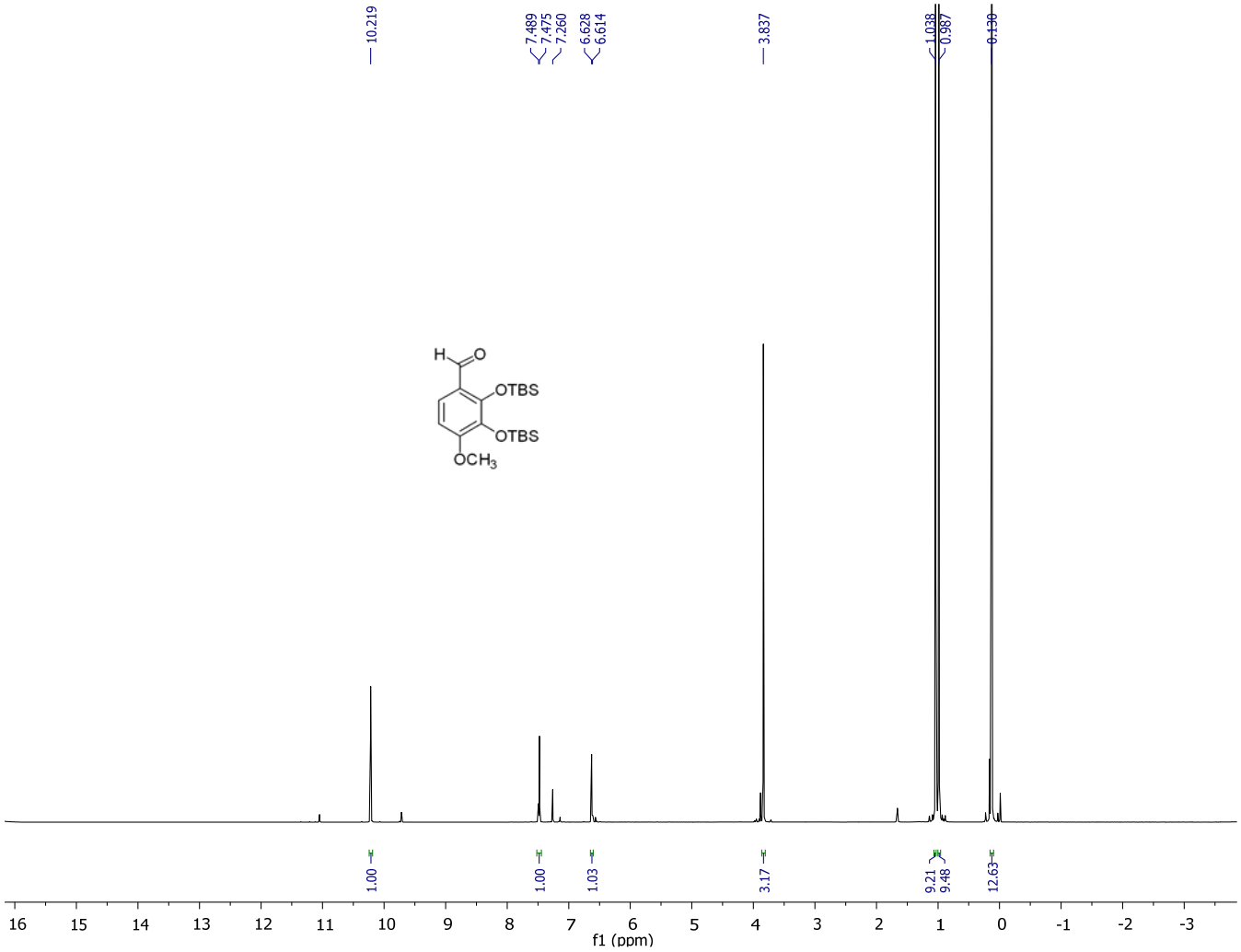
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **4**



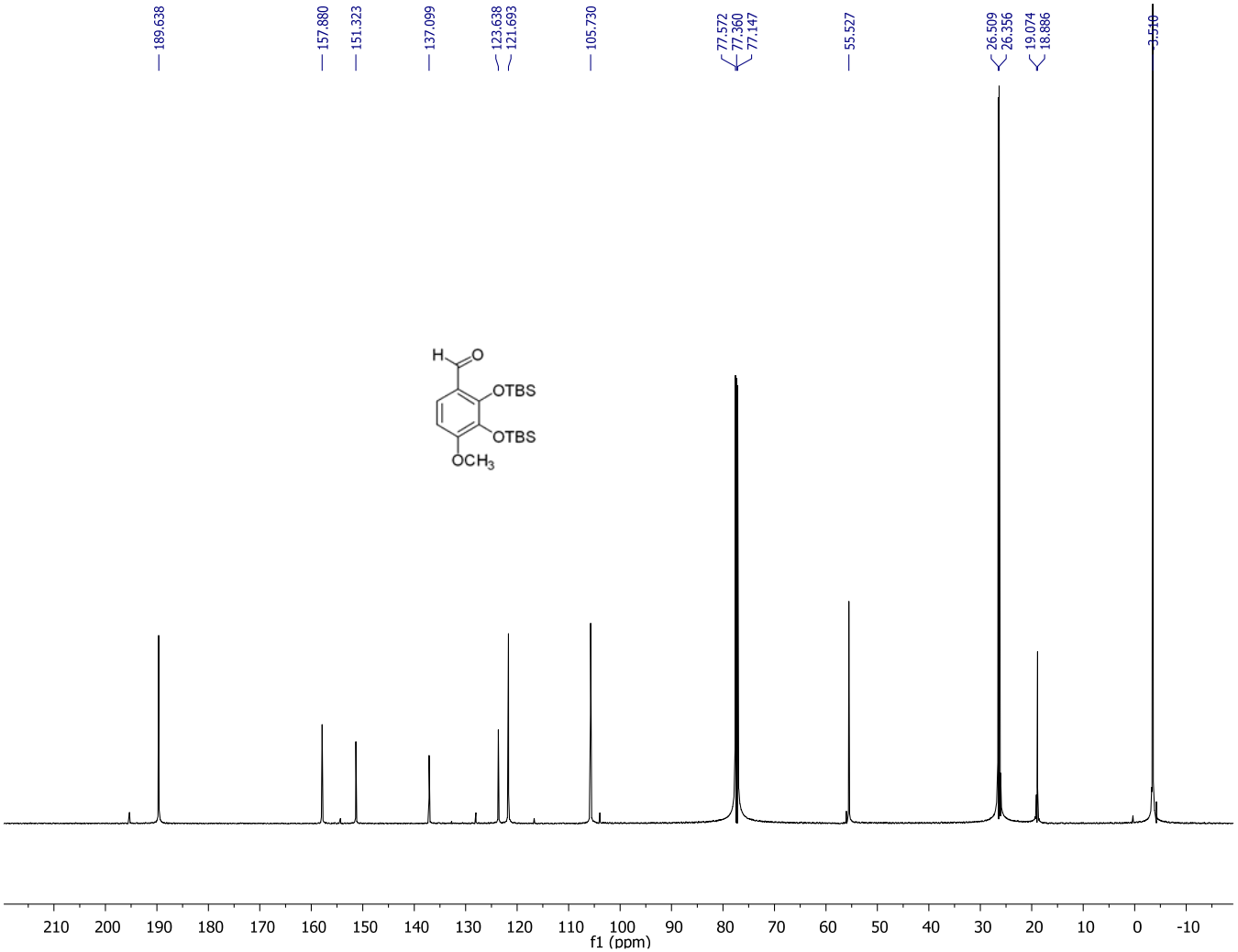
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **4**



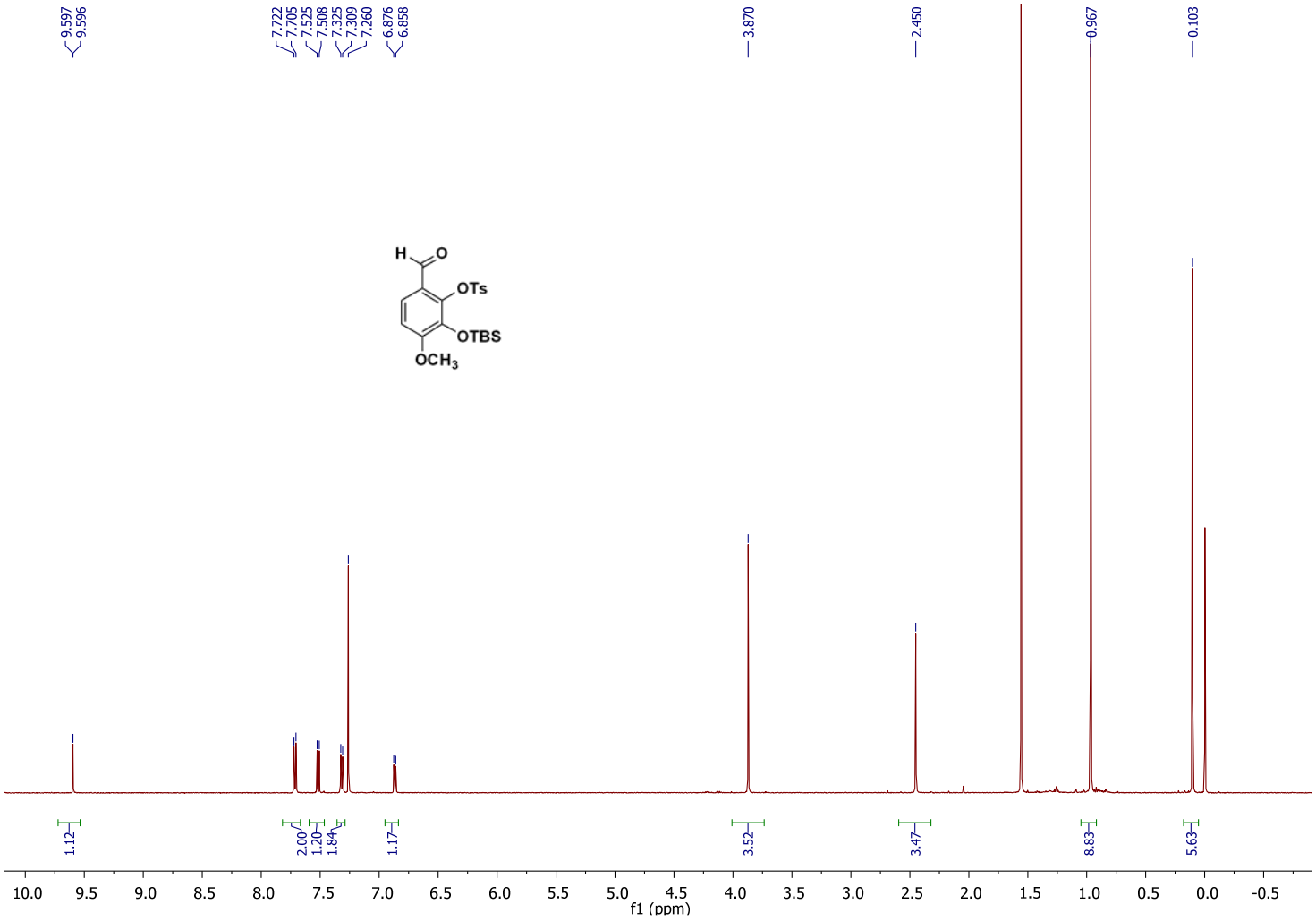
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **5**



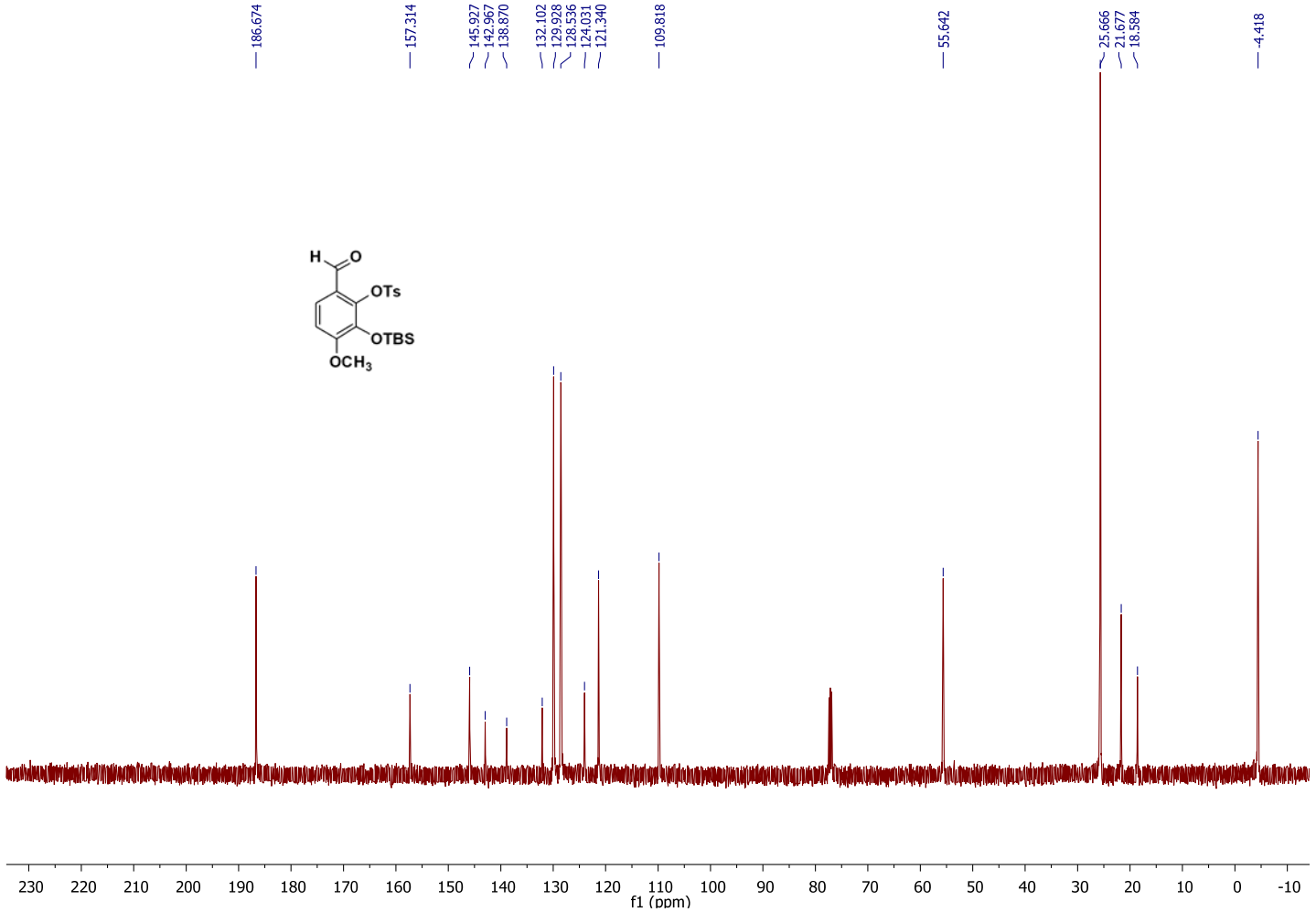
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **5**



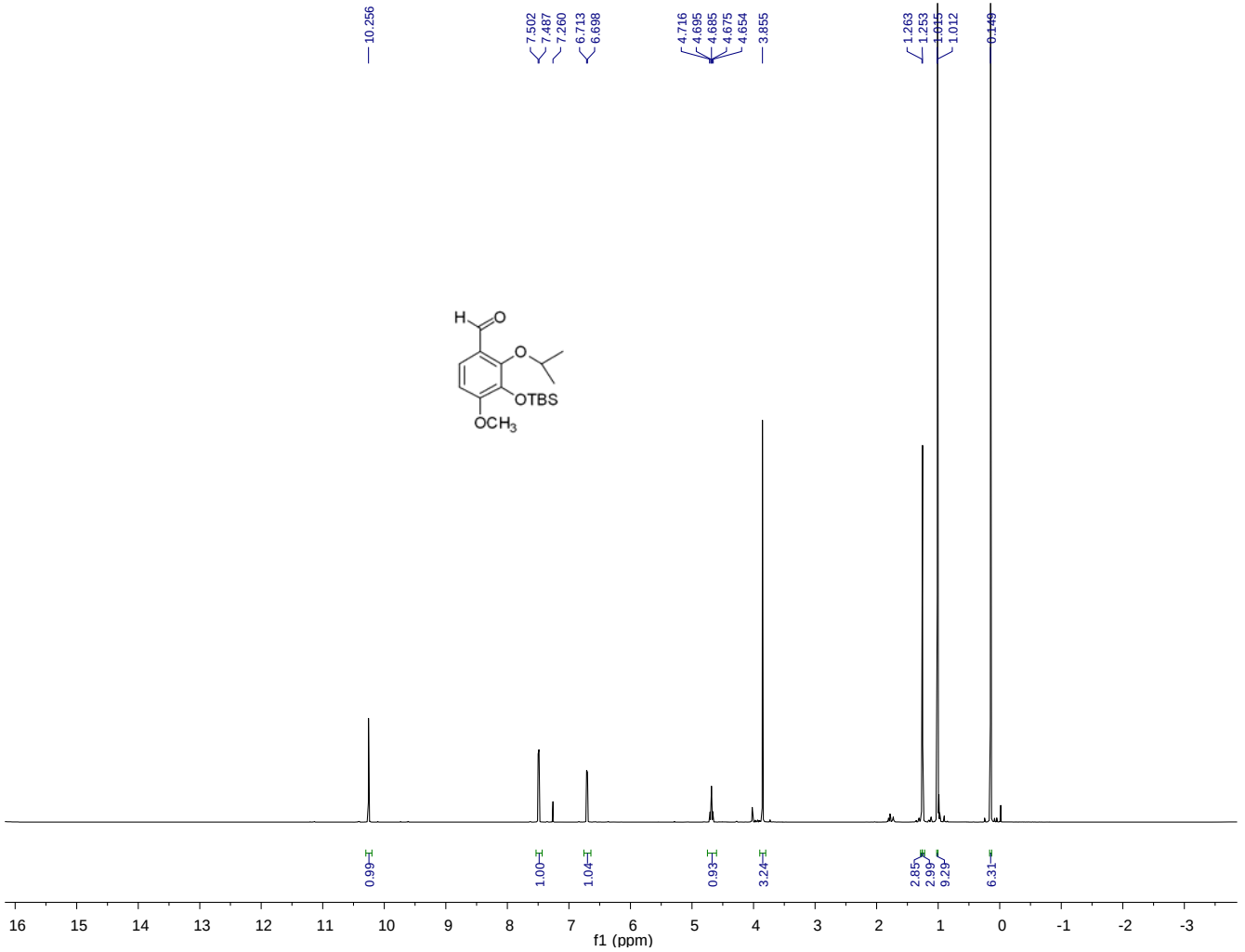
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **6**



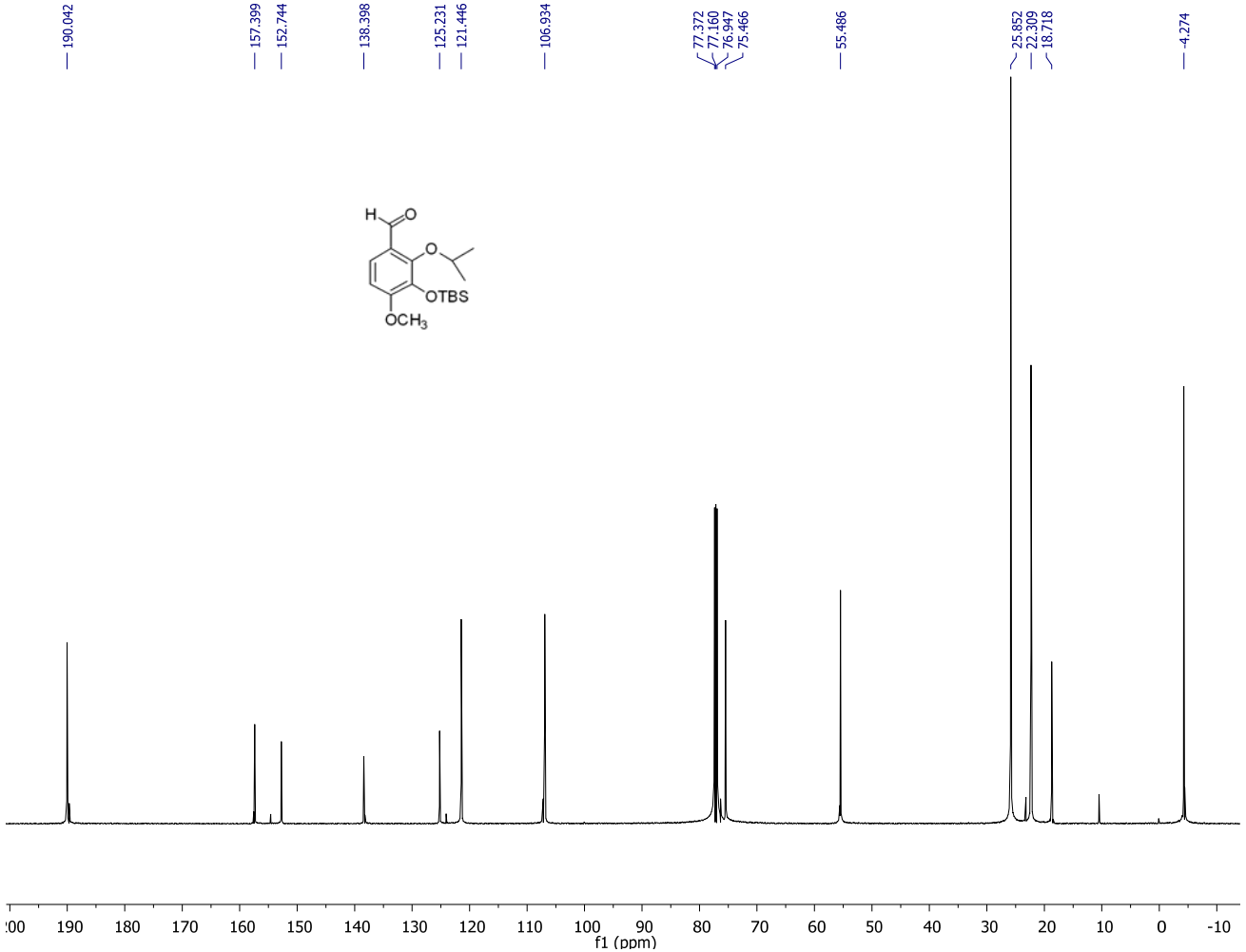
^{13}C NMR (126 MHz, $CDCl_3$) for Compound **6**



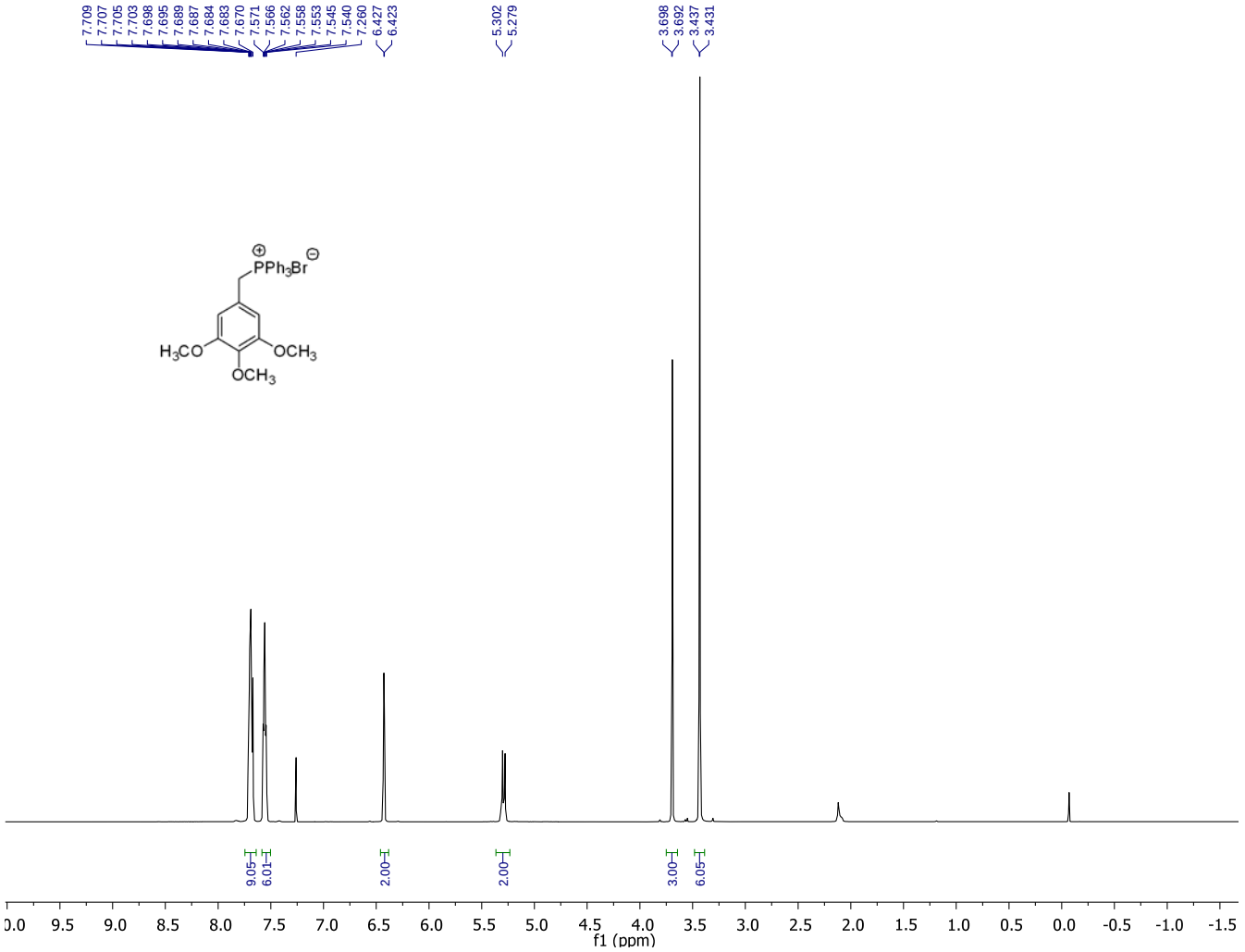
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **7**



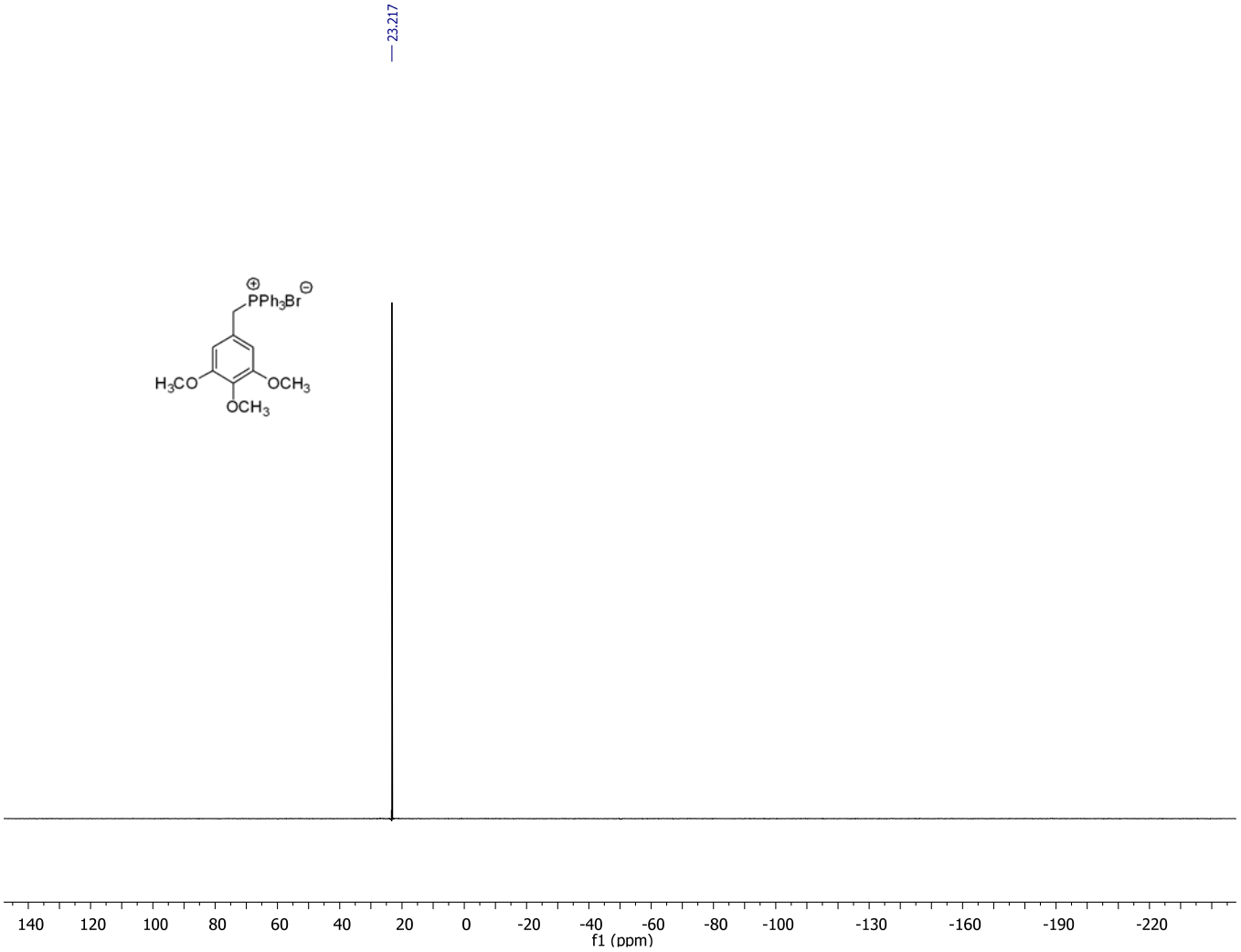
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **7**



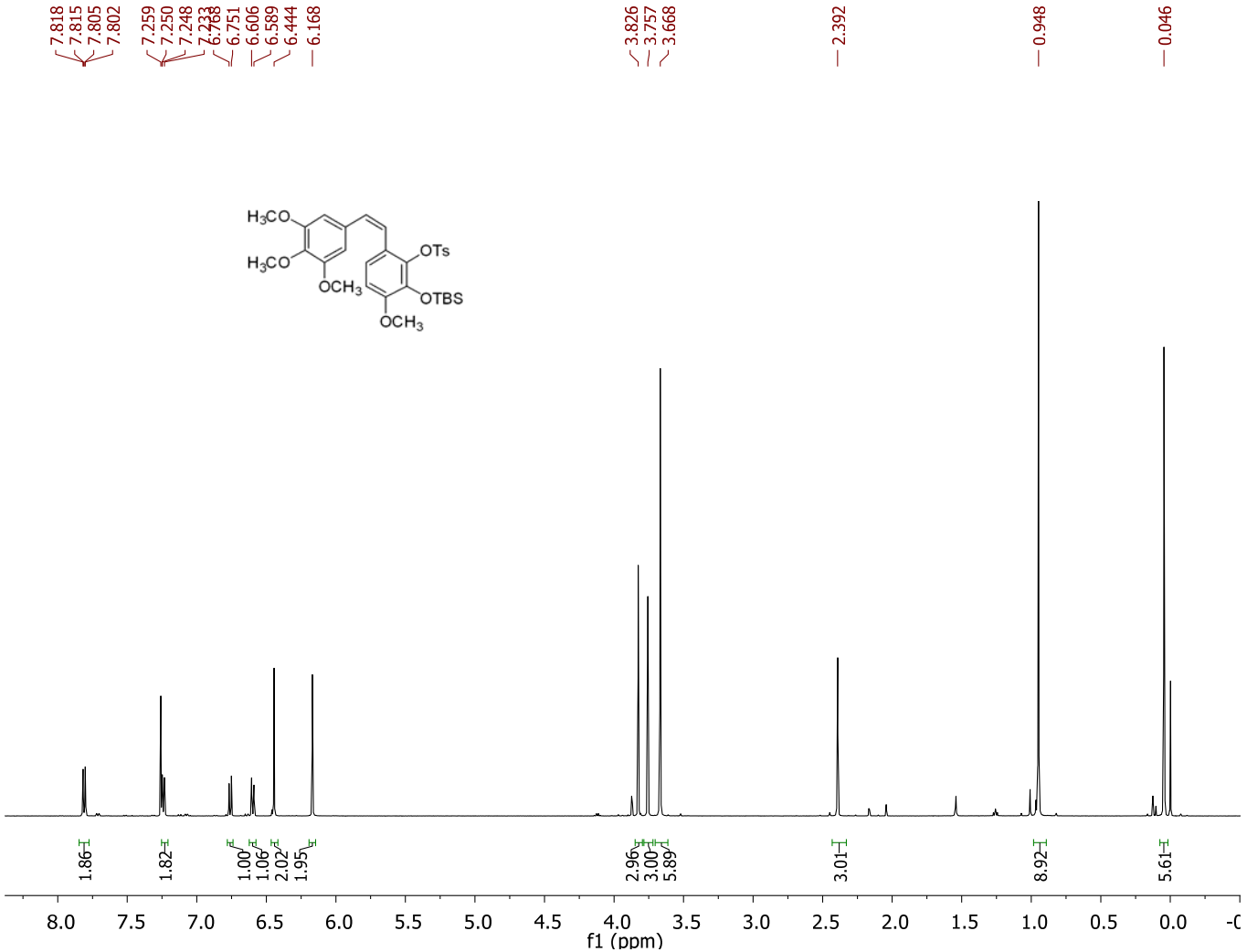
^{1}H NMR (600 MHz, $CDCl_3$) Compound **10**



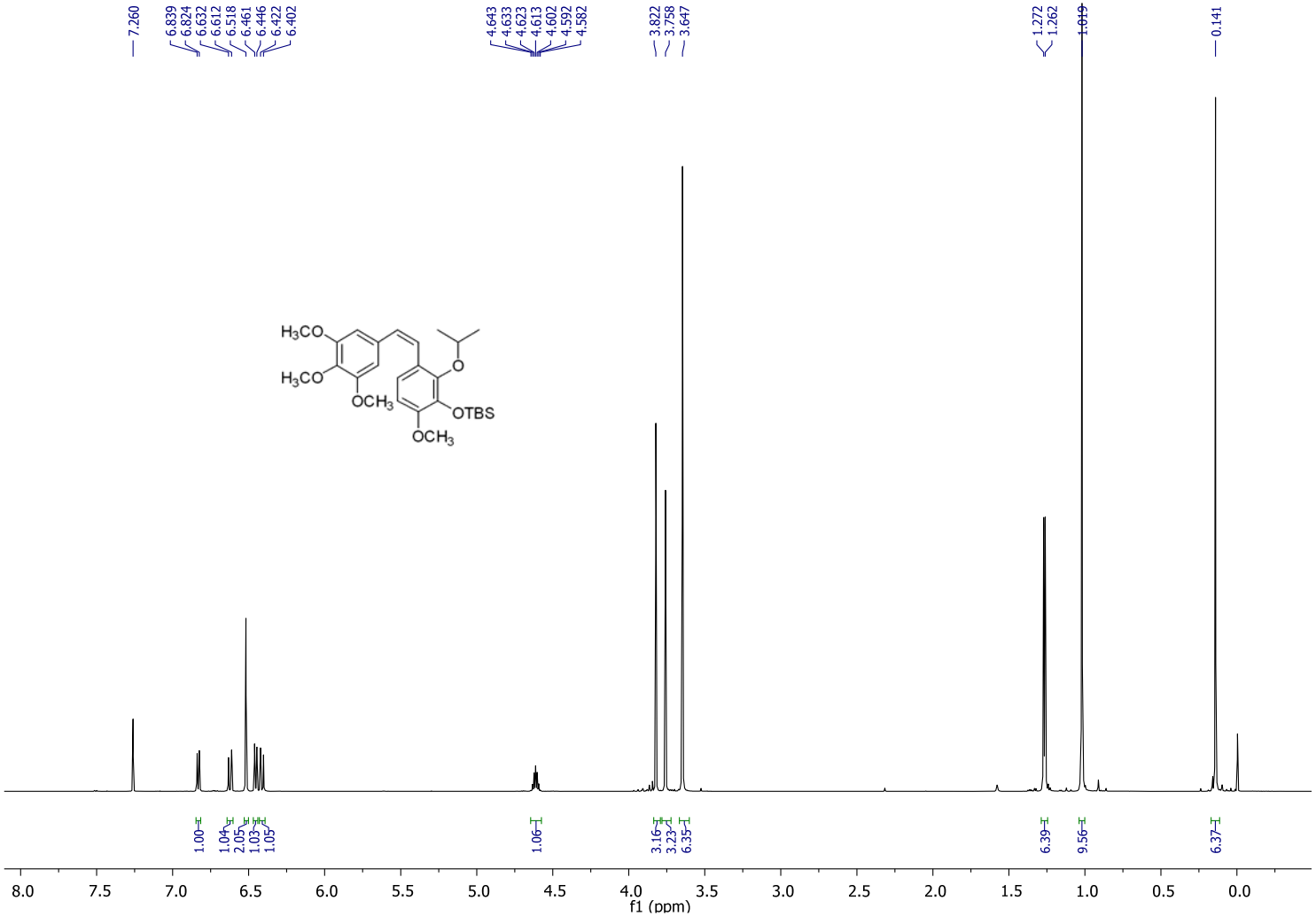
^{31}P NMR (240 MHz, $CDCl_3$) Compound **10**



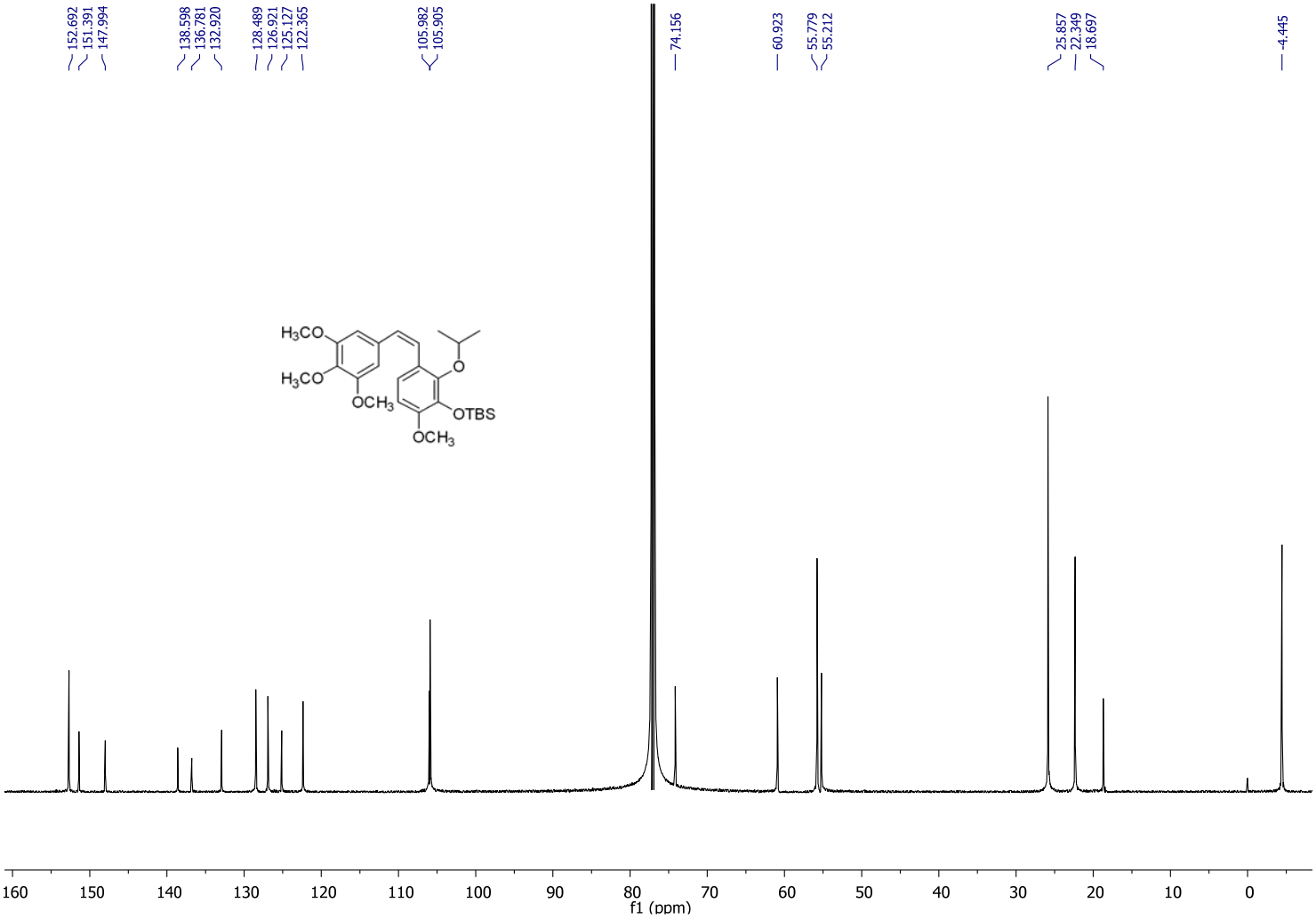
^{1}H NMR (500 MHz, $CDCl_3$) Compound **11**



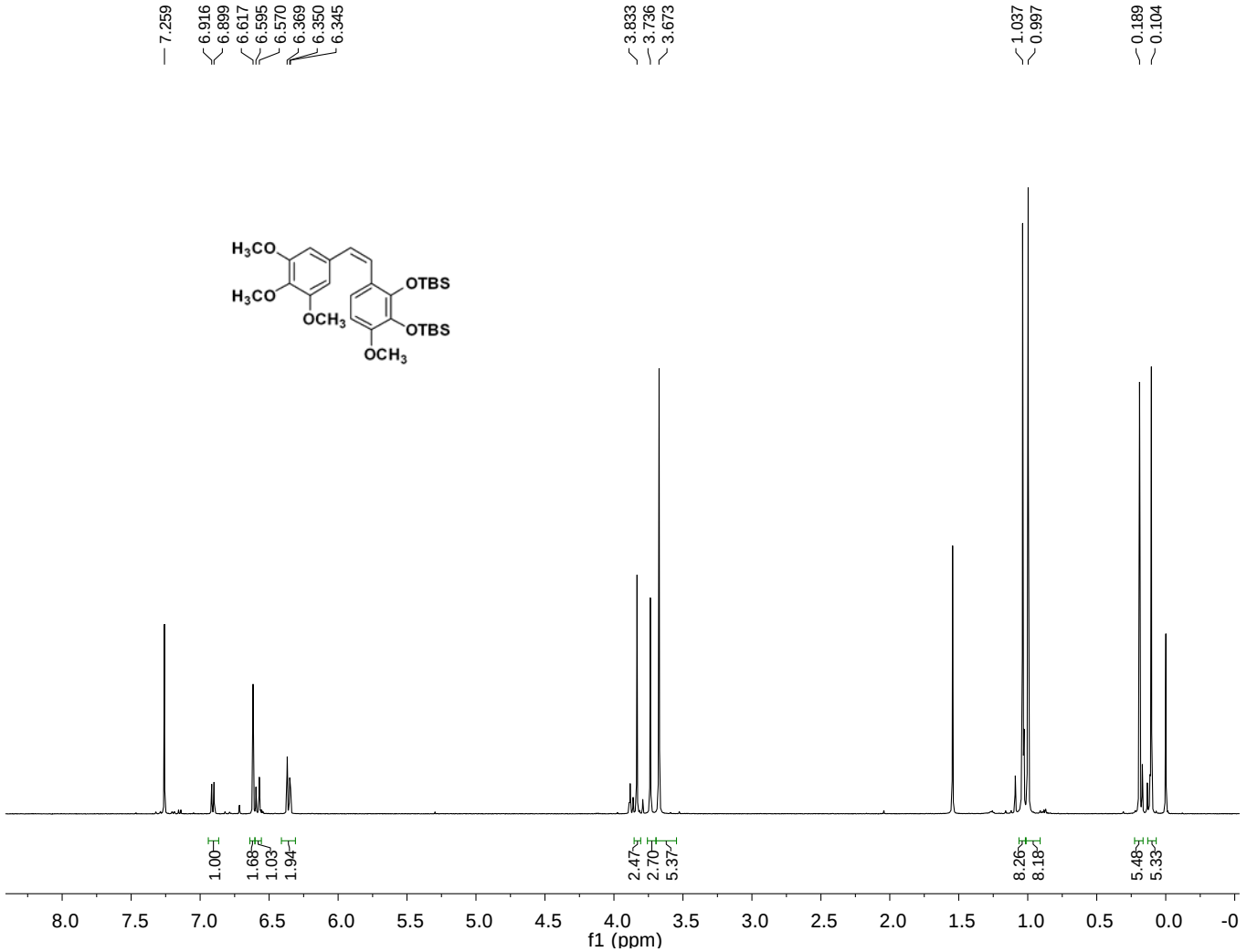
^{1}H NMR (600 MHz, $CDCl_3$) Compound **12**



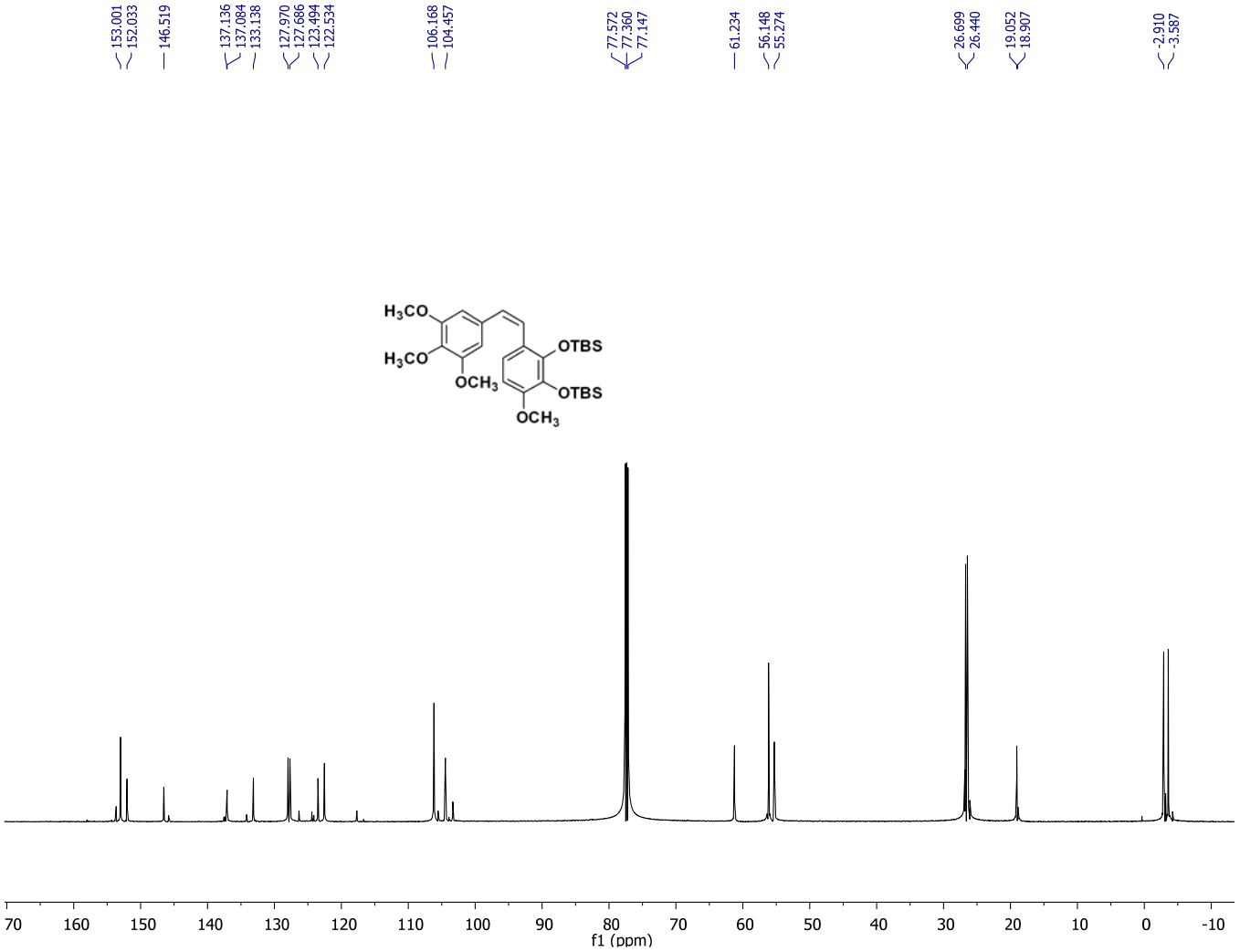
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **12**



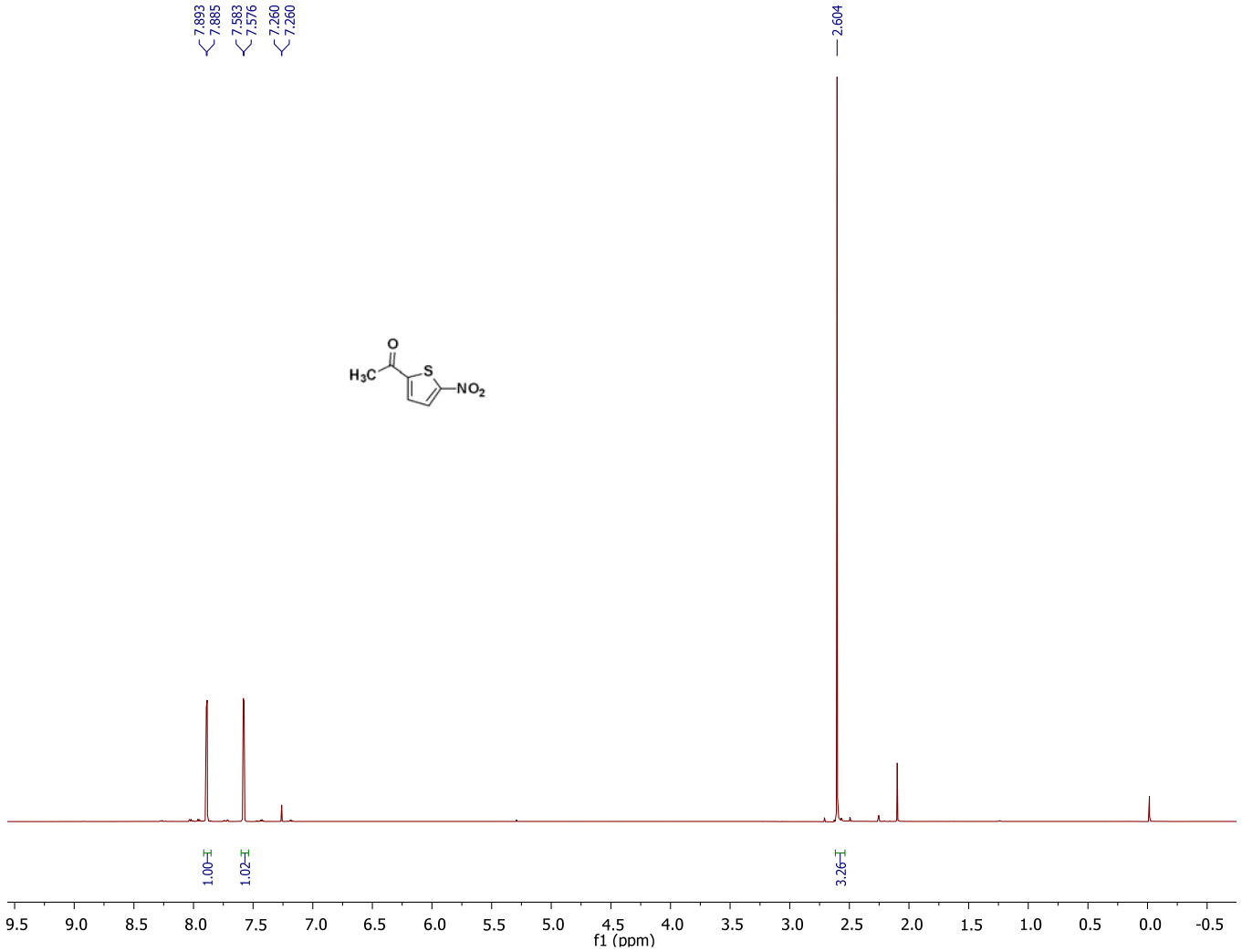
^{1}H NMR (600 MHz, $CDCl_3$) Compound **13**



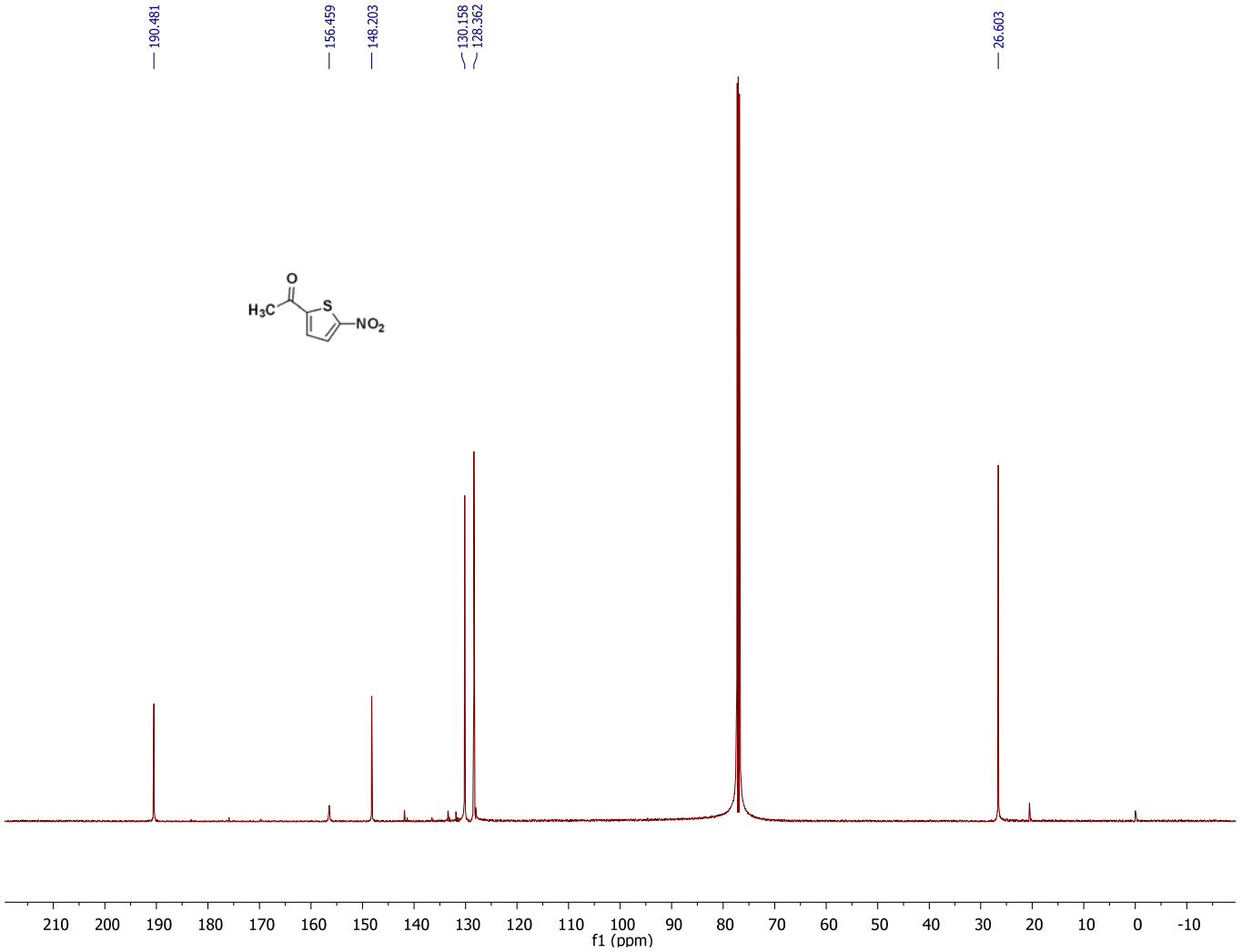
^{13}C NMR (125 MHz, $CDCl_3$) for Compound **13**



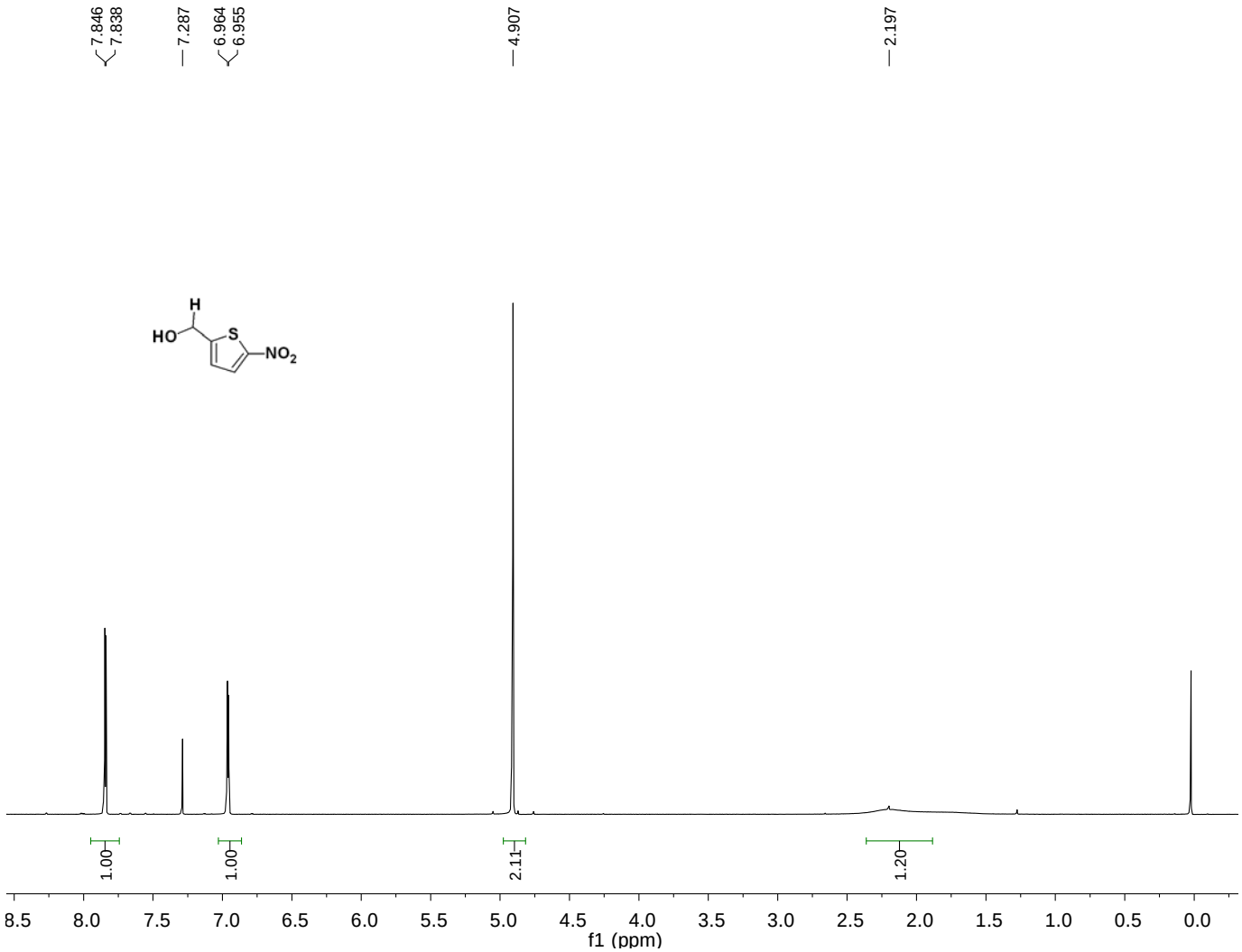
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **15**



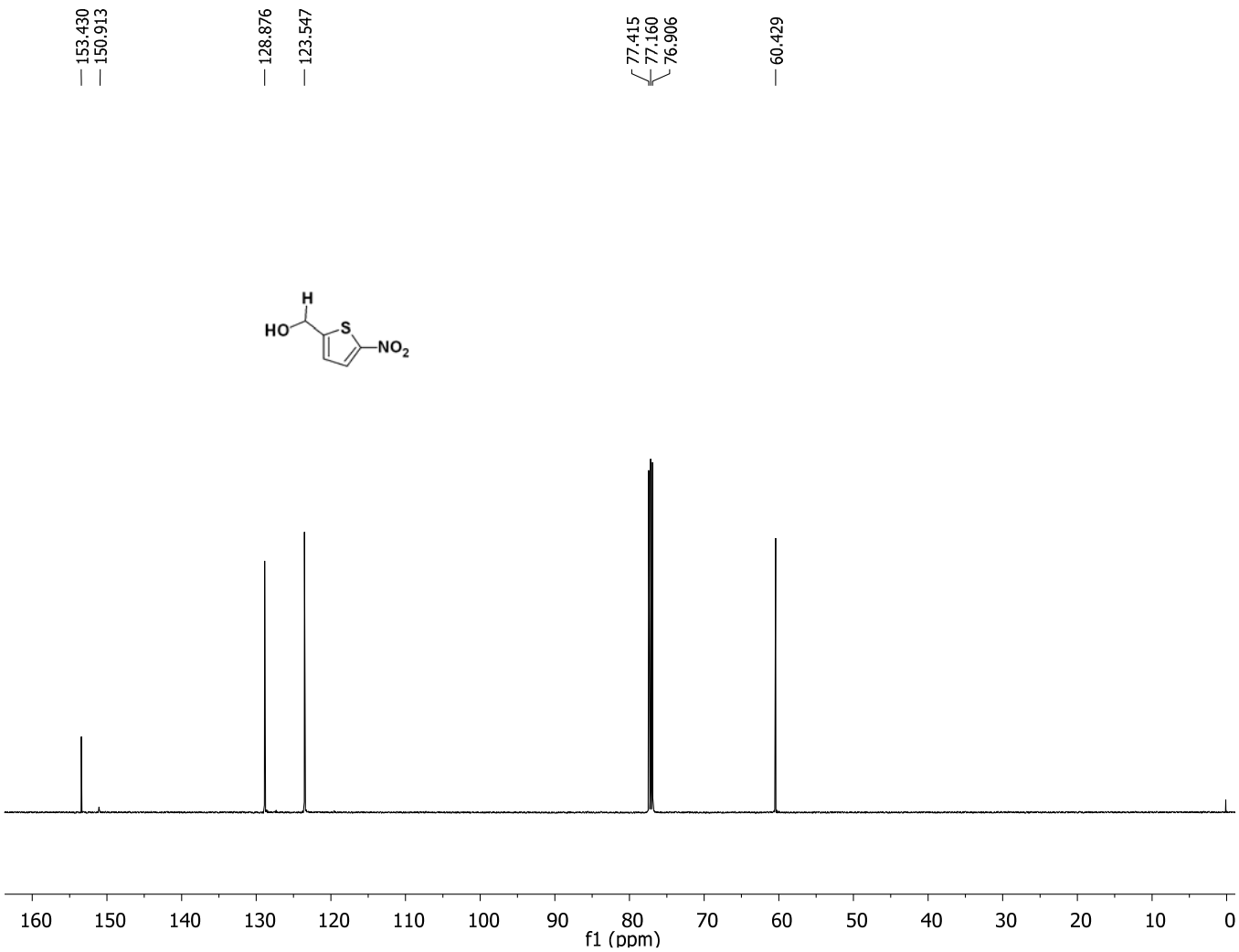
^{13}C NMR (126 MHz, $CDCl_3$) for Compound **15**



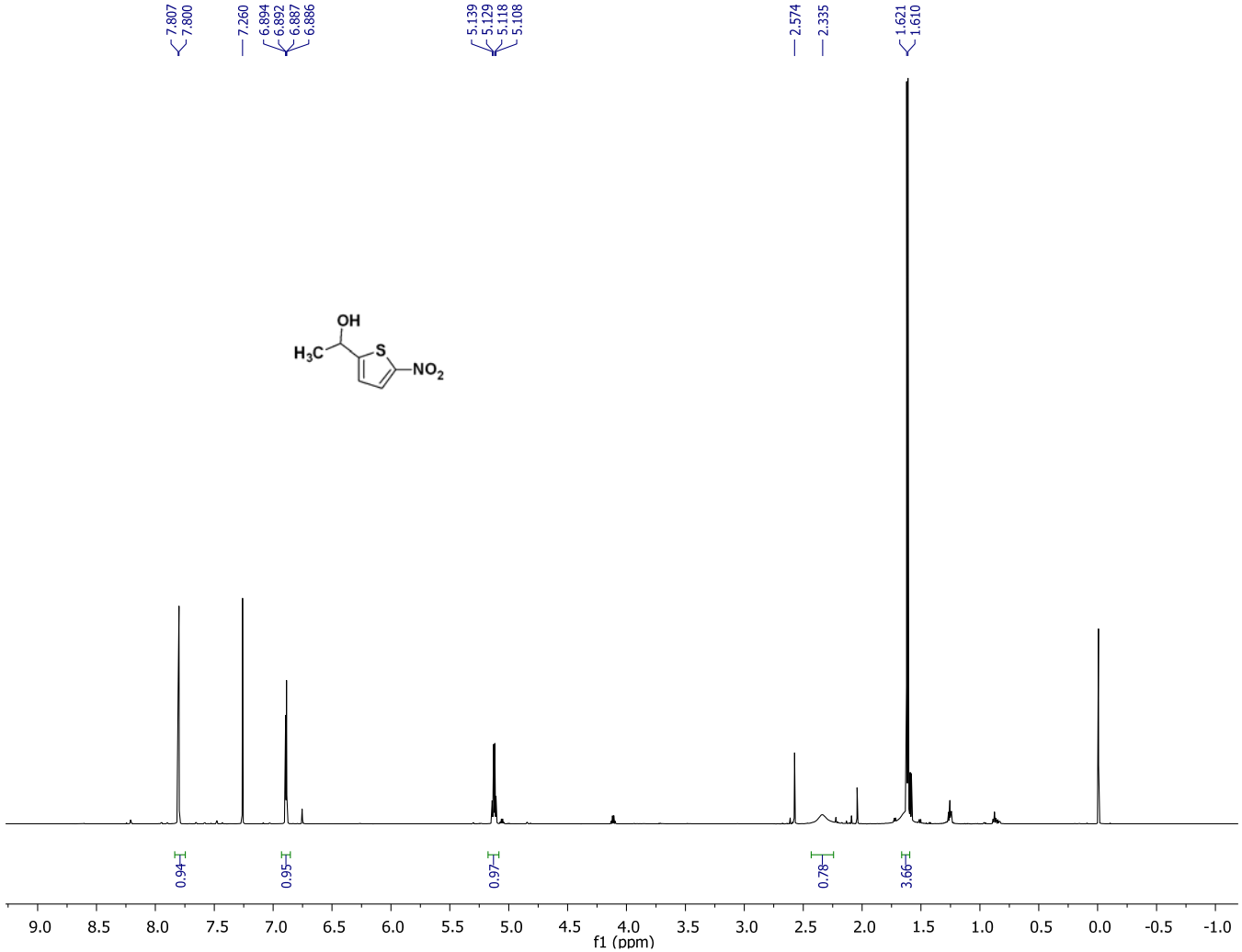
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **16**



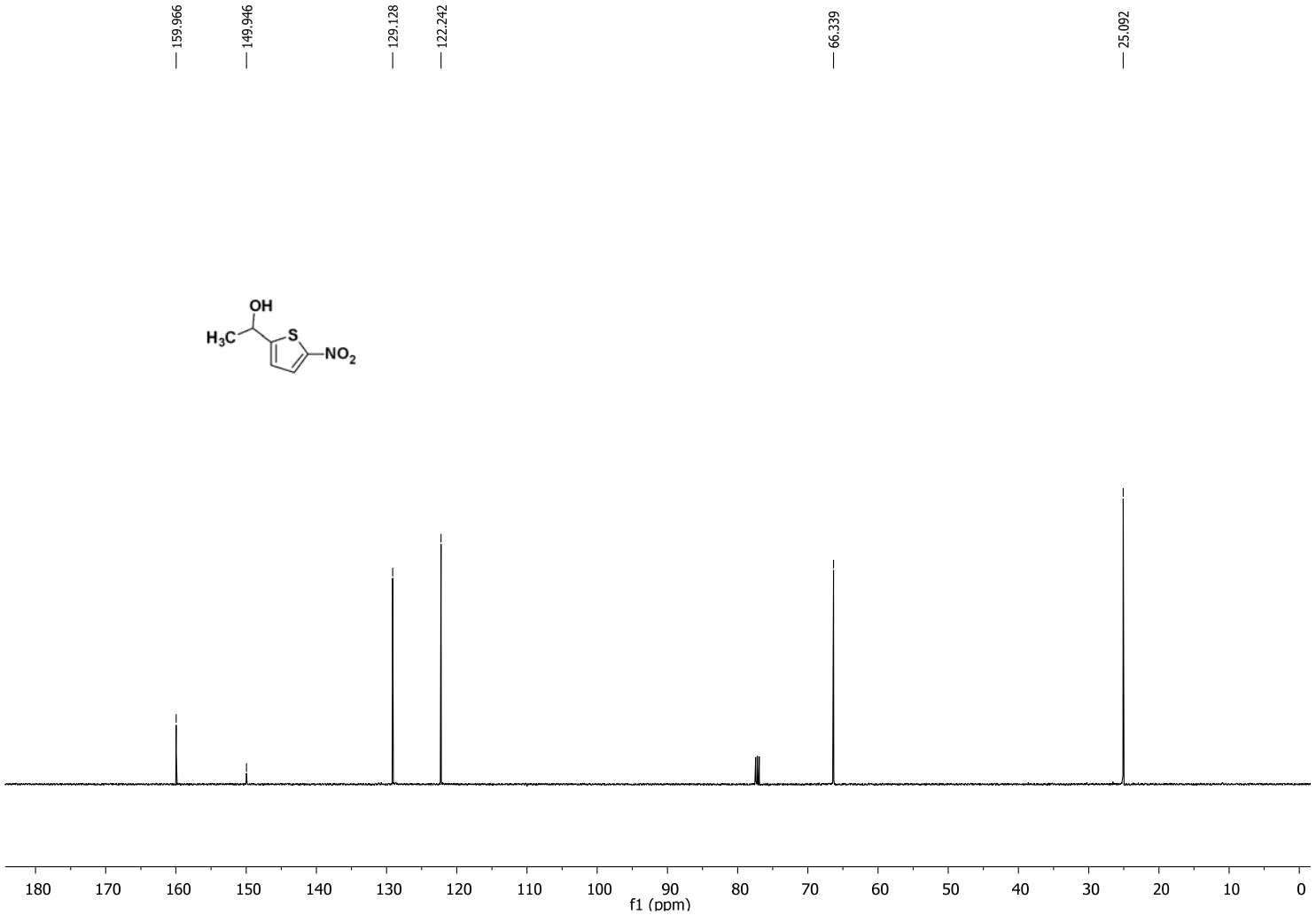
^{13}C NMR (126 MHz, $CDCl_3$) for Compound **16**



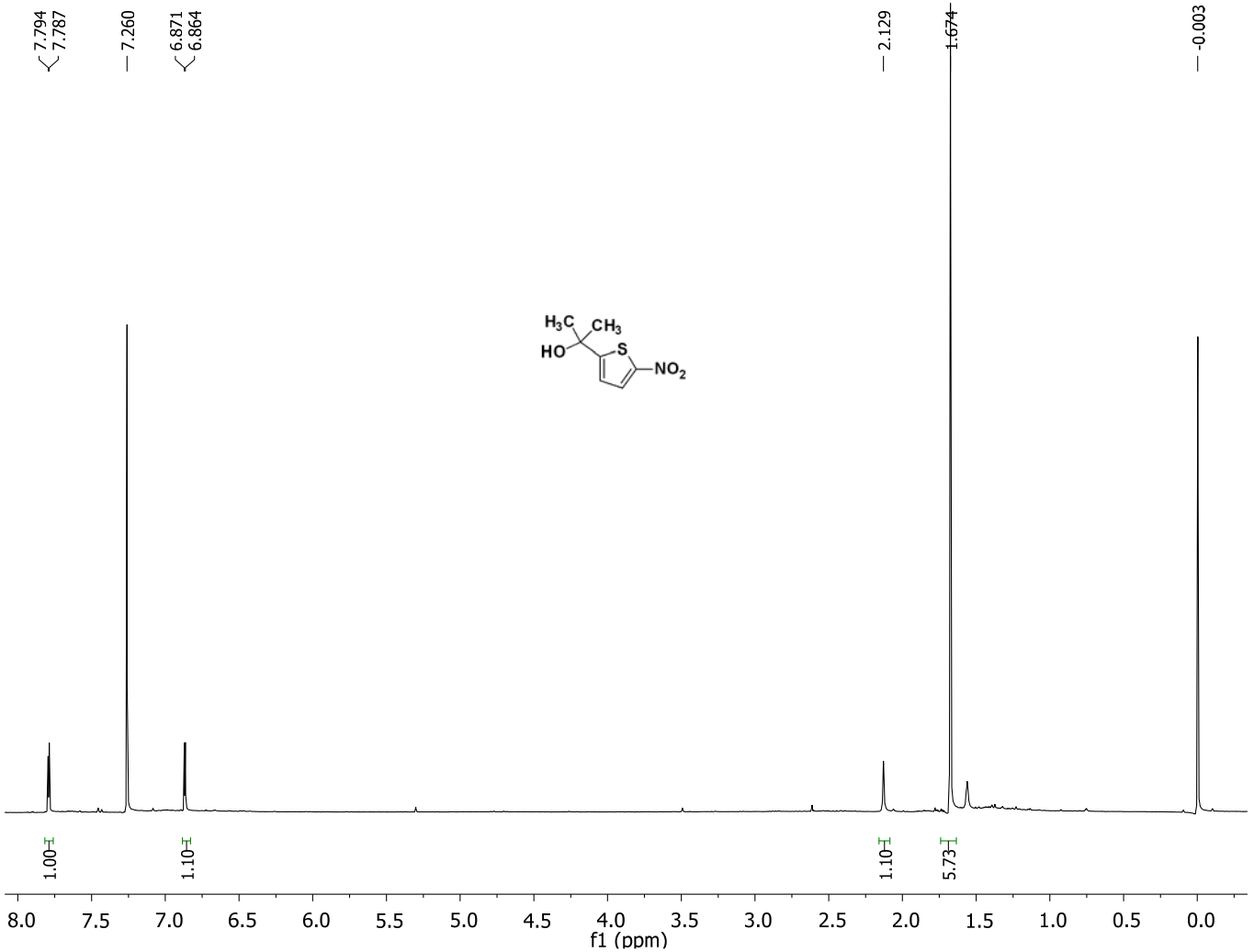
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **17**



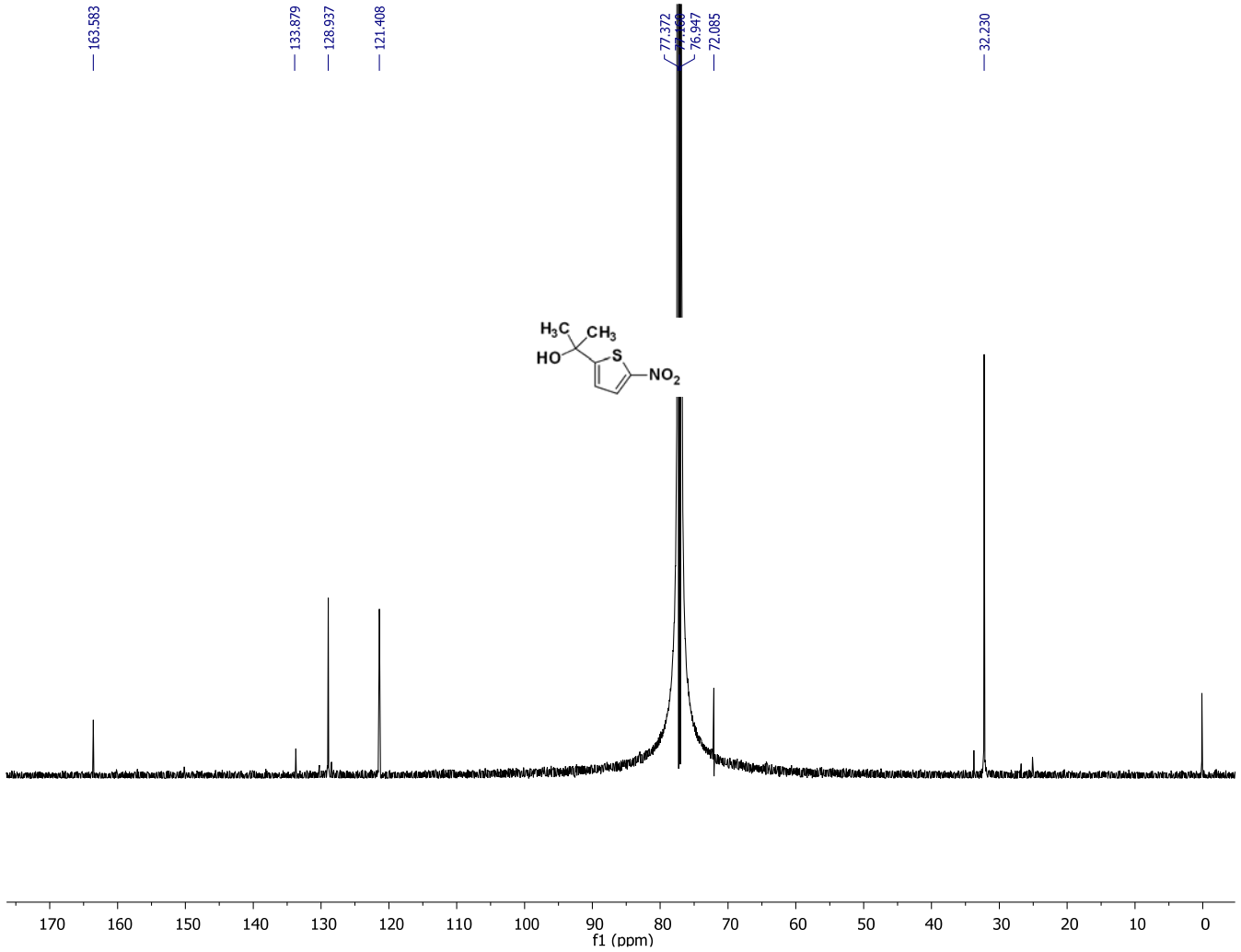
^{13}C NMR (126 MHz, $CDCl_3$) for Compound **17**



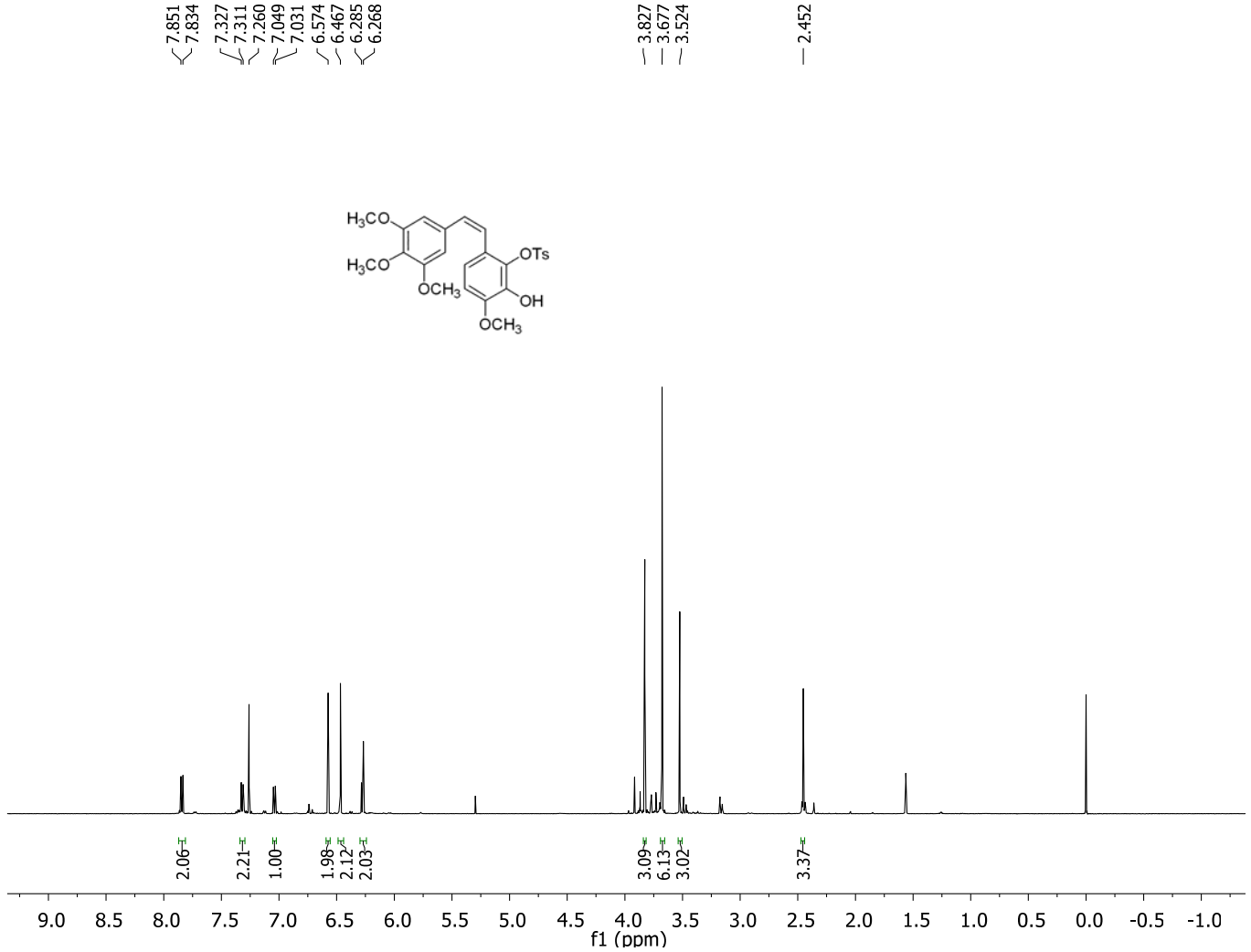
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **19**



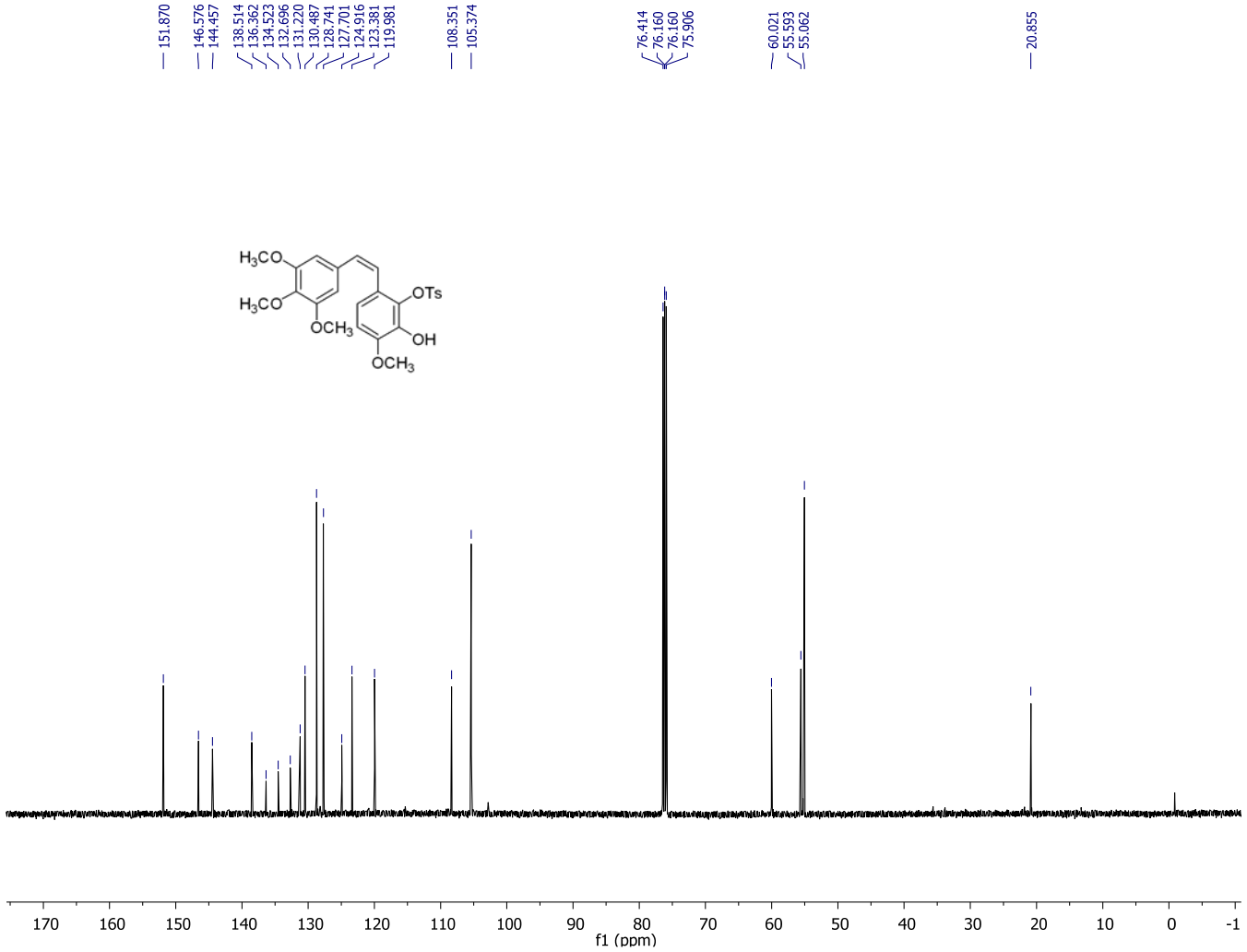
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **19**



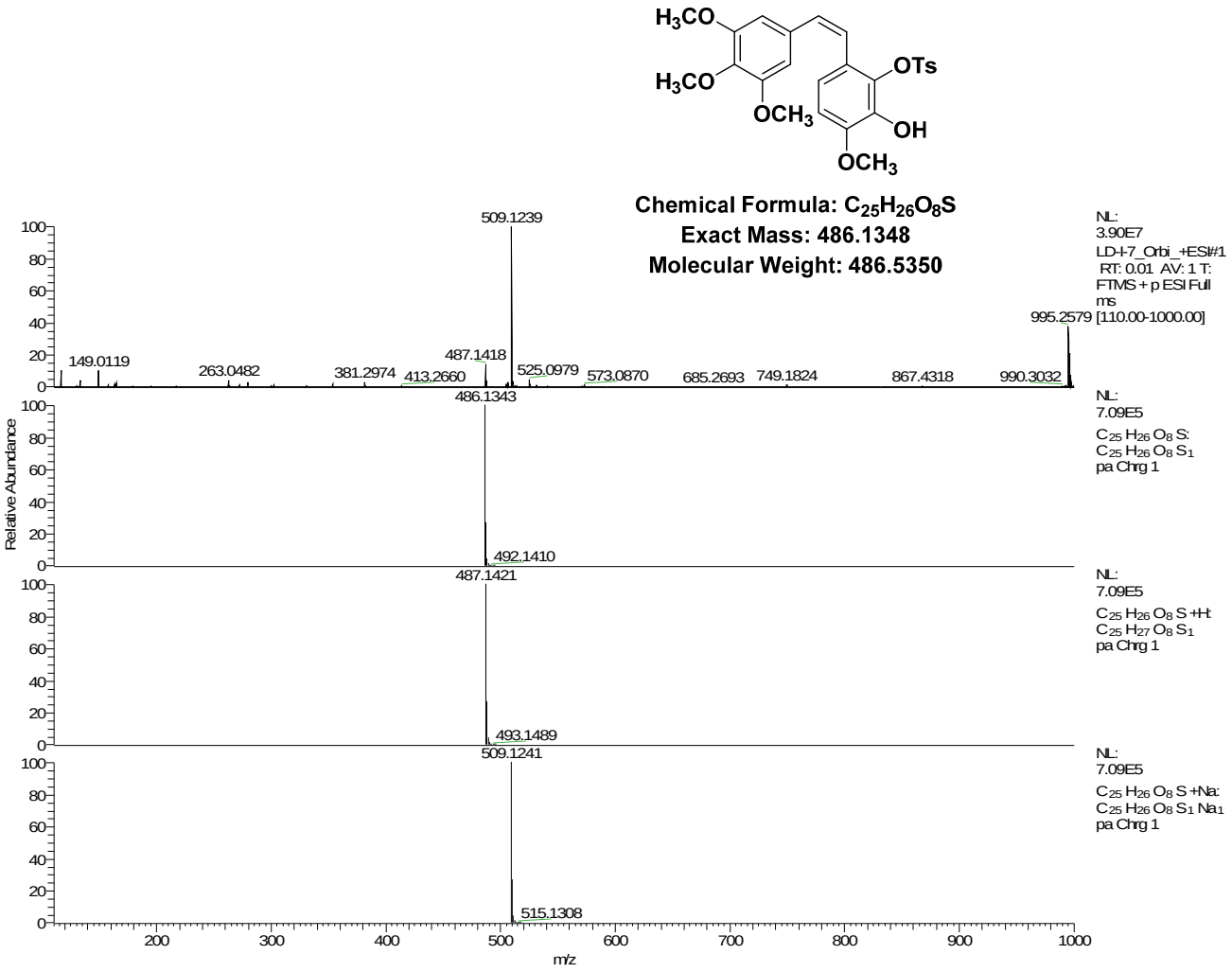
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **20**



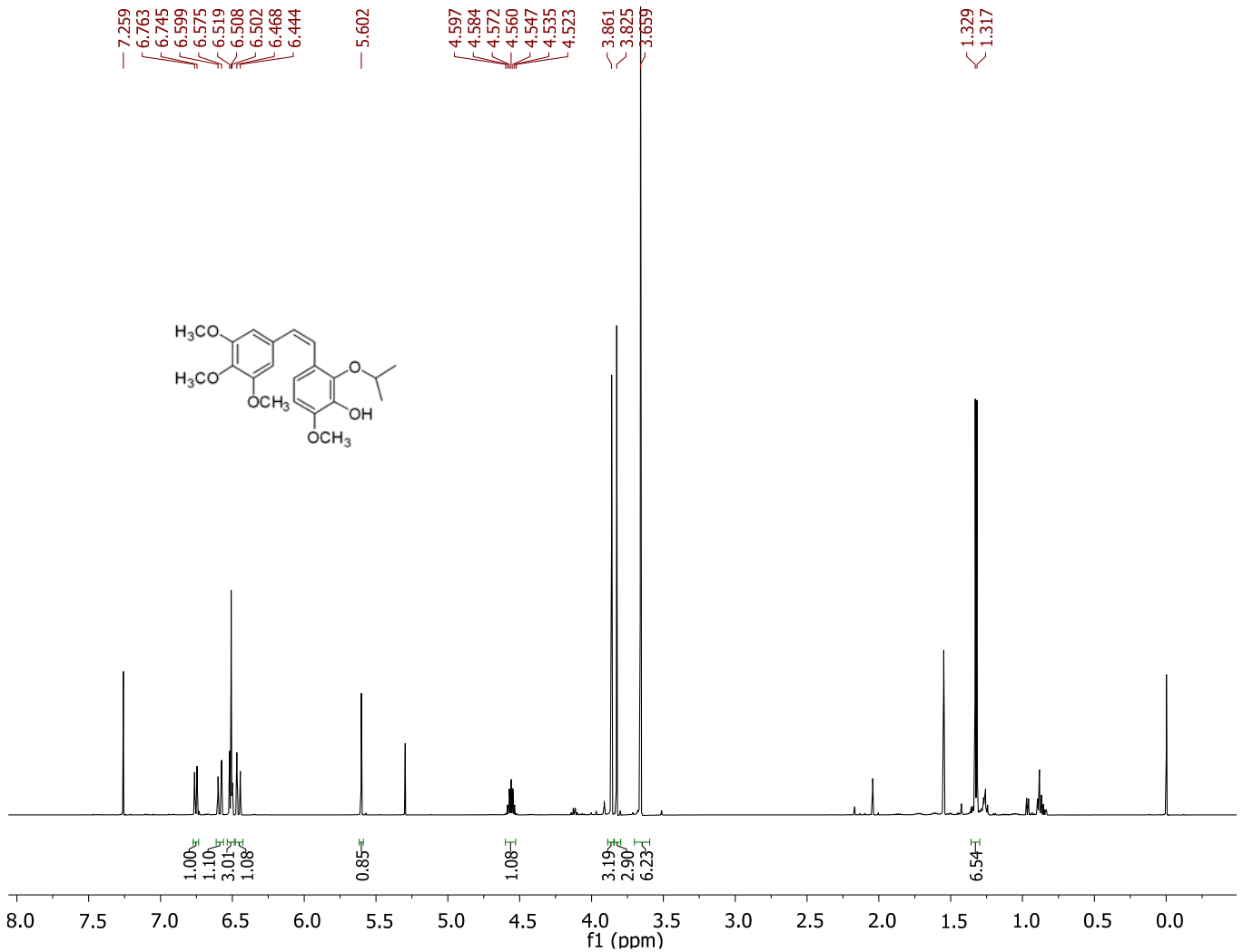
^{13}C NMR (125 MHz, $CDCl_3$) for Compound **20**



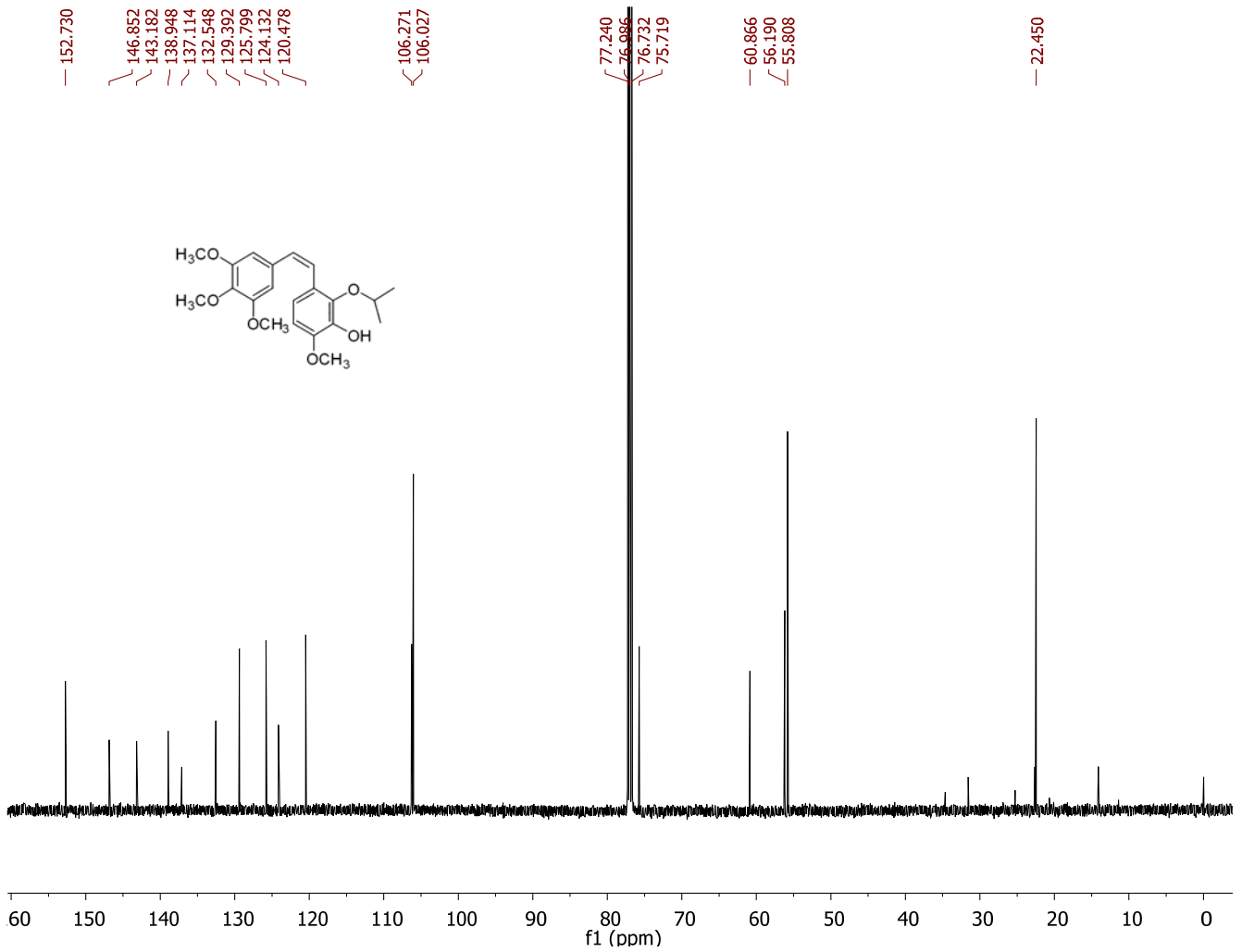
HRMS Traces for Compound **20**



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



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



HPLC Traces of Compound **21**

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

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    Acq. Instrument : Instrument 1                    Location :   -
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    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
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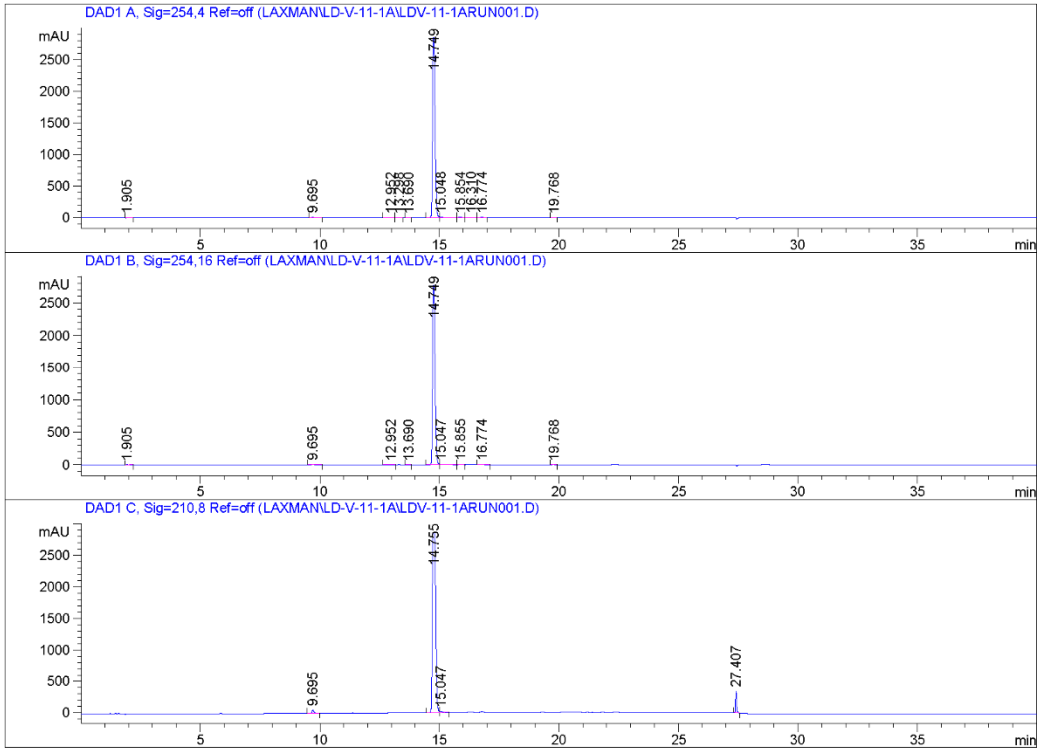
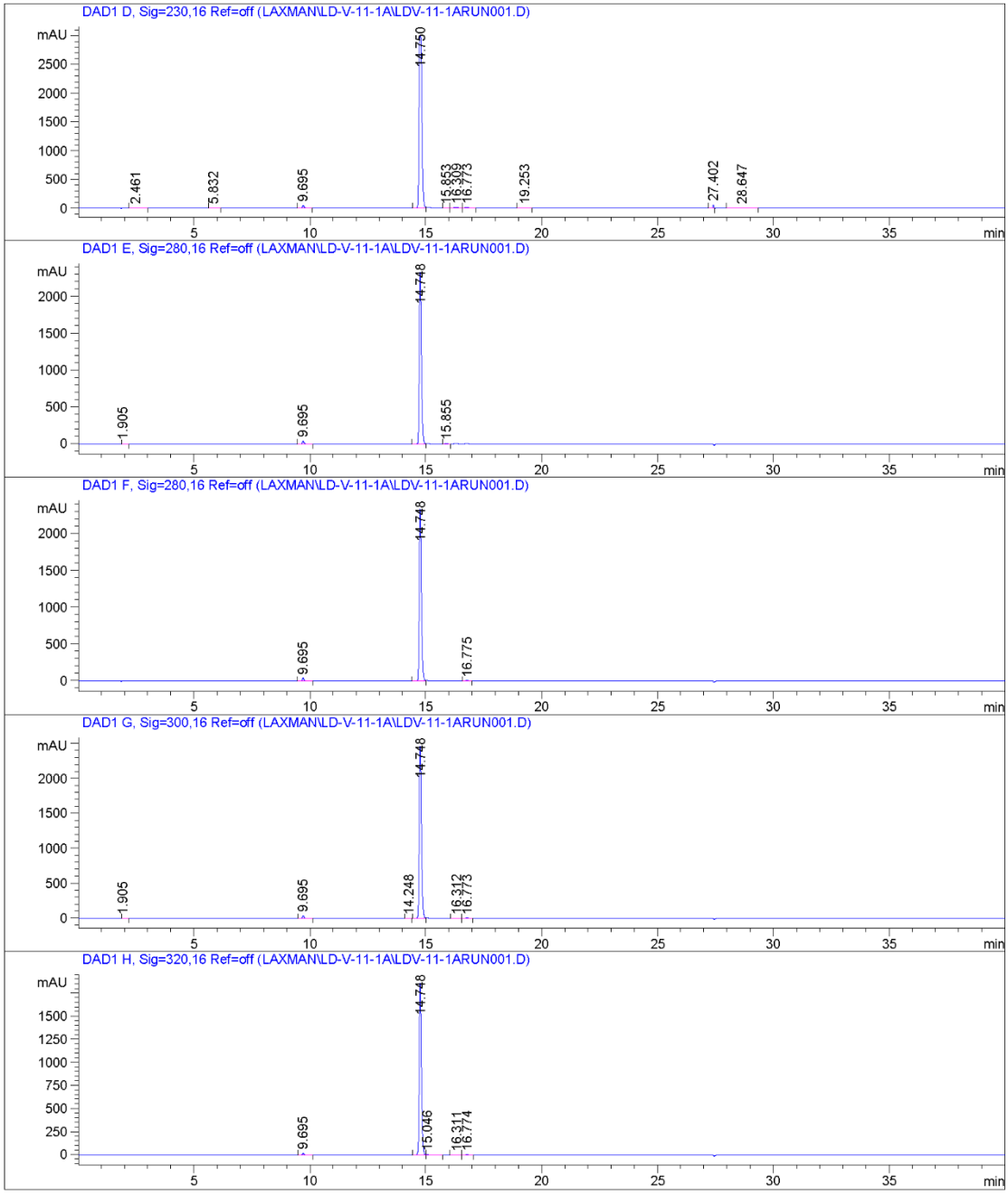


Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-11-1A\LDV-11-1ARUN001.D
Sample Name: LD-V-11-1A-run1



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

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Area Percent Report

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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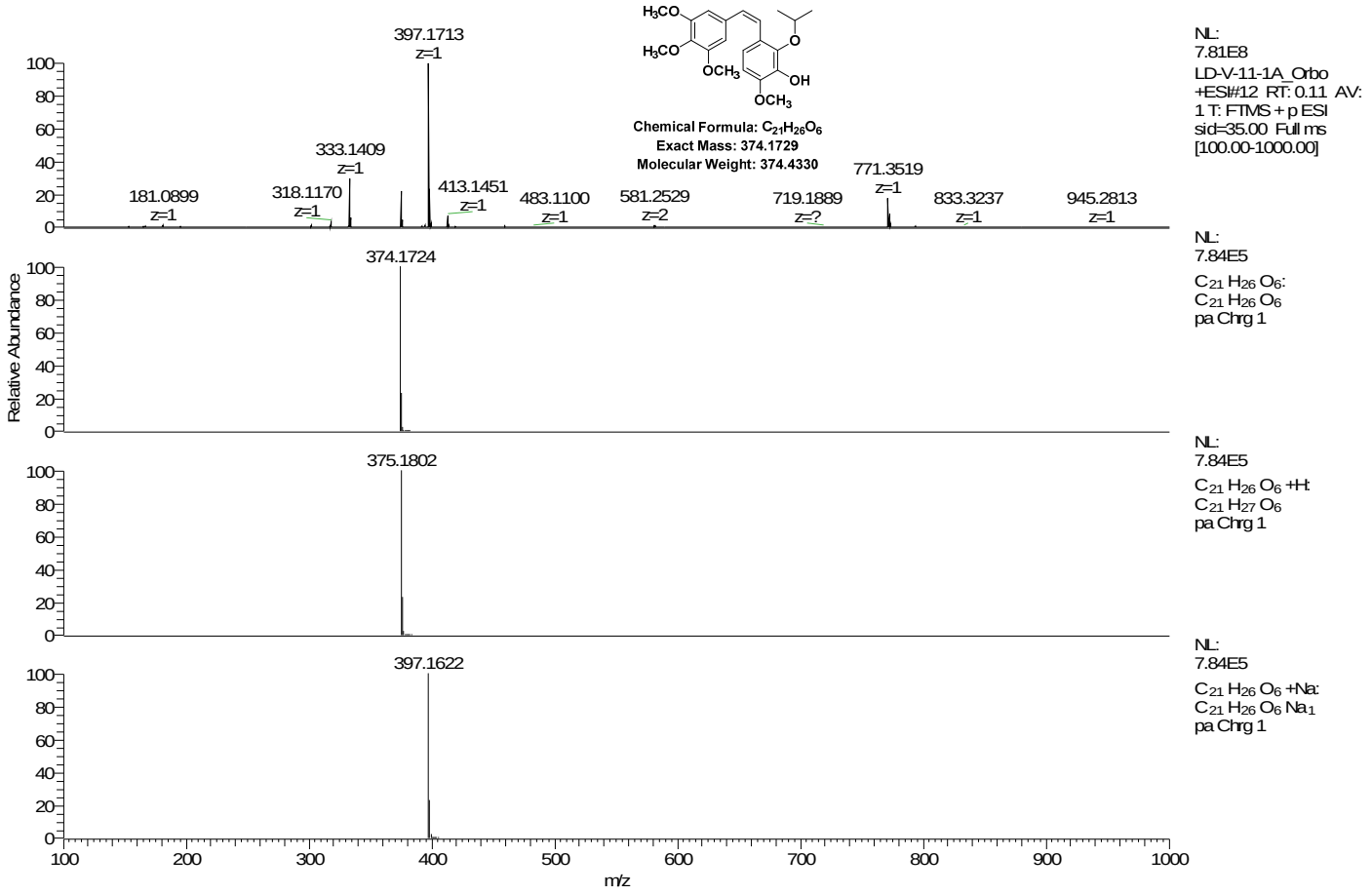
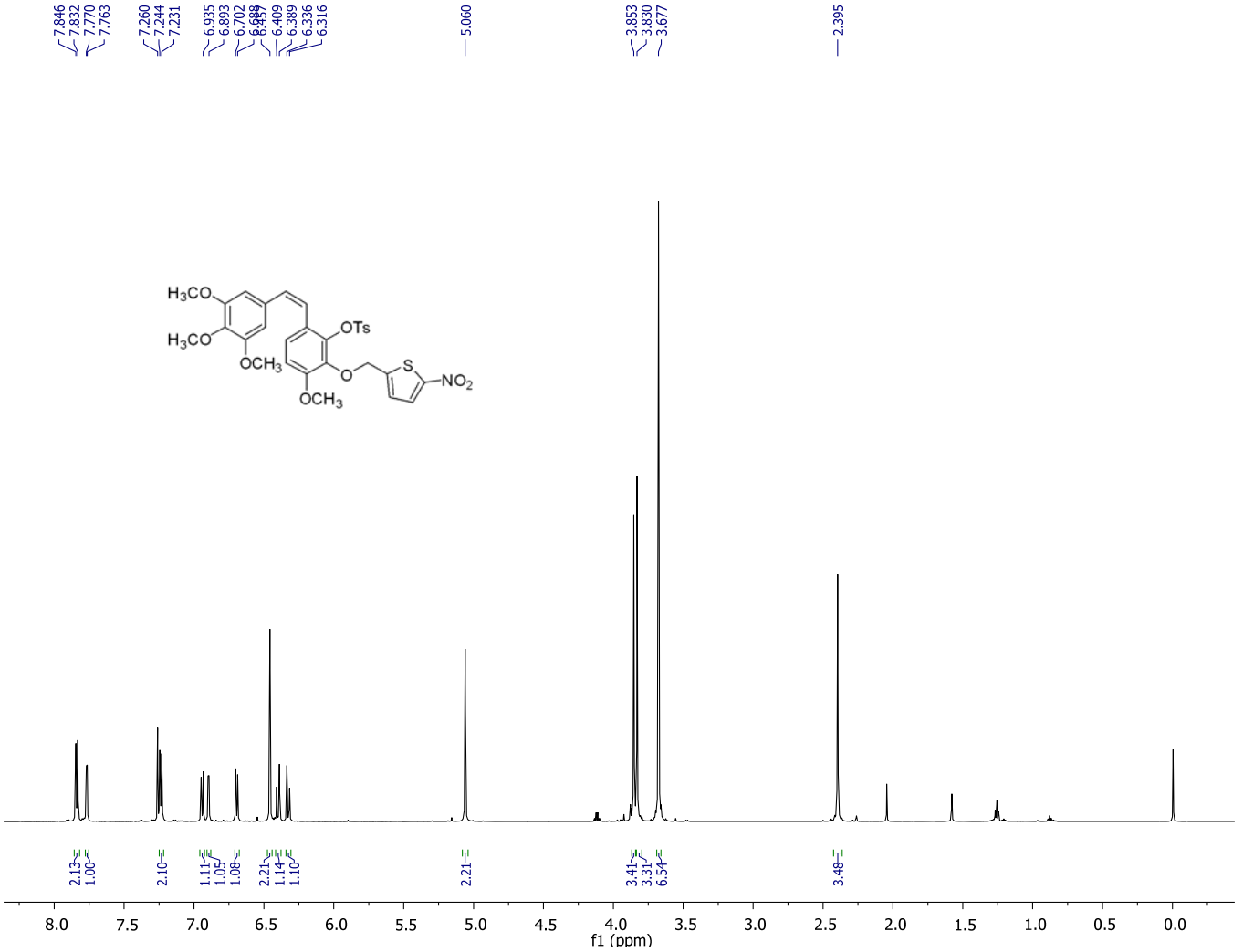
*** End of Report ***

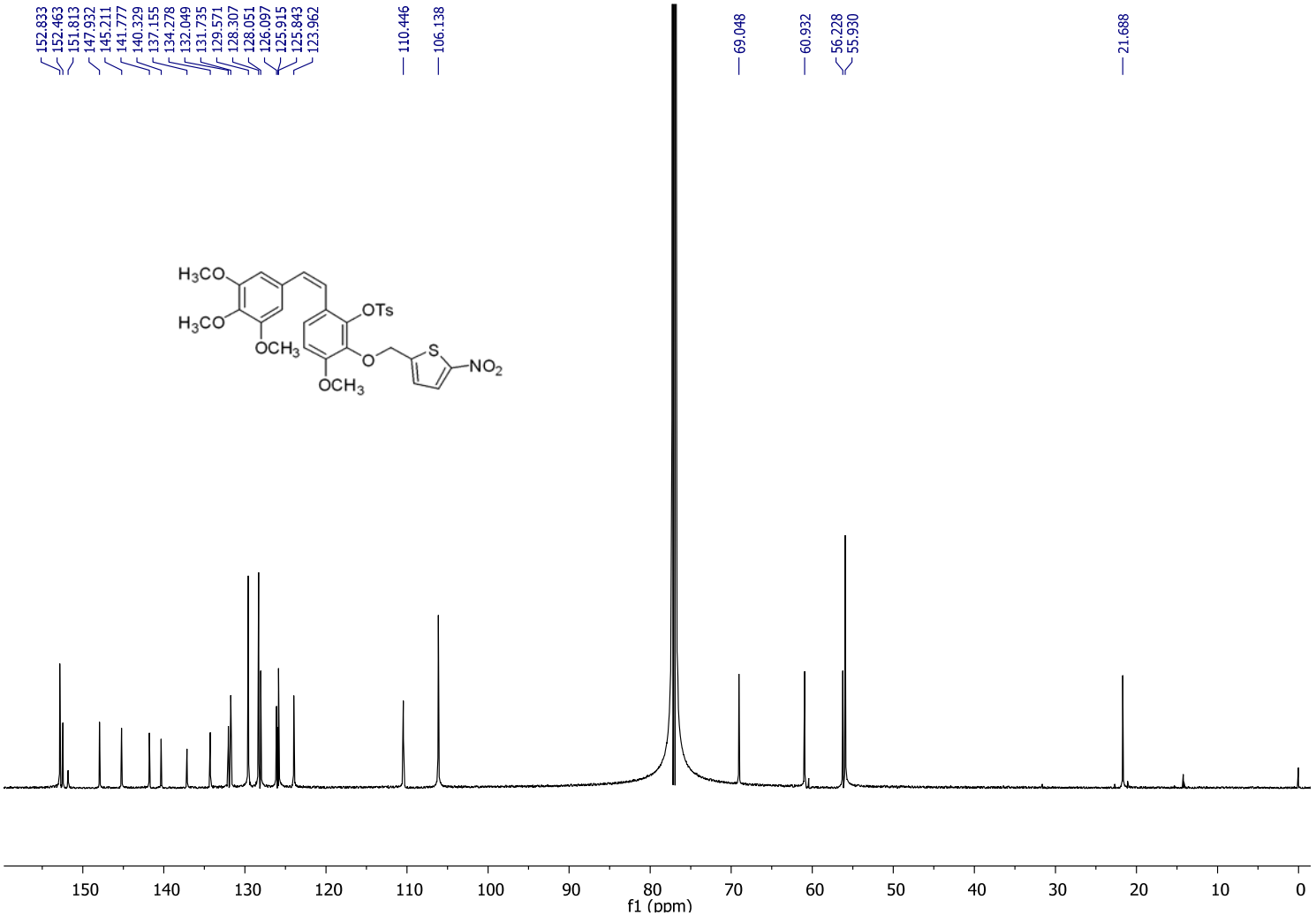
HRMS Traces of Compound **21**



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



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



HPLC Traces of Compound **22**

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

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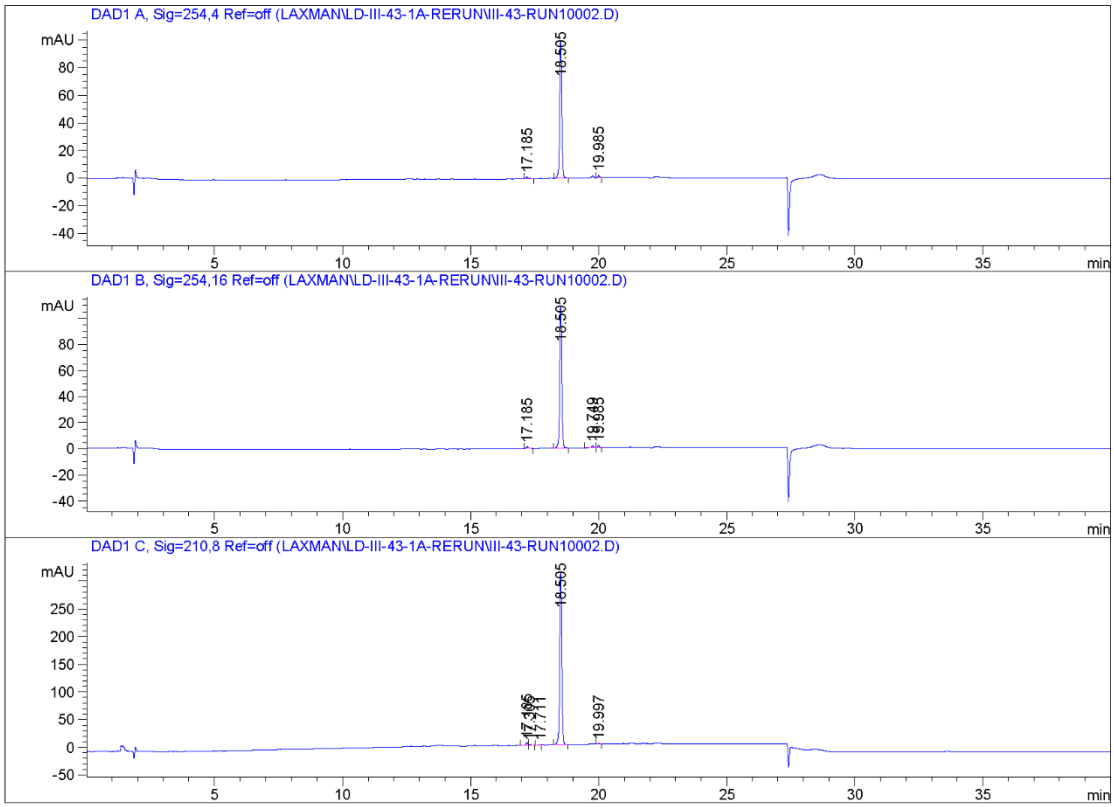
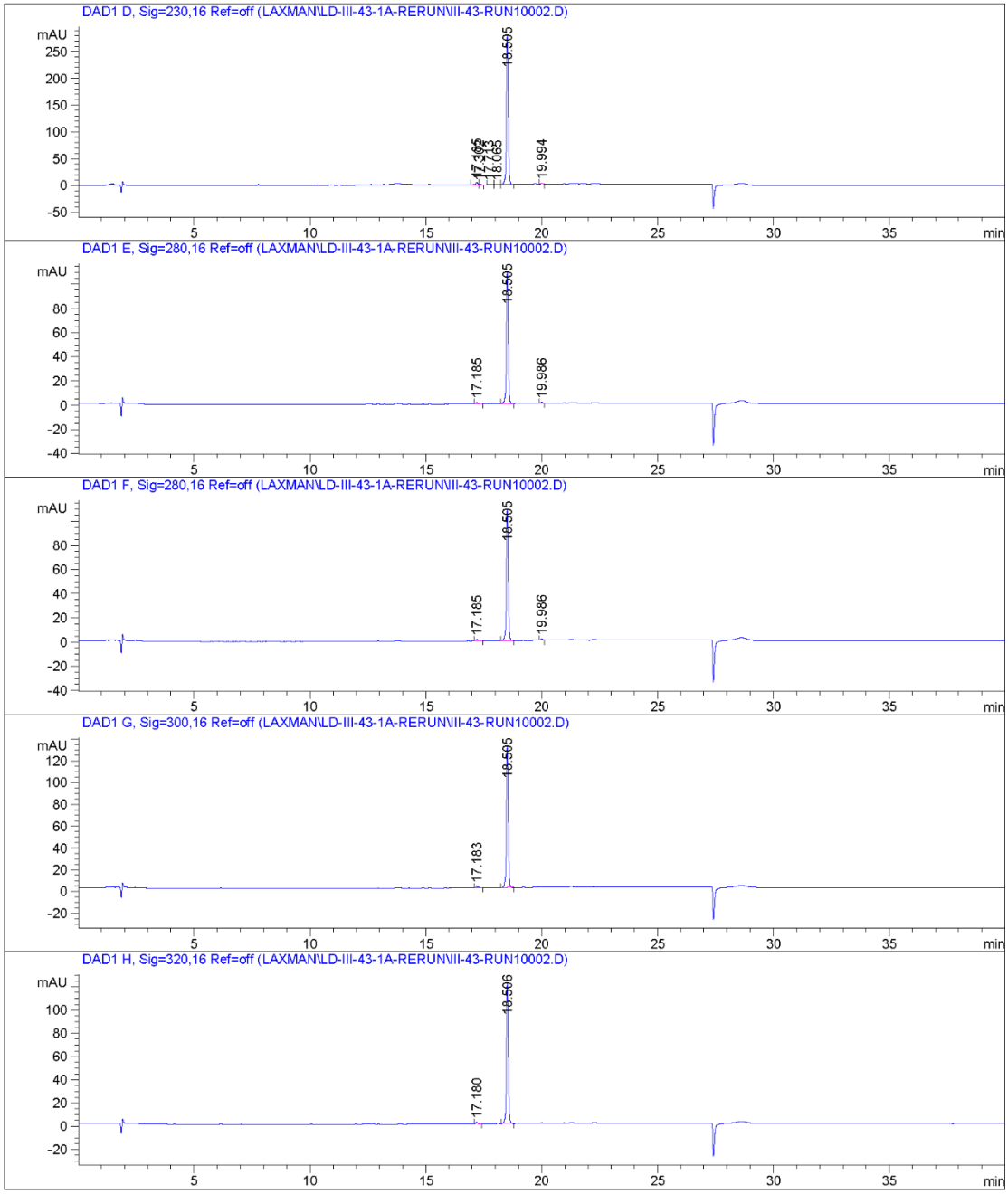


Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D
Sample Name: LD-III-43-1A-rerun-run1



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

Area Percent Report

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

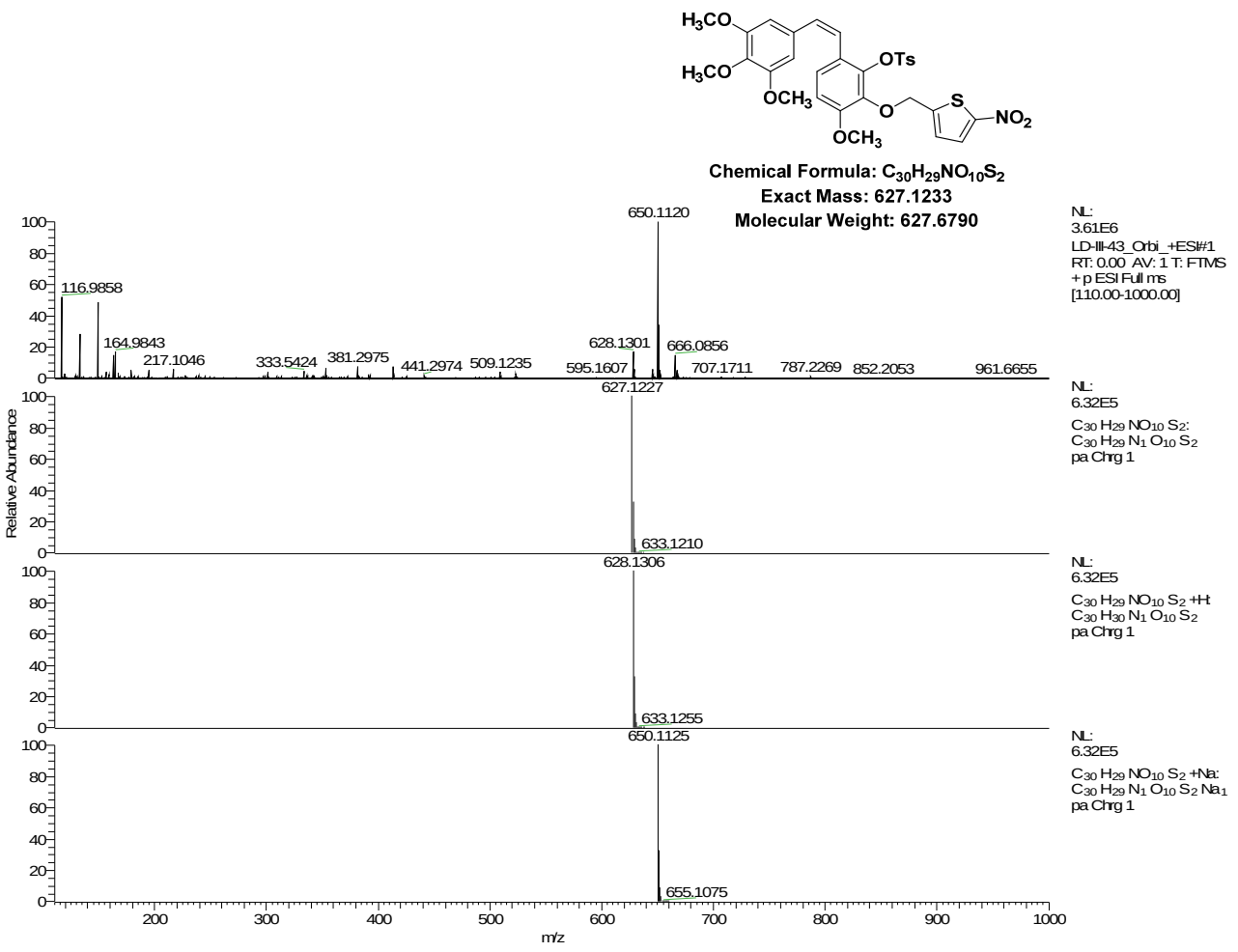
Data File C:\CHEM32\1\DATA\LAXMAN\LD-III-43-1A-RERUN\III-43-RUN10002.D
Sample Name: LD-III-43-1A-rerun-run1

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

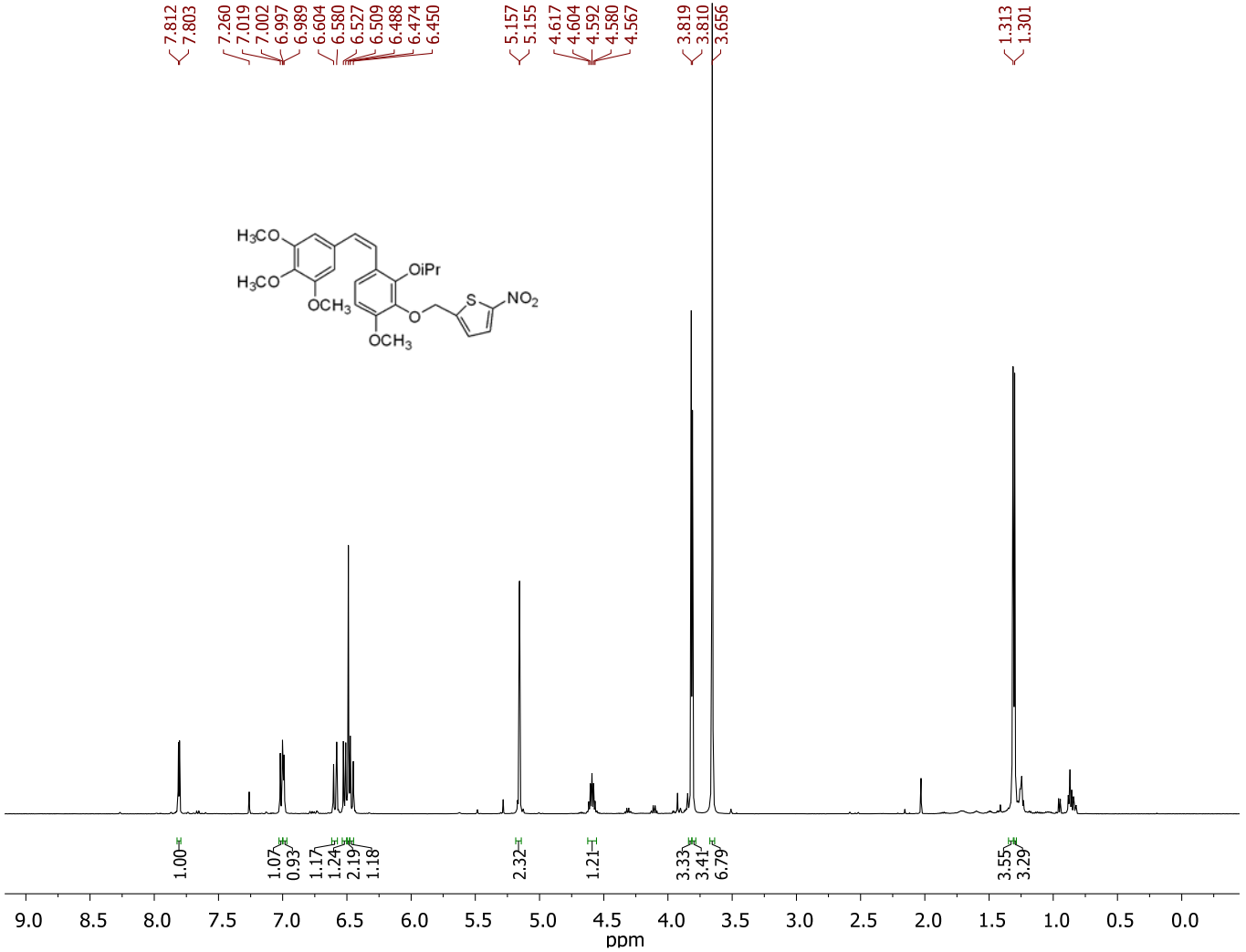
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.180	BB	0.0952	8.94699	1.42591	1.3034
2	18.506	BB	0.0853	677.49988	121.10182	98.6966
Totals :				686.44687	122.52773	

*** End of Report ***

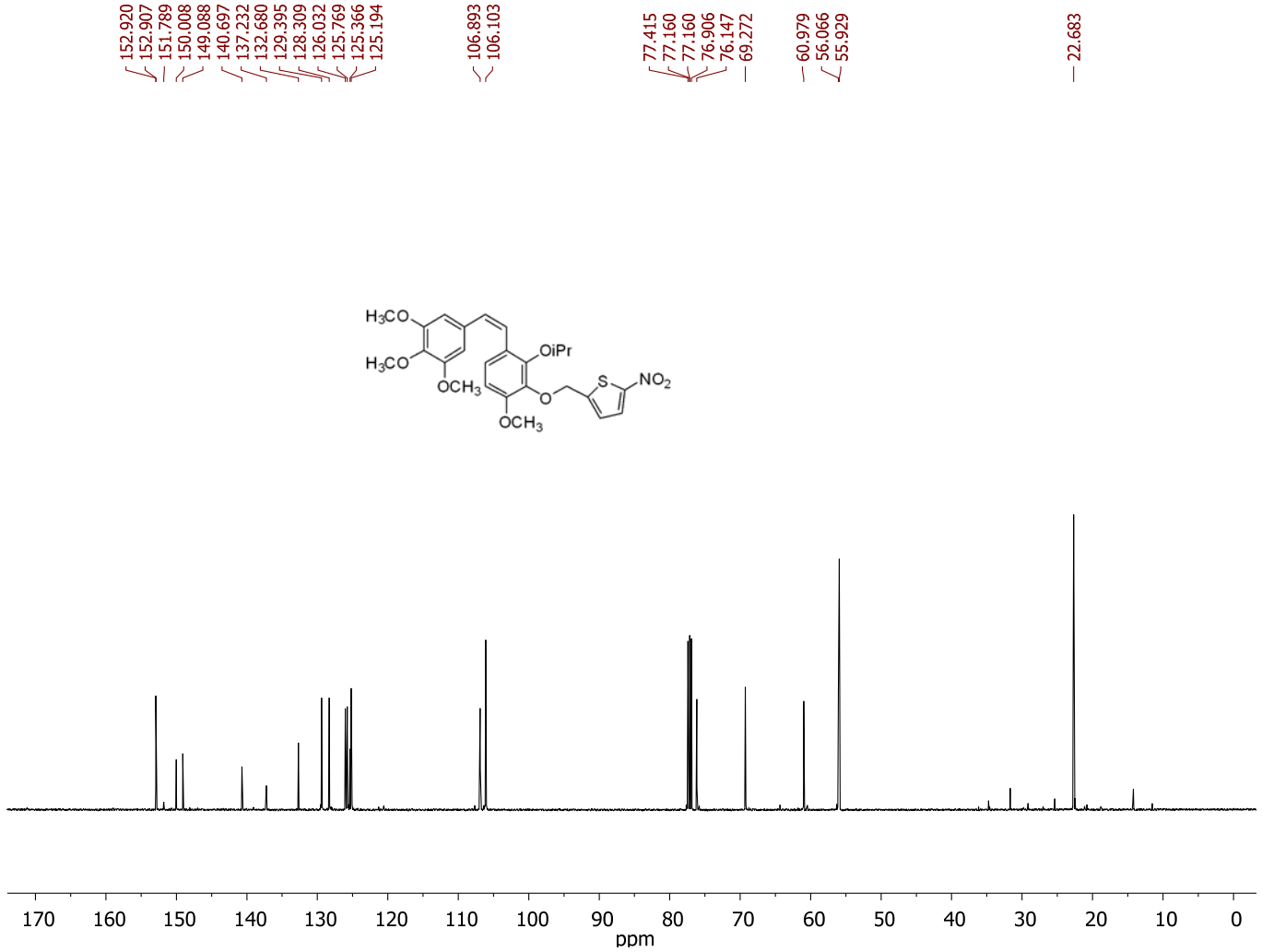
HRMS Traces for Compound **22**



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



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



HPLC Traces of Compound **24**

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

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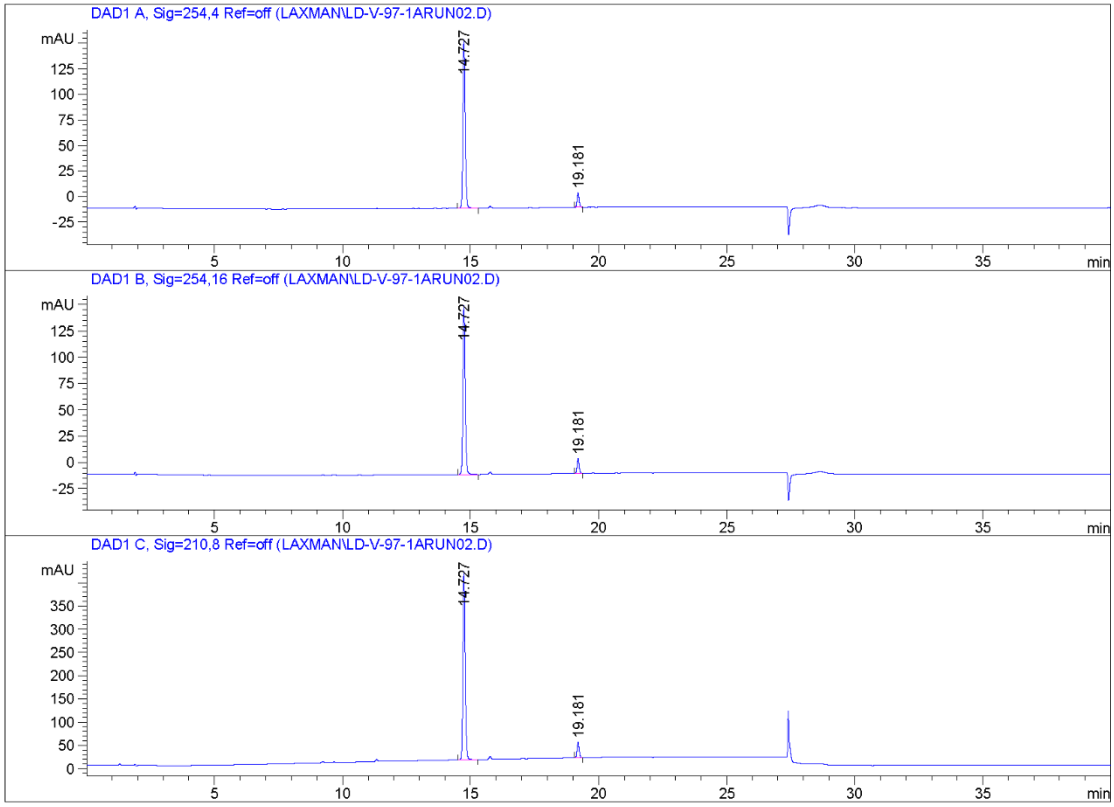
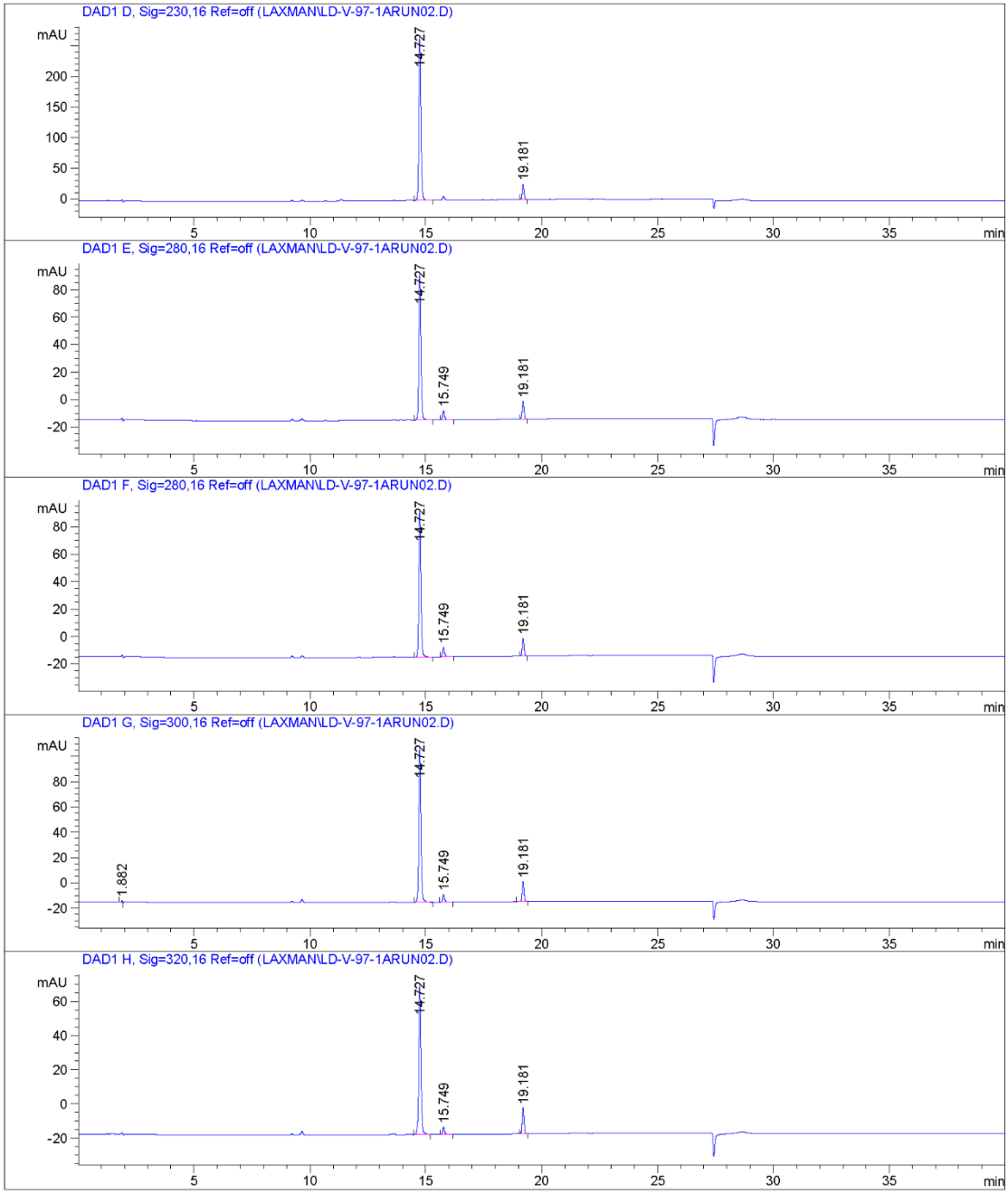


Data File C:\CHEM32\1\DATA\LAXMAN\LD-V-97-1ARUN02.D
Sample Name: LD-V-97-1A-run2



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

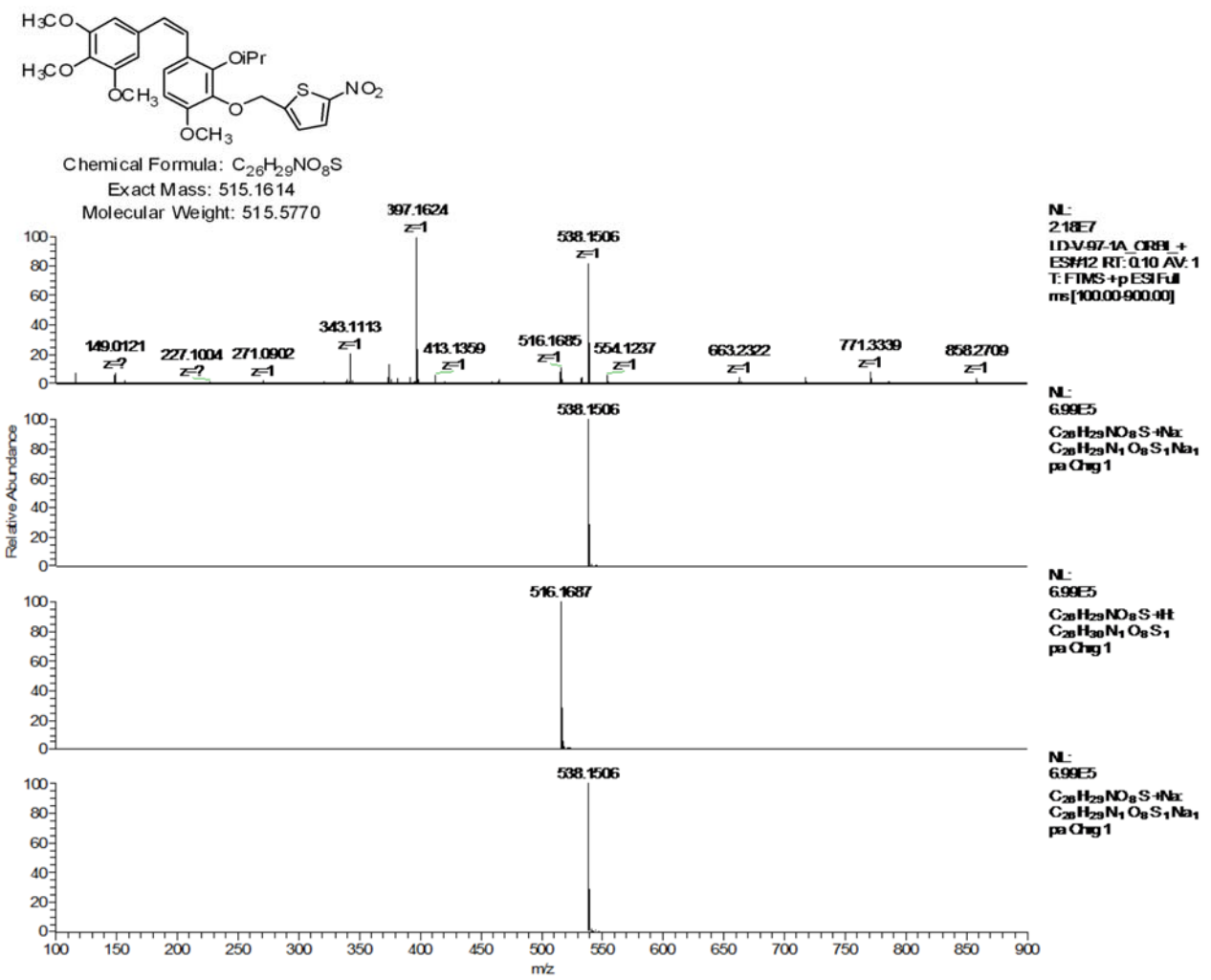
Signal 8: DAD1 H, Sig=320,16 Ref=off

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

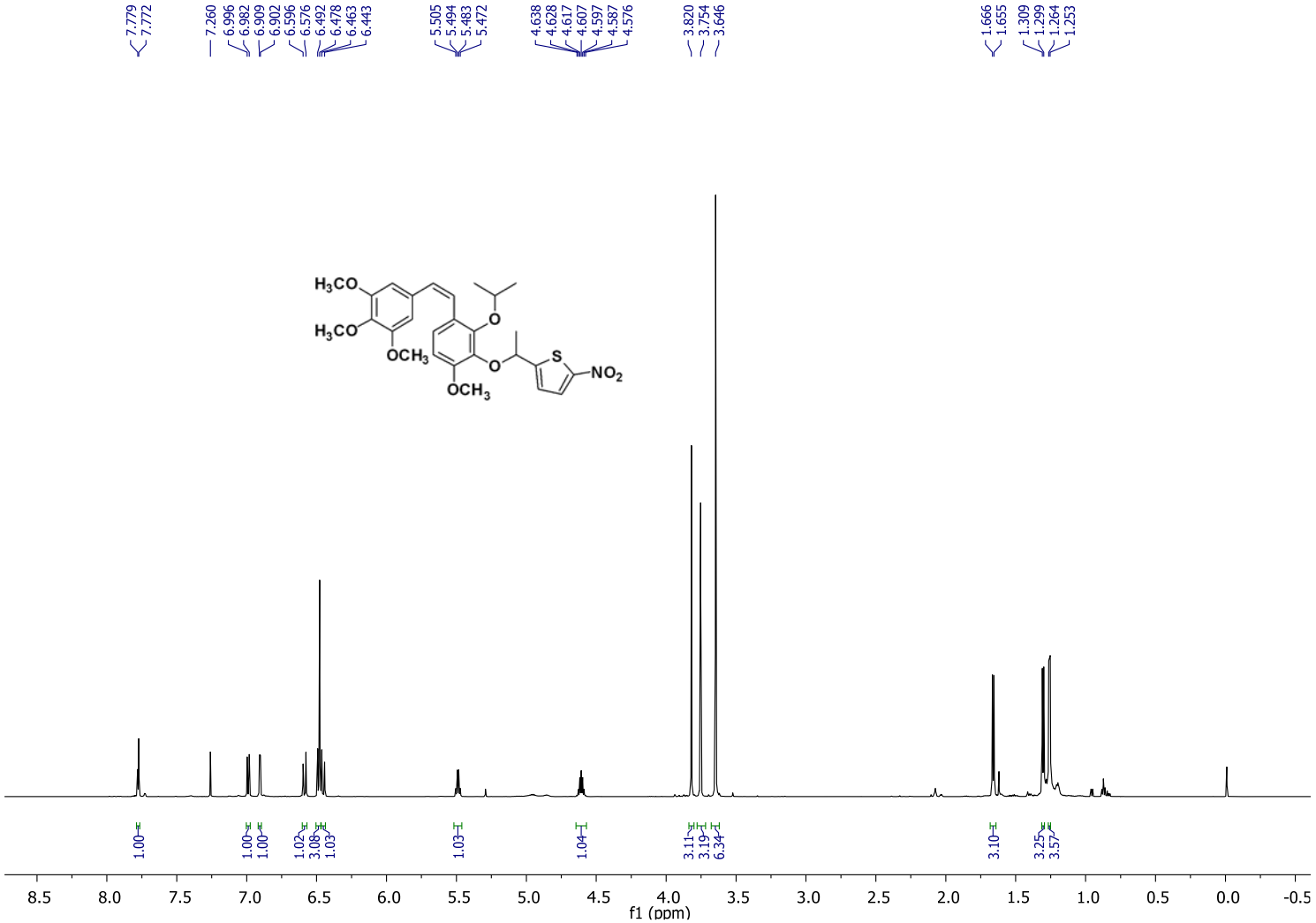
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.727	BB	0.0968	568.63849	88.65764	83.4444
2	15.749	BB	0.0922	27.48027	4.56638	4.0326
3	19.181	VB	0.0867	85.33914	15.39945	12.5230
Totals :				681.45790	108.62348	

*** End of Report ***

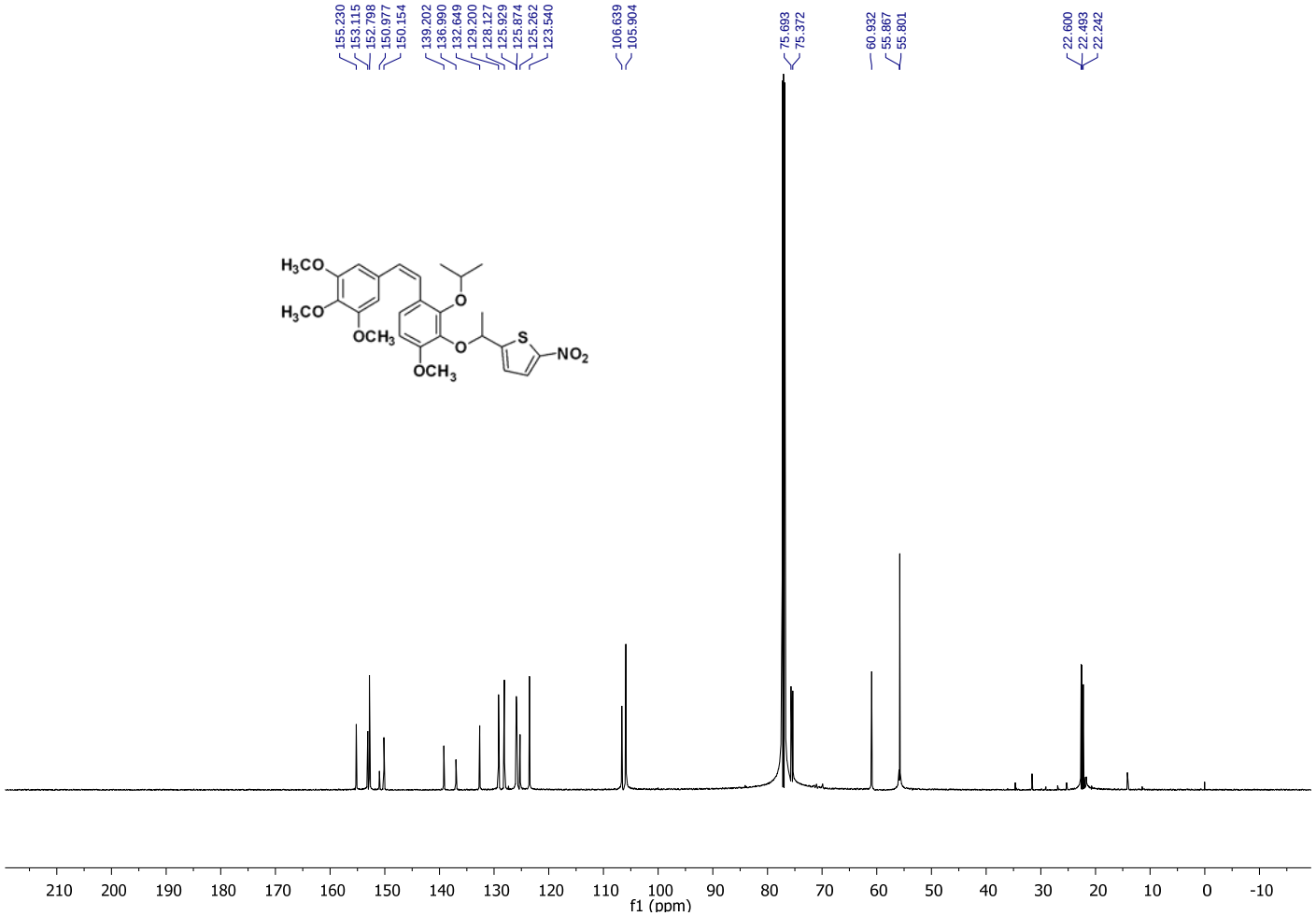
HRMS Traces of Compound **24**



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



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



HPLC Traces for Compound **25**

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

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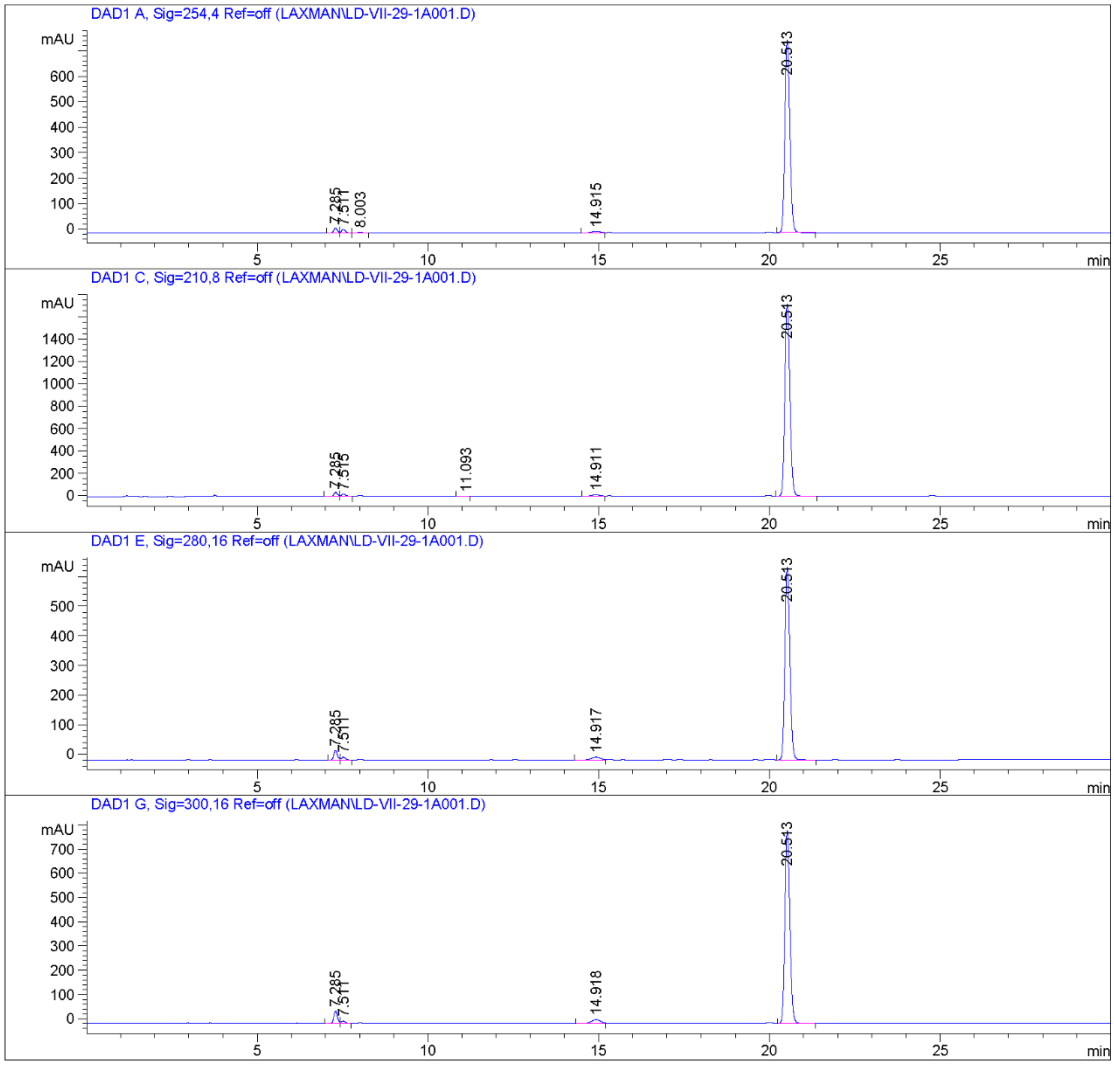
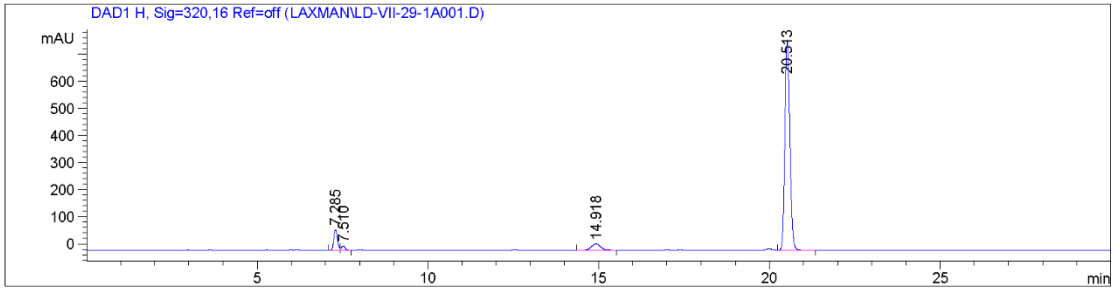


Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-29-1A001.D
Sample Name: LD-VII-29-1A



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Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.285	BV	0.1134	137.70985	18.81188	1.7190
2	7.511	VB	0.1180	108.58200	14.07325	1.3554
3	8.003	BB	0.1245	30.20725	3.72818	0.3771
4	14.915	BV	0.2773	103.74889	5.89437	1.2951
5	20.513	VB	0.1577	7630.70166	756.65521	95.2534
Totals :				8010.94966	799.16290	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.285	BV	0.1181	282.01685	36.51880	1.5456
2	7.515	VB	0.1310	187.78674	21.27100	1.0291
3	11.093	BV	0.1711	14.92364	1.34912	0.0818
4	14.911	BV	0.2798	225.43584	12.77740	1.2355
5	20.513	VB	0.1588	1.75367e4	1721.62097	96.1080
Totals :				1.82469e4	1793.53730	

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

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

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

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

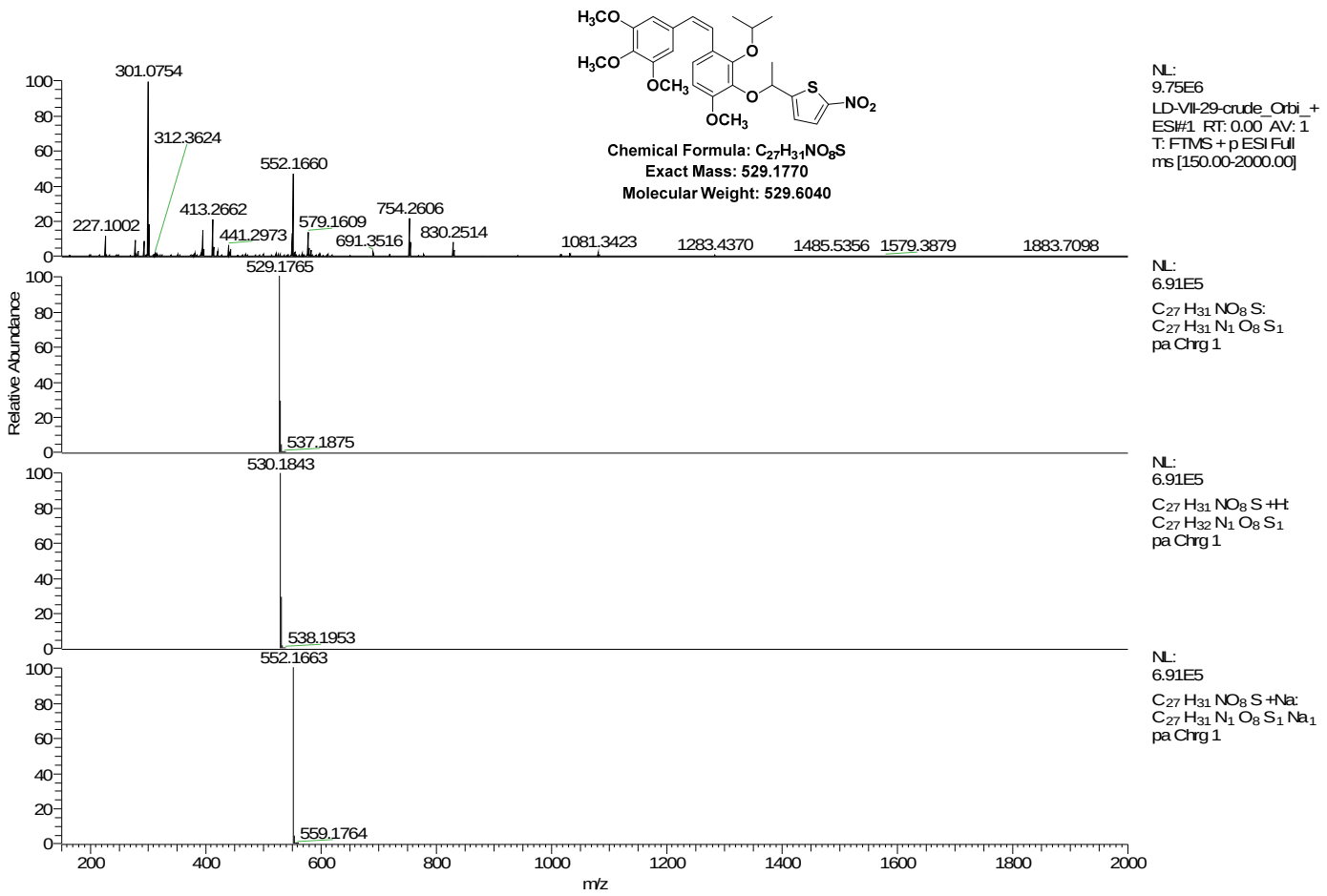
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.285	BV	0.1139	376.73462	51.17566	4.2972
2	7.511	VB	0.1162	81.67451	10.56421	0.9316
3	14.918	BV	0.2823	290.15118	16.09673	3.3096
4	20.513	VB	0.1556	8018.51367	795.31226	91.4617
Totals :				8767.07399	873.14884	

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

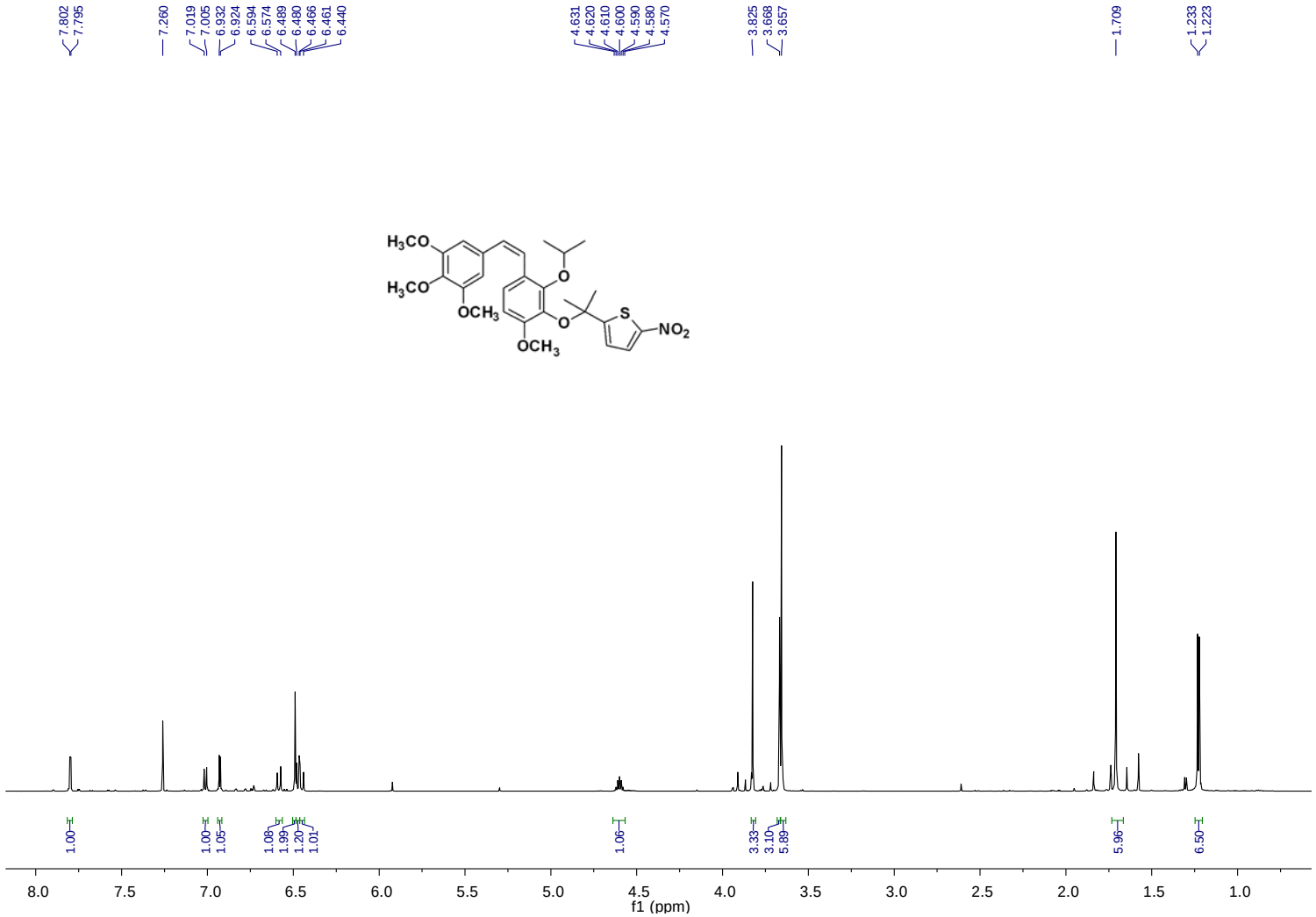
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.285	BV	0.1139	556.89746	75.66253	6.2256
2	7.510	VB	0.1160	116.93579	15.15809	1.3072
3	14.918	BB	0.2899	437.53549	23.64482	4.8912
4	20.513	VB	0.1578	7833.95703	775.83319	87.5760
Totals :				8945.32578	890.29863	

*** End of Report ***

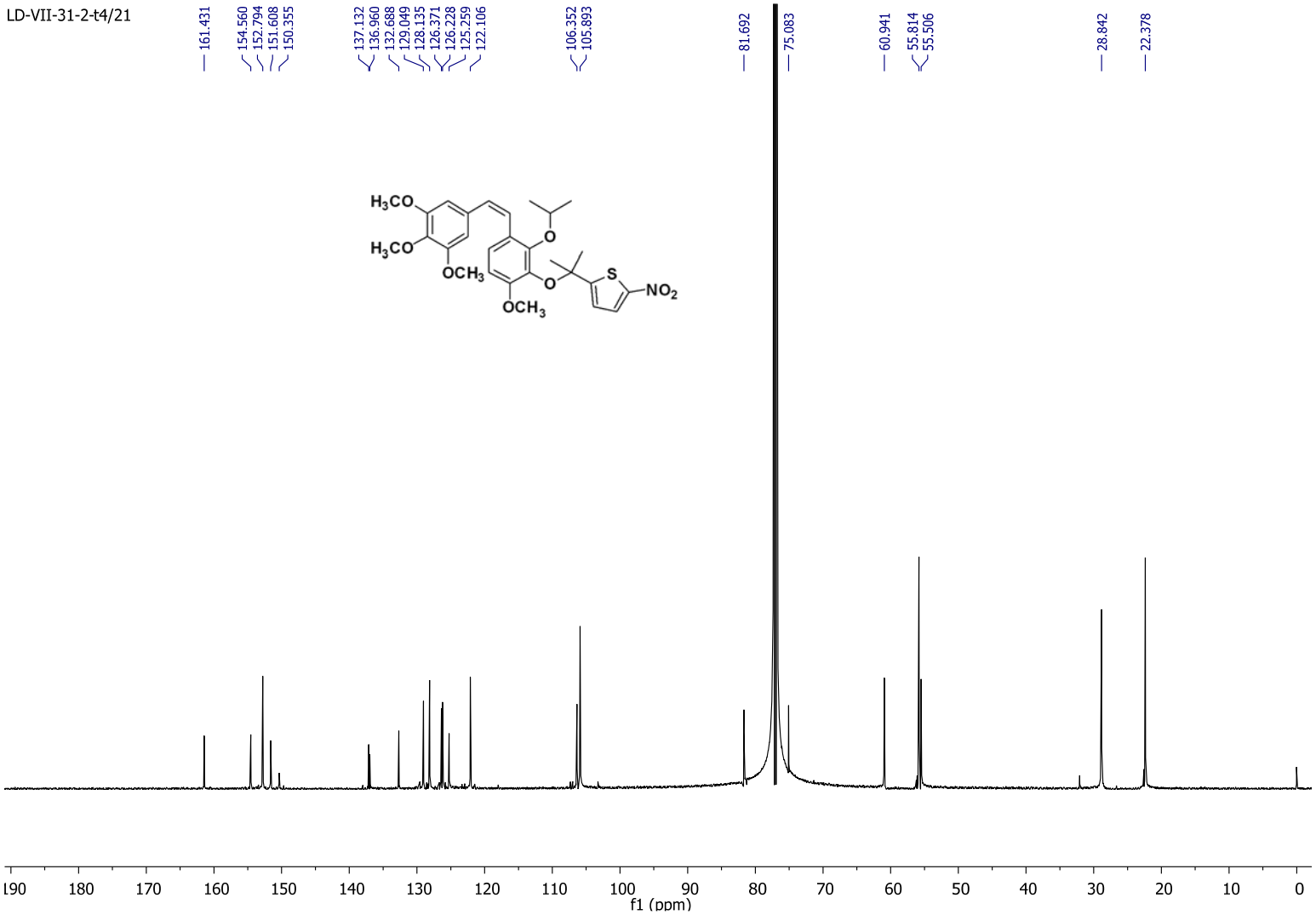
HRMS Traces of Compound **25**



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



^{13}C NMR (151 MHz, $CDCl_3$) for Compound **26**



HPLC Traces of Compound **26**

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

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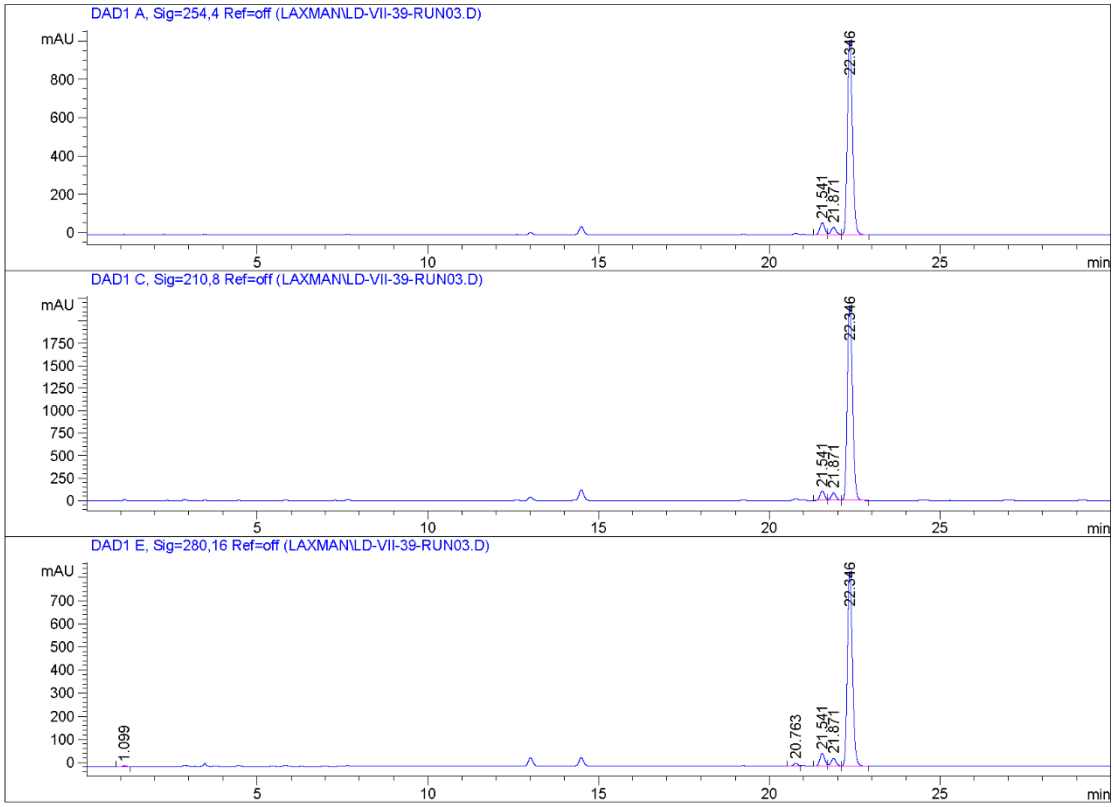
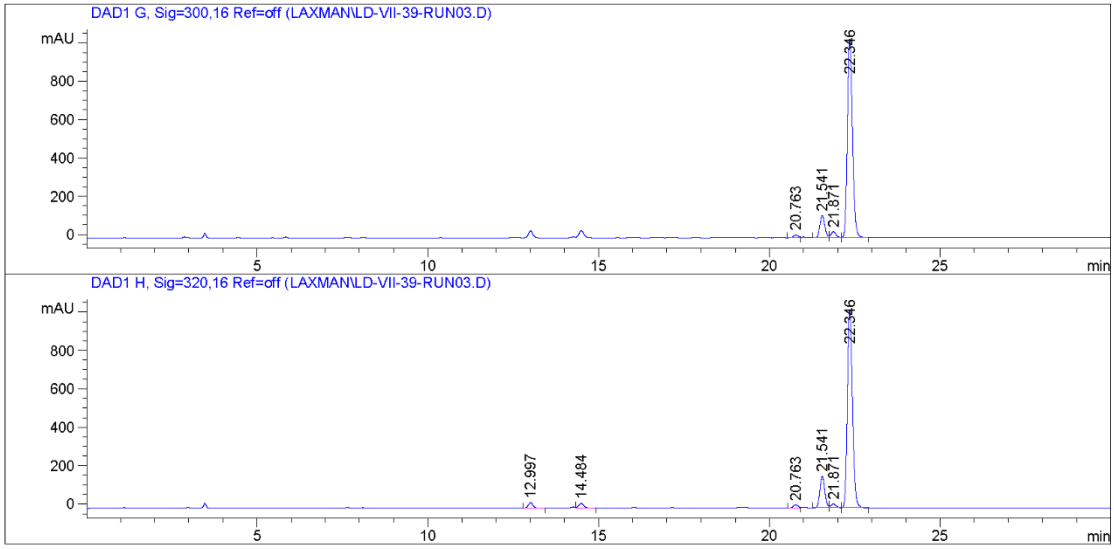


Data File C:\CHEM32\1\DATA\LAXMAN\LD-VII-39-RUN03.D
Sample Name: LD-VII-39-run3



==========

Area Percent Report

==========

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

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

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

Totals : 1.14983e4 1118.02668

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

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

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

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

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

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

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

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

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

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

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

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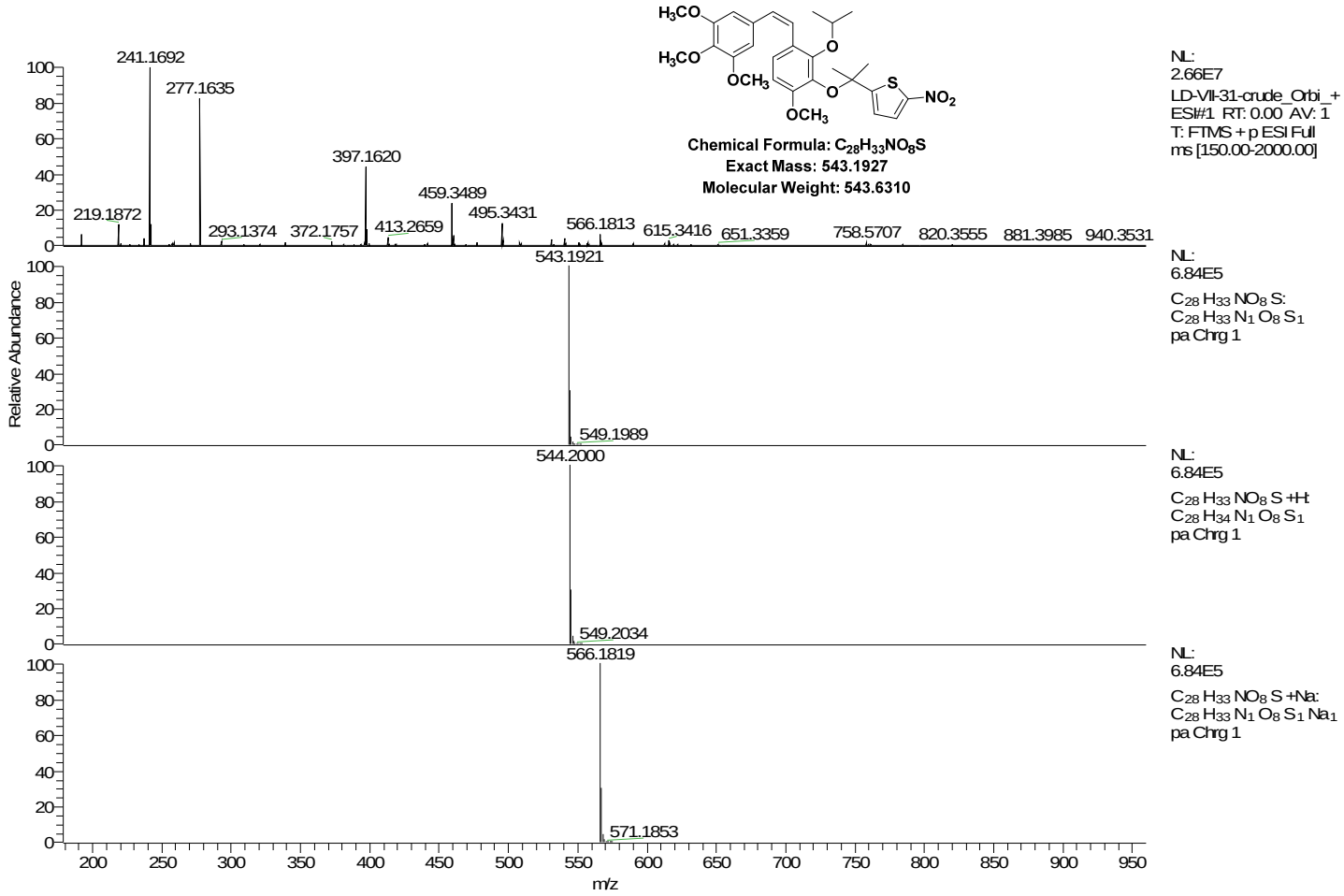
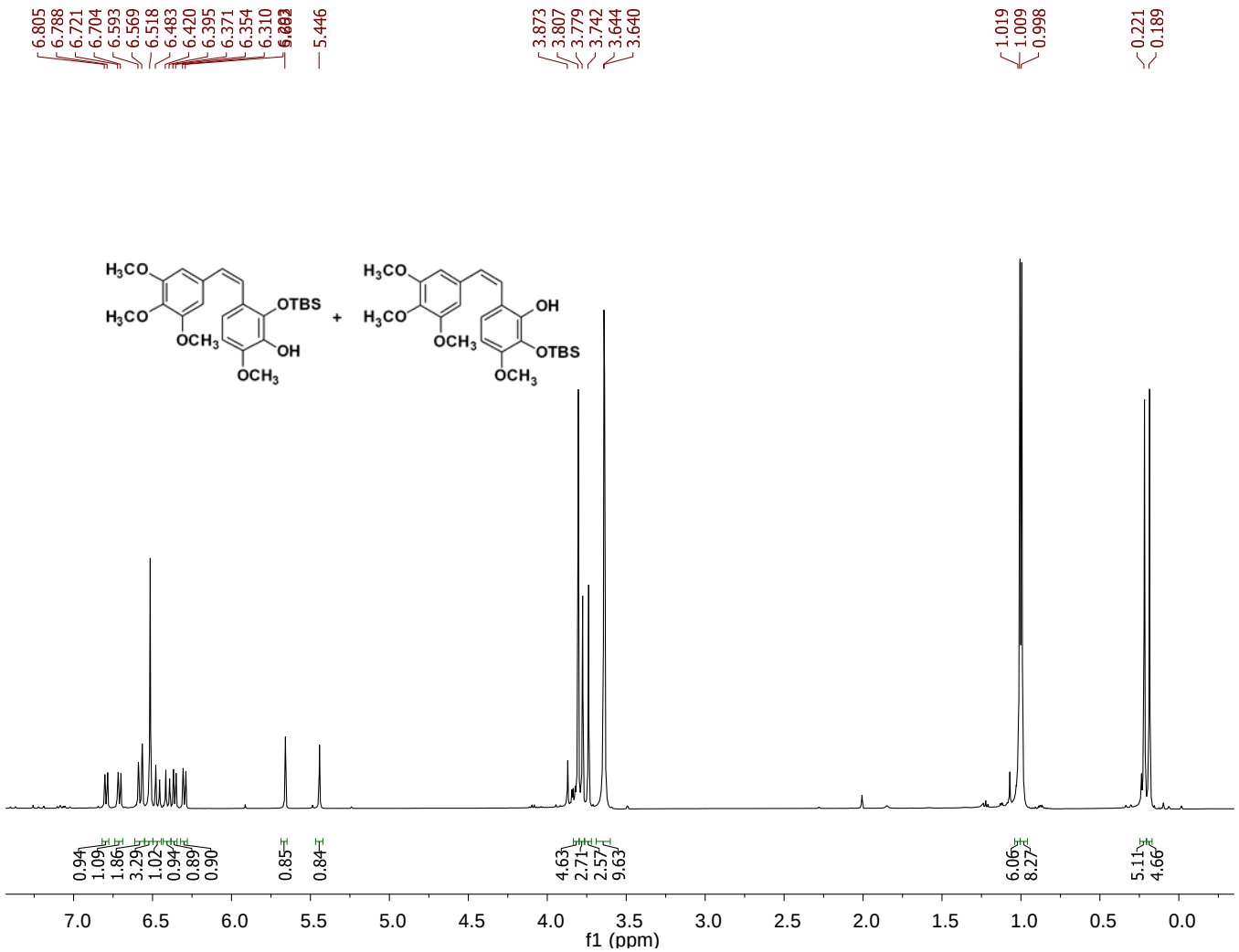
*** End of Report ***

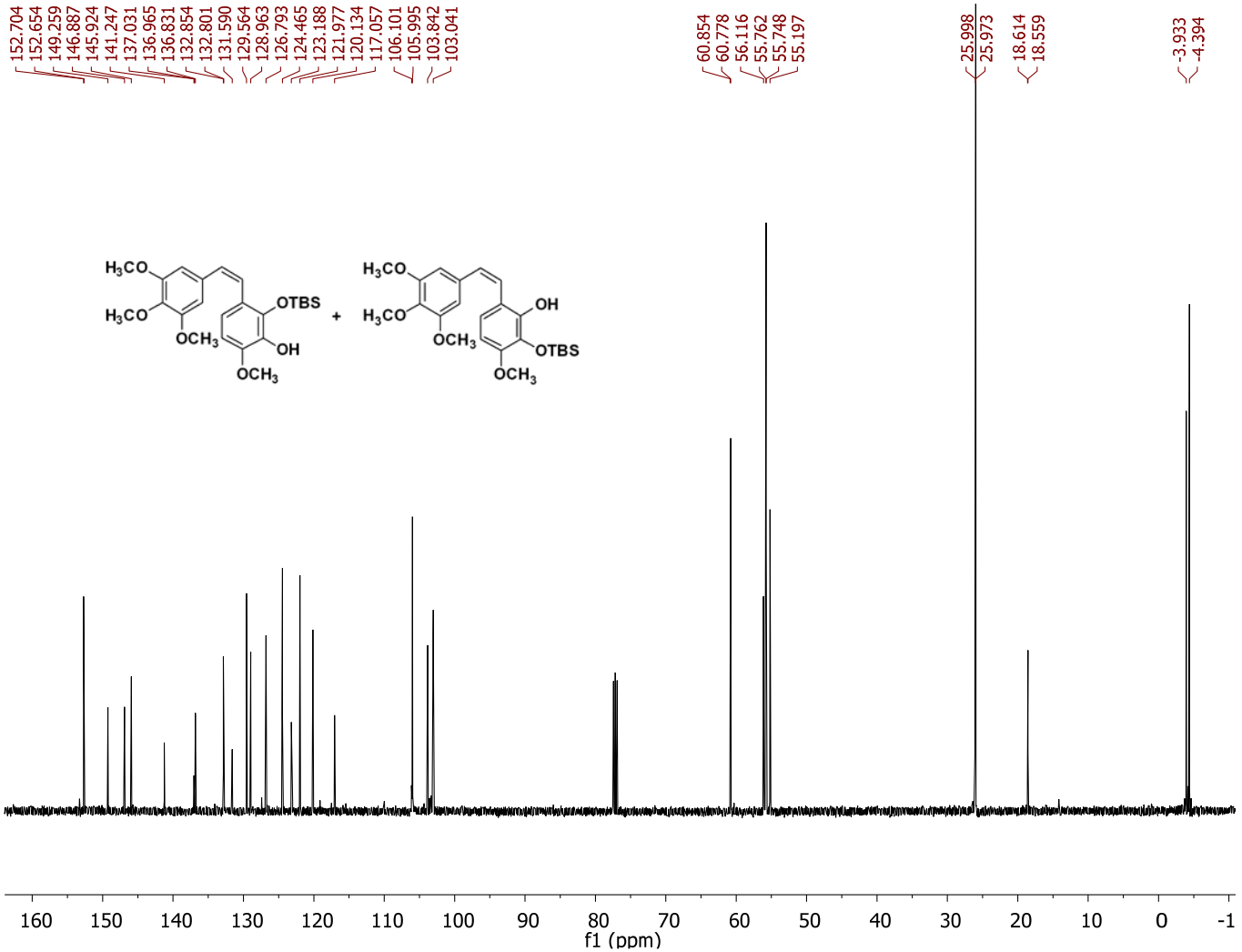
HRMS Trace of Compound **26**



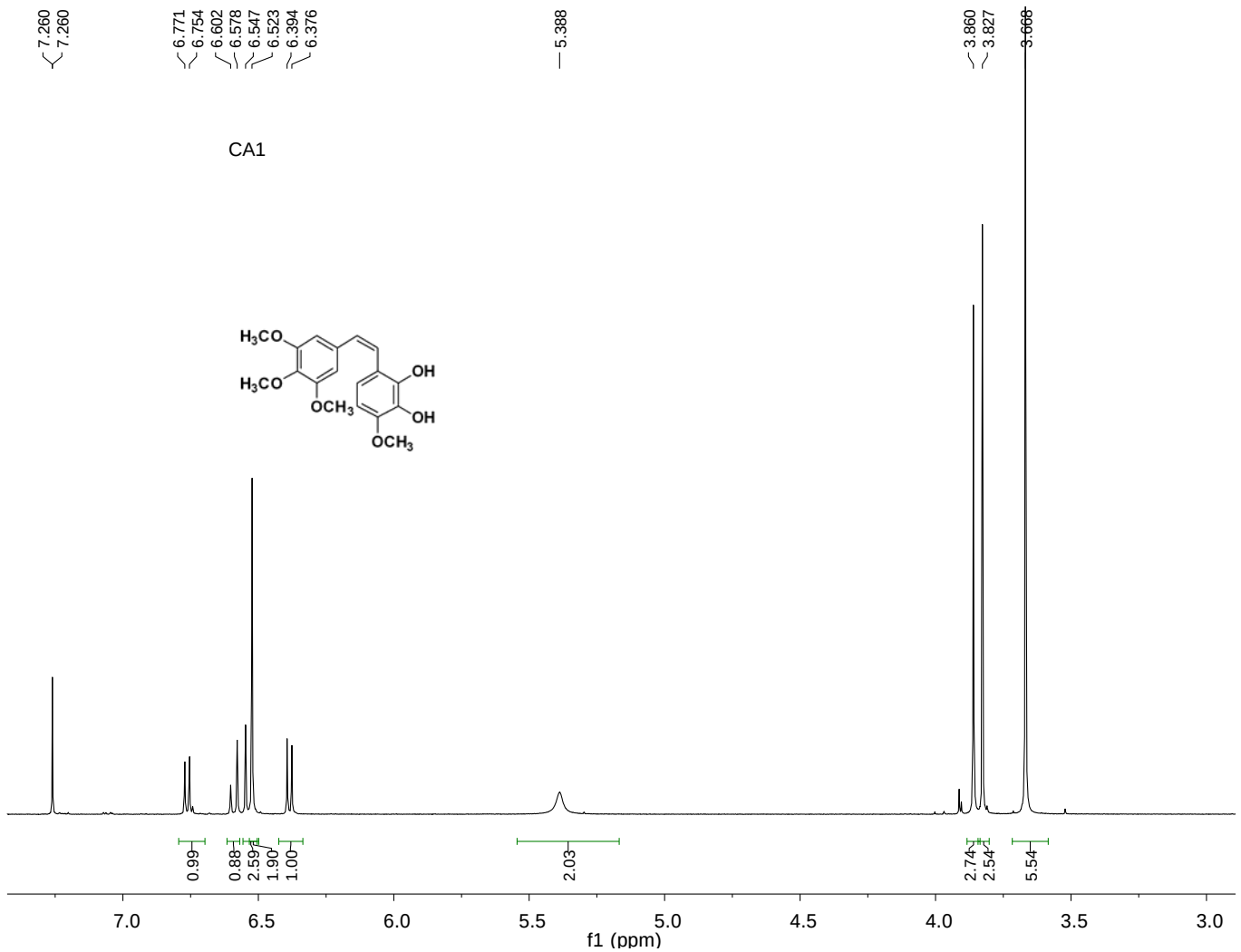
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **27** and **28**



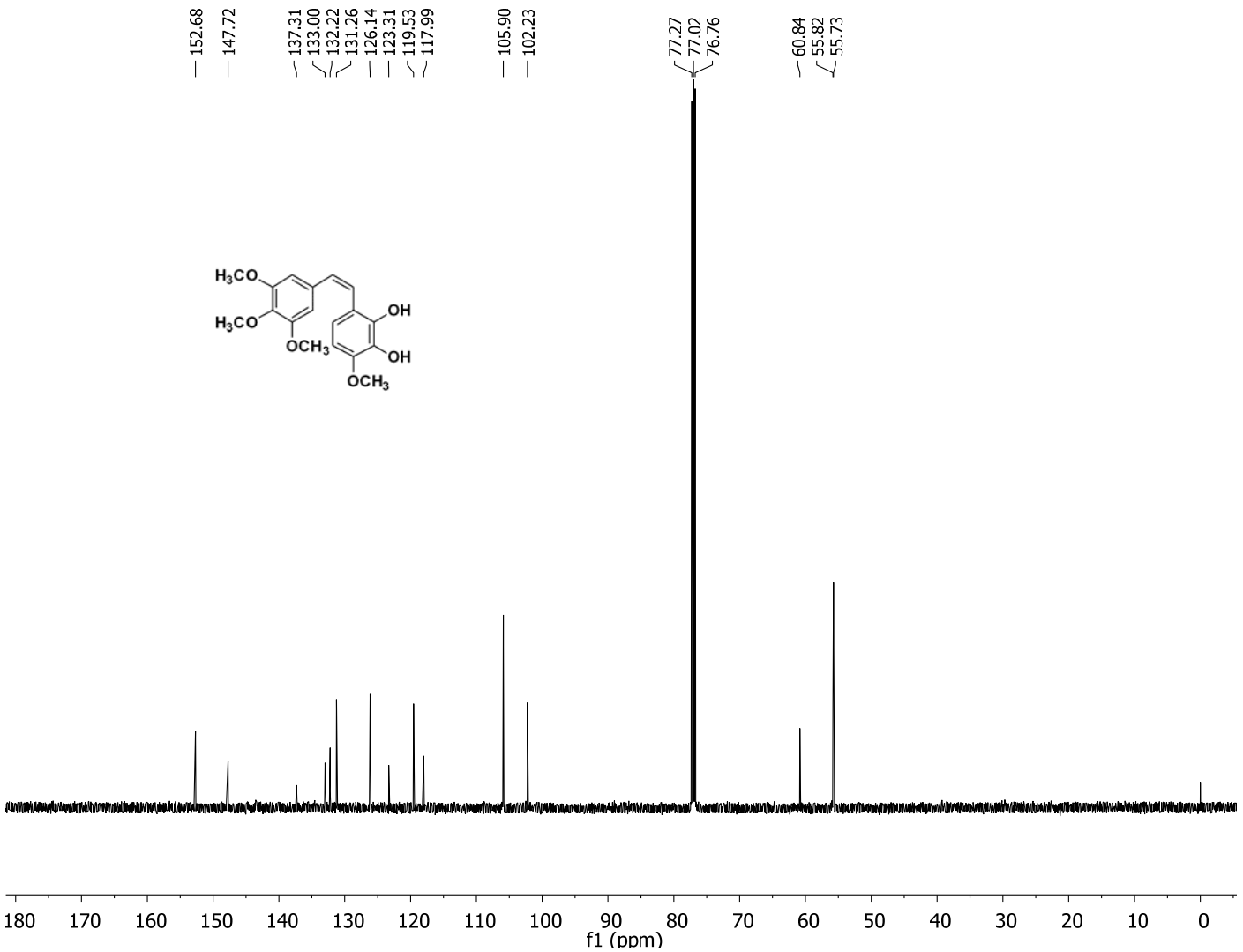
^{13}C NMR (125 MHz, $CDCl_3$) for Compound **27** and **28**



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



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



HPLC Traces of Compound **29**

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

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    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
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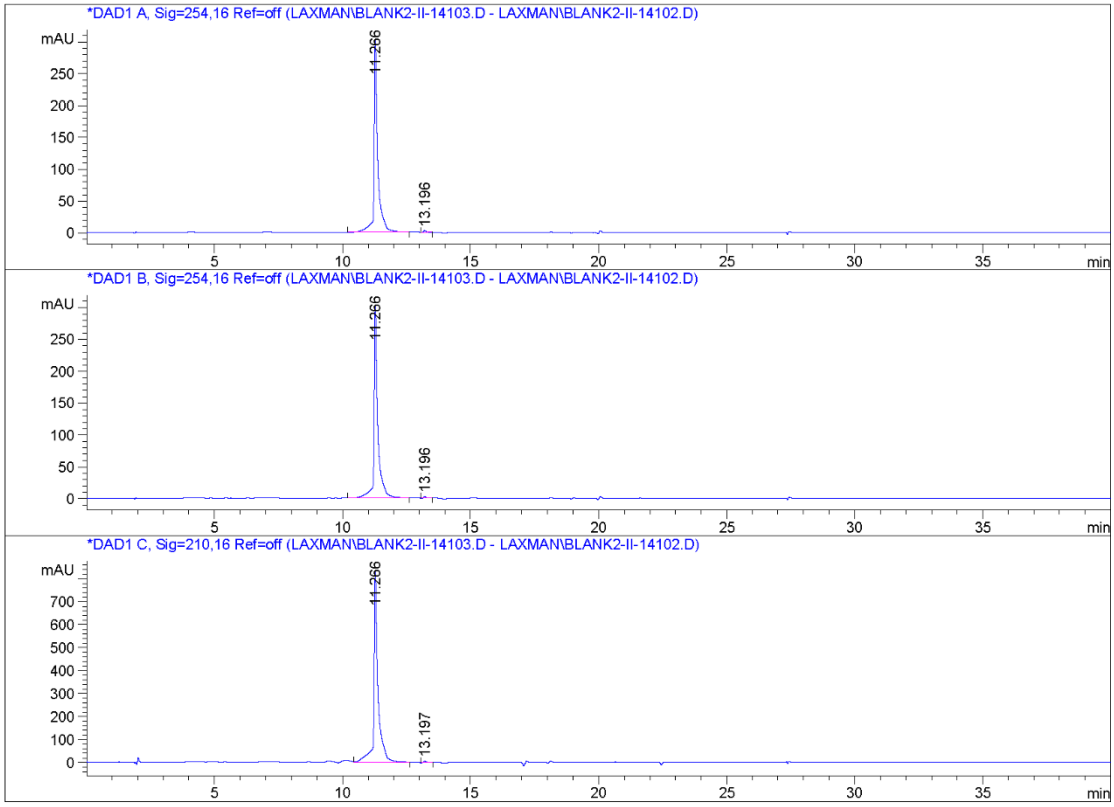
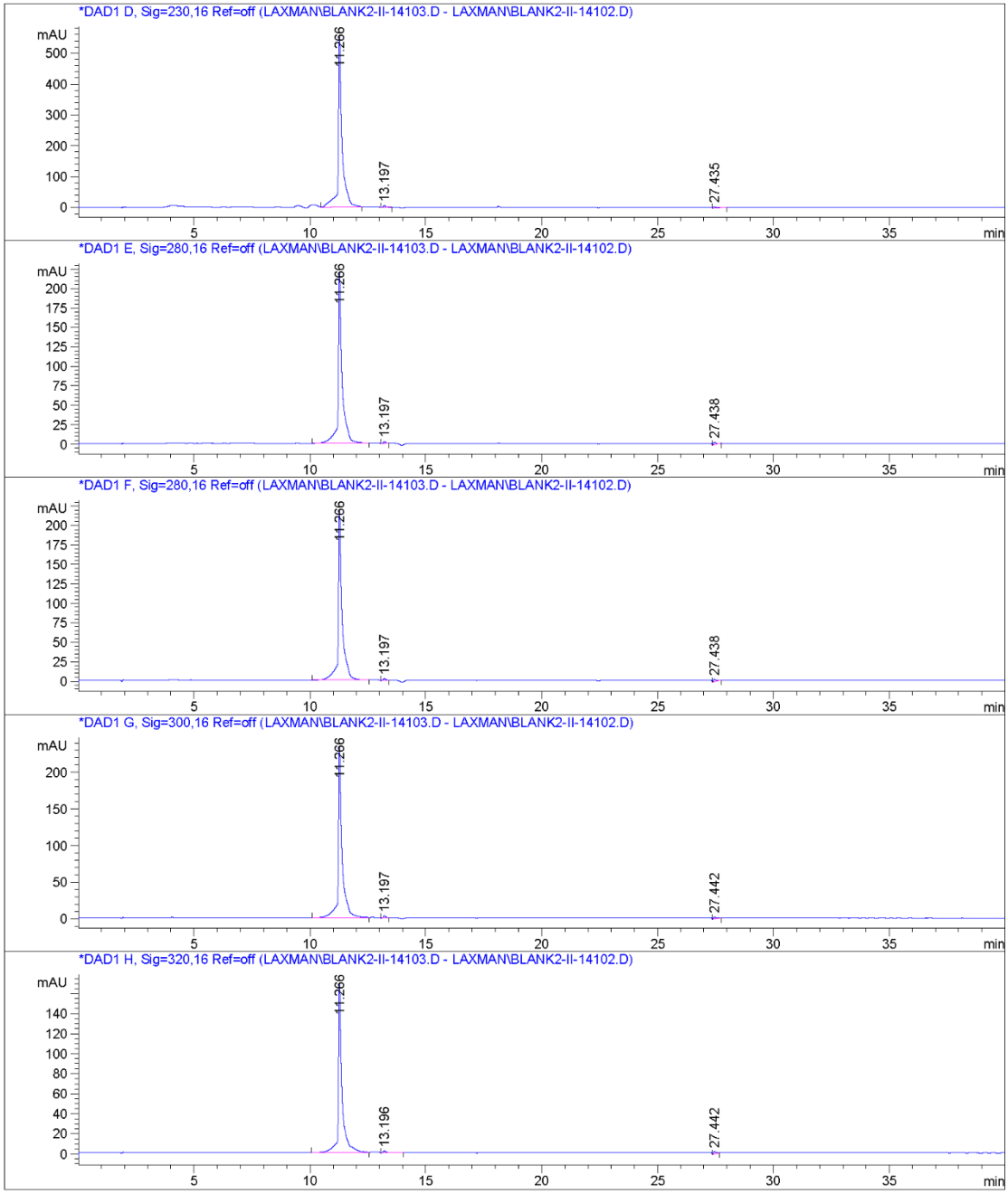


Data File C:\CHEM32\1\DATA\LAXMAN\BLANK2-II-14103.D
Sample Name: LD-II-141-1blank2



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

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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=254,16 Ref=off
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.266	BB	0.1372	3040.83838	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 [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
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 [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.266	VB	0.1514	9399.36523	834.36102	99.2611
2	13.197	BB	0.1183	69.97166	8.47993	0.7389
Totals :				9469.33689	842.84096	

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

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

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

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

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

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

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

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

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

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

Totals : 2694.67441 240.13445

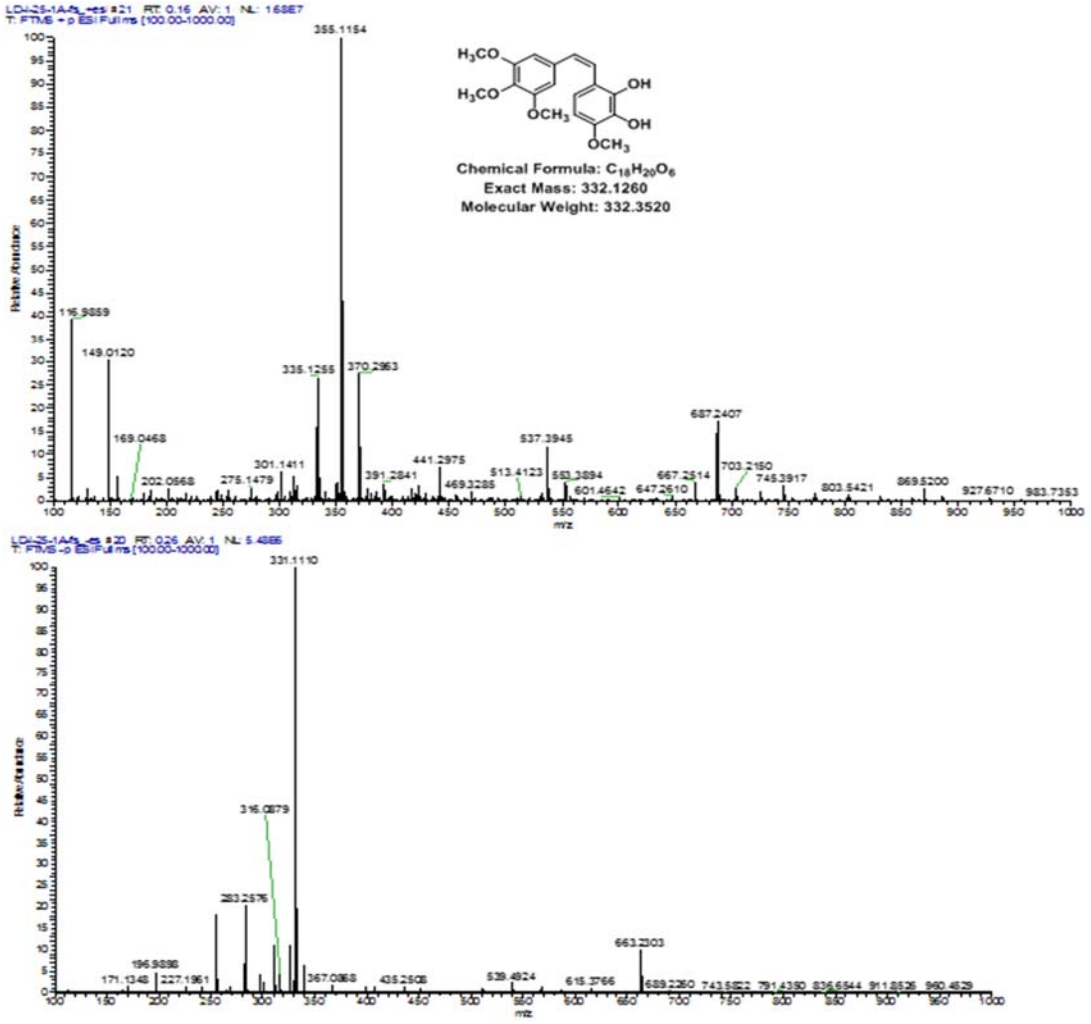
Signal 8: DAD1 H, Sig=320,16 Ref=off
Signal has been modified after loading from rawdata file!

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

Totals : 1897.94584 174.09949

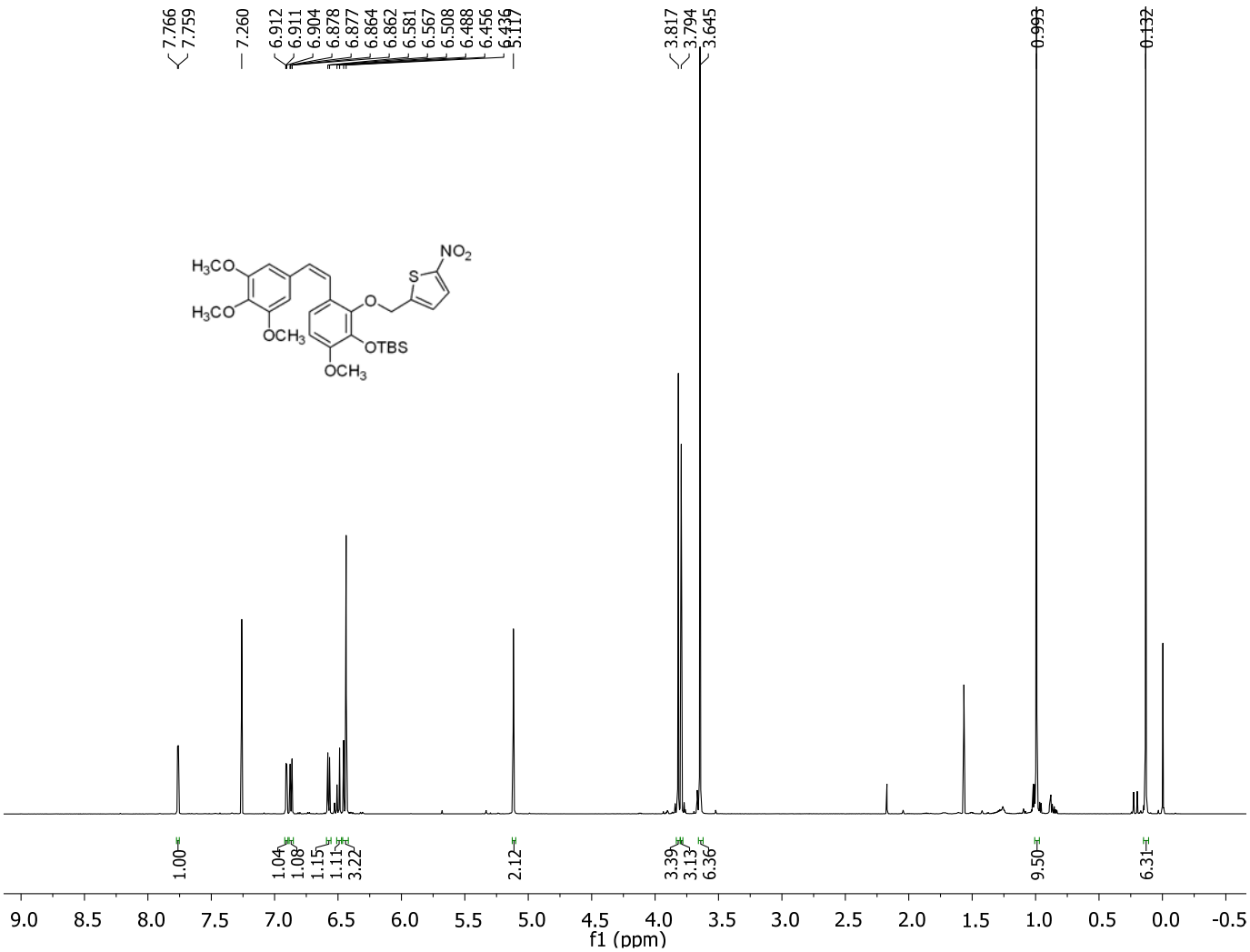
*** End of Report ***

HRMS Traces of Compound **29**

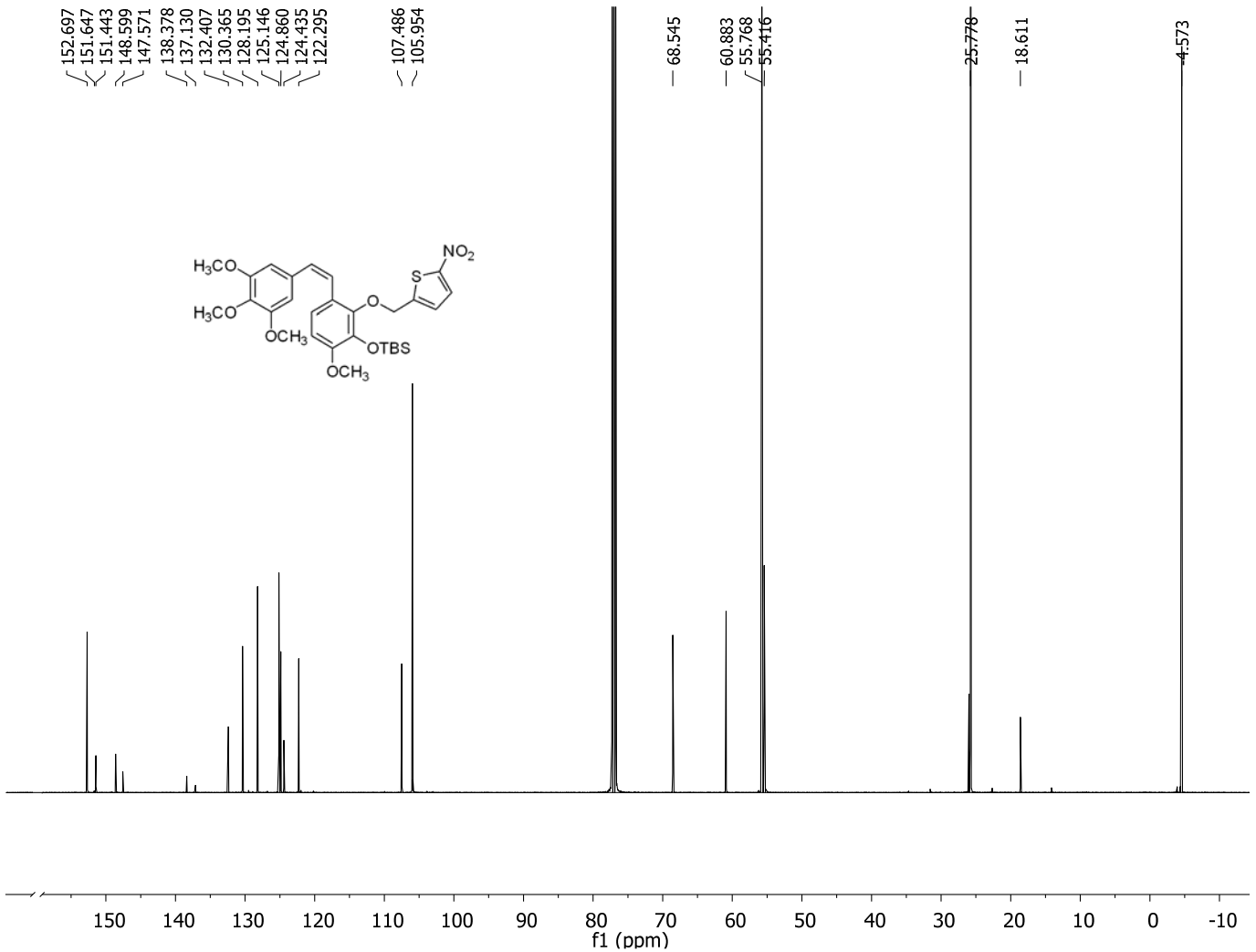


Chemical Formula: $C_{18}H_{20}O_6$
Exact Mass: 332.1260
Molecular Weight: 332.3520

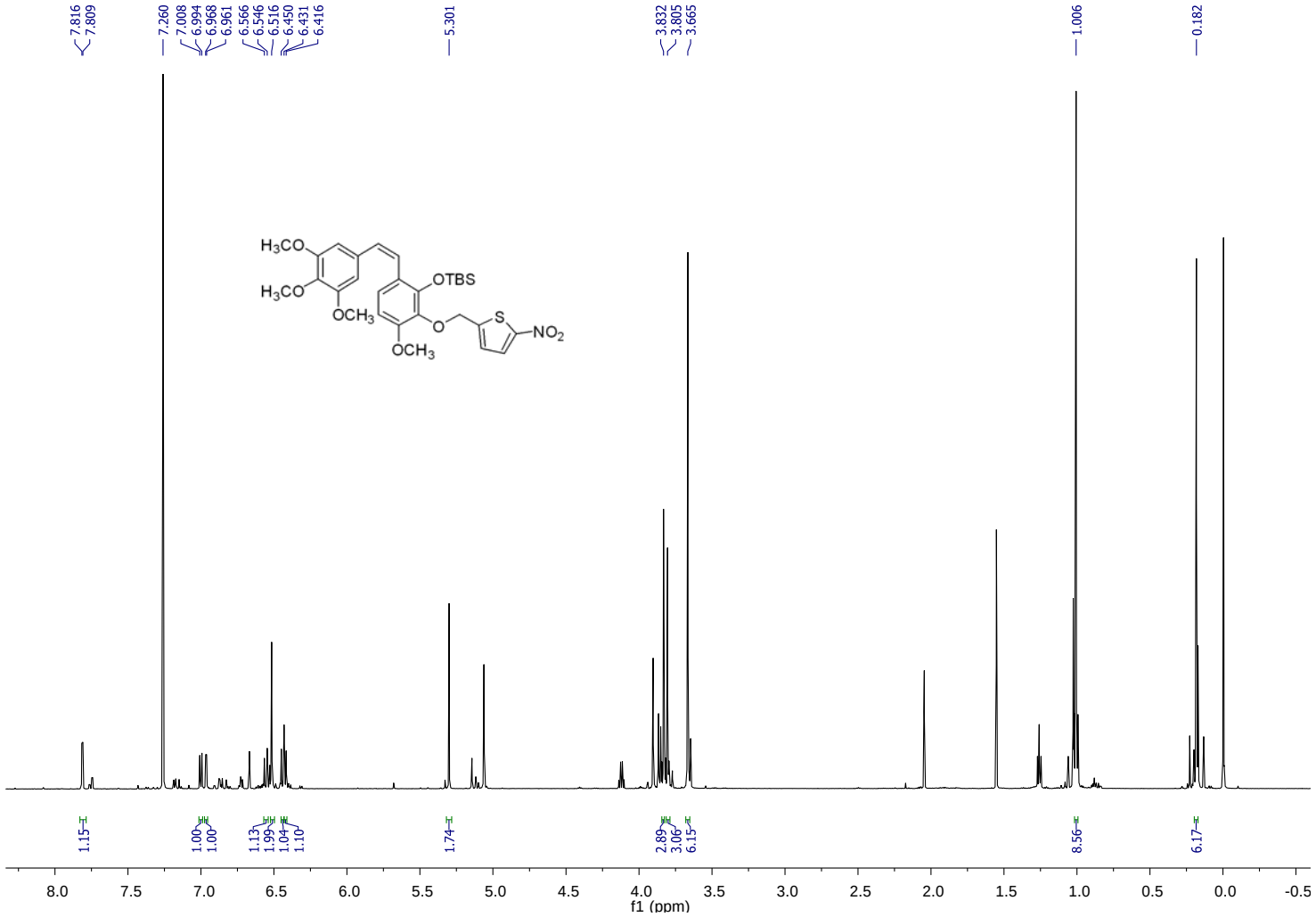
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **30**



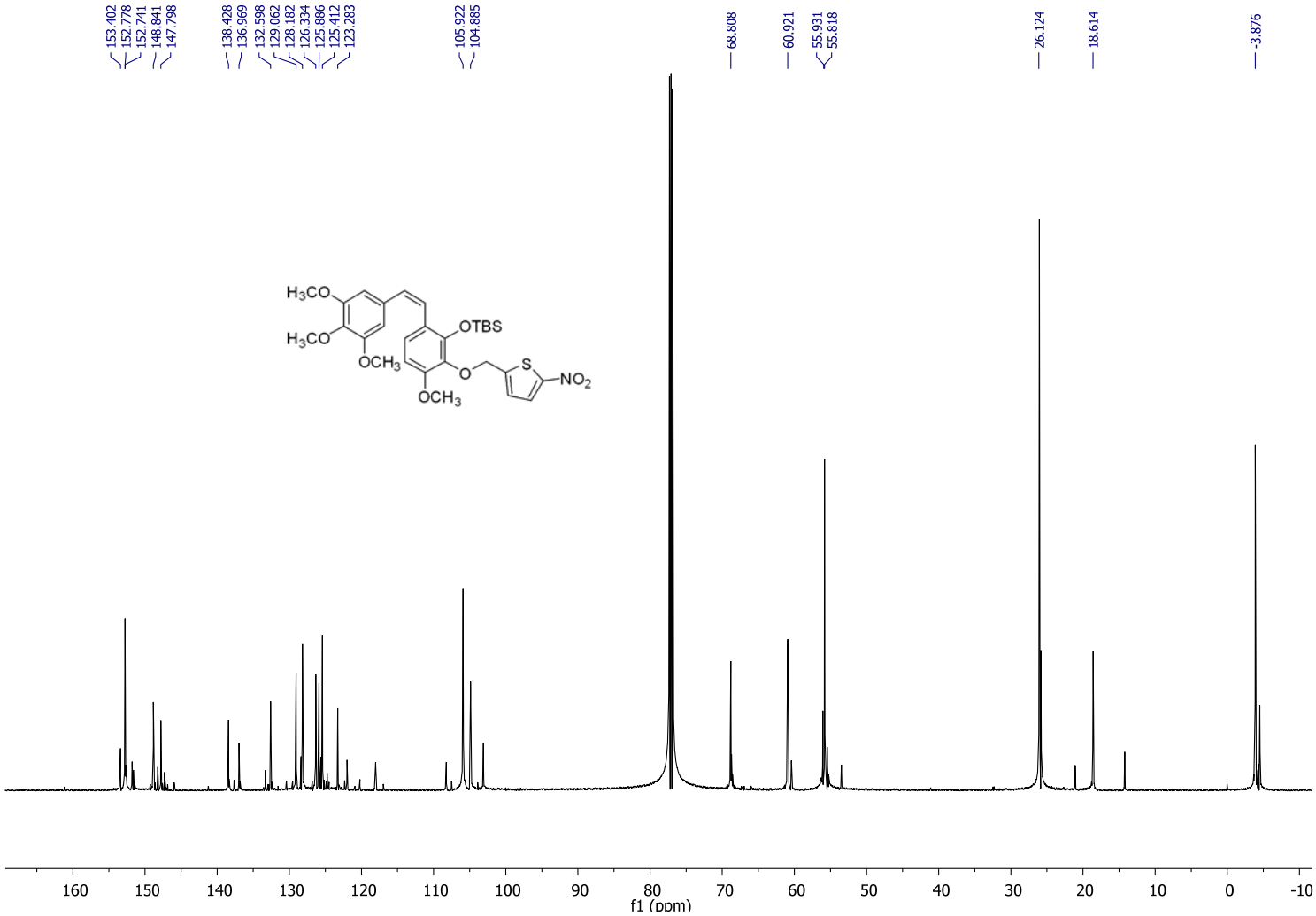
^{13}C NMR (125 MHz, $CDCl_3$) for Compound **30**



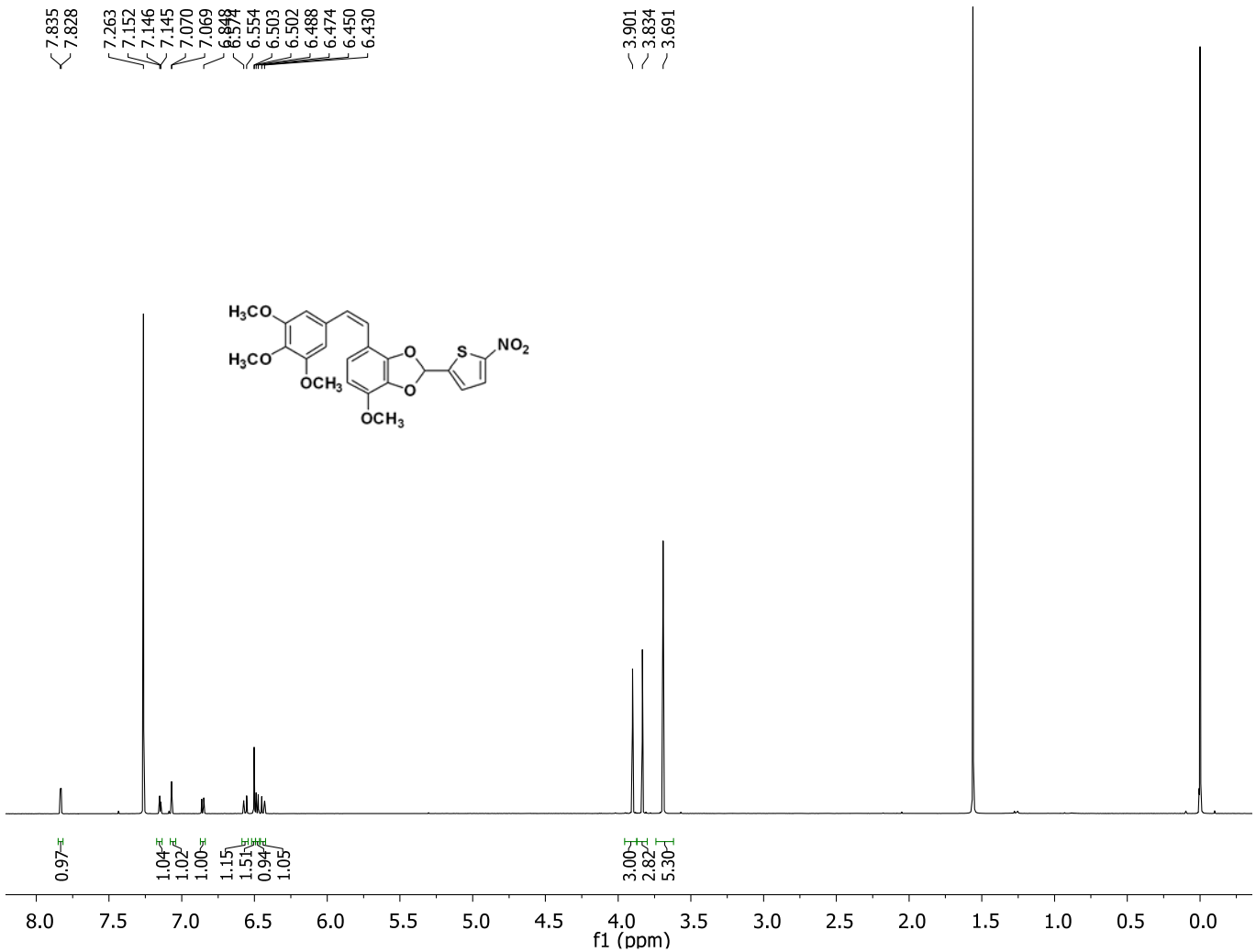
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **33**



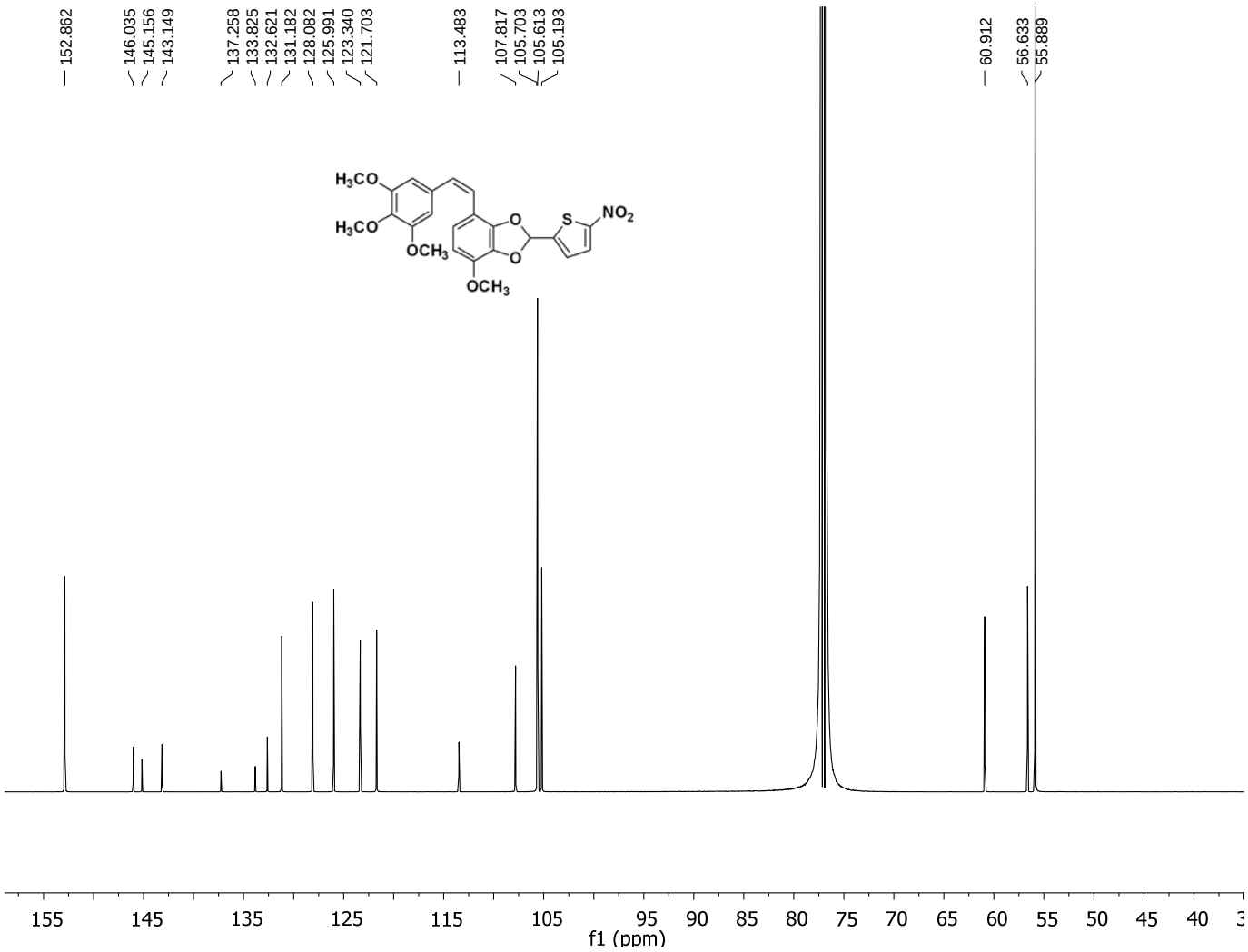
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **33**



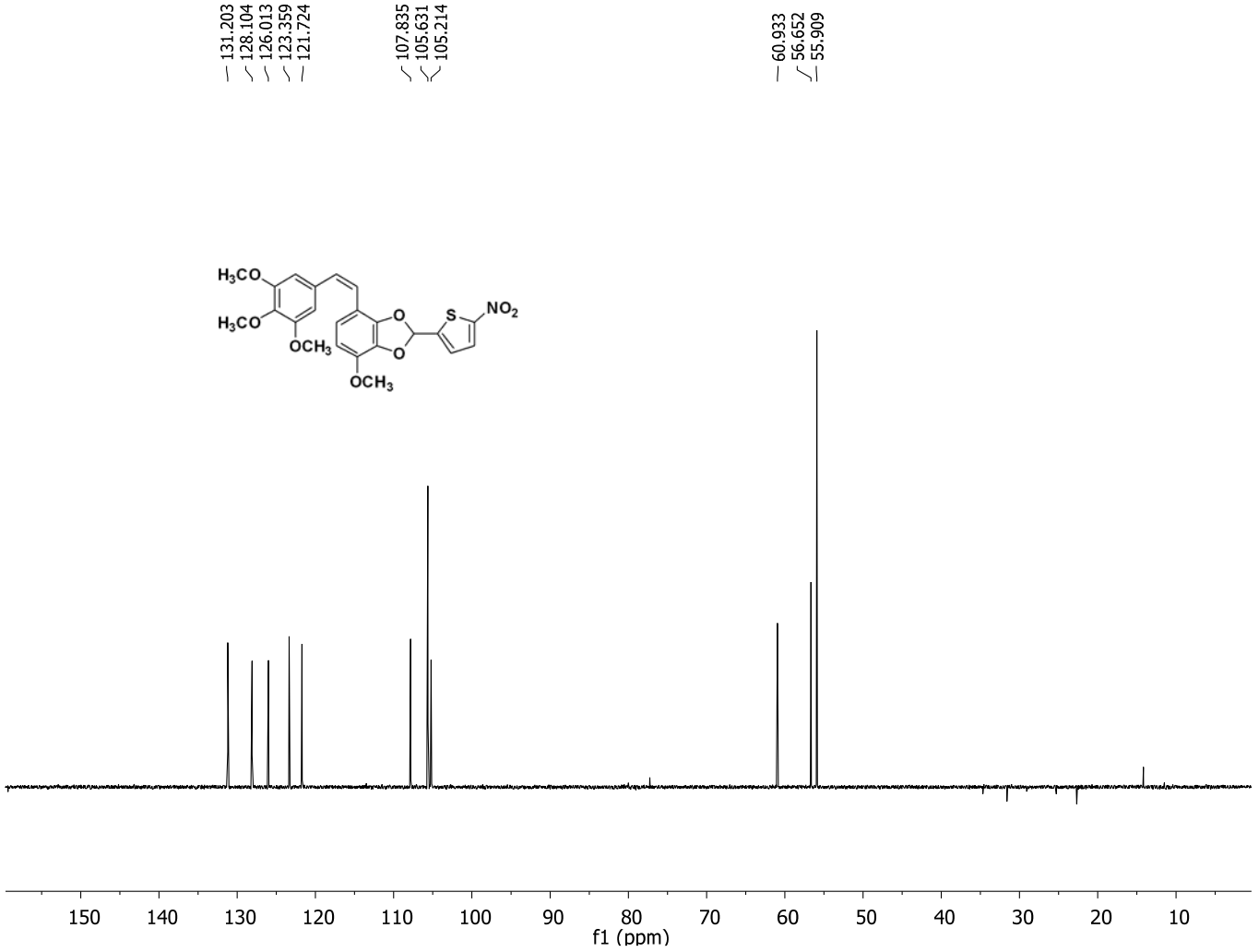
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **35**



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



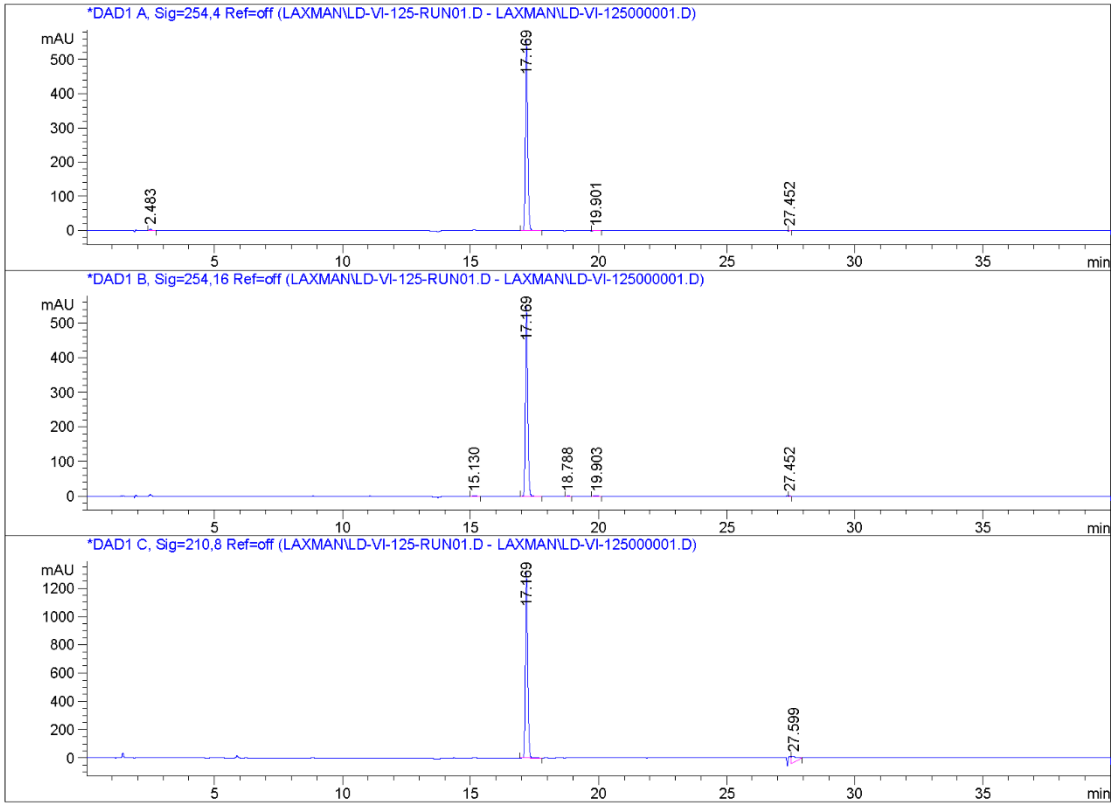
^{13}C DEPT NMR (125 MHz, $CDCl_3$) for Compound **35**



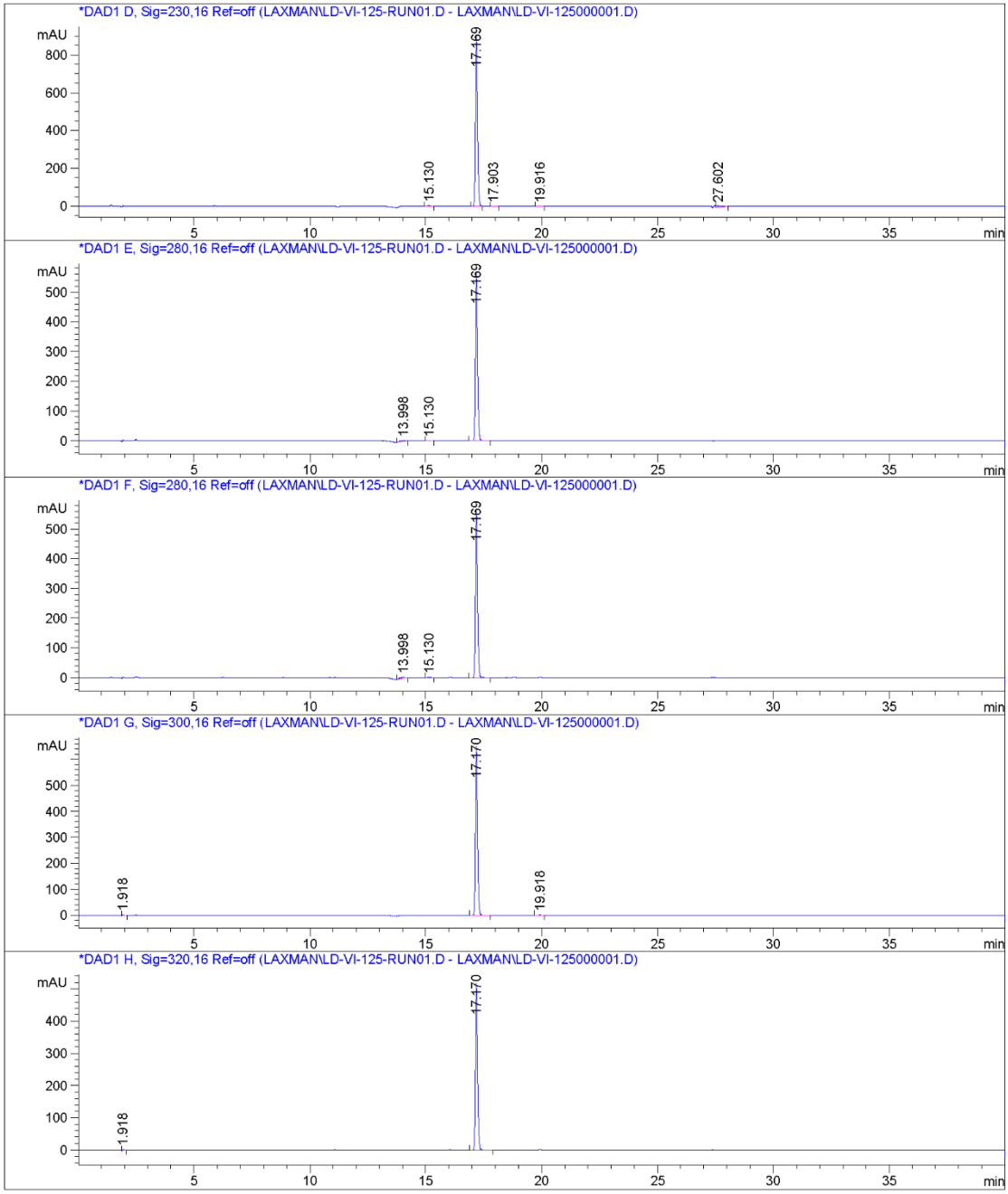
HPLC Traces for Compound **35**

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

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



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



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

Area Percent Report

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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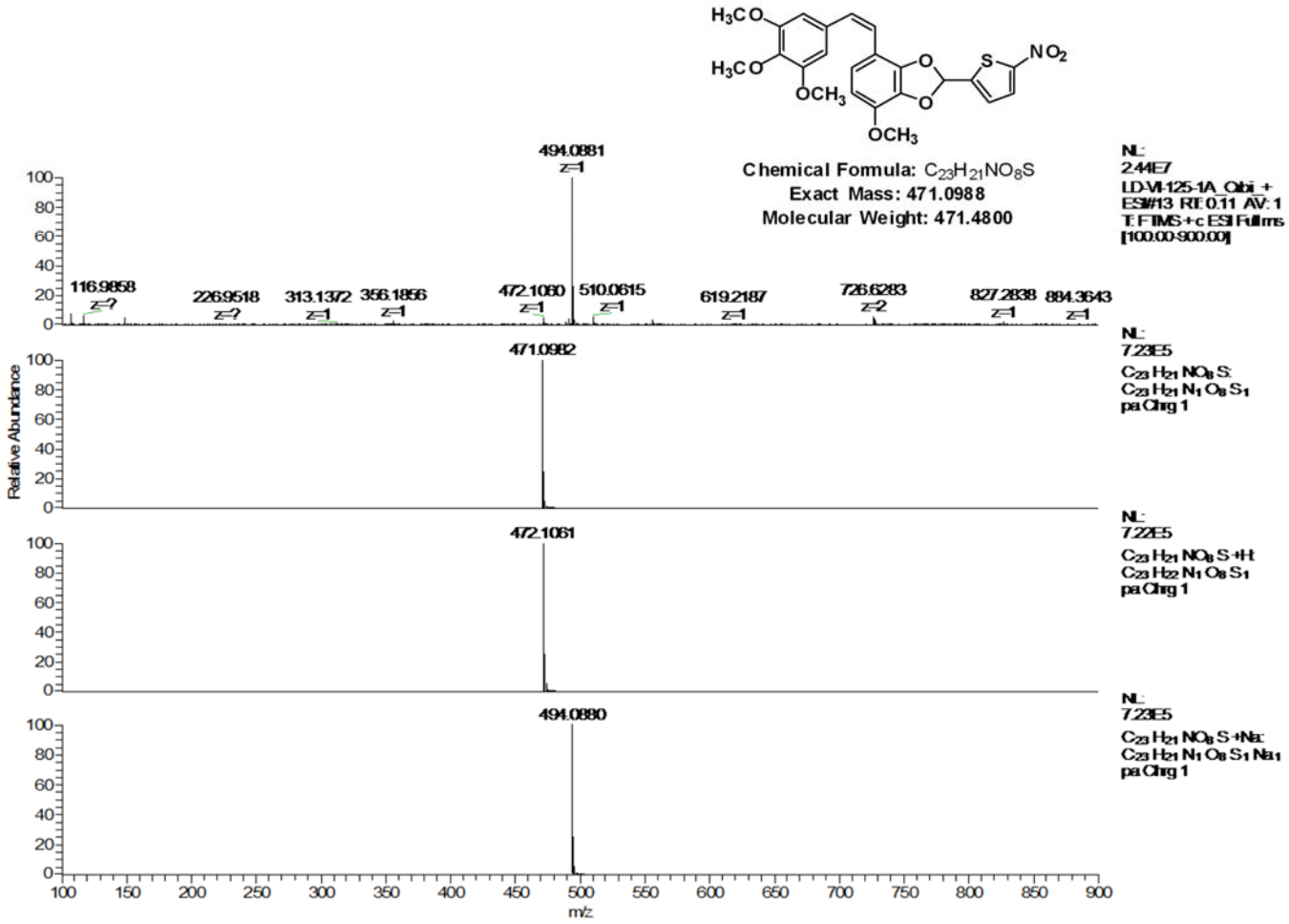
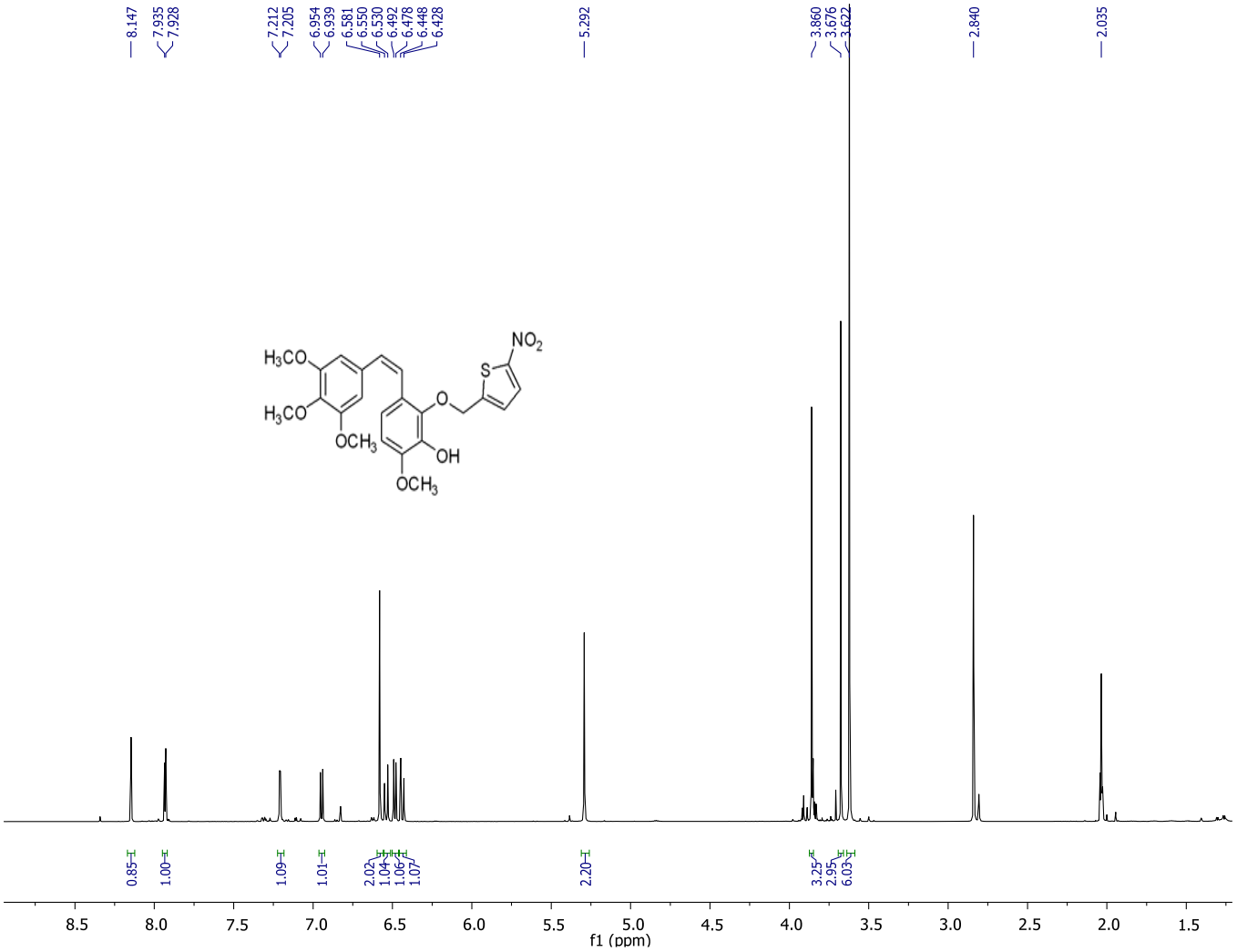
*** End of Report ***

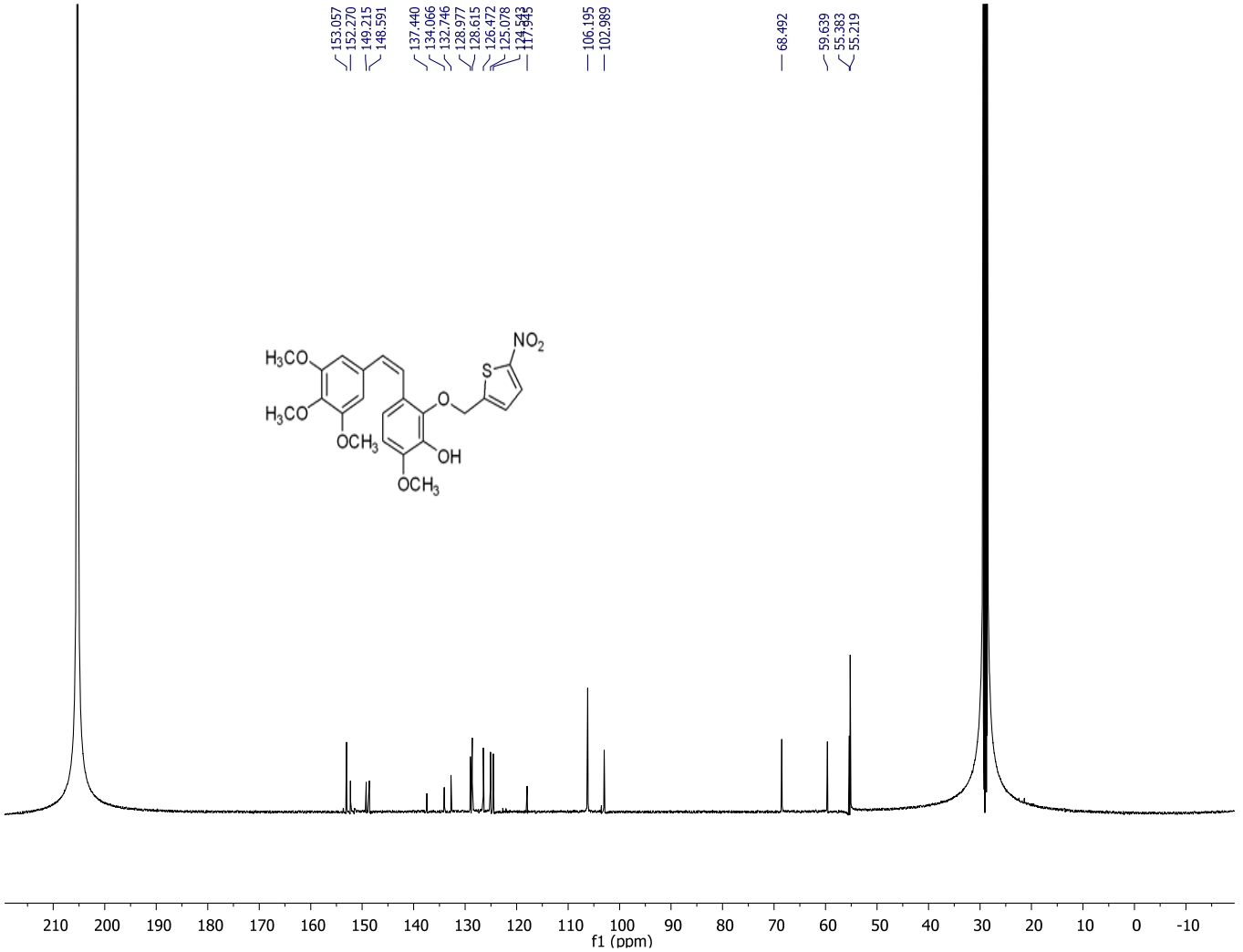
HRMS Traces for Compound **35**



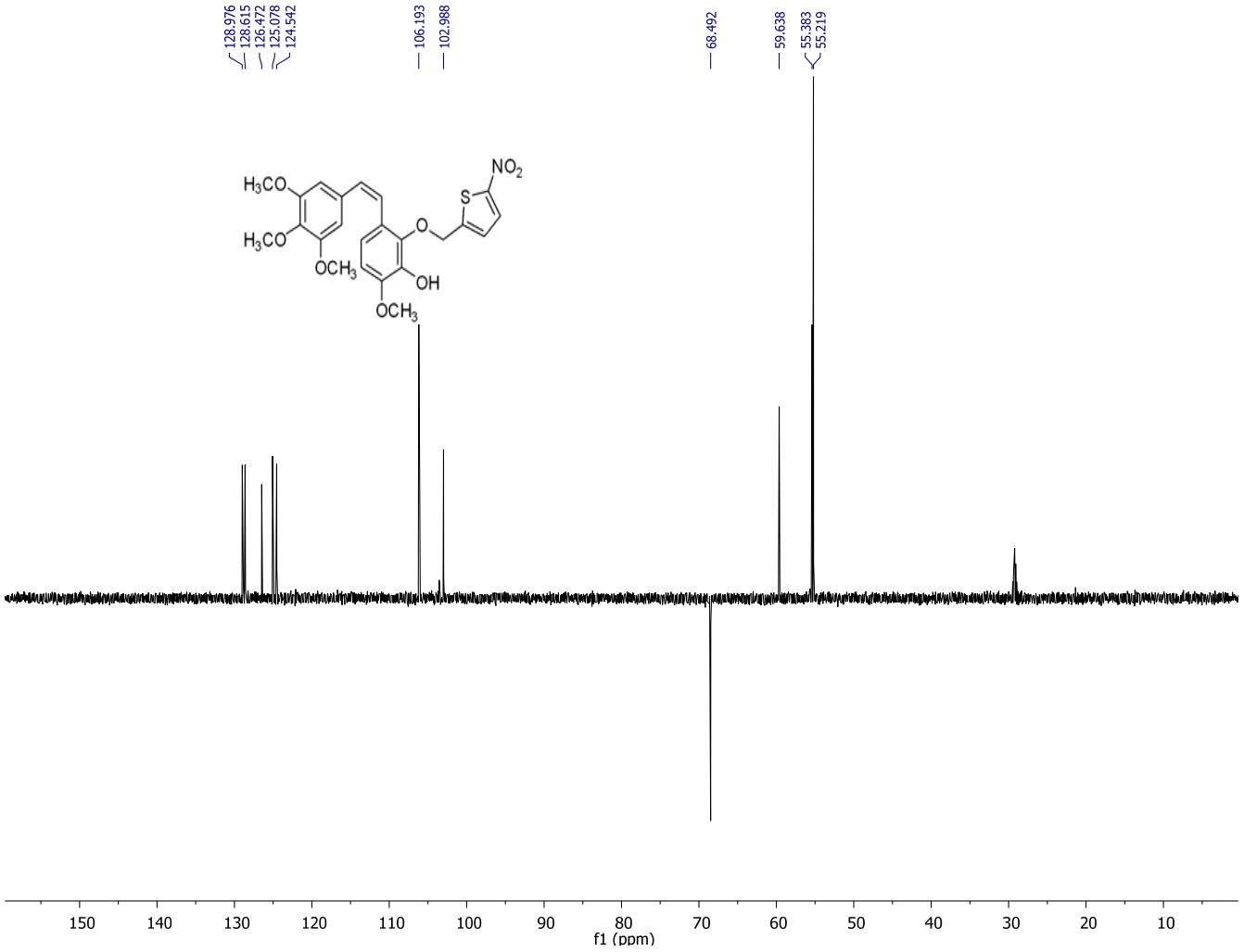
^{1}H NMR (600 MHz, Acetone) for Compound **37**



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



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



HPLC Traces for Compound **37**

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

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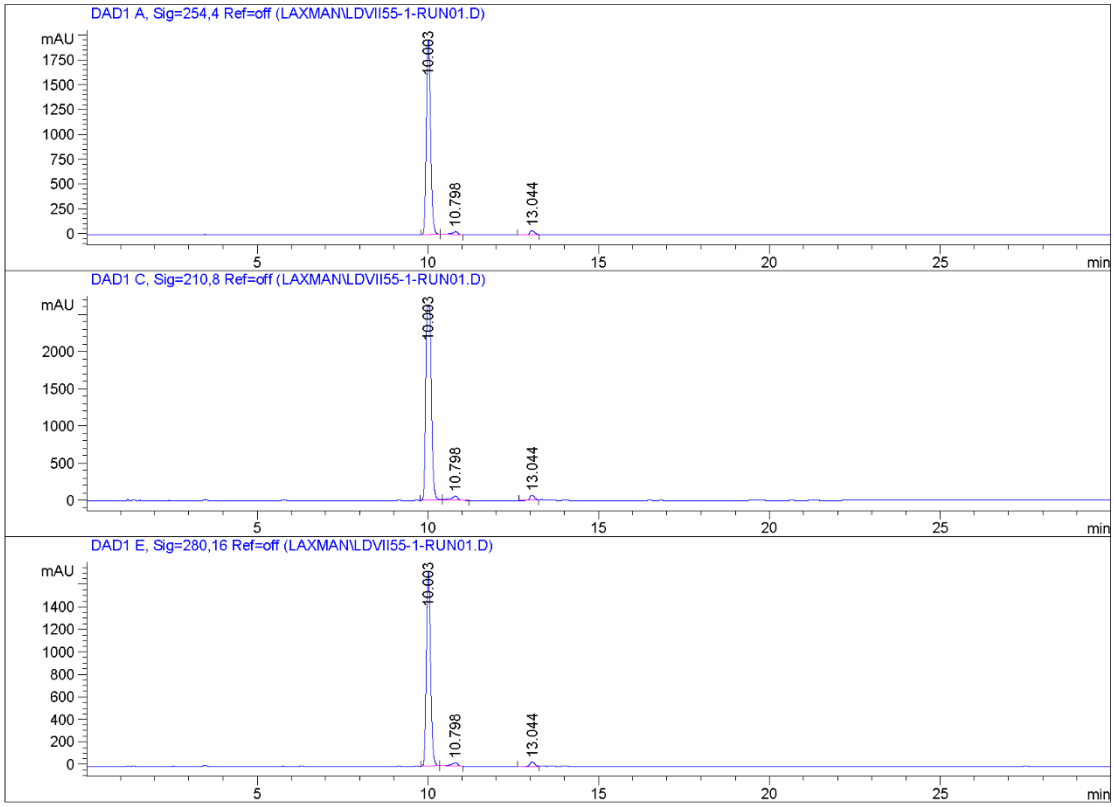
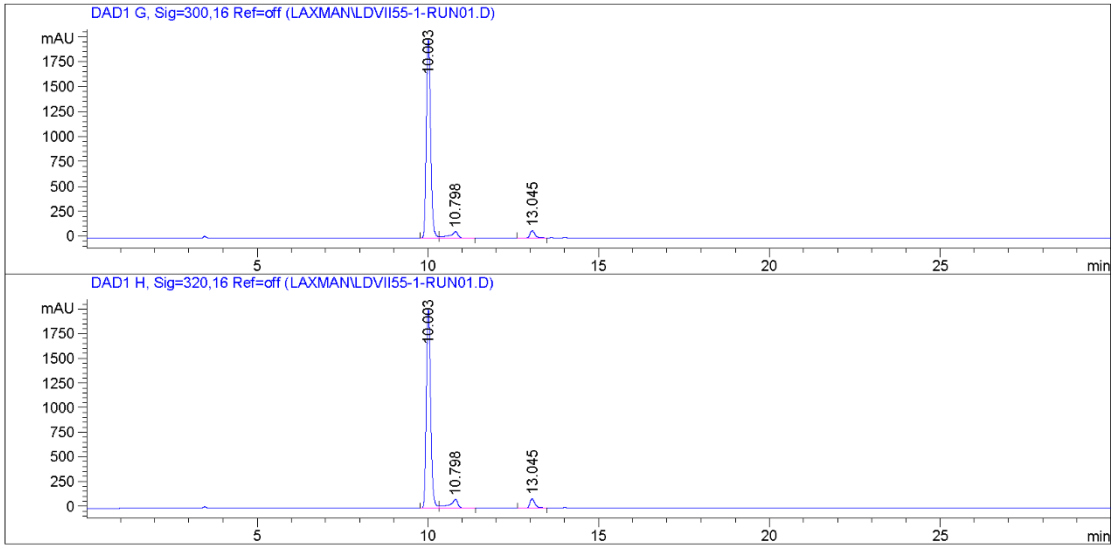


Data File C:\CHEM32\1\DATA\LAXMAN\LDVII55-1-RUN01.D
Sample Name: LD-VII-55-1A-run1




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

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


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

Peak RetTime 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
```

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

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

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

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

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

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

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

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

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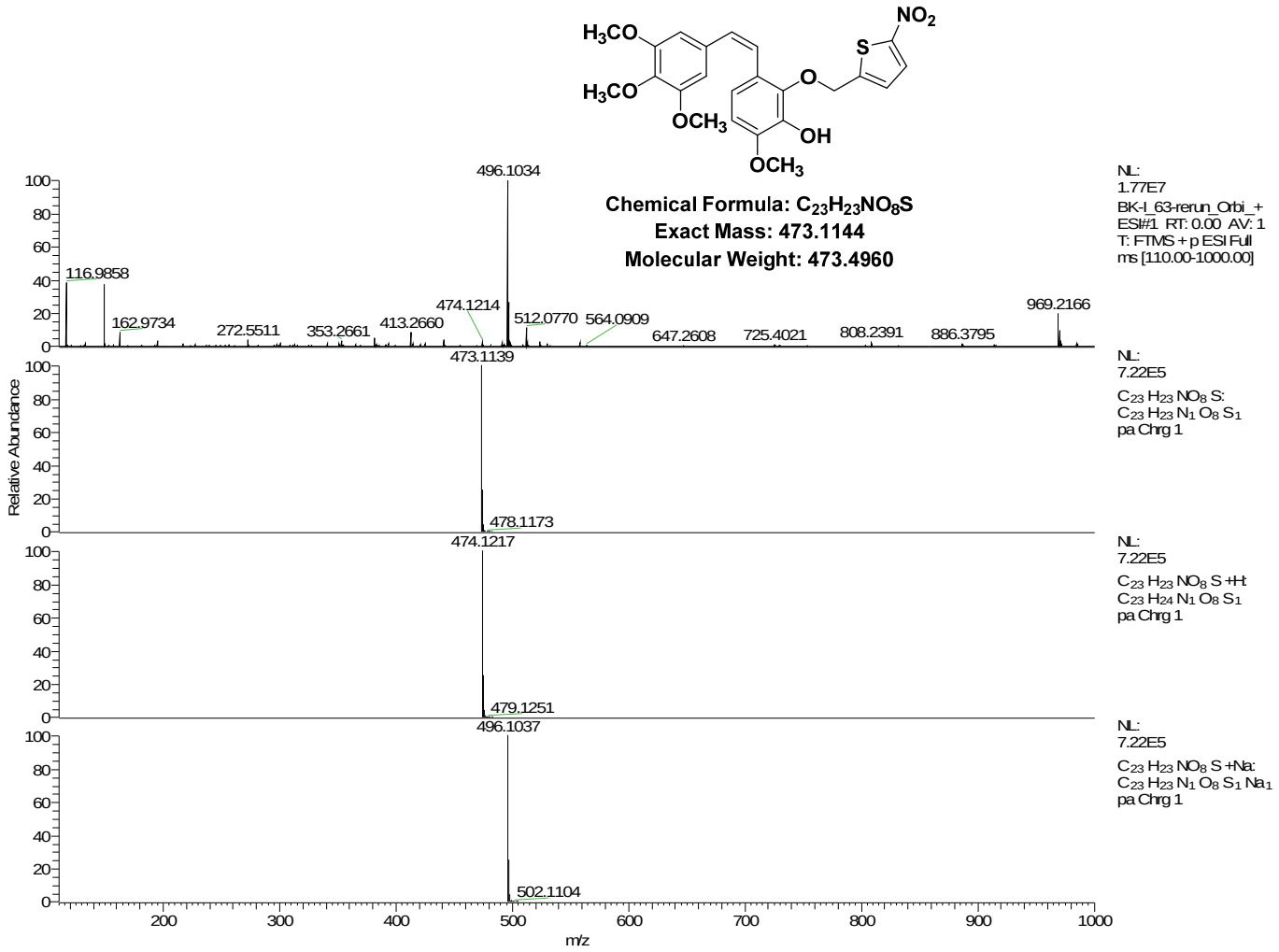
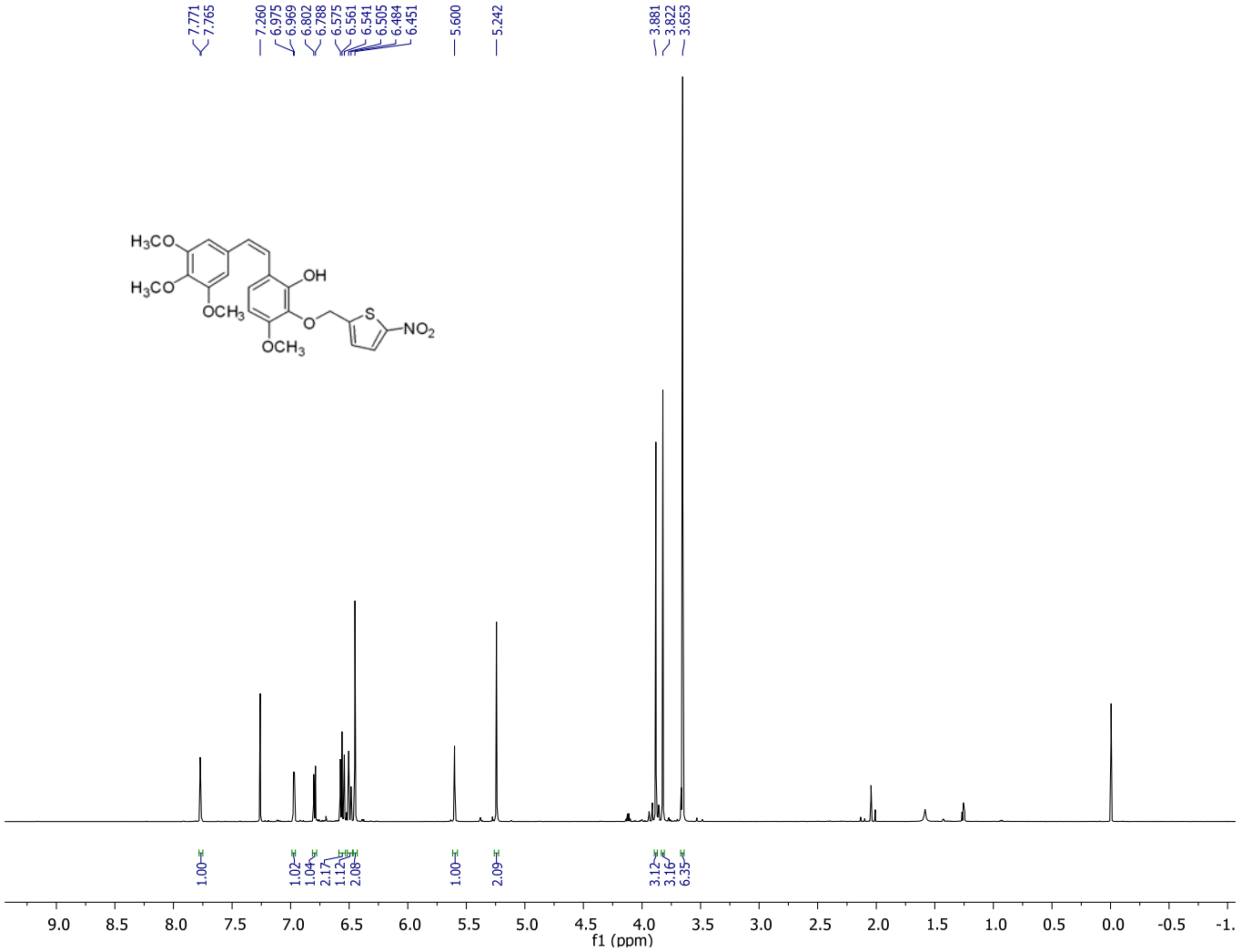
*** End of Report ***

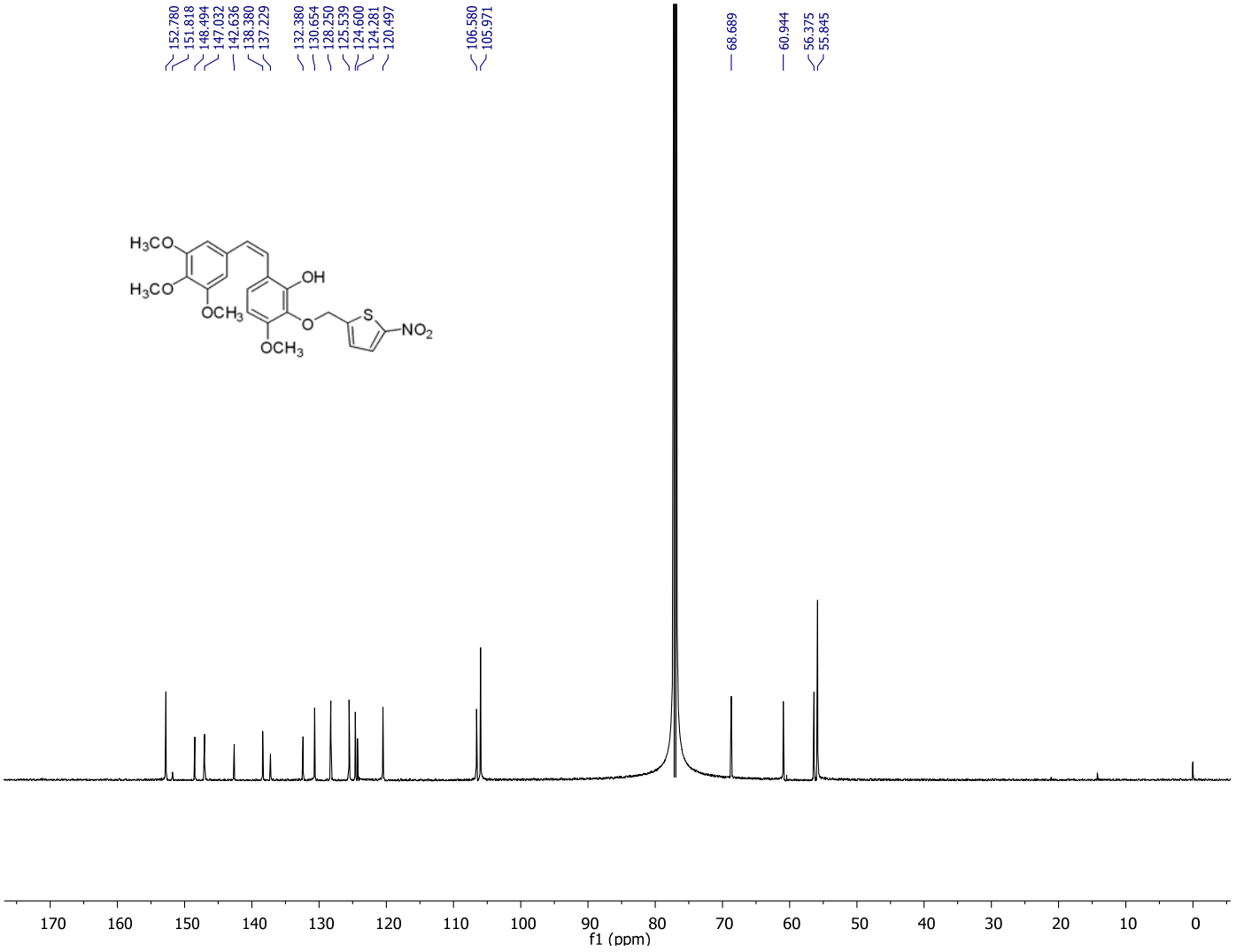
HRMS Traces for Compound **37**



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



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



HPLC Traces for Compound **39**

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

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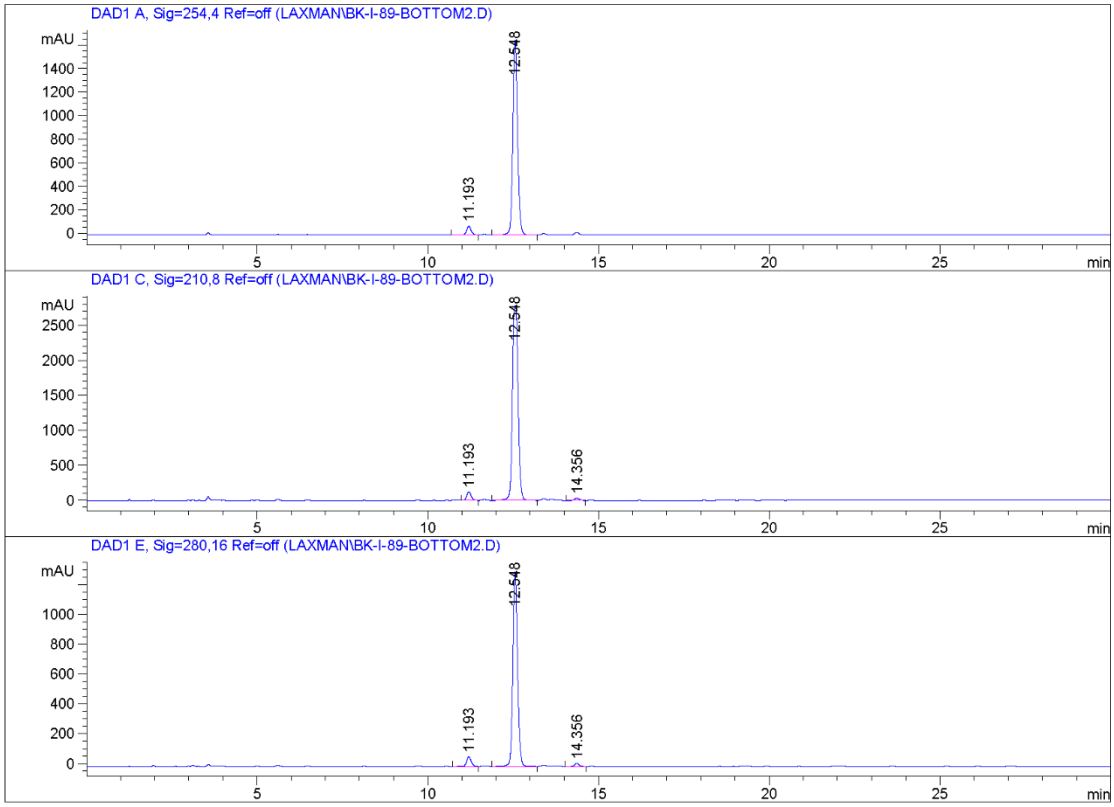
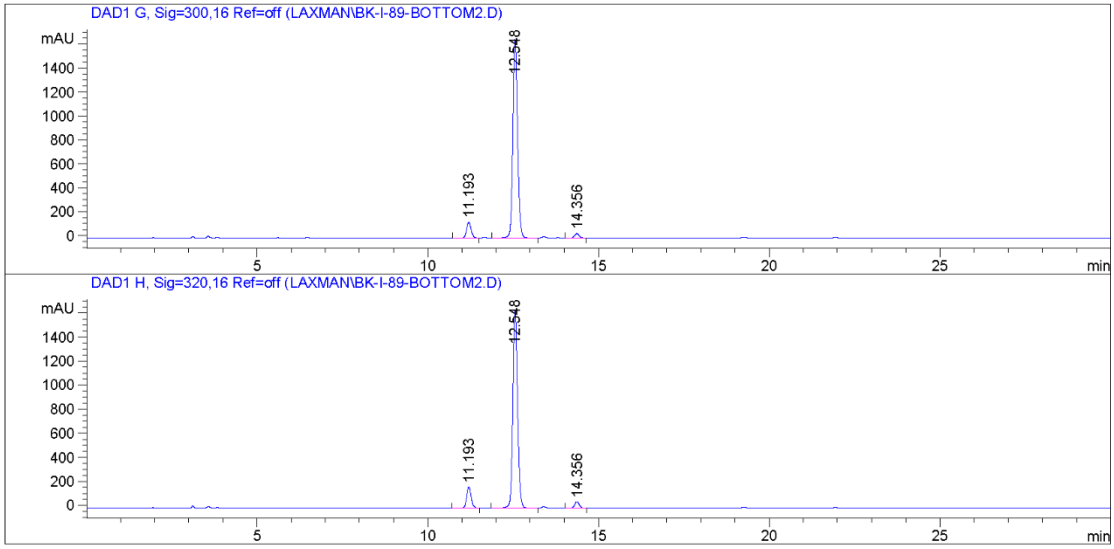


Data File C:\CHEM32\1\DATA\LAXMAN\BK-I-89-BOTTOM2.D
Sample Name: BK-I-89-bottom-isomer-rerun



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Area Percent Report

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Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

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

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

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

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

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

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

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

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

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2	12.548	BV	0.1473	1.58569e4	1661.58582	90.9187
3	14.356	BV	0.1442	355.36490	38.30421	2.0376
Totals :				1.74407e4	1834.63463	

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

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

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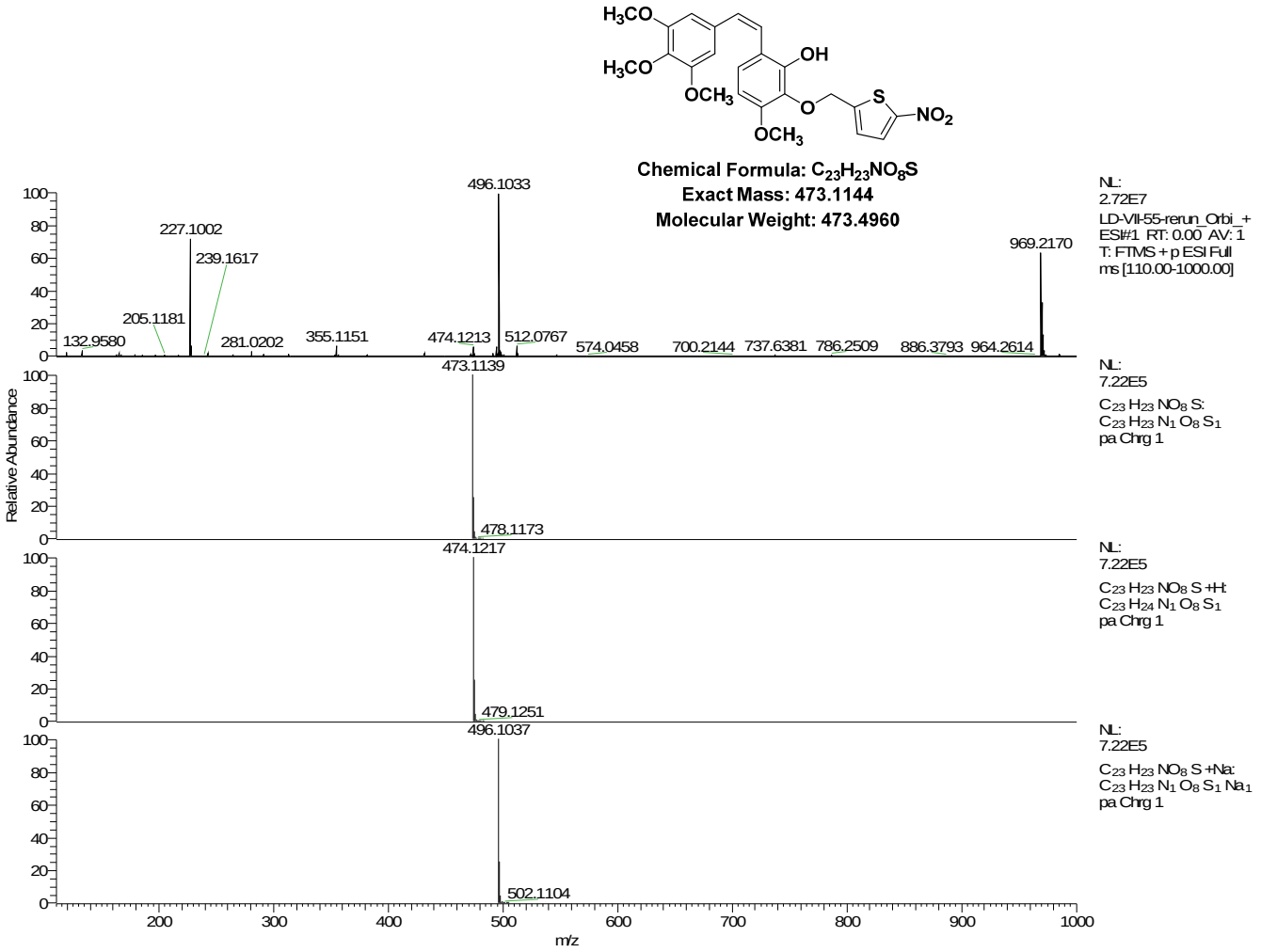
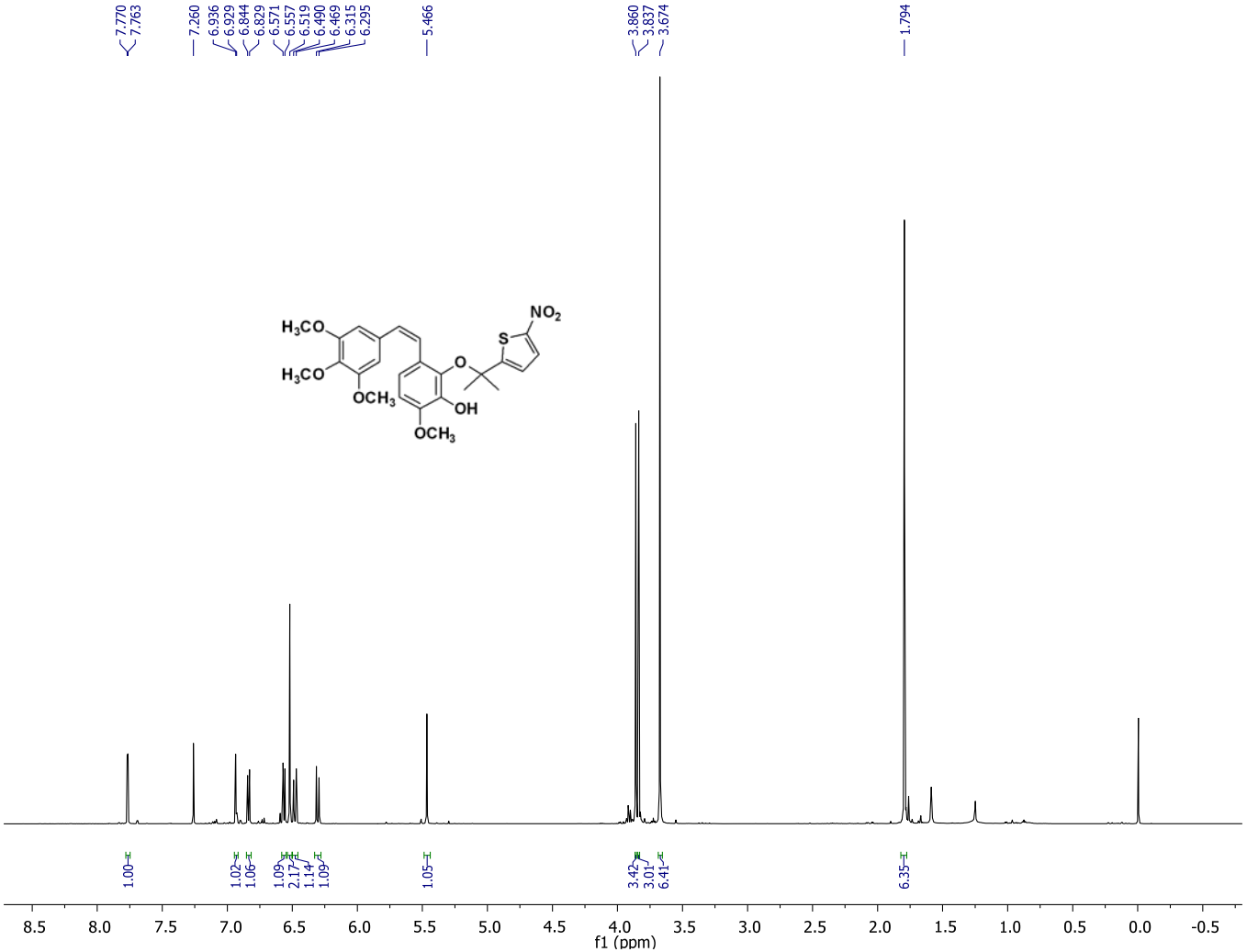
*** End of Report ***

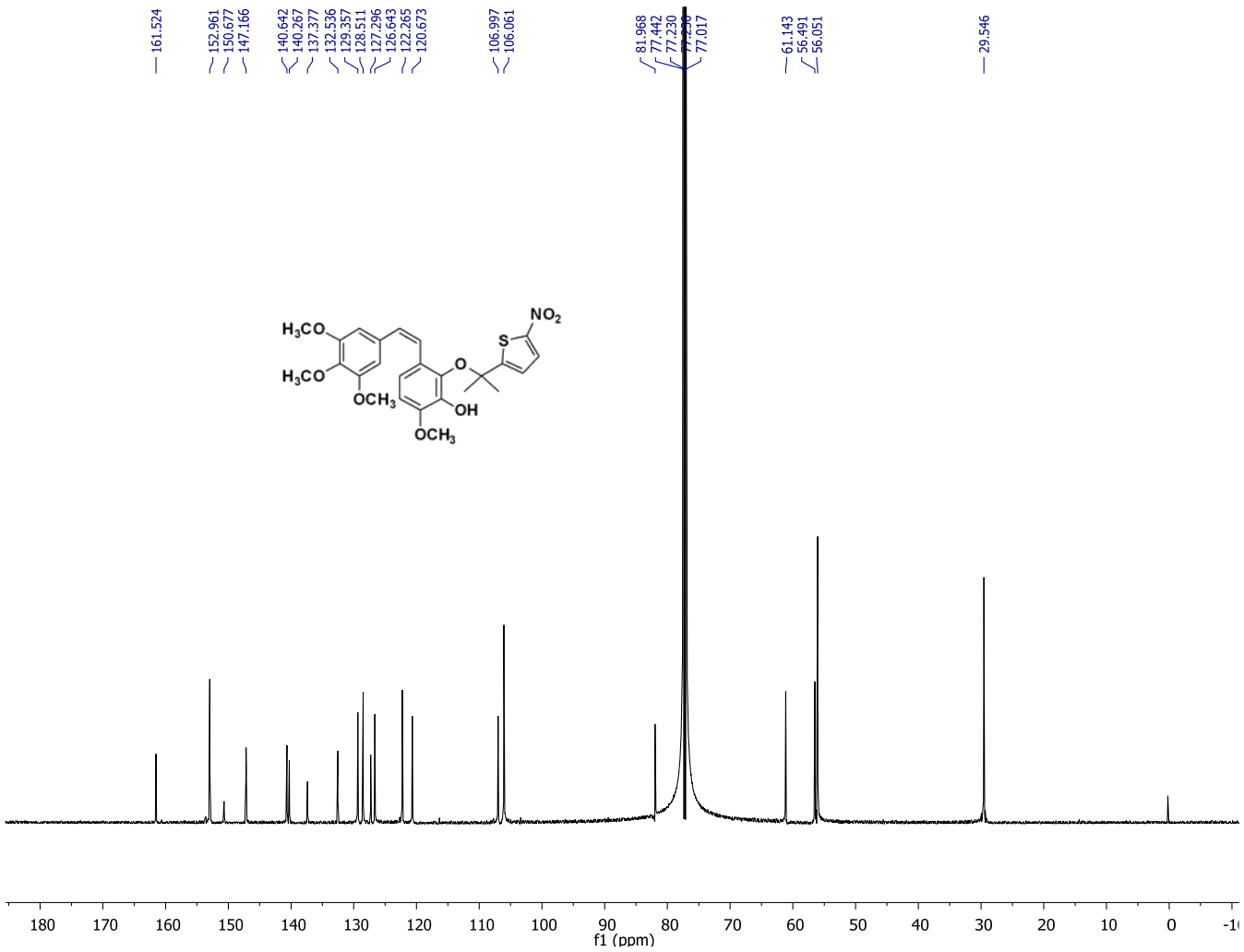
HRMS Traces for Compound **39**



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



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



HPLC Traces for Compound **41**

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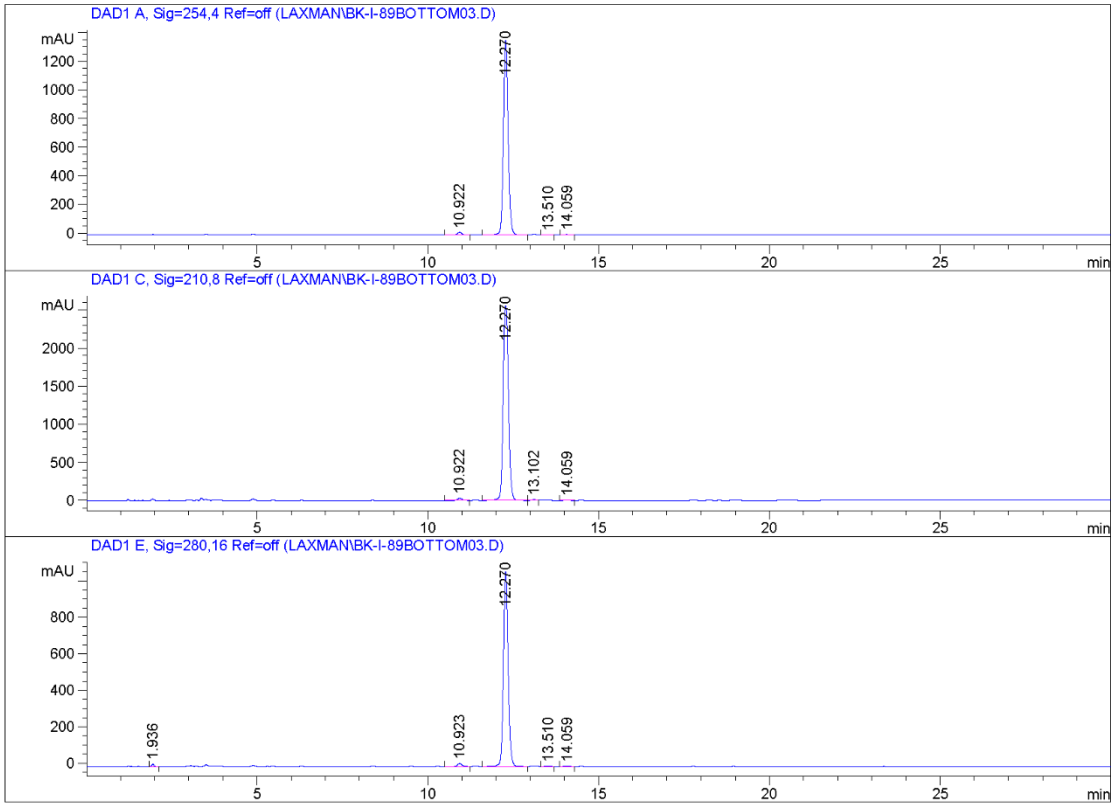

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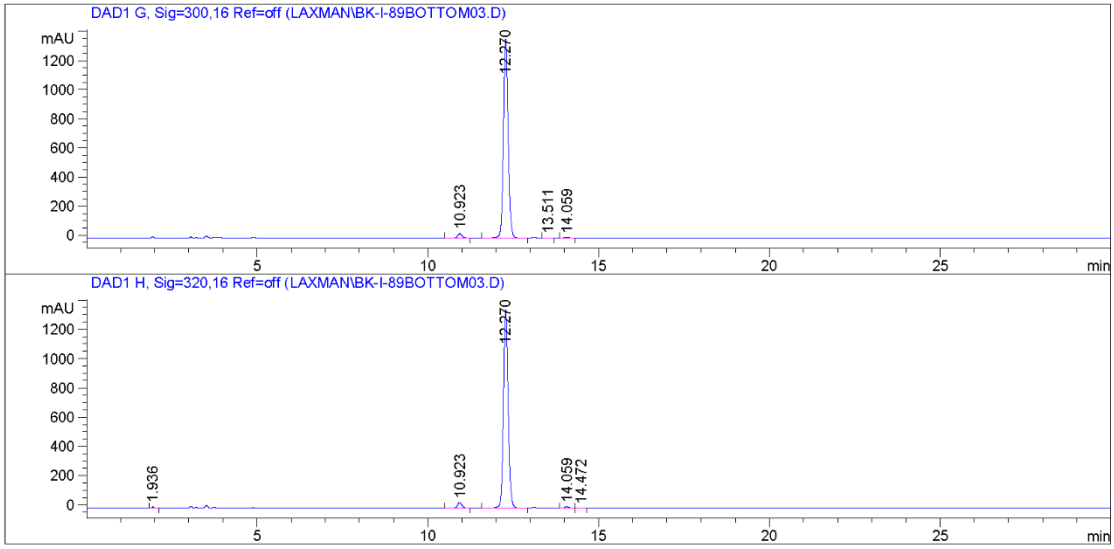

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Area Percent Report

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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 [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.922	BV	0.1421	168.96503	18.23570	1.2853
2	12.270	BB	0.1469	1.29245e4	1359.97986	98.3160
3	13.510	BB	0.1399	17.83705	1.96581	0.1357
4	14.059	BB	0.1396	34.57308	3.82064	0.2630
Totals :				1.31458e4	1384.00200	

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

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

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

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

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

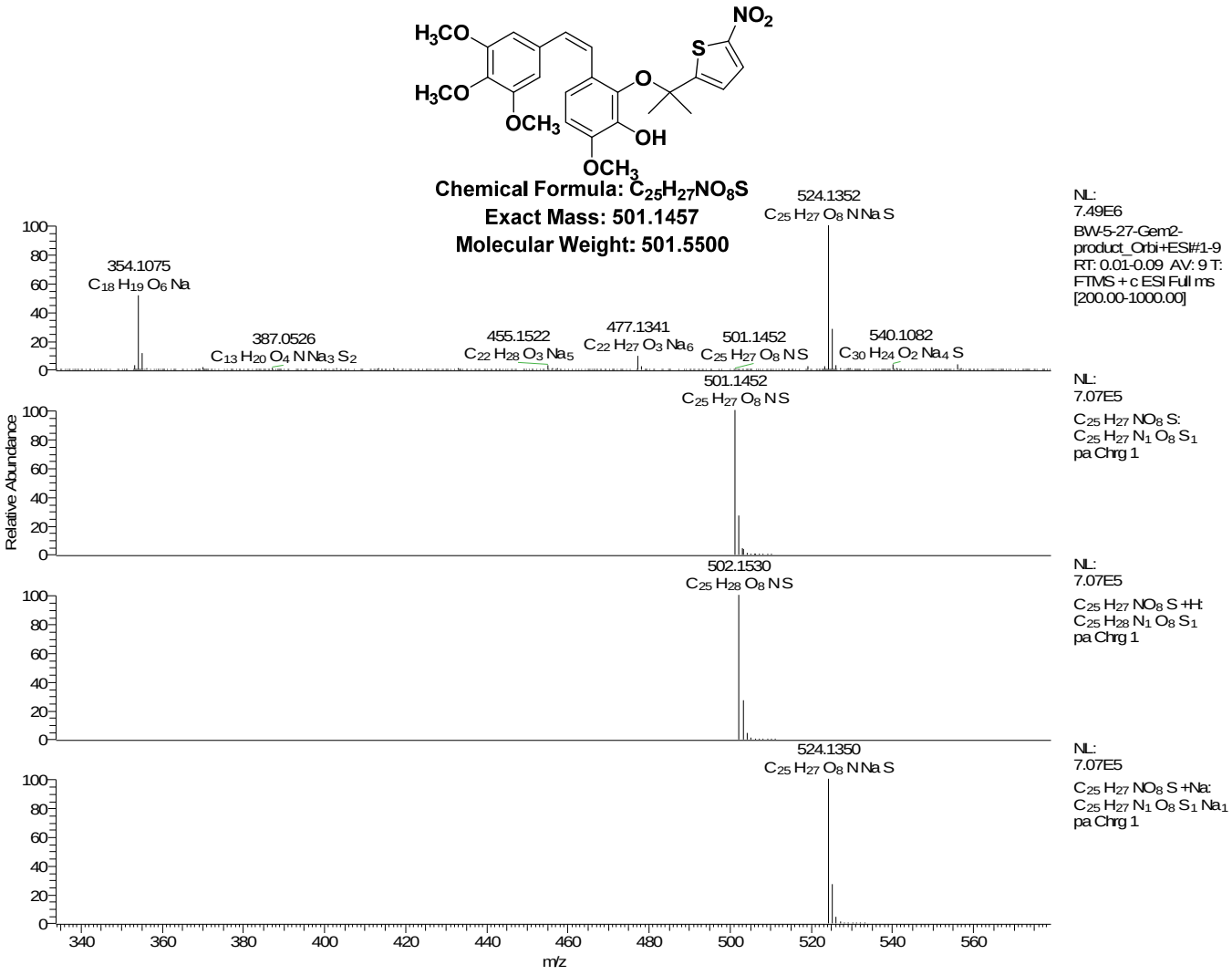
Signal 4: DAD1 G, Sig=300,16 Ref=off

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

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

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

HRMS Traces for Compound **41**



APPENDIX C

Benzosuberene-Based Tumor-Vascular Disrupting Agents

Some portion of this appendix is published as supplementary data: Tanpure, R. P.; George, C. S.; Strecker, T. E.; Devkota, L.; Tidmore, J. K.; Lin, C.-M.; Herdman, C. A.; MacDonough, M. T.; Sriram, M.; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. Synthesis of Structurally Diverse Benzosuberene Analogues and Their Biological Evaluation as Anti-Cancer Agents. *Bioorg. Med. Chem.* **2013**, *21* (24), 8019–8032.

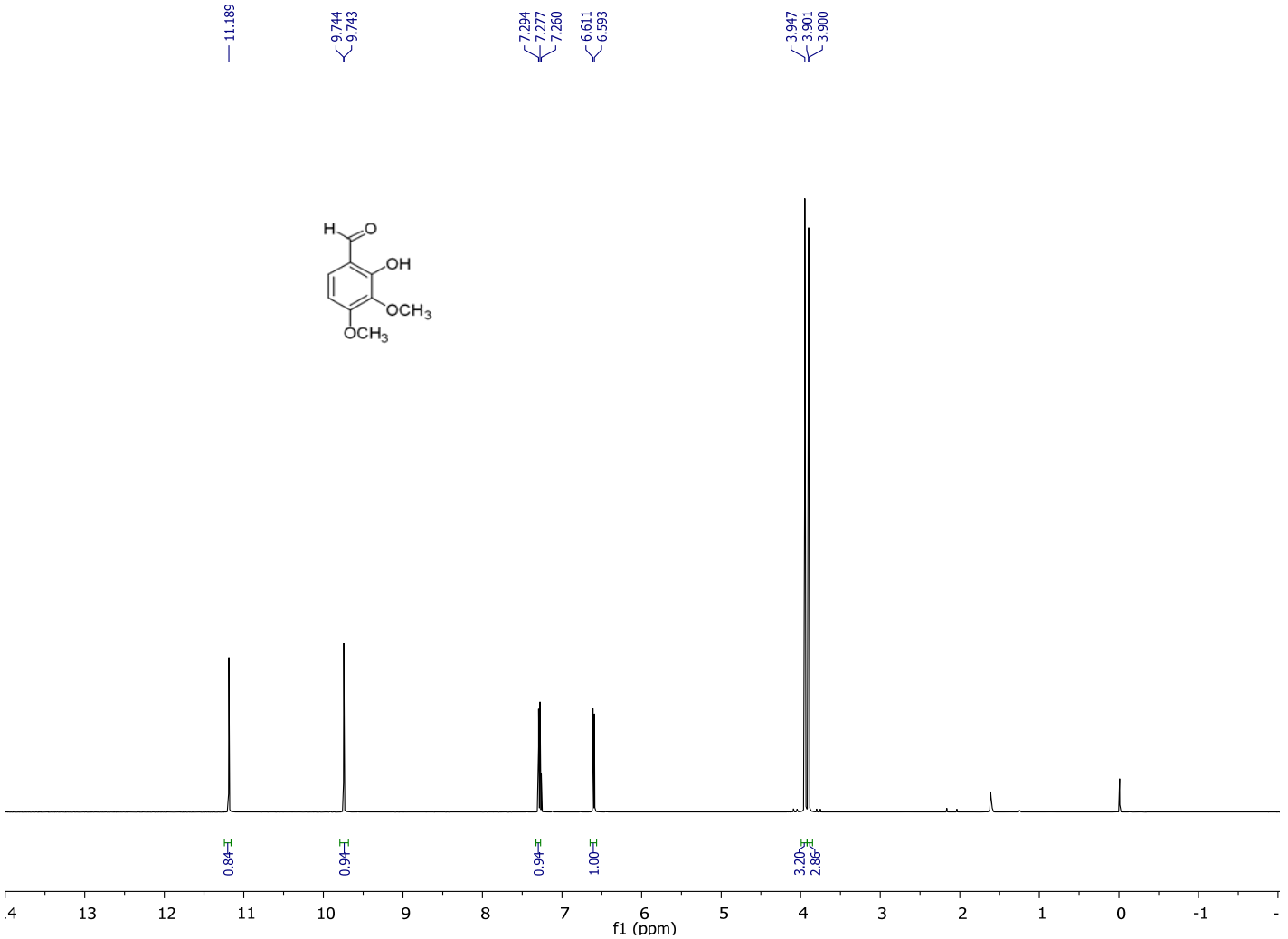
The author L. Devkota contributed in this manuscript by synthesizing a final compound **32** and all of its intermediates. Also, the author L. Devkota characterized and verified the compounds synthesized by R. P. Tanpure. In addition, L. Devkota contributed significantly to the preparation of the manuscript.

Rest of this appendix is published as supplementary data: Herdman, C. A.; Devkota, L.; Lin, C-M.; Niu, H.; Strecker, T. E.; Lopez, R.; Liu, L.; George, C. S.; Tanpure, R. P.; Hamel, E.; Chaplin, D. J.; Mason, R. P.; Trawick, M. L.; Pinney, K. G. Structural Interrogation of Benzosuberene-Based Inhibitors of Tubulin Polymerization. *Bioorg. Med. Chem.* **2015**, doi:10.1016/j.bmc.2015.10.012

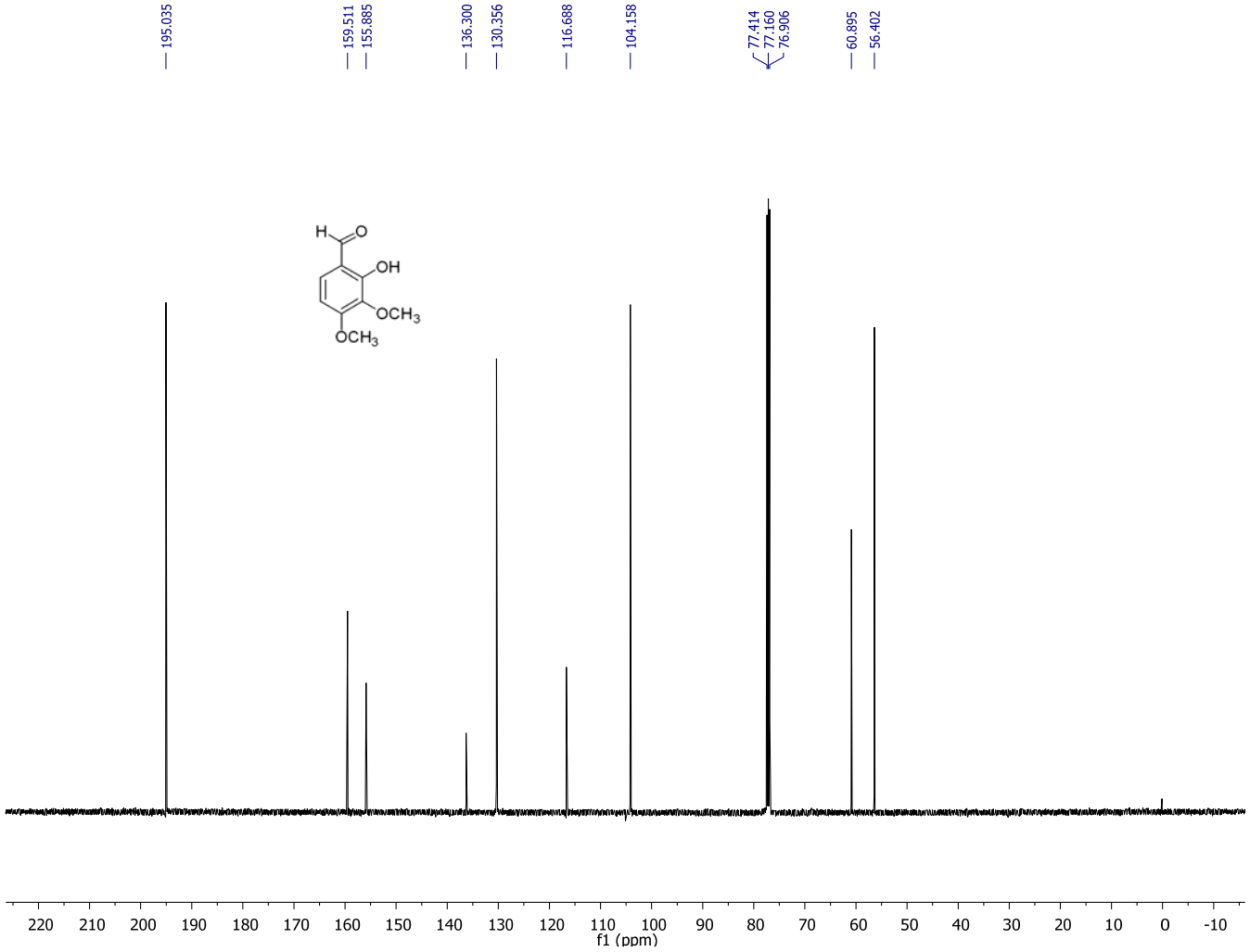
The author L. Devkota contributed in this manuscript by synthesizing five final compounds **27**, **30**, and **33**-**36**, and all of their intermediates. Also, the author L. Devkota contributed significantly to the preparation of the manuscript.

Table of Contents

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



^{13}C NMR (126 MHz, $CDCl_3$) for Compound **1**



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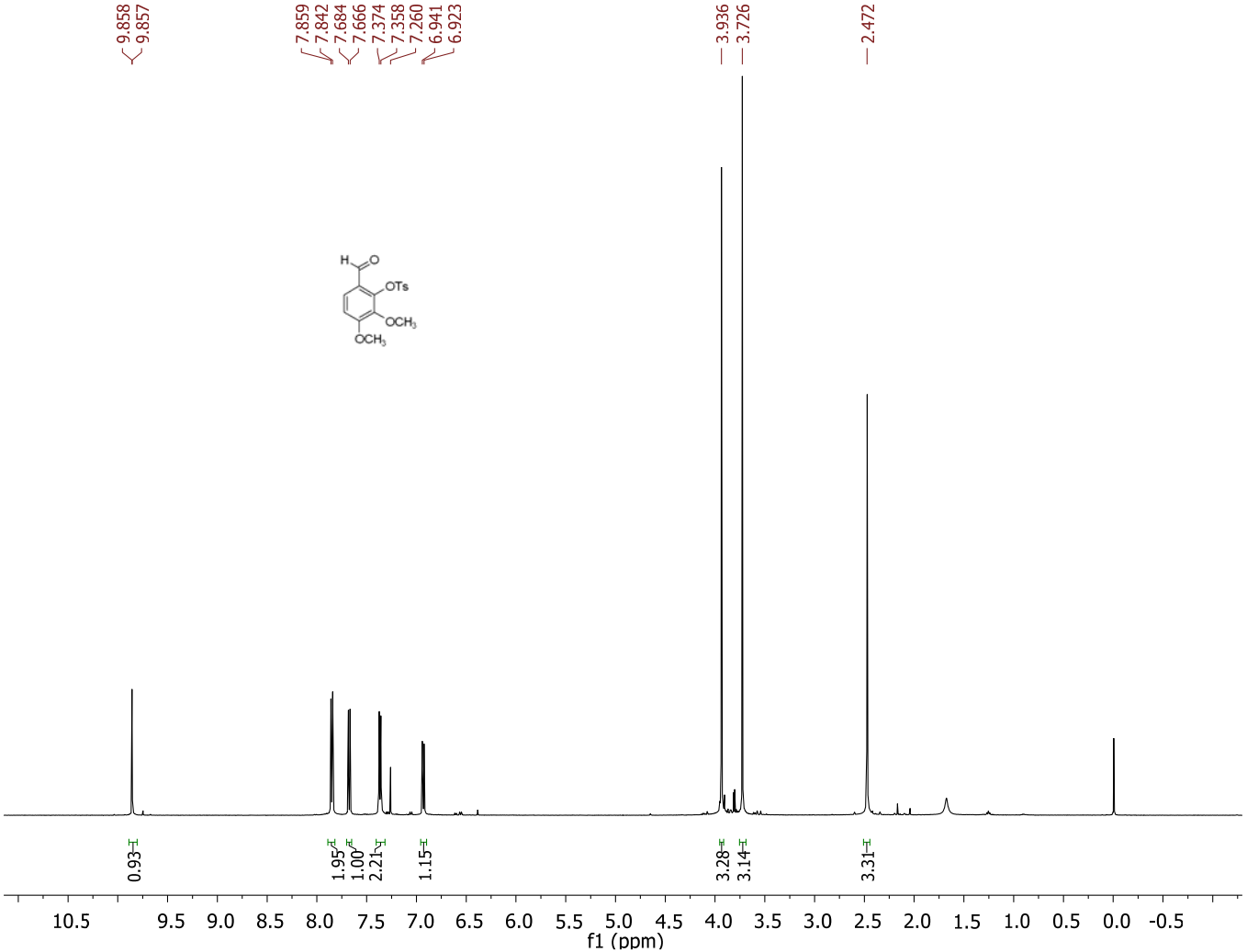

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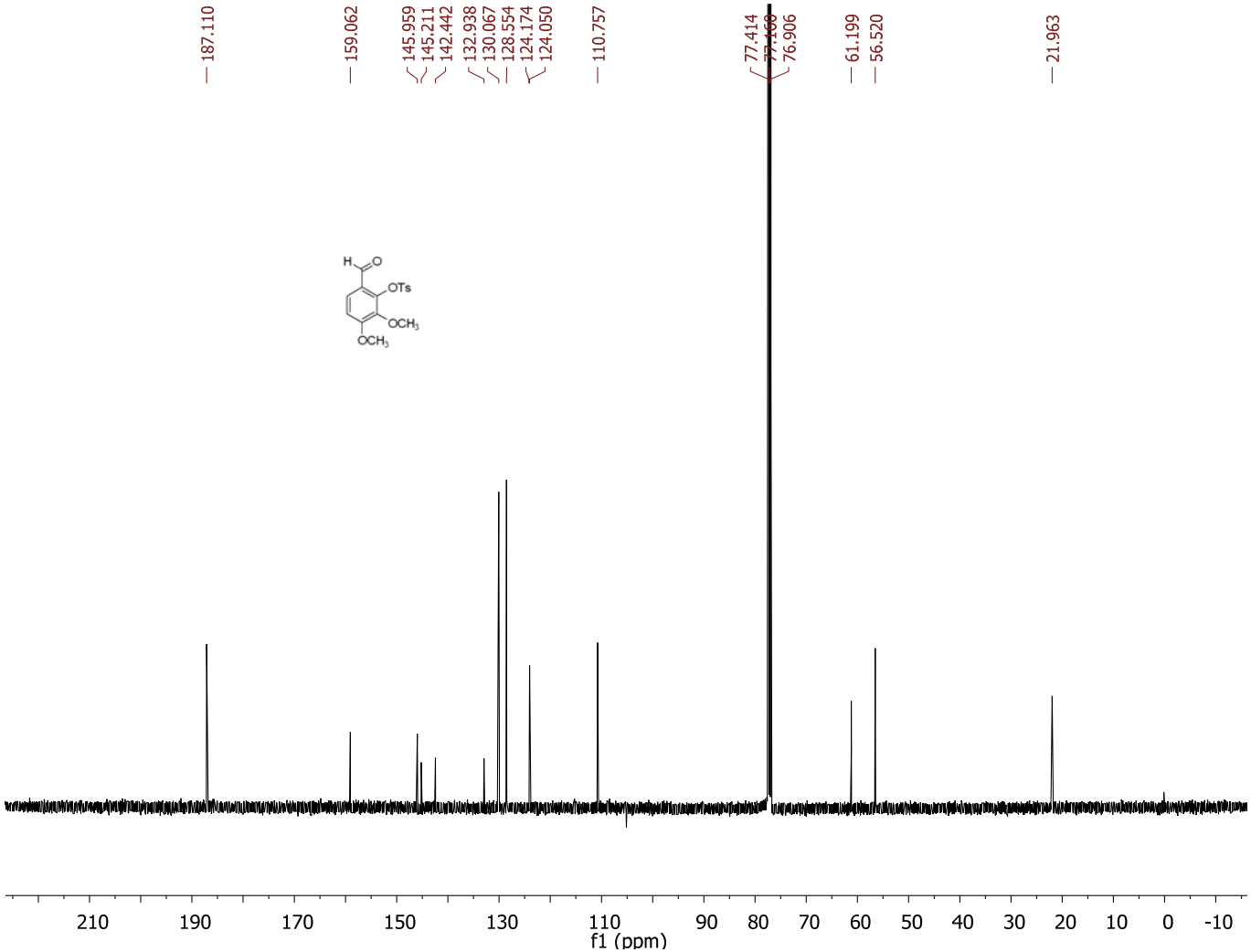

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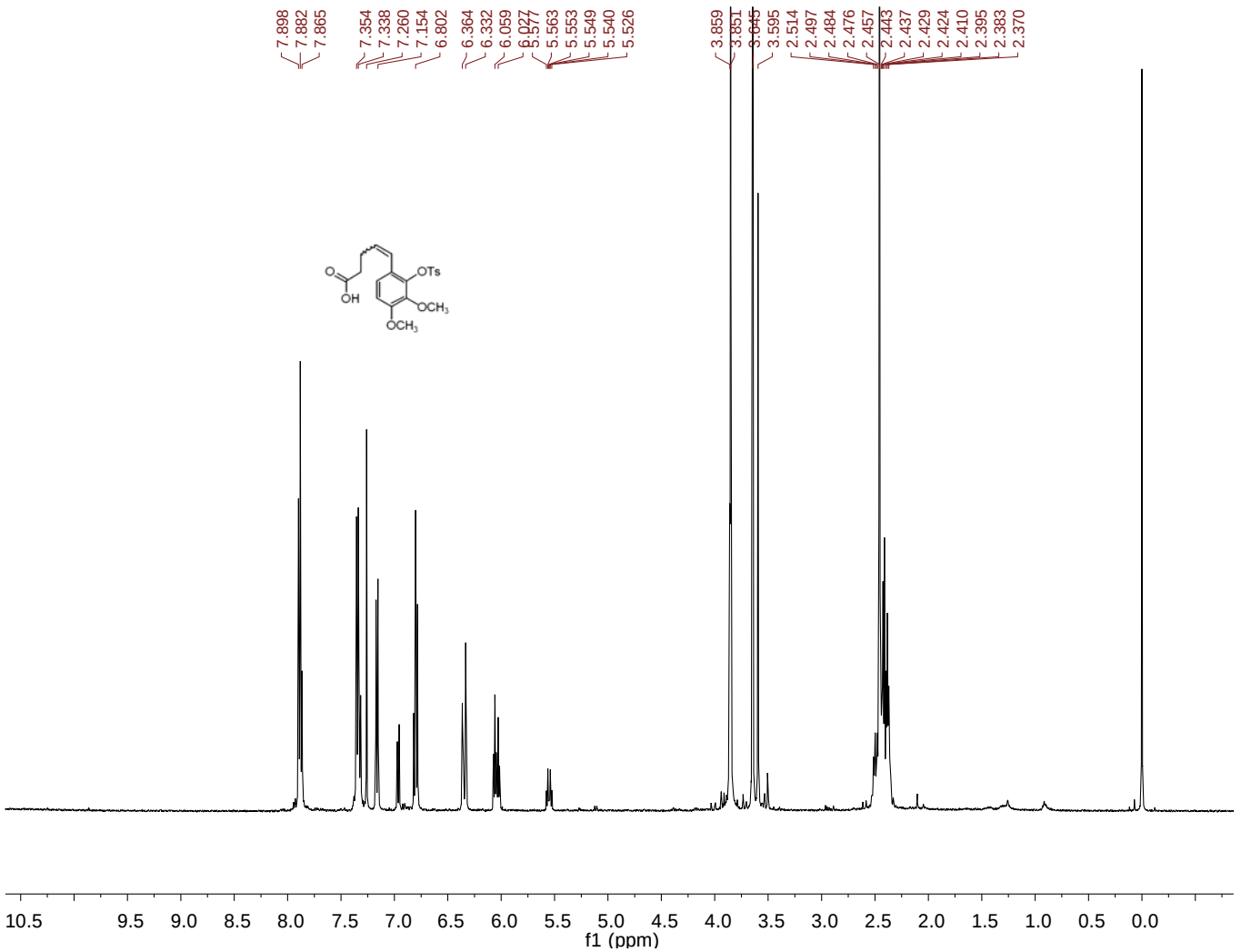

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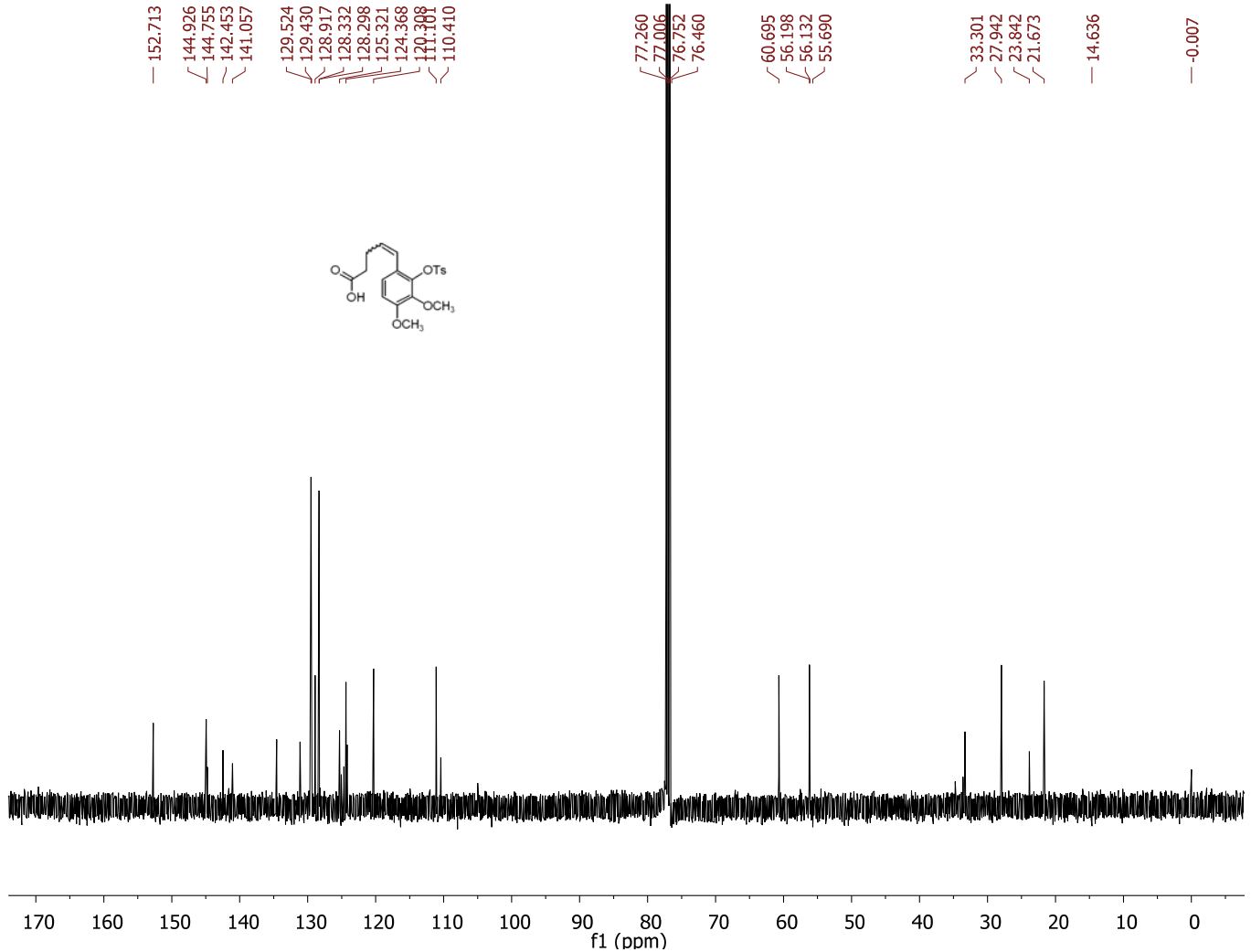
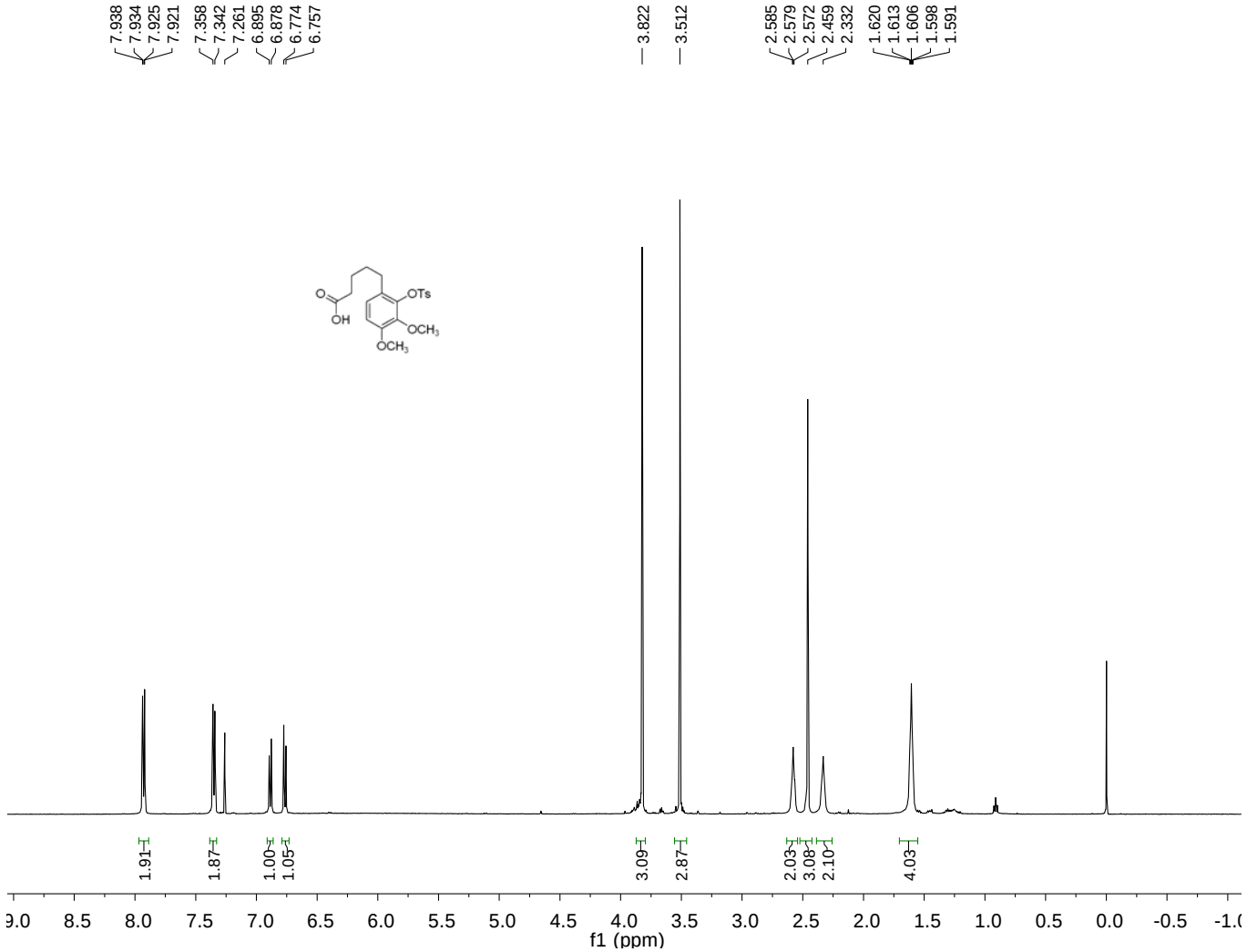


^{1}H NMR (500 MHz, $CDCl_3$) for Compound **8**



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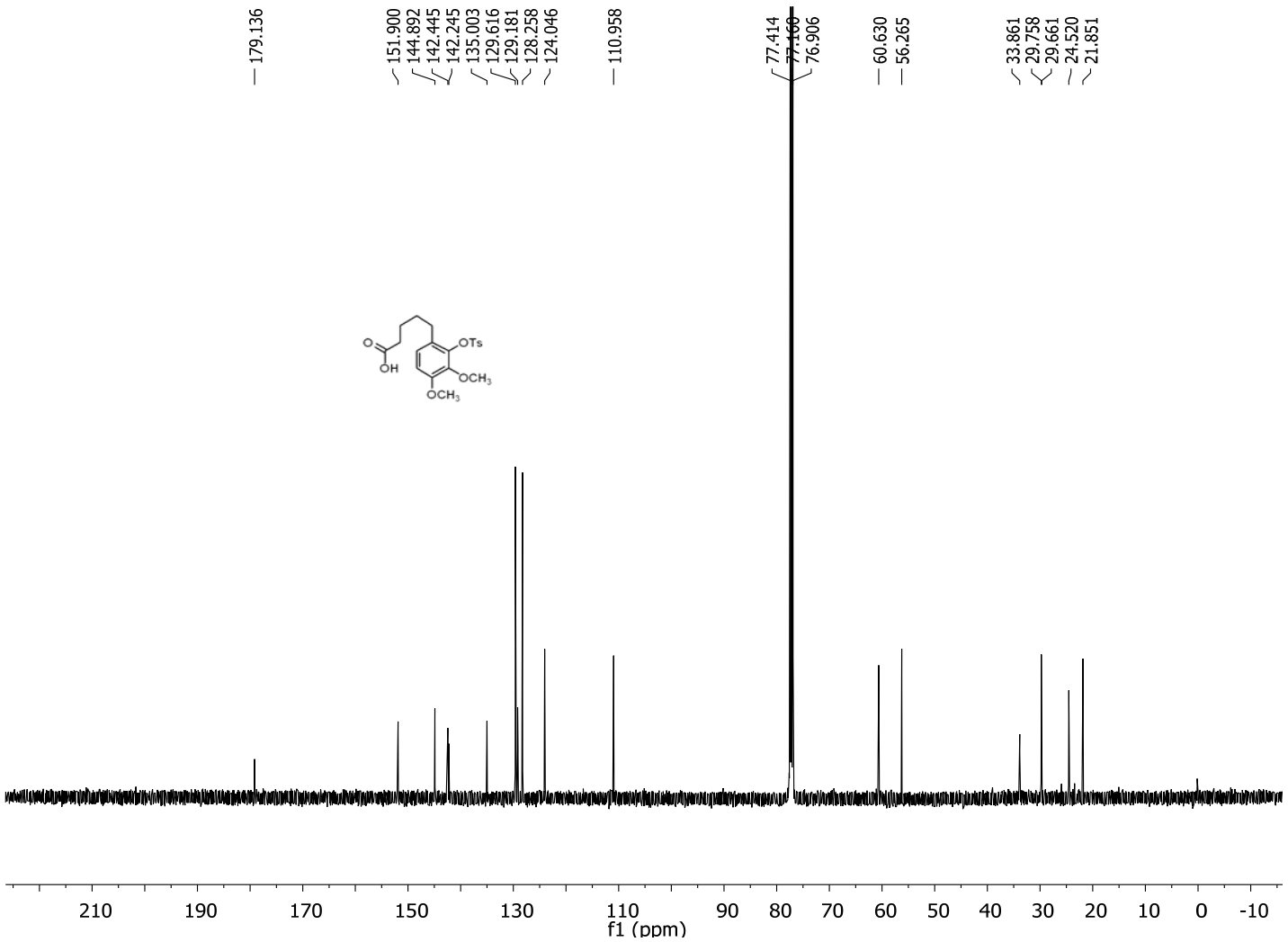

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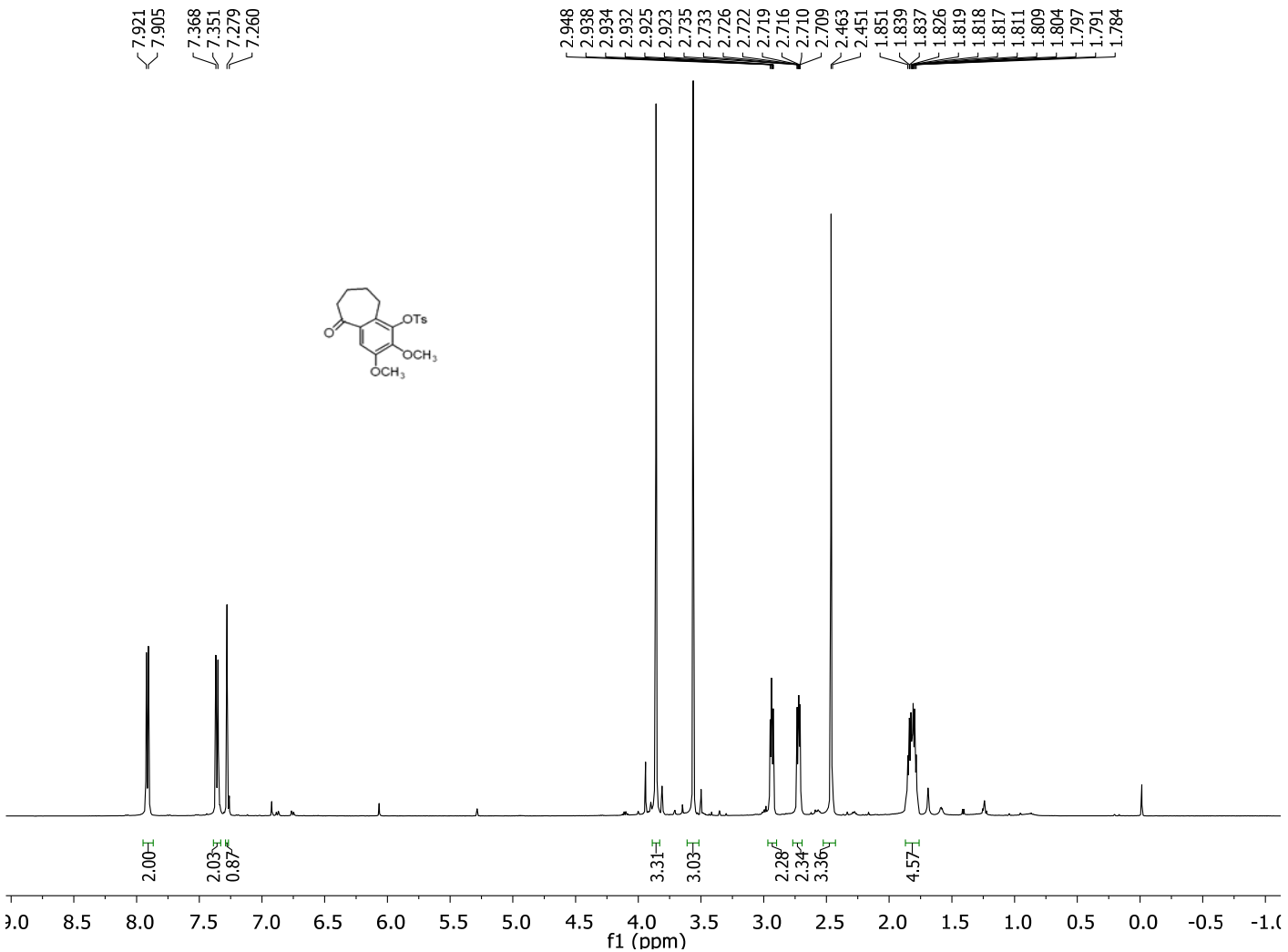

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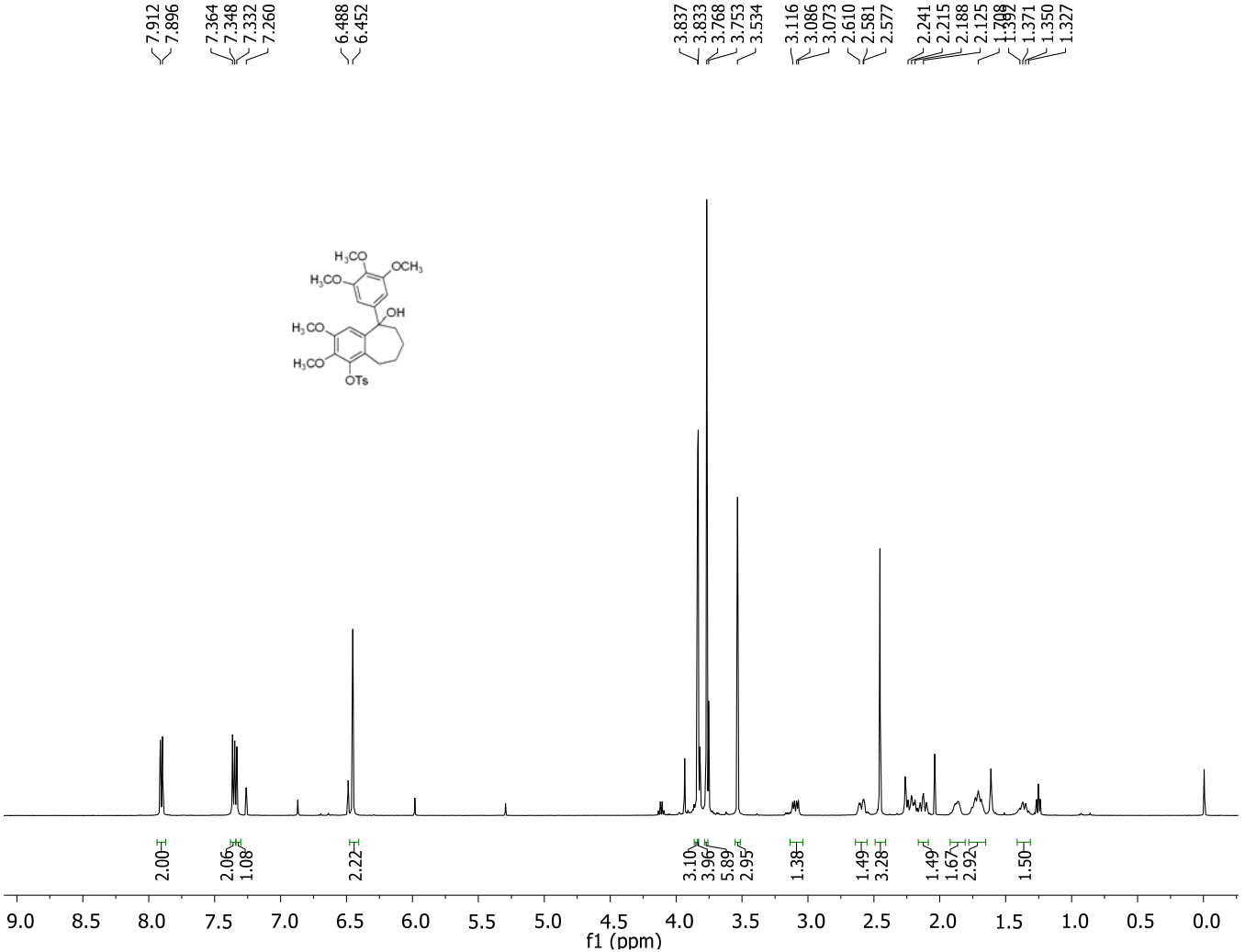
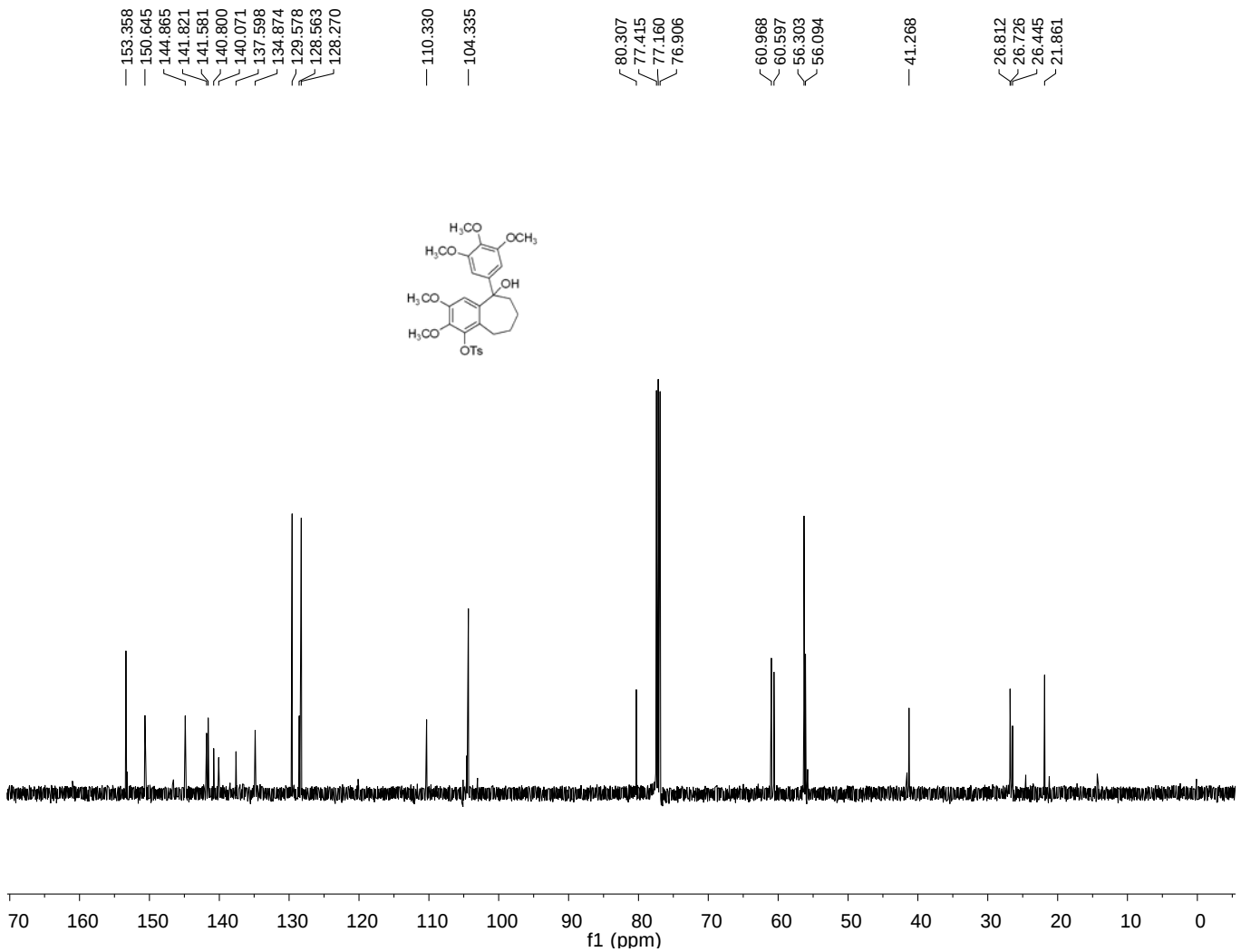


^{13}C NMR (126 MHz, $CDCl_3$) for Compound **20**



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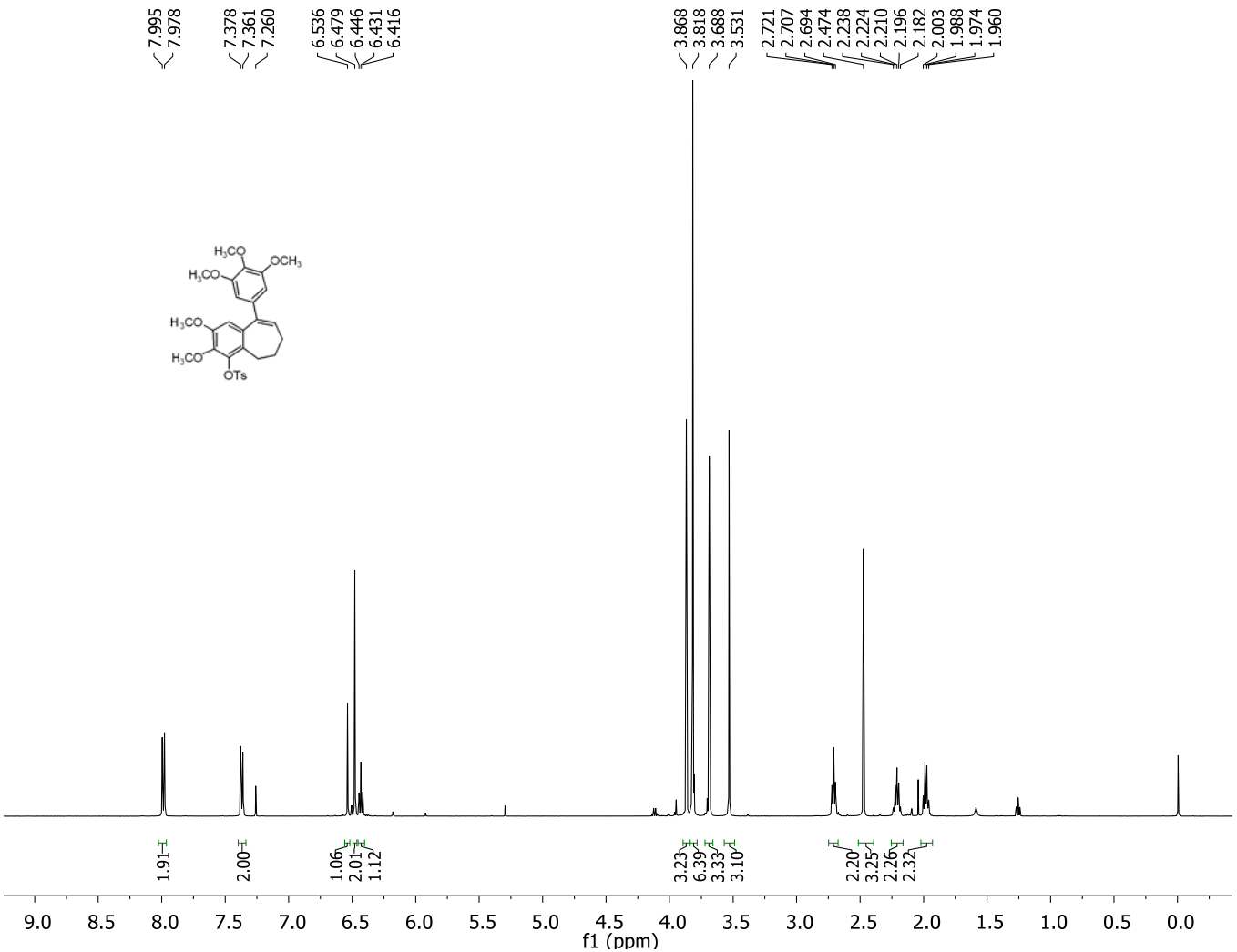

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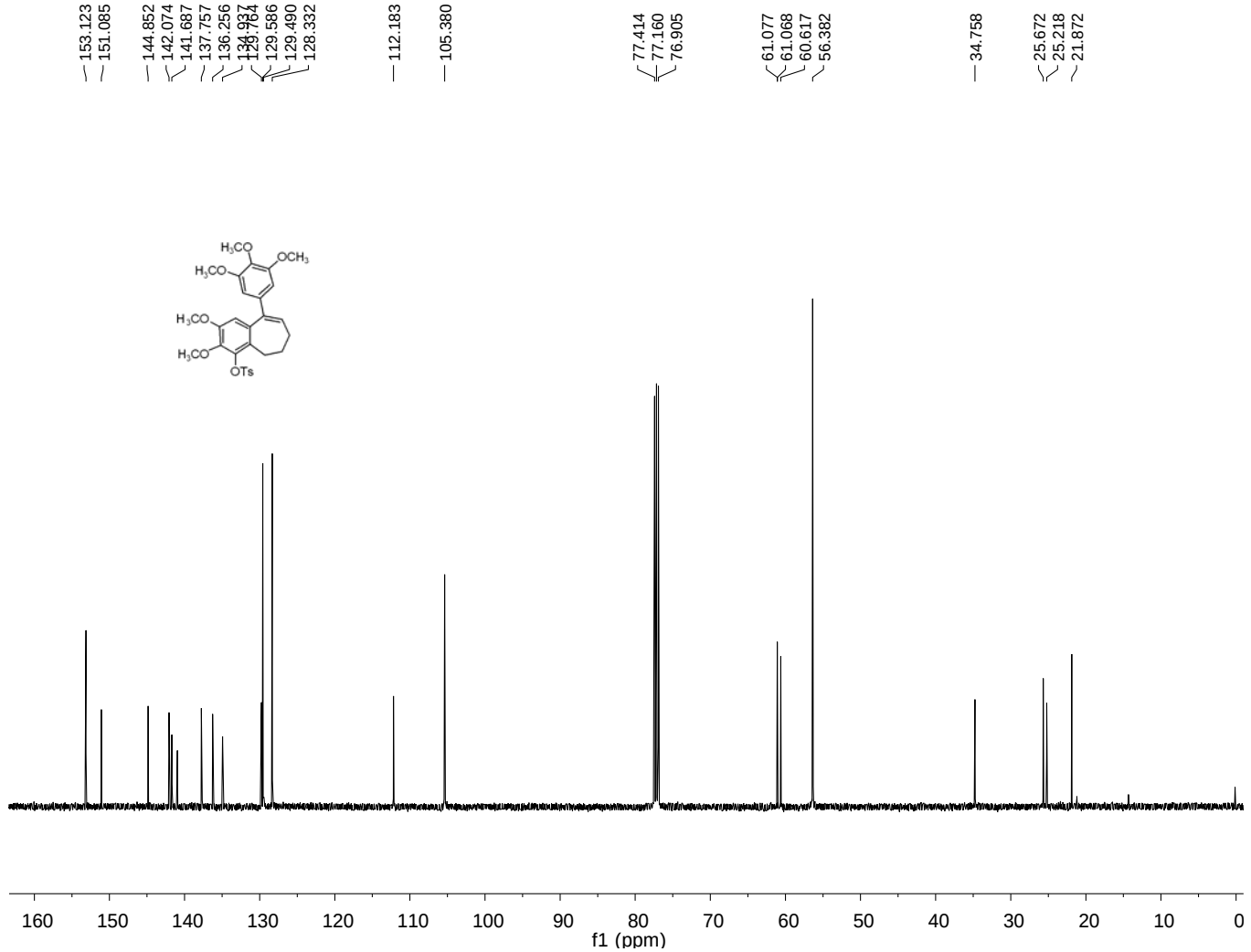

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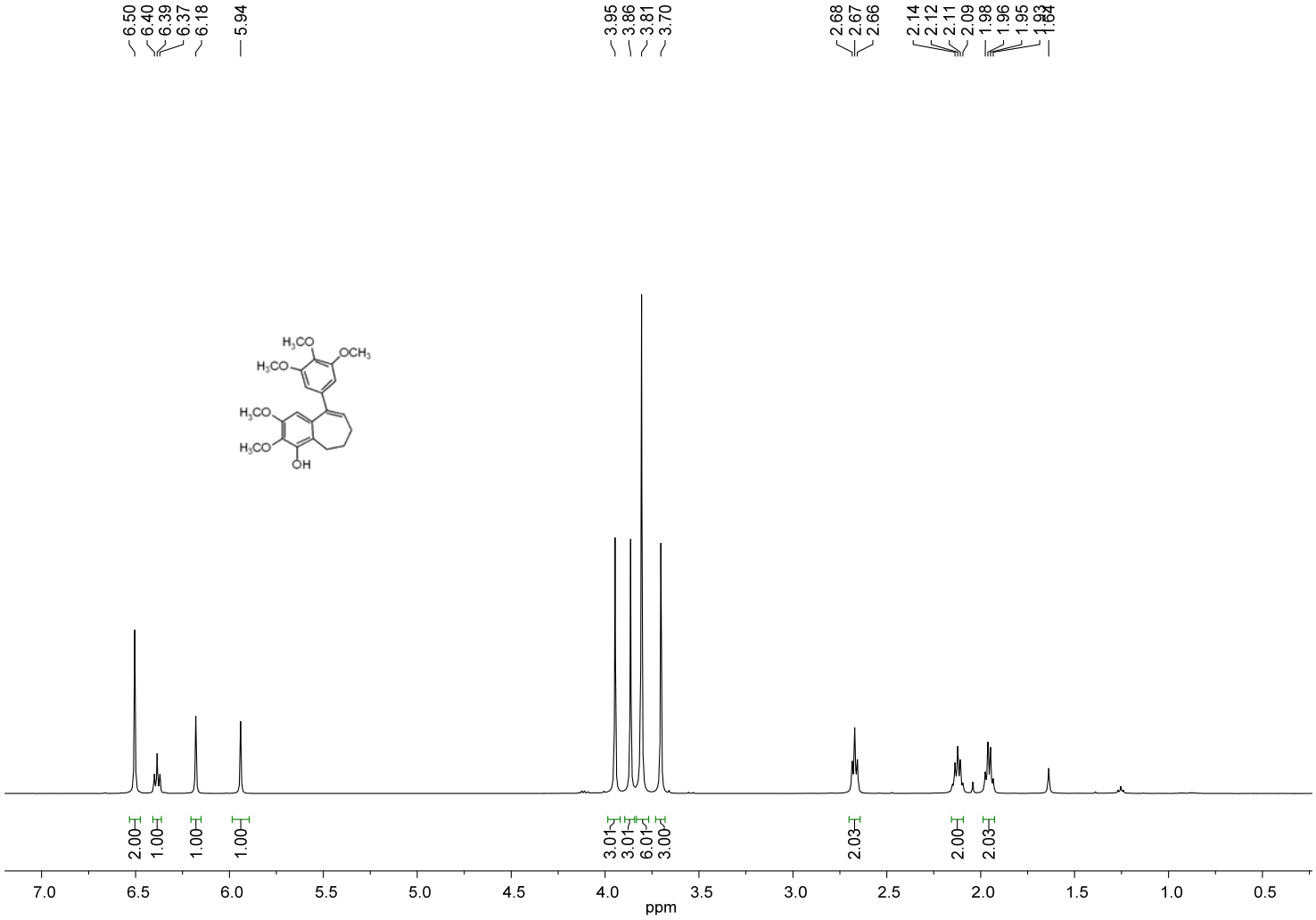

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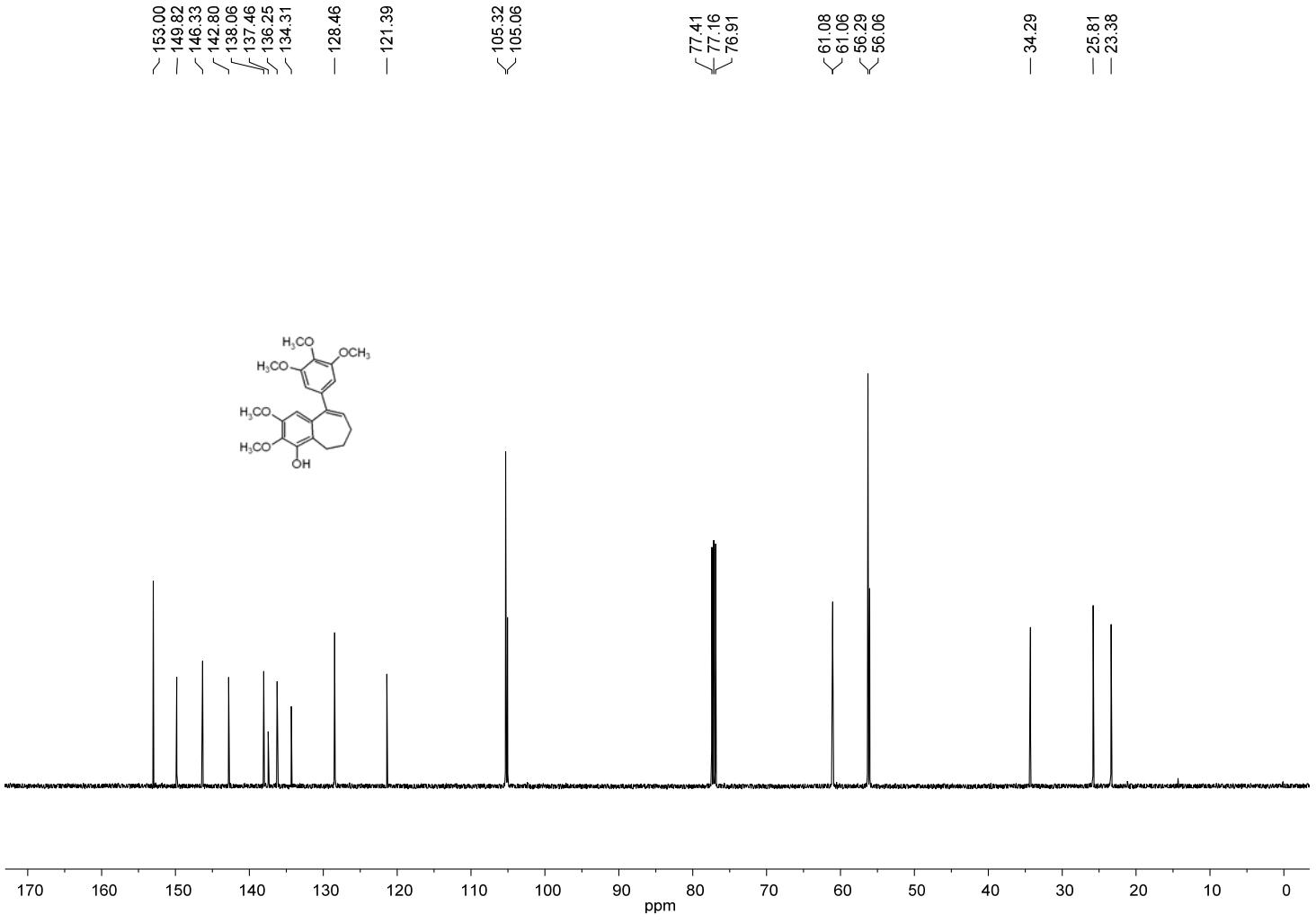

HPLC Traces of Compound **32**

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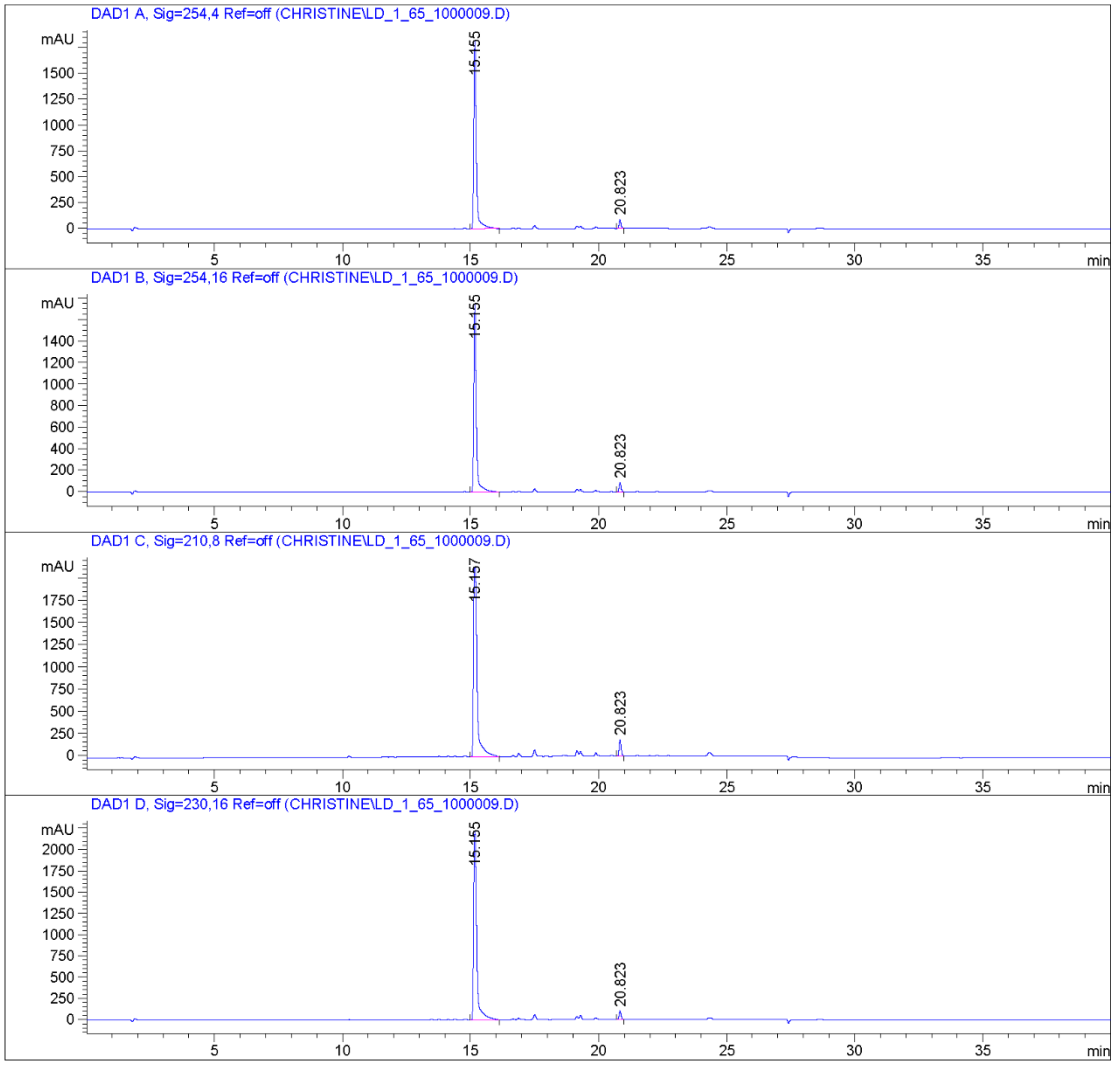

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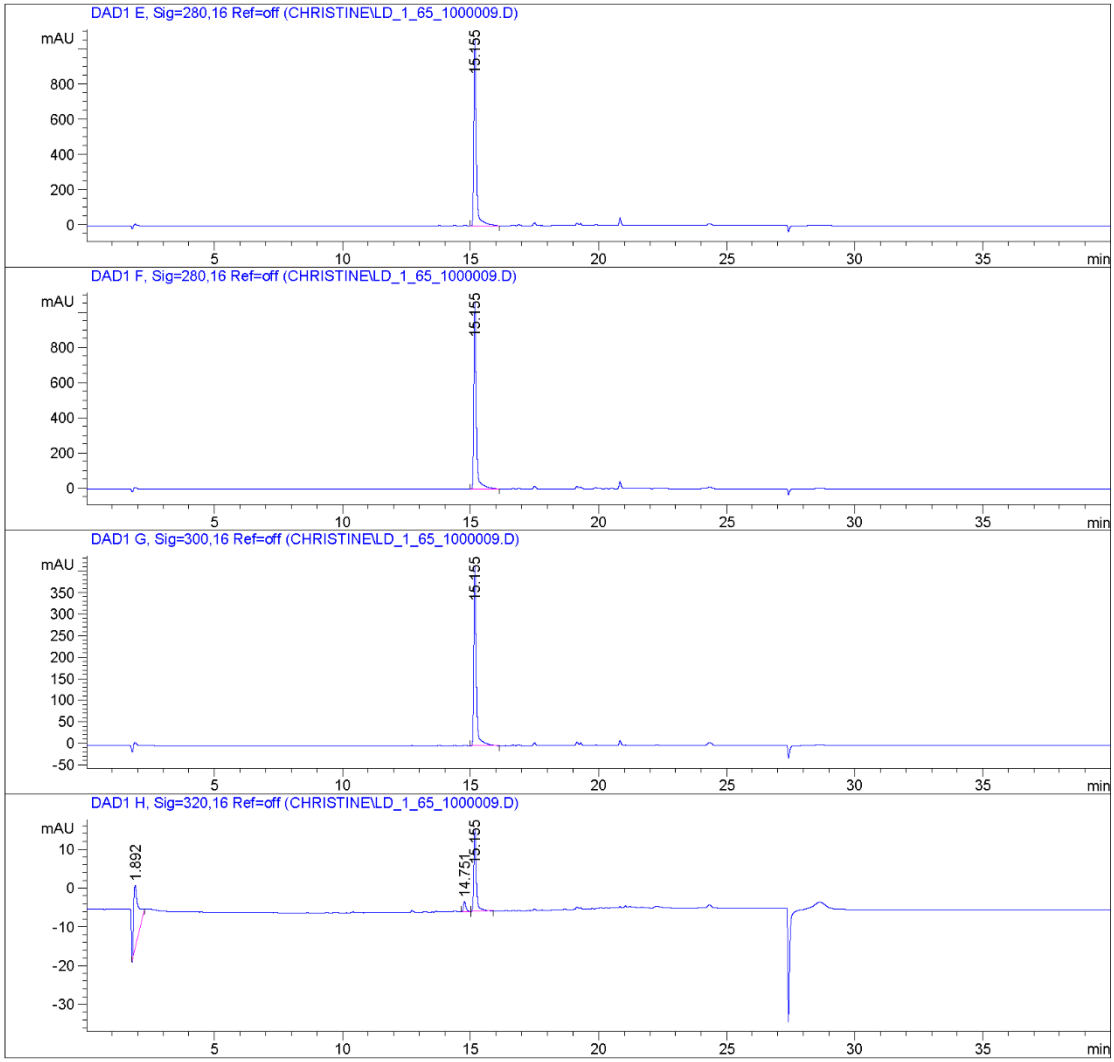

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Area Percent Report

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Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Data File C:\CHEM32\1\DATA\CHRISTINE\LD_1_65_1000009.D
Sample Name: run1

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.155	BB	0.1035	1.27558e4	1826.01721	96.6533
2	20.823	BB	0.0802	441.68518	85.54213	3.3467
Totals :				1.31975e4	1911.55934	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.155	BB	0.1029	1.21415e4	1751.75439	96.5072
2	20.823	BB	0.0782	439.43024	85.11578	3.4928
Totals :				1.25809e4	1836.87017	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.157	BV	0.1538	2.12365e4	2140.21191	95.6725
2	20.823	BV	0.0805	960.57239	185.28012	4.3275
Totals :				2.21971e4	2325.49203	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.155	BV	0.1315	1.89474e4	2221.88965	97.3141
2	20.823	BB	0.0802	522.95447	101.36359	2.6859
Totals :				1.94703e4	2323.25323	

Data File C:\CHEM32\1\DATA\CHRISTINE\LD_1_65_1000009.D
Sample Name: run1

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.155	BB	0.1009	7177.51318	1061.98120	100.0000
Totals :				7177.51318	1061.98120	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.155	BB	0.1009	7177.51318	1061.98120	100.0000
Totals :				7177.51318	1061.98120	

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

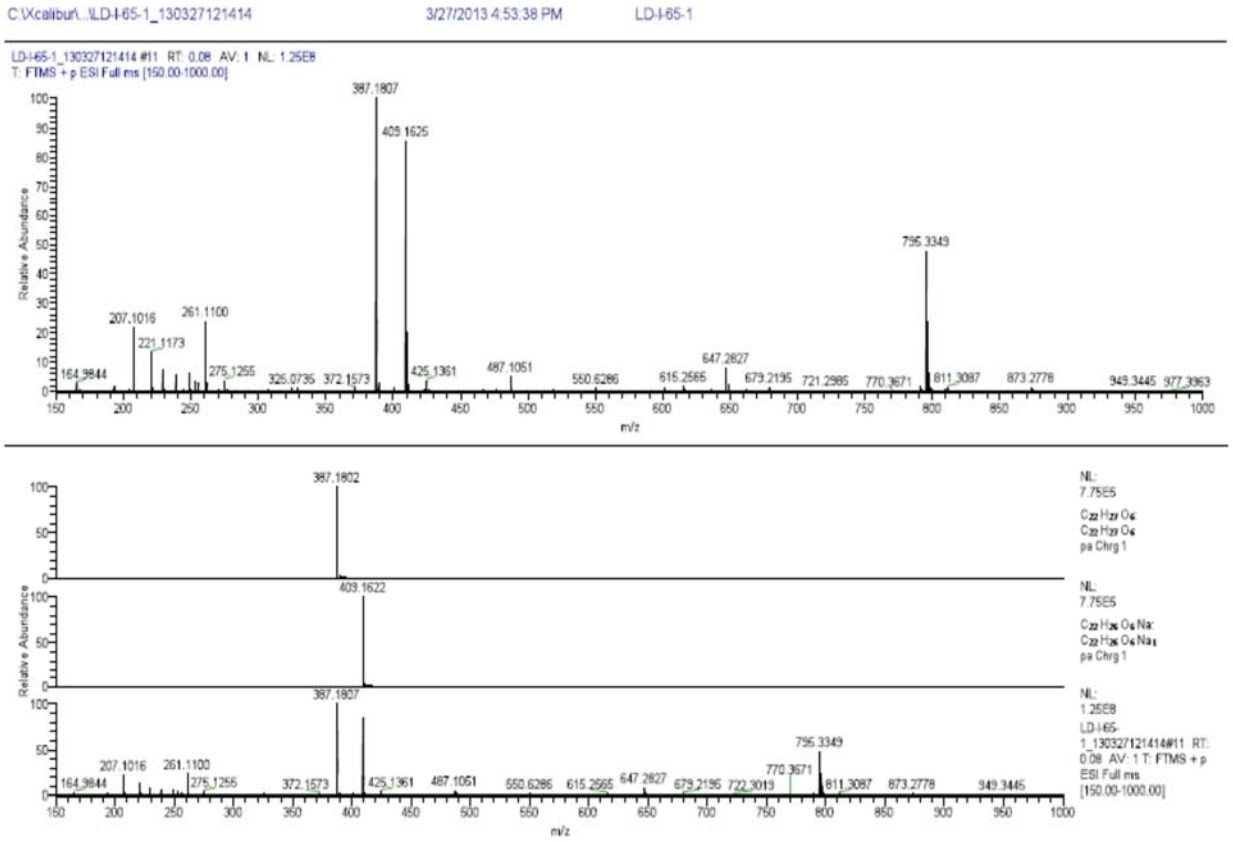
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	15.155	BB	0.1006	2817.50952	418.16830	100.0000
Totals :				2817.50952	418.16830	

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

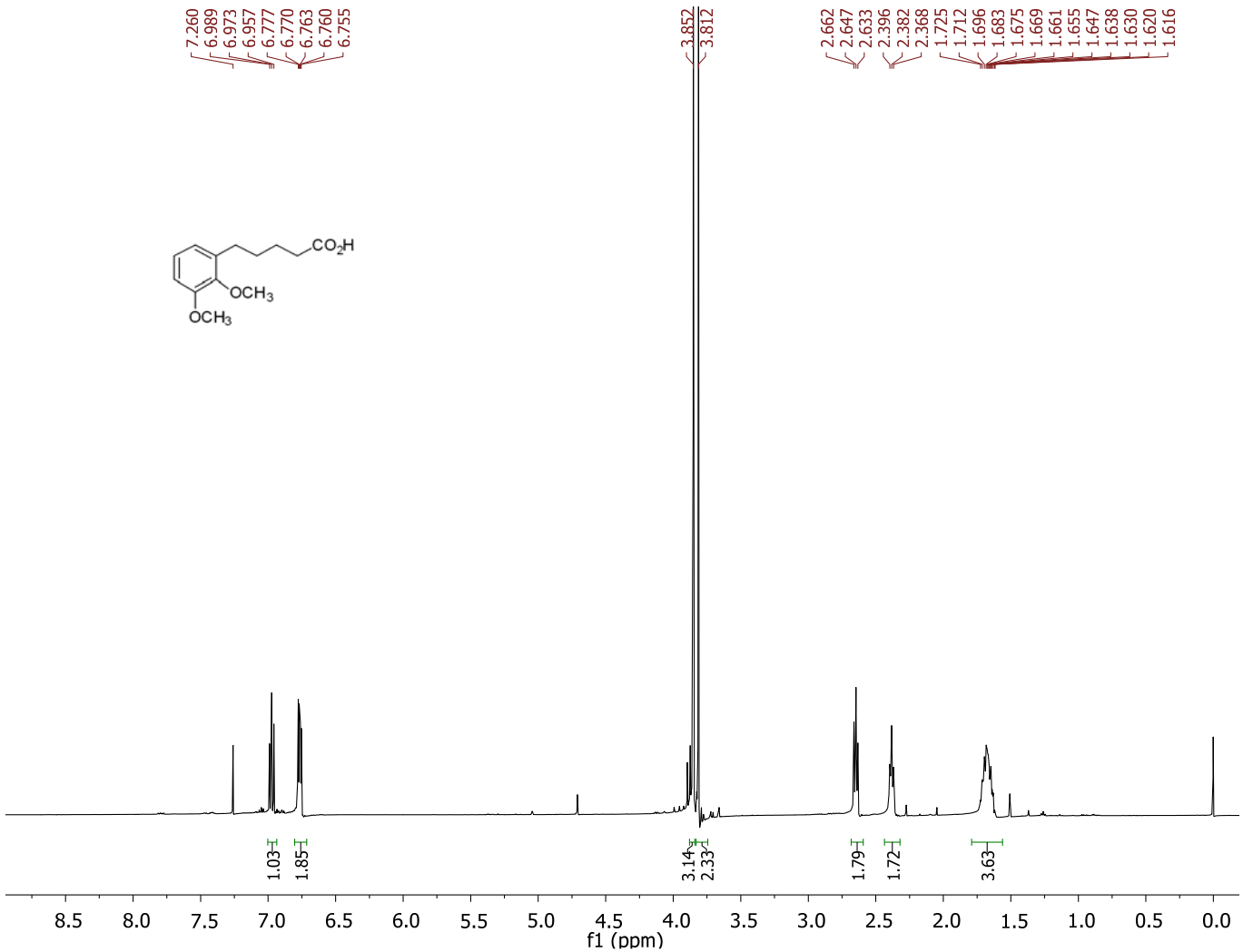
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.892	BB	0.1832	212.84036	16.64317	57.0213
2	14.751	BB	0.0976	16.61544	2.56333	4.4514
3	15.155	BB	0.1010	143.80884	21.22739	38.5273
Totals :				373.26464	40.43389	

*** End of Report ***

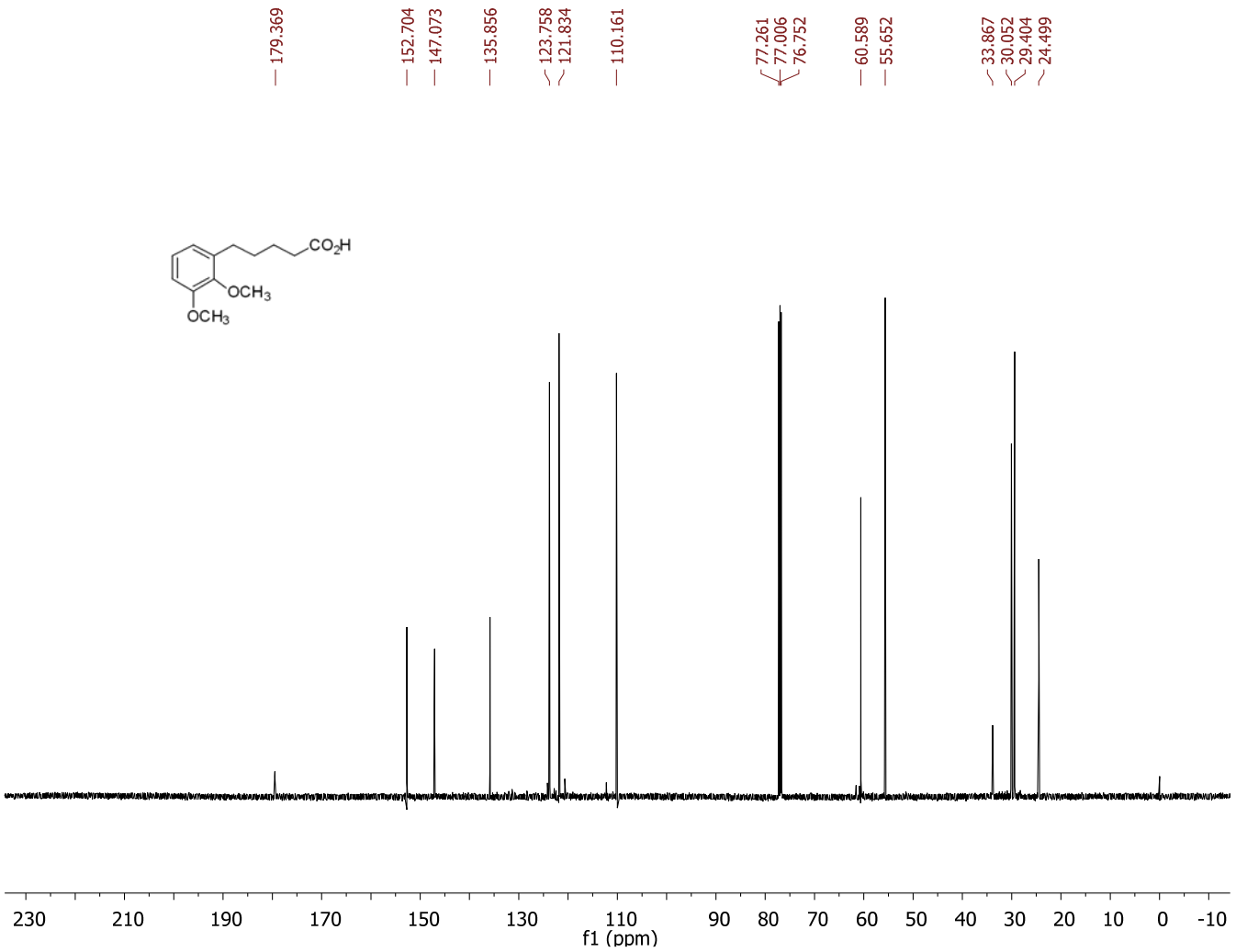
HRMS Trace for Compound **32**



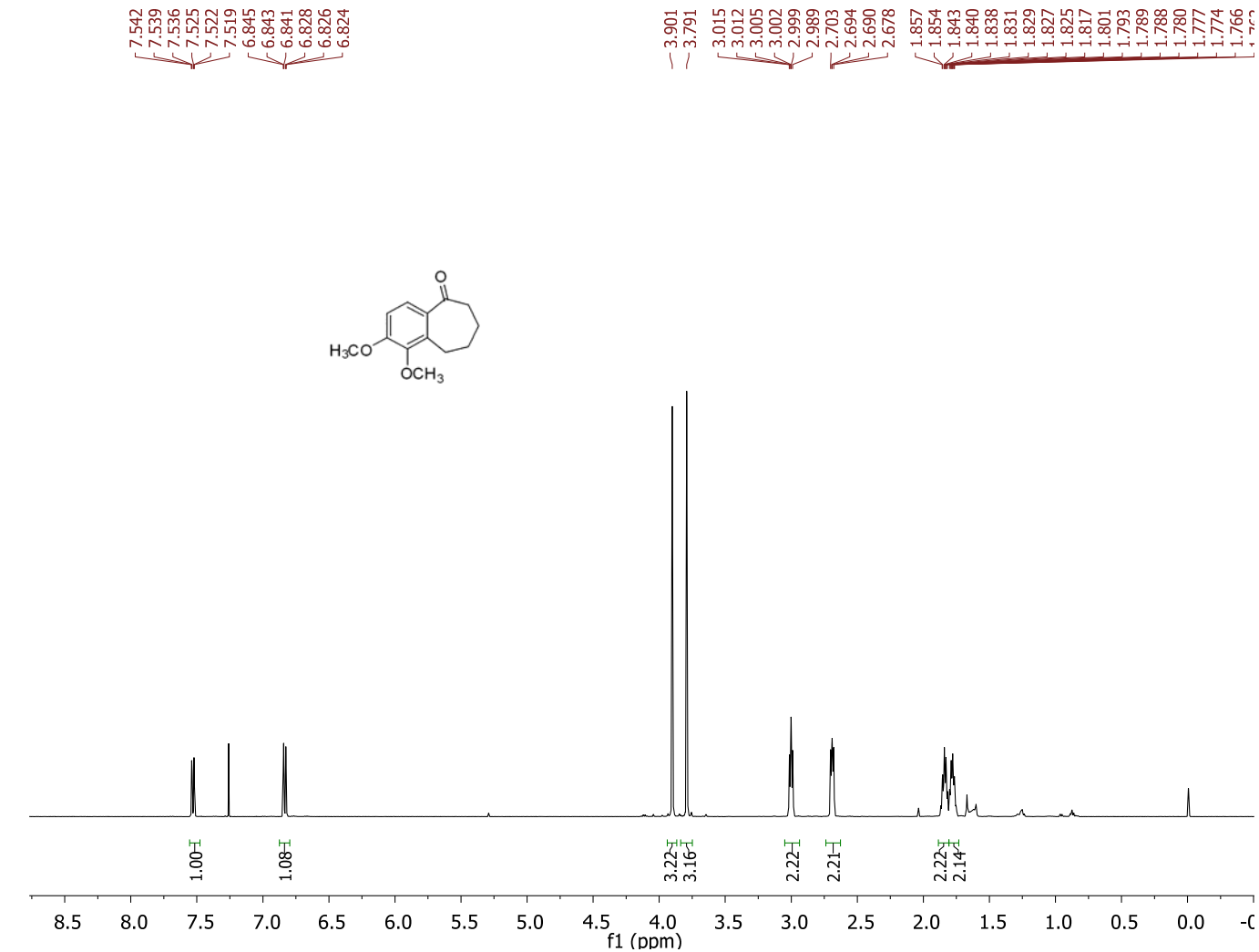
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **9**



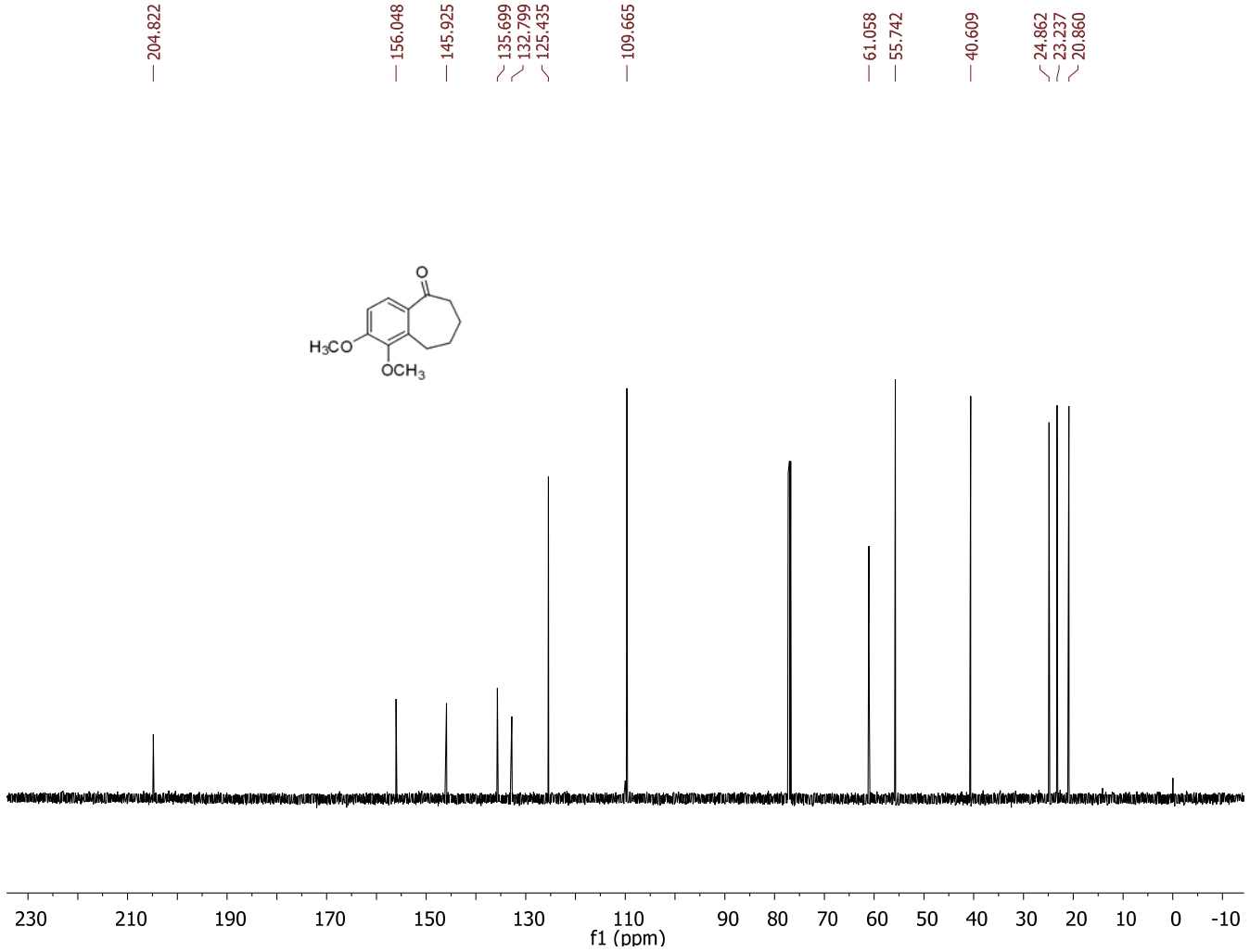
^{13}C NMR (126 MHz, $CDCl_3$) of Compound **9**



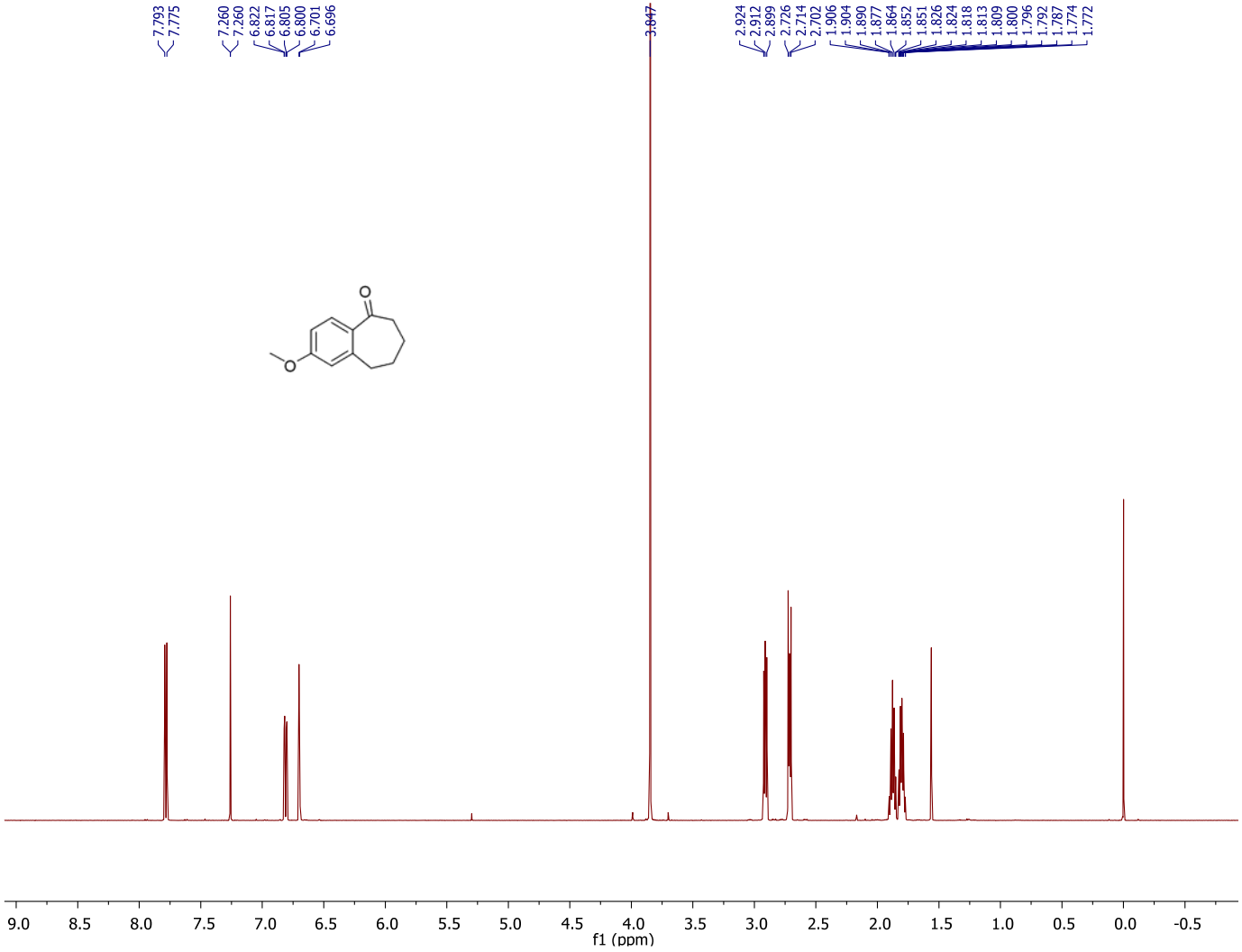
^{1}H NMR (500 MHz, $CDCl_3$,) of Compound **12**



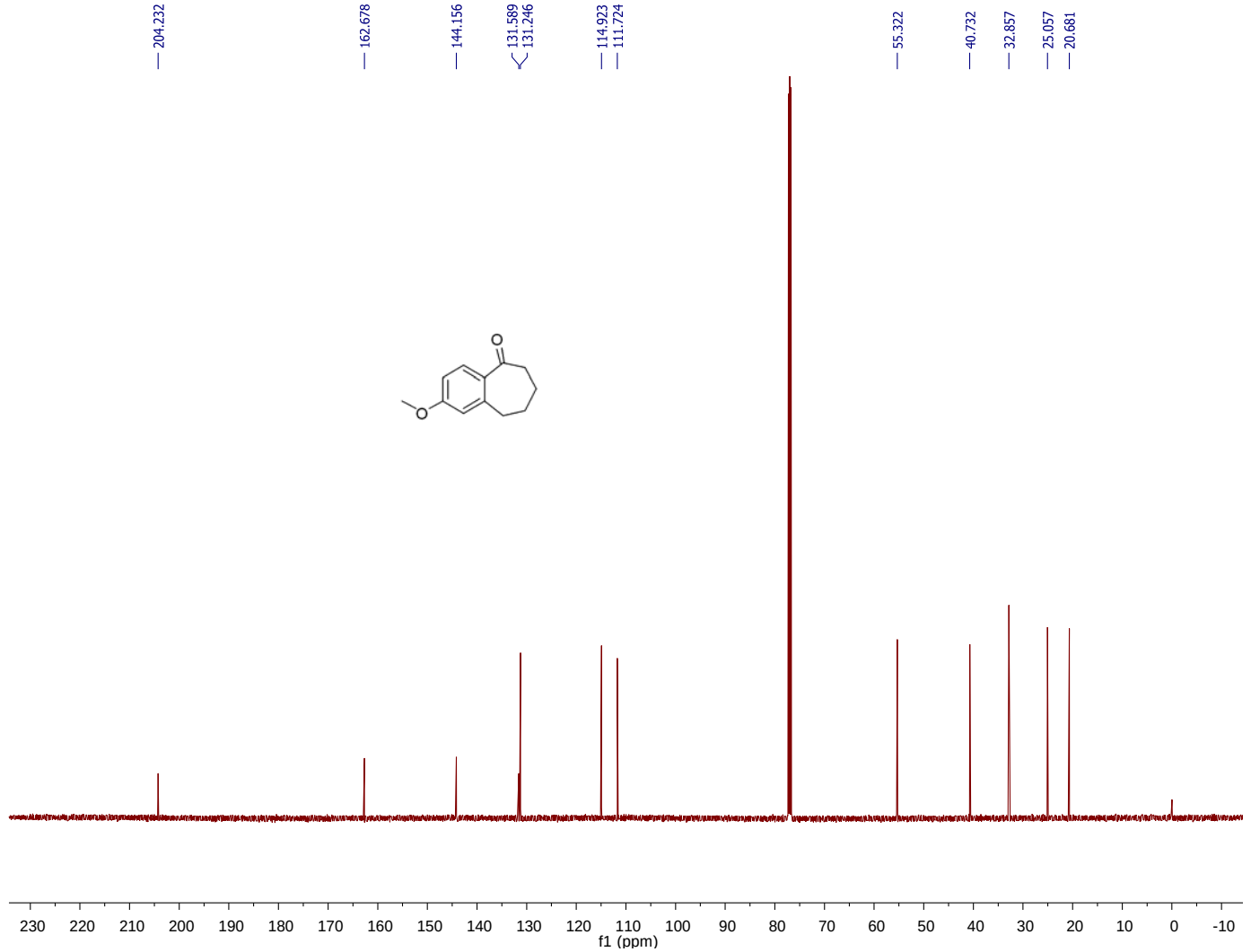
^{13}C NMR (500 MHz, $CDCl_3$,) of Compound **12**



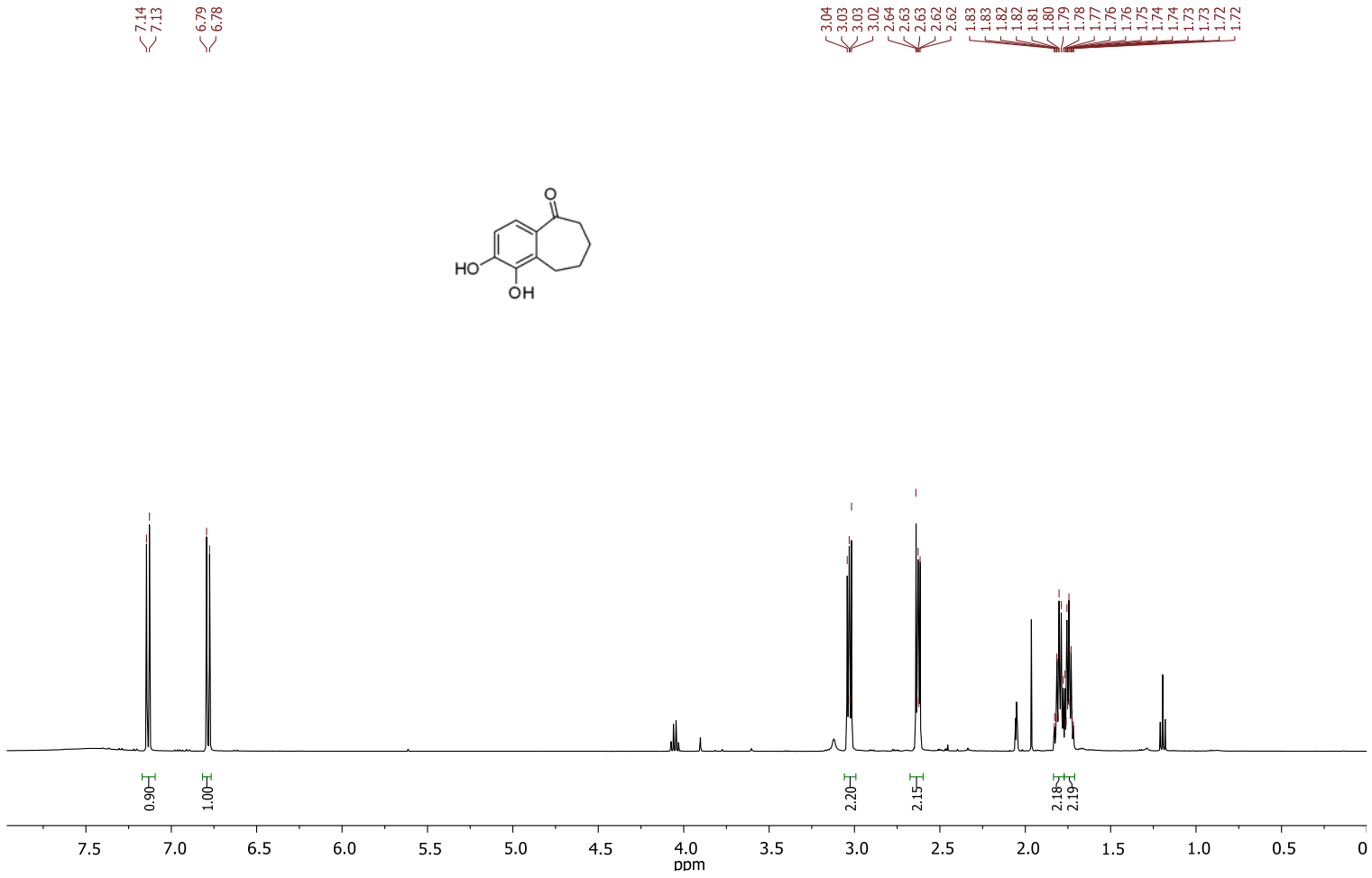
^{1}H NMR (500 MHz, $CDCl_3$,) of Compound **13**



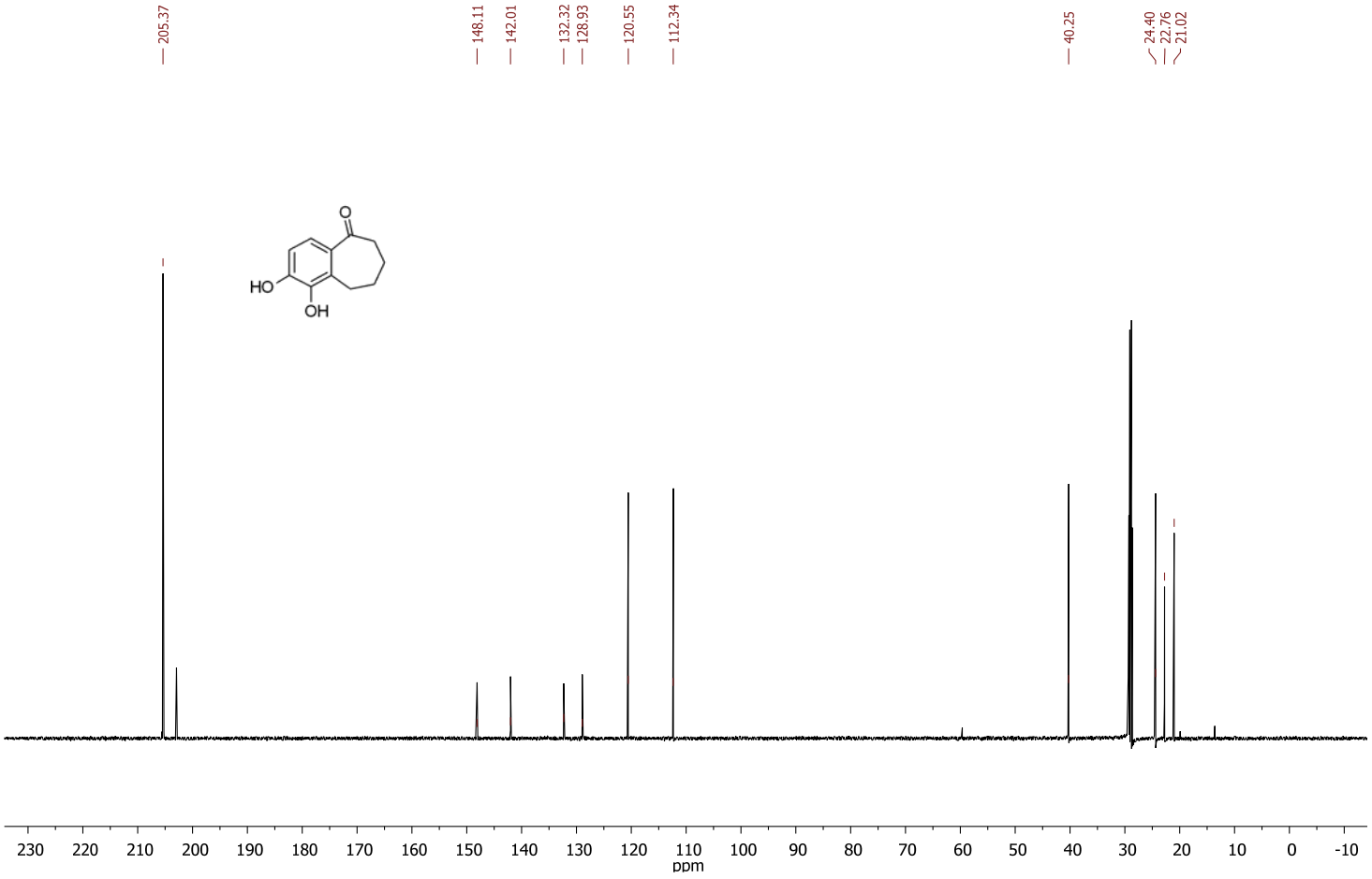
^{13}C NMR (500 MHz, $CDCl_3$,) of Compound **13**



^{1}H NMR (500 MHz, $(CD_3)_2CO$) of Compound **14**

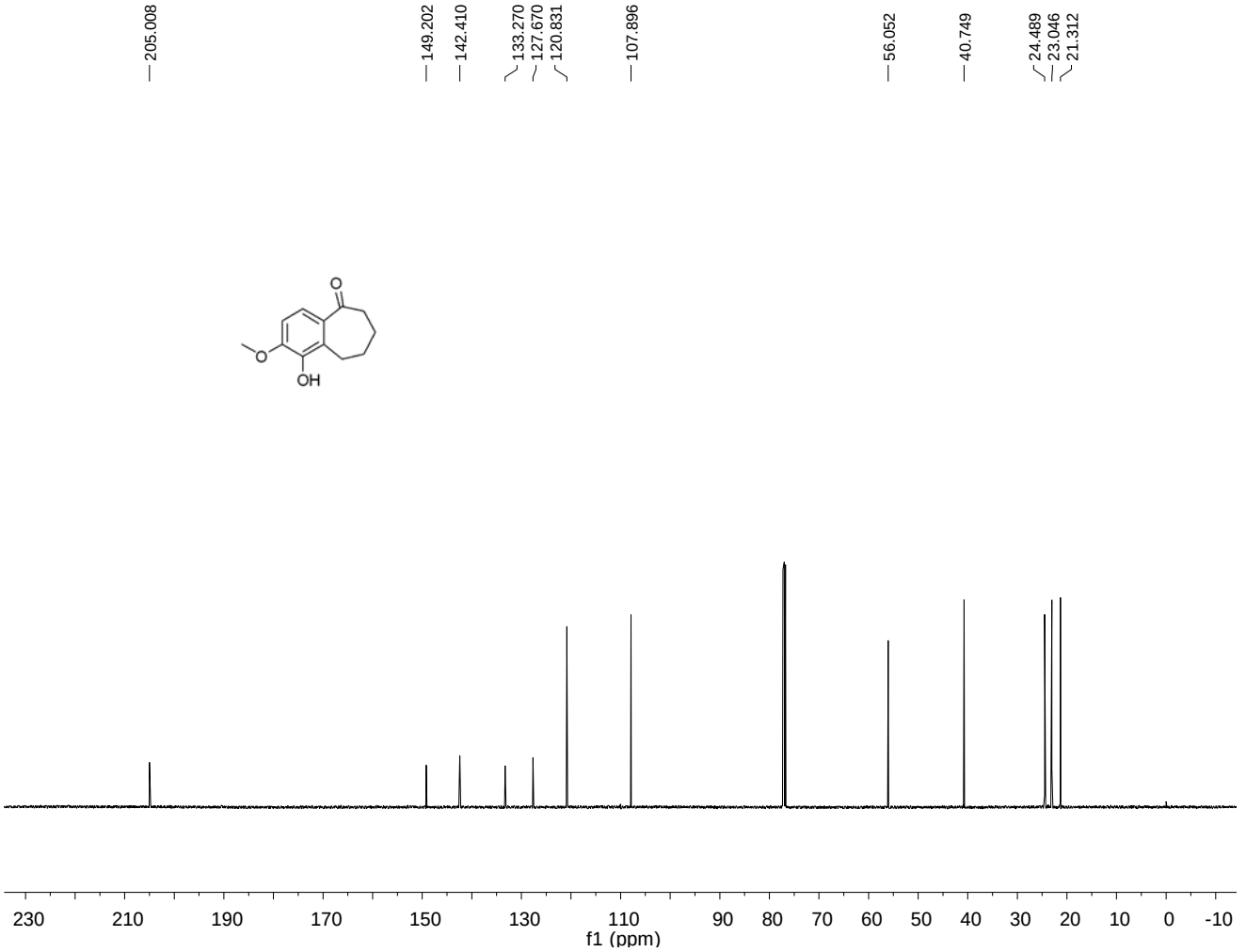


^{13}C NMR (126 MHz, $(CD_3)_2CO$) of Compound **14**

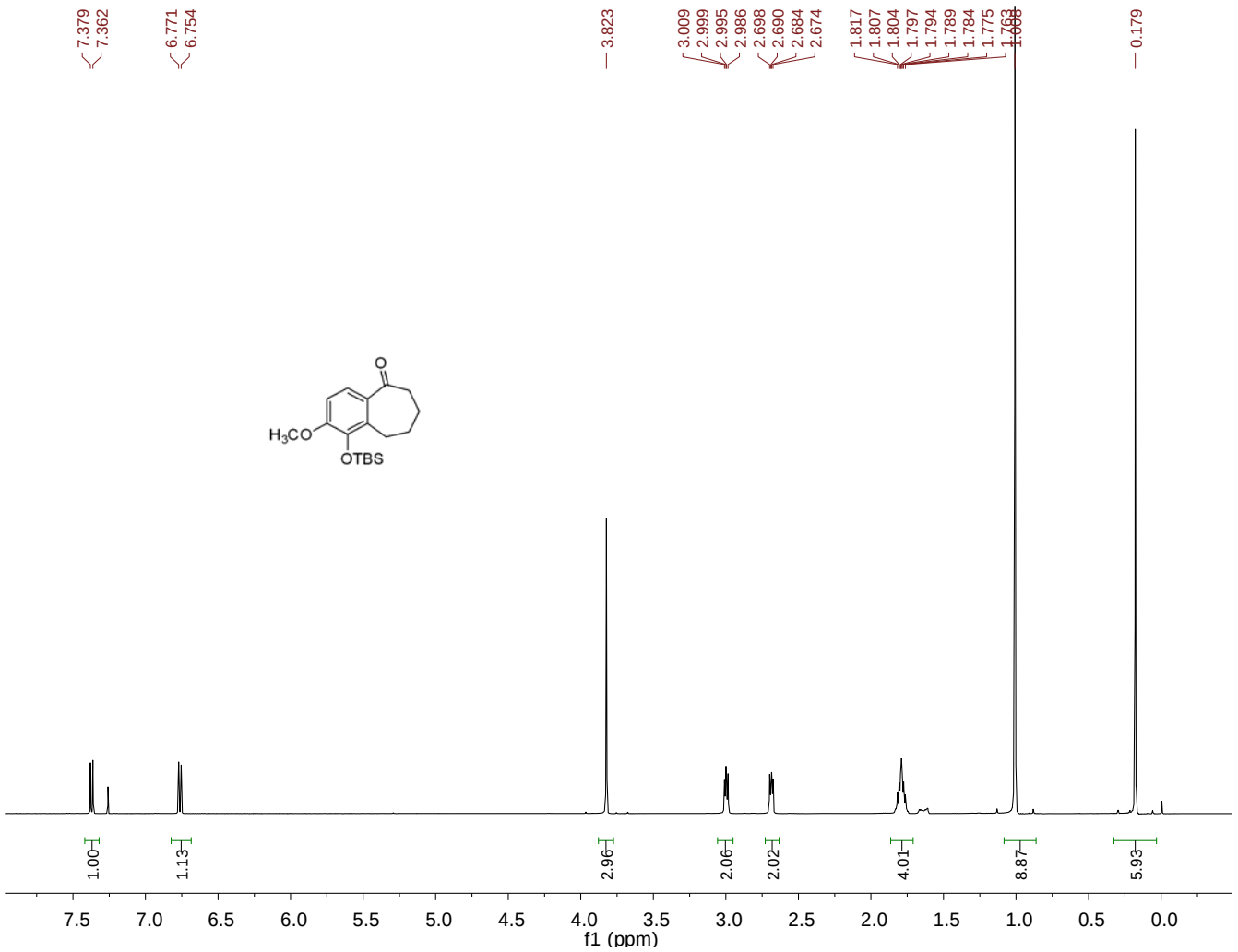


^{1}H NMR (500 MHz, $(CD_3)_2CO$) of Compound **15**

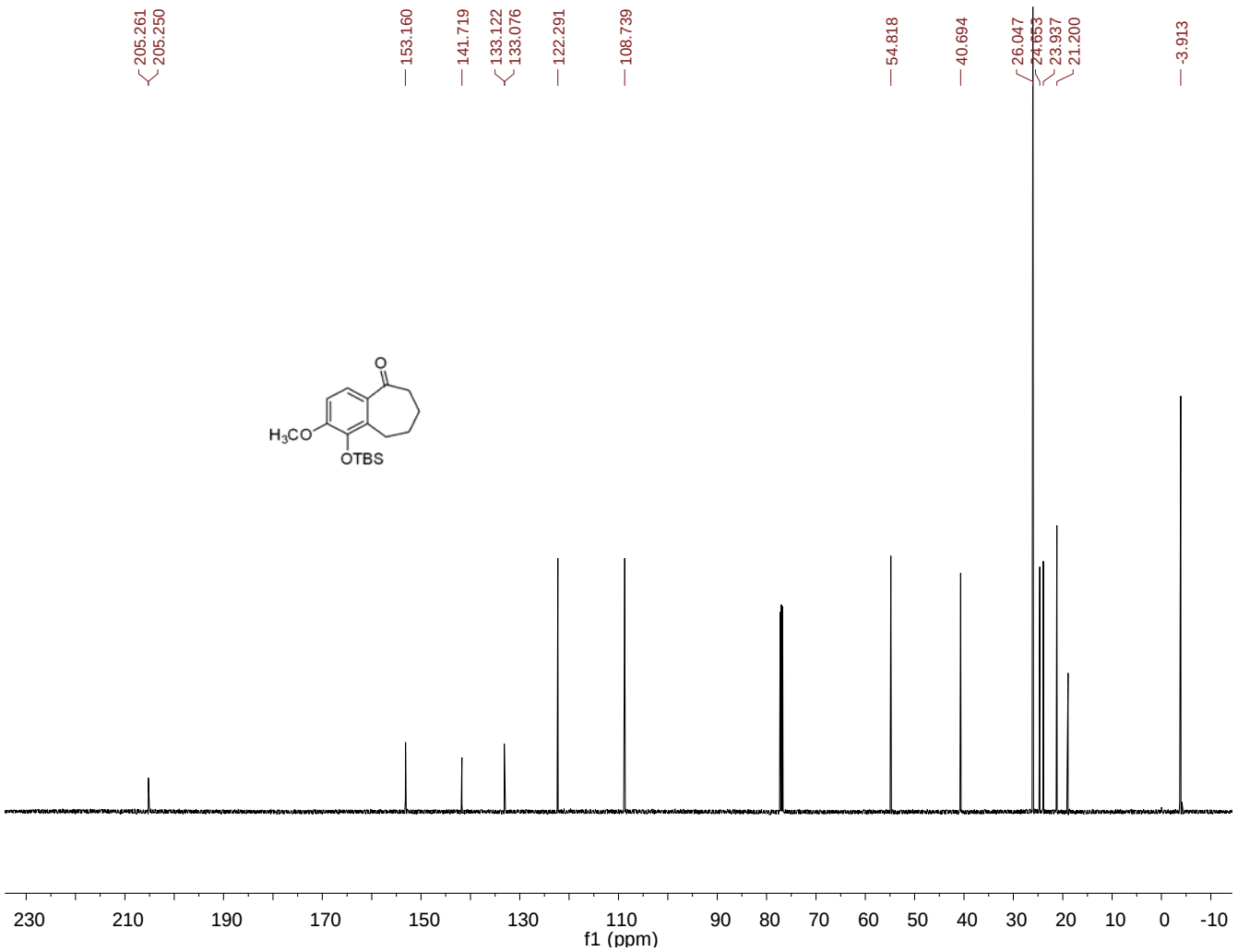
^{13}C NMR (126 MHz, $(CD_3)_2CO$) of Compound **15**



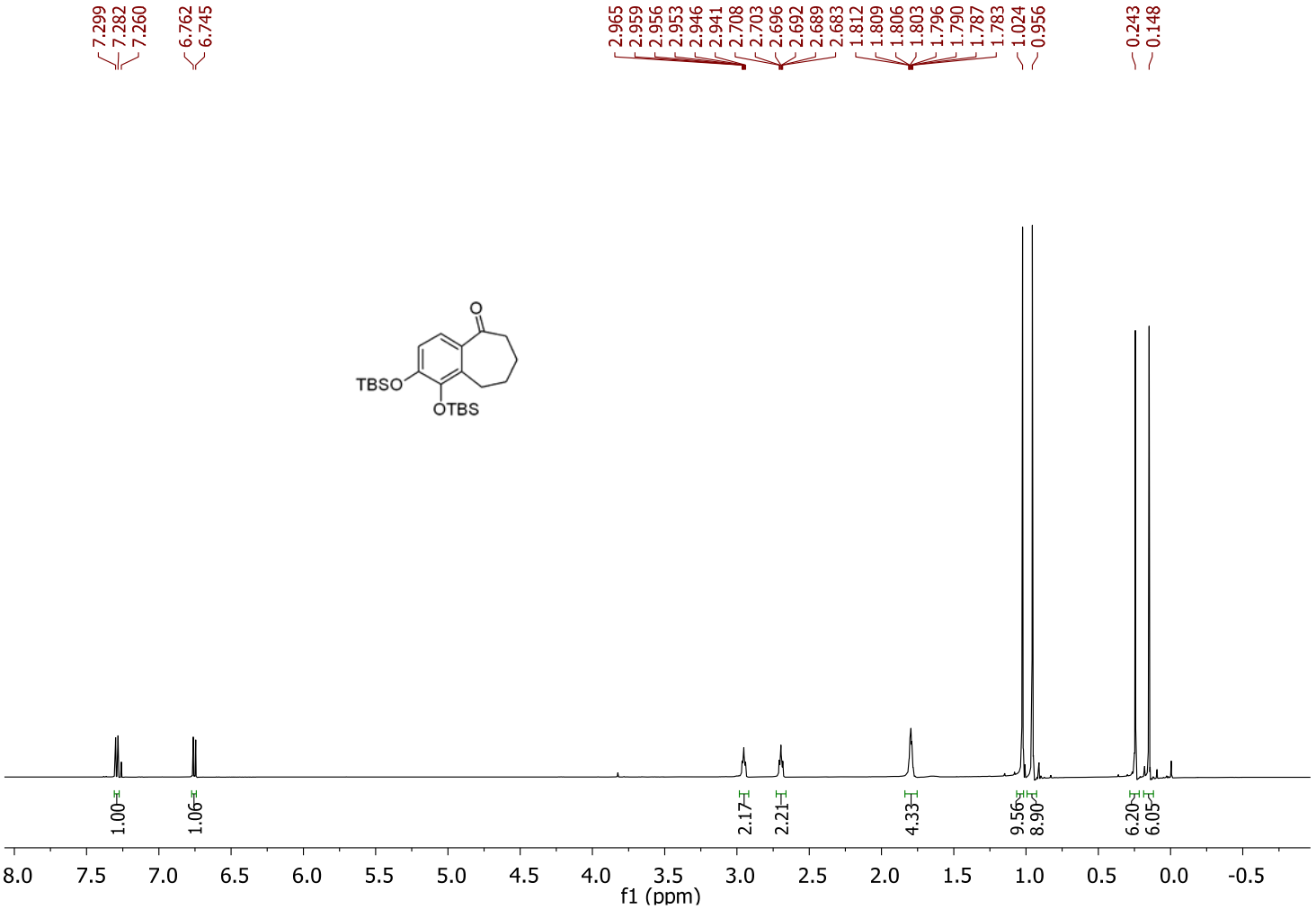
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **17**



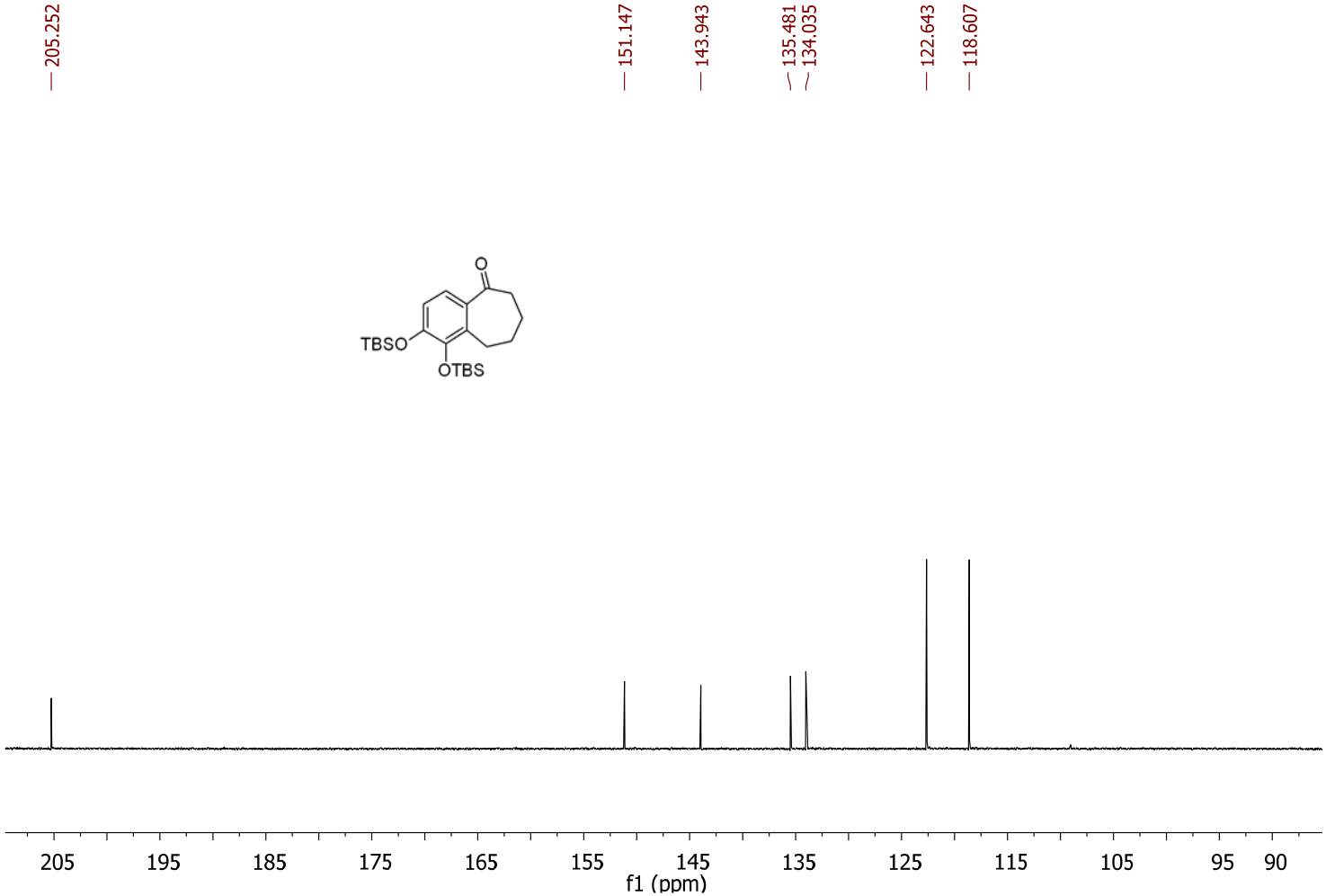
^{13}C NMR (126 MHz, $CDCl_3$) of Compound **17**



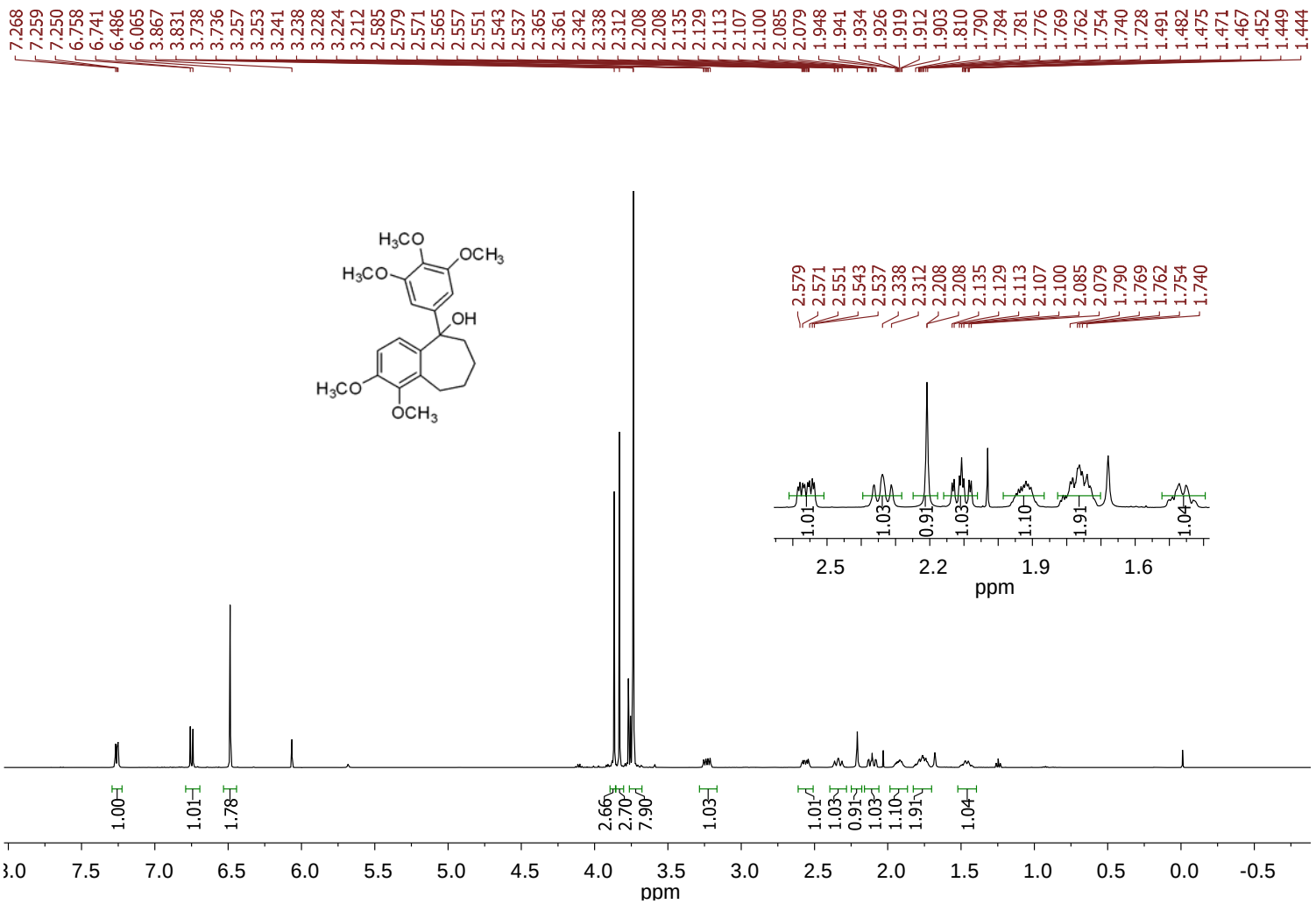
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **16**



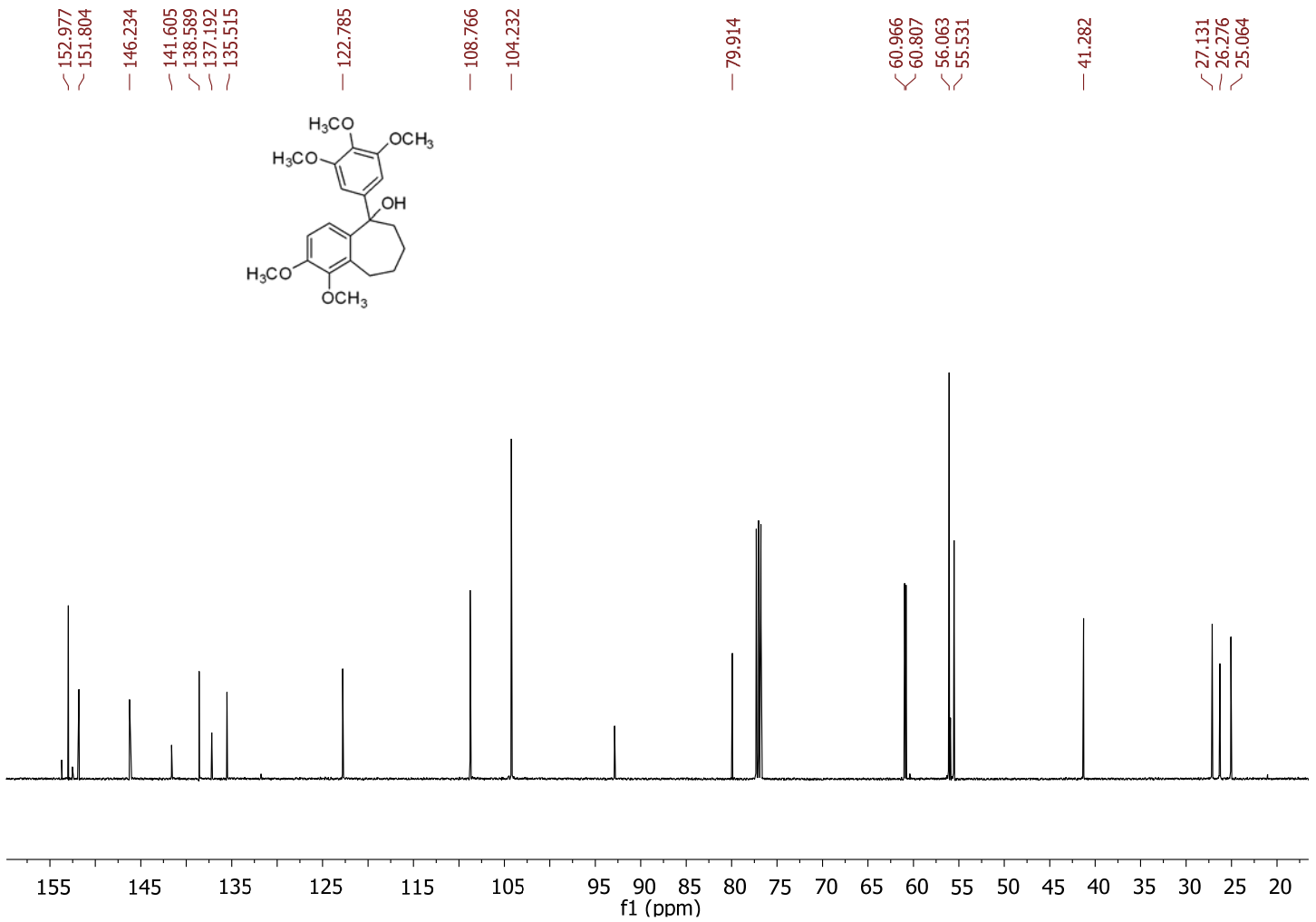
^{13}C NMR (126 MHz, $CDCl_3$) of Compound **16**



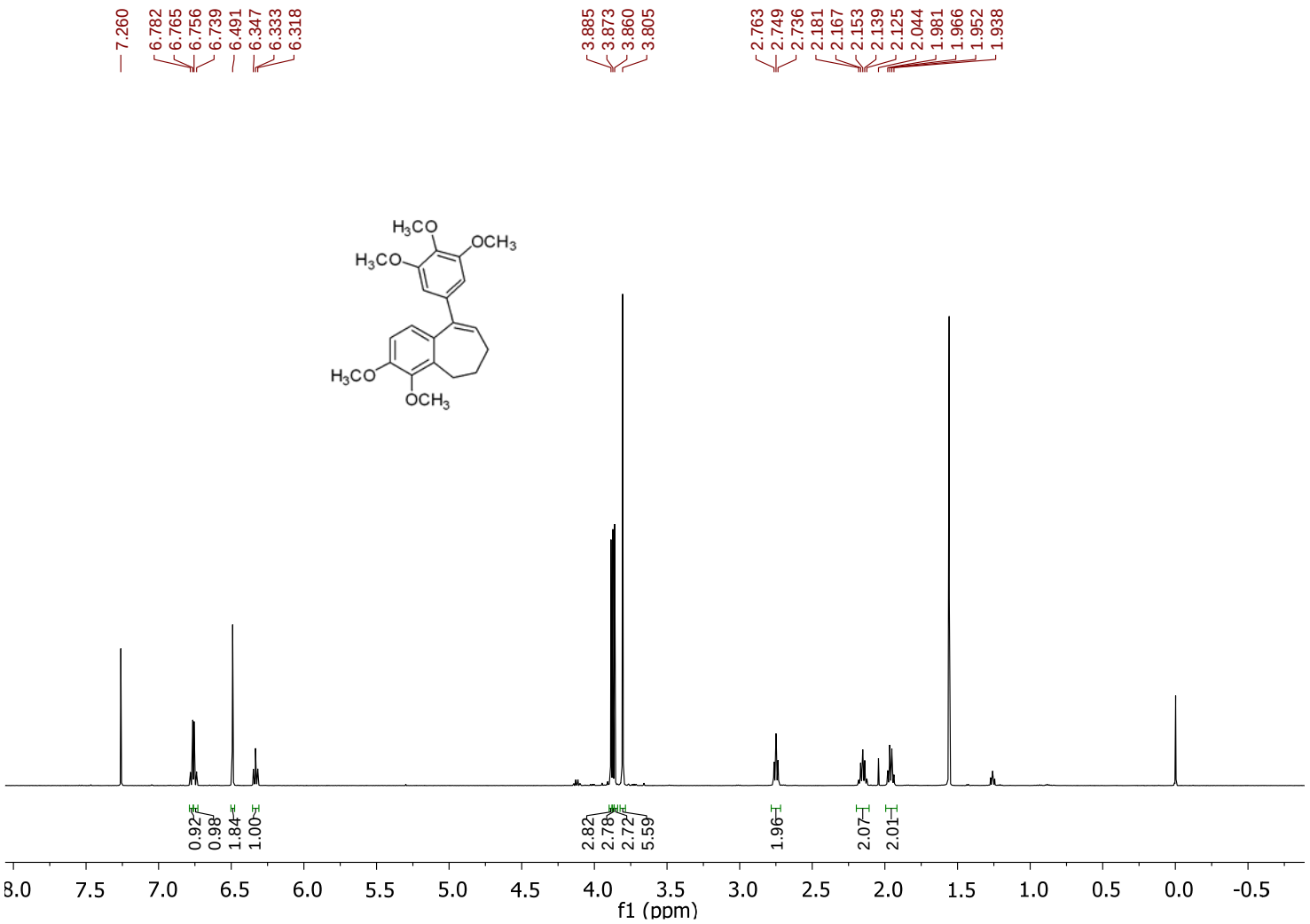
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **21**



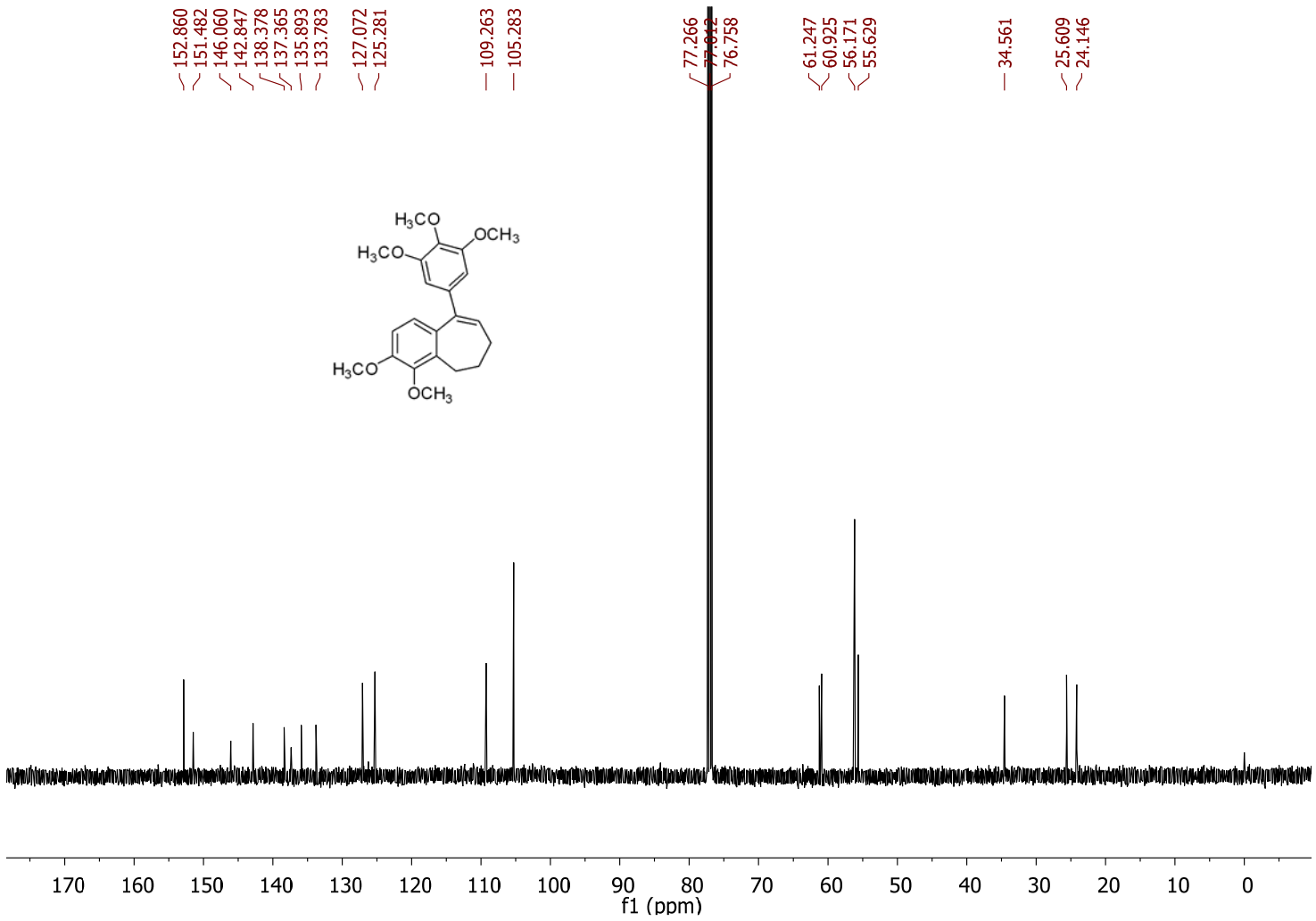
^{13}C NMR (126 MHz, $CDCl_3$) of Compound **21**



^{1}H NMR (500 MHz, $CDCl_3$) of Compound 27



^{13}C NMR (126 MHz, $CDCl_3$) of Compound **27**



HPLC Traces of Compound **27**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LDIV53-1RUN2002.D
Sample Name: LD-IV-53-1A-run2

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    Acq. Operator   : Laxman
    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 1/28/2014 4:36:52 PM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 1/28/2014 4:31:37 PM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LDIV53-1RUN2002.D\DA.M (MASTERMETHOD.M)
    Last changed    : 1/28/2014 5:32:55 PM by Laxman
    Sample Info     : run2
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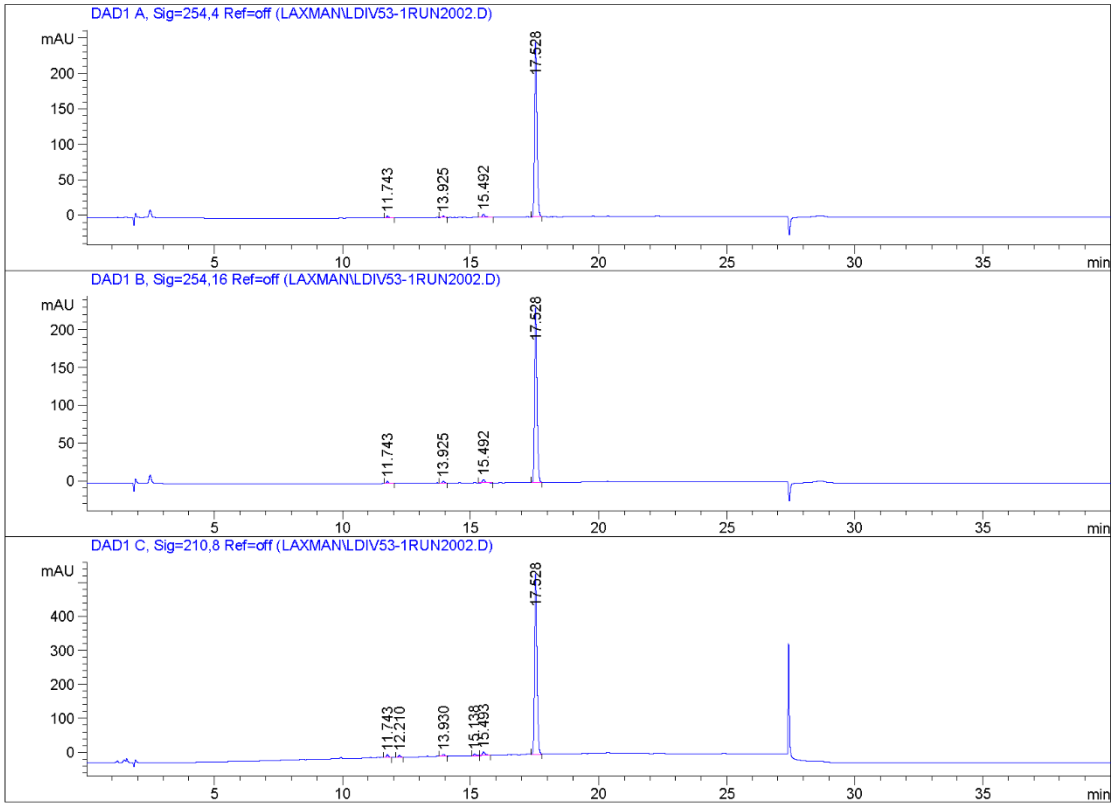
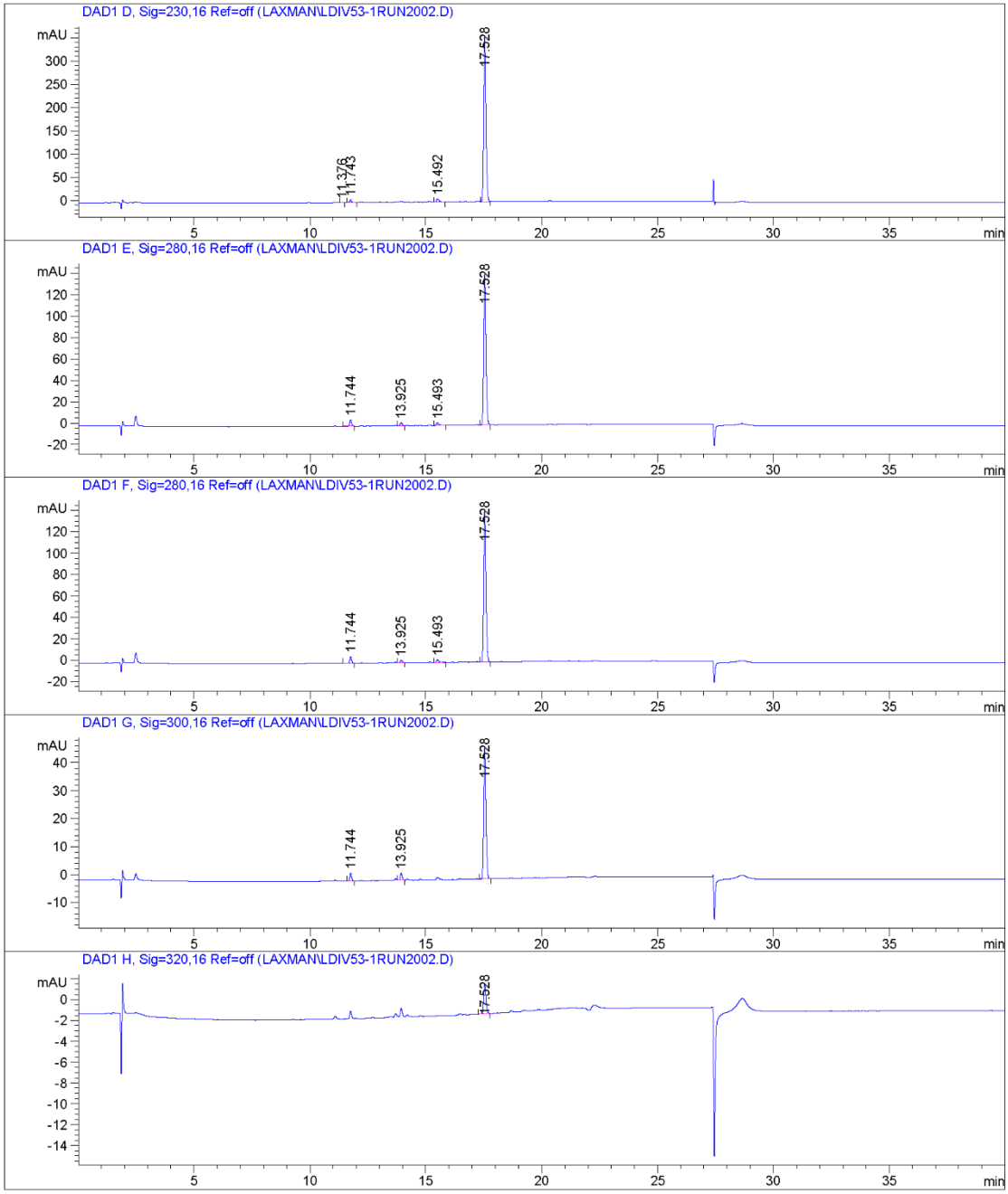


Data File C:\CHEM32\1\DATA\LAXMAN\LDIV53-1RUN2002.D
Sample Name: LD-IV-53-1A-run2



Data File C:\CHEM32\1\DATA\LAXMAN\LDIV53-1RUN2002.D
Sample Name: LD-IV-53-1A-run2

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.743	BB	0.0826	14.26201	2.65778	0.8361
2	13.925	BB	0.0929	13.40179	2.14591	0.7857
3	15.492	BB	0.1162	36.18954	4.57916	2.1215
4	17.528	BB	0.1009	1641.95728	249.16393	96.2567
Totals :				1705.81062	258.54678	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.743	BB	0.0825	15.31448	2.85651	0.9551
2	13.925	BB	0.0930	13.93967	2.22854	0.8693
3	15.492	BB	0.1162	34.20804	4.32975	2.1333
4	17.528	BB	0.1009	1540.03906	233.65747	96.0423
Totals :				1603.50126	243.07228	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.743	VB	0.0846	46.10949	8.32722	1.2122
2	12.210	BB	0.0942	31.44279	5.22819	0.8266
3	13.930	VB	0.1058	44.68492	6.07767	1.1747
4	15.138	BB	0.1074	40.66746	5.55600	1.0691
5	15.493	BB	0.1166	84.69407	10.67318	2.2265
6	17.528	VV	0.1012	3556.32837	537.31000	93.4910
Totals :				3803.92710	573.17226	

Data File C:\CHEM32\1\DATA\LAXMAN\LDIV53-1RUN2002.D
Sample Name: LD-IV-53-1A-run2

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.376	BB	0.0778	5.07398	1.02429	0.2062
2	11.743	BB	0.0837	34.19037	6.25711	1.3895
3	15.492	VB	0.1148	57.49661	7.38936	2.3366
4	17.528	VV	0.1010	2363.93970	358.03088	96.0677
Totals :				2460.70065	372.70165	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.744	BB	0.0836	33.69853	6.18095	3.3237
2	13.925	BB	0.0929	16.22744	2.59767	1.6005
3	15.493	BB	0.1161	21.33865	2.70186	2.1046
4	17.528	BB	0.1010	942.63556	142.84668	92.9712
Totals :				1013.90018	154.32717	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.744	BB	0.0836	33.69853	6.18095	3.3237
2	13.925	BB	0.0929	16.22744	2.59767	1.6005
3	15.493	BB	0.1161	21.33865	2.70186	2.1046
4	17.528	BB	0.1010	942.63556	142.84668	92.9712
Totals :				1013.90018	154.32717	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.744	BB	0.0831	15.00270	2.77378	4.3629
2	13.925	BB	0.0900	14.52386	2.41986	4.2237
3	17.528	BB	0.1013	314.33951	47.44872	91.4134
Totals :				343.86607	52.64236	

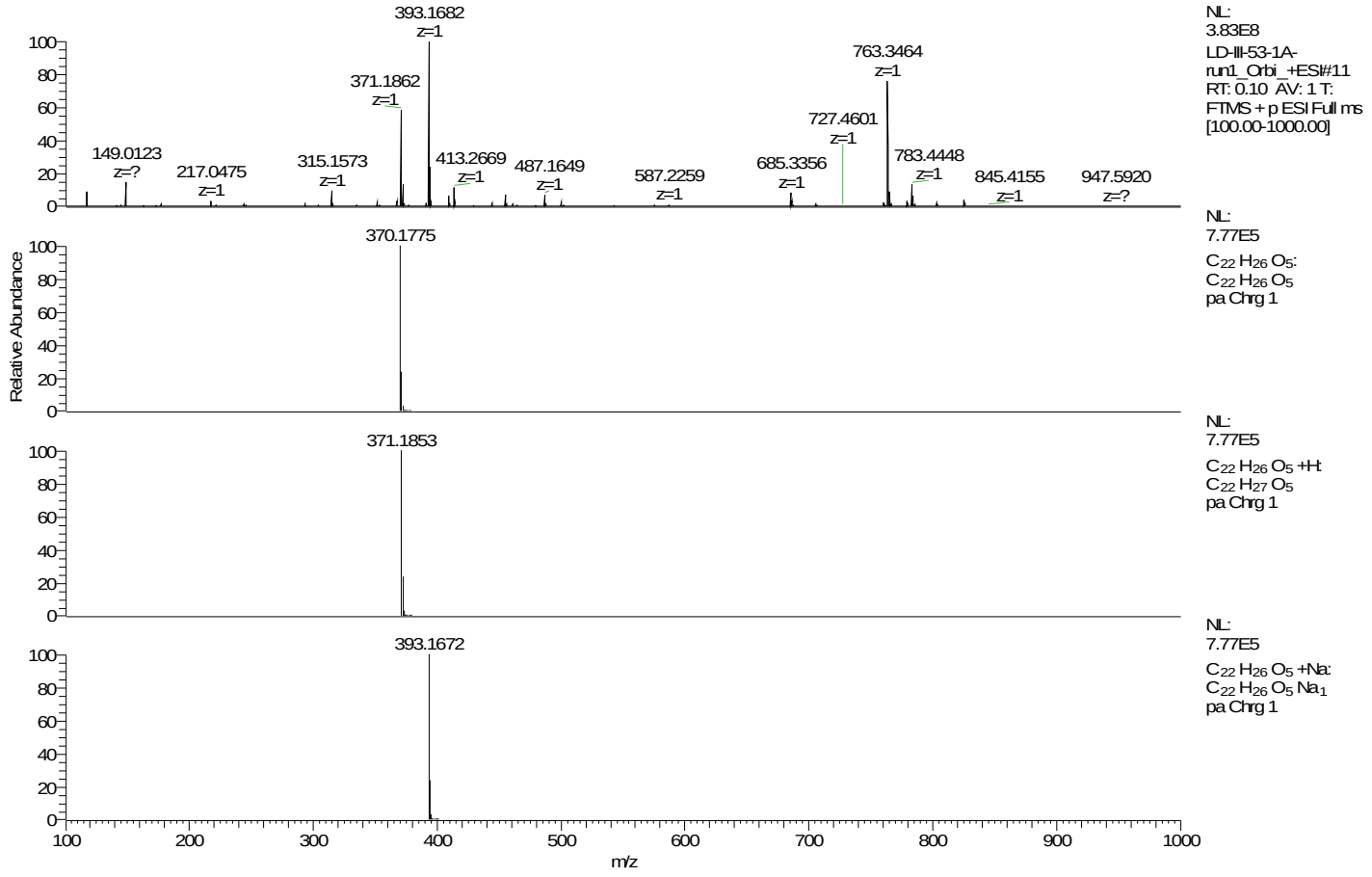
Data File C:\CHEM32\1\DATA\LAXMAN\LDIV53-1RUN2002.D
Sample Name: LD-IV-53-1A-run2

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

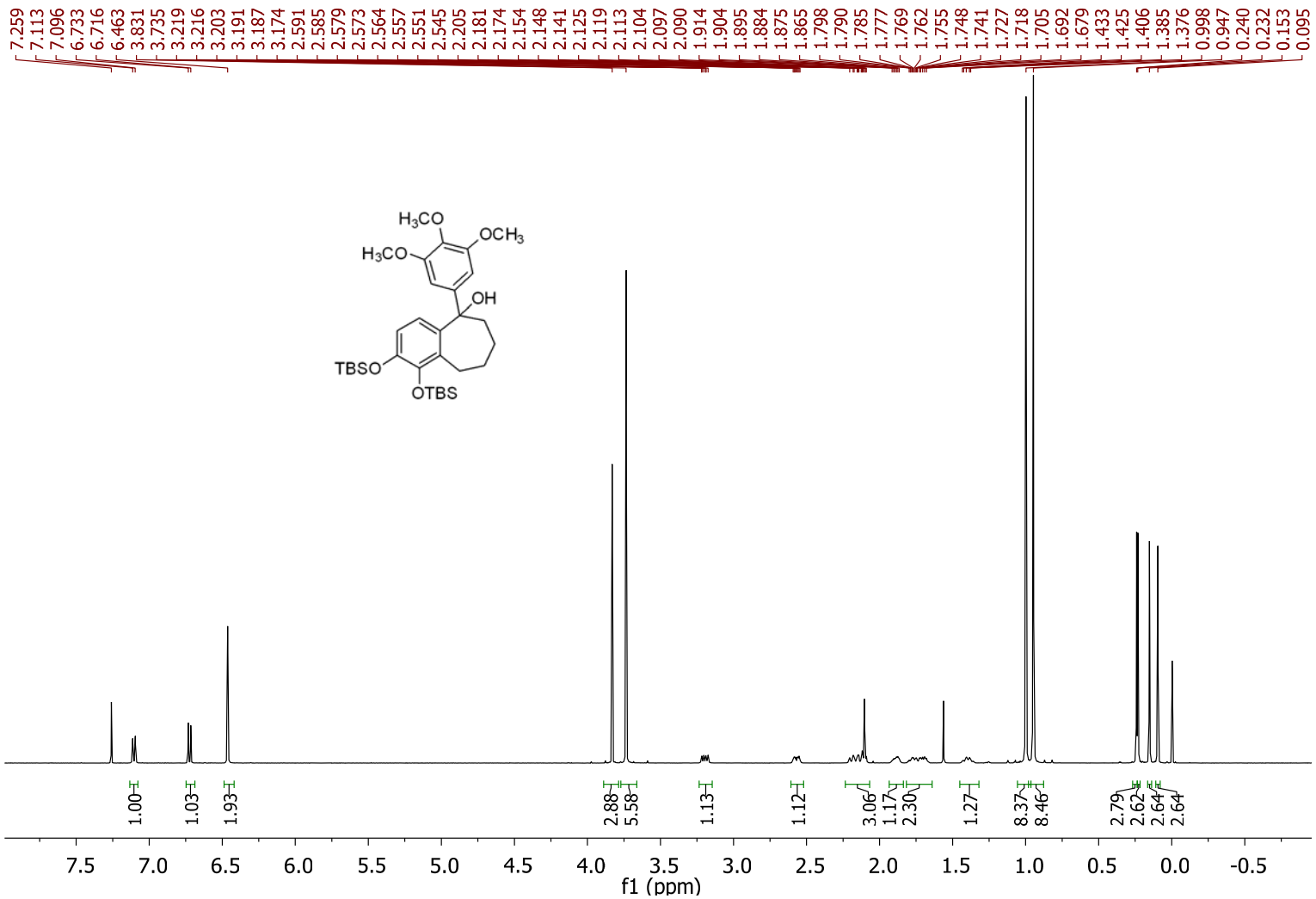
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.528	BB	0.1072	20.50176	2.94557	100.0000
Totals :				20.50176	2.94557	

*** End of Report ***

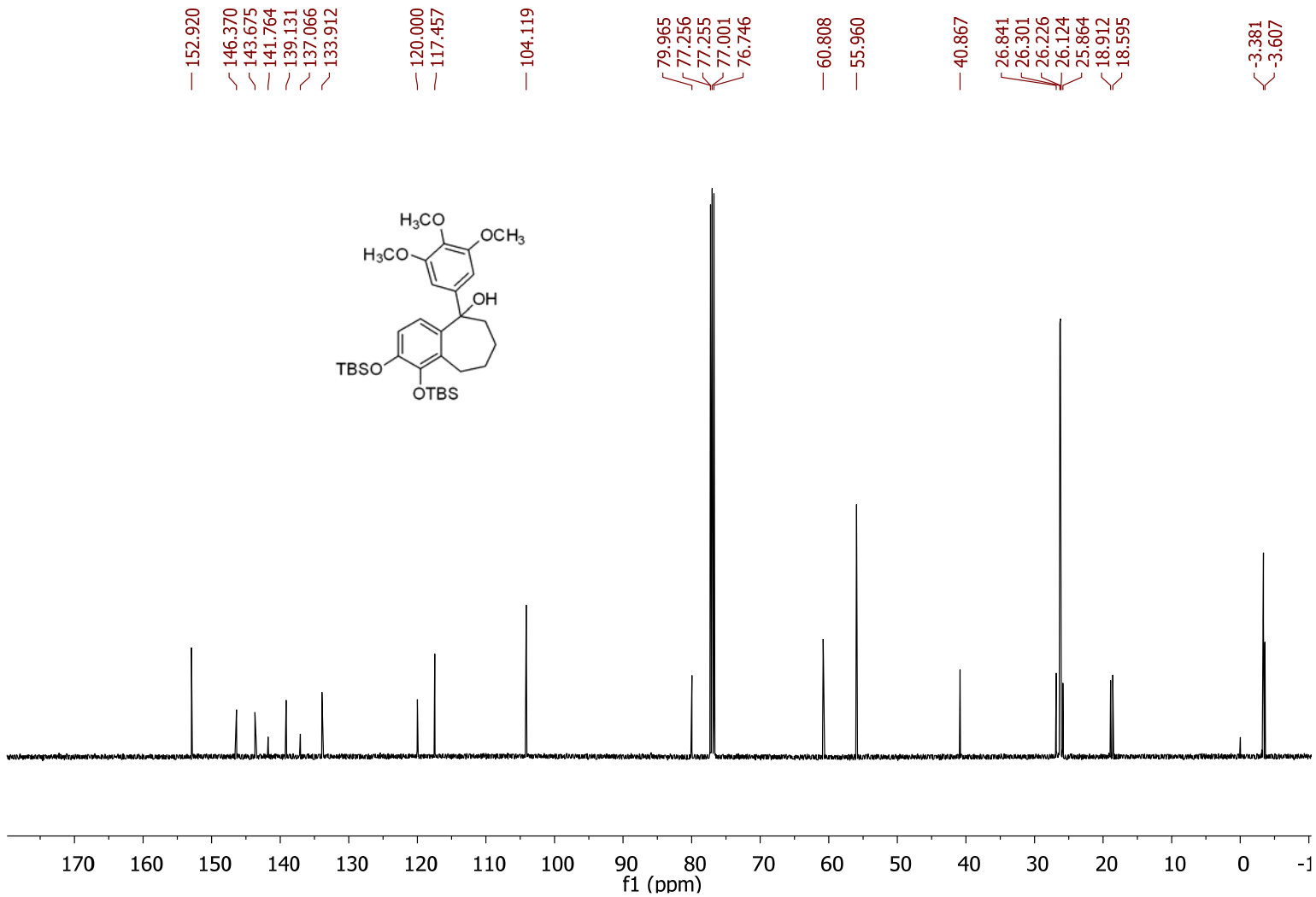
HRMS of Compound **27**



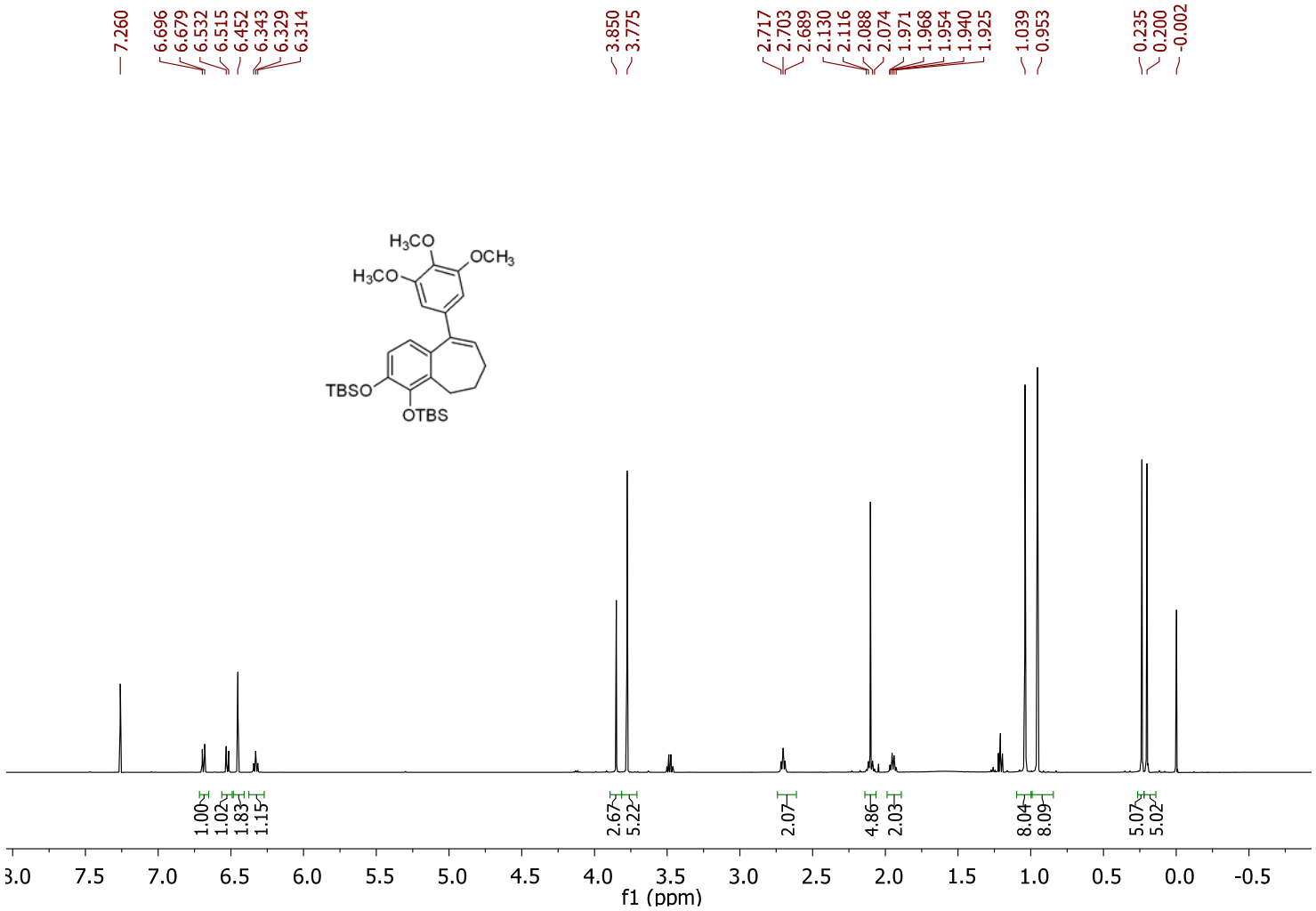
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **22**



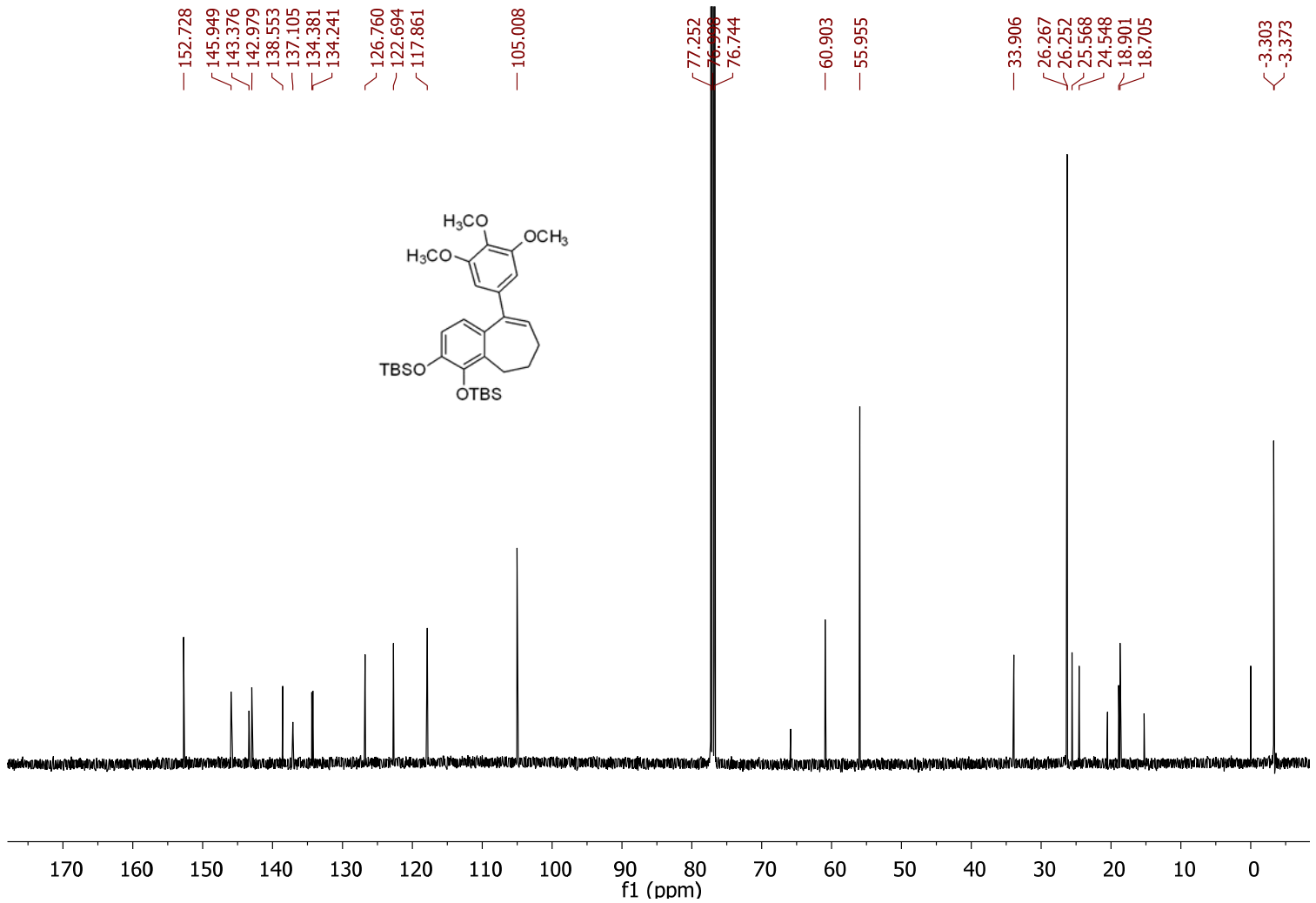
^{13}C NMR (126 MHz, $CDCl_3$) of Compound **22**



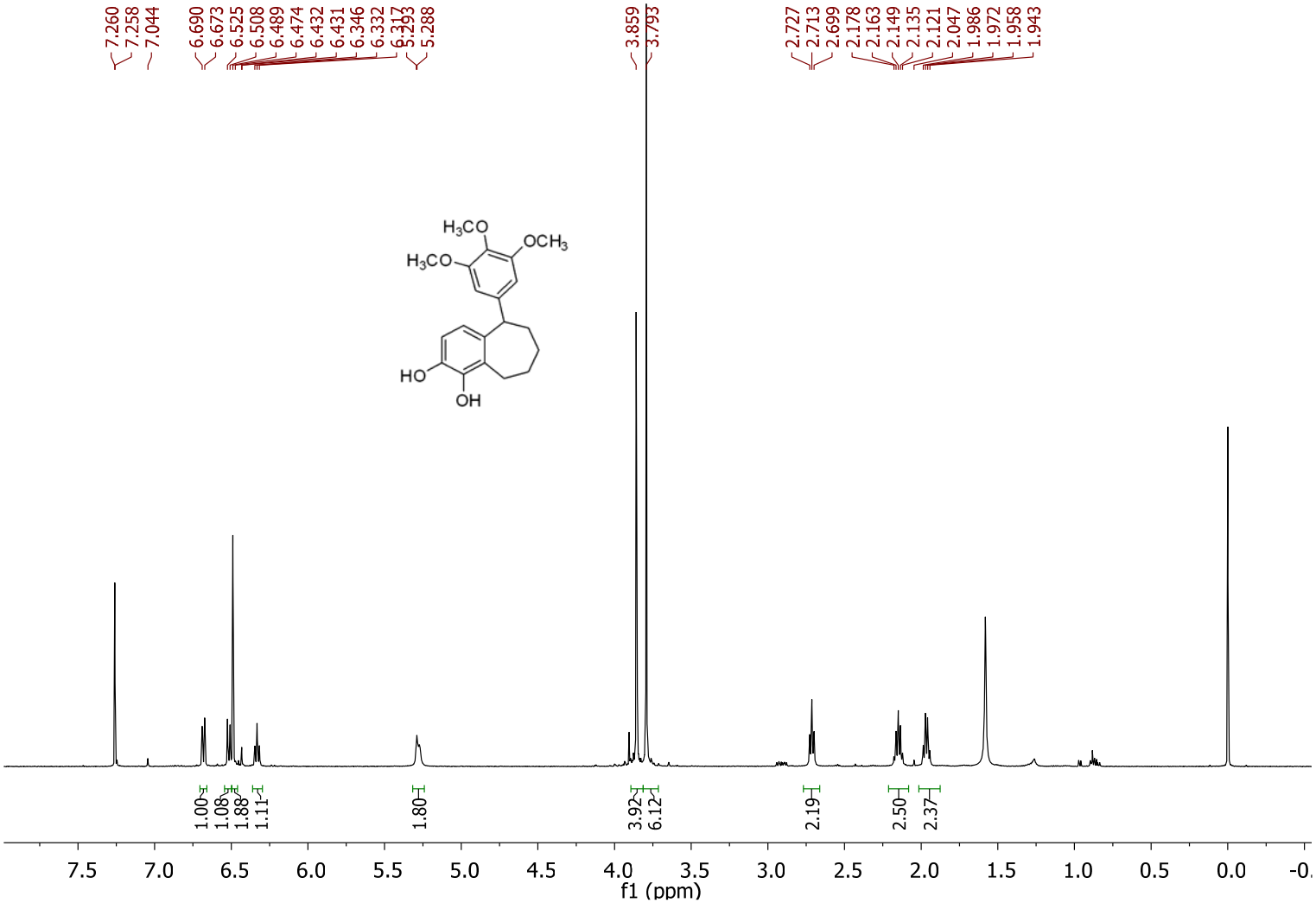
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **28**



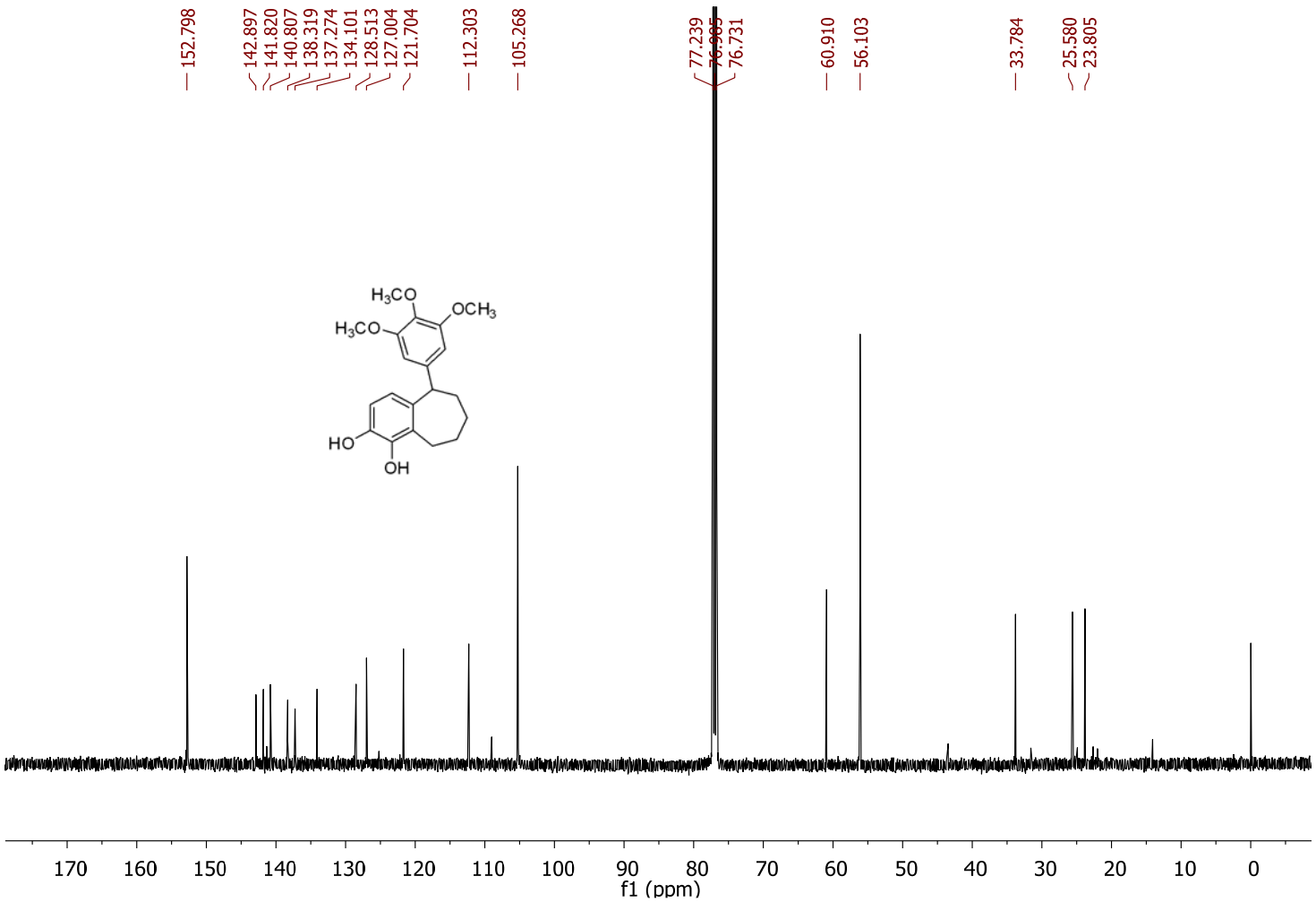
^{13}C NMR (126 MHz, $CDCl_3$) of Compound **28**



^{1}H NMR (500 MHz, $CDCl_3$) of Compound **33**



^{13}C NMR (126 MHz, $CDCl_3$) of Compound **33**



HPLC Traces of Compound **33**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-73000001.D
Sample Name: LD-VI-73-1A-run1

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    Acq. Instrument : Instrument 1                  Location :   -
    Injection Date  : 9/12/2014 2:43:59 PM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 9/12/2014 2:31:31 PM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-VI-73000001.D\DA.M (MASTERMETHOD.M)
    Last changed    : 9/12/2014 5:02:51 PM by Eric Lin
    Sample Info     : Method-Mastermethod
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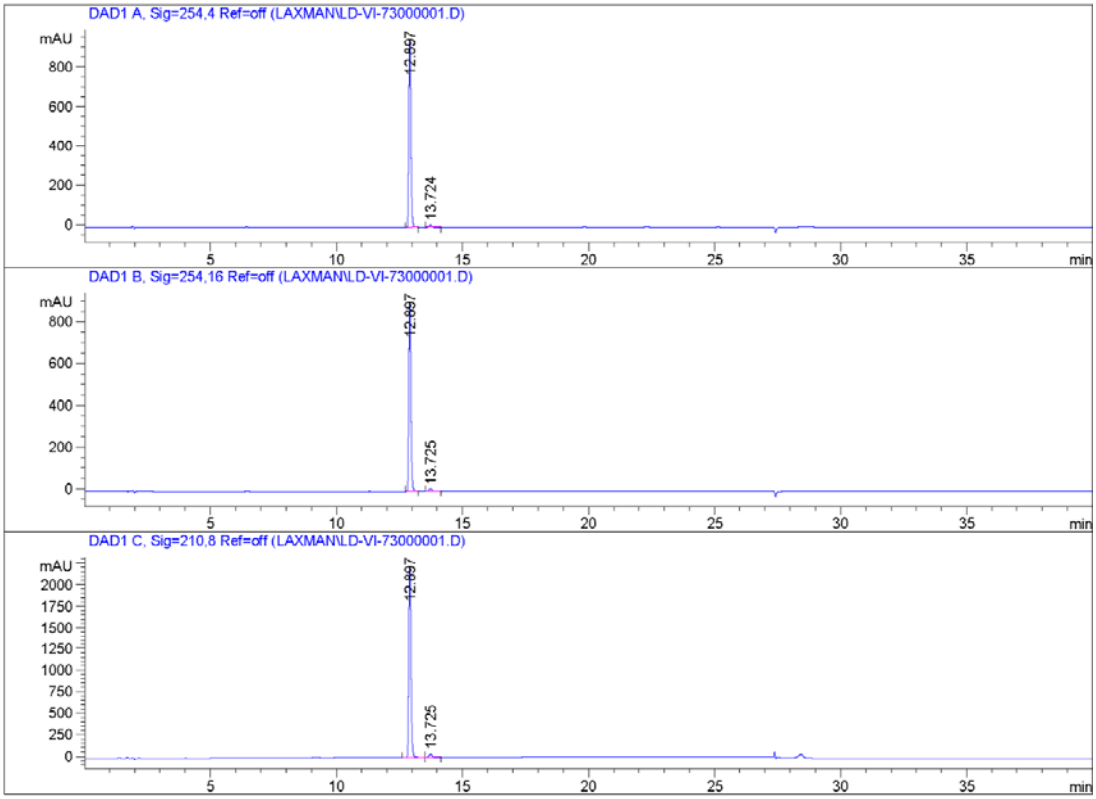
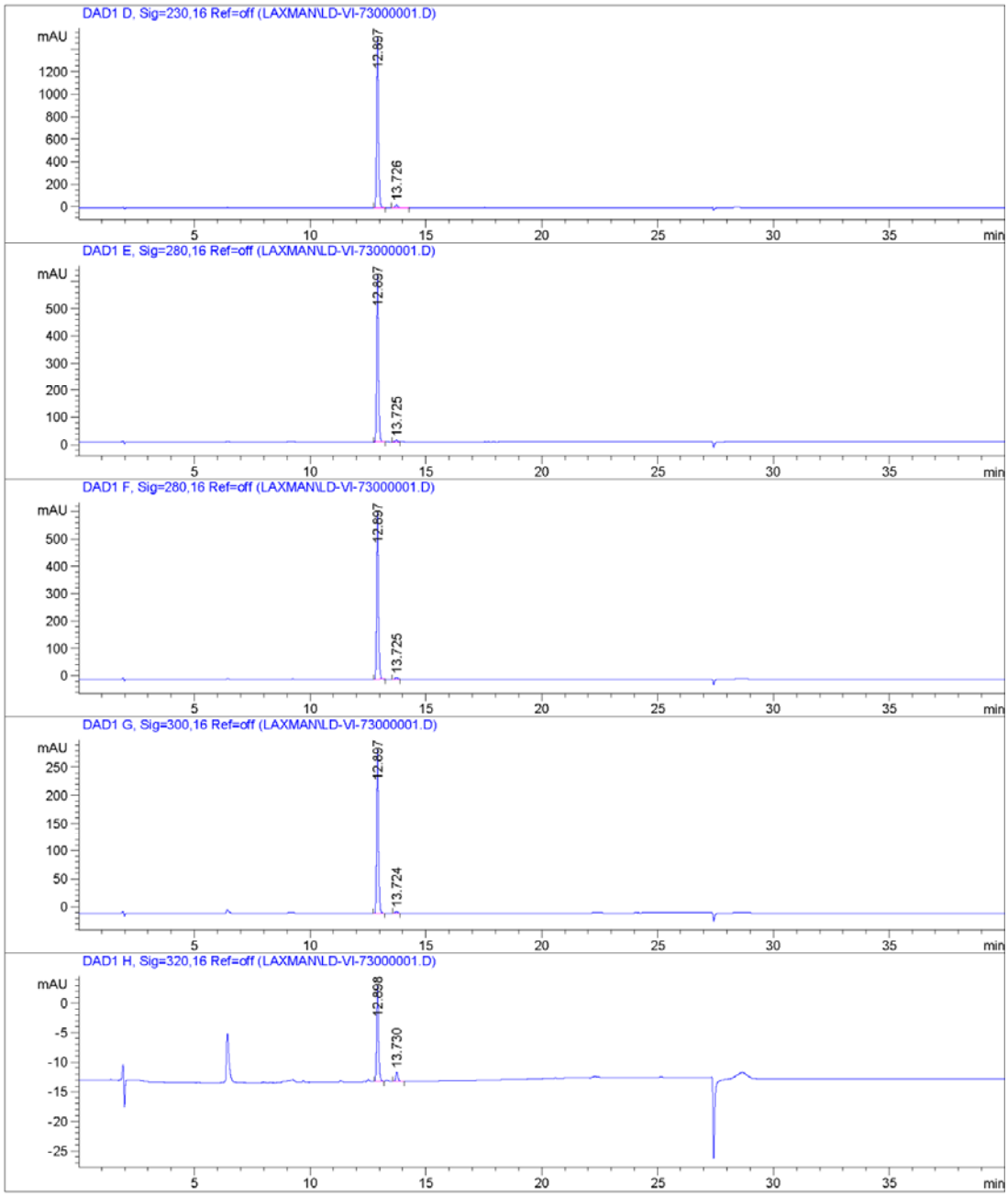


Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-73000001.D
Sample Name: LD-VI-73-1A-run1



Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-73000001.D
Sample Name: LD-VI-73-1A-run1

==========

Area Percent Report

==========

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.897	BB	0.0842	5219.16211	948.45355	98.1260
2	13.724	BB	0.1178	99.67384	11.89176	1.8740
Totals :				5318.83595	960.34531	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.897	BB	0.0842	4968.39111	902.85382	98.0270
2	13.725	BB	0.1175	100.00153	11.96880	1.9730
Totals :				5068.39264	914.82262	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.897	BV	0.0898	1.29026e4	2218.69849	97.3838
2	13.725	VB	0.1184	346.62473	41.94163	2.6162
Totals :				1.32492e4	2260.64012	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.897	BB	0.0850	8459.47461	1519.36890	97.5900
2	13.726	BB	0.1176	208.90370	25.50353	2.4100

Data File C:\CHEM32\1\DATA\LAXMAN\LD-VI-73000001.D
Sample Name: LD-VI-73-1A-run1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				8668.37831	1544.87242	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.897	BB	0.0842	3387.88330	615.90149	98.2865
2	13.725	BV	0.1117	59.06462	7.51596	1.7135
Totals :				3446.94792	623.41745	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.897	BB	0.0842	3387.88477	615.90149	98.2865
2	13.725	BV	0.1137	59.06182	7.51595	1.7135
Totals :				3446.94659	623.41744	

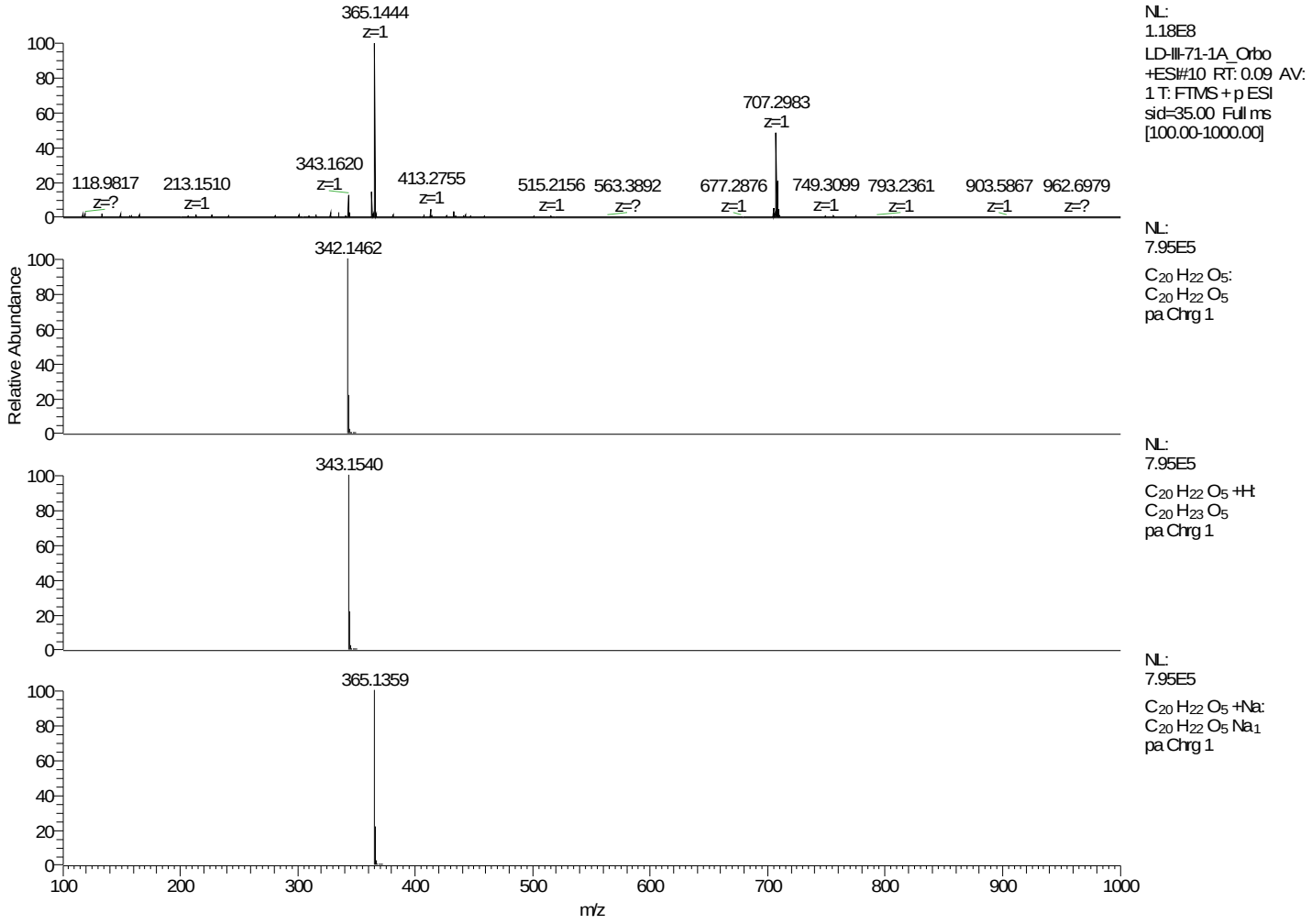
Signal 7: DAD1 G, Sig=300,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.897	BV	0.0844	1630.89148	295.33459	98.6910
2	13.724	BB	0.1092	21.63143	2.82855	1.3090
Totals :				1652.52291	298.16315	

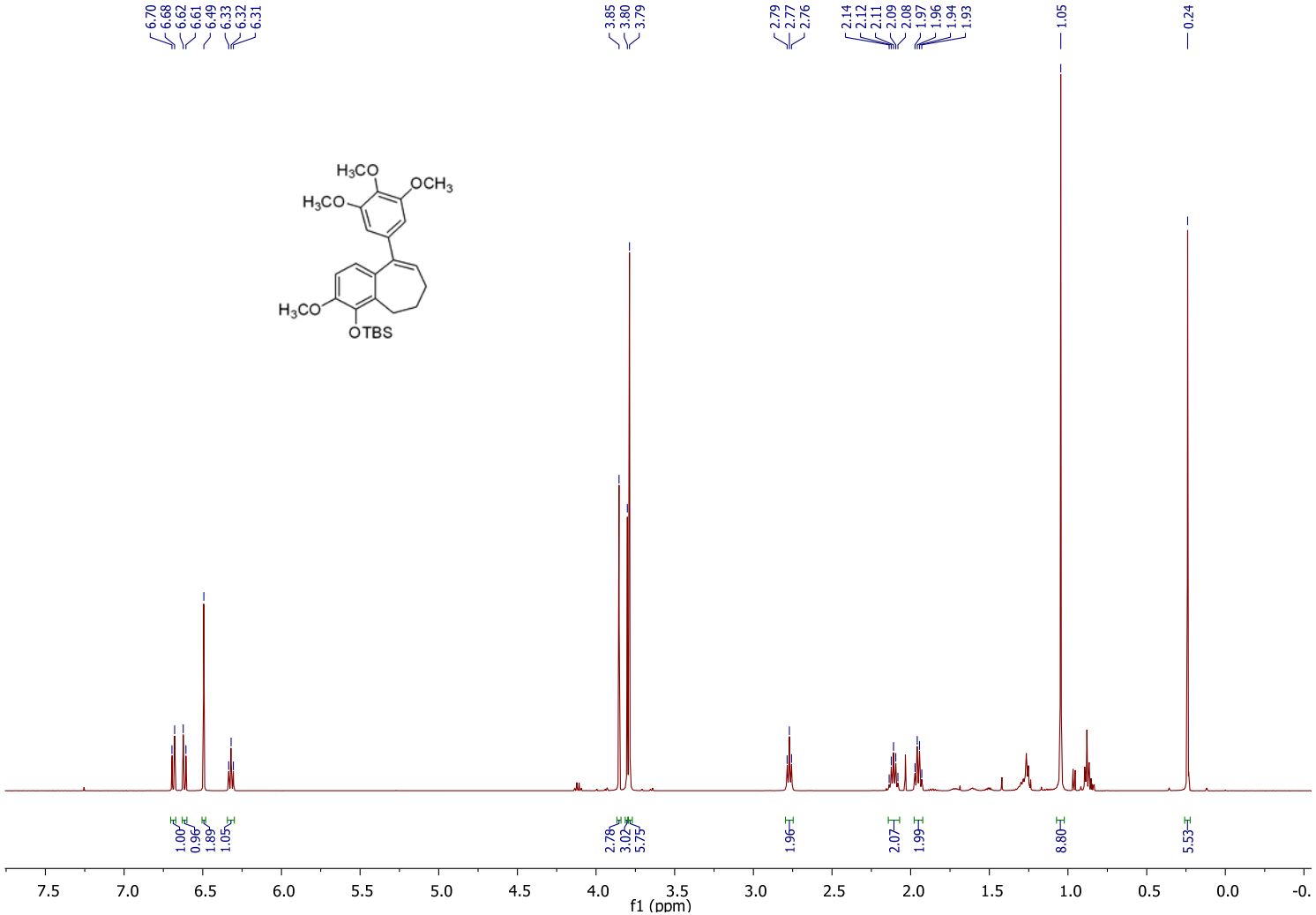
Signal 8: DAD1 H, Sig=320,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.898	BB	0.0895	94.41895	16.31277	90.0631
2	13.730	BB	0.0986	10.41746	1.58729	9.9369
Totals :				104.83641	17.90006	

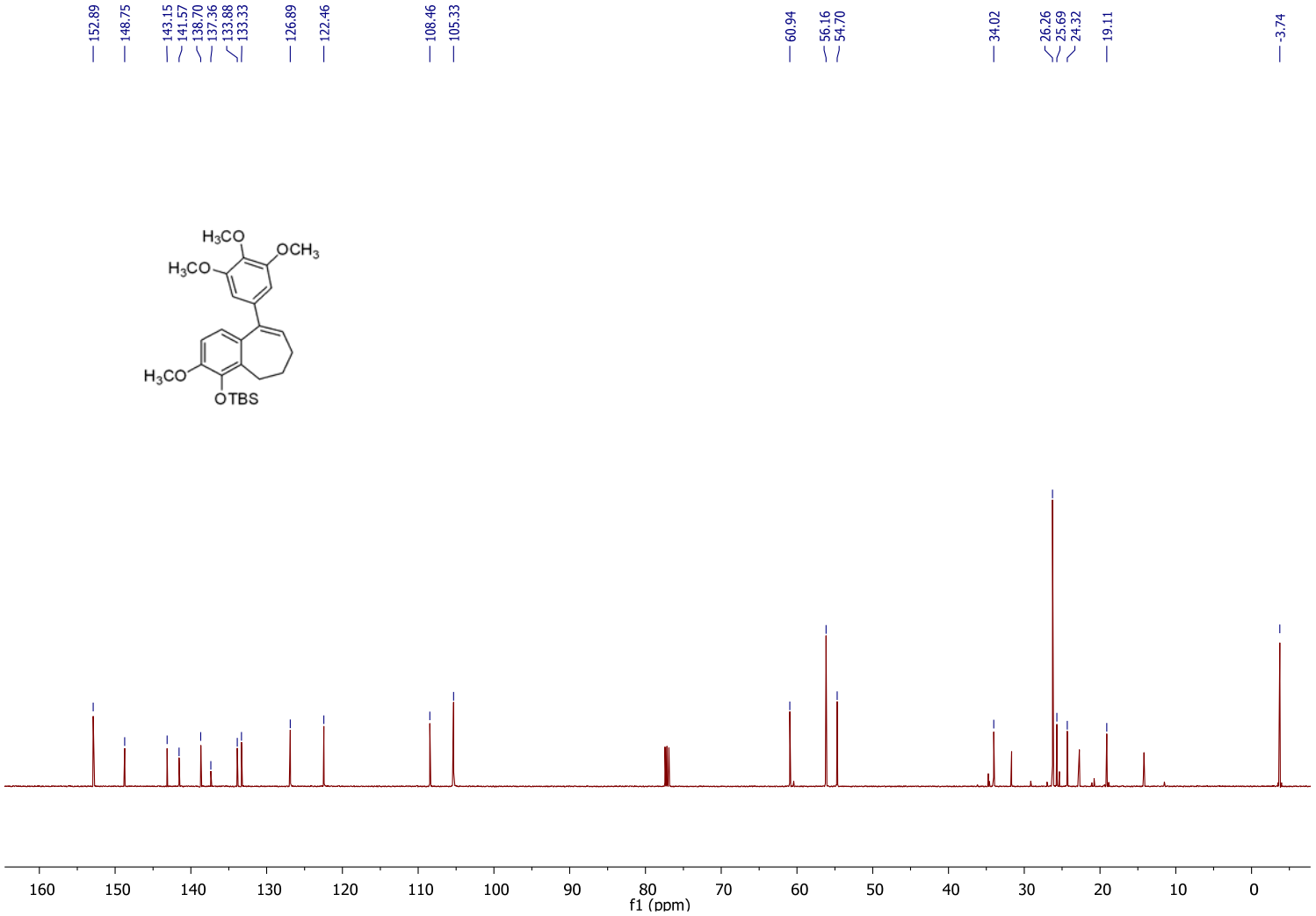
HRMS of Compound **33**



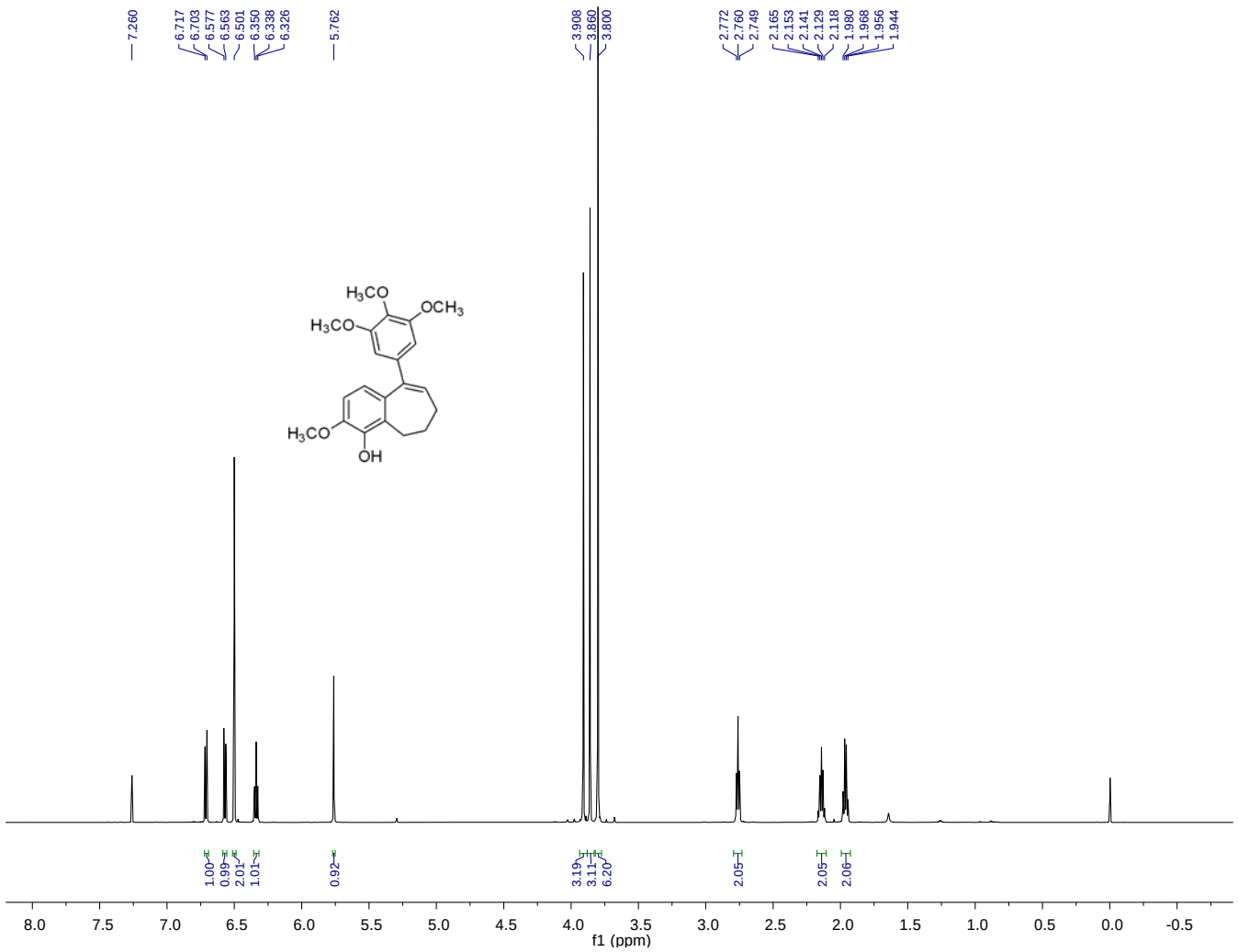
^{1}H NMR (600 MHz, $CDCl_3$) for Compound **29**



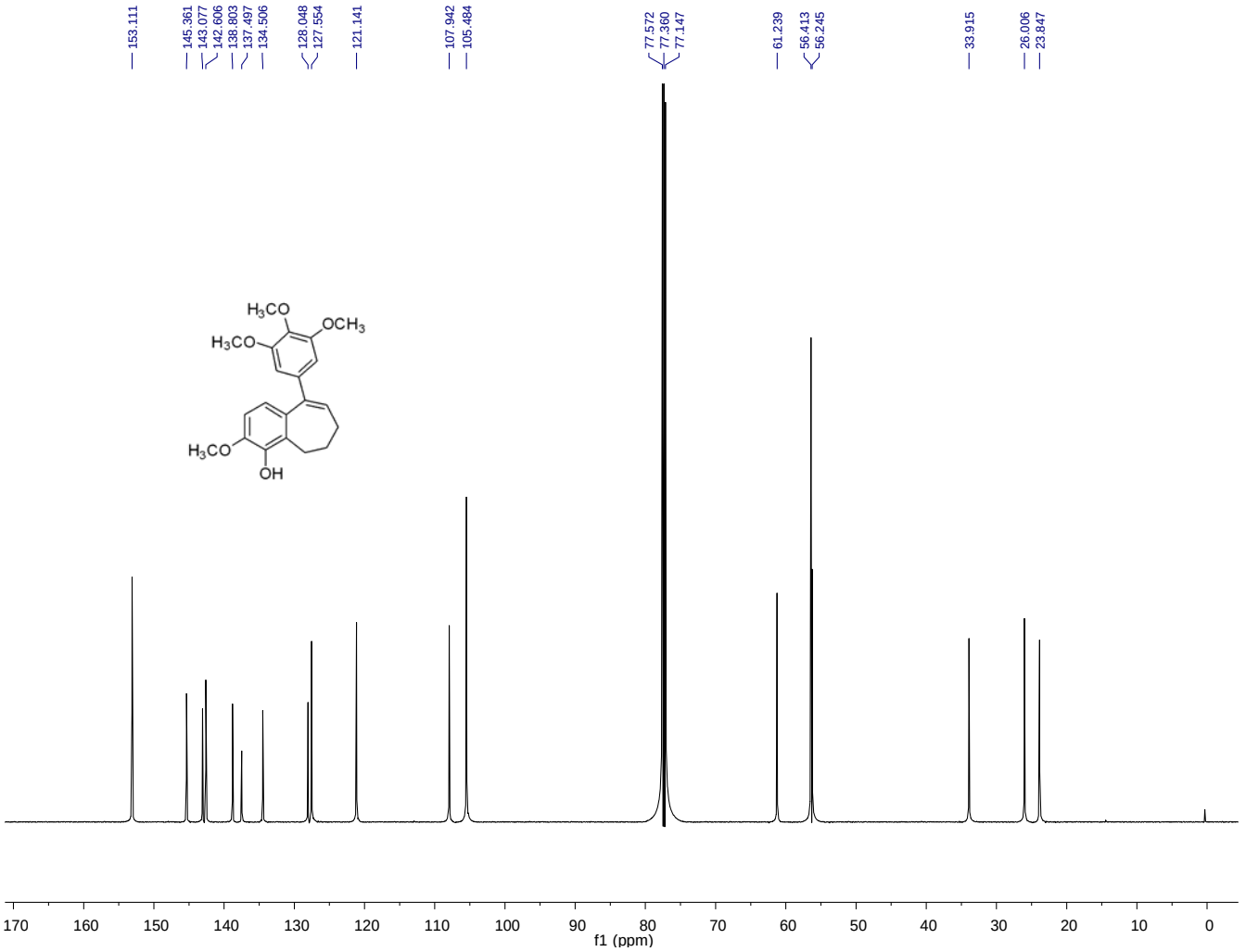
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **29**



^{1}H NMR (600 MHz, $CDCl_3$) for Compound **34**



^{13}C NMR (151 MHz, $CDCl_3$) for Compound **34**



HPLC Traces of Compound **34**

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Data File C:\CHEM32\1\DATA\LAXMAN\LD-IV-155-RUN01.D
Sample Name: LD-IV-155-KGP1-run1

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    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 5/21/2015 10:21:27 AM
    Acq. Method     : C:\CHEM32\1\METHODS\GRAD 2 50-90 ACN.M
    Last changed    : 5/21/2015 9:36:26 AM by lAXMAN
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-IV-155-RUN01.D\DA.M (GRAD 2 50-90 ACN.M)
    Last changed    : 5/21/2015 11:01:44 AM by lAXMAN
    Sample Info     : Method-Grad2 50-90% ACN
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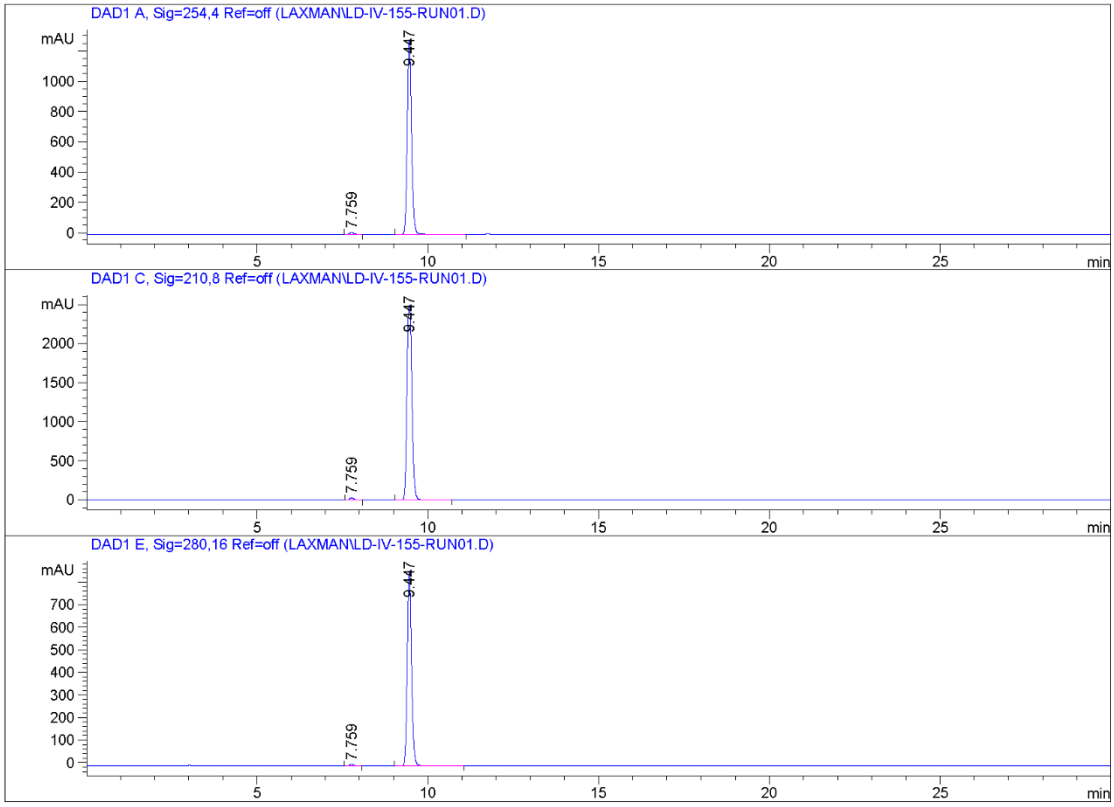
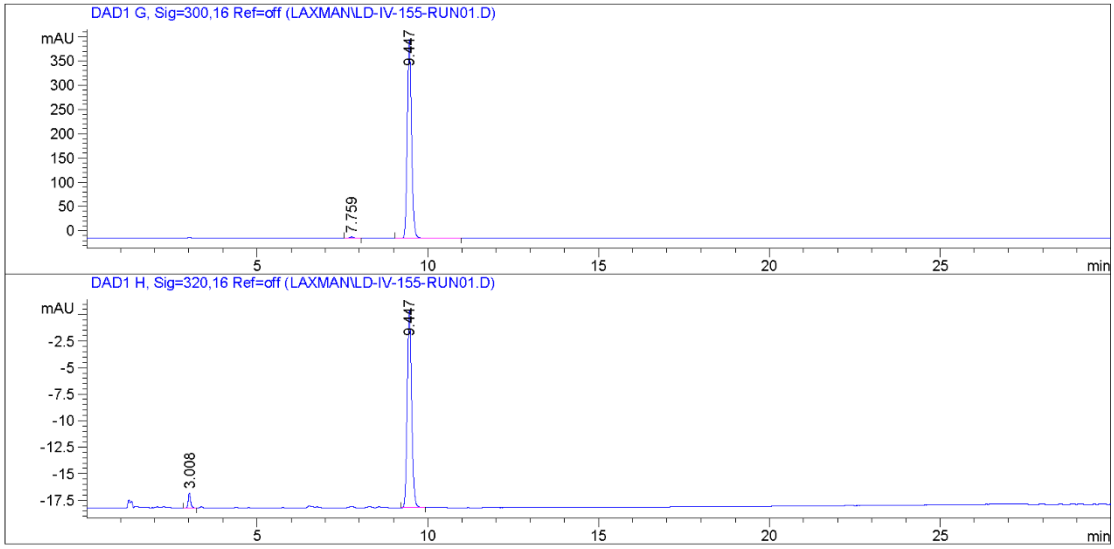


Data File C:\CHEM32\1\DATA\LAXMAN\LD-IV-155-RUN01.D
Sample Name: LD-IV-155-KGP1-run1



==========

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	7.759	BB	0.1216	95.23830	12.12386	0.8471
2	9.447	BB	0.1351	1.11470e4	1286.25732	99.1529
Totals :				1.12423e4	1298.38119	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.759	BB	0.1215	204.49210	26.08730	0.8069
2	9.447	BB	0.1616	2.51377e4	2494.85083	99.1931

Data File C:\CHEM32\1\DATA\LAXMAN\LD-IV-155-RUN01.D
Sample Name: LD-IV-155-KGP1-run1

Totals : 2.53422e4 2520.93813

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.759	BB	0.1216	53.38469	6.79636	0.7081
2	9.447	BB	0.1329	7485.58984	865.29053	99.2919

Totals : 7538.97453 872.08689

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.759	BB	0.1224	18.61229	2.34957	0.5217
2	9.447	BB	0.1331	3548.94580	409.53812	99.4783

Totals : 3567.55809 411.88769

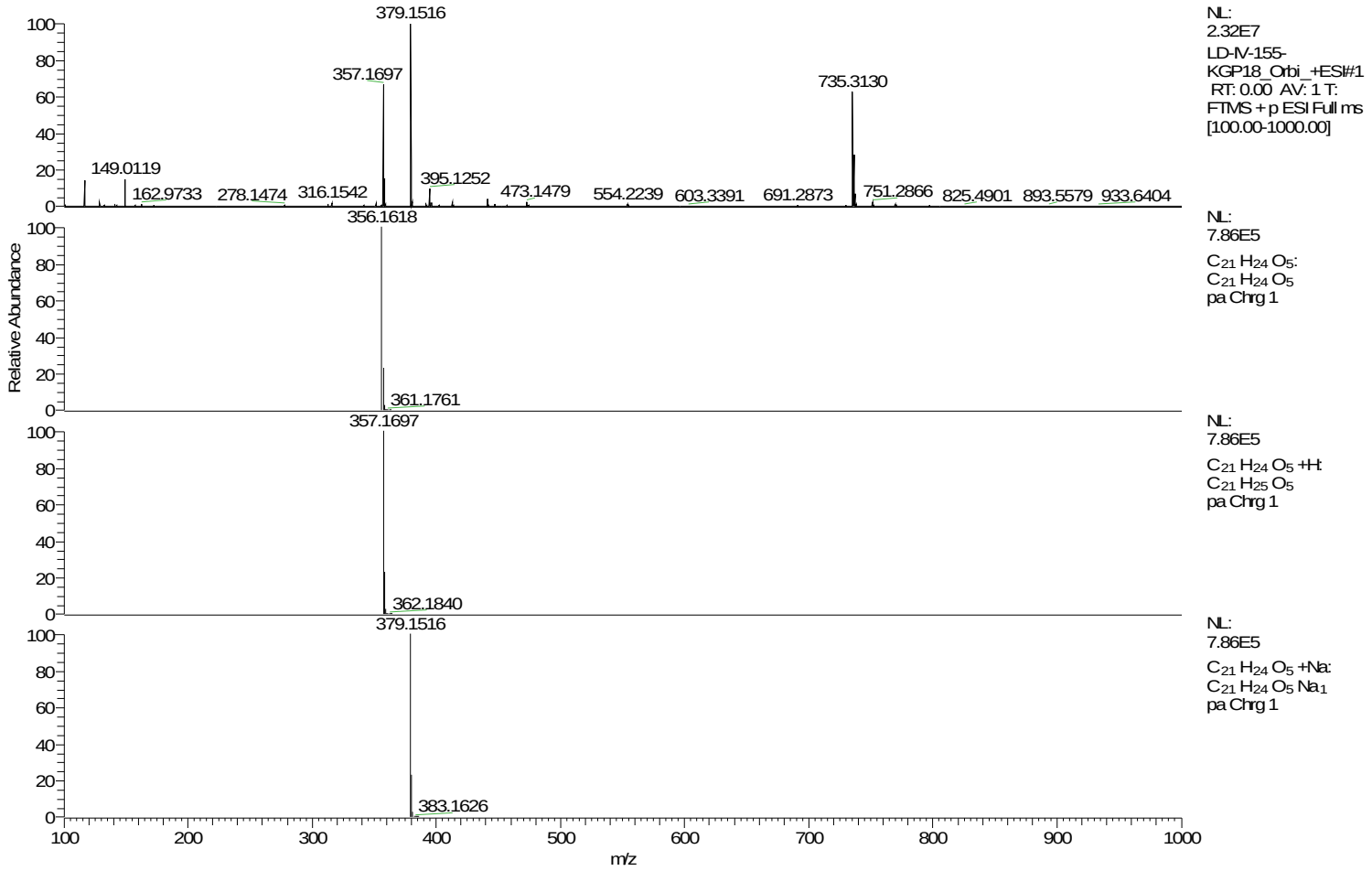
Signal 5: DAD1 H, Sig=320,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.008	BB	0.0743	6.87490	1.42403	3.9585
2	9.447	BB	0.1381	166.80150	18.68763	96.0415

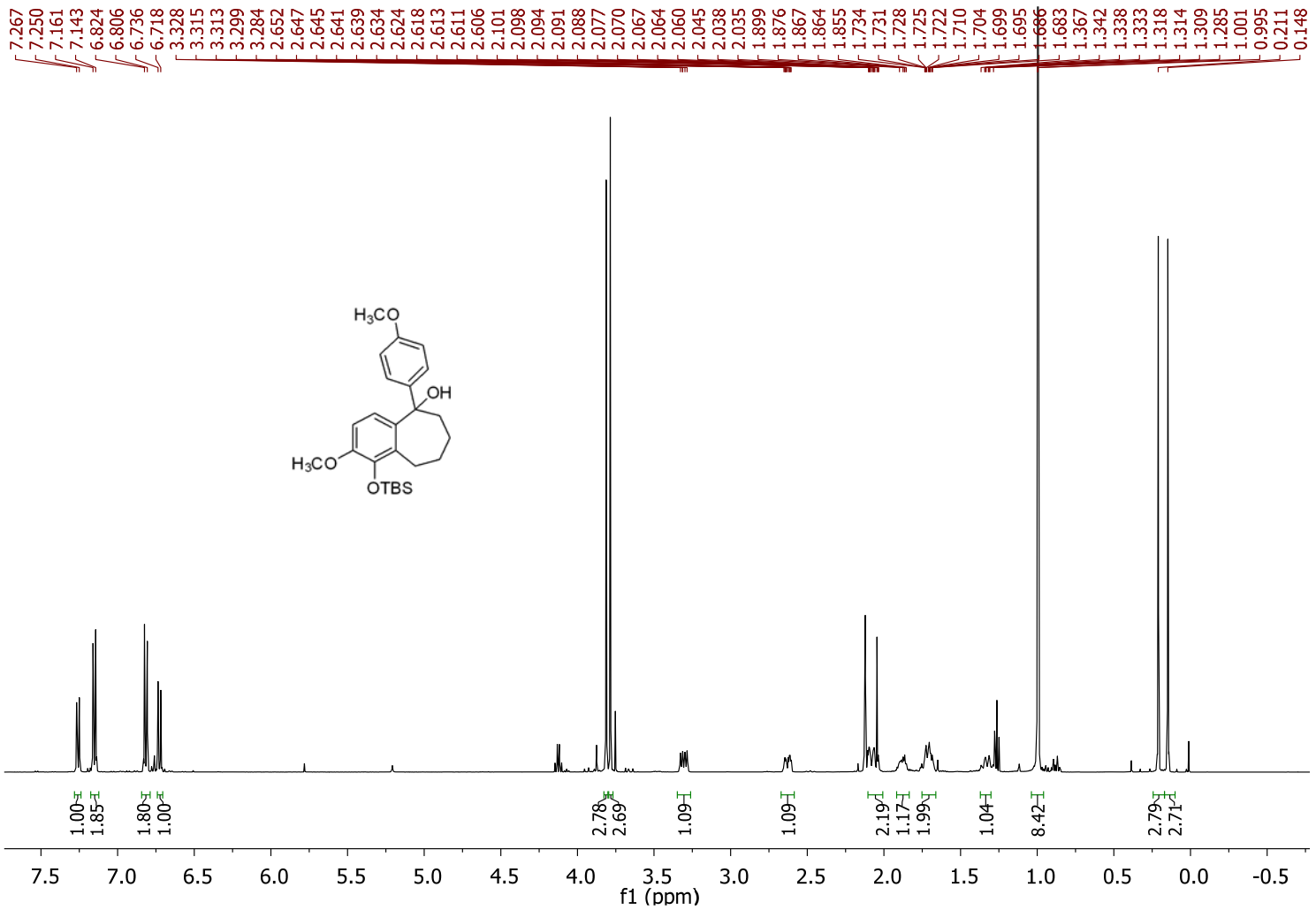
Totals : 173.67639 20.11166

*** End of Report ***

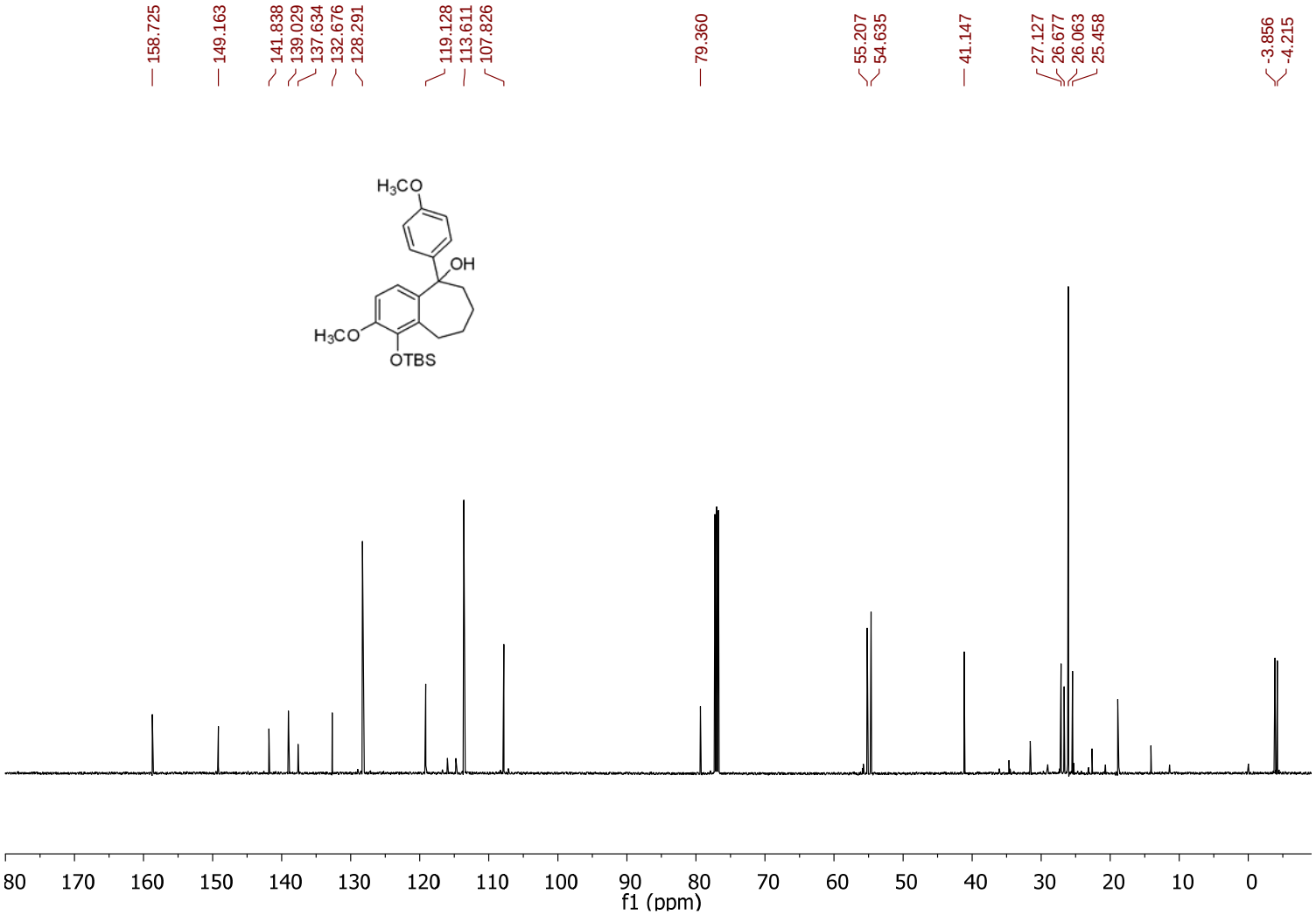
HRMS of Compound **34**



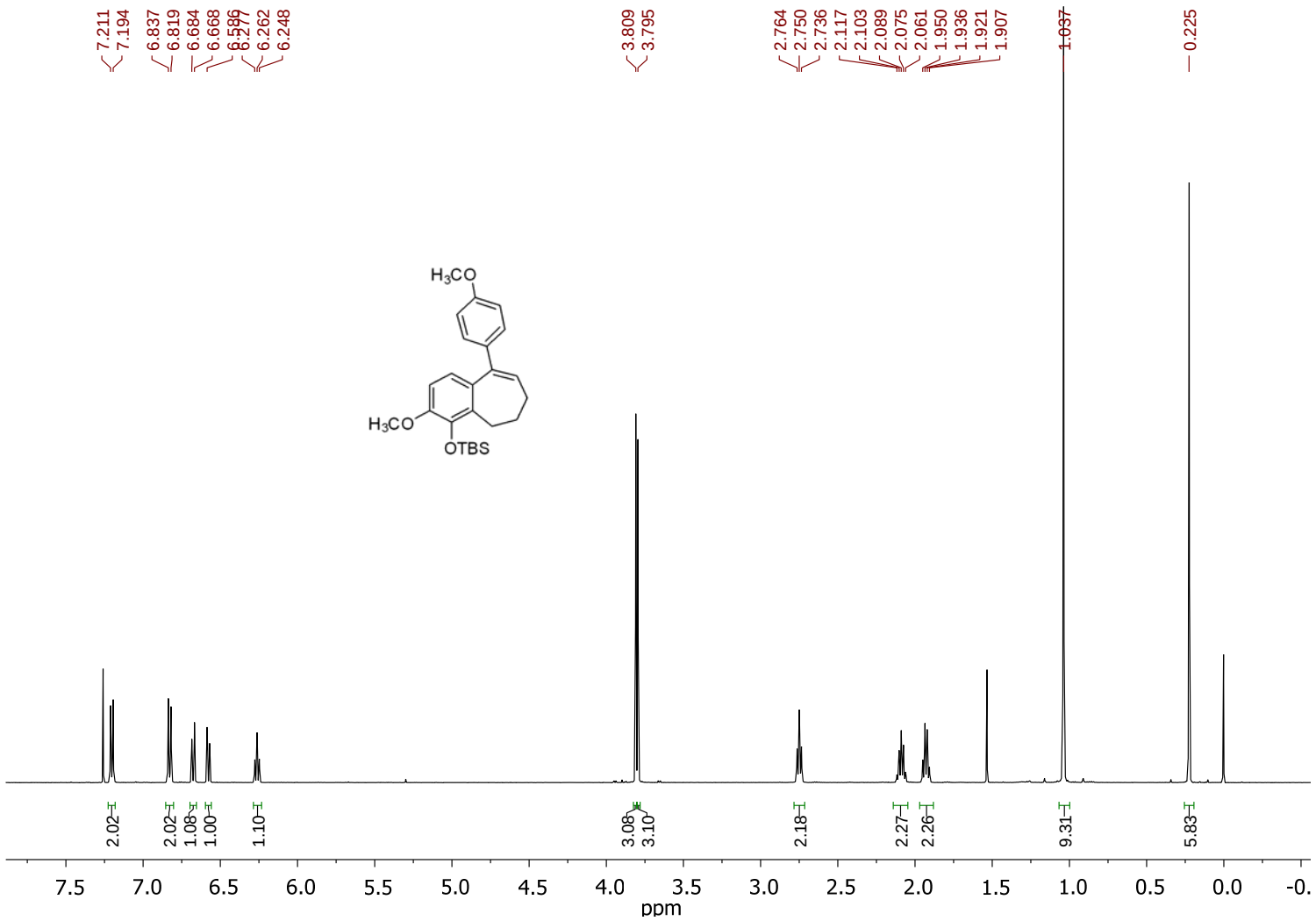
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **25**



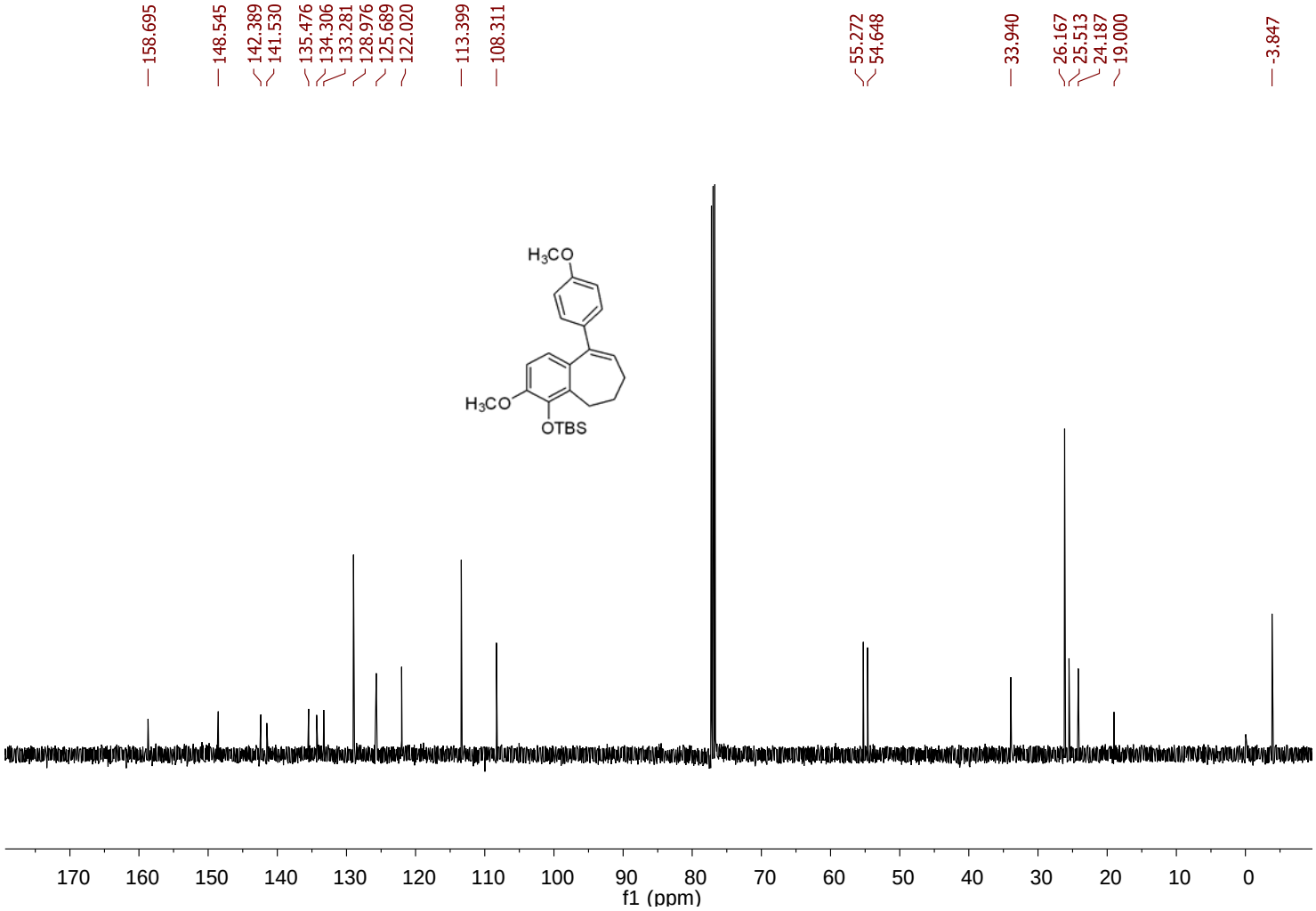
^{13}C NMR (126 MHz, $CDCl_3$) of Compound **25**



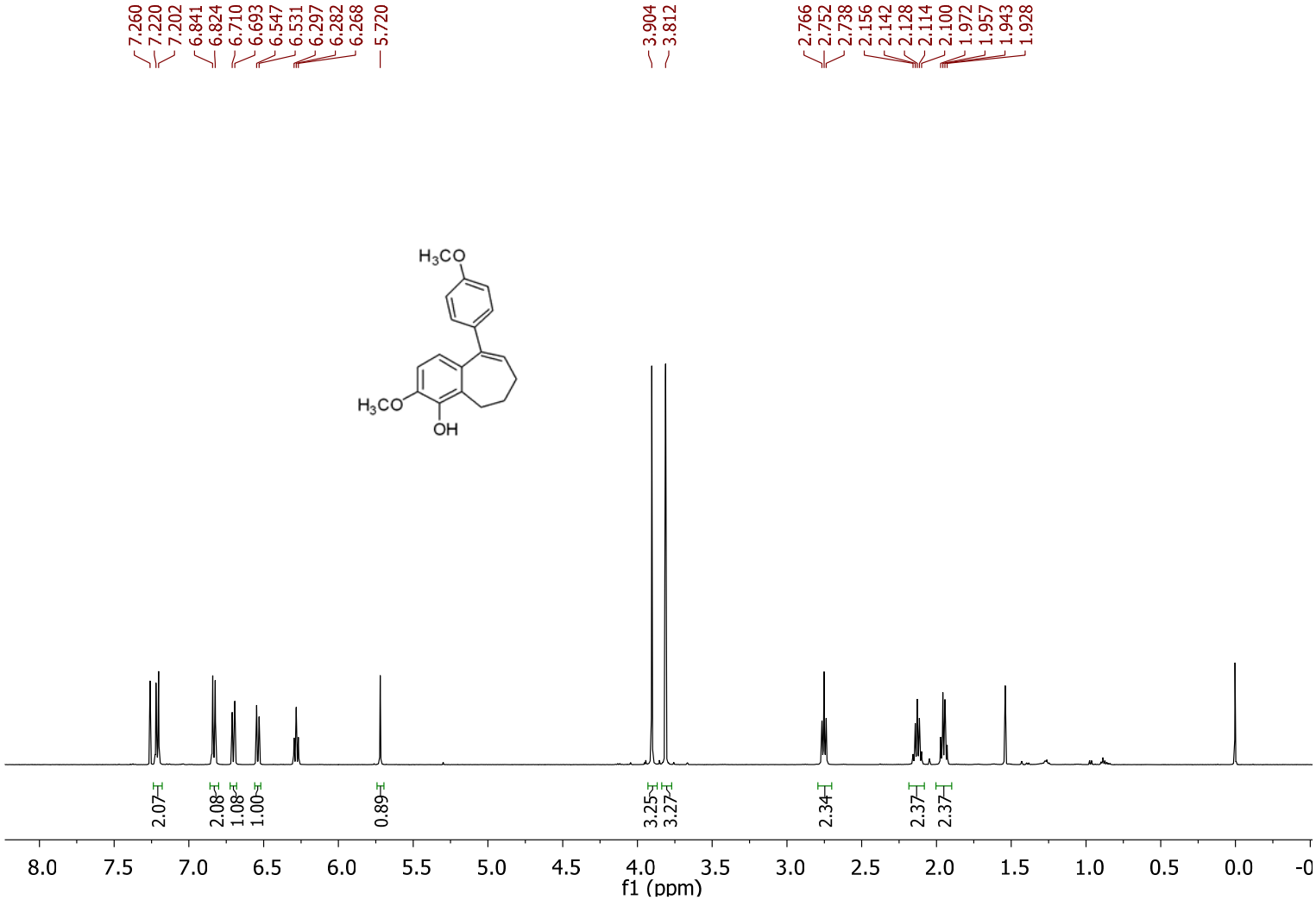
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **31**



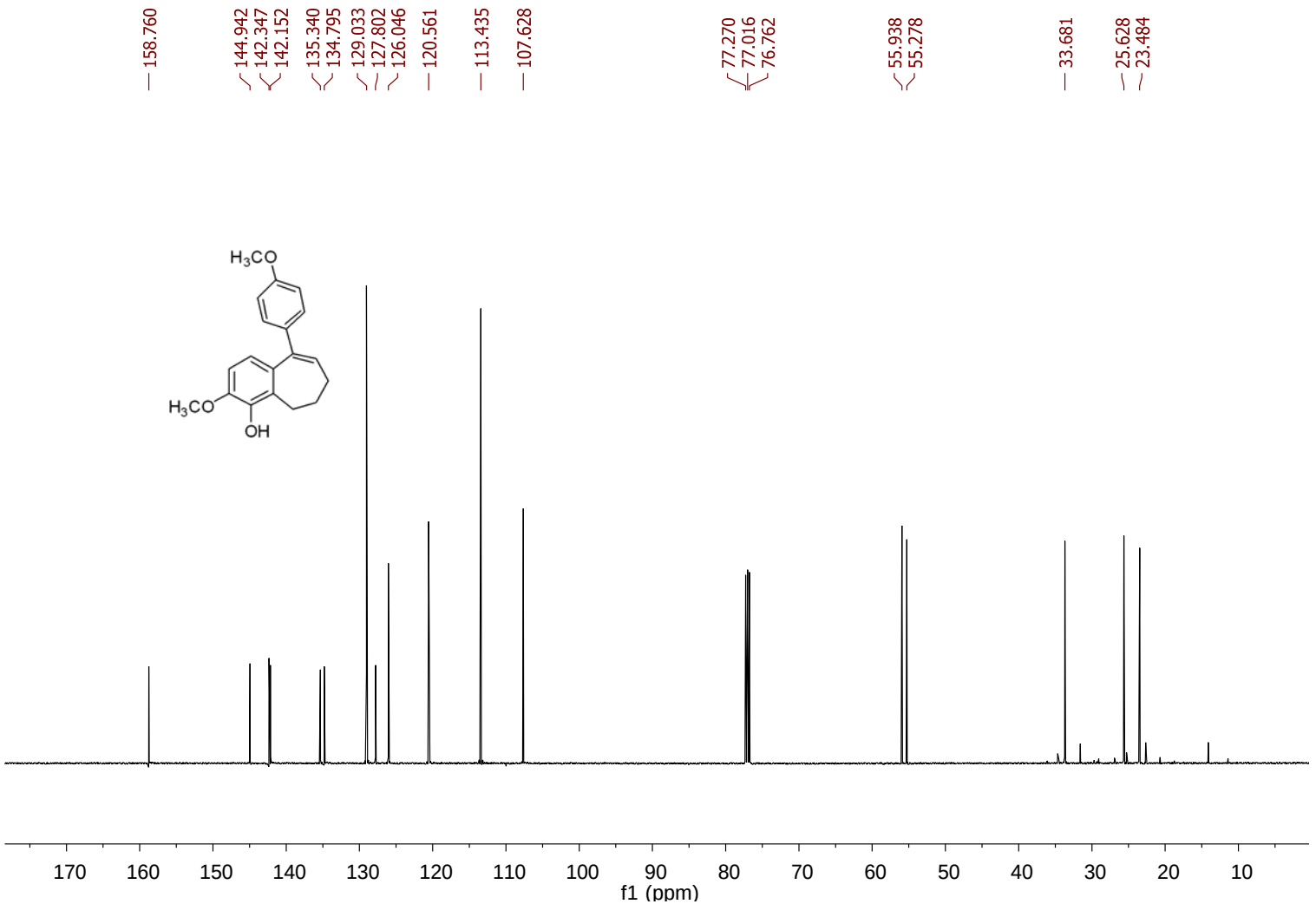
^{13}C NMR (126 MHz, $CDCl_3$) of Compound **31**



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



^{13}C NMR (126 MHz, $CDCl_3$) of Compound **35**



HPLC Traces of Compound **35**

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Data File C:\CHEM32\1\DATA\LAXMAN\LD-IV-131-1A\LDIV131-1ARUN02.D
Sample Name: LD-IV-131-1A-rerun-run1

    ======================================================================
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    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 1/27/2014 5:37:35 PM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 1/27/2014 5:33:50 PM by Laxman
    Analysis Method : C:\CHEM32\1\DATA\LAXMAN\LD-IV-131-1A\LDIV131-1ARUN02.D\DA.M (MASTERMETHOD.M)
    Last changed    : 1/28/2014 10:47:18 AM by Laxman
                      (modified after loading)
    Sample Info     : run1
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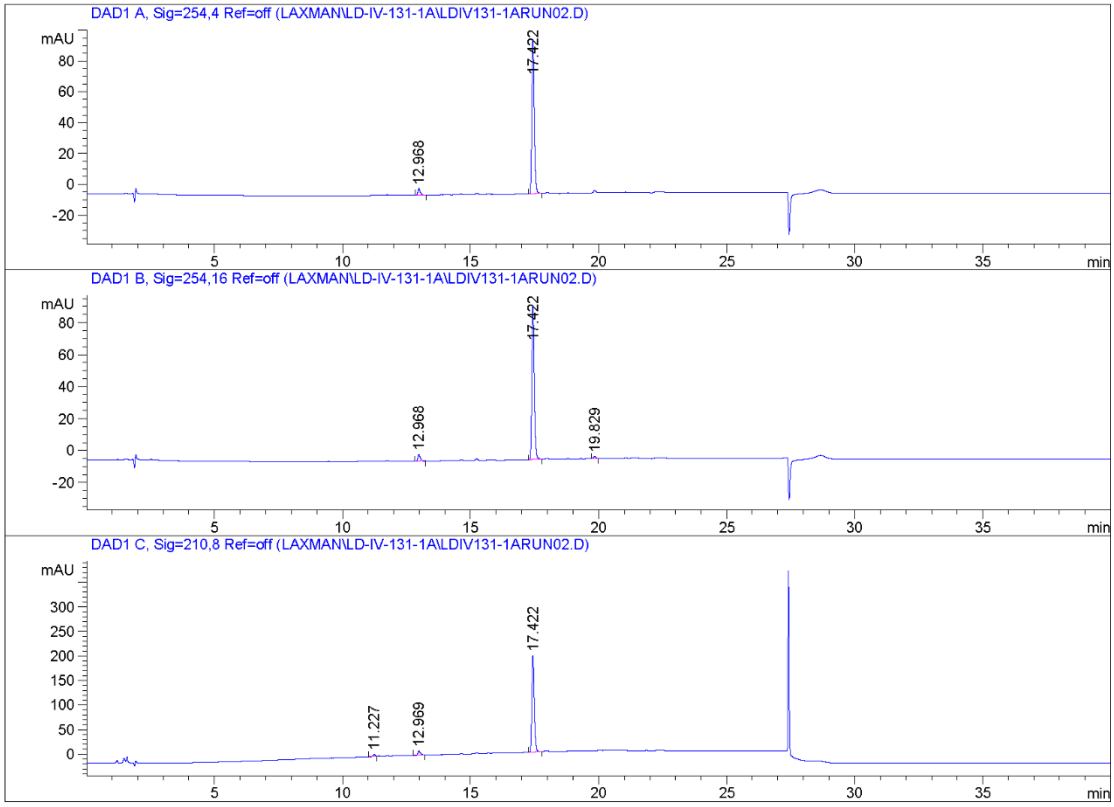
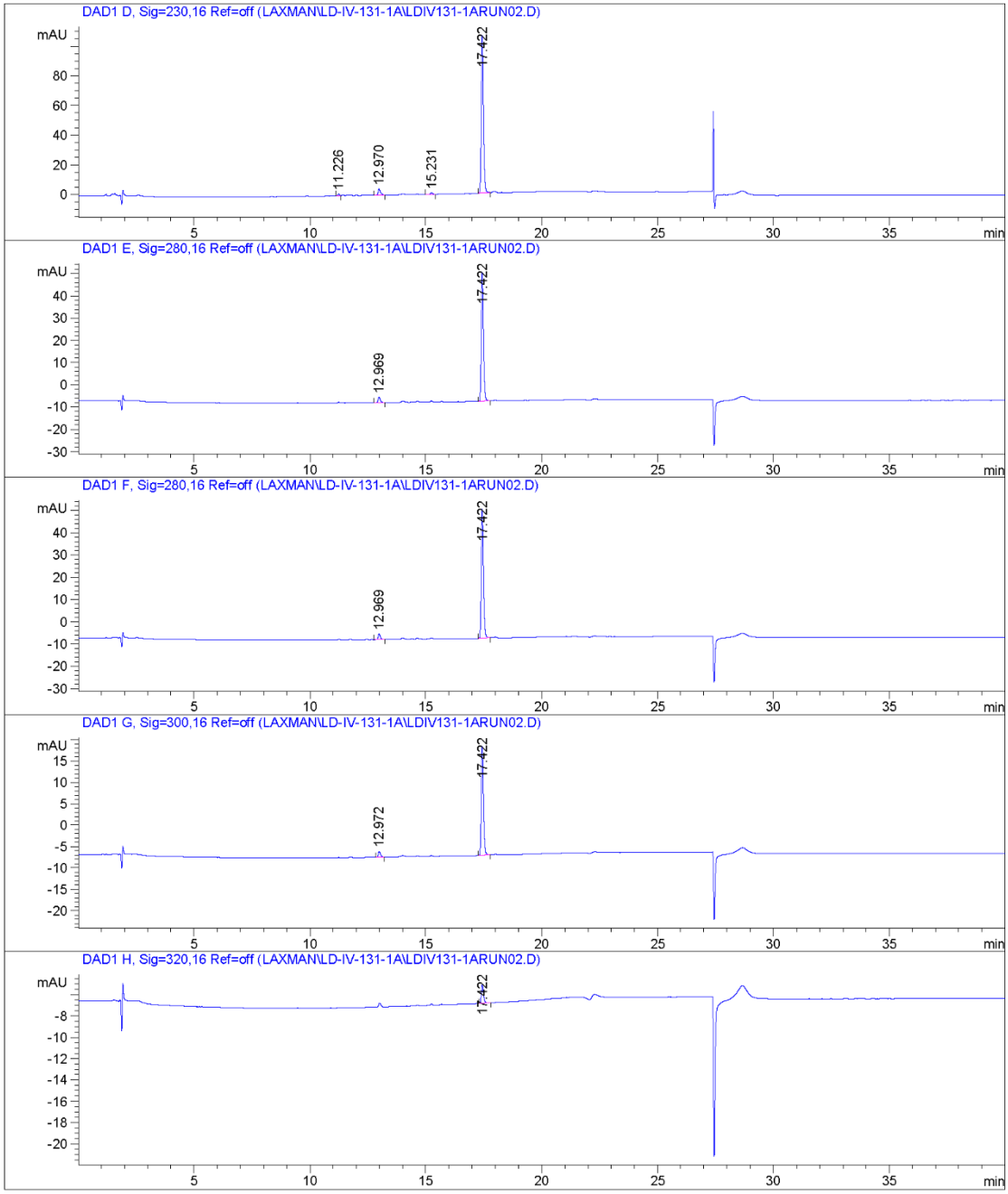


Data File C:\CHEM32\1\DATA\LAXMAN\LD-IV-131-1A\LDIV131-1ARUN02.D
Sample Name: LD-IV-131-1A-rerun-run1



Data File C:\CHEM32\1\DATA\LAXMAN\LD-IV-131-1A\LDIV131-1ARUN02.D
Sample Name: LD-IV-131-1A-rerun-run1

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.968	BB	0.0937	27.01311	4.27345	4.0000
2	17.422	BB	0.0976	648.31488	100.04857	96.0000
Totals :				675.32799	104.32202	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.968	BB	0.0938	26.28994	4.15377	3.9796
2	17.422	BB	0.0976	626.04687	96.60343	94.7661
3	19.829	BB	0.0944	8.28639	1.33497	1.2543
Totals :				660.62320	102.09217	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.227	BV	0.0829	24.89905	4.61790	1.8119
2	12.969	BB	0.1030	61.09814	8.58504	4.4460
3	17.422	BV	0.1000	1288.22766	197.75839	93.7421
Totals :				1374.22486	210.96132	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-IV-131-1A\LDIV131-1ARUN02.D
Sample Name: LD-IV-131-1A-rerun-run1

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	11.226	BB	0.0760	5.55864	1.15744	0.7525
2	12.970	BB	0.1029	31.45657	4.42585	4.2583
3	15.231	BB	0.1021	7.81155	1.13753	1.0575
4	17.422	BV	0.0980	693.87982	106.45760	93.9317
Totals :				738.70657	113.17843	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.969	BB	0.0958	17.15126	2.64083	4.3596
2	17.422	BB	0.0977	376.26514	58.01117	95.6404
Totals :				393.41640	60.65200	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.969	BB	0.0958	17.15126	2.64083	4.3596
2	17.422	BB	0.0977	376.26514	58.01117	95.6404
Totals :				393.41640	60.65200	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	12.972	BB	0.1010	9.45148	1.39534	5.3948
2	17.422	BB	0.0977	165.74431	25.52812	94.6052
Totals :				175.19579	26.92346	

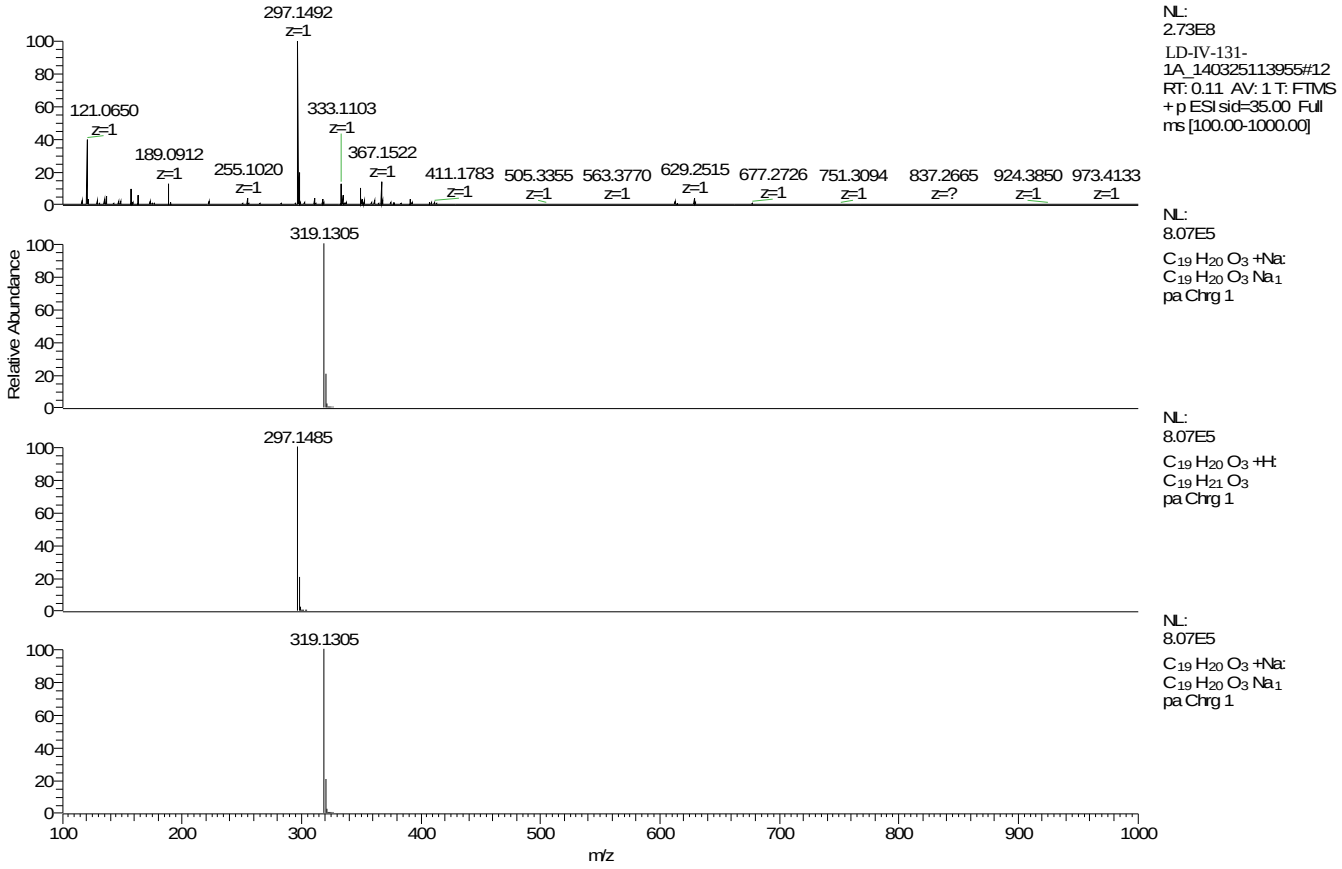
Data File C:\CHEM32\1\DATA\LAXMAN\LD-IV-131-1A\LDIV131-1ARUN02.D
Sample Name: LD-IV-131-1A-rerun-run1

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

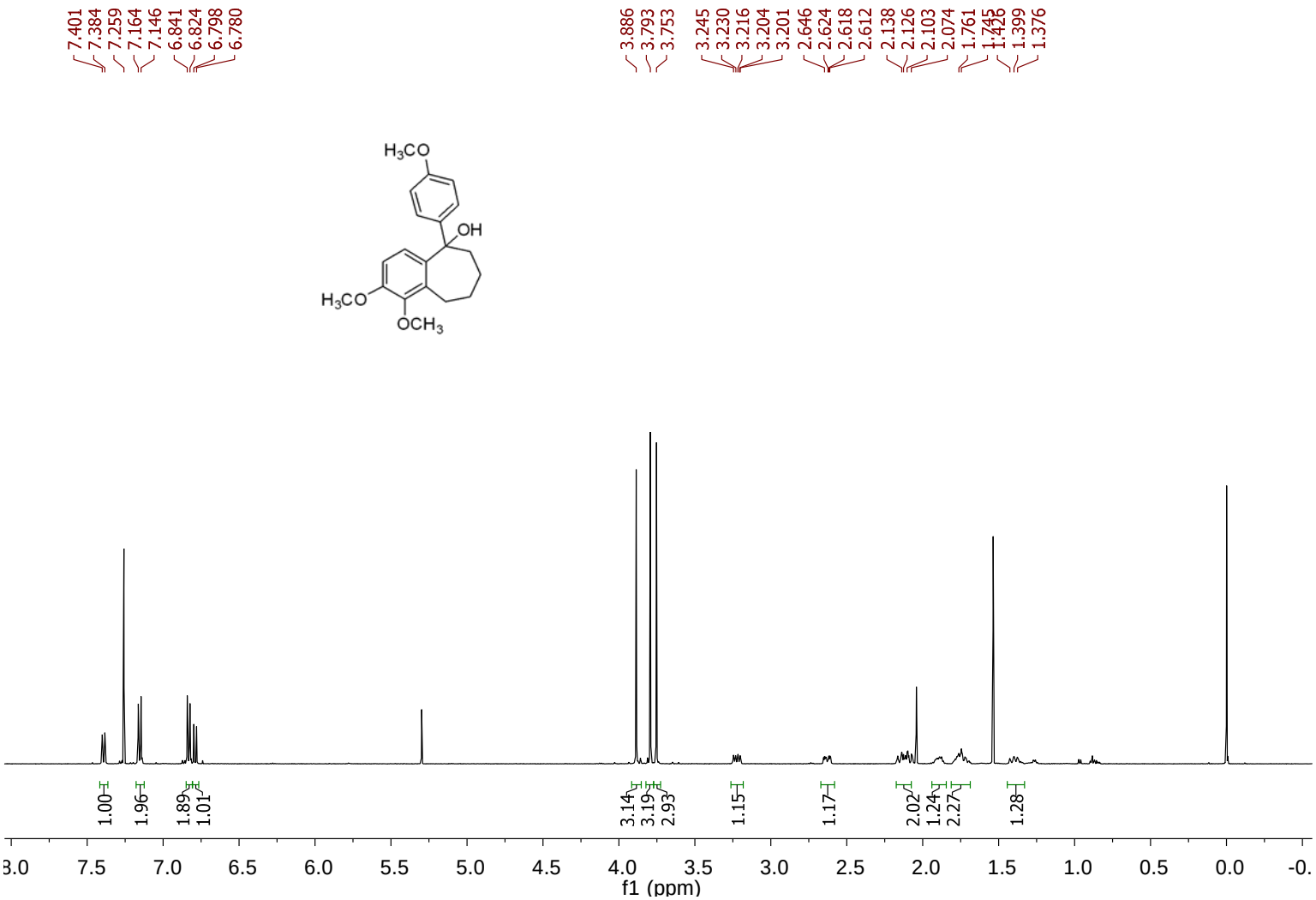
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	17.422	BB	0.1003	11.65500	1.73621	100.0000
Totals :				11.65500	1.73621	

*** End of Report ***

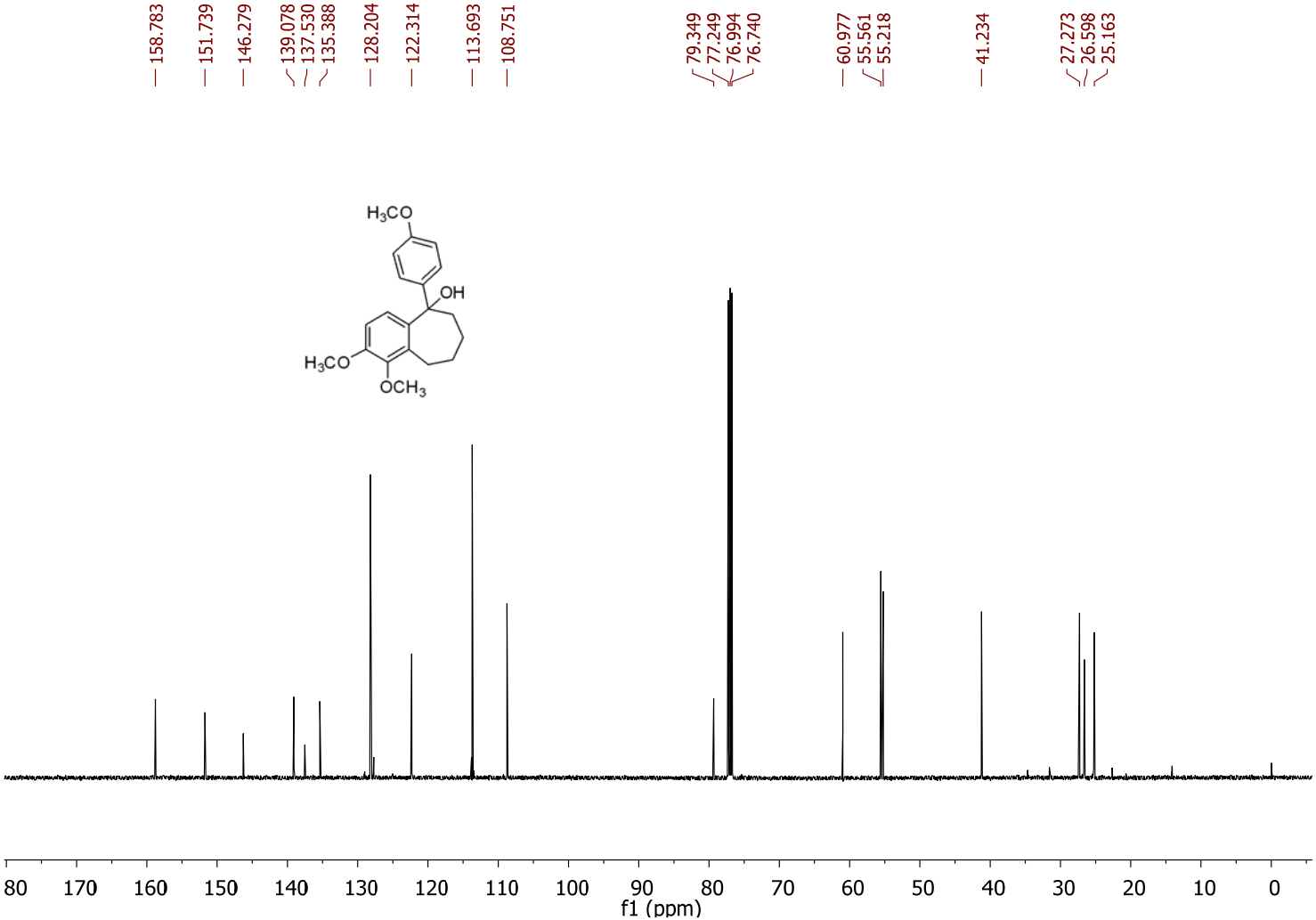
HRMS Traces of Compound **35**



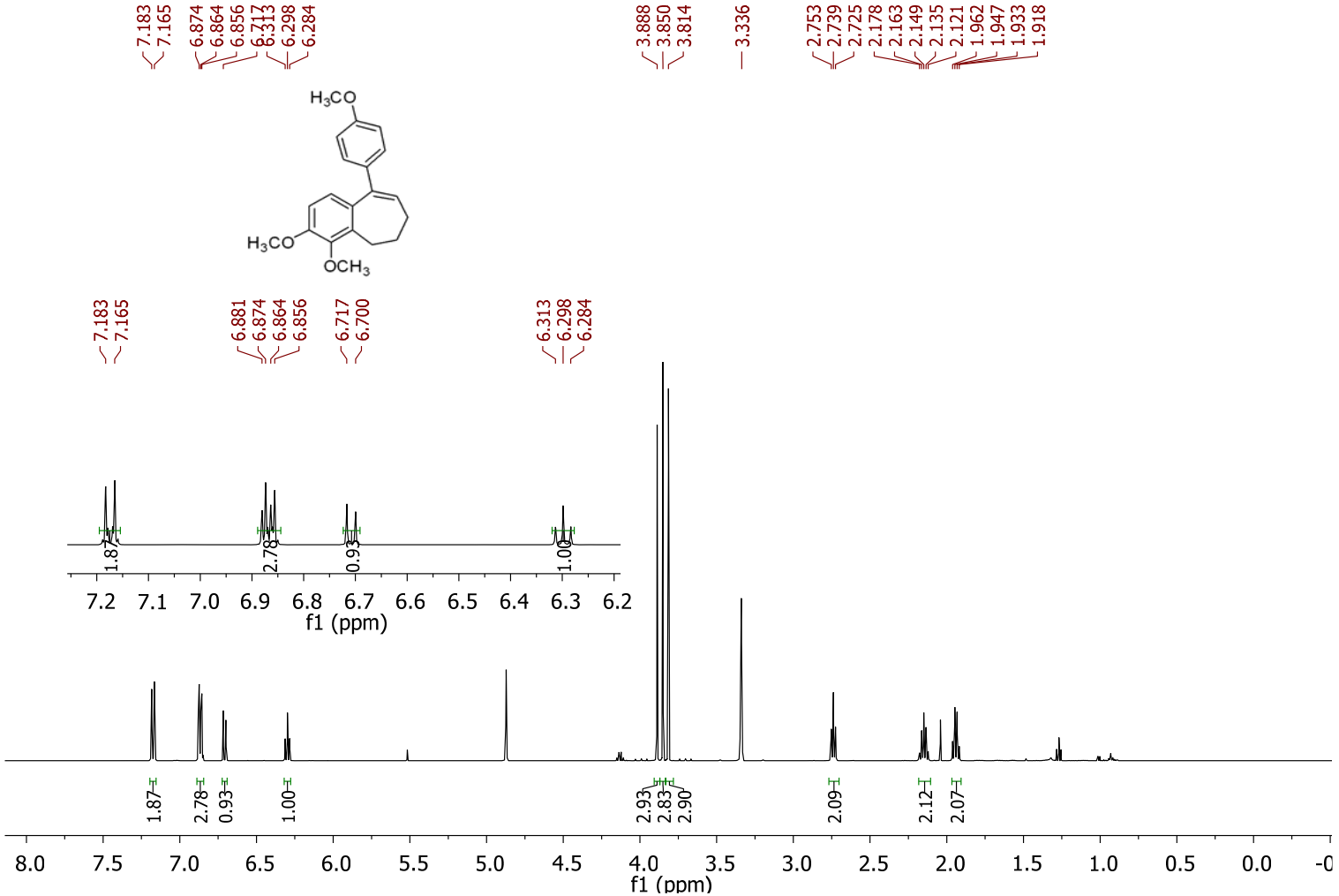
^{1}H NMR (500 MHz, $CDCl_3$) of Compound **24**



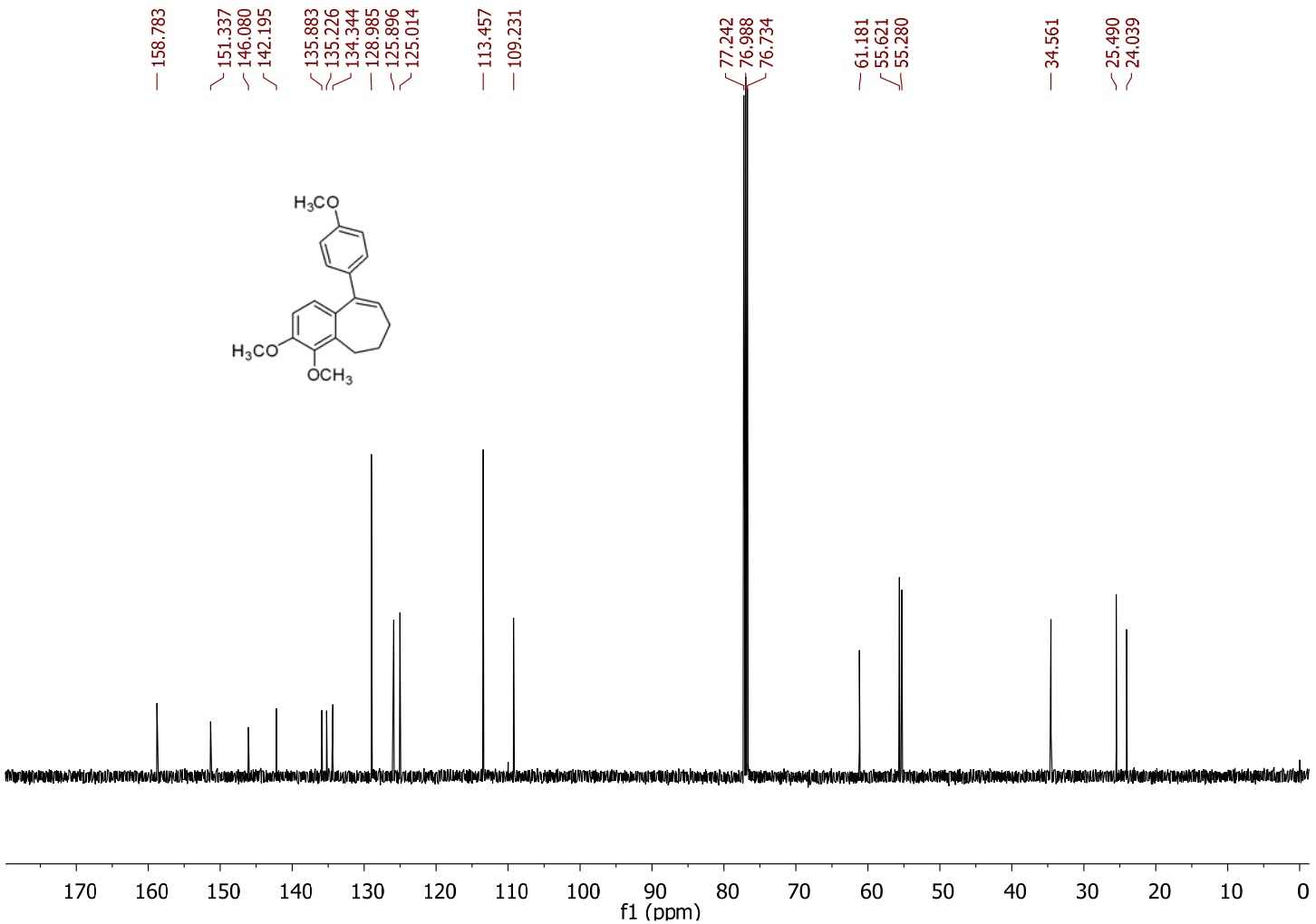
^{13}C NMR (126 MHz, $CDCl_3$) of Compound **24**



^{1}H NMR (500 MHz, CD_3OD) of Compound **30**



^{13}C NMR (126 MHz, $CDCl_3$) of Compound **30**



HPLC Traces of Compound **30**

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Data File C:\CHEM32\1\DATA\LAXMAN\LDIV139-1ARUN01.D
Sample Name: LD-IV-139-1A-1

    ======================================================================
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    Acq. Instrument : Instrument 1                    Location :   -
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    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 1/30/2014 9:43:27 AM by Laxman
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    Last changed    : 1/30/2014 11:06:05 AM by Laxman
    Sample Info     : Run1
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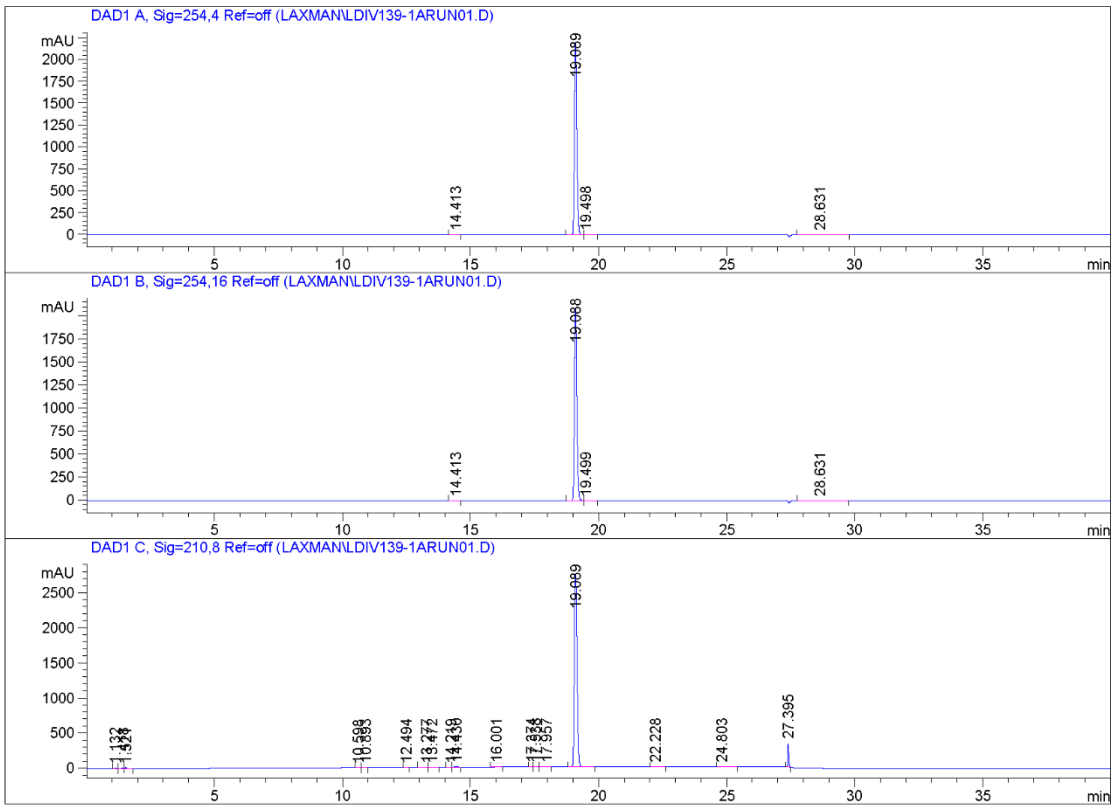
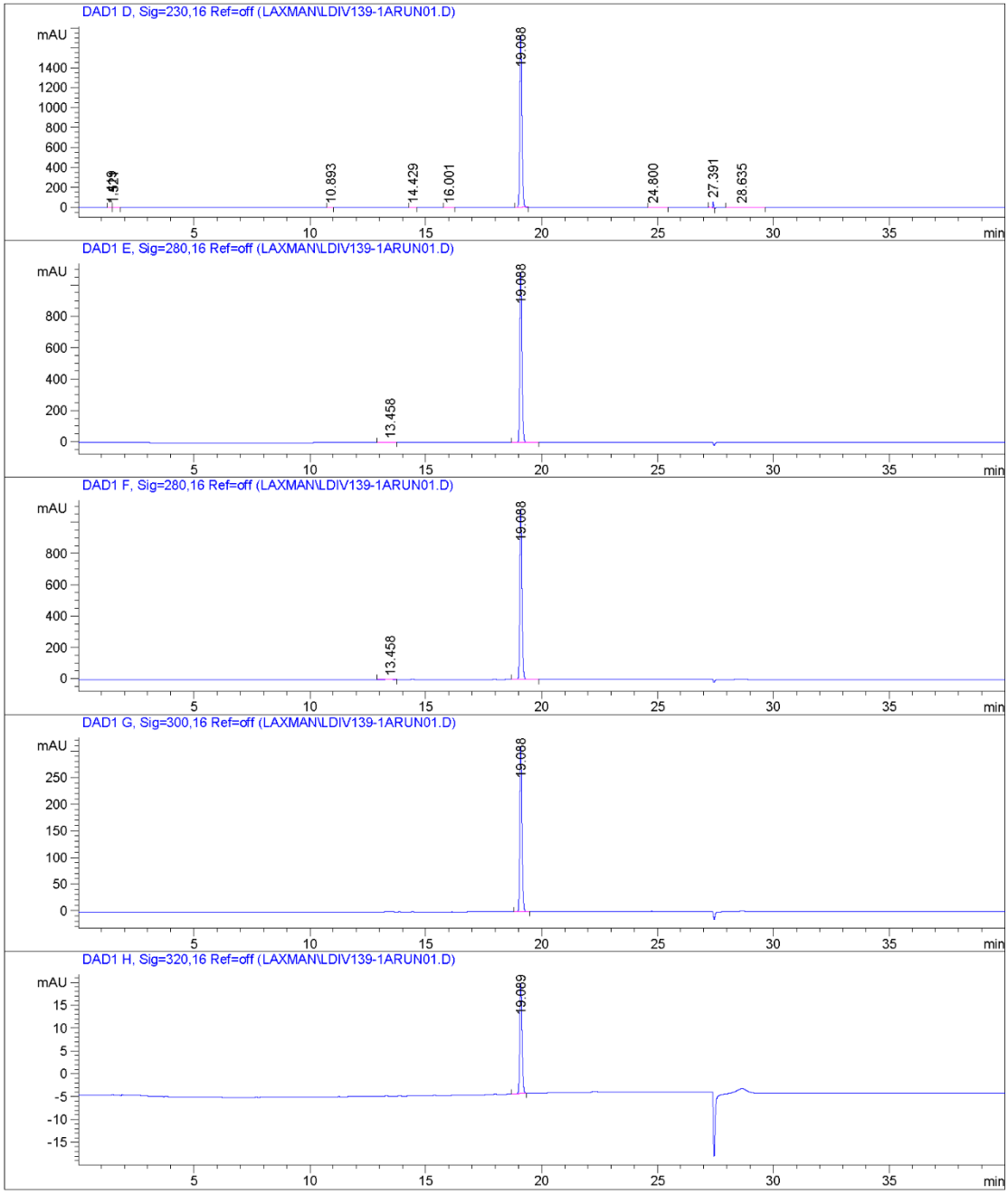


Data File C:\CHEM32\1\DATA\LAXMAN\LDIV139-1ARUN01.D
Sample Name: LD-IV-139-1A-1



Data File C:\CHEM32\1\DATA\LAXMAN\LDIV139-1ARUN01.D
Sample Name: LD-IV-139-1A-1

Area Percent Report

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.413	BB	0.1604	21.42000	2.07520	0.1499
2	19.089	BV	0.0989	1.41765e4	2207.01196	99.1916
3	19.498	VB	0.1340	18.92470	1.93655	0.1324
4	28.631	BB	0.4860	75.18730	2.19272	0.5261
Totals :				1.42921e4	2213.21642	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.413	BB	0.1601	19.95540	1.93899	0.1477
2	19.088	BV	0.0986	1.34034e4	2097.21265	99.1985
3	19.499	VB	0.1348	17.67035	1.79536	0.1308
4	28.631	BB	0.4580	70.67089	2.11384	0.5230
Totals :				1.35117e4	2103.06084	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.132	BB	0.0614	14.57730	3.58883	0.0679
2	1.418	BV	0.0759	48.01664	10.02032	0.2238
3	1.521	VB	0.0610	54.63629	13.01121	0.2547
4	10.598	BB	0.0938	8.46140	1.41429	0.0394
5	10.893	BB	0.0874	14.87960	2.57484	0.0694
6	12.494	BB	0.1000	8.76446	1.38110	0.0409
7	13.277	BV	0.1336	24.42828	2.55433	0.1139
8	13.472	VV	0.2102	32.02443	2.02785	0.1493
9	14.219	BV	0.0878	7.31985	1.29744	0.0341
10	14.430	VB	0.1658	35.88203	3.38270	0.1673

Data File C:\CHEM32\1\DATA\LAXMAN\LDIV139-1ARUN01.D
Sample Name: LD-IV-139-1A-1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
11	16.001	BB	0.1367	17.37272	1.73647	0.0810
12	17.374	BV	0.0930	7.81489	1.32111	0.0364
13	17.538	VB	0.0939	12.18892	1.97756	0.0568
14	17.957	BB	0.1325	13.73361	1.53351	0.0640
15	19.089	BB	0.1154	2.01518e4	2751.83130	93.9339
16	22.228	BB	0.1848	28.84549	2.29175	0.1345
17	24.803	BB	0.1325	21.64288	2.41514	0.1009
18	27.395	BB	0.0445	950.77936	341.76141	4.4319
Totals :				2.14531e4	3146.12117	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.419	BV	0.0785	8.55047	1.64809	0.0759
2	1.521	VB	0.0891	11.86712	1.79461	0.1053
3	10.893	BB	0.0867	7.44412	1.30266	0.0661
4	14.429	VB	0.1630	15.97235	1.53950	0.1418
5	16.001	BB	0.1503	12.82694	1.16594	0.1138
6	19.088	BB	0.0976	1.09185e4	1729.82043	96.9030
7	24.800	BB	0.1450	15.16185	1.53794	0.1346
8	27.391	BB	0.0488	207.03316	65.66882	1.8374
9	28.635	BB	0.4285	70.09544	2.34659	0.6221
Totals :				1.12674e4	1806.82458	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.458	BB	0.2502	33.25743	1.77782	0.4806
2	19.088	BB	0.0978	6887.35742	1088.69373	99.5194
Totals :				6920.61486	1090.47154	

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

Data File C:\CHEM32\1\DATA\LAXMAN\LDIV139-1ARUN01.D
Sample Name: LD-IV-139-1A-1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.458	BB	0.2502	33.25743	1.77782	0.4806
2	19.088	BB	0.0978	6887.35742	1088.69373	99.5194
Totals :				6920.61486	1090.47154	

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

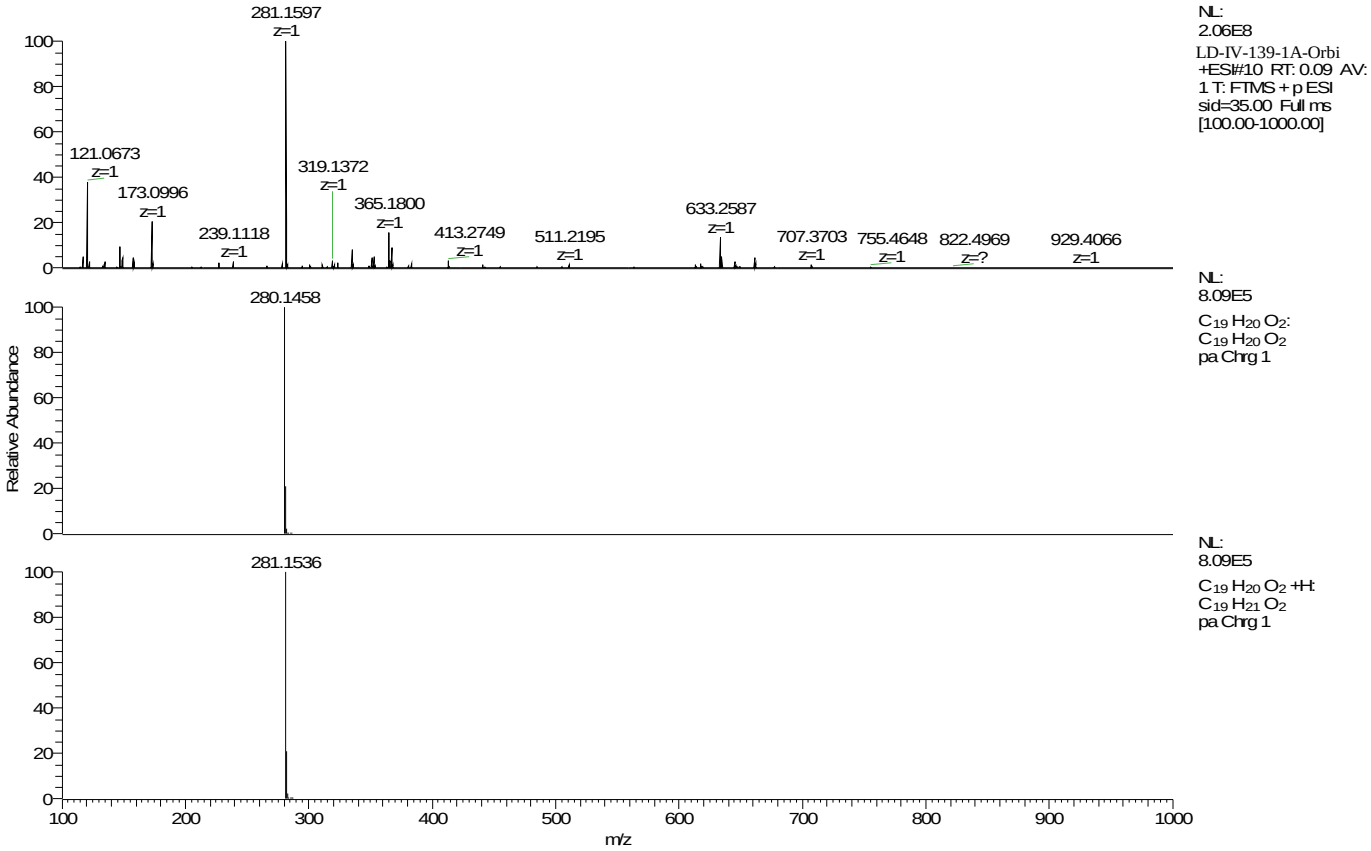
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.088	BB	0.0978	1975.91748	312.50003	100.0000
Totals :				1975.91748	312.50003	

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

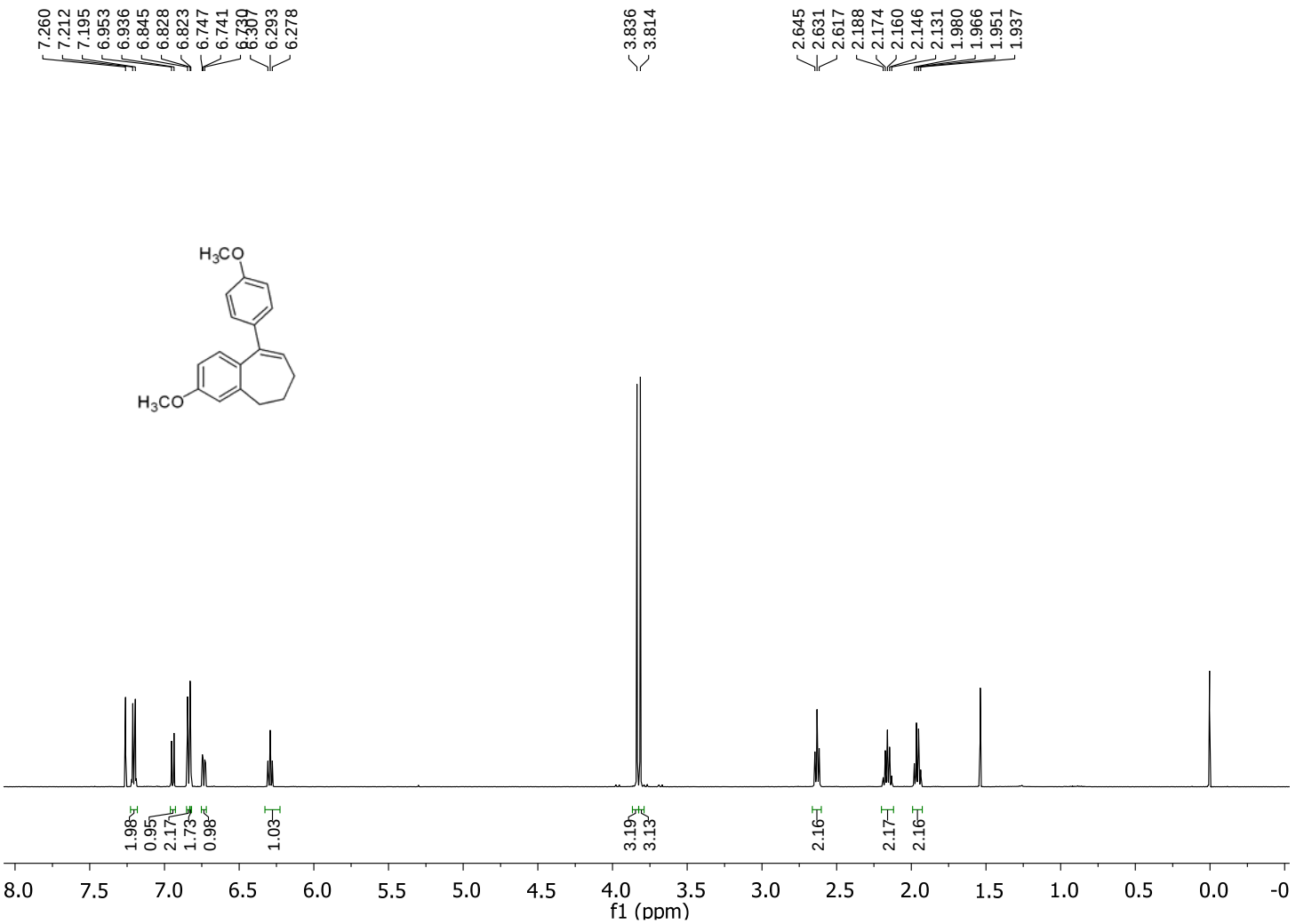
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	19.089	BB	0.1021	162.21338	24.24150	100.0000
Totals :				162.21338	24.24150	

*** End of Report ***

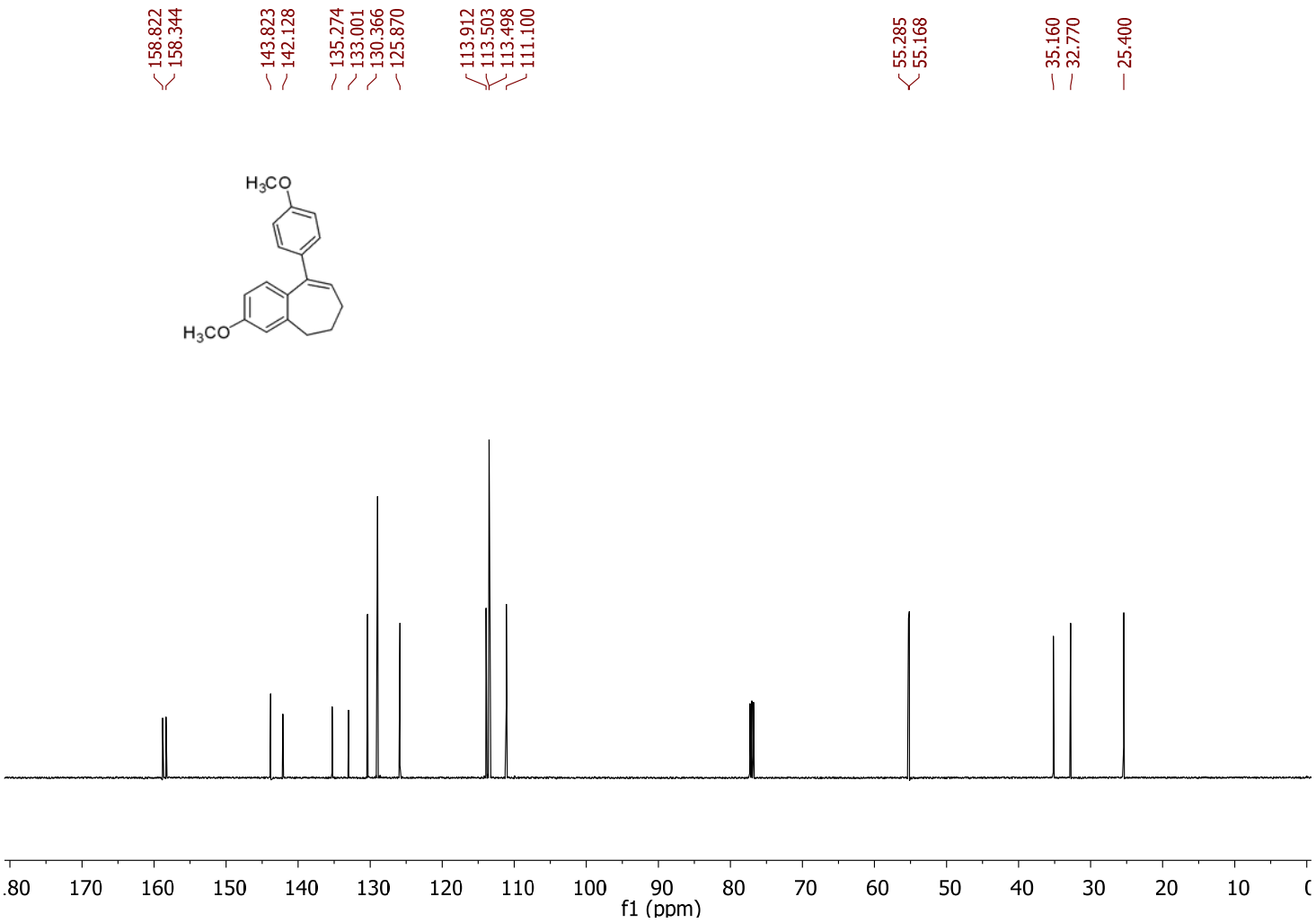
HRMS of Compound **30**



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



^{13}C NMR (126 MHz, $CDCl_3$) of Compound **36**



HPLC Traces of Compound **36**

```
Data File C:\CHEM32\1\DATA\LAXMAN\LDIV137-1A-RUN1.D
Sample Name: LD-IV-137-1A-Run1

    ======================================================================
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    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 1/24/2014 4:55:16 PM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 1/24/2014 4:30:40 PM by Laxman
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    Last changed    : 1/24/2014 6:21:25 PM by Laxman
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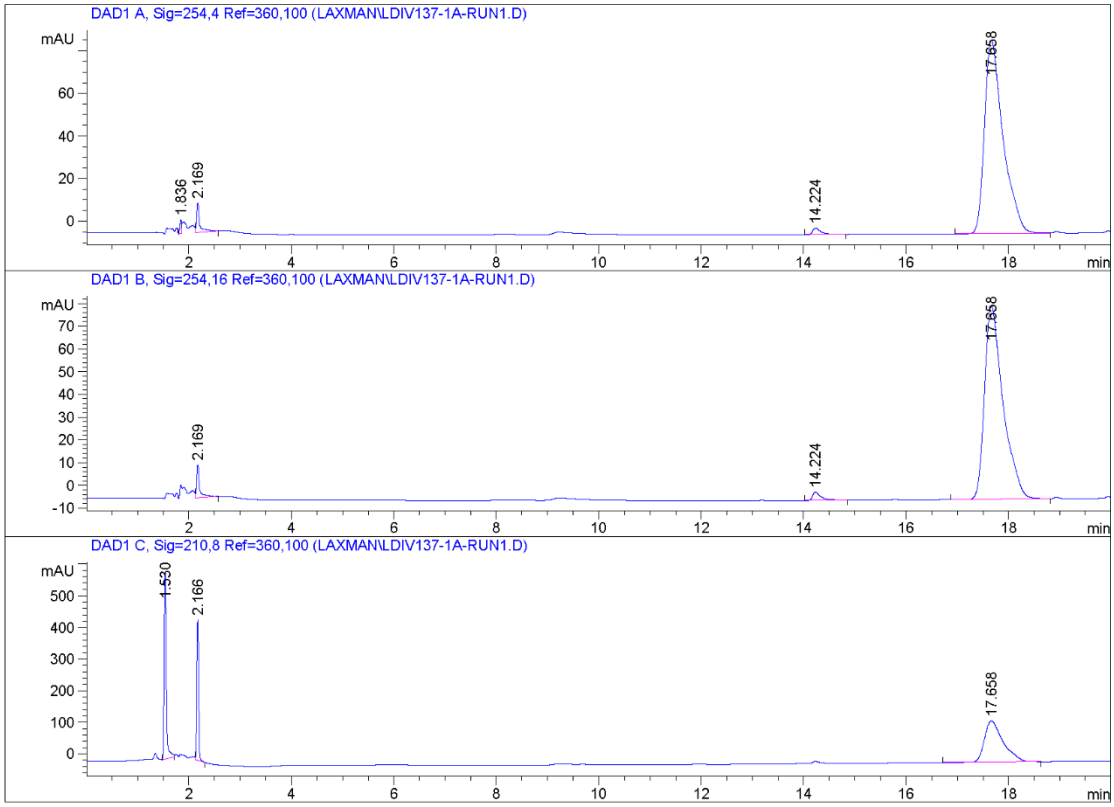
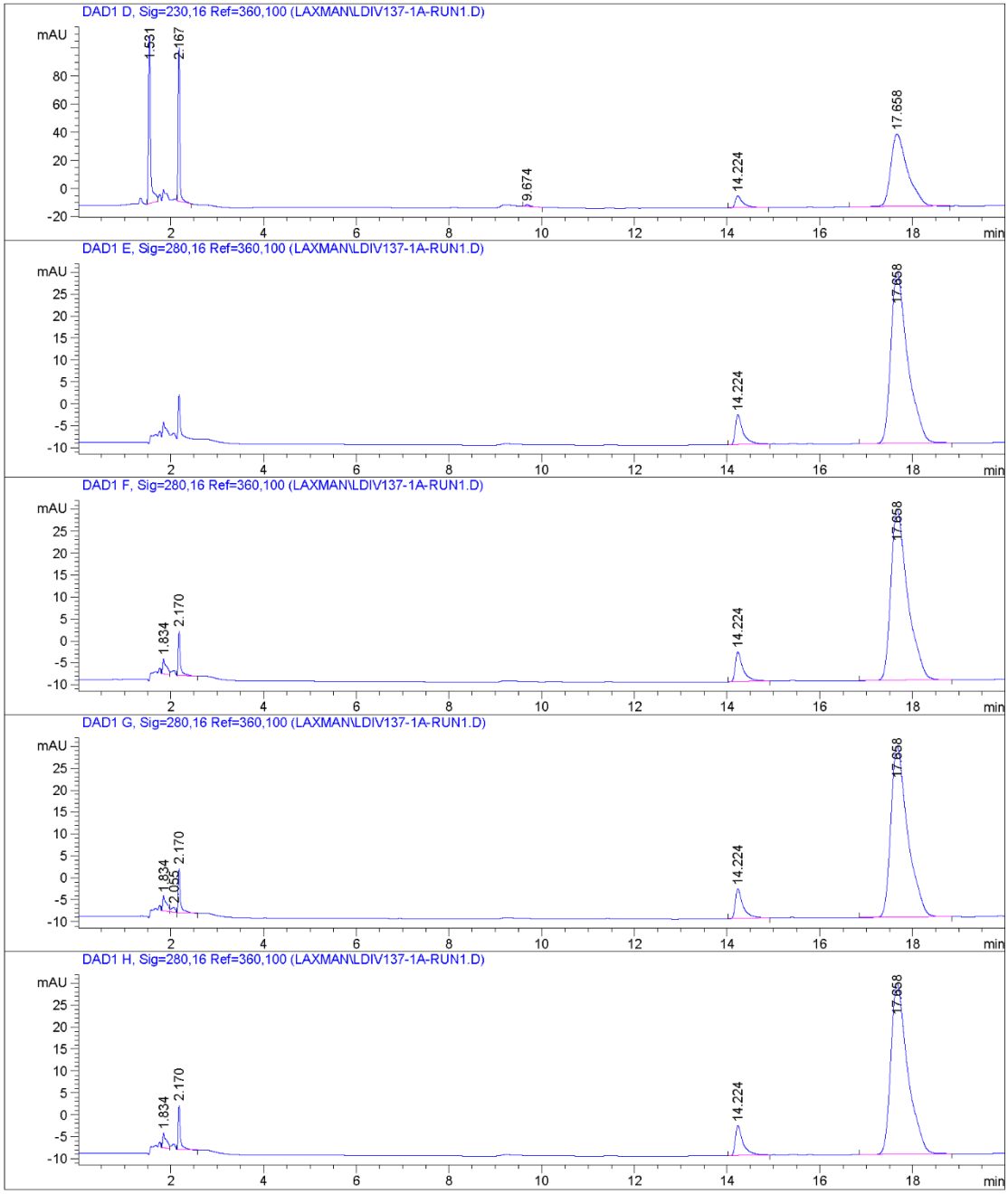


Data File C:\CHEM32\1\DATA\LAXMAN\LDIV137-1A-RUN1.D
Sample Name: LD-IV-137-1A-Run1



Data File C:\CHEM32\1\DATA\LAXMAN\LDIV137-1A-RUN1.D
Sample Name: LD-IV-137-1A-Run1

==========

Area Percent Report

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

Signal 1: DAD1 A, Sig=254,4 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.836	BV	0.0409	18.19539	6.45327	0.7590
2	2.169	VB	0.0613	58.36508	13.82253	2.4345
3	14.224	BB	0.1651	36.58915	3.20765	1.5262
4	17.658	BV	0.3693	2284.27368	90.85317	95.2804
Totals :				2397.42330	114.33662	

Signal 2: DAD1 B, Sig=254,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.169	VB	0.0590	58.52003	14.53531	2.6126
2	14.224	BB	0.1630	43.06221	3.77694	1.9225
3	17.658	BV	0.3693	2138.35132	85.04366	95.4649
Totals :				2239.93356	103.35591	

Signal 3: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.530	BV	0.0460	1748.86523	600.82697	28.3435
2	2.166	VB	0.0416	1125.40662	444.25931	18.2392
3	17.658	BB	0.3717	3295.98486	130.90834	53.4173
Totals :				6170.25671	1175.99461	

Data File C:\CHEM32\1\DATA\LAXMAN\LDIV137-1A-RUN1.D
Sample Name: LD-IV-137-1A-Run1

Signal 4: DAD1 D, Sig=230,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.531	BV	0.0484	378.01849	121.24623	18.1187
2	2.167	VB	0.0430	291.49344	109.53181	13.9715
3	9.674	BB	0.1070	11.05938	1.48353	0.5301
4	14.224	BB	0.1651	97.43915	8.54247	4.6703
5	17.658	BB	0.3736	1308.33740	51.63680	62.7095
Totals :				2086.34786	292.44084	

Signal 5: DAD1 E, Sig=280,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.224	BB	0.1659	78.54280	6.84231	7.4125
2	17.658	BB	0.3691	981.05591	39.04969	92.5875
Totals :				1059.59871	45.89200	

Signal 6: DAD1 F, Sig=280,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.834	BV	0.0789	19.77675	3.44678	1.7756
2	2.170	BB	0.0512	34.41515	10.23419	3.0899
3	14.224	BB	0.1659	78.54280	6.84231	7.0518
4	17.658	BB	0.3691	981.05591	39.04969	88.0826
Totals :				1113.79061	59.57297	

Signal 7: DAD1 G, Sig=280,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.834	BV	0.0789	19.77675	3.44678	1.7652
2	2.055	VB	0.0840	6.56384	1.16043	0.5859
3	2.170	BB	0.0512	34.41515	10.23419	3.0718
4	14.224	BB	0.1659	78.54280	6.84231	7.0105
5	17.658	BB	0.3691	981.05591	39.04969	87.5666

Data File C:\CHEM32\1\DATA\LAXMAN\LDIV137-1A-RUN1.D
Sample Name: LD-IV-137-1A-Run1

Totals :	1120.35444	60.73339

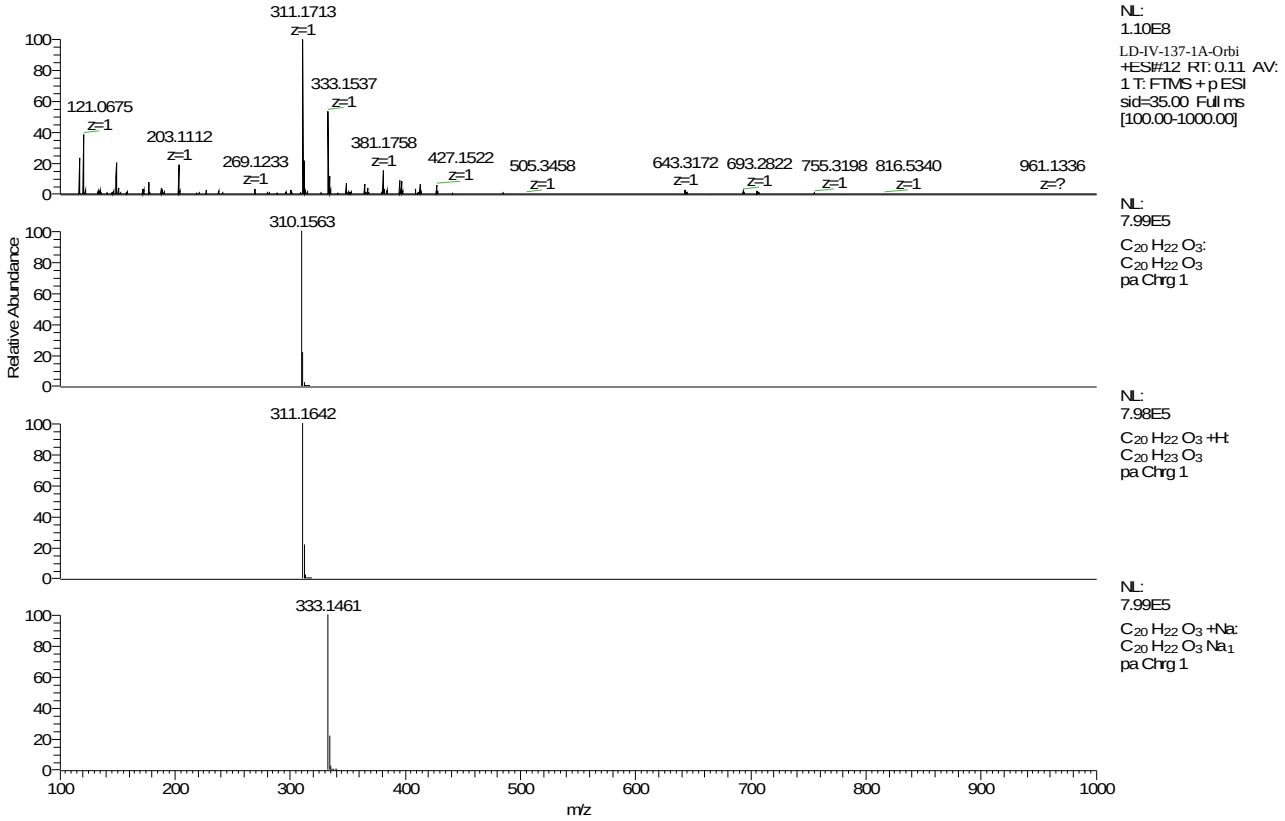
Signal 8: DAD1 H, Sig=280,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.834	BV	0.0789	19.77675	3.44678	1.7756
2	2.170	BB	0.0512	34.41515	10.23419	3.0899
3	14.224	BB	0.1659	78.54280	6.84231	7.0518
4	17.658	BB	0.3691	981.05591	39.04969	88.0826

Totals :	1113.79061	59.57297

*** End of Report ***

HRMS Traces of Compound **36**

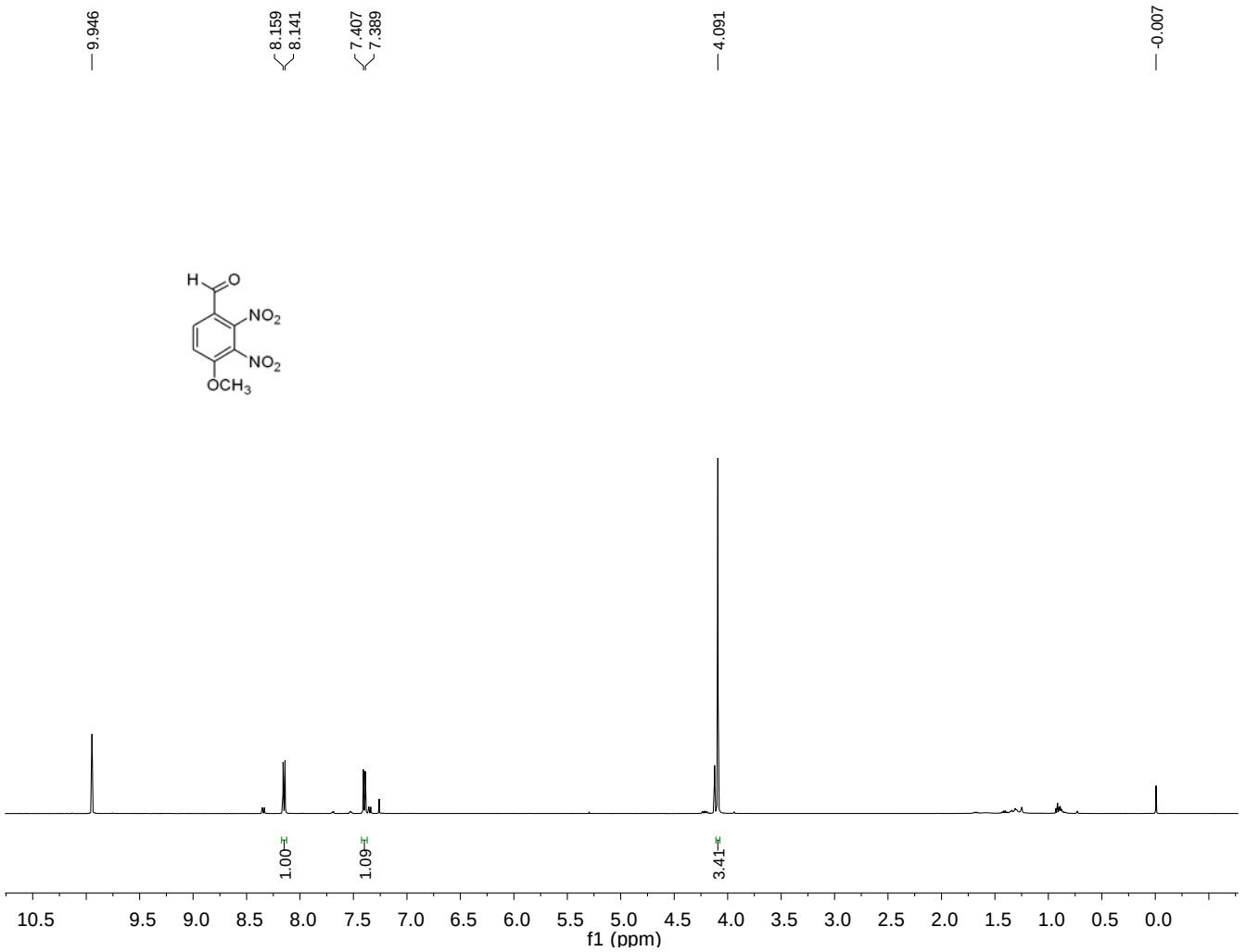


APPENDIX D

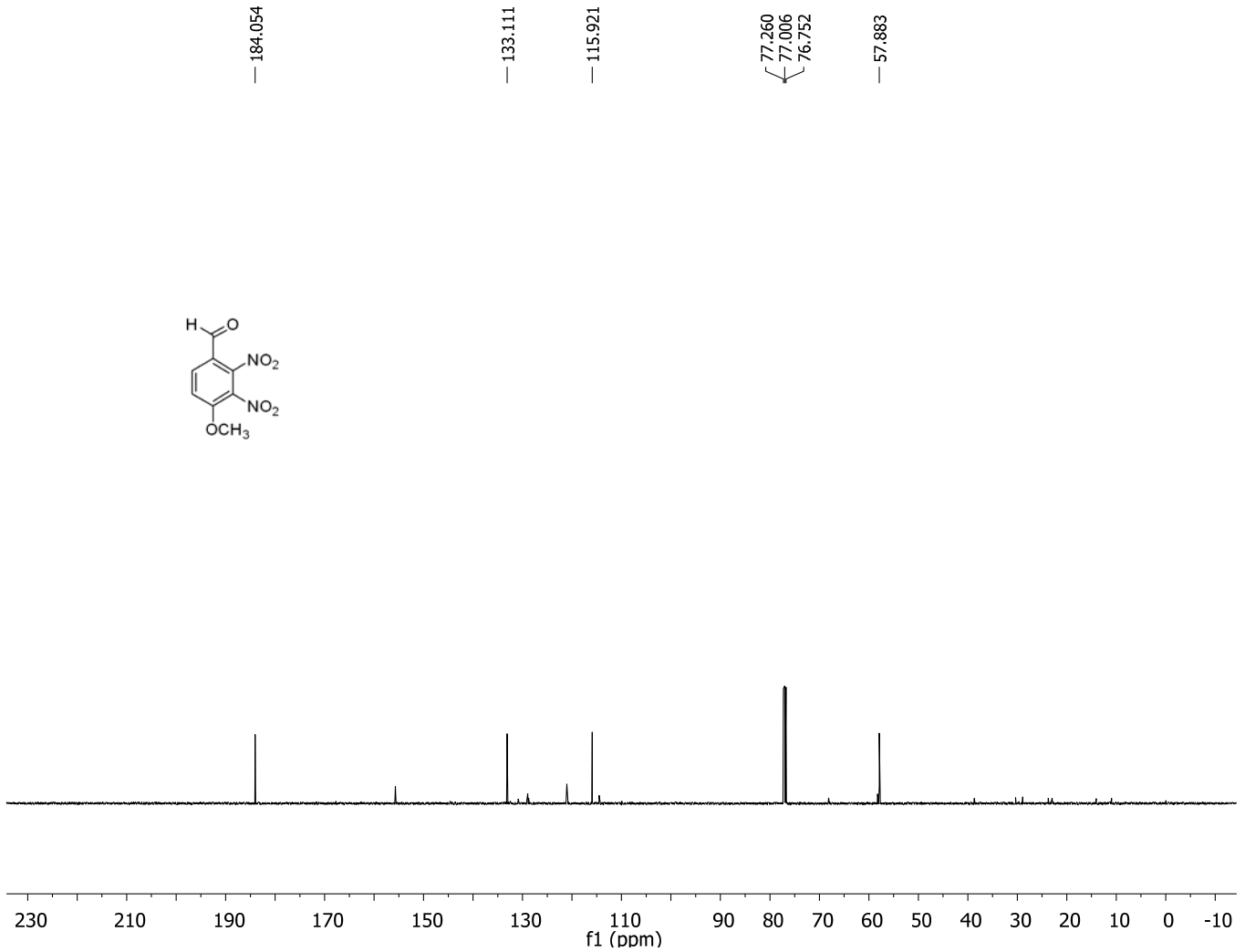
Synthesis of ^{19}F Analogues of Amino-Based Combretastatins

Table of Contents

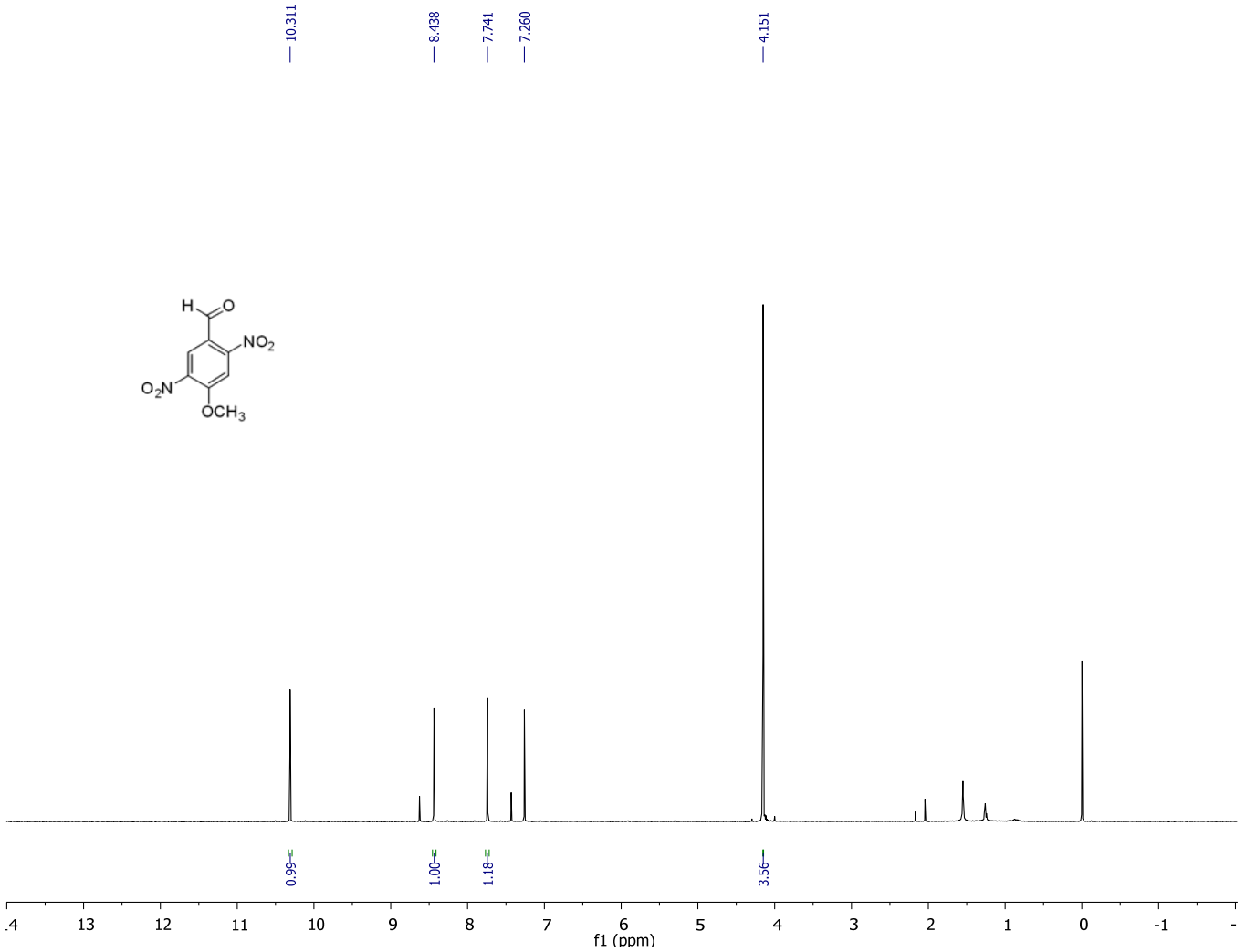
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **2**



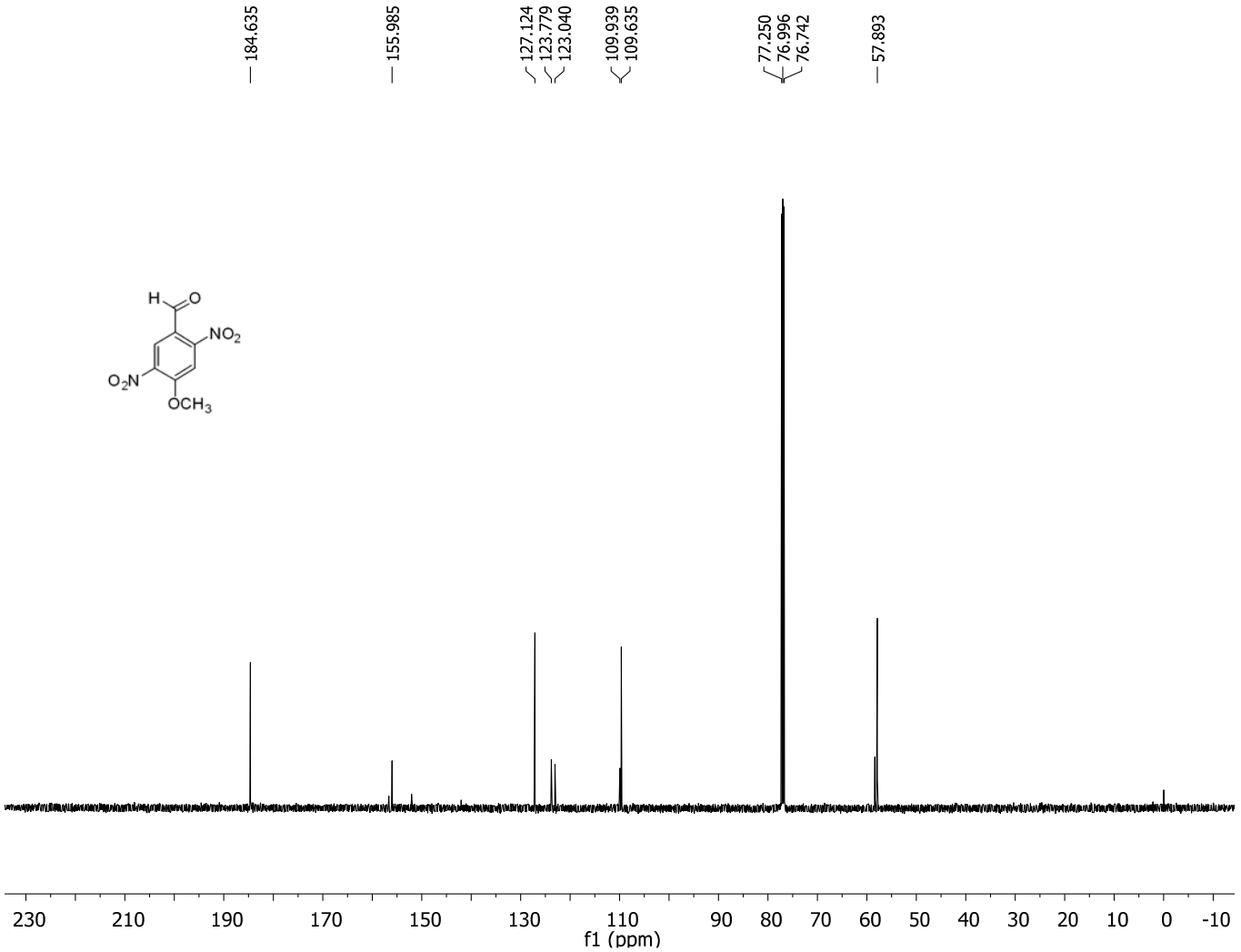
^{13} C NMR (126 MHz, $CDCl_3$) for Compound **2**



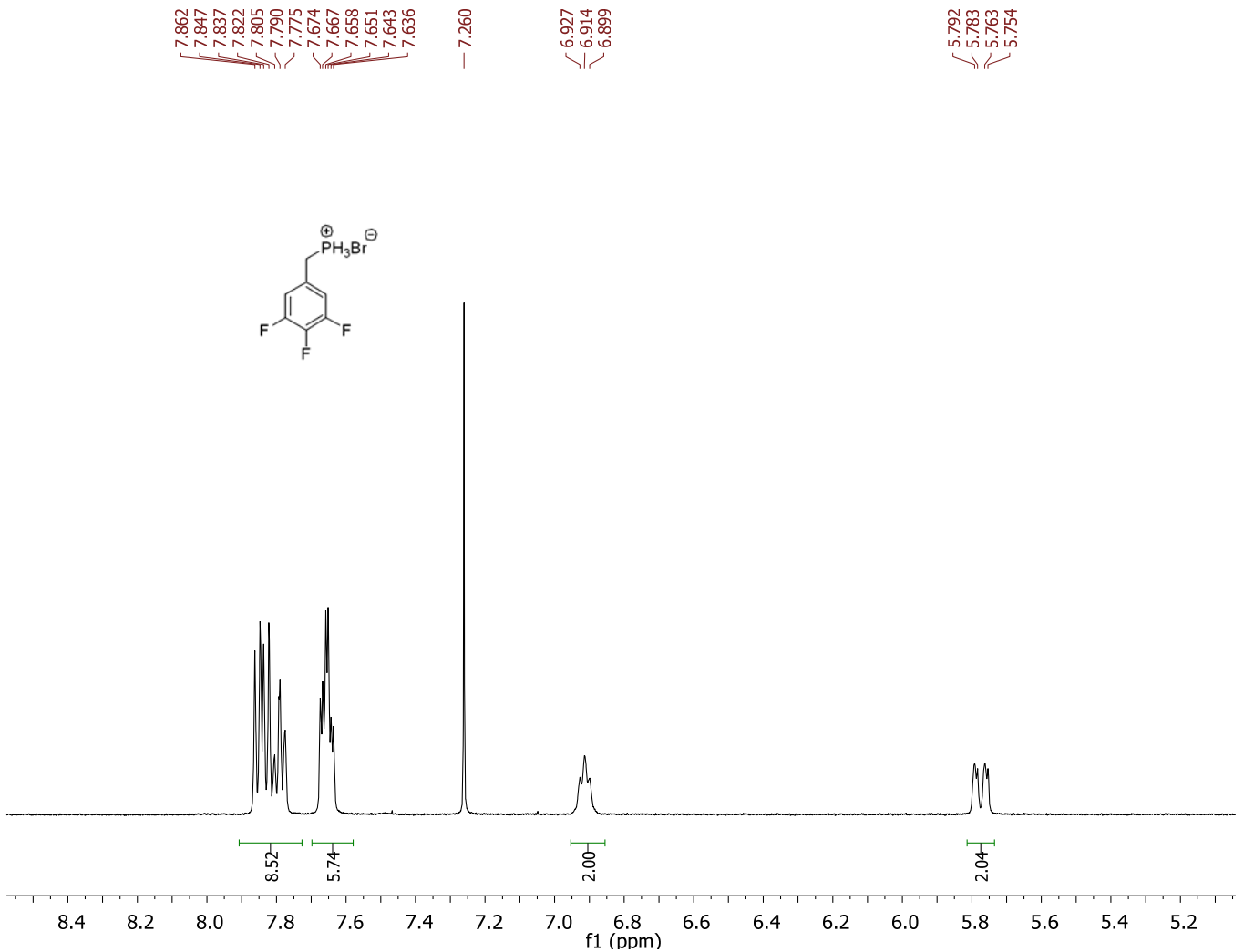
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **3**



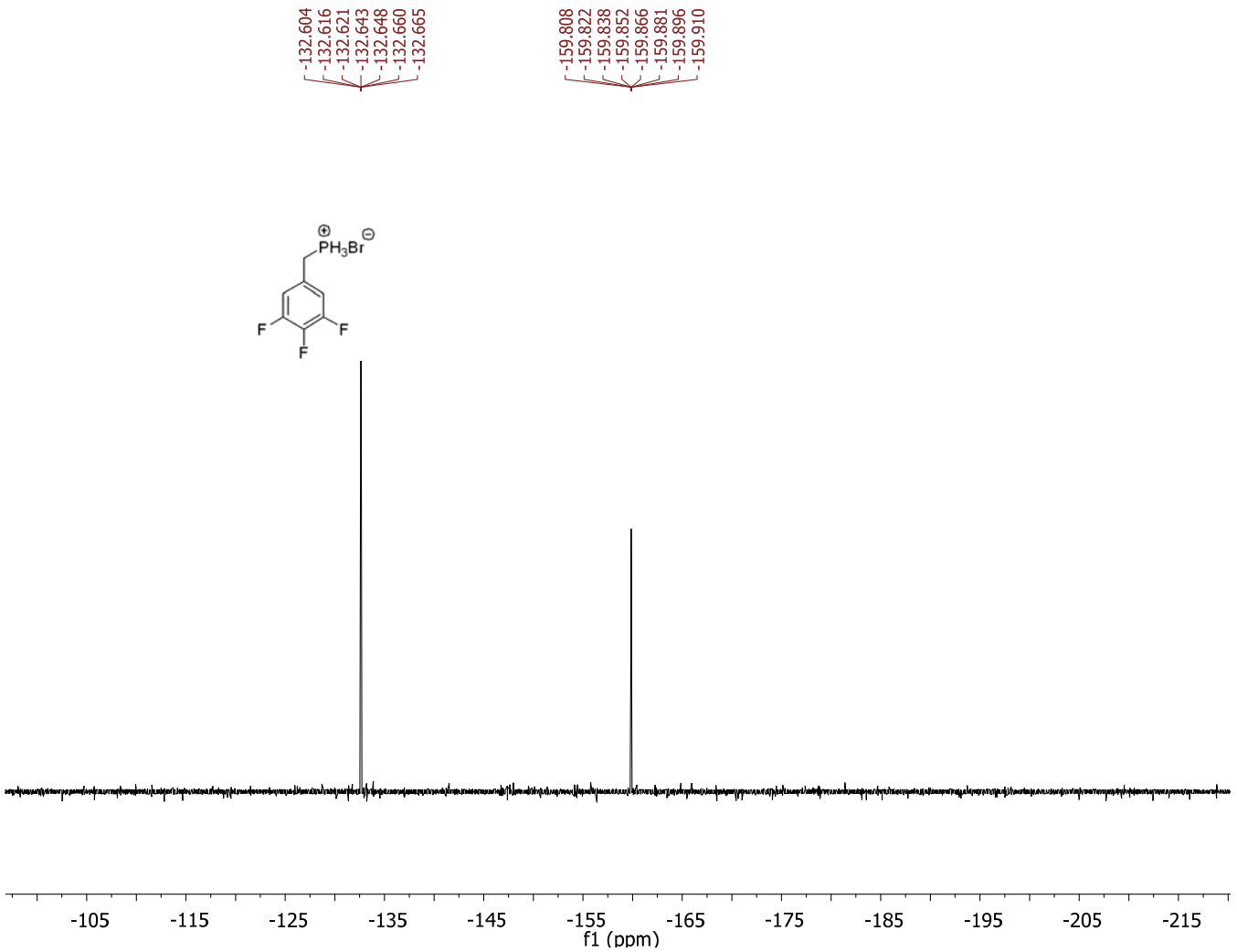
^{13} C NMR (126 MHz, $CDCl_3$) for Compound **3**



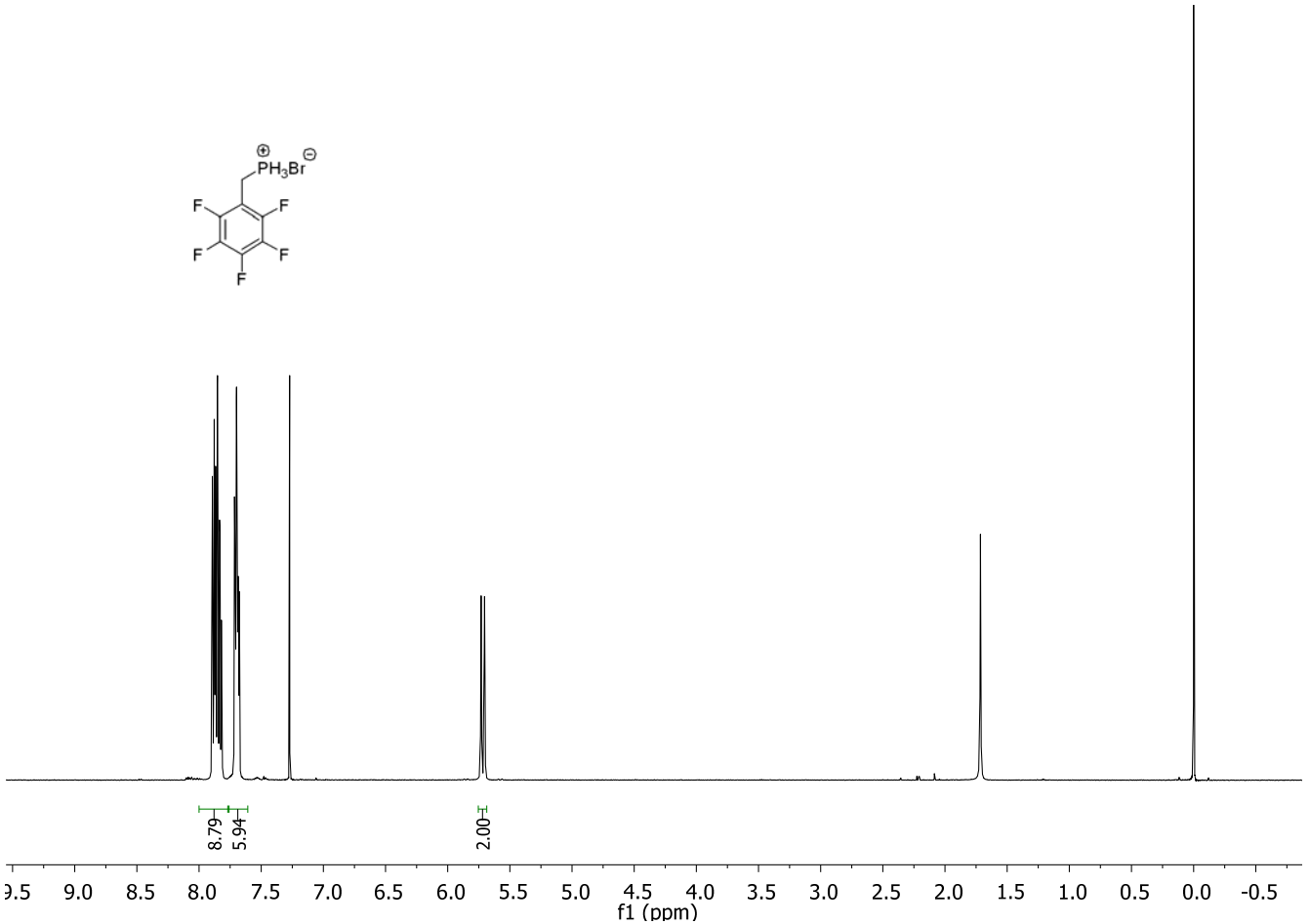
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **4**



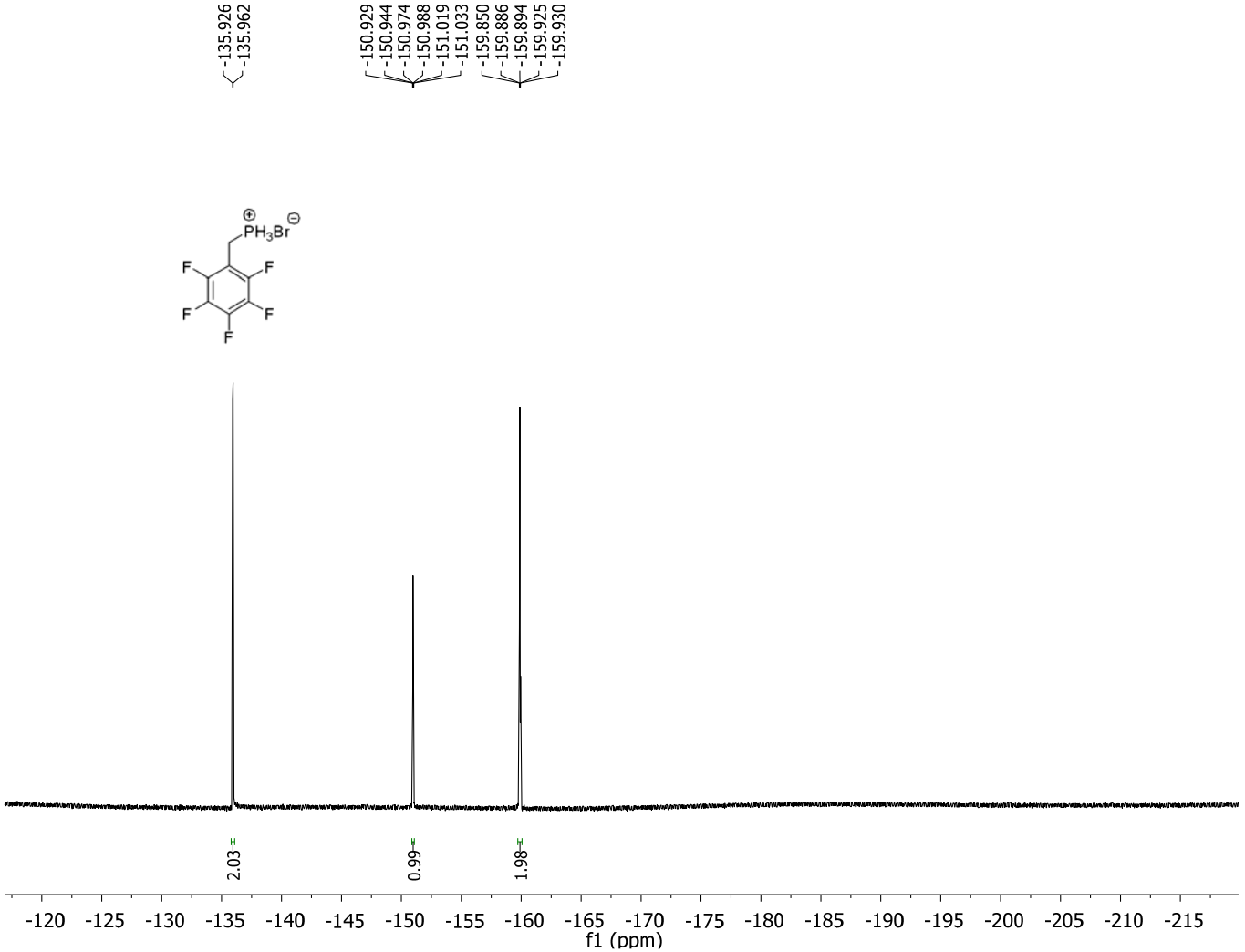
^{19}F NMR (470 MHz, $CDCl_3$) for Compound **4**



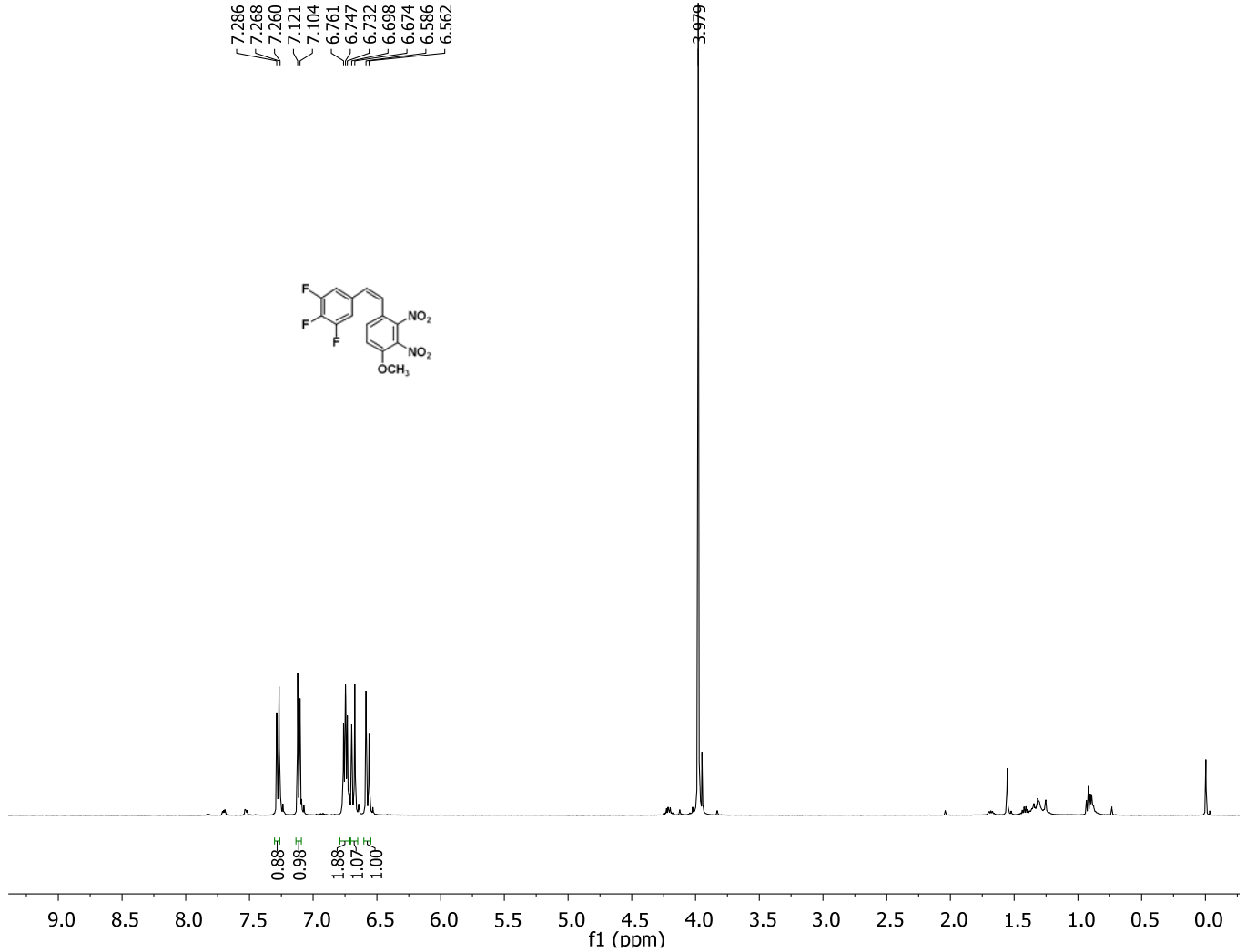
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **5**



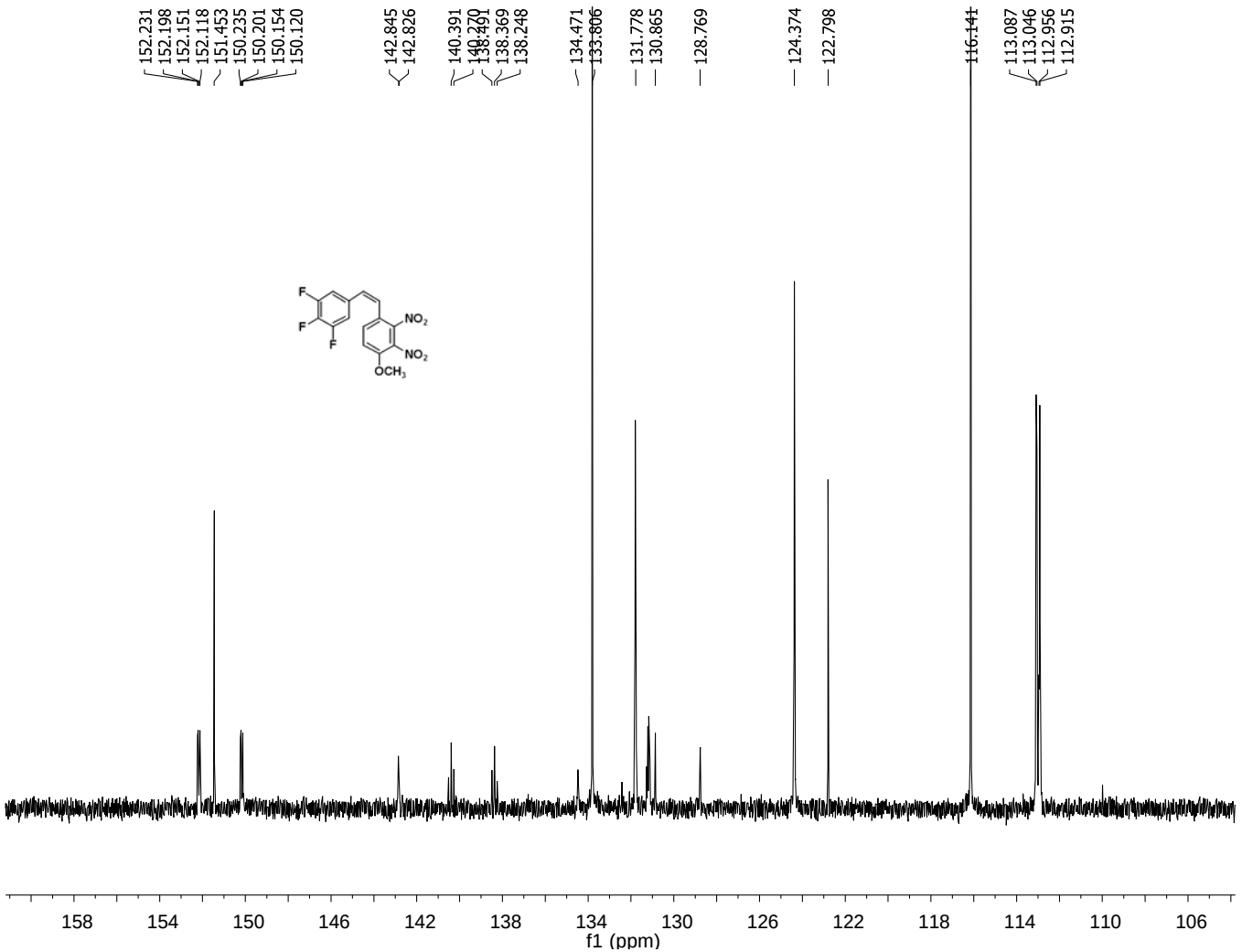
^{19}F NMR (470 MHz, $CDCl_3$) for Compound **5**



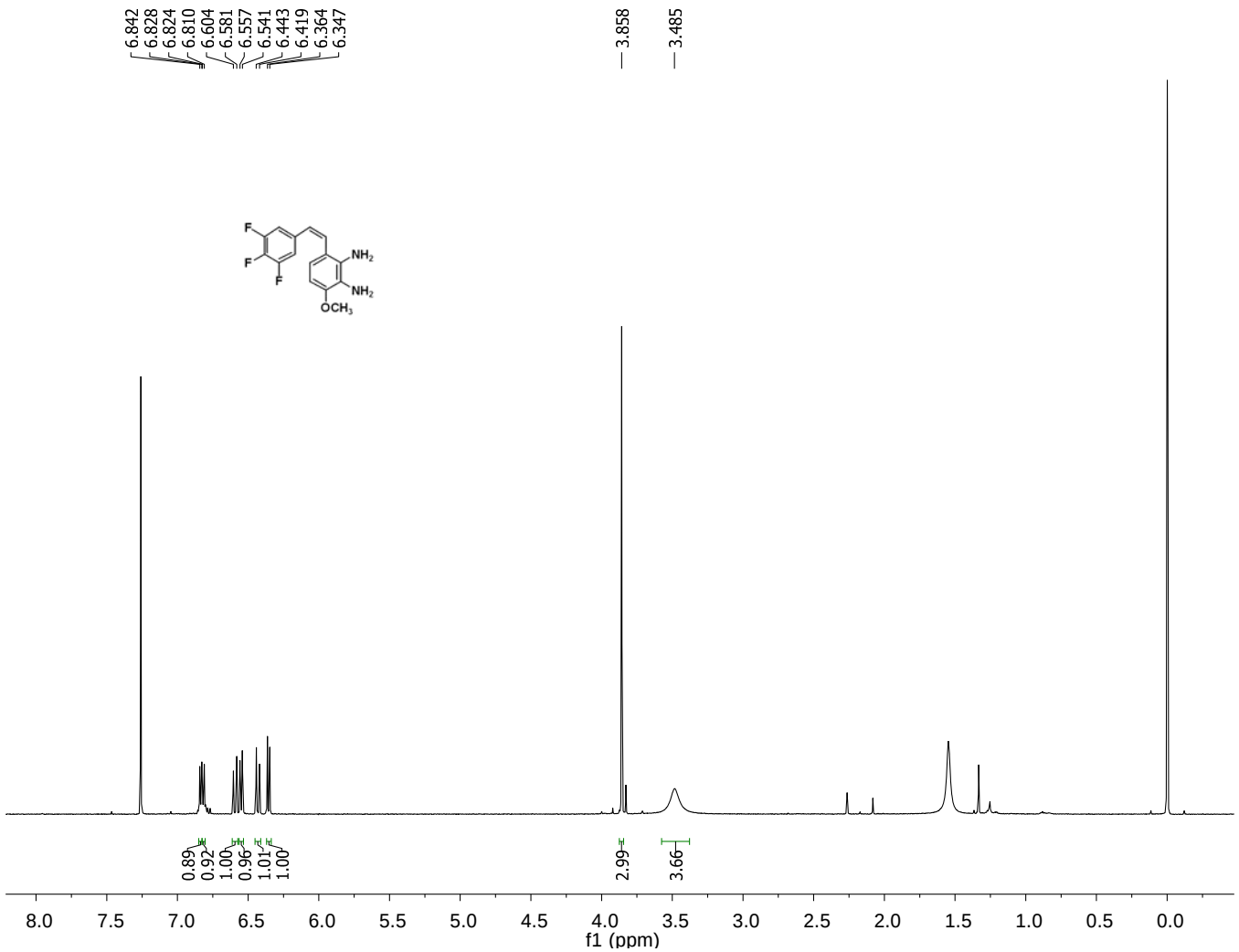
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **6a**



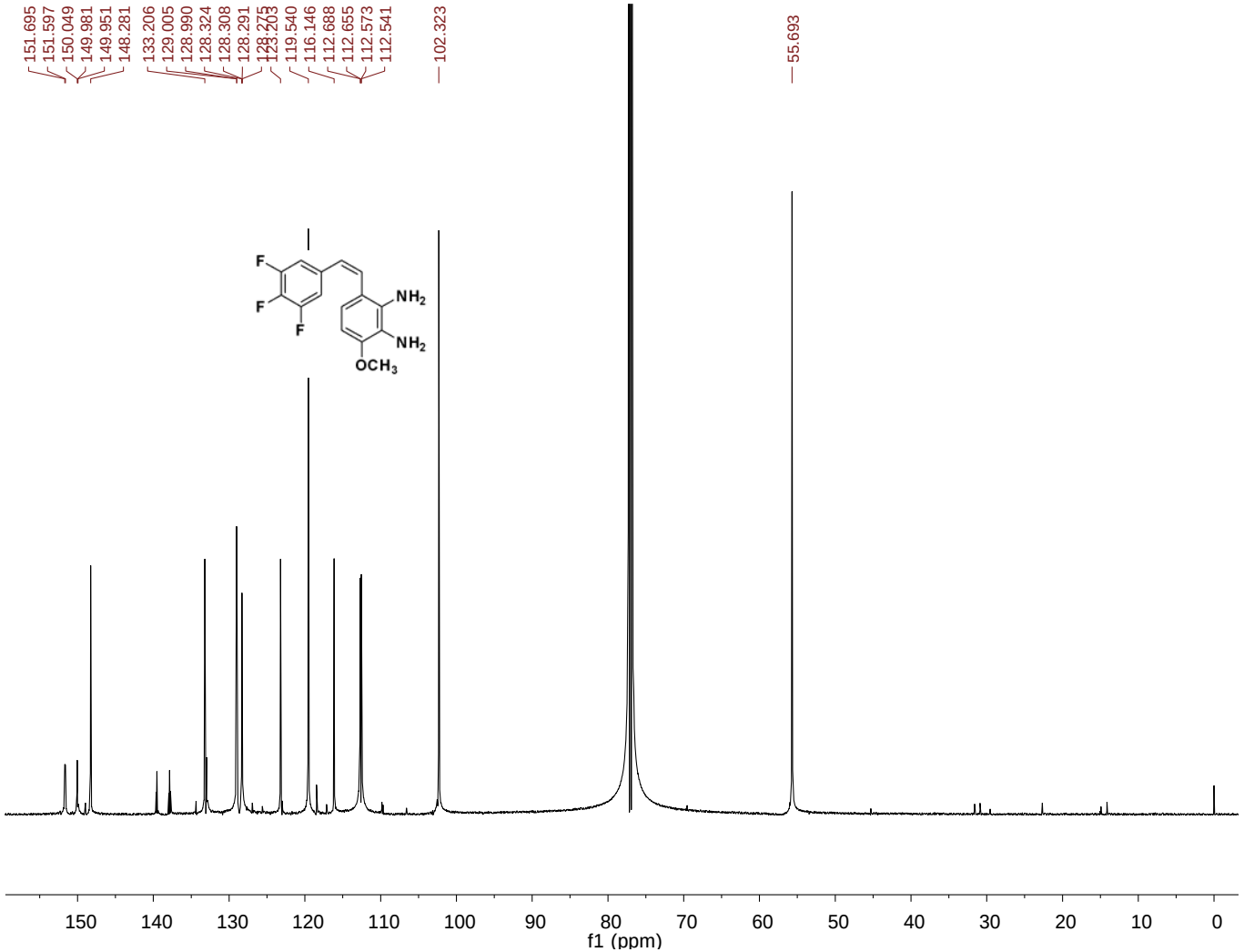
^{13} C NMR (126 MHz, $CDCl_3$) for Compound **6a**



^{1}H NMR (500 MHz, $CDCl_3$) for Compound **9**



^{13}C NMR (151 MHz, $CDCl_3$) for Compound **9**



HPLC Traces for Compound **9**

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    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
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                      (modified after loading)
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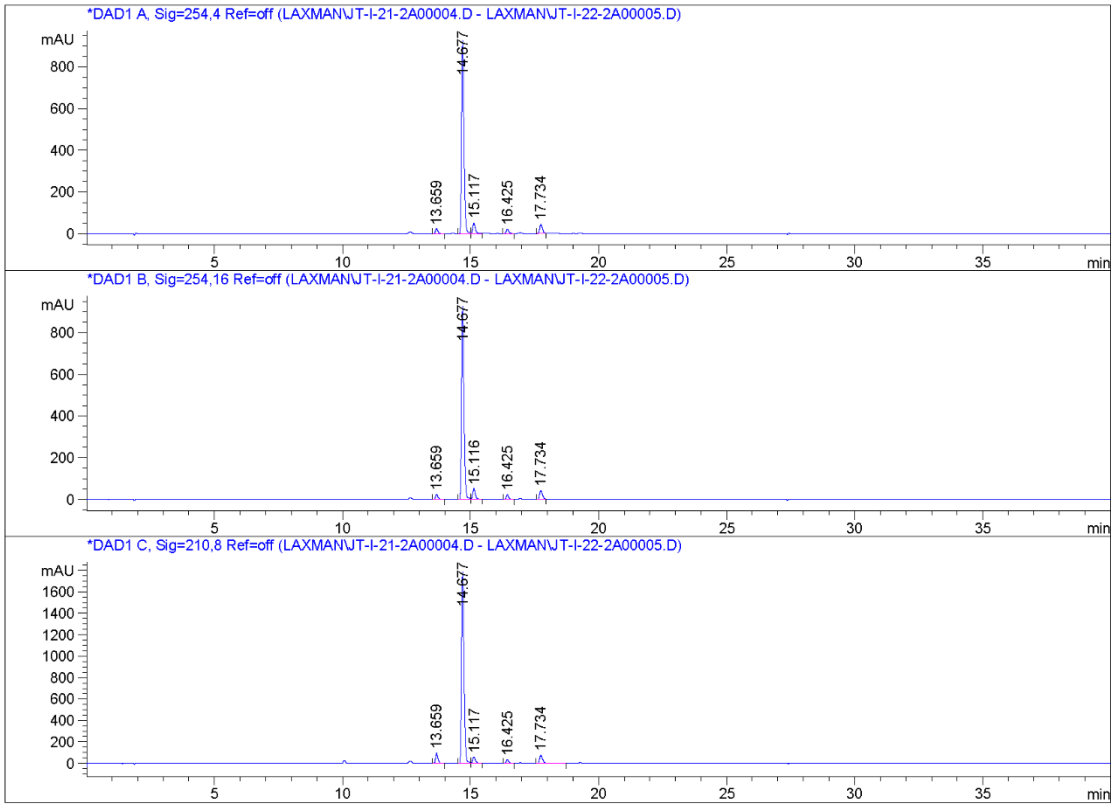
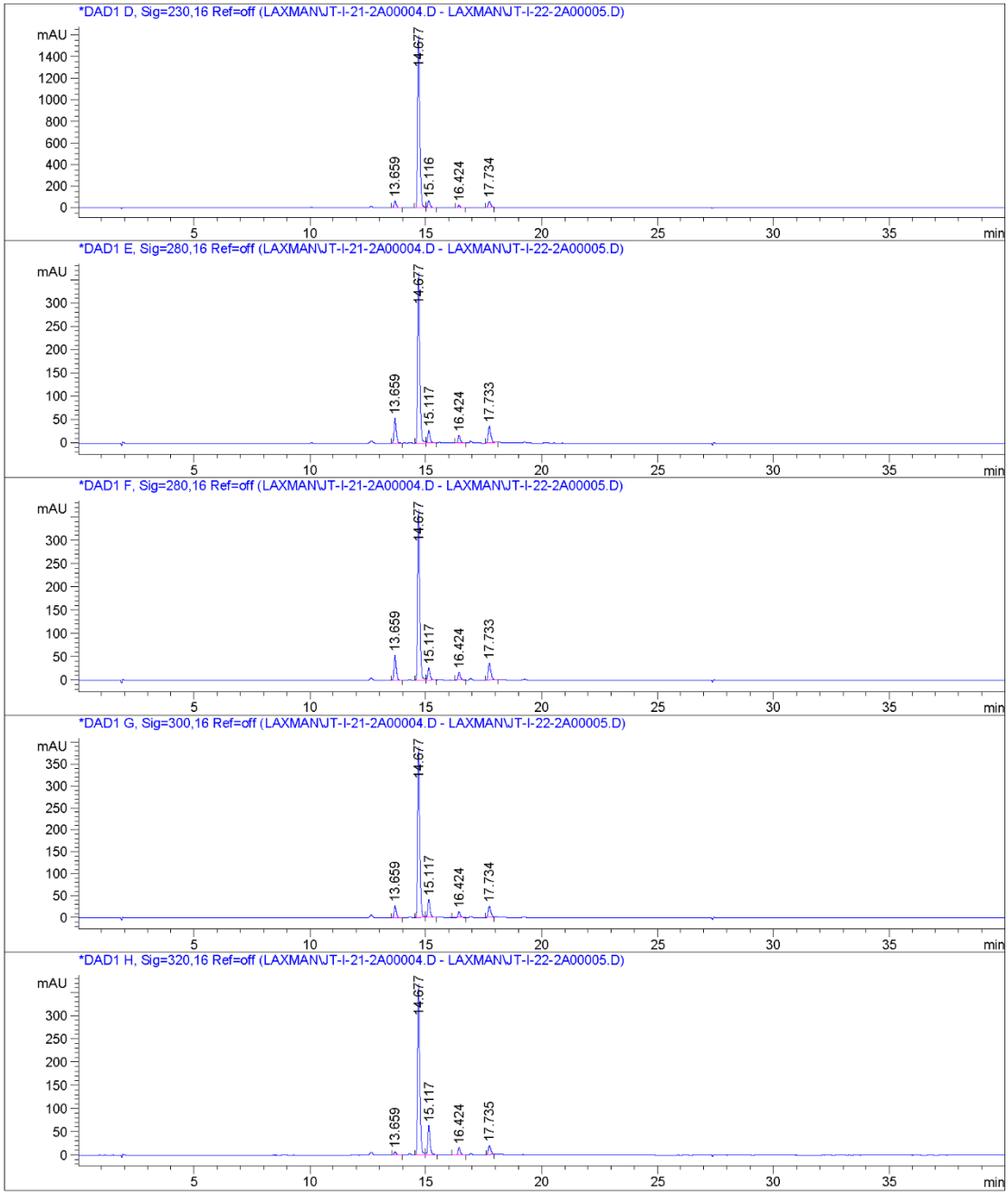


Data File C:\CHEM32\1\DATA\LAXMAN\JT-I-21-2A00004.D
Sample Name: Run1 JT-I-21-2A



Data File C:\CHEM32\1\DATA\LAXMAN\JT-I-21-2A00004.D
Sample Name: Run1 JT-I-21-2A

==========

Area Percent Report

==========

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.659	BB	0.0991	162.13081	24.53632	2.3543
2	14.677	BV	0.0960	5894.12646	928.71790	85.5894
3	15.117	VV	0.1031	364.67896	52.43297	5.2956
4	16.425	BB	0.0953	141.26567	22.49835	2.0513
5	17.734	BB	0.1155	324.31000	43.24024	4.7094
Totals :				6886.51190	1071.42577	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.659	BB	0.0991	165.57846	25.05725	2.4047
2	14.677	BV	0.0960	5890.34814	928.09528	85.5451
3	15.116	VV	0.1033	364.30359	52.30087	5.2908
4	16.425	BB	0.0953	139.68668	22.23136	2.0287
5	17.734	BB	0.1154	325.74994	43.46316	4.7308
Totals :				6885.66681	1071.14791	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.659	BB	0.0988	623.91852	94.80615	4.5163
2	14.677	BV	0.1029	1.17900e4	1787.53845	85.3431
3	15.117	VV	0.1075	452.73615	61.80439	3.2772
4	16.425	BV	0.0958	218.76558	34.59695	1.5836
5	17.734	BB	0.1359	729.40509	78.90025	5.2799

Data File C:\CHEM32\1\DATA\LAXMAN\JT-I-21-2A00004.D
Sample Name: Run1 JT-I-21-2A

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
Totals :				1.38148e4	2057.64620	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.659	BB	0.0987	427.35291	64.97440	3.6347
2	14.677	BV	0.0972	1.02954e4	1597.63464	87.5639
3	15.116	VV	0.1062	453.48181	62.82598	3.8569
4	16.424	BV	0.0988	146.08319	22.19318	1.2425
5	17.734	BB	0.1157	435.26248	57.92679	3.7020
Totals :				1.17575e4	1805.55499	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.659	BB	0.0983	351.87100	53.82482	10.8368
2	14.677	BV	0.0961	2324.20068	365.90540	71.5802
3	15.117	VV	0.1044	186.44841	26.40283	5.7422
4	16.424	BB	0.0967	105.24044	16.44383	3.2412
5	17.733	BB	0.1176	279.22800	36.37391	8.5996
Totals :				3246.98853	498.95078	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.659	BB	0.0983	351.87100	53.82482	10.8368
2	14.677	BV	0.0961	2324.20068	365.90540	71.5802
3	15.117	VV	0.1044	186.44841	26.40283	5.7422
4	16.424	BB	0.0967	105.24044	16.44383	3.2412
5	17.733	BB	0.1176	279.22800	36.37391	8.5996
Totals :				3246.98853	498.95078	

Data File C:\CHEM32\1\DATA\LAXMAN\JT-I-21-2A00004.D
Sample Name: Run1 JT-I-21-2A

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

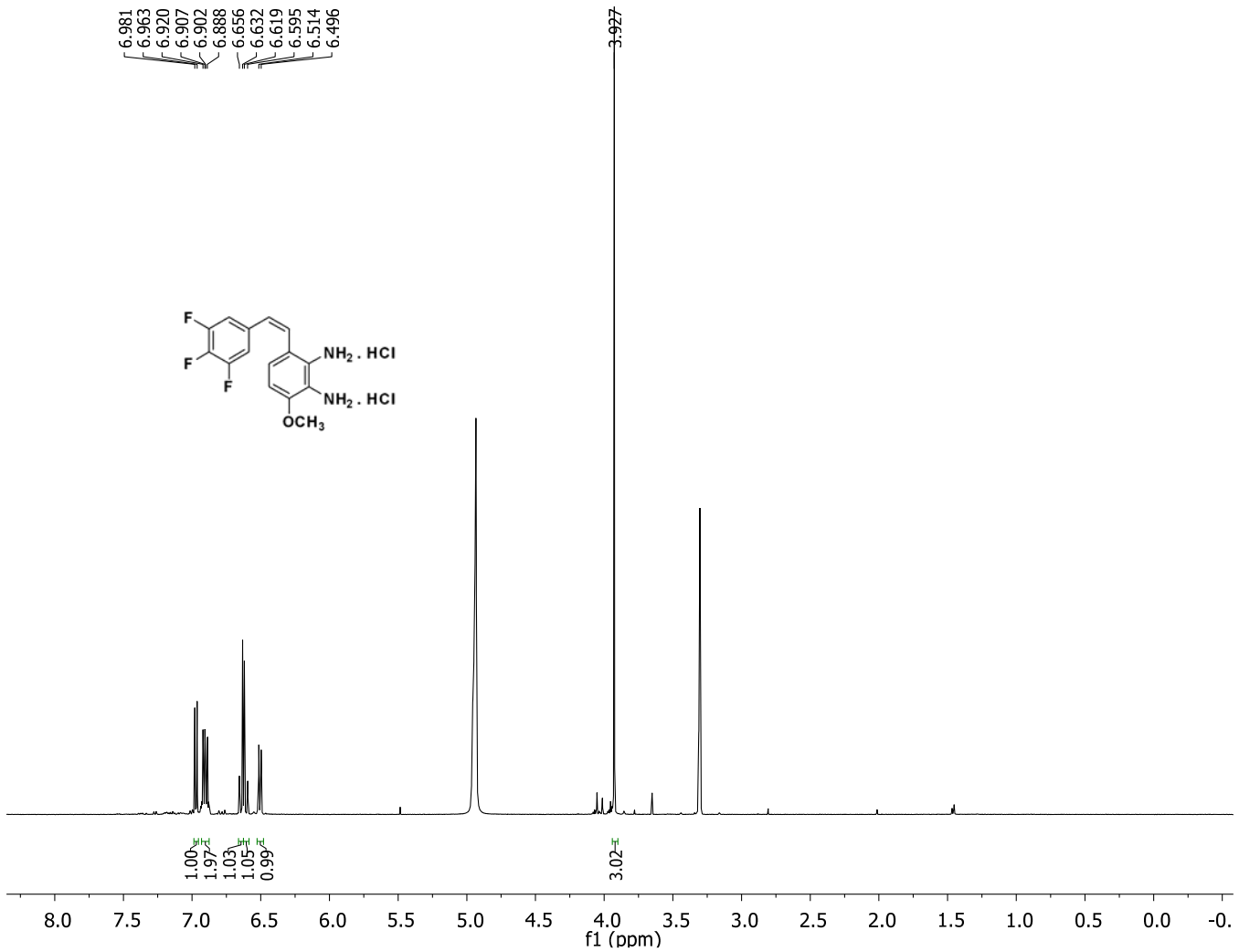
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.659	BB	0.0987	185.64053	28.22444	5.7361
2	14.677	BV	0.0961	2475.58276	389.73145	76.4923
3	15.117	VV	0.1025	290.10934	42.03966	8.9640
4	16.424	BB	0.0989	91.09869	13.82672	2.8148
5	17.734	BB	0.1155	193.94939	25.87396	5.9928
Totals :				3236.38071	499.69622	

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

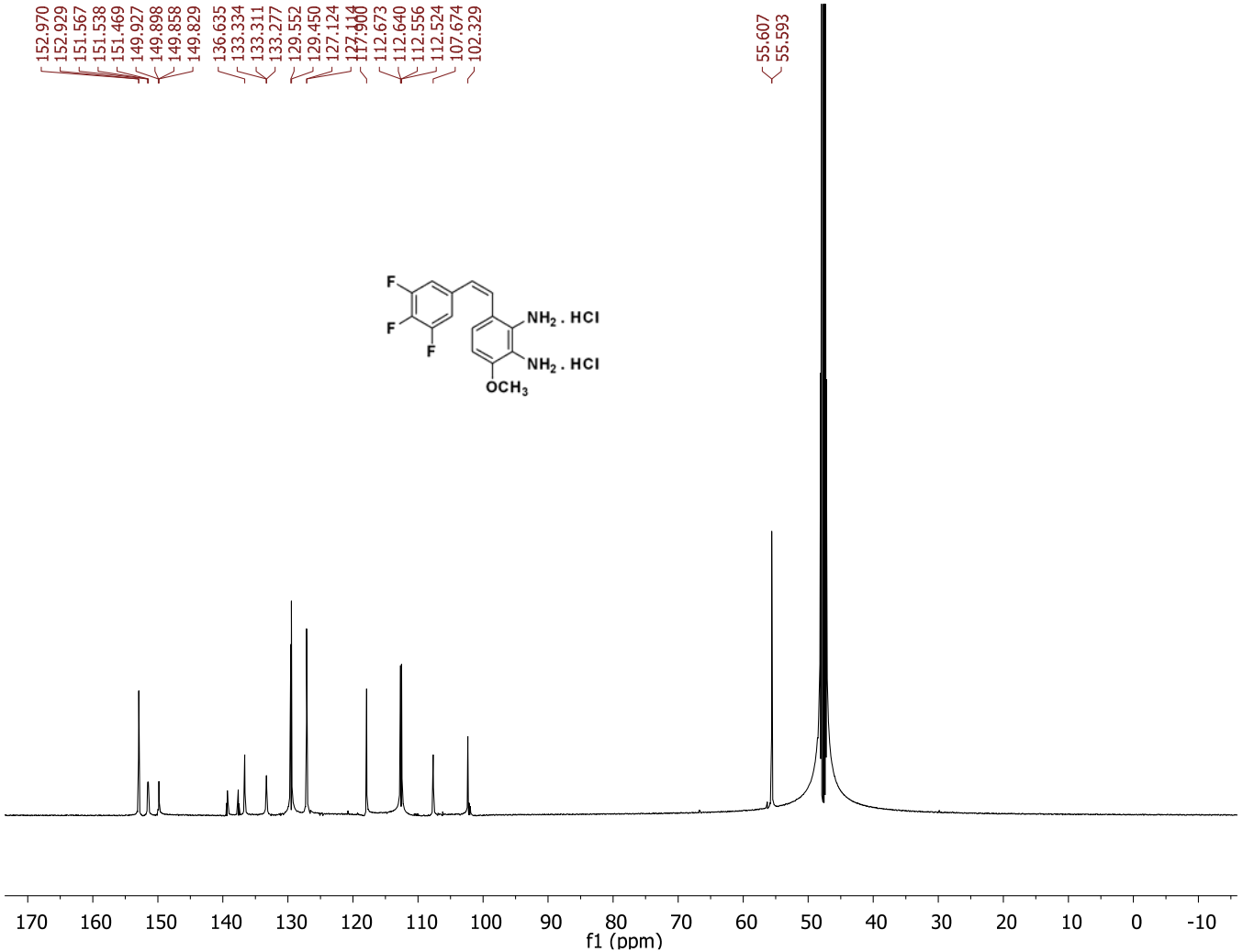
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.659	BB	0.1013	46.44753	7.00805	1.5162
2	14.677	BV	0.0962	2344.52319	368.62598	76.5309
3	15.117	VV	0.1000	423.56235	63.38282	13.8261
4	16.424	BB	0.0971	101.18475	15.71365	3.3029
5	17.735	BB	0.1155	147.78171	19.70036	4.8240
Totals :				3063.49952	474.43085	

*** End of Report ***

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



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



HPLC Traces for Compound **13**

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Data File C:\CHEM32\1\DATA\LAXMAN\JT-I-22-2A00006.D
Sample Name: run1-salt

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    Acq. Instrument : Instrument 1                    Location :   -
    Injection Date  : 9/21/2012 4:44:32 PM
    Acq. Method     : C:\CHEM32\1\METHODS\MASTERMETHOD.M
    Last changed    : 9/21/2012 3:43:30 PM by Laxman
                      (modified after loading)
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    Last changed    : 2/20/2013 3:22:17 PM by Laxman
    Sample Info     :
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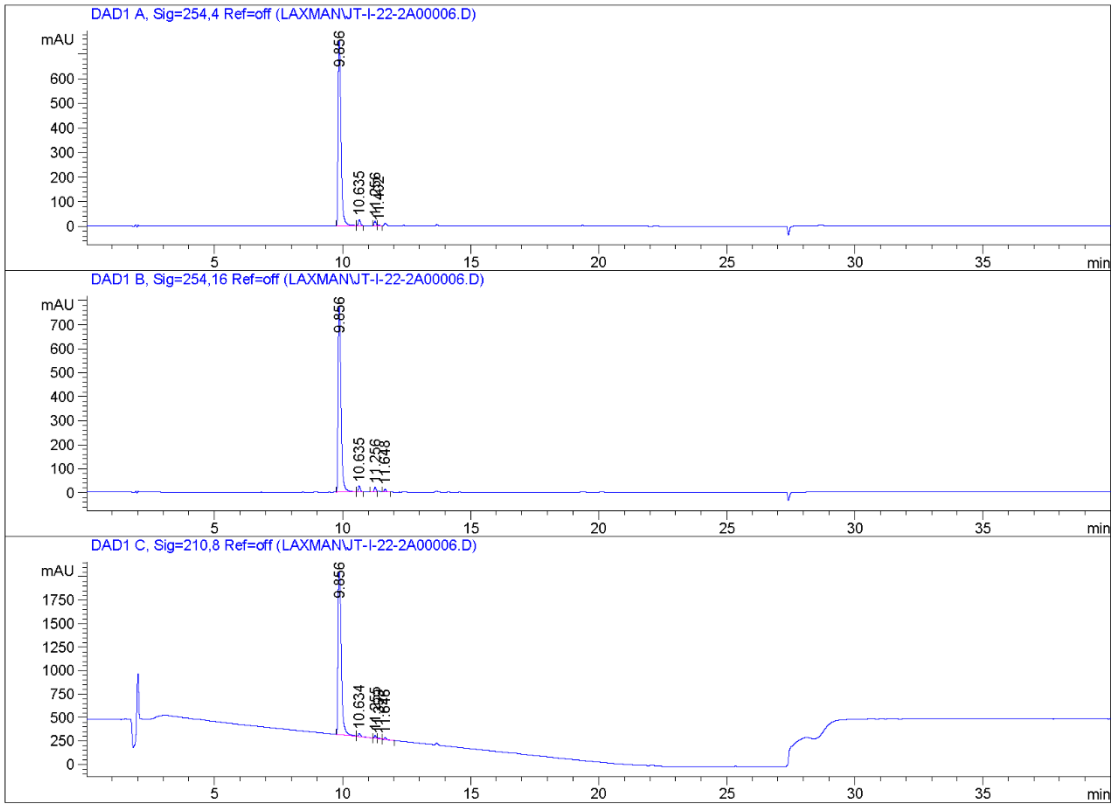

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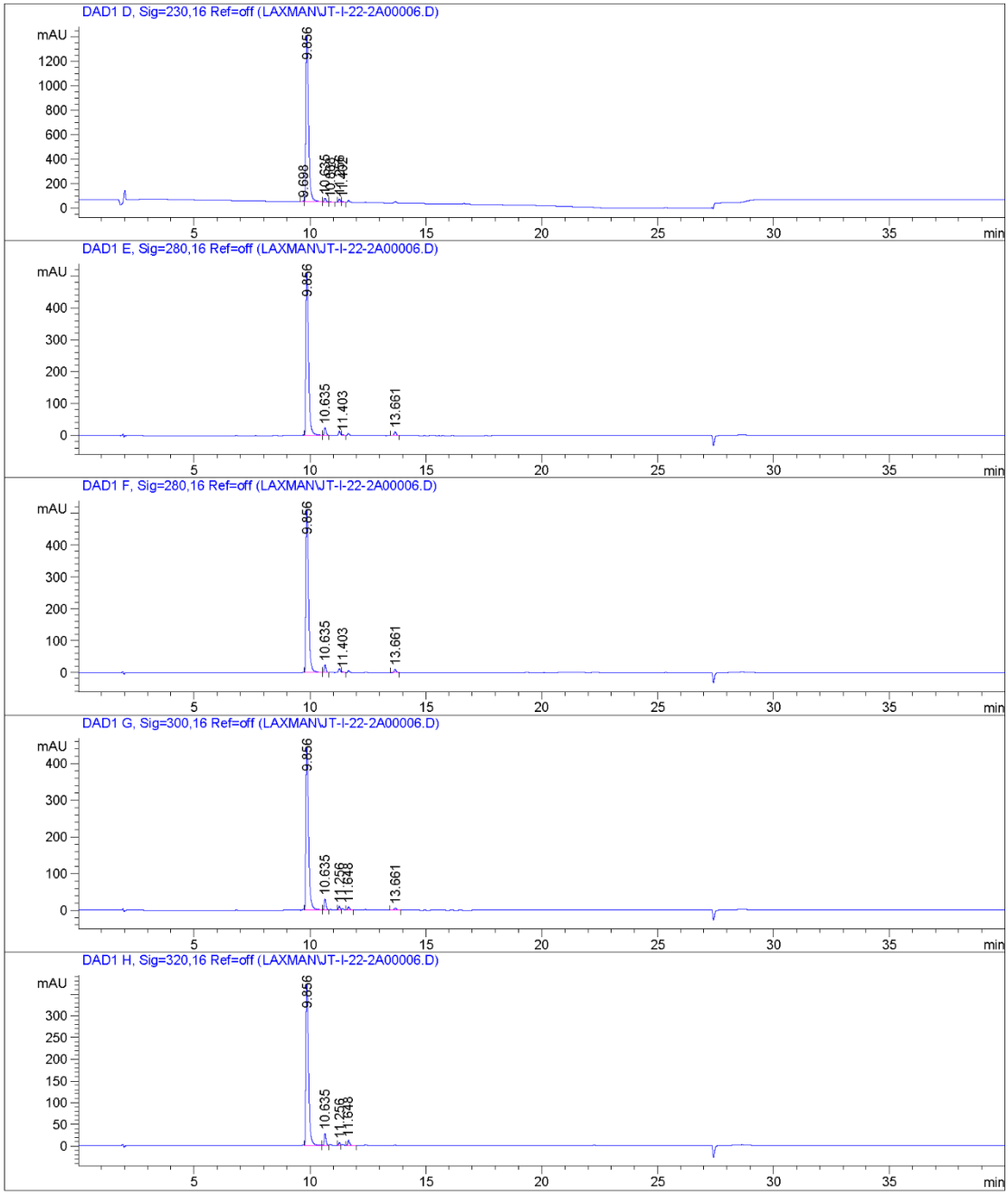
Sample Name: run1-salt



Data File C:\CHEM32\1\DATA\LAXMAN\JT-I-22-2A00006.D
Sample Name: run1-salt

==========

Area Percent Report

==========

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

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.856	VV	0.1215	5924.17090	755.57947	95.6460
2	10.635	VV	0.0832	143.86528	25.75566	2.3227
3	11.256	BV	0.0796	102.32201	19.37108	1.6520
4	11.402	VV	0.0834	23.49303	4.19086	0.3793
Totals :				6193.85122	804.89706	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.856	VV	0.1215	6085.08984	775.90594	95.0144
2	10.635	VV	0.0833	142.33685	25.44177	2.2225
3	11.256	BV	0.0808	105.61462	19.61191	1.6491
4	11.648	VB	0.0828	71.34705	12.84008	1.1140
Totals :				6404.38836	833.79970	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.856	VV	0.1416	1.54228e4	1736.95813	96.4466
2	10.634	VB	0.0956	259.50131	39.00152	1.6228
3	11.255	BV	0.0789	148.64664	28.47282	0.9296
4	11.399	VB	0.0793	42.90706	8.17255	0.2683
5	11.646	BB	0.0856	117.16740	20.22860	0.7327
Totals :				1.59910e4	1832.83362	

Data File C:\CHEM32\1\DATA\LAXMAN\JT-I-22-2A00006.D
Sample Name: run1-salt

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.698	BV	0.0685	25.98634	5.76979	0.2317
2	9.856	VV	0.1228	1.08293e4	1361.54736	96.5643
3	10.635	VV	0.0849	179.02850	31.24263	1.5964
4	10.868	VB	0.0936	15.27435	2.29633	0.1362
5	11.256	BV	0.0788	129.79822	24.90076	1.1574
6	11.402	VB	0.0800	35.21987	6.63243	0.3141
Totals :				1.12147e4	1432.38931	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.856	VV	0.1219	4036.54590	512.68793	94.6037
2	10.635	VV	0.0824	137.31654	24.88067	3.2183
3	11.403	VB	0.0801	17.49596	3.28632	0.4100
4	13.661	BB	0.0956	75.43528	11.95968	1.7680
Totals :				4266.79369	552.81460	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.856	VV	0.1219	4036.54590	512.68793	94.6037
2	10.635	VV	0.0824	137.31654	24.88067	3.2183
3	11.403	VB	0.0801	17.49596	3.28632	0.4100
4	13.661	BB	0.0956	75.43528	11.95968	1.7680
Totals :				4266.79369	552.81460	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.856	VV	0.1220	3513.60327	445.56769	91.8976
2	10.635	VV	0.0817	166.20305	30.46274	4.3470
3	11.256	BV	0.0782	49.79693	9.65365	1.3024

Data File C:\CHEM32\1\DATA\LAXMAN\JT-I-22-2A00006.D
Sample Name: run1-salt

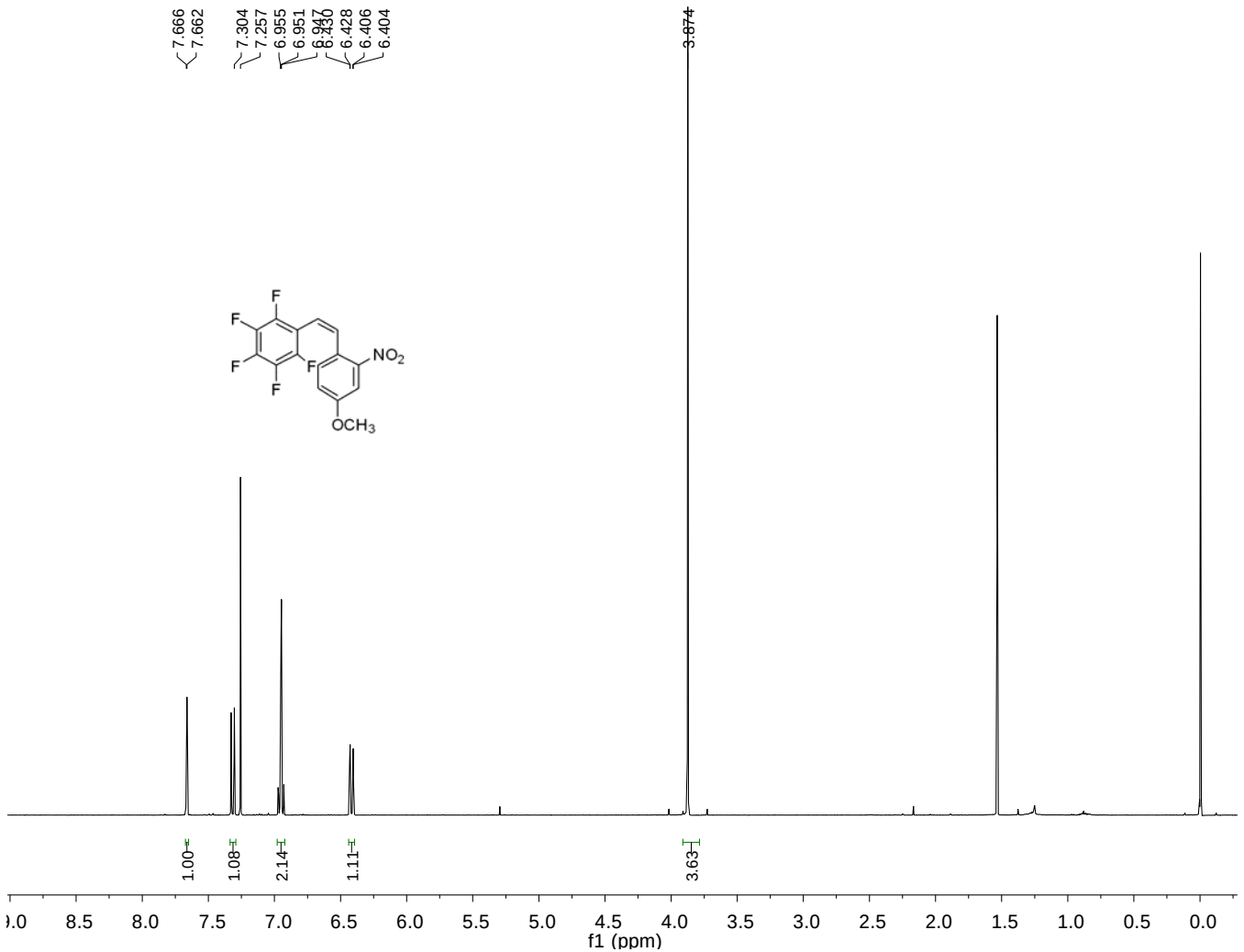
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
4	11.648	BB	0.0829	53.77131	9.66843	1.4064
5	13.661	BB	0.0965	40.01443	6.26962	1.0466
Totals :				3823.38898	501.62213	

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

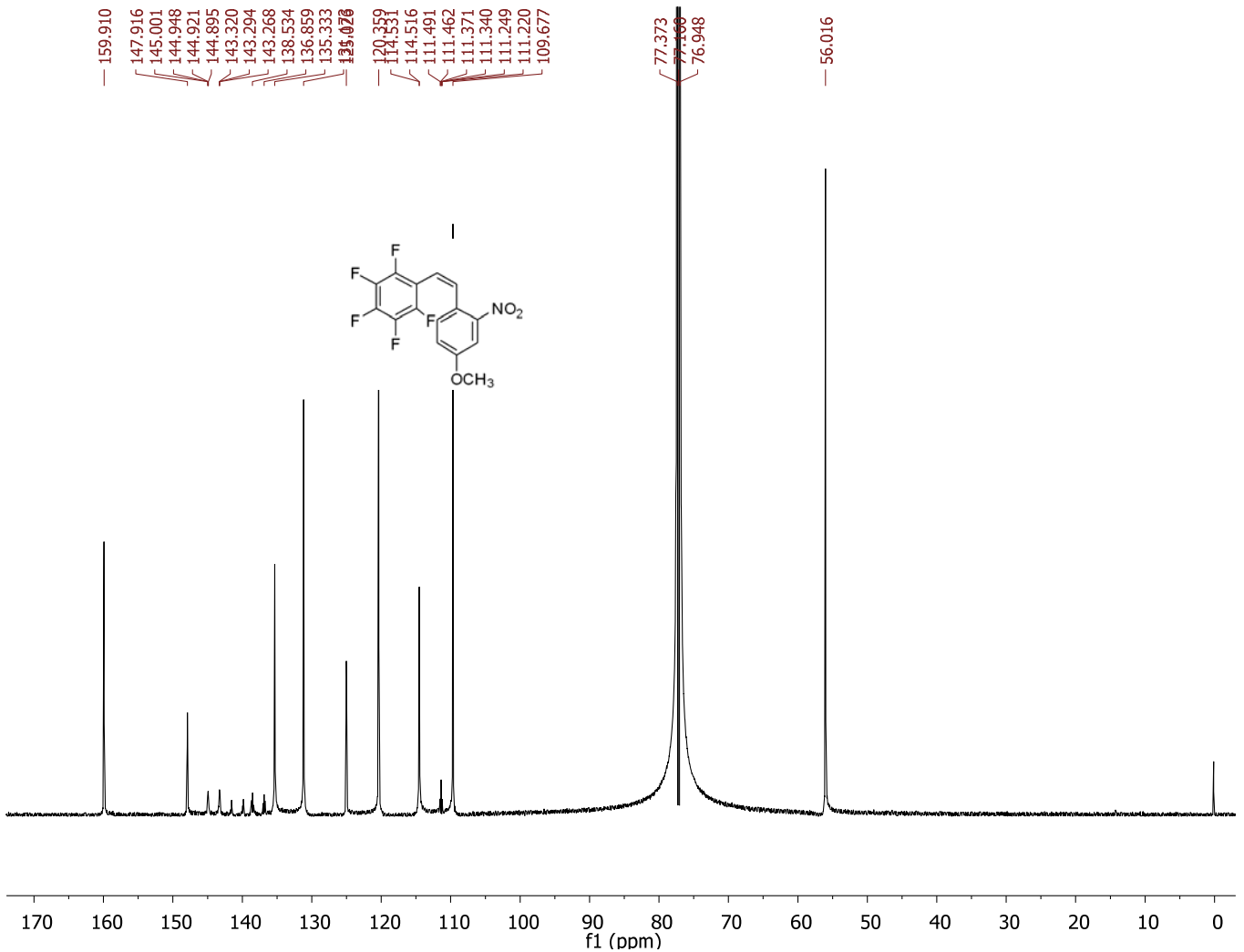
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.856	VV	0.1216	2925.48853	372.75449	91.7959
2	10.635	VV	0.0820	155.77783	28.41552	4.8880
3	11.256	BV	0.0776	35.15205	6.88268	1.1030
4	11.648	BB	0.0845	70.52934	12.37988	2.2131
Totals :				3186.94775	420.43257	

*** End of Report ***

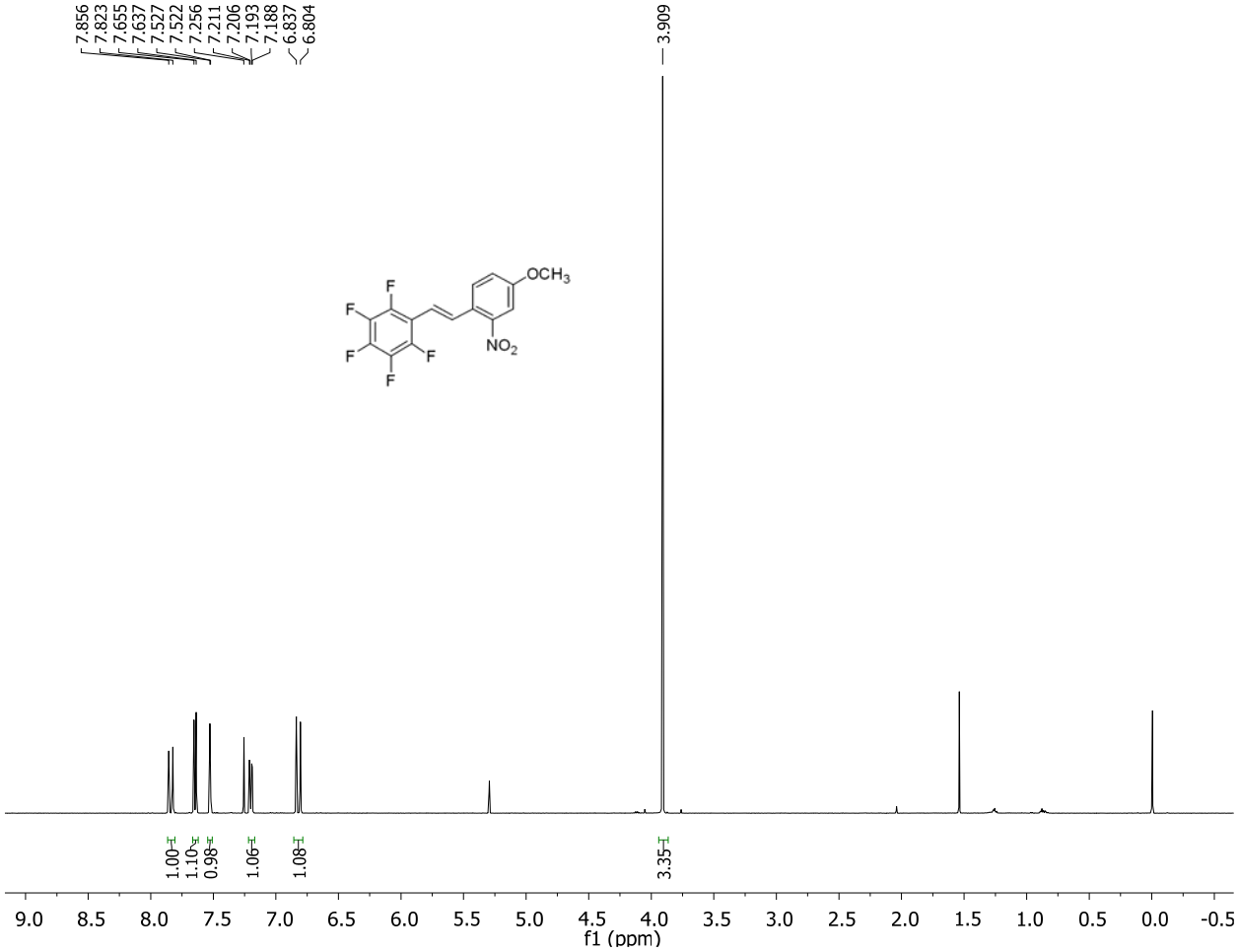
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **7a**



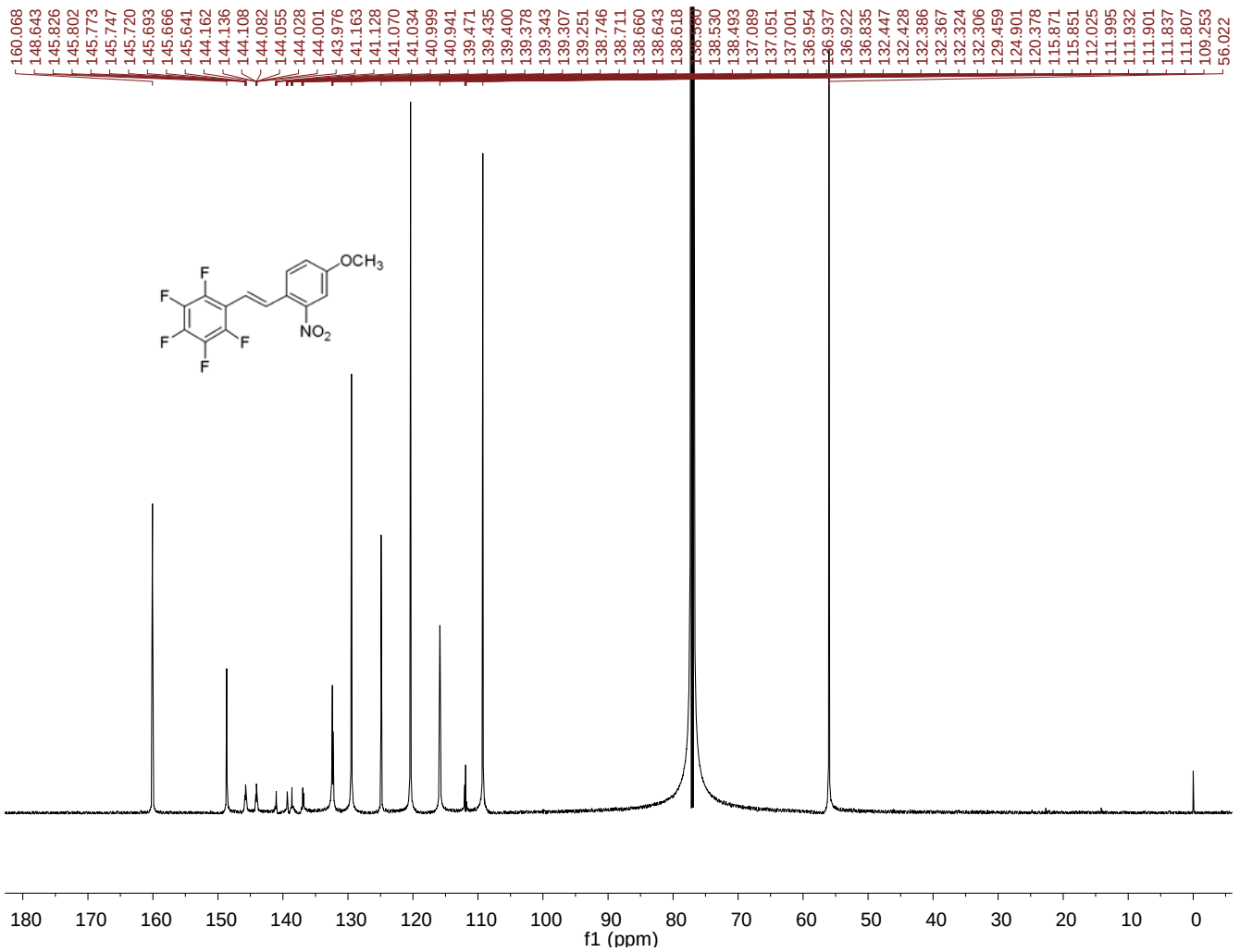
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **7a**



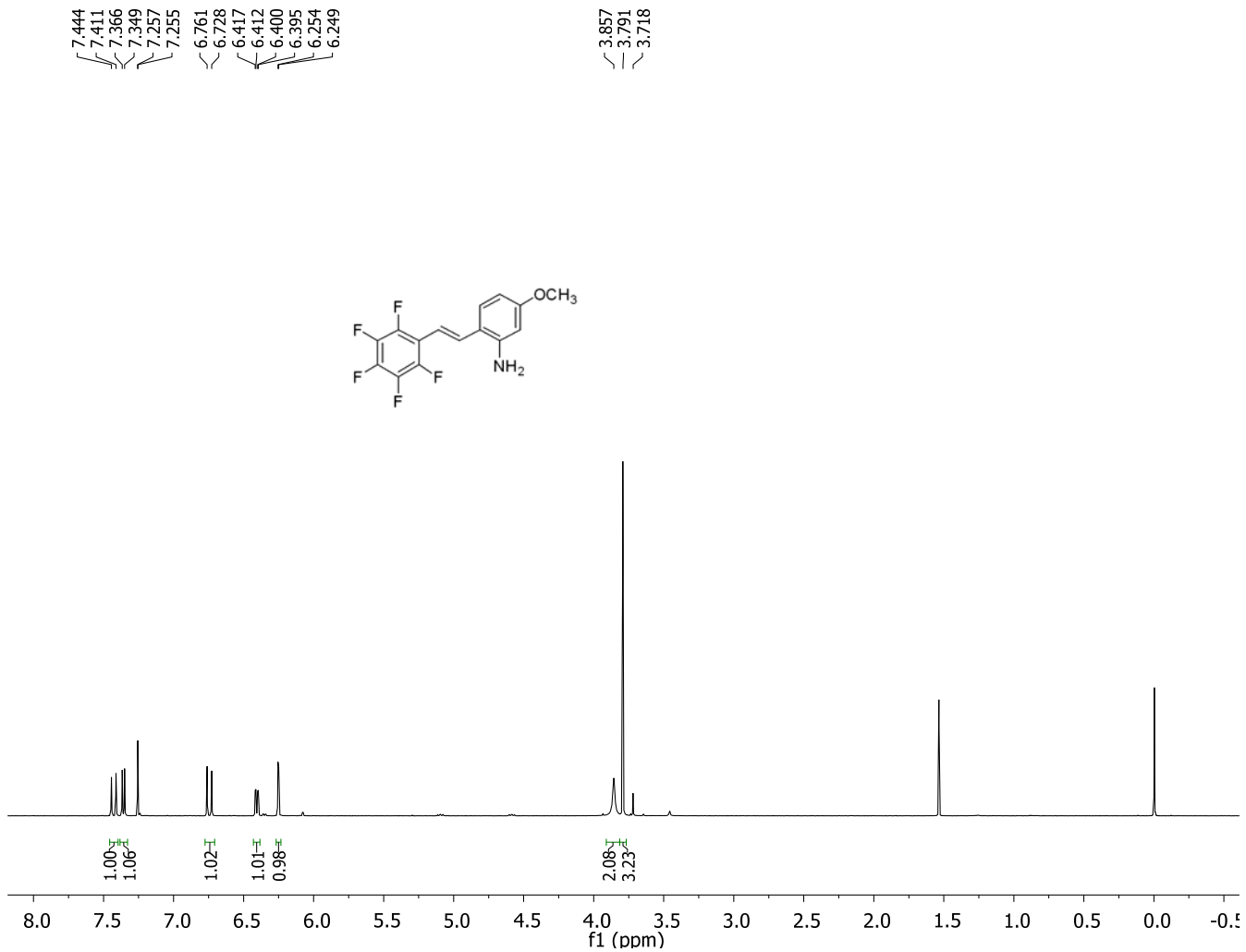
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **7b**



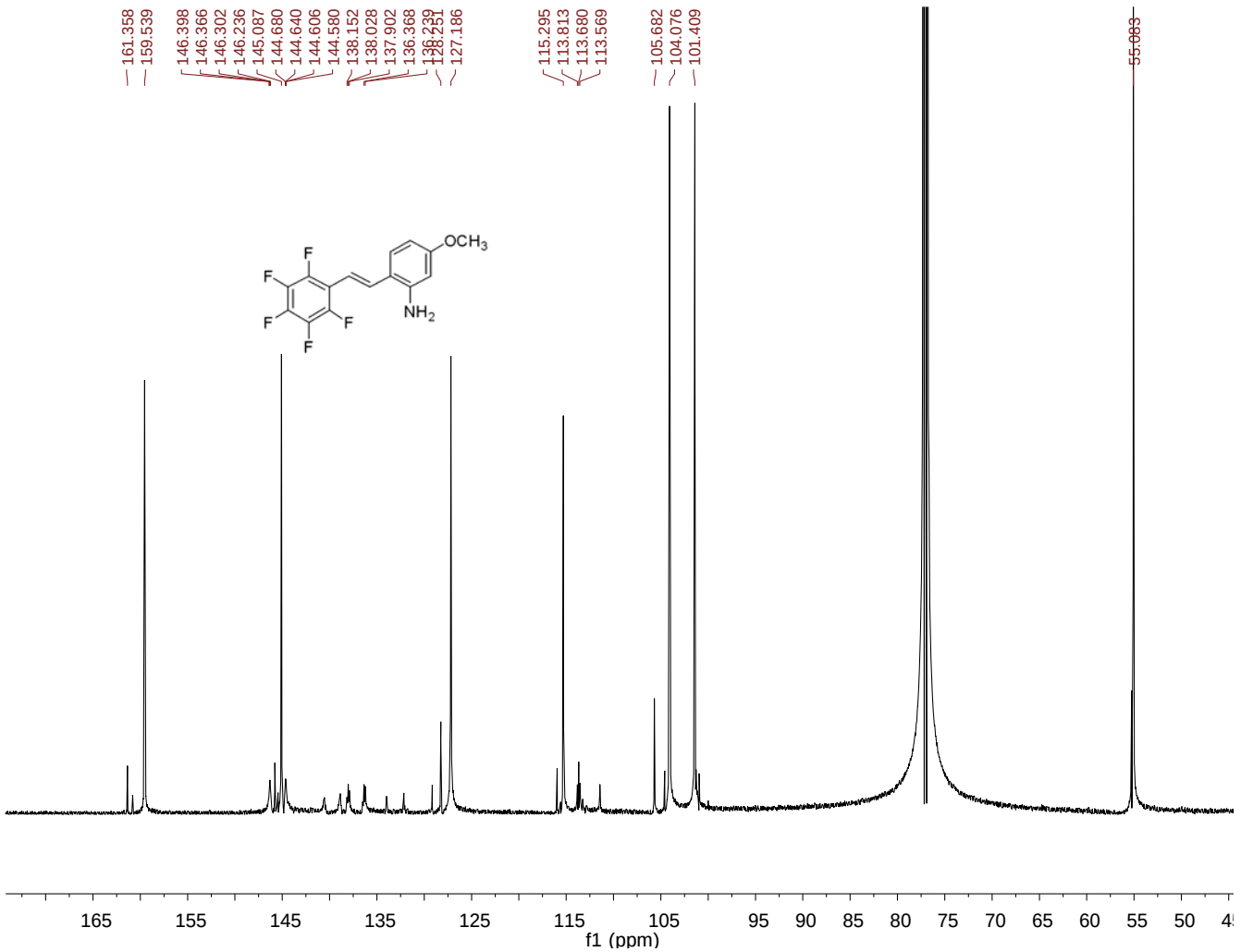
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **7b**



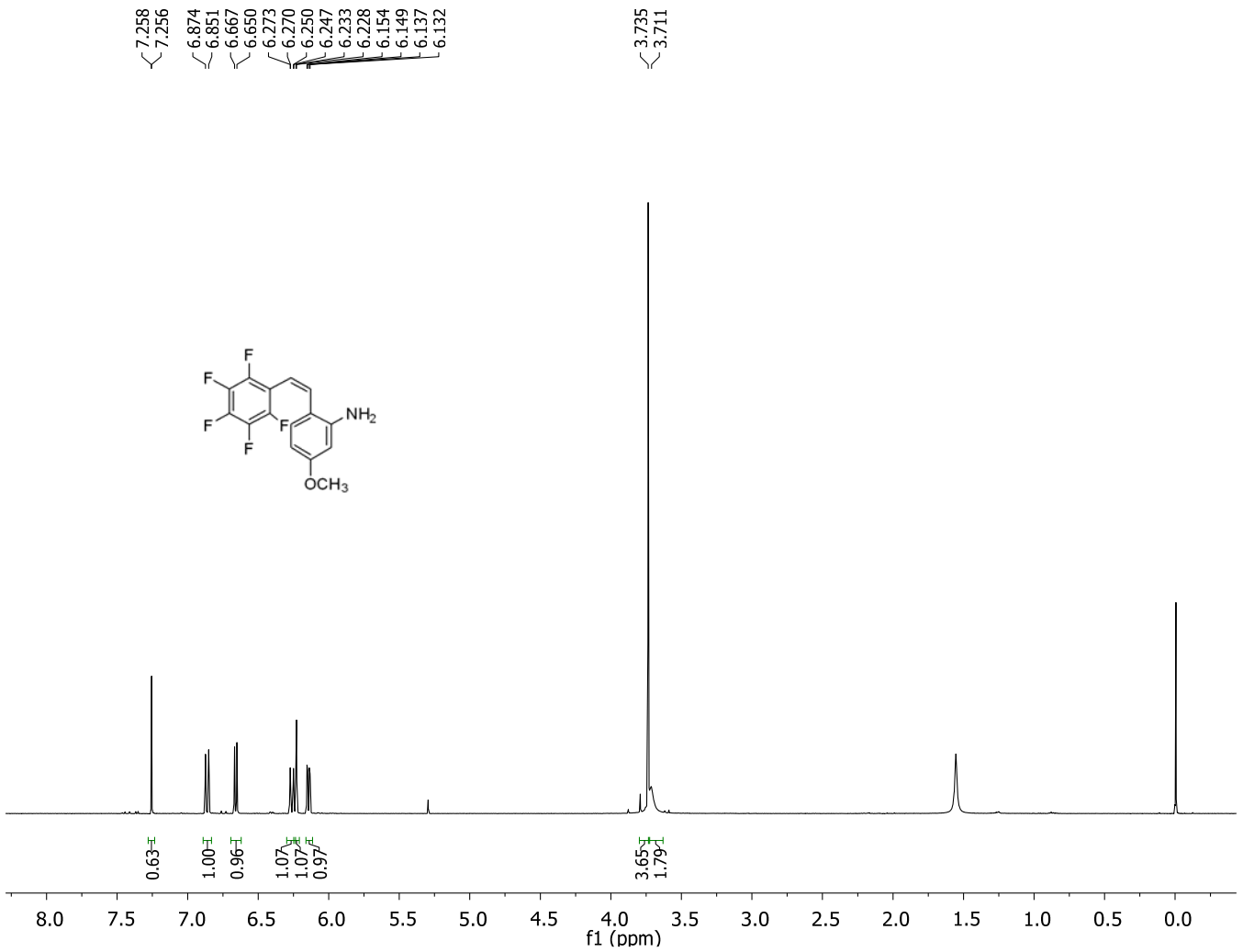
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **11**



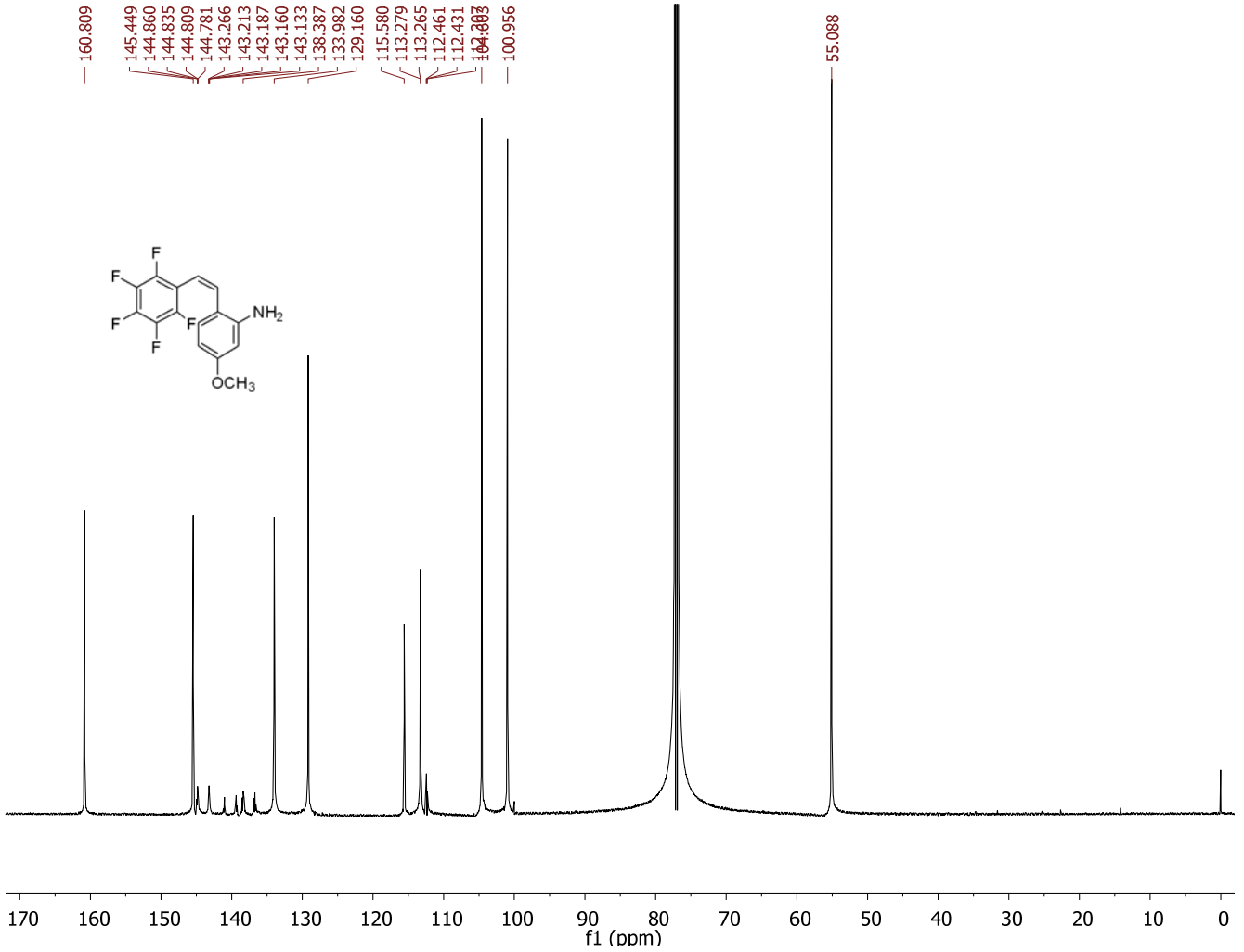
^{13} C NMR (151 MHz, $CDCl_3$) for Compound **11**



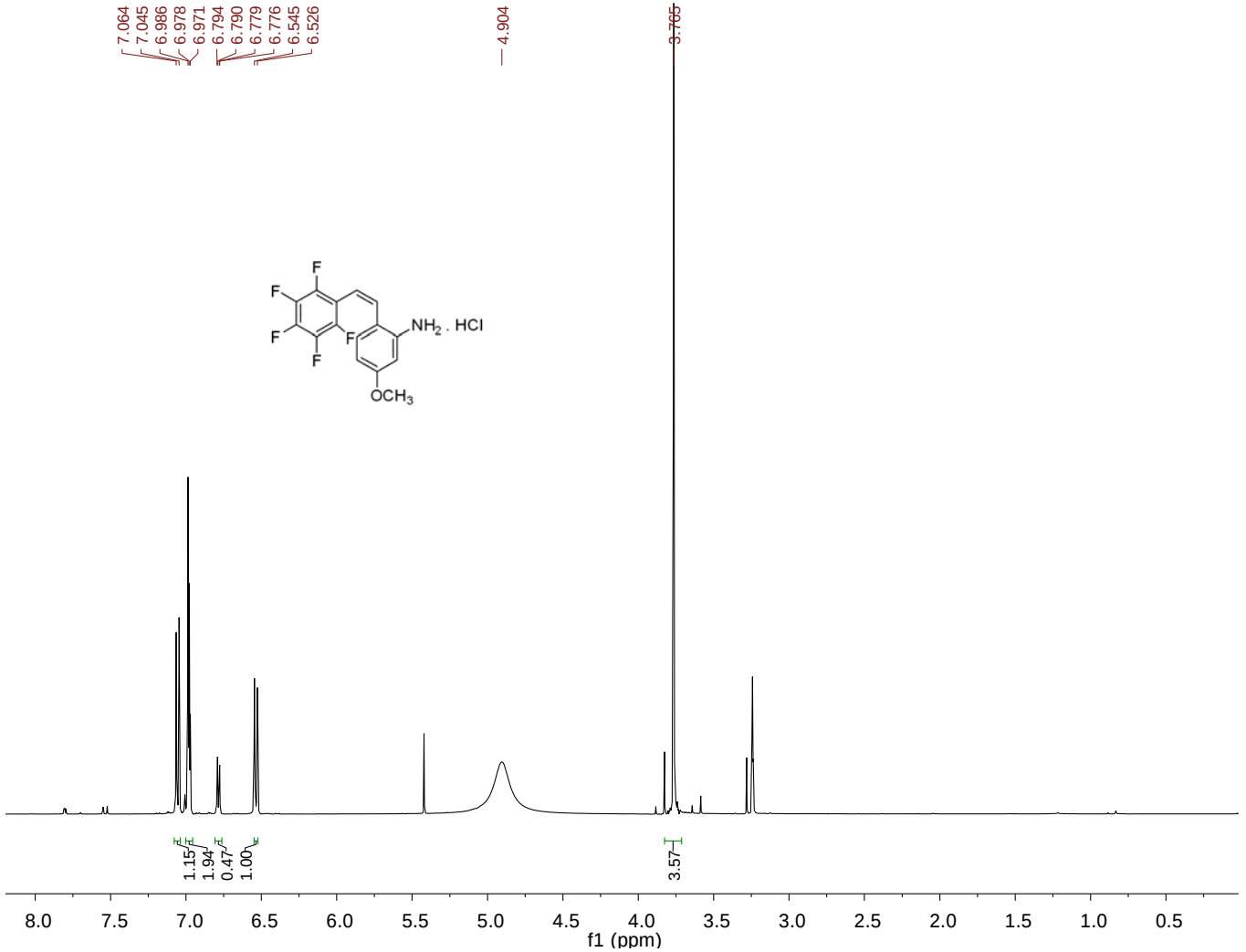
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **10**



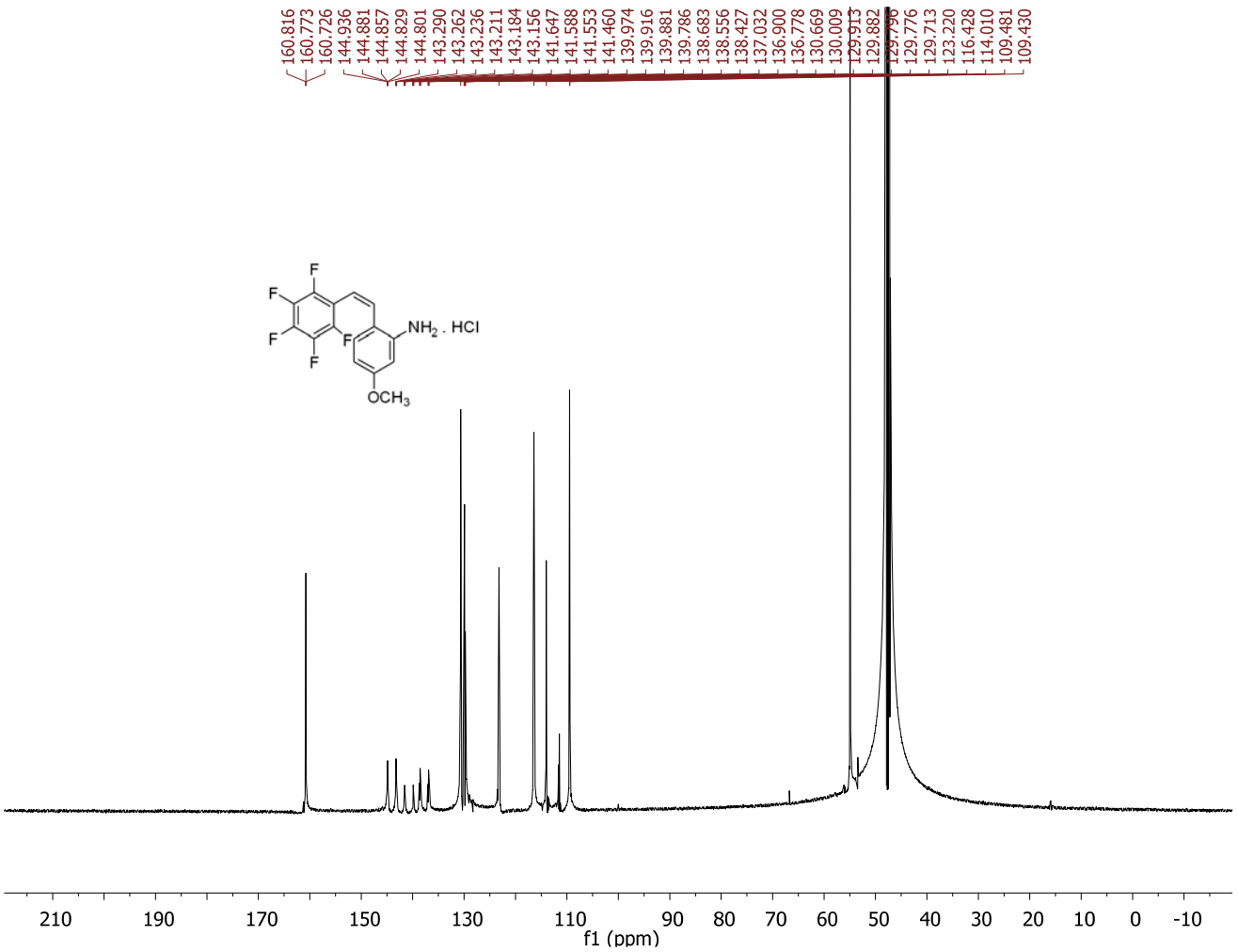
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **10**



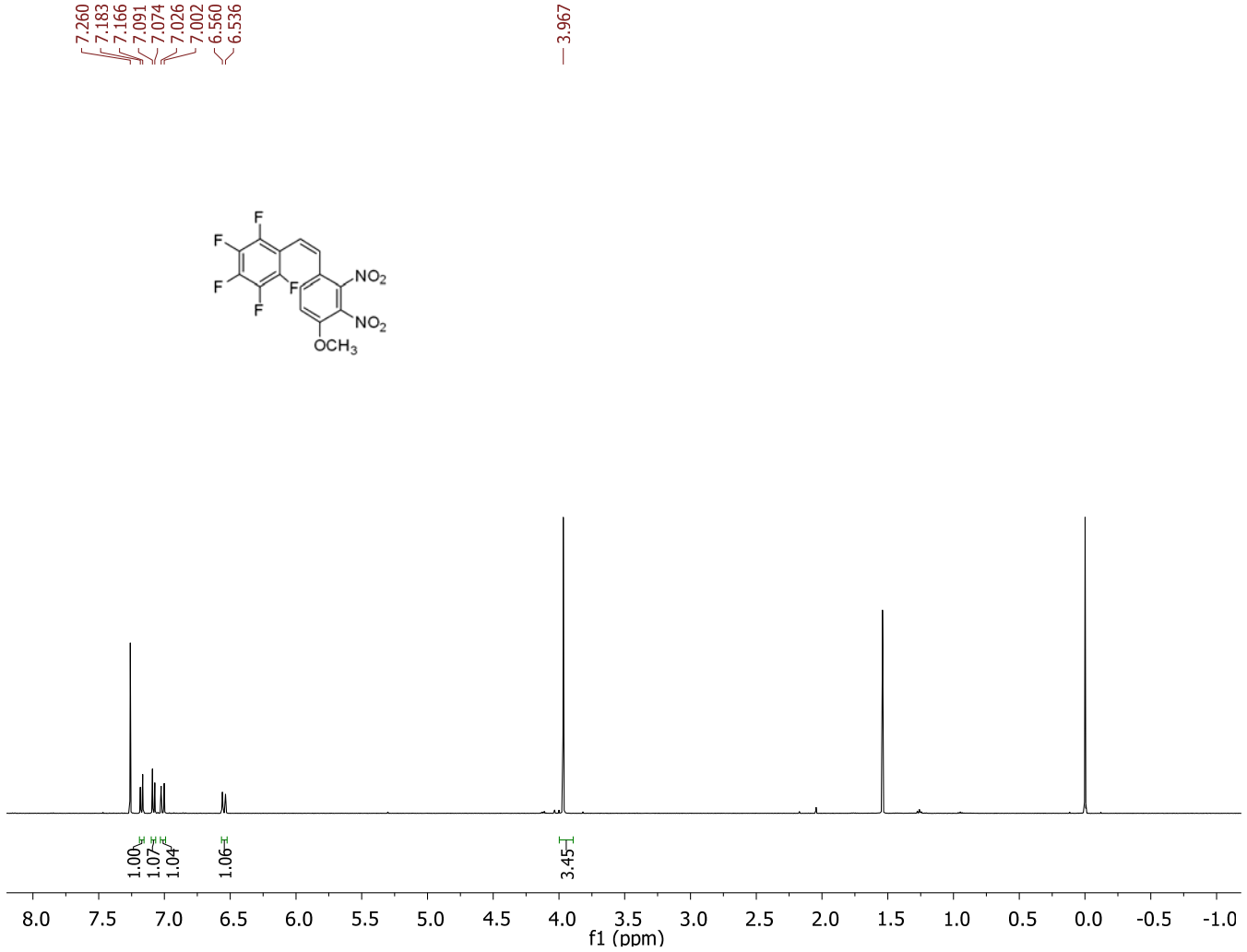
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **14**



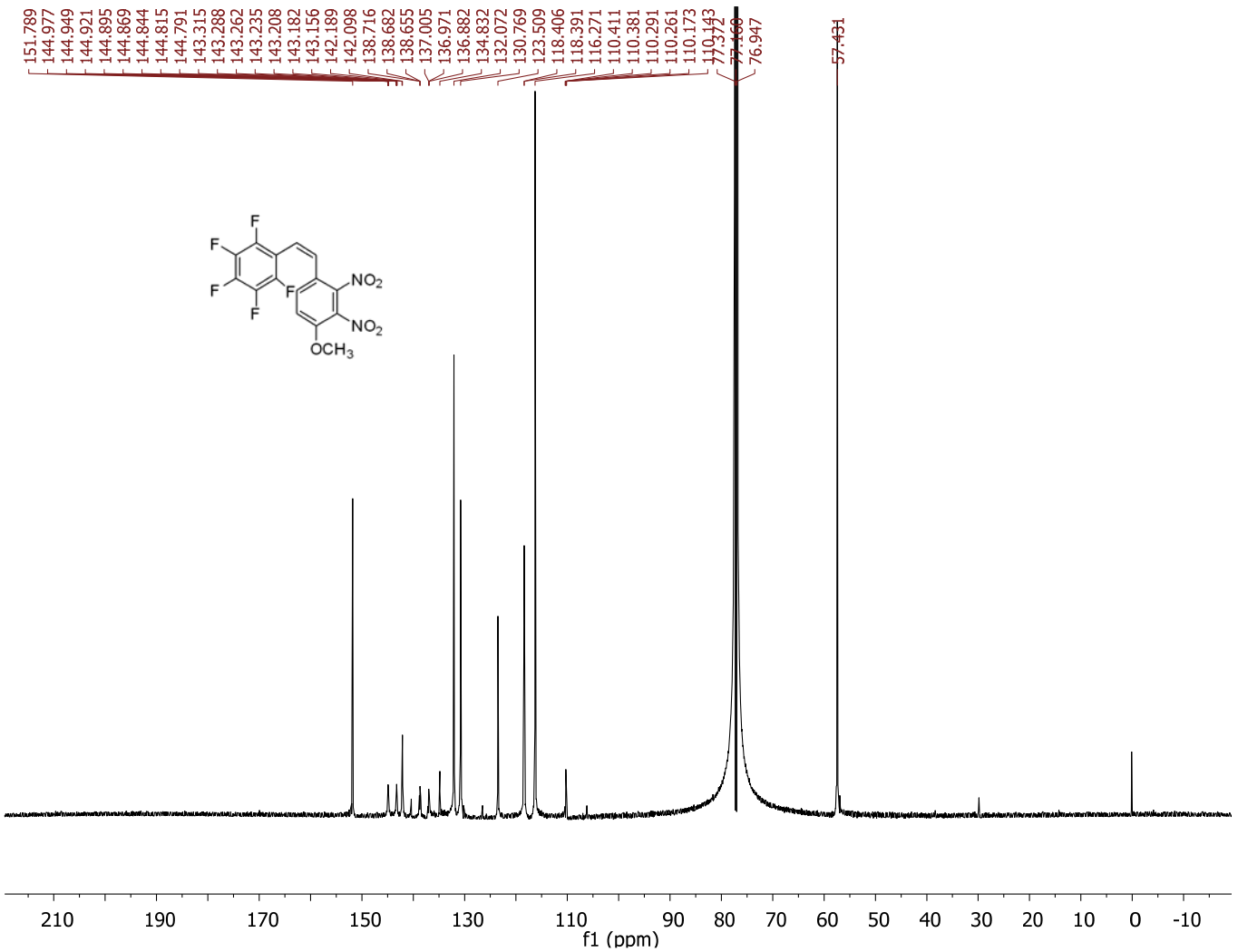
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **14**



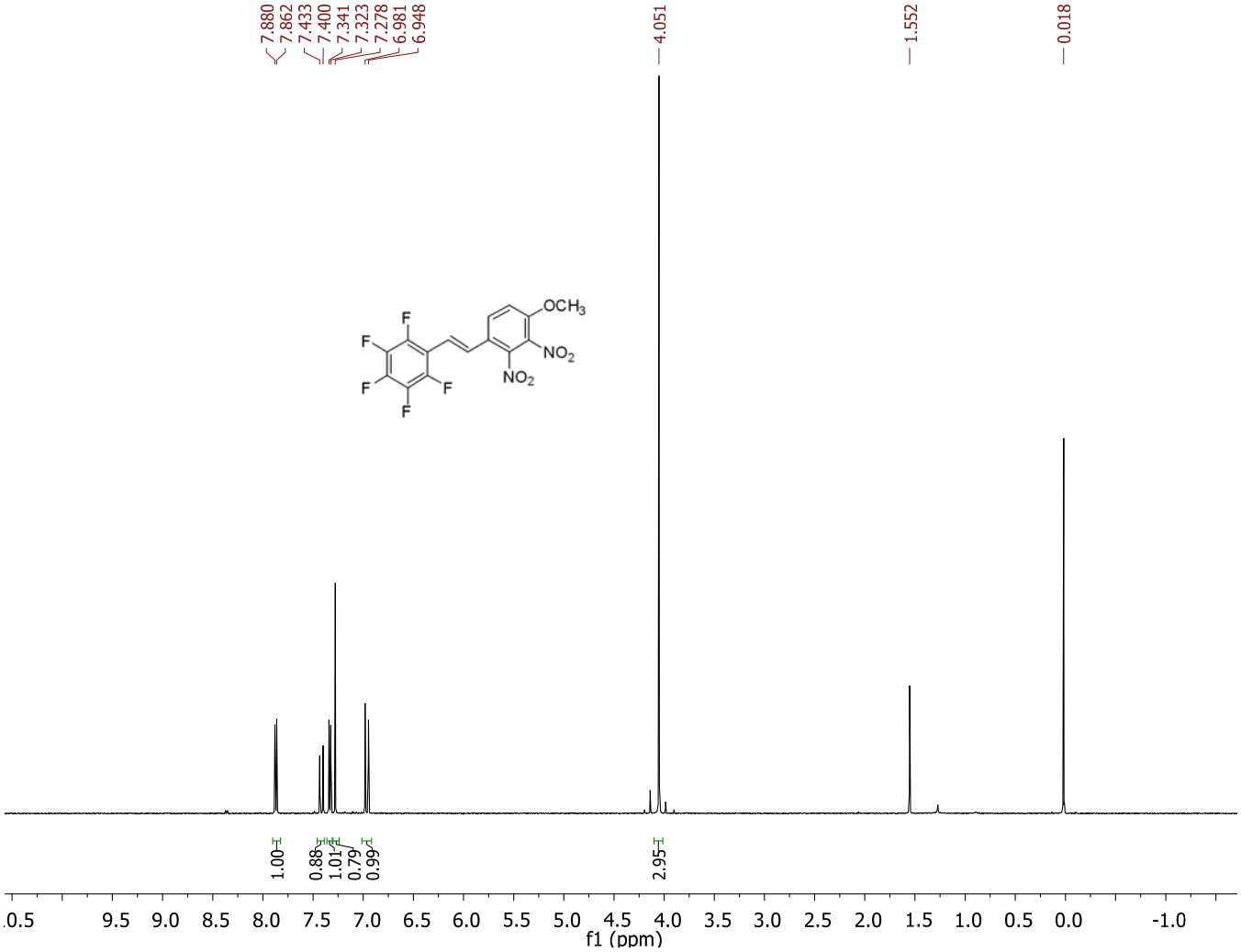
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **8a**



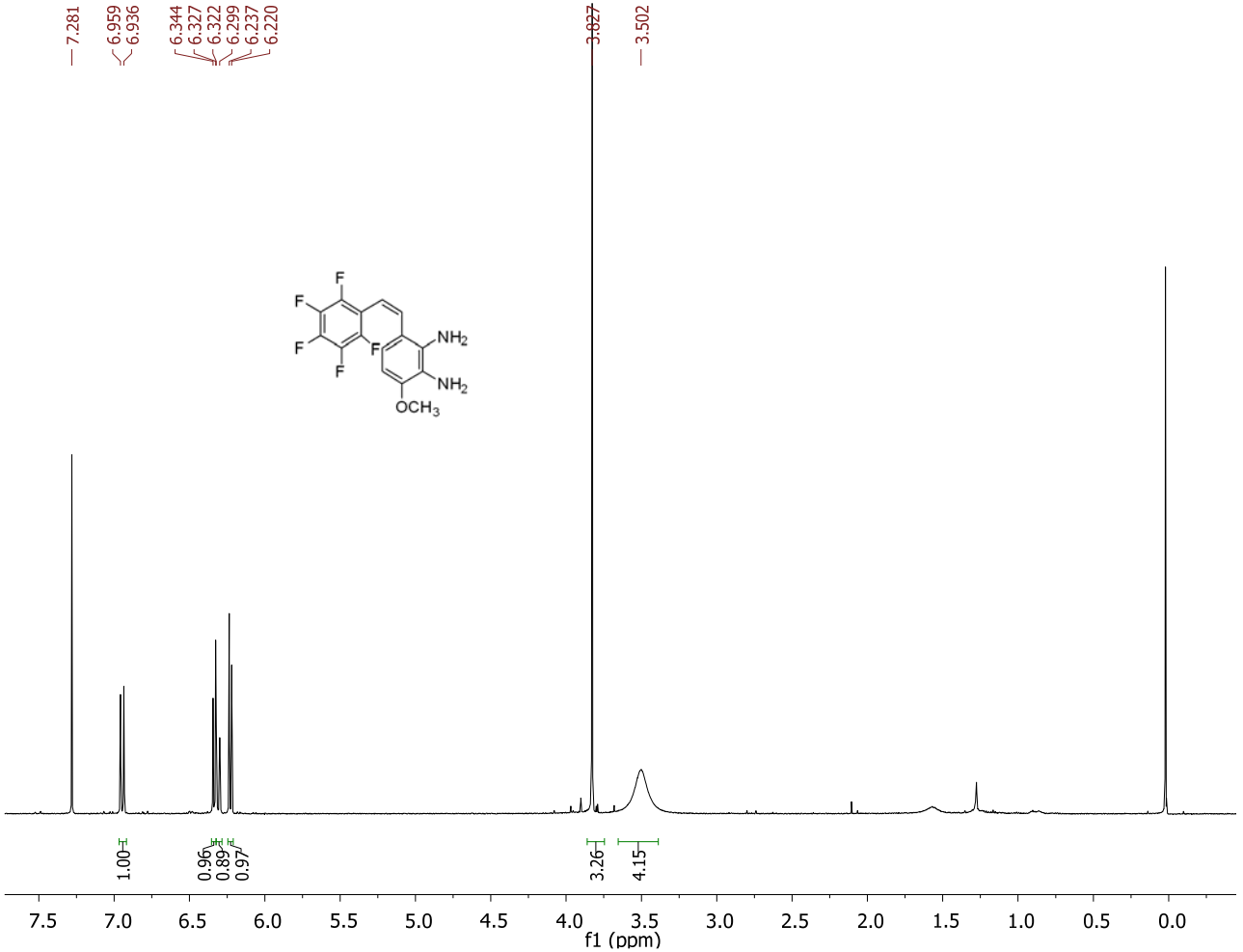
^{13}C NMR (151 MHz, $CDCl_3$) for Compound **8a**



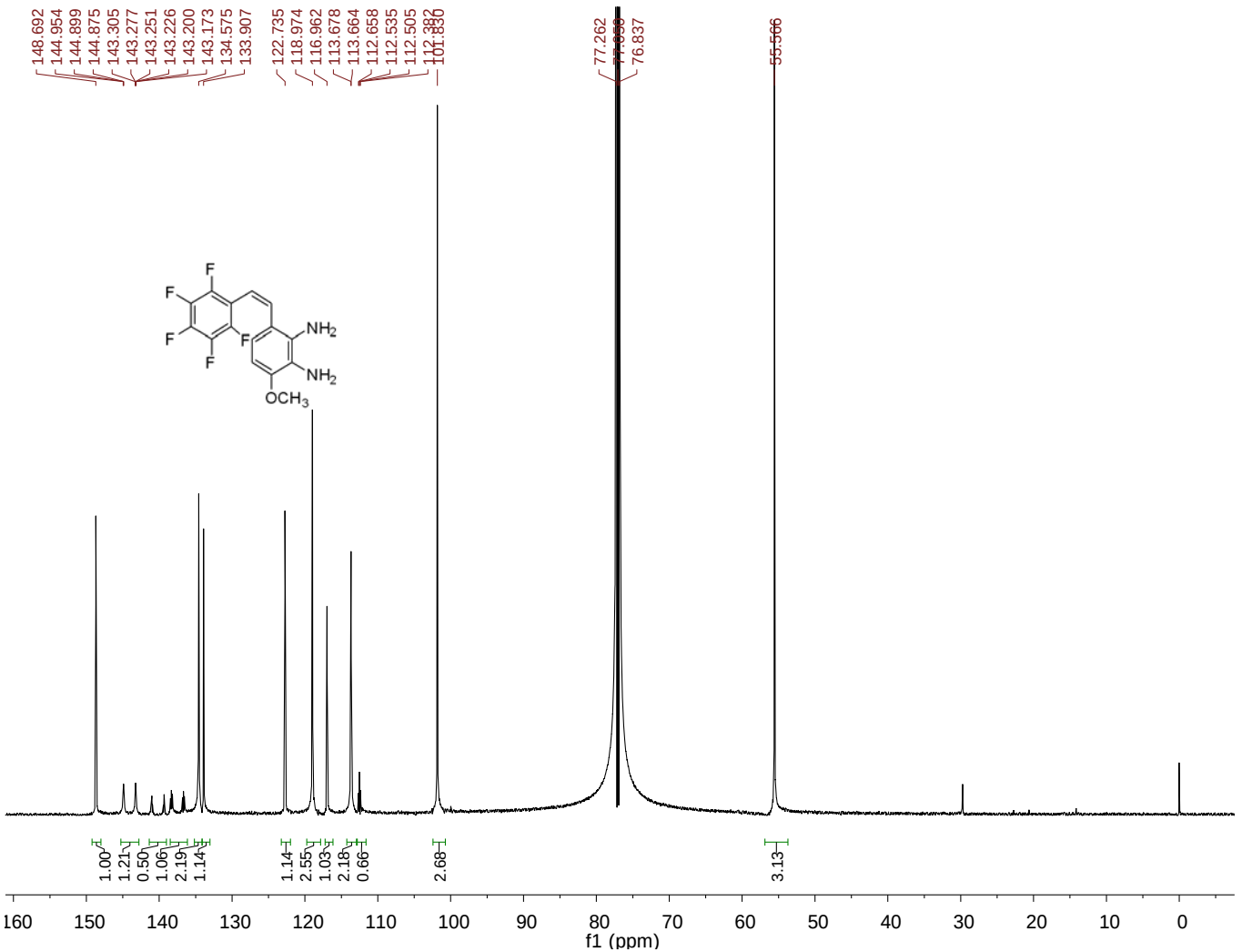
^{1}H NMR (500 MHz, $CDCl_3$) for Compound **8b**



^{1}H NMR (500 MHz, $CDCl_3$) for Compound **12**



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



HPLC Traces for Compound **12**

Data File C:\CHEM32\1\DATA\LAXMAN\RUN1000043.D
Sample Name: LD-II-119-1A

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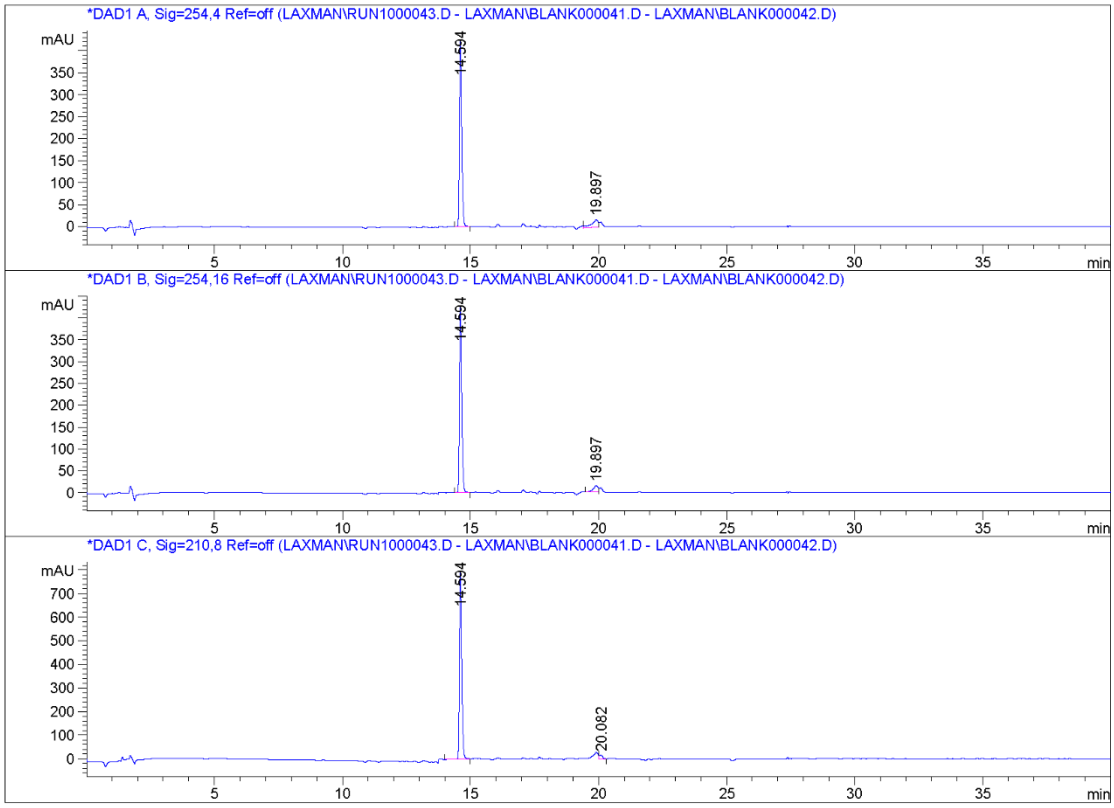
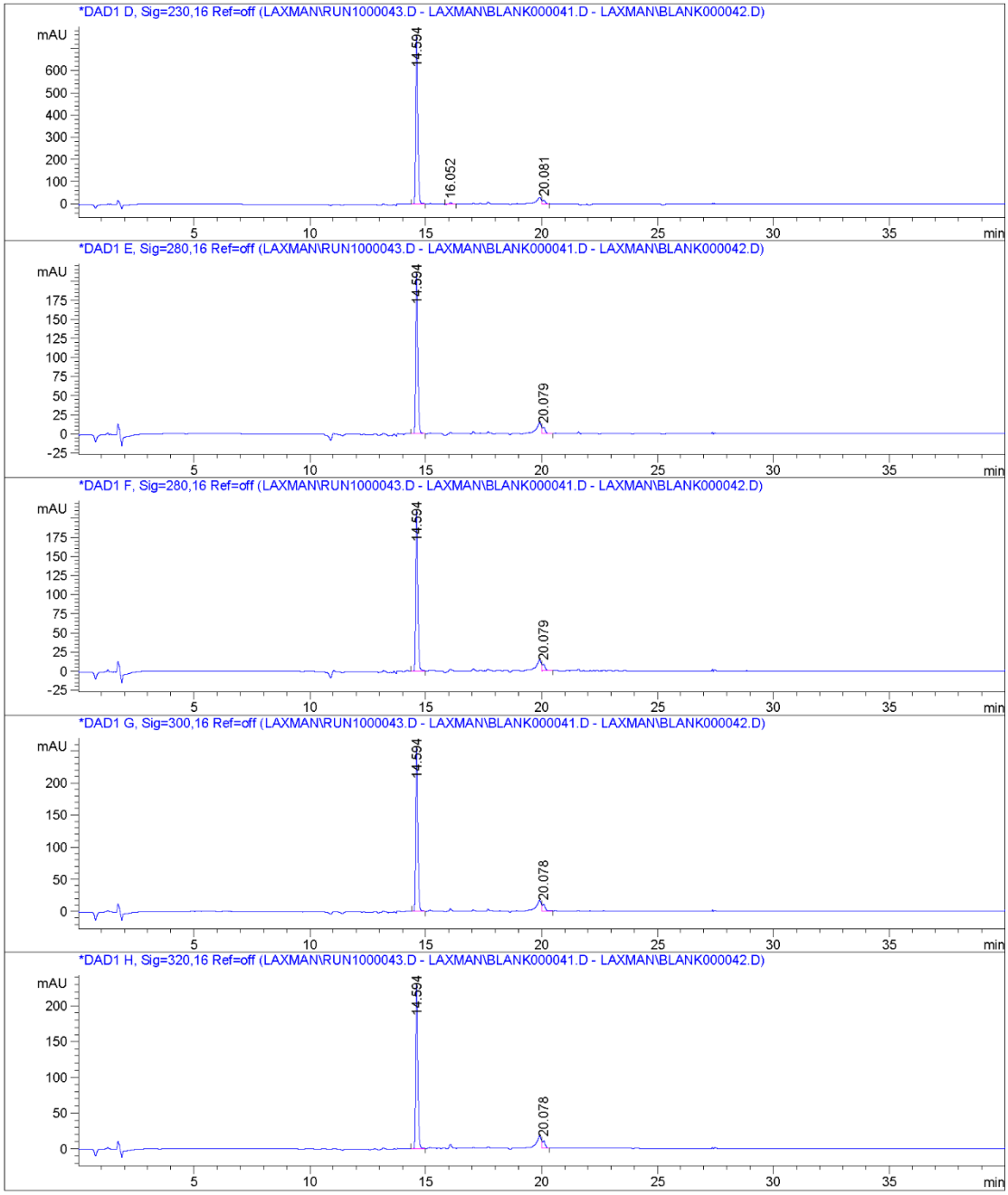


Data File C:\CHEM32\1\DATA\LAXMAN\RUN1000043.D
Sample Name: LD-II-119-1A



Data File C:\CHEM32\1\DATA\LAXMAN\RUN1000043.D
Sample Name: LD-II-119-1A

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
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.594	BB	0.0941	2622.45142	424.43347	88.3852
2	19.897	VV	0.2586	344.61978	17.74589	11.6148
Totals :				2967.07120	442.17936	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.594	BB	0.0941	2647.82861	428.55783	92.6464
2	19.897	BV	0.1906	210.16573	15.46235	7.3536
Totals :				2857.99434	444.02018	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.594	BB	0.0950	4978.99170	796.27325	97.5650
2	20.082	VB	0.1148	124.26624	15.97464	2.4350
Totals :				5103.25794	812.24789	

Data File C:\CHEM32\1\DATA\LAXMAN\RUN1000043.D
Sample Name: LD-II-119-1A

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.594	BB	0.0941	4697.77246	760.06799	95.7956
2	16.052	BB	0.1144	53.14312	6.71140	1.0837
3	20.081	VV	0.1239	153.03746	17.87930	3.1207
Totals :				4903.95304	784.65870	

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	14.594	BB	0.0944	1312.73535	211.60797	95.1735
2	20.079	VB	0.1163	66.57233	8.41418	4.8265
Totals :				1379.30769	220.02215	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.594	BB	0.0944	1312.73535	211.60797	95.1735
2	20.079	VB	0.1163	66.57233	8.41418	4.8265
Totals :				1379.30769	220.02215	

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	14.594	BB	0.0943	1592.02637	256.74332	94.9873
2	20.078	VB	0.1189	84.01540	10.32688	5.0127
Totals :				1676.04176	267.07020	

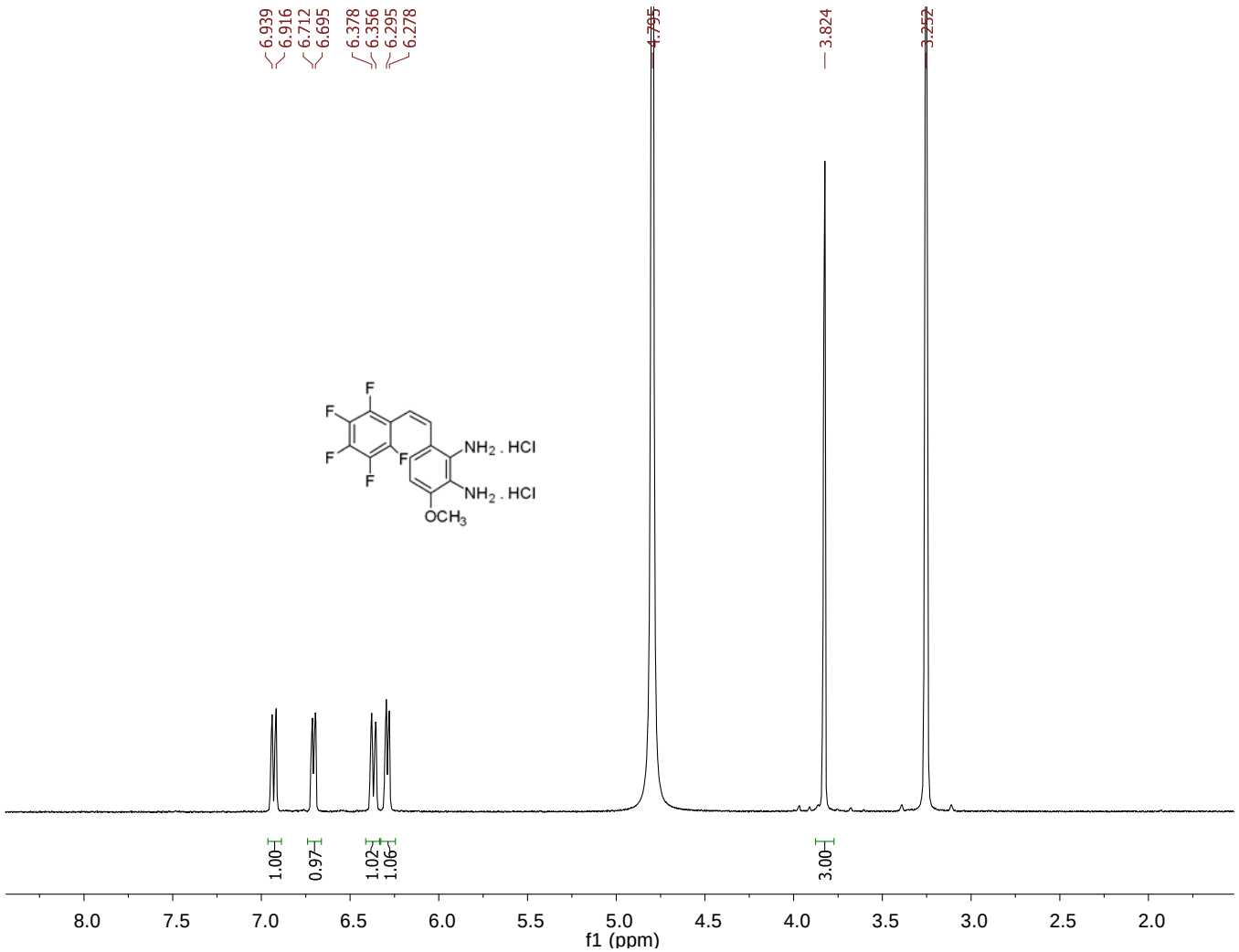
Data File C:\CHEM32\1\DATA\LAXMAN\RUN1000043.D
Sample Name: LD-II-119-1A

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

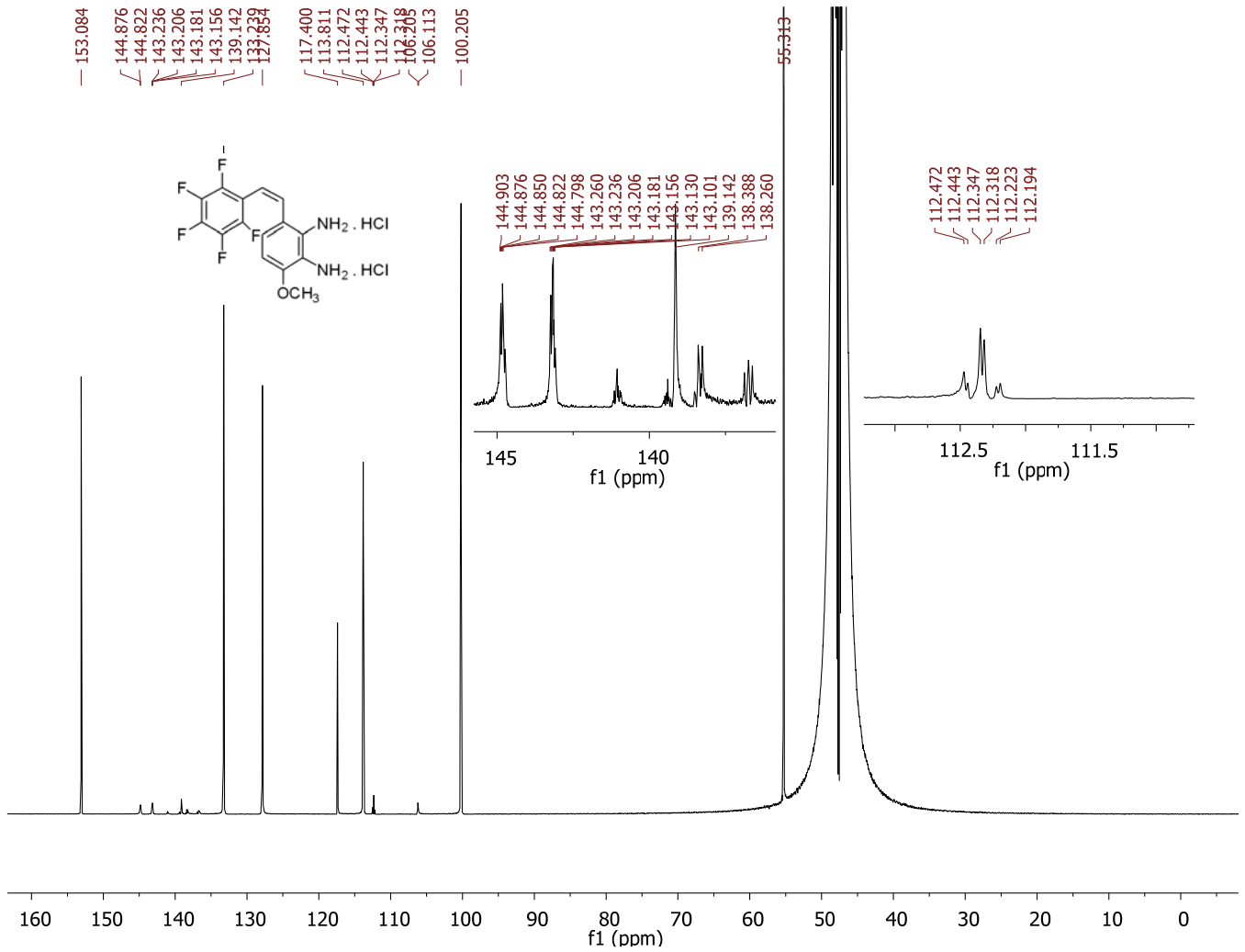
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	14.594	BB	0.0943	1430.27234	230.88907	94.8844
2	20.078	VV	0.1128	77.11158	10.13453	5.1156
Totals :				1507.38392	241.02360	

*** End of Report ***

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



^{13}C NMR (151 MHz, $CDCl_3$) for Compound **15**



HPLC Traces for Compound **15**

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Data File C:\CHEM32\1\DATA\LAXMAN\LD-II-121-1A\RUN1-121-1A0002.D
Sample Name: LD-II-121-1A

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    Last changed    : 10/15/2012 12:56:31 PM by Laxman
                      (modified after loading)
    Sample Info     :
                      Run1 LD-II-121-1A
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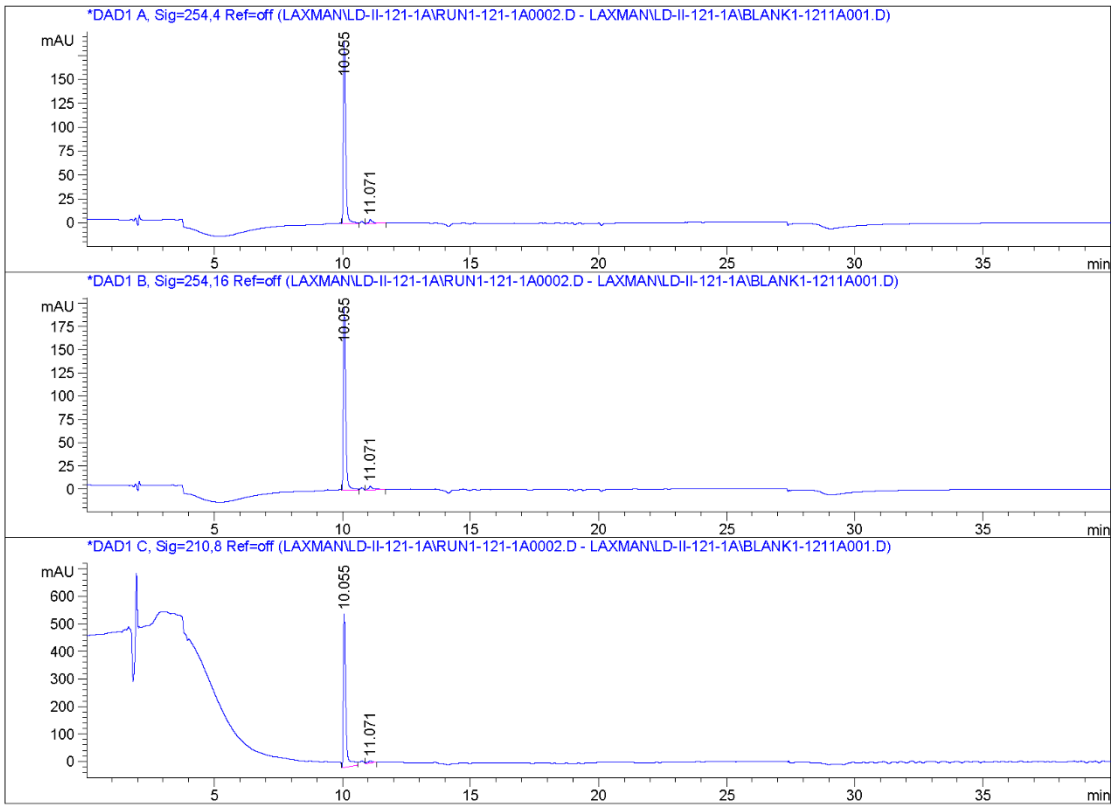
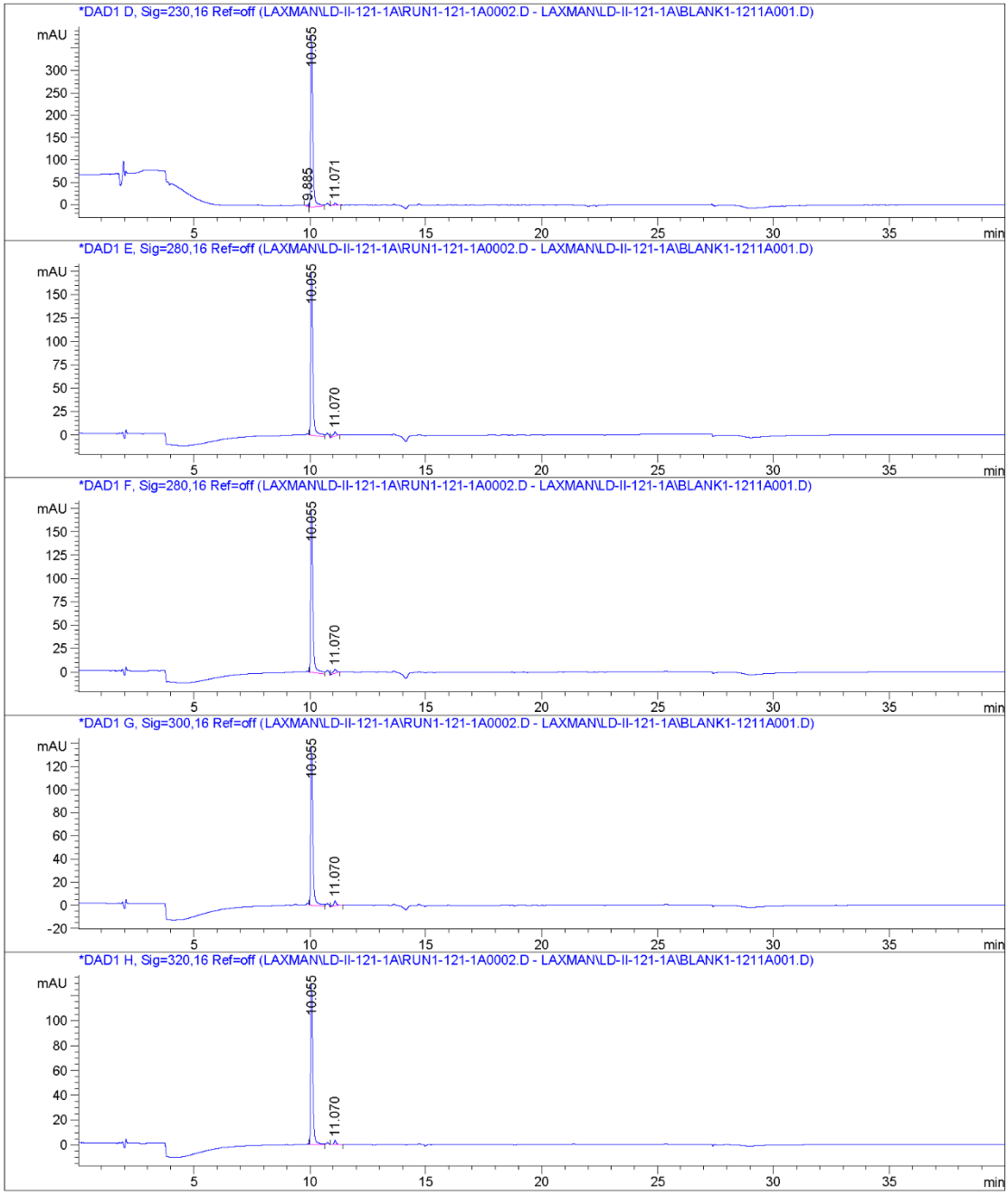


Data File C:\CHEM32\1\DATA\LAXMAN\LD-II-121-1A\RUN1-121-1A0002.D
Sample Name: LD-II-121-1A



Data File C:\CHEM32\1\DATA\LAXMAN\LD-II-121-1A\RUN1-121-1A0002.D
Sample Name: LD-II-121-1A

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
Signal has been modified after loading from rawdata file!

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.055	BV	0.0942	1182.25732	191.10005	95.5444
2	11.071	BB	0.1586	55.13276	4.57589	4.4556
Totals :				1237.39008	195.67594	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.055	BV	0.0942	1219.21594	197.02242	95.7010
2	11.071	BB	0.1594	54.76902	4.51838	4.2990
Totals :				1273.98497	201.54080	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.055	BV	0.0995	3714.97290	559.20099	97.0351
2	11.071	VB	0.1785	113.50977	8.24869	2.9649
Totals :				3828.48267	567.44968	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-II-121-1A\RUN1-121-1A0002.D
Sample Name: LD-II-121-1A

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	9.885	BB	0.1112	30.21793	4.34214	1.2110
2	10.055	BV	0.0952	2418.88110	385.34790	96.9407
3	11.071	BB	0.1229	46.11797	5.12804	1.8483
Totals :				2495.21701	394.81808	

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	10.055	VV	0.0973	1128.59668	174.74995	95.7414
2	11.070	BB	0.1379	50.19968	4.88264	4.2586
Totals :				1178.79636	179.63260	

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

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.055	VV	0.0973	1128.59668	174.74995	95.7414
2	11.070	BB	0.1379	50.19968	4.88264	4.2586
Totals :				1178.79636	179.63260	

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	10.055	VV	0.0964	874.11151	137.15108	95.2244
2	11.070	BB	0.1250	43.83793	4.77962	4.7756
Totals :				917.94944	141.93070	

Data File C:\CHEM32\1\DATA\LAXMAN\LD-II-121-1A\RUN1-121-1A0002.D
Sample Name: LD-II-121-1A

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	10.055	VV	0.0936	795.26794	129.58533	97.4159
2	11.070	BB	0.0902	21.09530	3.40752	2.5841
Totals :				816.36325	132.99285	

*** End of Report ***

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