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PREPARATION OF NEOCLERODANE DITERPENES AS PROBES FOR THE OPIOID RECEPTOR SYSTEM

by

Anthony Lozama

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Pharmacy
in the Graduate College of
The University of Iowa

July 2010

Thesis Supervisor: Visiting Associate Professor Thomas E. Prisinzano

ABSTRACT

While there are a variety of therapeutics that interact with the opioid receptor system, they are not without side effects; including constipation, dysphoria and respiratory depression. A better understanding of the opioid receptor system may yield therapeutic agents with a limited side effect profile. The neoclerodane diterpene, salvinorin A, appears to interact at opioid receptors through a unique mode of action. A better understanding of its interactions with opioid receptors will yield valuable information about the opioid system.

In order to probe further how salvinorin A interacts at opioid receptors, a series of novel analogues modified at the C-2 and furan ring were synthesized and evaluated for their ability to interact at opioid receptors. Synthetic methods were identified to modulate the furan ring, including the synthesis of Diels-Alder cycloadducts and phenyl rings derived from a reductive elimination. The cycloadducts are one of the first reported examples of Diels-Alder chemistry being applied to modify a neoclerodane while the phenyl ring analogues are the first to have aromatic rings directly off the salvinorin A core. C-2 sulfonate analogues were found to interact differently then their ester counterparts at opioid receptors while several of the cycloadduct analogues maintained affinity and efficacy demonstrating the furan is not required for opioid receptor activity. These findings demonstrate that salvinorin A is amenable for chemical modification, illustrating its potential as a novel scaffold for the development of opioid ligands.

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Date

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Graduate College
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CERTIFICATE OF APPROVAL

PH.D. THESIS

This is to certify that the Ph.D. thesis of

Anthony Lozama

has been approved by the Examining Committee
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This work is dedicated to those who stood by me and those who walked away from me and to the people of East New York, Brooklyn.

If you can keep your head when all about you are losing theirs and blaming it on you, if you can trust yourself when all men doubt you, but make allowance for their doubting too, if you can wait and not be tired by waiting; or being lied about, don't deal in lies, or being, hated, don't give way to hating, and yet don't look too good; nor talk too wise; if you can dream and not make dreams your master; if you can think and not make thoughts your aim; if you can meet with triumph and disaster and treat those two imposters just the same; if you can bear to hear the truth you've spoken twisted by knaves to make a trap for fools, or watch the things you gave your life to broken, and stoop and build them up with worn-out tools, if you can make one heap of all your winnings and risk it on one turn of pitch and toss and lose, and start again at your beginnings and never breath a word about your loss; if you can force your heart and nerve and sinew to serve your turn long after they are gone, and so hold on when there is nothing in you except the will which says to them: "hold on"; if you can talk with crowds and keep your virtue, or walk with kings nor lose the common touch, if neither foes nor loving friends can hurt you, if all men count with you, but none too much; if you can fill the unforgiving minute with sixty seconds' worth of distance run yours is the earth and everything that's in it, and which is more you'll be a man my son.

Rudyard Kipling
If

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
ACE	ANGIOTENSIN-CONVERTING ENZYME
ATP	ADENOSINE TRIPHOSPHATE
AZT	AZIDOTHYMIDINE
CDP-ME	4-DIPHOSPHOCYTIDYL-2-C-METHYLERYTHRITOL
CDP-MEP	2-C-METHYL-D-ERYTHRITOL 2,4-CYCLOPYROPHOSPHATE
COSY	CORRELATION SPECTROSCOPY
CNS	CENTRAL NERVOUS SYTSTEM
DA	DOPAMINE
DDC	*N*'*N*-DICYCLOHEXYLCARBODIIMIDE
ddC	2'-3'-DIDEOXCYTIDINE
DEC	DECOMPOSITION
Δ^9-THC	DELTA-9-TETRAHYDROCANABINOL
DIBAL-H	DI-ISOBUTY LALUMINUM HYDRIDE
DMAP	DI-METHYL AMINO PYRIDINE
DMAPP	DIMETHYLALLYL PYROPHOSPHATE
DMT	DI-METHYL TRYPTAMINE
DOXP	1-DEOXY-D-XYLULOSE-5-PHOSPHATE
DSB	DIMETHYL SUCCINYL BETULINIC ACID
DTT	DITHIOTHREITOL
EC_{50}	HALF MAXIMAL EFFECTIVE CONCENTRATION
EDCI	1-ETHYL-3-(3-DIMETHYLAMINOPROPYL)CARBODIIMIDE
EDTA	ETHYLENEDIAMINE-TETRA-ACETIC ACID
EGFR	EPIDERMAL GROWTH FACTOR RECPTOR
FST	FORCED SWIM TEST
FPP	FARNESYL DIPHOSPHATE
GDP	GERANYL DIPHOSPHATE

GGPP ....GERANYLGERANYLDIPHOSPHATE

GPP .... GERANYL DIPHOSPHATE

GPCR .... G-PROTEIN COUPLED RECEPTOR

GPP ....GERANYL DIPHOSPHATE

GTP .... GUANOSINE-5'-TRIPHOSPHATE

HMBB-PP .... (*E*)-4-HYDROXY-3-METHYL-BUT-2-ENYL PYROPHOSPHATE

HMBC .... HETERONUCLEAR MULTIPLE BOND COHERENECE

HMG-CoA .... 3-HYDROXYL-3-METHYL-GLUTARYL CoA

HMGR ....HMG-CoA REDUCTASE

HOBt 1-HYDROXYBENZOTRIAZOLE

HPLC ....HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HRMS .... HIGH RESOLUTION MASS SPECTROMETRY

HTS ....HIGH THROUGHPUT SCREENING

IPP .... 3-ISOPENTENYL PYROPHOSPHATE

K_d ....DISASSOCIATION CONSTANT

K_i ....BINDING AFFINITY

LSD .... LYSERGIC ACID DIETHYLAMIDE

LUMO .... LOWEST UNOCUPPIED MOLECULAR ORBITAL

MEcPP 2-C-METHYL-D-ERYTHRITOL 2,4-CYCLOPYROPHOSPHATE

MEP 2C-METHYL-D-ERYTHRIOL 4-PHOSPHATE

MP .... MELTING POINT

MVA .... MEVALONIC ACID PATHWAY

m/*z* .... MASS-TO-CHARGE RATIO

NADPH ....................NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE

NBS .... *N*-BROMOSUCCINIMIDE

NCE ....NOVEL CHEMICAL ENTITY

NE ....NOREPINEPHRINE

NMR	NUCLEAR MAGNETIC RESONANCE
PET	POSITRON EMISSION TOPOGRAPHY
PGP	P-GLYCO PROTEIN
RAS	RENIN-ANGIOTENSIN SYSTEM
ROESY	ROTATING FRAME OVERHAUSE EFFECT SPECTROSCOPY
SAR	STRUCTURE-ACTIVITY RELATIONSHIP
5-HT	SEROTONIN
TIA	TUBULIN INTERACTING AGENT
THF	TETRAHYDROFURAN
TLC	THIN LAYER CHROMATOGRAPHY
TM	TRANSMEMBRANE
TMS	TRIMETHYLSILYL

PREFACE

The use of compounds derived from nature for medicinal purposes dates back to antiquity and continues to this day. Ginger root is used to soothe upset stomachs,[1-5] while green tea has risen in popularity due to its antioxidant properties[6, 7] and both were used by the ancient Chinese as remedies for various ailments. Along with their medicinally beneficial qualities, natural products have intrigued both organic chemists and pharmacologists alike. The structural complexity of natural products has challenged the organic chemists who try to advance the barriers of organic chemistry. The unique pharmacological properties of natural products have helped pharmacologists develop a better understanding of human and animal physiology. Their combined efforts have helped bring dozens of beneficial drugs derived from natural sources to the market as well as advancing science on a whole.

A prime example of a natural product that has caused the convergence of pharmacology and organic chemistry while simultaneously expanding both fields is the alkaloid morphine. Derived from the opioid poppy in 1805 by Sertuner,[8] morphine has been used as an analgesic for over 100 years.[9] The study of its interactions within the body led to the discovery of the endogenous opioid ligands and helped establish the entire field of receptor pharmacology. Morphine's complex structure has attracted many organic chemists like Nobel Laureate Sir Robert Robinson, who first elucidated its structure, and Marshall Gates who first synthesized the molecule. These and other efforts helped develop and advance the field of alkaloid synthesis and natural products synthesis. In addition, the synthesis of morphine gave valuable information on the structure-activity relationship (SAR) of morphine. These SAR studies themselves have led to the

development of several clinically relevant compounds derived from morphine like the analgesic buprenorphine and cough suppressant dextromorphan.

However, despite their utility, morphine and other opioid ligands are not without fault. Opioids have been shown to cause respiratory depression, constipation, and tolerance and in some cases may result in death.[10, 11] Due to these deleterious side effects, there remains a pressing need to develop novel opioid ligands that do not possess these negative characteristics. These attributes may yet to be found in a different structural class of molecule found in nature. One such class may be neoclerodane diterpenes. Neoclerodane diterpenes are a structural class of natural products that are found in various plants and some animals. Many neoclerodanes have interesting pharmacological properties including anti-proliferation and insect deterrent activity. [12-14] They have also been found to be potent antimicrobials as well as insect anti-feedants.[13, 15] Despite their widespread presence in nature however, neoclerodanes as a structural class, remain by comparison to alkaloids, an under-explored area of research. A limited amount of synthetic works has been published on neoclerodanes and fewer still dedicated to the SAR of any of these natural occurring compounds. [16-18] Recently, one neoclerodane has garnered great interest from pharmacologist and organic chemist alike due to its interesting chemical and pharmacological properties and may help to shed light on the structural class of neoclerodanes.

The neoclerodane, salvinorin A was found to possess both affinity and efficacy for opioid receptors despite bearing no structural similarity to morphine or other traditional opioid ligands.[19, 20] Furthermore, preliminary SAR studies conducted on

salvinorin A showed that chemical modification to its structure was tolerated at opioid receptors.[21-28]

In order to further establish the pharmacophore of salvinorin A continued modification to its core structure must be carried out. However, due to the limited chemical methodology for neoclerodanes, new methods must be developed that will allow further probing of its pharmacophore. It is also envisioned that the chemical methodology developed for molecular alteration of salvinorin A will be readily applied to other neoclerodanes. This methodology will provide pathways to establishing the pharmacophore for other members of this class of molecule.

CHAPTER I. INTRODUCTION

Throughout time, products derived from natural sources have been use by man to treat a variety of ailments.[29] Egyptian medicine, which relied heavily on the use of plant products, dates back to 3000 BCE.[30, 31] This practice continues to this day as an estimated 1 billion people use natural products for medicinal purposes.[32] Besides its folk value, natural products have come to both form and shape modern medicine. Today, more than half of all modern pharmaceuticals were discovered due to the investigation of a natural product.[33-35] The first example of this was by Friedrich Sertüner, who isolated the first pharmacologically active pure compound from a plant.[36] Morphine was isolated approximately 200 years ago from *Papaver somniferum* L.[8] and this initiated the field of natural products isolation of pure compounds for pharmaceutical development.

Natural products by one definition, are substances produced by an organism that are not essential for the survival of the organism which produces it.[37] These substances are referred to as secondary metabolites but have come to be known in the vernacular as natural products and are produced by plant, marine, animals and microorganisms. While seemingly not important for the organisms that produce them, from a health and quality of life perspective, the importance and significance of these metabolites/natural products are too large to be ignored. Natural products have helped reduce pain, treat disease and provide longer and healthier lives. The first antibiotics used to treat infections were derived from natural products.[38, 39] The immuno-modulator, cyclosporine was isolated from a natural source and this as well as the agents based upon it, allow for organ transplants to occur as they lower the ability of the body to reject new organs.[33, 40] The direct result of these medicinal breakthroughs has been the doubling of the life expectancy from the beginning of the 20-century to now.[36] Even now, the importance of natural products is clear when one examines the role natural products play in treating different afflictions.

Natural Products and Cancer

Over the last 50 years in the United States, cancer has become more and more prevalent. According to the American Cancer Society 50% of men and 35% of women will develop some form of cancer in their lifetime.[41] As of 2009 an estimated 1.4 million people in the United States were diagnosed as having cancer and approximately 600,000 deaths were attributed to the disease.[41] This makes cancer the 2nd highest cause for mortality in the country.[41] With cancer being such a serious health phenomenon, pharmaceutical companies have invested heavily into the development of anti-cancer therapeutics to the sum of an estimated 30 billion dollars a year.[35] Natural products have proved to be a valuable commodity in this regard as some of the most important and influential anti-cancer drugs are natural products. These include camptothecin[42-47] (Figure 1), doxorubicin,[48-52] vincristine,[47, 53-59] vinblastine,[47, 53-59] mitomycin,[60-63] and streptozocin[64, 65]. Etoposide and tenoposide, two cancer chemotherapeutics based off of the natural product podophyllotoxin[66-71] have proven to be extremely influential as the mechanisms of action of these two agents, topoisomerase II inhibitors, differs from that of the parent compound.[66, 67] This helped illustrate that structural similarity alone is not a reliable predictor of biological activity.[69]

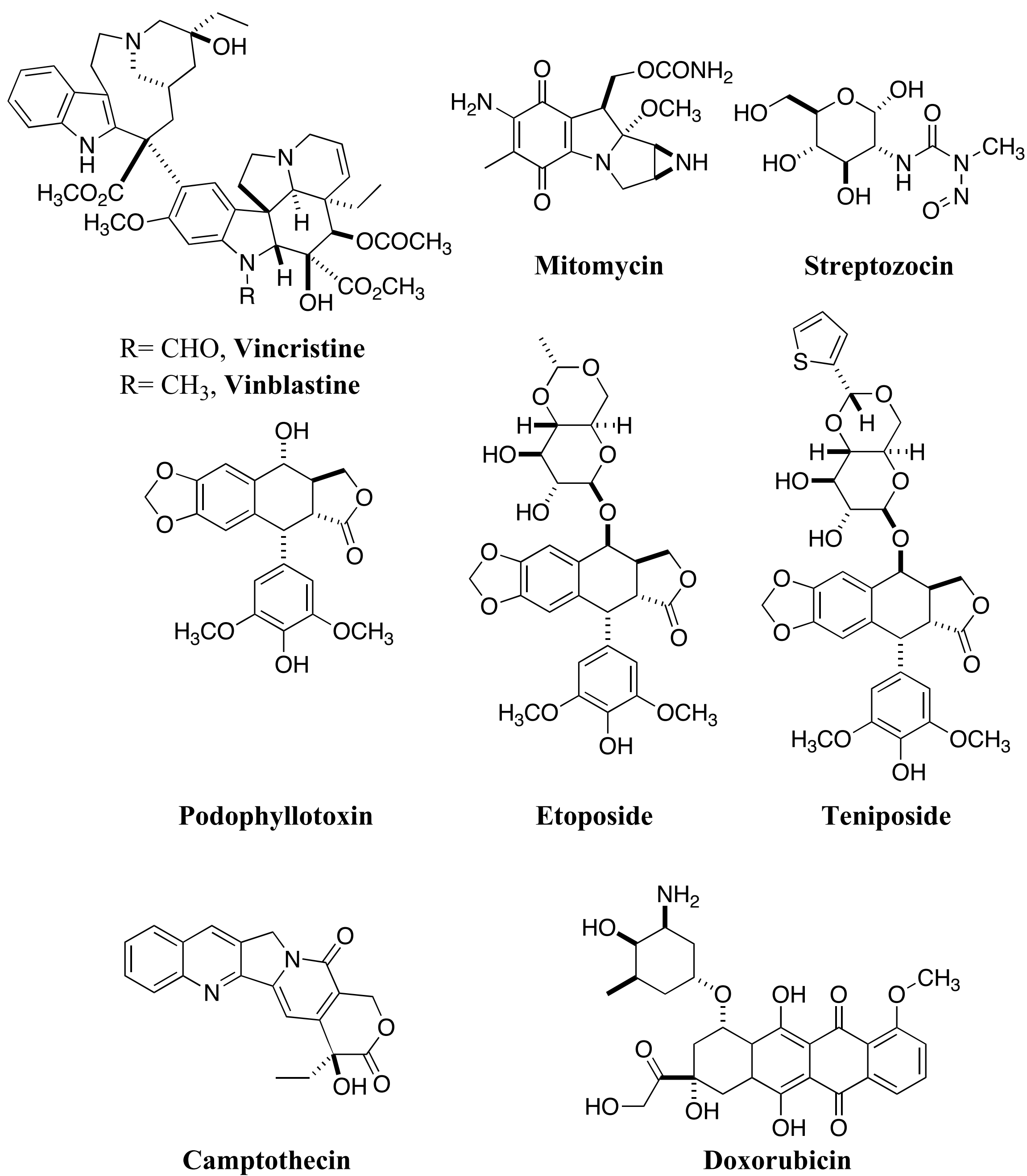


Figure 1: Structures of selected anti-cancer agents.

Natural Product Inspired Drug Target Discovery

Along with this lesson, natural products have also helped identify new targets for anticancer drug discovery. This was the case with paclitaxel (Taxol™) (Figure 2) as it was one of the first compounds observed with the ability to block depolymerization of microtubules.[72] Colchicine, another natural product isolated from *Colchicum autumnale*[73, 74], also displays this activity.[75-78] Since then, this mechanism has become a target for drug discovery efforts in both academia and industry. The discovery of new mechanisms of action is not uncommon with natural products. This can be seen with the case of pironetin. The majority of tubulin interacting agents (TIAs), which cause mitotic arrest through suppression of dynamic changes in microtubule functions, act as either reversible inhibitors or promoters of tubulin heterodimer polymerization. However, pironetin, which is derived from a strain of *Streptomyces*, forms covalent bonds to the α-tubulin chain.[29, 79, 80] This is the only known compound that acts in this manner and in addition to targeting a different site, the overall structure of pironetin is far less complicated then that of other TIAs.[79] This is significant as a less complex chemical structure makes pironetin much more amenable for total synthesis and/or analogue development which would only enhance its ability to be a commercially viable anti-cancer therapeutic.

Protein kinase inhibitors are another heavily investigated target for anti-cancer therapies. Several types of mutations in which kinases are constitutively active, have been associated with a variety of cancers. The key substrate for all kinases is ATP. However, with several thousand protein kinases believed to be in existence, the general belief was that selectivity with inhibitors could not be achieved due to the ubiquitous nature of ATP and enzymes.[81, 82] Gleevec, a natural product mimic, has shown to be a

selective and competitive inhibitor of ATP, illustrating the ability that protein kinases can indeed be inhibited and thus changing the overall consensus view in this field.[83, 84] Nakijiquinone C (Figure 2), which is isolated from an Okinawan sea sponge out of the Spongiidae family[85, 86], has also shown to be a protein kinase inhibitor as well as an inhibitor of epidermal growth factor receptor (EGFR) which plays a role in regulating cell growth, proliferation and differentiation.[87-89] Inhibition of this receptor has been shown to decrease cancer risk.[89, 90] Testing of a library of compounds derived from nakijiquinone C yielded four inhibitors of Tie-2 kinase, which is highly involved in angiogenesis and for which no inhibitors were known of before the library was constructed and tested.[29, 91, 92]

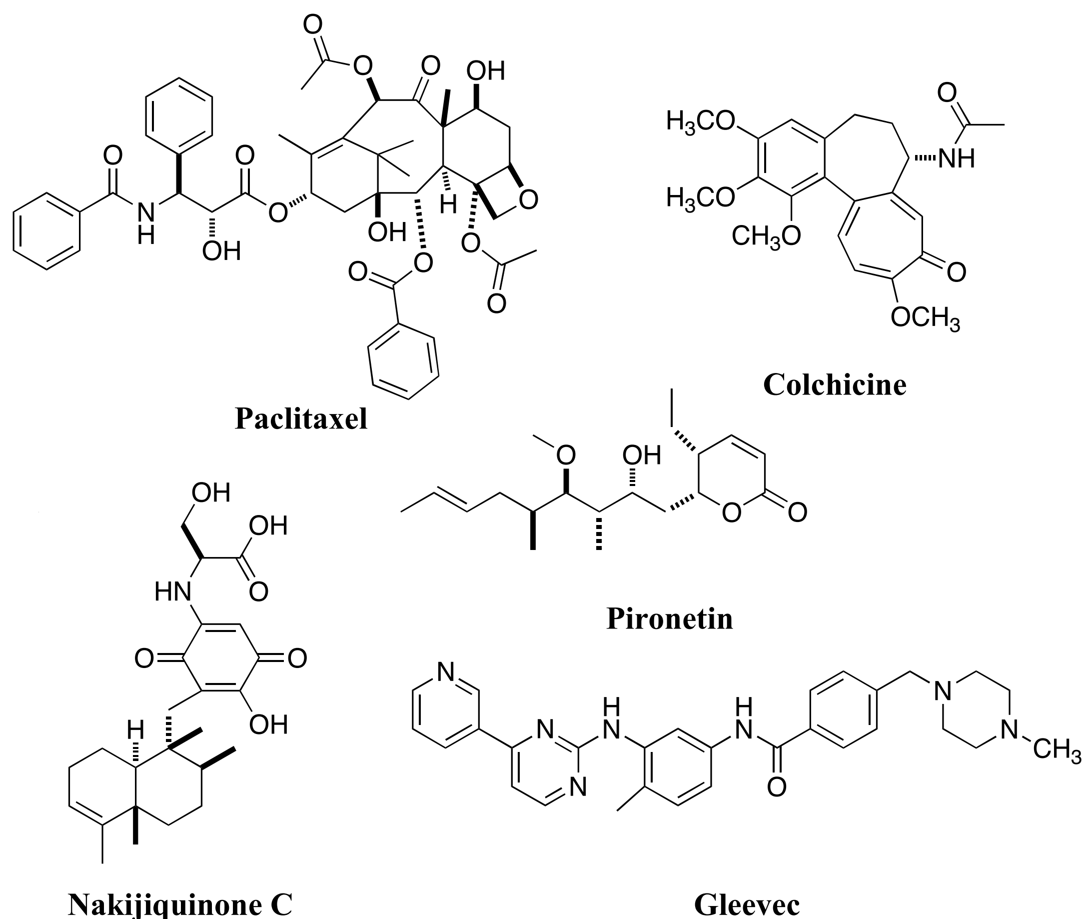


Figure 2: Structures of selected natural products with different anti-cancer activity.

<u>Natural Products Influence on Anti-Cancer Therapeutics</u>

These discoveries again illustrate the role and potential power that natural products can have in the anti-cancer drug discovery process. Besides being effective treatments, natural products have enhanced our overall understanding of cancer and have provided previously unknown target pathways to investigate in the effort to develop better therapeutics. Overall, natural products or compounds derived from natural

products represent approximately, 80% of all cancer drugs to come on the market from 1981-2006.[93] Furthermore, despite the advent of high throughput screening (HTS) of synthetic compounds for drug target leads, 74% of all novel chemical entities (NCEs) developed for cancer are related to natural products.[39, 82, 93]

The success of natural products in cancer therapeutics had a direct and overall positive effect on the survival rate of those diagnosed with cancer. From 1975 to 2004, the five-year survival rate of those diagnosed with cancer has gone from 50 to 70%.[41] It was during this time period that a host of new anti-cancer therapeutics reached the market thus allowing for higher survival rates and as stated before, a majority of these compounds were derived from natural products. While pharmaceutical companies on a whole have been or continue to downsize their emphasis in natural products, one need only look at the pipelines of big pharma to see that for the foreseeable future, the majority of anti-cancer therapeutics that pass through clinical trials will be either natural products or derived from natural products.[29, 35, 81, 93] If their past success is any indication of the future, the next generation of natural product therapeutics should be even more effective than the previous.

Natural Products and Heart Disease

While cancer is and remains a serious medical concern in the United States, the number one cause for mortality in the United States is heart disease.[94] Heart disease, which can encompass heart failure, high blood pressure, stroke and high cholesterol, causes anywhere from 600,000 to 1 million fatalities a year due to complications including stroke and heart attack.[94] Even more staggering then the death toll is the fact that an estimated 80 million Americans are categorized as having some form of heart

disease.[94] A closer look at the statistics show that 74 million people suffer from hypertension (high blood pressure), 6 million Americans suffer heart failure and 6.5 million Americans suffer from strokes annually.[94] These afflictions result in 500,000 deaths each year.[94]

Natural Products and Vasoconstriction

As seen in the numbers, hypertension affects a significant portion of Americans each year. The renin-angiotensin system (RAS) is a hormone system that sees over the regulation of blood pressure and fluid balance.[95] When blood volume is low, the kidneys secrete renin, which in turn causes the production of angiotensin I.[95] Angiotensin I in turns produces Angiotensin II, which is a protein that causes blood vessels to constrict which results in an increase in blood pressure.[95] Over activity of this system will lead to high blood pressure. High blood pressure may result in fainting, dizziness and significantly increases the chance of heart attack and stroke.[94] Angiotensin converting enzyme inhibitors (ACEs) such as enalapril (Figure 3) and fosinopril are some of the leading agents for the treatment of this condition.[96, 97] The role of natural products with ACE inhibitors is significant as the discovery of the pharmacological effects of a peptide found in the venom of the pit viper (*Bothrops jaraca*) led to the discovery of ACEs role in hypertension.[98, 99] Captopril (Figure 3), the first ACE inhibitor to come to market, was developed from this peptide.[33] Captopril led to the development of a series of ACE inhibitors, which has become an important class of therapeutics that help manage heart disease.[100]

Several other natural products have also shown ability to affect vasoconstriction. Reserpine[101-103] (Figure 3) and papaverine[104-107] have been clinically shown to act as

vasodilators.[33] Their mechanisms of action differ than that of ACE inhibitors, giving researchers other targets for treating this component of heart disease.

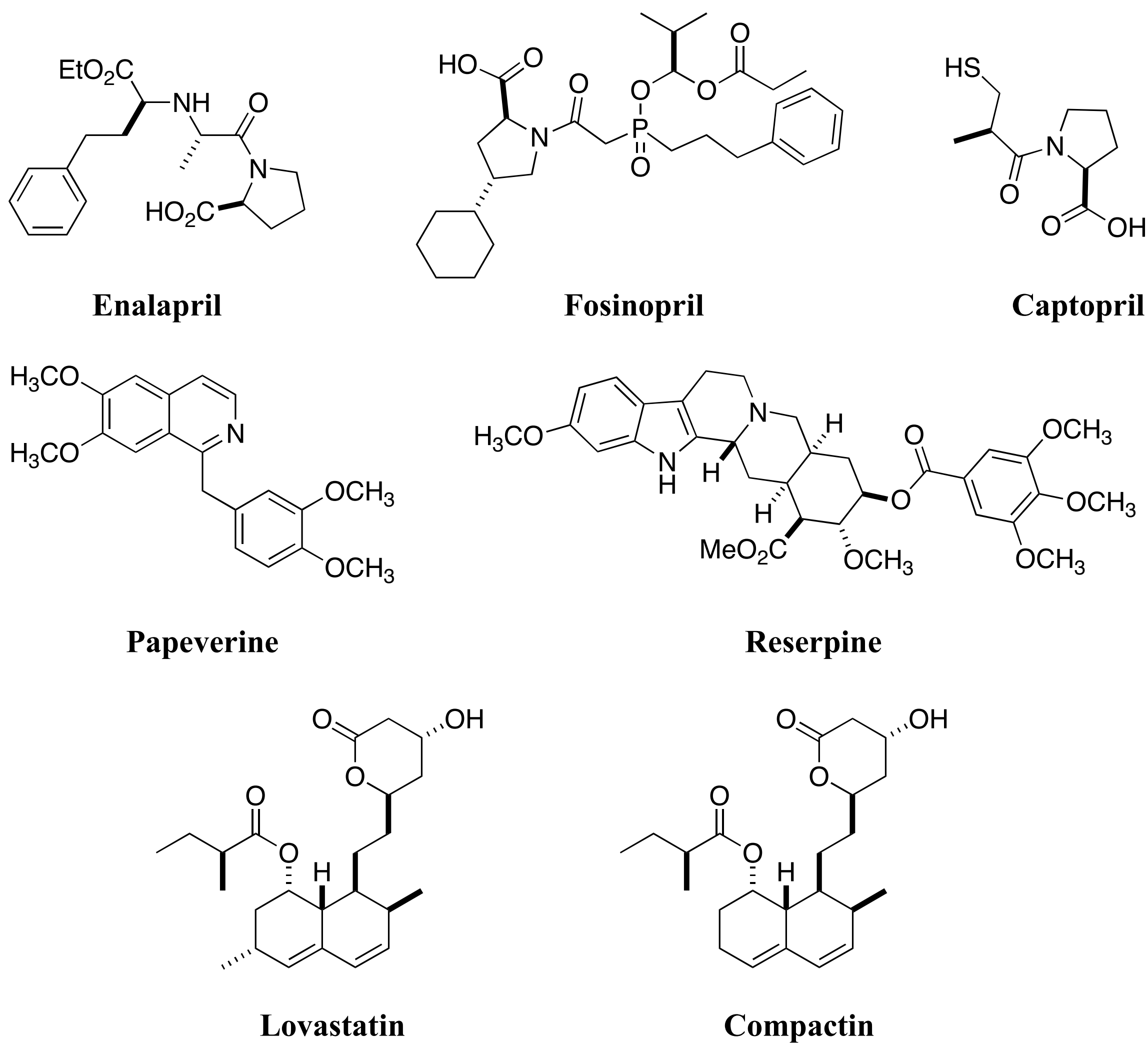


Figure 3: Structures of natural product derived treatments for heart disease.

Natural Product Derived Treatment of Arthrosclerosis

A major cause of both stroke and heart failure is atherosclerosis, which is the hardening of blood vessels.[87, 88]One risk factor implicated in this disorder is a high level of cholesterol which may lead to this condition.[87, 88] Cholesterol itself is an essential natural steroid that is require for the construction of cell membranes.[87, 88] It also has a role in the manufacturing of bile acids, steroidal hormones and several vitamins.[108] The consumption of foods high in fats can contribute to elevating the natural levels of cholesterol in the body. Coupled with this, certain segments of the populous cannot control their cholesterol levels even with the aid of a healthy and well balance diet due to an overproduction of cholesterol synthesis. The enzyme HMG-CoA reductase (HMGR) regulates the mevalonate pathway, which is responsible for the biosynthesis of cholesterol.[109] It allows the conversion of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) to be converted to mevalonic acid, which ultimately gets converted to cholesterol.[109] A group of therapeutics, known as statins has been able to inhibit this transformation and drastically lower the levels of cholesterol in the blood.

The first statins were natural products that were isolated from microorganisms. Compactin, which was isolated from broths of *Penicillum citrinum*, was found to directly inhibit HMGR.[110] Moreover, a methylated derivative of compactin, lovastatin was found in the broths of *Monascus rubber* and *Aspergillus terreus* respectively.[111, 112] These statins were found to reduce overall levels of cholesterol by 40%, more than double of what the previous therapies were capable of.[39] Lovastatin was the first statin to reach the market in 1987 and since then a series of derivatives based off its structure have come to market. Perhaps most famous of these is Lipitor® which has been consistently one of the

world's top selling drugs.[113] The ability of statins to lower cholesterol levels and decrease the risk for stroke and heart attack greatly enhances its medicinal benefits and helps many thousands of people to live longer lives.

Natural Products and HIV/AIDS

While heart disease and cancer are major medical concerns here in the United States, globally their mortality rates pale in comparison to infectious diseases such as malaria and HIV/AIDS. Since its discovery in 1983, HIV/AIDS has reached pandemic levels.[114-116] An estimated 35 million people worldwide are currently living with either HIV/AIDS[117] with 2 million deaths annually attributed to the disease.[117] Since the late 1980's, an intensive study into the development of therapeutics for this epidemic has yielded over 20 clinically approved drugs.[118] Natural products have played an important role in this cause as some of the first approved therapeutics were nucleotide and nucleoside bases. Cytarabine (Figure 4), an antiviral which was developed from a nucleoside found in the sea sponge *Cryptotethia crypta*[119, 120], led to the development of AZT[121-123], ddC[124, 125] and Videx™[124, 125], which act as inhibitors of reverse transcriptase. When a cell is infected with HIV, reverse transcriptase copies the viral RNA into the host cells DNA.[88] The host cell then replicates the virus, allowing it to infect other cells.[88] The reverse transcriptase inhibitors stop this process. These natural product derived inhibitors were the 1st generation of anti-HIV medications and inspired the development of the next generation of HIV treatment, protease inhibitors.[40] These compounds inhibit the protease that is responsible for releasing nascent proteins for the final assembly of new viral molecules and include Darunavir[126-130] and Tipranavir.[131-133]

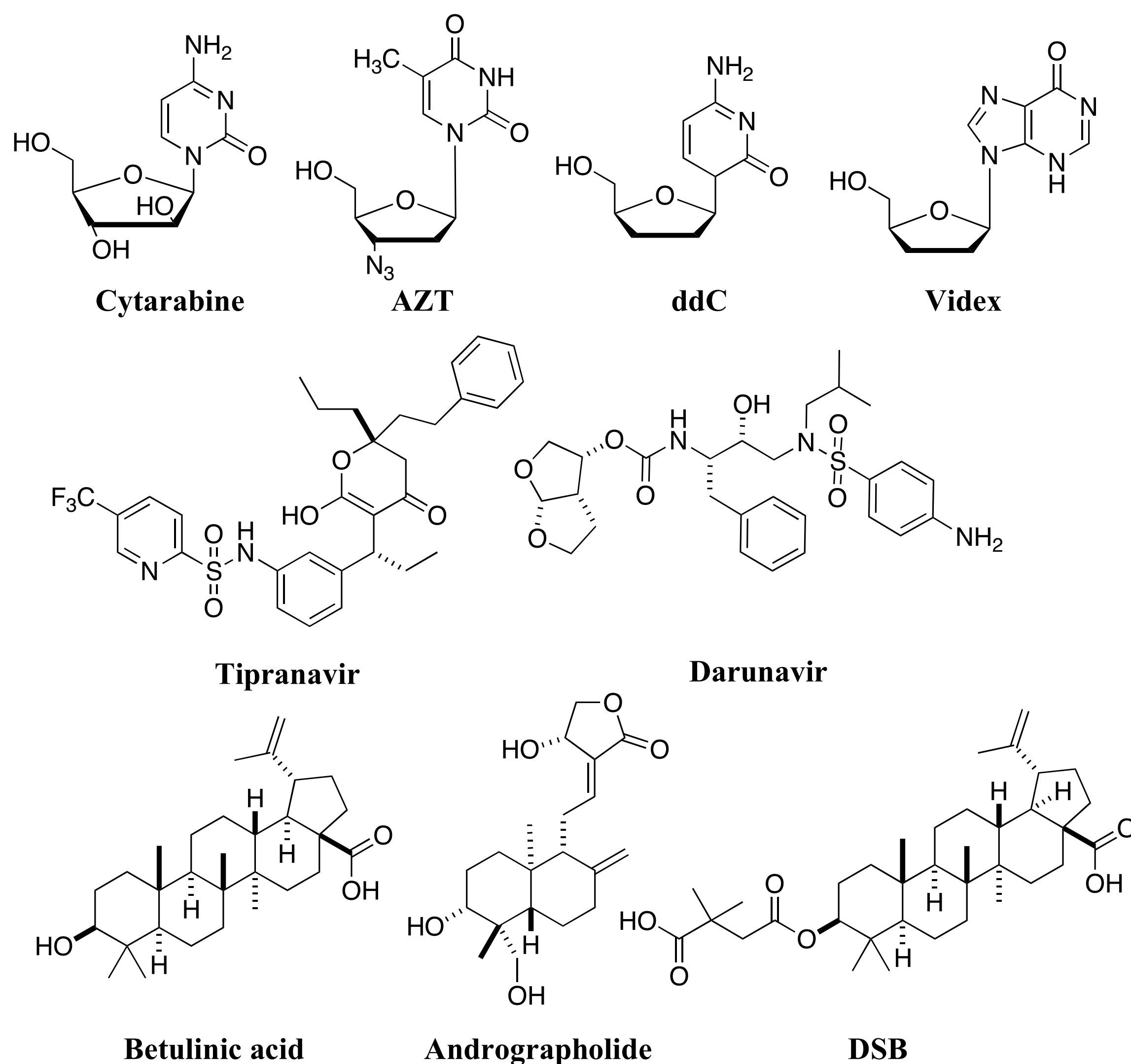


Figure 4: Structures of natural product derived HIV/AIDS therapeutics.

Natural Product Leads for HIV/AIDS

One factor that makes treating HIV difficult is the cost of the therapy. Many of the people infected with the virus live in economically undeveloped regions in the world

including sub-Sahara Africa and parts of the Pacific Rim. These regions simply do not have the type of economic infrastructure that could afford the price of HIV therapeutics. Cheaper, yet effective medicines are needed in order to truly make an impact in the HIV/AIDS epidemic. Natural products may yet provide the answer to this very difficult question, as they may be more economically viable treatments for these regions. Several promising natural product leads have been discovered to treat HIV and are currently being investigated. These include andrographolide (Figure 4), isolated from *Andrographis paniculata*[134], which during a phase I clinical trial, lowered viral load in subjects.[135] It is proposed that andrographolide inhibits cell cycle dysregulation instead of interrupting viral replication, which would make it very unique amongst HIV treatments.[136-141] Betulinic acid, derived from *Syzigium claviflorum*[142, 143], led to the development of an extremely potent analog, DSB, which is potent against mutant strains of HIV that are resistant to other drugs.[144] Also, DSB seems to inhibit replication in until this point, an unforeseen way making it a "first in class" inhibitor and is currently in clinical trials.[143, 145]

Natural Products and Malaria

While HIV/AIDS will remain a global health concern for years to come, malaria surpasses HIV/AIDS in mortality every year. Malaria is an infectious disease caused by a parasite (*Plasmodium falciparum*)[146] and it is estimated that upwards of 3 million people die annually from the disease.[146, 147] More alarming is that 40% of the world's population is at risk of contracting the disease as each year, there are approximately 350-500 million cases reported annually.[148, 149] Natural products have been and continue to be the leading source of therapeutics in dealing with the disease. Artemisinin, isolated from

Artemisia annua[150, 151], was found to be a very potent killer of the parasite that causes the disease while being safe for humans.[152] Analogues derived from artemisinin, including artemether and arteether have become widely used amongst areas prone to malaria due to its ability to be effective against resistant strains of malaria.[33, 55, 153, 154]Before artemisinin and its derivatives, the standard therapeutics used to treat malaria were, primaquine and chloroquine.[155] These were derived from quinine[156-158], which comes from the bark of the *Chichona* tree.[159] While effective, the rise of resistance led to the development of new and improved therapies. Besides artemisinin and its derivatives, a majority of anti-malarials currently in development are derived from natural products.[93]

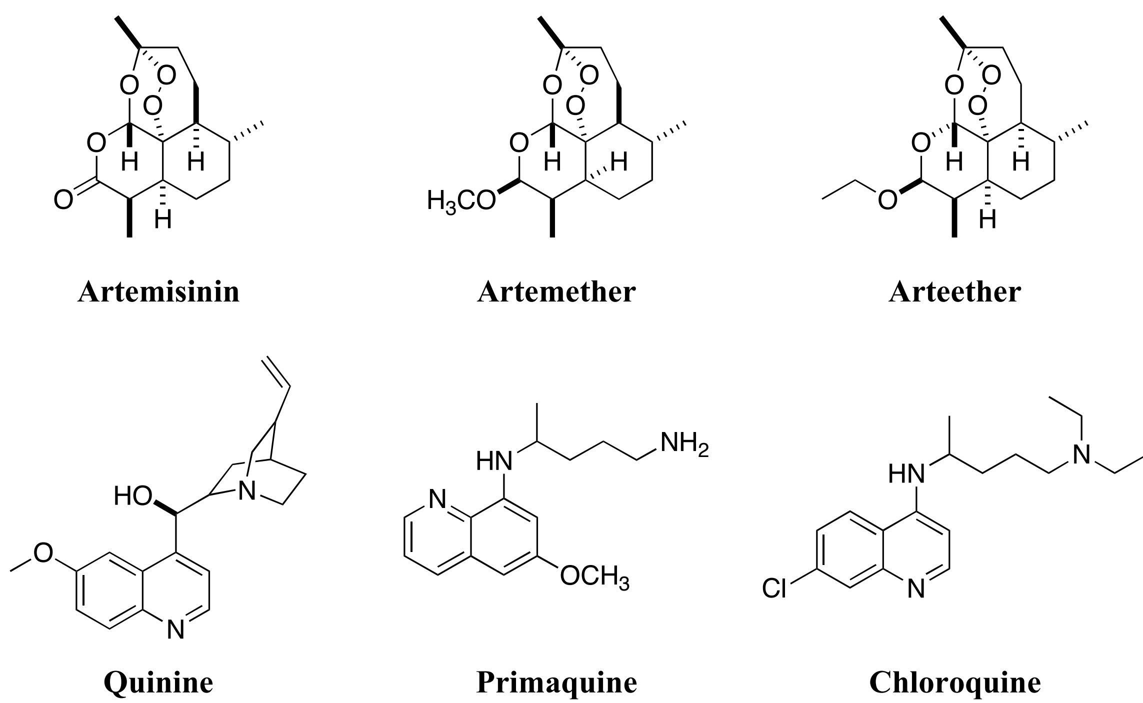


Figure 5: Structures of natural product derived malaria therapeutics.

Windfalls of Natural Product Investigation

While patent issues and lack of material may hinder the development of natural products by commercial interest, the effort may certainly be worth it as natural products and their derivatives account for over 40 billion dollars in sales.[39] This is further illustrated in the fact that the two top selling drugs in the world, Lipitor® and Advair®, were derived from natural products.[160] The sheer number of natural products, which has been estimated to be 600,000 from plants alone[39], most certainly ensures that an even larger number of useful therapeutics will be developed from this source.

Further incentive to develop natural products comes from the academic lessons learned from their investigation. Natural products have aided in the development of modern medicinal chemistry through analogue construction along with helping establish important concepts essential to drug development, such as chirality and its role in drug action. This was the case with quinidine, the anti-arrhythmic agent whose diastereomer was quinine, the first antimalarial.[33] Natural products have also aided with our understanding of the biological systems that they interact with. One of the best examples of this is in the field of neuroscience.

Natural Product Unearthing of the CNS (Cannabinoids)

Neuroscience is the branch of life science that deals with the structure, development, function, chemistry, pharmacology and pathology of the central nervous system (CNS)[161] and natural products that interact with the CNS have greatly enhanced our understanding of this extremely complex system. Many of the natural products that interact with the CNS are psychotropic and the study of these effects has helped us elucidate the mechanisms by which the CNS works. One such example of this can be

seen with the active component of *Cannabis sativa* L., Δ^9-tetrahydrocannabinol (Δ^9-THC) (Figure 6).[162, 163] Δ^9-THC has long been considered the chemical in marijuana that accounts for the "high" and investigations into its activity led to the discovery that it bound to a specific G protein-coupled receptor (GPCR) in the brain.[164] This led to the discovery of the endogenous ligand for this receptor, anandamide.[165, 166] The discovery of anandamide led to the ongoing elucidation of the cannabinoid receptors and the endocannabinoid system.[166-168] The endocannabinoid system has been and continues to be investigated for its role in nausea. Nabilone, a synthetic mimic of Δ^9-THC, has proven to be an effective anti-emetic agent, especially for people who suffer from cancer chemotherapy induced nausea.[169-171] Along with its involvement with nausea, the cannabinoid receptors are currently being investigated as a target for obesity due to its role in regulating appetite and satiety.[172-176]

According to the World Health Organization (WHO), obesity has become a global epidemic, as an estimated 700 million adults will be obese by 2015.[177, 178] There are various amounts of co-morbidities associated with this condition that further jeopardize public health, including heart disease, diabetes and osteoarthritis.[179] Rimonabant, an endocannabinoid receptor antagonist, was shown to cause weight loss in clinical trials[180]. However, it was later pulled off of the market due to side effects.[181] Despite this setback, endocannabinoid antagonists are still being investigated for anti-obesity therapeutics.[182] Thus without the discovery of the natural product, Δ^9-THC and its pharmacological site of action, the knowledge that we now possess of the endocannabinoid receptor system would have greatly diminish our ability to develop therapeutics that target it. This is of great significance as the endocannabinoid receptors are also a target for the treatment of

several other afflictions, including stroke[183-185], Parkinson's[186-188], Huntington's[189-191], anxiety[192, 193] and Tourette's.[194-196]

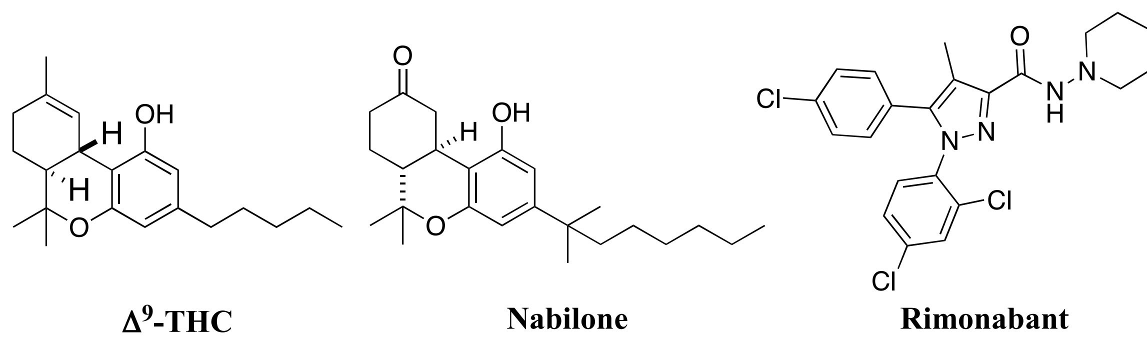


Figure 6: Structures of selected cannabinoid ligands.

Natural Products and Neurotransmission

Neurotransmission, and the function of the monoamine neurotransmitters serotonin (5-HT), norepinephrine (NE), and dopamine (DA) affects on brain function is one of the central areas in neuroscience. Natural products have been instrumental in understanding how these compounds affect the brain. Studies on compounds isolated from *Rauvolfia serpentina* like reserpine[197-199], (Figure 3) have given us insight on neurotransmitters and the underlying mechanisms of human depression.[200] While being investigated for its effects on hypertension, reserpine was found to alter the mood of patients, causing sedation and depression.[201, 202] Further investigation into this

phenomenon found that reserpine depleted NE levels in the brain[197, 198] and this led to the formulation of the catecholamine hypothesis of depression.[203] From this initial hypothesis, monoamines were heavily investigated resulting in further elucidation of their syntheses, release processes and biological function.[204] This enhanced understanding has been instrumental in the development of therapeutics that target this system in the effort to treat depression and other neurotransmitter related disorders.[200] The elucidation of the mechanisms behind neurotransmission has also aided our understanding of sensations such as pleasure as well as some insight into its counterpart, pain.

Natural Products, Pain and the Opioid Receptors

The CNS oversees many physiological tasks including the sensation of pain in the body. Pain can be defined as "an unpleasant sensory experience due to noxious stimuli" and its role is that of protection: warning us from imminent or actual tissue damage in order for us to respond and keep said damage to a minimum.[205] Pain is a symptom of many medical conditions and is the most common reason for the seeking of medical attention in the United Sates.[206] Much of the insight that we have on pain is due to the investigation of a natural product derived from the opium poppy *Papaver somniferum* L., the alkaloid morphine[8] (Figure 7).

Many useful natural products are represented in this class including the previously mentioned reserpine (Figure 3) and the antitussive/analgesic agent, codeine.[207, 208] In addition to being part of this structural class of molecules, morphine was the first alkaloid isolated from a natural source and has been heavily investigated ever since.[209] This investigation was prompted by morphine's profound pharmacological profile. Morphine is a powerful analgesic and has been used clinically for the treatment of pre and post-

operative pain[210-212] as well as chronic pain associated with cancer. Along with its analgesic profile, morphine also causes respiratory depression, constipation, tolerance and dependence.[9] Due to these deleterious side effects, morphine's utility as an analgesic was compromised, so in the effort to limit these, morphine's chemical structure was extensively probed. This probing led to the discovery of many other useful compounds that shared morphine's core structure, including the big-game tranquilizer etorphine[213, 214], the analgesic hydrocodone[215] and the opioid antagonist nalorphine[216]. The discovery of nalorphine was historically significant as it was able to block the pharmacological actions of morphine while displaying pharmacological activity as well.[216, 217] This discovery caused speculation of multiple opioid receptors.[218] This idea was indeed verified as studies using morphine derived ligands led to the discovery of the opioid receptors.[219-227] Following a reoccurring theme in science, this discovery led to further investigation, which uncovered the endogenous opioid ligands.[228-234] The discovery of the opioid receptors and the endogenous ligands has helped give insight into the underlying mechanisms of pain along with the development of tolerance to drugs of abuse such as cocaine and methamphetamine as opioid receptors have been implicated in these complex processes as well.[235-239]

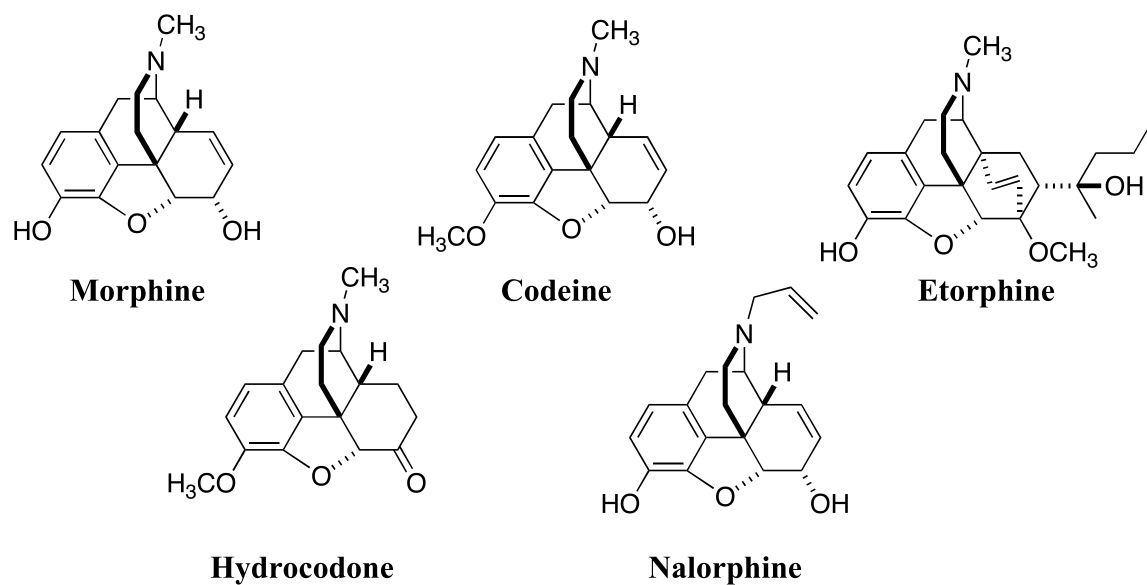


Figure 7: Structures of selected opioid ligands.

The natural product morphine has been instrumental in the discovery of the opioid receptor system and SAR studies on morphine has yielded information on how opioid receptors function along with deriving several useful compounds used today. However, a common problem with many opioid ligands, including morphine and its derivatives, are the negative side effects associated with opioid receptor activation, mainly respiratory depression and tolerance.[9] Due to this and along with the fact that opioid receptors may have utility in a variety of afflictions including depression[240-243] and gastrointestinal disorders[244-246], there remains a need to develop compounds that do not share the negative consequences of other opioid ligands. Novel scaffolds that interact at the opioid receptors may present an opportunity to develop therapeutics that can circumvent the negative side effects of typical opioid ligands, therefore exponentially increasing their

value as medicinal agents. To this end, one such scaffold may be the neoclerodane skeleton, a subset of a broad class of natural products categorized as terpenes.

Terpenes

Terpenes represent the most structurally and stereochemically diverse group of natural products known as more than 55,000 terpenes have been identified from all living organisms.[247, 248] Terpenes consist of 5-carbon building blocks, termed isoprene.[249] Isoprene itself is actually not part of the building process of terpenes but rather; chemically activated forms of isoprene undergo the synthesis of terpenes. These activated isoprenes; dimethylallyl pyrophosphate (DMAPP) (Figure 8) and 3-isopentenyl pyrophosphate (IPP) are themselves biosynthesized through two pathways.[250-254] The first pathway to be elucidated was the mevalonic acid pathway.[250, 251] This pathway consists of two acetyl-CoA units condensing via acetyl-CoA transferase to form acetoacetyl-CoA. Acetyl-CoA then condenses with acetoacetyl-CoA to form 3-hydroxy-3-methyglutaryl-CoA otherwise known as HMG-CoA via the actions of HMG-CoA synthase. HMG-CoA is then reduced by HMG-CoA reductase and 2 units of NADPH to mevalonate. Mevalonate is then subsequently phosphorylated by mevalonate kinase and phosphomevalonate kinase to give mevalonate-5-pyrophosphate. Reaction of a decarboxylase then yields IPP. IPP isomerase catalyzes a reaction of IPP to give DMAPP. This pathway is believed to be the mechanism of how eukaryotes, higher plants and archaebacteria produce terpenes.[255]

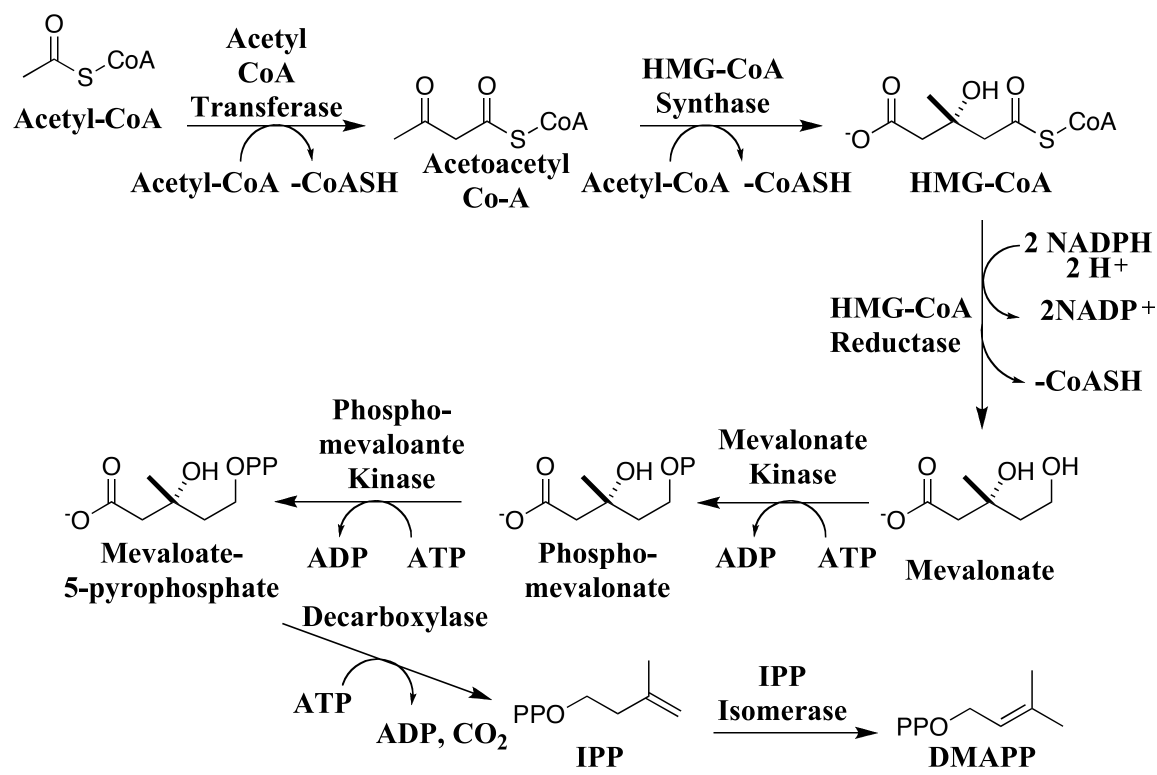


Figure 8: Mevalonate pathway for the biosynthesis of IPP and DMAPP.

Recently, another pathway to obtain IPP and DMAPP has been elucidated.[255-258] The non-mevalonate/1-Deoxy-D-xylulose-5-phosphate (MEP/DOXP) (Figure 9) pathway is the proposed mechanism for the production of terpenes by many eubacteria, green algae and plants.[255-258] The pathway starts with the condensation of D-glyceraldehyde 3-phosphate and pyruvate through DOXP synthase (Dxs) to yield DOXP.[259] A reduction and rearrangement of DOXP by DOXP reductase (IspC) gives 2C-methyl-D-erythriol 4-phosphate (MEP). A cytidine moiety is introduced into MEP by the actions of 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase (IspD) to produce 4-

diphosphocytidyl-2-C-methylerythritol (CDP-ME). CDP-ME is then phosphorylated by 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (IspE) to yield 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate (CDP-MEP), which is the substrate for 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (IspF). The result of this reaction is 2-C-methyl-D-erythritol 2,4-cyclopyrophosphate (MEcPP)[259] which, is a substrate for HMBB-PP (IspG) synthase and this reaction produces (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP). The pathway concludes with HMB-PP being reduced by HMB-PP reductase (IspH) to either 3-isopentenyl pyrophosphate (IPP) or dimethylallyl pyrophosphate (DMAPP).[260]

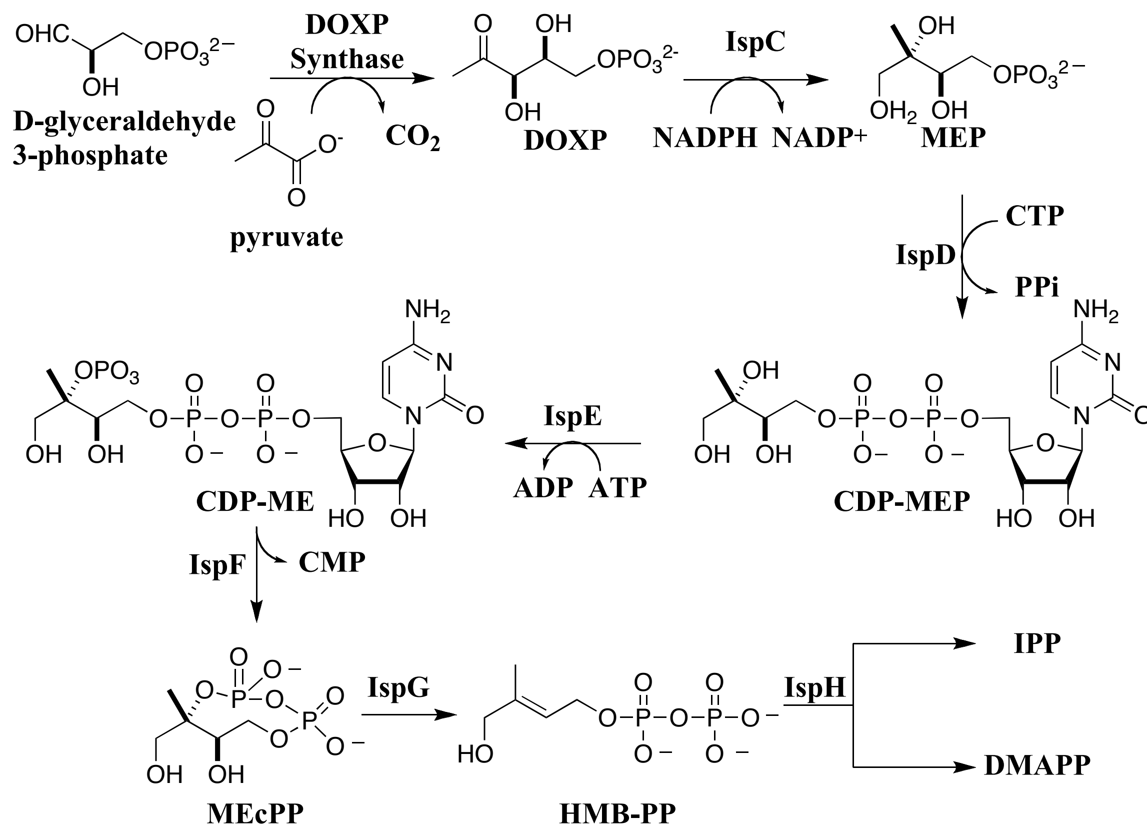


Figure 9: MEP/DOXP pathway for the biosynthesis of IPP and DMAPP.

Construction of Terpenes

With these two building blocks in hand, the synthesis of terpenes can be facilitated. Chemically, chain elongation typically consists of a series of electrophilic alkylations that occur via a three-step process.[261] The first step involves the dissolution of the carbon-oxygen bond of IPP/DMAPP to from a carbocation.[262] This intermediate then proceeds to alkylate at the double bond of another IPP which generates a second carbocation at the C-3 position of the isopentyl unit.[261] Stereoselective elimination of a

proton at C-2 produces a new allylic diphosphate which has now been elongated by one isoprene unit.[261] This reaction is carried out in a head to tail manner resulting in the formation of a 10-carbon chain referred to as geranyl diphosphate (GPP).[262] Successive additions of IPP yields farnesyl diphosphate (FPP), which contains 15 carbons, geranylgeranyl diphosphate (GGPP), 20 carbons, and a series of even longer chain products.[263] A family of prenyltransferases carries out the elongation of the chain. The individual members of this enzyme family determine the chain length and the stereochemistry of the new allylic bonds generated.[261] The double bonds of the elongated chain are either *E* or *Z* where with the (*E*) designation; the carbon atoms of the isoprene chain are opposite sides of the double bond (t*rans*). An example of an enzyme that functions in this manner is farnesyl diphosphate synthase (FPPSase). FPPSase functions by adding two units of IPP to DMAPP to form FPP with all three double bonds in the (*E*) configuration.[261] The (*Z*) designation is when the carbon atoms in the isoprene unit are on the same side of the double bond (*cis*). Often times in longer isoprene chains there is a mixture of (*E*) and (*Z*) stereochemistry. Prenyltransferases are responsible for this and an example can be seen with dehydrodolichyl diphosphate synthase, which is involved in the synthesis of dolichols.[264] These molecules are important for transportation of sugar molecules during the biosynthesis of glycoproteins. Alternatively, further structural complexity and diversity can be introduced into the elongation of isoprene chains, by enzymes that can also couple IPP and DMAPP in different ways to create products with branch points, cyclizations or both.[263]

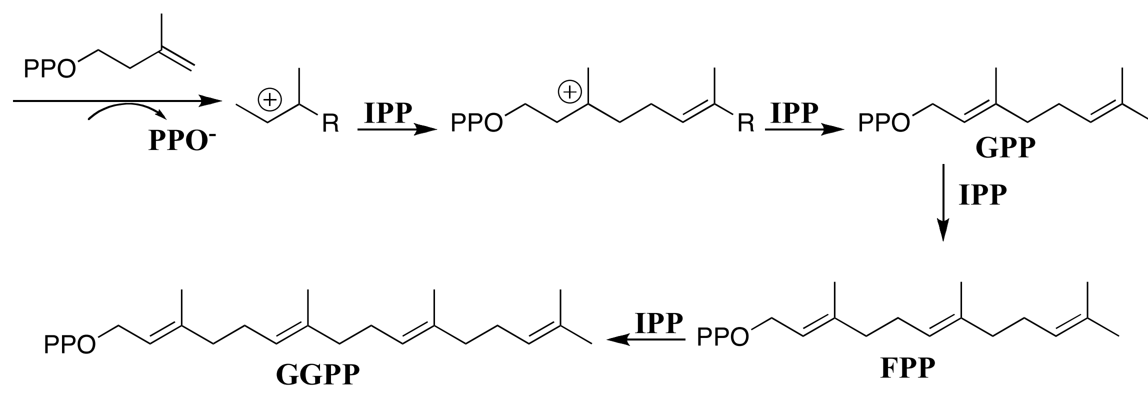


Figure 10: Construction of terpene backbones.

Classification of Terpenes

It is this chain elongation, rearrangement and cyclization of GPP, FPP and GGPP that leads to the construction of other terpenes. Due to their ubiquitous nature, there is a standard classification system for terpenes based on the number of 10 carbon units possessed in their skeleton.

Monoterpenes contain 10 carbons in their structure and like their more complex counterparts, can be cyclic or acyclic in structure.[263] This is represented in the dozens of different skeletal structures that fall under monoterpenes. Many monoterpenes have been found to have biological functions as insecticides and antiseptics. Monoterpenes have also found use in the flavor and fragrance industry for their aromas. A well known acyclic terpene is (+)-citronellal (Figure 11), isolated from *Cymbopogon citratus*[265], and is used as an insect repellant.[266] It is also believed that (+)-citronellal contributes to the

aroma of ripe fruit.[267] (-)-Menthol, a cyclic monoterpene from *Mentha arvensis*[268], has found use as an analgesic[269, 270] as well as for its mint like odor.[271]

Sesquiterpenes are the next class of terpenes and they typically consist of a 15-carbon backbone.[263] This backbone is derived from *trans*, *trans*-farnesyl pyrophosphate which can undergo a variety of rearrangements and cyclizations.[263]As a result of this, there are over 80 different types of sesquiterpene skeletons that have been elucidated and hundreds of sesquiterpenes have been found to have biological activity.[272] Several examples of these are the American cockroach sex pheromone, periplanone B (Figure 11), and the previously mentioned antimalarial artemisinin (Figure 5).

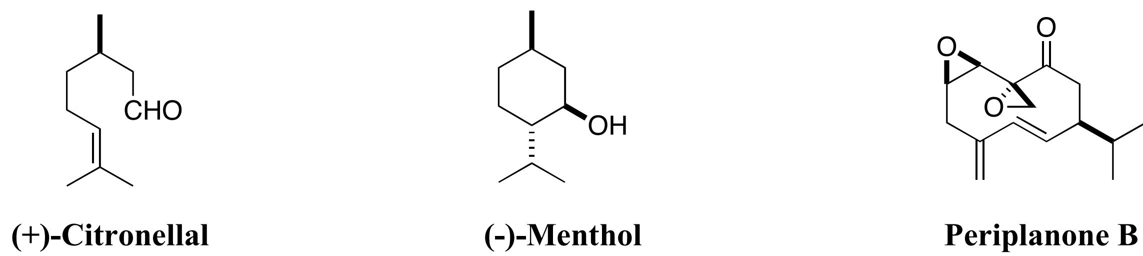


Figure 11: Structures of selected mono- and sesquiterpenes.

Diterpenes are compounds that contain 20 carbons in their backbone as they arise from GGPP. This class of terpene arises through various rearrangements, and cyclizations to produce over 50 known skeletons.[263] Several of these will be discussed later. Some of the known biologically significant diterpenes are the aforementioned anti-

cancer therapeutic paclitaxel (Figure 2) as well as retinal (Figure 12), one of the components involved in vision.[273]

Sesterterpenes are compounds that contain a 25-carbon backbone.[263] Several examples of biologically active sesterterpenes are the cytotoxic compounds ophiobolin A[274, 275], isolated from *Ophiobolus miyabeanus*[276], as well as the antimicrobial compound scytoscalarol[277] which was isolated from *Scytonema sp.*[277]

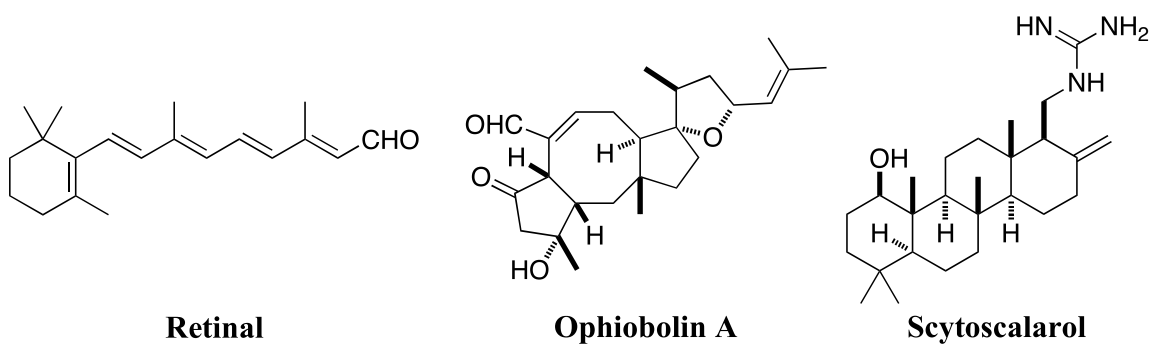


Figure 12: Structures of selected di- and sesterterpenes.

The largest number of terpenes known consist of the triterpenes.[263] These are terpenes that have a 30-carbon backbone. Triterpenes are unique in terms of their biosynthesis when compared to monoterpenes, sesquiterpenes and diterpenes in the fact that they are generated from squlaene.[88] Squlaene (Figure 13) itself is generated from the condensation of two units of FPP, instead of the sequentially adding on of IPP to an existing chain.[263] Squlaene is biologically significant as it is the precursor to cholesterol

and the steroid hormone precursors progesterone and pregnenolone. Other triterpenes with biological activity include the previously mentioned betulinic acid (Figure 4), quassin, an antimalarial[278-280],from *Quassia amara*[278], and celastrol, an Hsp-90 inhibitor[281, 282], isolated from *Celastrus scandens*[282]. Hsp-90 inhibitors have generated significant interest recently due to their anti-cancer properties.[29]

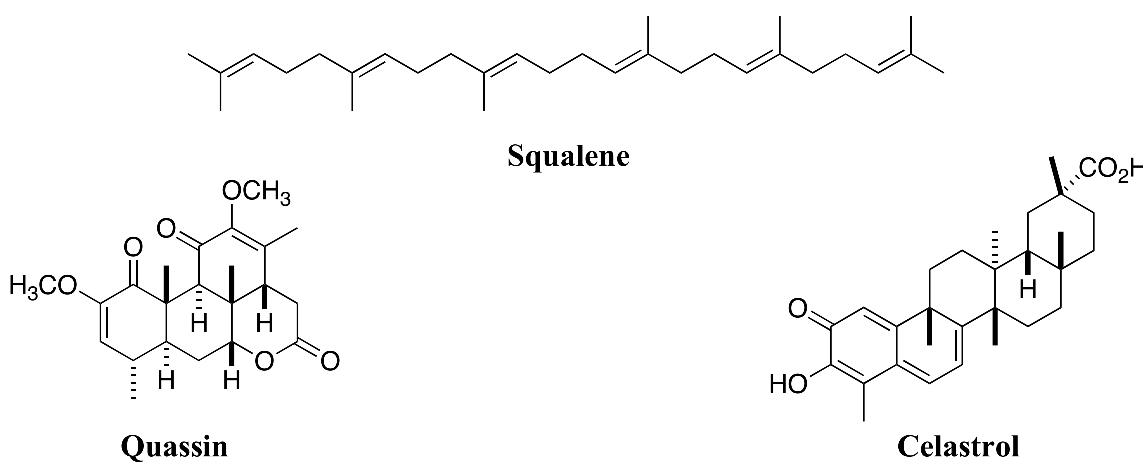


Figure 13: Structures of selected triterpenes.

Tetraterpenes contain 40-carbon backbones and one the more famous examples of this class is β-carotene which is the precursor of the previously mentioned diterpene retinal (Figure 14). Lycopene, which is responsible for the pigment in tomatoes[88] and has been shown to have antioxidant activity[263], also falls into this class.

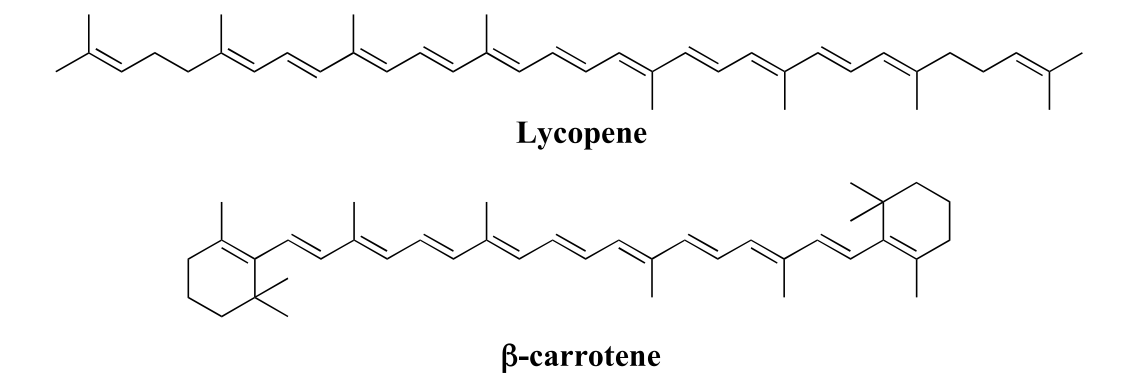


Figure 14: Structures of selected tetraterpenes.

Terpenes with longer chains also exist and are classified as polyterpenes. The best example of this class is perhaps natural rubber, which is a polymer of repeating isoprene units.[263] Meroterpenes are classified as natural products with mixed biosynthetic origins that are partially derived from terpenes.[283] Several natural products previously discussed fall under this category, including the anti-cancer therapeutics vinblastine and vincristine (Figure 1) as well as the cannabinoid Δ^9-THC (Figure 6). Meroterpenes have very diverse structures due to the incorporation of multiple biosynthetic pathways, however, they can be classified into two distinct categories: polyketide-terpenes and non-polyketide terpenes.[284] Polyketides are an extremely large class of natural products consisting of thousands of known compounds. Bacteria, fungi and plants through the condensation of acetic acid like moieties produce polyketides. This reaction is carried out through the actions of polyketide synthases.[88] Polyketide-terpenes meroterpenes are classified by associating the compounds 1st by their acyl units which are contributed to

the polyketide chain and then to type of cylclization the compound has gone through.[284] The non-polyketide groups of meroterpenes are formed from compounds that arise from the shikimic acid pathway and are linked to a terpene moiety by at least one carbon bond.

Diterpenes

The many different classes of terpenes have provided an abundance of topics to be investigated by scientists. Also, the many different subclasses of terpenes have and continue to provide interesting targets for the study of their biosynthesis and pharmacological profile. The structural complexity and diversity seen in all the different classes of terpenes also inspire interest in the total synthesis of these compounds by organic chemists. As mentioned previously, diterpenes consist of 20-carbon skeletons and many of these compounds have been investigated for their pharmacological properties including the previously mentioned andrographolide (Figure 4). Diterpenes themselves have over 50 different subclasses of skeletons and many of these are biologically active compounds. Some of the better-known subclasses of diterpenes include labdanes, kauranes, gibberlins, beyeranes, aphidicolins, cembranes and abietanes.[263] Another interesting subclass of diterpenes that have displayed biological activity are clerodanes. Several members of this class with biological activity include, columbin (Figure 15), isolated from *Columbae radix*[285], and clerocidin, isolated from *Oidiodendron truncatum*[286]. Columbin has shown to have cancer chemo-preventive properties[287] while clerocidin has antibiotic activity.[286]

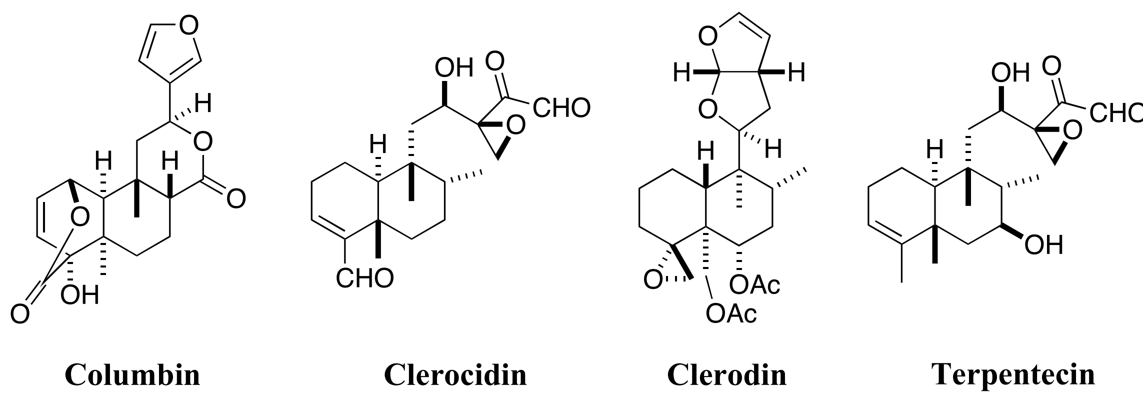


Figure 15: Structures of selected clerodanes.

Clerodanes

Clerodanes are found throughout nature in various plants, fungi and microorganisms and are classified by the presence of four contiguous stereocenters contained in a *cis* or *trans* decalin ring (Figure 16).[16] Biosynthetically, clerodanes are formed from the rearrangement of another diterpene backbone, labdanes, which is produced by the cylclization of GGPP.[16] When the rearrangement occurs concertedly, a methyl group shifts to create a trans-ring fusion.[288] An example of this *trans*-ring fusion can be seen in the compound clerodin (Figure 15), an antifeedant that also inhibits insect growth.[289] Approximately, 75% of clerodanes have this trans ring fusion, however, the rest have a *cis*-configuration similar to columbin.[16] The presence of these *cis*-ring configurations suggests an alternative route to the biosynthesis of clerodanes. A proposed pathway involves a common intermediate derived from labdane precursor and

the *cis* or *trans* configuration is determined by which one of the methyl groups at C4 shifts.[288]

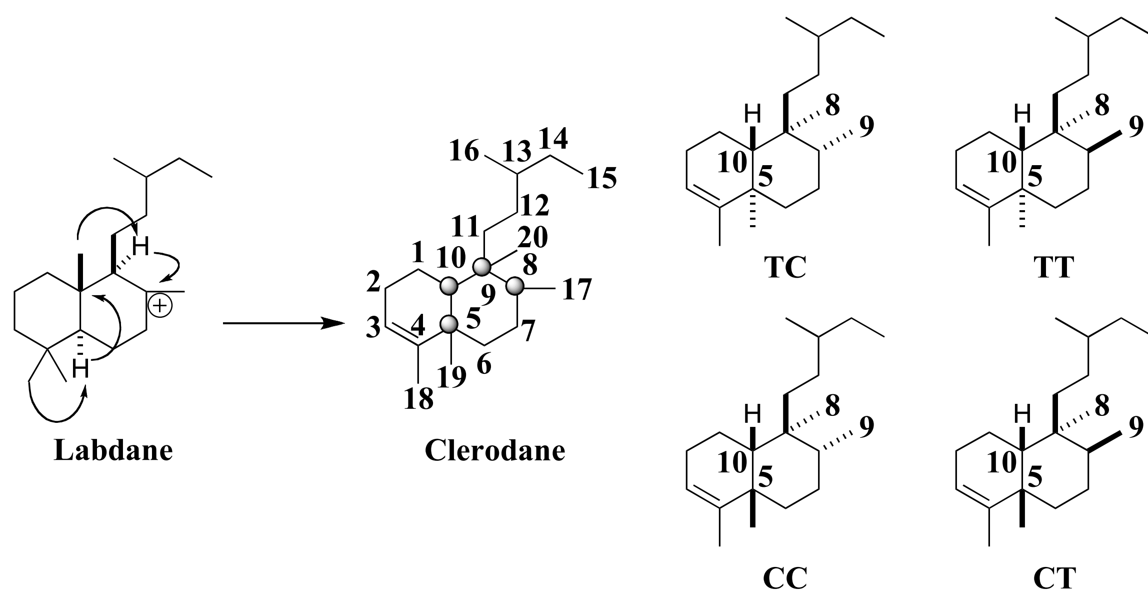


Figure 16: Structures of clerodane skeletons.

Classification of Clerodanes

A second classification of clerodane configuration is determined by the relative configuration of C1-unit groups at C-8 and C-9.[16] While most clerodanes possess a *cis* relationship at these 2 carbons, there are a few that are *trans*. Several of these display biological activity, including clerocidin and the antibiotic terpentecin[15], which was originally isolated from *Streptomyces* sp.[15] This extra clarification results in four types of clerodane skeletons as defined with respect to configuration of ring fusion and the

substitution pattern at C-8 and C-9, *trans-cis (TC), trans-trans (TT), cis-cis (CC) and cis-trans* (CT) (Figure 16). Clerodanes are further classified by their absolute stereochemistry. Compounds that have the same absolute stereochemistry as clerodin are termed neoclerodanes.[290] Compounds that are enantiomeric to clerodin are referred to as *ent*-neoclerodanes.[290]

Investigation of Neoclerodanes

Hundreds of clerodane diterpenes have been investigated for their biological activity. Dozens of these and closely related compounds have been created in the lab via total synthesis often while developing new synthetic methodology. An example of this was the synthesis of arenarol. While arenarol is classified as a drimane sesquiterpene, its rearranged skeleton resembles that of a *cis*-clerodane like structure. Wiemer and co-workers were able to synthesized arenarol through the use of a $NiCl_2$-mediated neopentyl coupling, the first demonstrated use of this in natural product synthesis.[291] However, even with the great strides made in methodology development for total synthesis, neoclerodanes as a structural class have not been synthesized as frequently as many of their other terpene counterparts including clerodanes. While many neoclerodanes have interesting pharmacological profiles, their chemical complexity often times hinders efforts for both total syntheses as well as for pharmacophore exploration through structure-activity relationship studies (SAR). These difficulties include some of the classic problems faced in organic chemistry including: diastereoselective ring formation and performing diastereoselective reactions on the ring. Despite this, there are examples of successful total synthesis of neoclerodanes as well as probing of their pharmacophores through SAR studies.

Total Synthesis of Neoclerodanes

While there is not a plentiful amount of total syntheses of neoclerodanes, there are several excellent examples including the complete synthesis of tanabalin (Figure 17) by Watanabe[292] and the previously mentioned clerocidin by Theodorakis[293]. More often then not, total synthesis calls for the construction of uncommon structures; many times these are oxygenated ring systems in unusual configurations. This feature is often found in neoclerodanes. However, there are examples of this obstacle being overcome en route to the total synthesis of a neoclerodane. Ajugarin I, an antifeedant[294] from *Ajuga remota*[295], is one of these neoclerodanes and was synthesized by Ley and co-workers.[296] Ajugarin I contains a 3-substituted-Δ^2-butenolide and during the course of the synthesis, Ley established a previously unprecedented way to synthesize this functionality using a synthetic retron approach.

On occasion, total synthesis of complex natural products can be achieved through the use of an advanced starting material. Hagiwara and co workers employed this strategy in their total synthesis of (-)-Methyl Barbascoate.[17] Although its exact biological activity is unexplored, (-)-methyl barbascoate, which is found in the leaves of *Croton californicus*[297], has been used as a pain reliever for rheumatism as well as a paralyzing agent of fish.[17] (-)-Methyl barbascoate also has a furo-lactone moiety to which few natural products have been successfully synthesized.[17] Hagiwara's synthesis used the commercial available (*R*)-(-)-Wieland-Miescher ketone to establish the trans decalin core. Once the core was established, multiple functionalization strategies were employed to complete the synthesis of (-)-methyl barbascoate.

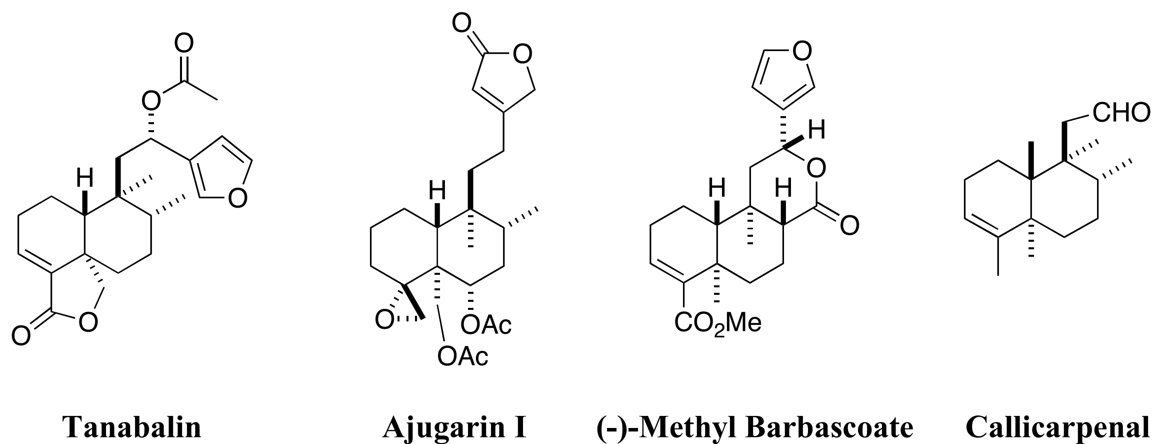


Figure 17: Structures of chemically investigated neoclerodanes.

Structure-Activity Relationship Studies of Neoclerodanes

The pharmacological profile of biologically active neoclerodanes has prompted the investigation of their pharmacophore through SAR studies. However, some of the same obstacles that hinder total synthesis efforts of these molecules increase the difficulty of conducting such studies, mainly structural complexity and subsequent chemo-sensitivity. Finding reactions that are chemoselective for molecules that are often polyfunctionalized and contain multiple chiral centers can prove extremely difficult. Another obstacle that must be overcome in the effort to conduct SAR studies on neoclerodanes, are potential supply problems. The compound must be available in enough quantities to conduct chemical investigations into its pharmacophore. Also, the lack of precedent for the chemical methodology needed to transform neoclerodanes also has contributed to the limited SAR studies conducted on these molecules. From a

pharmacological standpoint, often times, the site of action of the neoclerodane diterpene, i.e. the receptor, is unknown which also increases the difficulty in determining SAR. However, there are several examples of neoclerodanes that have had SAR studies conducted on their structures and these studies have given insight on their mechanism of biological action.

Neoclerodanes are probably best known for their antifeedant activity as dozens of neoclerodanes that have been discovered possess this attribute including the previously discussed clerodin and ajugarin I.[263] The antifeedant properties of neoclerodanes have attracted great interest as natural insect pest deterrents. Often times, neoclerodanes display significant antifeedant activity at low concentrations and they often seem specific for certain insects, leaving beneficial insects and other species unharmed.[298] Furthermore, it is thought that no problems with persistence in the environment would come about due to the natural origin of neoclerodanes.[298] These qualities, along with the ever-growing need to develop environmentally friendly, yet effective, pest deterrents have helped spur the total synthesis of several neoclerodanes. These attributes also spurred the investigation of neoclerodanes pharmacophore through SAR studies.

Callicarpenal

Callicarpenal (Figure 17) is a neoclerodane isolated from the leaves of *Callicarpa americana*[298]. Callicarpenal has shown to be a mosquito bite detterant[12] and a repellant of the blacklegged tick *Ixodes scapularis* and the lone star tick, *Amblyomma americanum*,[298, 299]which have been linked with the spreading of Lyme Disease.[299] Lyme Disease is an infectious condition that causes rashes, joint arthritis and bladder problems. Currently, callicarpenal is being investigated by the United States Department of

Agriculture's Agricultural Research Service (USDA-ARS) as an alternative insect repellant.[299] Callicarpenal can be extracted on gram scale and while it possesses multiple chiral centers, it is not as chemically complex as other neoclerodanes, making it an attractive target for analog development. Investigation into the SAR of callicarpenal by Cantrell and co-workers[298] showed that the alkene was not necessary for activity. They also showed that oxidation was also tolerated at this position and that the aldehyde functionality was not necessary for activity against mosquitoes.

The antifeedant activity of callicarpenal led to investigation of its SAR. Another neoclerodane derived from the Mexican sage, *Salvia divinorum*, salvinorin A, has also shown antifeedant activity[300], however, this was not the pharmacological attribute that has garnered attention for this molecule.

CHAPTER II. SALVINORIN A

Salvia divinorum, one of the various members of the Salvia genus of plants, is a hallucinogenic mint indigenous to Oaxaca, Mexico that has been used by the Mazatec Indians of this region for their divination ceremonies as well as for headaches, rheumatism and panzón de barrego which roughly translates to swollen belly.[301] While used in this region for generations, *S. divinorum* did not surface in western culture until the late 1930's.[302] *S. divinorum* was officially catalogued by Carl Epling in the 1960's, but it would be another 20 years before a chemical compound was isolated from the plant.[302] The main active component of *Salvia divinorum* was found to be the neoclerodane diterpene salvinorin A (**1**) (Figure 18).[303, 304] Originally isolated by Ortega in 1982[305], its structure was confirmed two years later by Valdes.[303] Once isolated, salvinorin A was found to be a potent hallucinogen with an active dose in the range of 200-500 μg.[19, 304, 306] Interestingly, further investigation showed that salvinorin A has no activity at the serotonin 5-HT_{2A} receptor which is the site of action for classical hallucinogens such as LSD and DMT.[306] Salvinorin A was also found to be a full agonist at κ opioid receptors despite bearing no structural similarity to other non-peptide opioid ligands like cyclazocine.[19] These discoveries prompted extensive investigations into the pharmacological effects of salvinorin A.

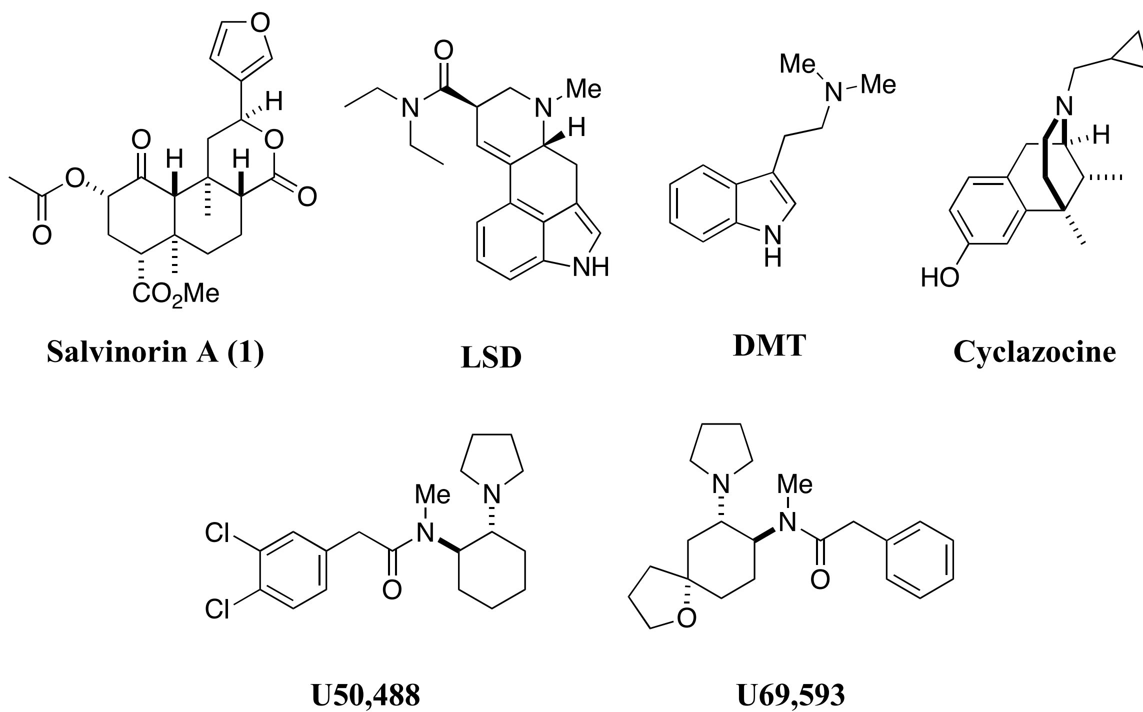


Figure 18: Structures of Salvinorin A (**1**), LSD, DMT, cyclazocine, U50, 488 & U69, 593.

Salvinorin A Pharmacological Testing

Mowry and co-workers, using rodents, examined the effects of chronic and acute dosing of salvinorin A.[307] While previous studies had used tinctures and extracts from the plant, this was one of the earliest studies that used pure salvinorin A, extracted from the plant. In their study, they examined the consequences of acute administration in rats and chronic dosing in mice, focusing on any effects on heart rate and blood pressure. They also looked for histological changes in the kidneys and liver. Their study concluded that there was relatively low toxicity associated with salvinorin A.

Studies conducted in the lab of Mary Jeane Kreek demonstrated that salvinorin A had similar discriminative stimulus effects as other known κ opioid agonist such as U69, 593 (Figure 18).[308] This study was the first to administer salvinorin A to non-human primates and it also verified Roth's initial findings that salvinorin A was a κ opioid receptor agonist as the behavioral effects of salvinorin A were attenuated by an opioid antagonist. Continued studies with non-human primates in the Kreek lab provided more evidence that salvinorin A interacts at κ opioid receptors as it produced neuroendocrine effects similar to other κ opioid agonists like U69,593.[10] Salvinorin A was found to increase the levels of prolactin, a biomarker used to measure κ opioid receptor activity, in the primate test subjects.[309] To determine if these effects were being mediated through an opioid mechanism and not a serotonergic pathway, opioid antagonists were administered. Opioid antagonists did in fact block the increase of prolactin levels, which implicates the ability of salvinorin A to act at κ opioid receptors as the cause of prolactin level increase.[309] Further work with non-human primates from the Kreek lab showed that salvinorin A when injected intravenously, quickly reached the CNS and caused the rapid onset of facial relaxation and ptosis (drooping of the eye lids) in nonhuman primates which is consistent with reports of rapid effects of salvinorin A in humans.[310] These effects were attributed to activation of opioid receptors as ptosis and facial relaxation were prevented by the treatment of opioid antagonist, nalmefene. The most recent study from the Kreek lab compared salvinorin A with serotonergic hallucinogen psilocybin and the N-methyl-D-aspartic acid antagonists, ketamine along with several opioid agonists including U69,593 and U50,488.[10] These compounds were administered to non-human primates and it was found that despite being a hallucinogen, the discriminatory effects of

salvinorin are mediated by agonistic activity at κ opioid receptors as these effects were negated by opioid antagonist administration.[311] This has led to the conclusion that the observed discriminatory effects are mechanistically distinct from hallucinogens that interact at serotonergic receptors. These non-human primate studies are of significant interest as humans and primates have similar homologies for these systems and the studies provide data that may help translational studies in humans.

France and co-workers also studied the effects of salvinorin A in non-human primates.[312] Salvinorin A was compared to a variety of compounds including hallucinogens. They found that despite its hallucinogenic activity, salvinorin A was not able to substitute for other drugs with the same activity including LSD. The study concluded that different classes of hallucinogens exert different discriminatory effects in non-human primates and further illustrates that the hallucinogenic activity seen in salvinorin A does not proceed through the same mechanism as other hallucinogenic compounds.

In another comparative study, this time involving salvinorin A and other κ opioid agonists in rodents, salvinorin A was found to be as potent as U50, 488 in stimulating activation of the κ opioid receptor.[313] Of note, however, is the fact that salvinorin A was 40-fold less effective in promoting the internalization of the κ opioid receptor in comparison to U50, 488.[313] This happening is of significant interest, as it is believed that receptor internalization is a part of the mechanism that causes tolerance to opioid therapeutics like morphine.[314]

Being a κ opioid agonist, salvinorin A was examined for activity in nociception. A study by Liu-Chen and co-workers initially investigated salvinorin A antinociceptive

activity.[317] Using rodents, they showed that salvinorin A was not antinociceptive in the acetic acid abdominal constriction test. Liu-Chen and co-workers felt that this might be due to metabolism of salvinorin A *in vivo*.[313] Prisinzano and co-workers screened salvinorin A for antinociception as well.[26] This study found that while not active in the hot plate assay, salvinorin A demonstrated antinociceptive properties in the tail-flick and *p*-phenylquinone writhing assay.[26] Investigation by McCurdy and co-workers demonstrated that salvinorin A produced antinociceptive effects in three different pain assays: murine tail flick, hot plate and abdominal constriction.[315] These actions were blocked by the selective κ opioid antagonist, norBNI, indicating that the antinociceptive properties of salvinorin A are mediated through κ opioid receptors and not some other unknown mechanism.

Ansonoff and co-workers further investigated the antinociceptive ability of salvinorin A.[316] In their study, κ opioid receptor knockout mice were compared to wild type and their results show that intracerebroventricularly administered salvinorin A had antinociceptive effects in the tail flick assay with wild type mice but not with the κ opioid receptor knockout mice. These results also implicate that salvinorin A is able to act as an antinociceptive through the activation of κ opioid receptors.

Erlichman and co-workers also found that salvinorin A had antinociceptive ability in rodents.[317] These studies showed that salvinorin A was able to increase the latency time in the tail flick assay and this behavior was attenuated with the opioid antagonist, norBNI. Furthermore, their study showed that μ and δ opioid receptor antagonist did not significantly affect the antinociceptive response of salvinorin A, further supporting the

notion that salvinorin A exerts its antinociceptive actions exclusively through the κ opioid receptor.

Opioid receptors have been linked with gastrointestinal function.[246] Izzo and co-workers investigated the effects of salvinorin A on gastrointestinal function and found that salvinorin A inhibits intestinal motility and this effect is blocked by opioid antagonist nor-BNI, illustrating that salvinorin A exerts its effects on gastrointestinal motility through opioid receptors.[318] These results are of potential interest as salvinorin A could potentially be investigated for the use of gastrointestinal disorders such as diarrhea or IBS.

Along with its investigations into the potential utility in antinociception and gastrointestinal function, the behavioral effects of salvinorin A were examined. This was in part due to salvinorin A being reported as a hallucinogenic compound. Carlezon and co-workers conducted a study in rats where they found that salvinorin A increased immobility behavior in the force swim test (FST), an animal model of depression.[319] This increased immobility was accompanied by a decrease in overall swimming behavior, thus suggesting that salvinorin A was producing prodepressant-like effects in the FST. These effects are the opposite of what is typically seen from antidepressants as well as κ antagonists. Further investigation of the prodepressant effects led to microdialysis analysis of neurotransmitters in the brain which showed that salvinorin A decreased the levels of DA in the dorsal striatum region of the brain, this activity however was reversed with the κ opioid receptor antagonist norBNI.[319] The decrease in DA levels by salvinorin A was attributed to the activation of κ opioid receptors and their inhibition of DA release, however salvinorin A did not have any impact on DA reuptake. These results were also

seen in another κ opioid receptor agonist, U69,593. This finding has raised interest for potential clinical utility of salvinorin A and other κ opioid agonists as a controlled DA decrease may have use for treating mania because this and other related disorders are linked to hyper functioning DA systems.[319]

In a contrasting report, Sala and co-workers demonstrated that salvinorin A had antidepressant like effects.[320] They found when given subcutaneously, salvinorin A acted as an antidepressant and these effects were prevented by treatment with the opioid antagonist nor-BNI. Interestingly, they found that salvinorin A, reduced fatty acid amide hydrolase's activity in the amygdala, linking it to the cannabinoid system, despite the fact salvinorin A does not directly interact with cannabinoid receptors. These findings provide some evidence that along with its effects on neurotransmission, κ opioid receptors may have secondary interactions with other receptors of the CNS.

Shippenberg and co-workers utilized salvinorin A in their study of κ opioid receptors links with schizophrenia and bipolar disorder, two conditions that have been attributed to neurotransmitter dysregulation.[321] They were looking to probe the reported link between κ opioid receptors and neurotransmitter regulation. The acoustic startle reflex, a sensorimotor gating process, is disrupted in different psychiatric disorders including these. Using salvinorin A to activate κ opioid receptors, they demonstrated that κ opioid receptors does not affect the acoustic startle reflex, providing evidence that psychiatric disorders that result in altered acoustic startle reflexes are not linked to κ opioid receptors. In order to determine however, if these results are exclusive to salvinorin A or all κ opioid agonists, future studies using this testing paradigm must be conducted.

Further investigations into the neurological effects of salvinorin A by Butterweck and co-workers continued to link salvinorin A and κ opioid receptors with dopamine.[322] Their study demonstrated that salvinorin A has dopaminergic activity as it caused compulsive gnawing in rodents, an animal model for dopaminergic activity. The compulsive gnawing behavior was blocked by DA antagonist, haloperidol. Of interest, was the observation by Butterweck that the opioid antagonist, norBNI, did not reverse this behavior, suggesting that salvinorin A can exert its effects on dopaminergic activity through a non-opioid mediated mechanism. Further studies need to be conducted to determine if salvinorin A indeed has the ability to activate dopamine or other neurotransmitter receptors through a non-opioid mediated mechanism.

Continued studies into the effects of salvinorin A on neurotransmitters was carried out by Pittaluga and co-workers.[323] Their work showed that salvinorin A has the ability to presynaptically modulate norepinephrine, serotonin and dopamine exocytosis by acting at presynaptic opioid receptors. Opioid antagonists stopped this action, indicating a link between opioid receptors and the modulation of neurotransmitters. Staying with neurotransmission, Hirbec and co-workers screened multiple psychostimulants and hallucinogens, including modafinil, phencyclidine and salvinorin A, and showed that salvinorin A, amongst other compounds, can stimulate dopamine receptors.[324] Furthermore, salvinorin A inhibited the binding of the selective dopamine ligand, domperidone, indicating that along with being an agonist at κ opioid receptors, salvinorin A may have other sites of action.

Szechtman and co-workers conducted additional investigations on the effects of salvinorin A on dopamine neurotransmission.[325] Their study showed that high does of

salvinorin A (2 mg/kg) potentiated the locomotor sensitization seen with the dopamine agonist quinpirole. Interestingly, a low dose of salvinorin A (0.04 mg/kg) was found to attenuate sensitization to quinpirole. The opposite effects due to dosing suggest that salvinorin A has the ability to produce bidirectional modulation to dopamine agonists.

Another study by Shippenberg and co-workers looked to probe the effects of salvinorin A on the actual function of dopamine in a region of the brain, the dorsal striatum.[326] This region of the brain has been implicated in a number of conditions including Parkinson's and the development of addiction, particularly cocaine.[327] Shippenberg's study showed that acute administration of salvinorin A decreased dopamine neurotransmission in a dose dependent manner, which was in agreement with the previously mentioned study conducted by Carlezon and co-workers.[319] The administration of an opioid antagonist reversed this effect, linking the regulation of dopamine neurotransmission with the activation of κ opioid receptors.

Evidence of salvinorin A decreasing levels of dopamine in the brain prompted Carlezon and co-workers to examine the effects of salvinorin A exposure to cocaine taking rats.[328] They found that acute administration of salvinorin A blocked the locomotor stimulant effects of cocaine. Acute administration of salvinorin A also blocked the locomotor effects of the dopamine receptor agonist SKF 82958. Interestingly, repeated administration of salvinorin A along with placing the animals in an activity cage instead of their home cage potentiated the locomotor effects of cocaine. The same results were seen with SKF 82598. These findings suggest that salvinorin A can regulate the stimulant effects seen with cocaine and SKF 82958 through interactions with dopamine neurotransmission.

Further investigations into the effects of salvinorin A on cocaine taking rats were conducted by Schenk and co-workers.[329] In a cocaine-produced drug-seeking paradigm, where exposure to cocaine is meant to trigger cocaine-seeking behavior, salvinorin A was found to attenuate drug-seeking behavior in a similar fashion as other κ opioid agonists.

Along with exploring what behavioral effects are produced by salvinorin A and how they come about, studies have also been conducted to determine the metabolic and pharmacokinetic profile of salvinorin A. Using positron emission tomography (PET), Fowler and co-workers demonstrated that salvinorin A has rapid uptake in the baboon brain, reaching a peak concentration in 40 seconds.[330] They also showed the half-life of salvinorin A to be 8 minutes. The study showed that salvinorin A was distributed in the brain with the highest concentration being in the cerebellum and visual cortex, where the authors think may account for the visual hallucinations associated with use. Fowler's study suggests that salvinorin A has two modes of metabolism and excretion, the biliary and renal systems. This study also found that the rapid uptake noted along brief duration in the brain, match a time-course study of visual hallucinations for *S. divinorum* when smoked.

Inoue and co-workers attempted to determine the metabolic products of salvinorin A in rat plasma.[331] They found that salvinorin A was degraded by esterases and two main degradation products were detected. Inoue described a deacetylated C-2 compound, whose mass corresponds to the deacetylated salvinorin A derivative, salvinorin B along with the opened lactone ring.

Cohen and co-workers examined if the metabolism of salvinorin A could be manipulated to increase its half-life.[332] They found that derivatives of salvinorin A that

were more resistant to metabolism, such as ethers, would increase the half-life of salvinorin A, however, these compounds must be administered through routes giving slow absorption as rapid absorption led to rapid metabolism. Further studies with these and other metabolism resistant derivatives showed that they remained efficacious at opioid receptors and that several of these were actually more potent than salvinorin A,[24] demonstrating that the pharmacokinetic properties of salvinorin A can be improved without sacrificing pharmacological activity.

Further investigations into the rapid metabolism of salvinorin A was conducted by Eddington and co-workers.[333] Their studies found that salvinorin A may be a substrate for various oxidative enzymes as well as p-glyco-protein (pgp), which they feel explains the rapid metabolism and short duration of action seen in salvinorin A. Continued studies are needed to determine which metabolic enzymes salvinorin A is a substrate for, and furthermore, continued studies need to be conducted to investigate the ability of pgp to act upon salvinorin A.

The interesting pharmacological profile of salvinorin A is perhaps further magnified by its unique structure. While other κ opioid agonists share some of the same characteristics as salvinorin A, i.e. decrease of DA release, antinociceptive properties, and discriminatory behavior; there are none that are similar in structure.

Chemical Makeup and Biosynthesis of Salvinorin A

Structurally, salvinorin A is classified as a tri-cyclic *trans-cis* neoclerodane and contains 7 chiral centers.[334] Along with this, salvinorin A also contains multiple functionalities including esters at the C-2 and C-4 position, a carbonyl at C-1 and a furano-lactone moiety.[334] While structurally complex, other neoclerodanes including

some found in *Salvia divinorum*, were found to share similarities to salvinorin A[335-337] and an investigation into the biosynthetic pathway of salvinorin A was undertaken to determine how it and these related compounds are potentially formed in the plant.

Several studies showed that salvinorin A was localized in the glandular trichomes of the leaves of *Salvia divinorum*[338] and terpenes typically located in this region are derived via the DOXP pathway.[339] With this, Kutrzeba and co-workers hypothesized that salvinorin A too was formed in this manner (Figure 19).[340] It was decided that by incorporating ^{13}C tagged glucose, the biosynthetic pathway to salvinorin A could be determined due to the differences in both the DOXP and MVA pathways utilizations of glucose. Kutrzeba and co-workers proposed that in the DOXP pathway, the ^{13}C glucose is converted into IPP, which undergoes elongation to GGPP, which is a prerequisite of diterpene formation, and then cyclization to the labdane core. This is the believed intermediate towards the synthesis of the clerodane core.[340] Subsequent methyl shifts would generate said clerodane core, which would then undergo enzymatic oxidations and functionalizations to create salvinorin A. In order to validate their findings with ^{13}C glucose, Kutrzeba also used $^{13}C/^{2}H_2$ labeled deoxy-D-xylulose in place of ^{13}C glucose.[340] After structural analysis through NMR and high-resolution mass spectrometry (HRMS) it was found that deoxy-D-xylulose was incorporated in the same fashion as glucose as the heavy atoms were utilized in the same manner that was indicative of the DOXP pathway and not an alternative biosynthetic route that may incorporate glucose.

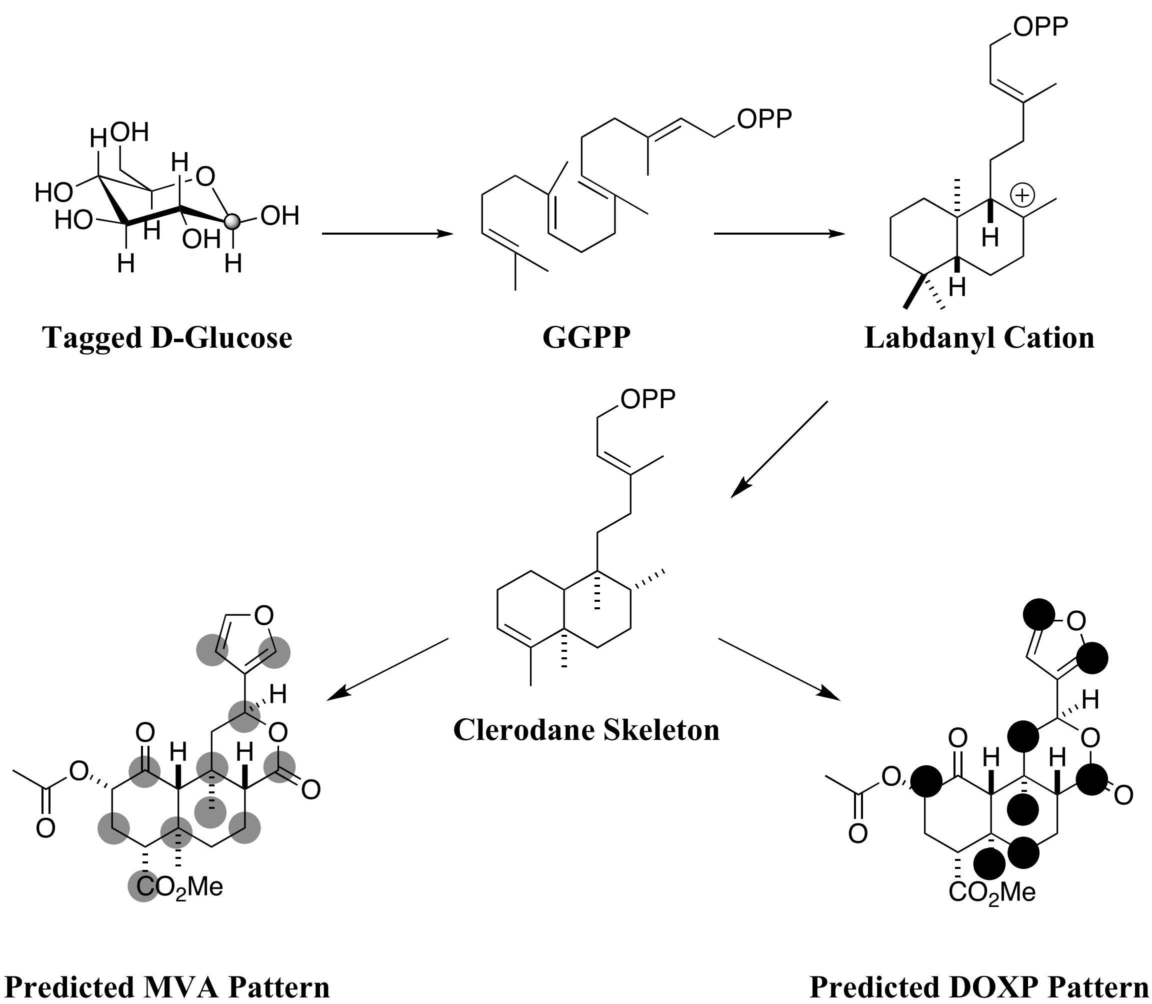


Figure 19: Proposed biosynthesis of salvinorin A adapted from Kutrzeba & Co-workers.[340]

Proposed Binding Sites for Salvinorin A

Upon the determination that salvinorin A bound to κ opioid receptors, proposed binding models were generated to explain its interactions.[19, 341-344] The κ opioid receptor, like its counterparts μ and δ, is a member of the 7-transmembrane (7-TM) GPCR super

family.[87] Often times, the binding site for a molecule with a GPCR is deep within the receptor and it is held in place by amino acid residues in the surrounding area. This often happens through ionic interactions, hydrophobic interactions or hydrogen bonding.[87] Typical opioid ligands have an ionizable nitrogen, as with the alkaloid morphine, that when charged, can form a salt bridge with an aspartate residue in TM3 which facilitates binding to the receptor.[345] However, salvinorin A does not contain an ionizable group thus it was the early hypothesis that it bound through hydrogen bonding and/or hydrophobic interactions.[19, 343, 344]

Roth and co-workers conducted the initial investigations of how salvinorin A binds to the κ opioid receptor.[19] Using the previously reported complexing of U69,593, a κ opioid receptor agonist, as a starting point, Roth proposed that salvinorin A interacts through hydrogen bonding between the carbonyls of the C-17 lactone and the C-2 and C-4 esters and the phenolic side chain of 3 tyrosine residues (139, 312, 313).[19] It was also proposed that the oxygen of the furan ring participates in hydrogen bonding with a glutamine residue (115).[19]

Approximately three years later, Roth proposed a refined binding site that incorporated site directed mutagenesis studies as well as computer modeling.[341] This model hypothesized that salvinorin A was interacting with multiple residues in 3 different extracellular loops of the receptor. The C-2 ester was still believed to be interacting with a tyrosine residue (313, TM7), however, instead of hydrogen bonding with the carbonyl, there may be a hydrophobic interactions occurring.[342] Instead of another tyrosine residue interacting with the C-4 ester (312, TM2), Roth proposed that an isoleucine (294, TM6) and glutamic acid (297, TM6) residue was participating in hydrogen bonding with the

methyl ester of C-4.[342] Also, the tyrosine residue (139,TM2) that was believed to be involved in hydrogen bonding with the lactone carbonyl was determined to not be essential for binding.[342] This new model was similar to the previous as it hypothesized that the furan ring interacts with the receptor through hydrogen bonding. However, instead of the originally proposed glutamine residue (115, TM3), it was postulated that the oxygen of the furan ring participates in hydrogen bonding with two tyrosine residues (119, TM2) (320, TM7). In 2007, Roth proposed a model of salvinorin A binding that explains its selectivity for κ opioid receptors.[341] In his model, he suggests that the selectivity seen for κ over μ and δ in salvinorin A is attributed to the rotation of TM2 of the κ opioid receptor that accommodates for salvinorin A binding.

Another recent model of salvinorin A binding to the receptor was proposed by Kane and co-workers.[343] Their model bears some similarity to the two previously proposed, however there are some key differences. Kane's model is in agreement with both of Roth's models in terms of interactions with the C-2 ester as they too propose that this moiety interacts with tyrosine 313 of TM7. Kane's model also proposes that tyrosine 320 of TM7 interacts through hydrogen bonding with the oxygen of the furan ring. This is in agreement with Roth's latest model as he too proposes this interaction. Interestingly, Kane's model incorporates several residues that were proposed by Roth's previous works to interact with salvinorin A but, Kane proposes that these residues are interacting at different functionalities then those postulated by Roth. While Roth's revised model predicts that tyrosine 119 of TM2 interacts with the furan ring, Kane suggests that it actually interacts with the C-4 carbomethoxy group.[343] Kane also proposes that the glutamine residue Roth initially hypothesized in his first model to

interact with the furan ring is actually interacting with the carbonyl of the lactone.[343] Kane's model also postulates that an isoleucine 316 of TM7 interacts with the furan ring along with the previously suggested tyrosine moiety (320, TM7).[343] While there are some differences in the proposed modes of salvinorin A binding, the general consensus between these hypothesized models is that both the C-2 and C-4 esters as well as the furan ring are involved in binding at the opioid receptor.

Structure-Activity Relationship Studies

Investigation of salvinorin A showed that it was more efficacious at κ opioid receptors than the standard κ opioid receptor agonist U50, 488.[19] Structural modifications to salvinorin A have been conducted to establish SAR. The most extensively studied position on the salvinorin A core is the C-2 ester. This is partly due to the relative ease to hydrolyze at this position to form the C-2 hydroxyl intermediate salvinorin B (**2**); which is present in *S. divinorum*, however, it has been found to have negligible psychotropic activity along with low affinity for opioid receptors.[19, 306, 346]

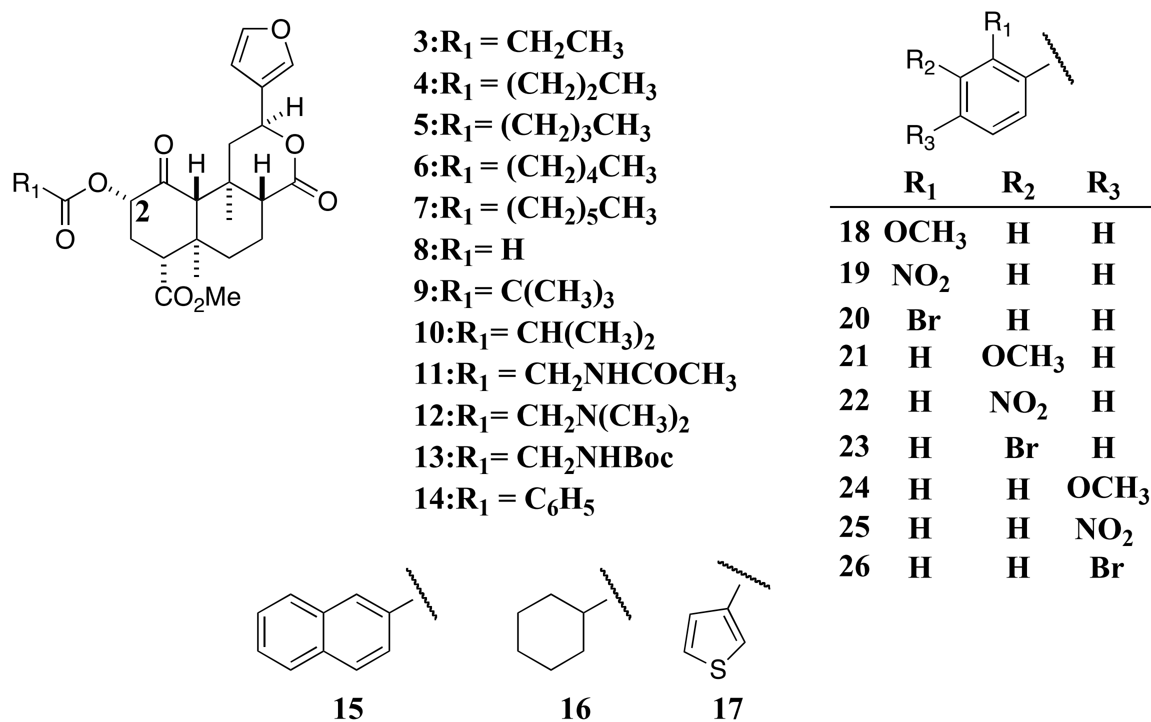
C-2 Structure-Activity Relationship

Alterations to the acetate moiety were tolerated at opioid receptors as it was found that the propionate (**3**) (Figure 20) and heptanoate (**7**) esters were partial agonists at κ opioid receptors but with reduced affinity than **1**.[347] The butyl (**4**), pentyl (**5**) and hexanoate (**6**) esters had reduced affinity at κ opioid receptors as well.[347] Substitution of the C-2 acetyl group for a formate (**8**) resulted in a 5-fold loss in affinity compared to **1**. The *tert*-butyl ester (**9**) of **1** had no affinity at μ, κ or δ opioid receptors; however, the isobutyl ester (**10**) possessed affinity for κ opioid receptors, though 10-fold lower than **1**.[26] Incorporation of an acetamido (**11**) or dimethylamino (**12**) group to **1** was not well

tolerated, as affinity for κ opioid receptors was lost. (**1** K_i = 1.9 ± .06 nM vs. **11** and **12** K_i = > 10,000 nM)[346] The addition of a *tert*-butoxycarbonylamino (**13**) group was tolerated at κ opioid receptors, however with reduced affinity then **1** (K_i = 90 nM vs. K_i =1.9 nM). A benzoyl ester (**14**) caused reduced affinity at κ opioid receptors, however, it increased affinity 25-fold at μ opioid receptors when compared to **1**. Furthermore, ester **14** was found to be a full agonist at μ opioid receptors.[26] This was the first reported example of a non-nitrogenous μ agonist. Additional pharmacological studies conducted on ester **14** showed that it did not promote internalization of the μ opioid receptor,[314, 348] a believed factor in the development of tolerance.[349, 350] Due to ester **14**'s unique pharmacological profile, studies were conducted to further understand its affinity and efficacy for μ over κ opioid receptors.

Replacement of the benzoyl group with the 2-naphthoyl (**15**) (Figure 3) reduced affinity 10-fold at μ opioid receptors.[347] Reduction of the benzene ring to a cyclohexane ring (**16**) also reduced affinity at μ opioid receptors compared to esetr **14**.[347] Isosteric replacement of the benzene ring with the 3-thiophene (**17**) was well tolerated as it had similar μ opioid receptor affinity as ester**14**.[28] Compound **17** was also found to be fully efficacious at μ opioid receptors.[28] Substitution of the benzene ring of ester **14** was also explored with substitution at the 2,3 and 4 positions with nitro, bromo or methoxy substituents.[28] Briefly, introduction of a methoxy (**18**) or nitro (**19**) group at the 2-position decreased affinity for μ opioid receptors in comparison to ester **14**. The 3-methoxy (**21**) decreased affinity for μ opioid receptors but it also increased selectivity for μ over κ in comparison to ester **14**. Compound **21** also had mixed efficacy, as it was a partial agonist at μ and full agonist at κ. The 3-nitro (**22**) substitution was not well

tolerated at μ opioid receptors, as affinity was lost. The 4-methoxy (**24**) has reduced affinity for μ but similar selectivity as ester **14**. The 4-nitro (**25**) decrease affinity for μ opioid receptors 20-fold. The 2 and 3-bromo compounds (**20** and **23**) were tolerated at opioid receptors but with less affinity than **14**. The 4-bromo group had similar affinity as **14** for μ opioid receptors. Compound **26** was also found to be fully efficacious at μ opioid receptors. The stereochemical configuration at C-2 and its impact on activity was also explored. Inversion at this center resulted in decreased affinity for esters **1, 3** and **4**.[351, 352]



	R_1	R_2	R_3
18	OCH_3	H	H
19	NO_2	H	H
20	Br	H	H
21	H	OCH_3	H
22	H	NO_2	H
23	H	Br	H
24	H	H	OCH_3
25	H	H	NO_2
26	H	H	Br

Figure 20: Selected C-2 ester analogues of salvinorin A.

Replacement of the C-2 acetyl group was also investigated. Substitution with a carbamoyl group (**27**) (Figure 21) was well tolerated at the κ opioid receptor as it was similar in affinity and efficacy to **1**.[353] Extension of carbamate **27** to its methyl **28** and ethyl **29** counterparts resulted in lowered affinity and efficacy.[353] Following the trend seen in the esters, introduction of a *N*-phenyl (**30**) group resulted in increased affinity for μ opioid receptors. [26] Compound **30** was found to be a full agonist at μ as well. Compounds **28** and **29** were converted to the corresponding carbonates **31** (Figure 4) and **32**, but this change was not well tolerated, as all affinity for opioid receptors was lost.[27]

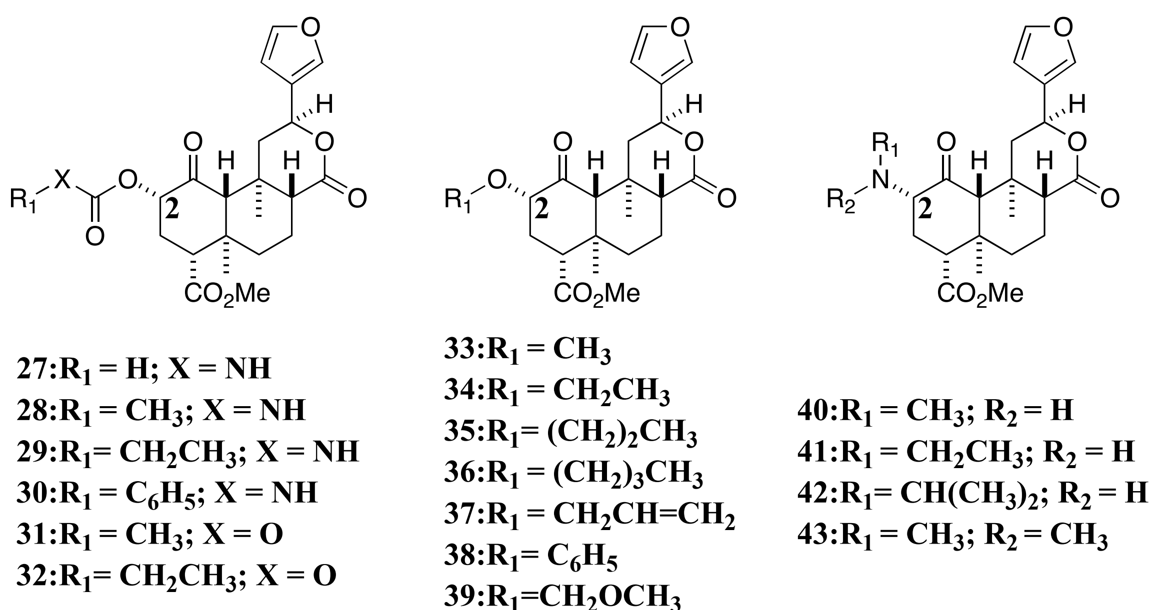


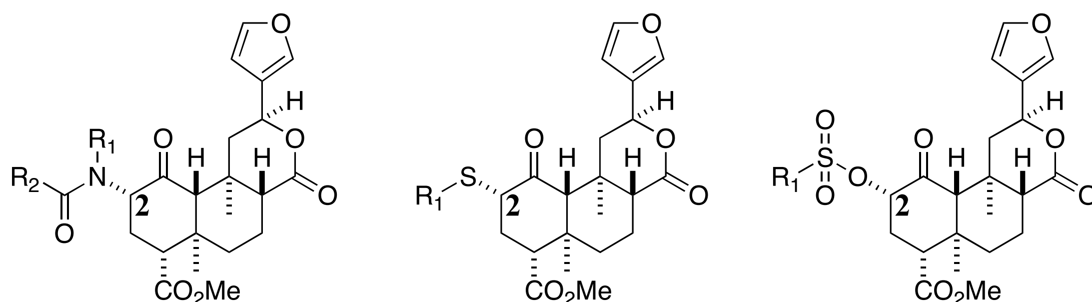
Figure 21: Selected C-2 carbamate, carbonate, ether & amine analogues of salvinorin A.

Various ether derivatives of **1** were studied for activity. Addition of a methyl group (**33**) (Figure 21) to **1** was well tolerated, as affinity for κ was comparable to **1**.[353] Elongation of the chain to the corresponding ethyl ether (**34**) increased affinity compared to ether **33**. Both compounds were fully efficacious at κ opioid receptors.[353] Increasing the chain length to the corresponding propyl (**35**) and butyl (**36**) ethers, decreased affinity and efficacy compared to ether **34**.[353] Both the allyl (**37**) and benzyl (**38**) ethers were found to have affinity and efficacy but not to the magnitude of ether **34**.

Amines at C-2 have also explored. The methyl amino compound (**40**) (Figure 21) maintained affinity for κ opioid receptors, but it did cause a reduction in activity.[352] Lengthening the chain to the ethyl group (**41**) increased both affinity and efficacy of amine **40**. An isopropylamino group (**42**) was incorporated into **1** at the C-2 position and this compound had an increase in activity than amine **41**. The *N*, *N*-dimethyl compound (**43**) was found to be the most potent of this series as it was a full agonist. The stereochemistry at C-2 of these compounds was also inverted and this change was tolerated at κ opioid receptors as it increased efficacy. Inversion of the stereochemistry at C-2 of amine **42** had comparable efficacy to that of **1**.[352]

Following up upon observations made with several of the various esters at C-2 of **1**. Replacement of the acetoxy group with bioisosteres was embarked on. Exchange of the acetoxy group of **1** for an acetamido group (**44**) (Figure 22) diminished affinity and efficacy at κ opioid receptors.[352] This was also seen with the extended propionamido group (**45**). Incorporating an *N*-methyl into acetamido **44** (**46**) increased activity at κ opioid receptors, a phenomenon also seen with the propionamido (**47**) counterpart of acetamido **46**. Compound **46** was found to be even more potent κ agonist than **1**.[352] *N*-

Ethyl groups were incorporated into compounds **44**(**48**) and **45**(**49**) and these resulted in increased activity at κ opioid receptors. They were not as potent as their *N*-methyl counterparts **46** and **47**, however. Like several of the amine and ester derivatives, the stereochemistry at C-2 of these compounds were inverted which subsequently resulted in decreased activity at κ opioid receptors. The amide counterpart of **14**(**50**) was found to be fully efficacious at μ and slightly more potent than ester **14** (**14** EC_{50} = 500 nM vs. **50** EC_{50} = 360 nM).



44:R_1= H; R_2= CH_3
45:R_1 = H; R_2 = CH_2CH_3
46:R_1 = CH_3; R_2 = CH_3
47:R_1= CH_3; R_2 = CH_2CH_3
48:R_1 = CH_2CH_3; R_2 =CH_3
49:R_1 = CH_2CH_3; R_2 = CH_2CH_3
50:R_1= H; R_2 = C_6H_5

51:R_1 = $COCH_3$
52:R_1 = H
53:R_1= COC_6H_5

54:R_1 = CH_3
55:R_1 = C_6H_5
56:R_1= 4-$CH_3C_6H_4$

Figure 22: Selected C-2 amide, thioester & sulfonate analogues of salvinorin A.

Replacement of the C-2 acetoxy for a thioacetoxy group (**51**) (Figure 22) resulted in a decrease in both affinity and activity for κ opioid receptors.[28, 354] Complete removal of the acetyl group yielded **52**, which decreased affinity and efficacy at κ opioid receptors. Following the trend previously shown in both the ester and amide series, introduction of a benzene ring to thioester **51** increased affinity and selectivity for μ opioid receptors over κ receptors.[28]

Sulfonate esters were also used as replacements for the acetyl group at C-2. The mesylate ester of **1** (**54**) was well tolerated at κ opioid receptors and was also slightly more potent then **1** as an agonist (**54** EC_{50} = 30 nM vs. **1** EC_{50} = 40 nM).[26] The benzenesulfonate ester (**55**) caused reduced affinity for κ opioid receptors, which followed the trend seen in the ester and amide series. However, unlike those compounds, the benzenesulfonate did not result in affinity or activity for μ opioid receptors. A 4-methyl group (**56**) was introduced to the benzenesulfonate and this change resulted in a 50-fold increase for affinity at μ compared to **55**.[26]

Furan Ring (C-12) Structure-Activity Relationship

The furan ring of **1** has also been a site of investigation. Reduction of the furan ring, followed by separation resulted in the tetrahydrofuran counterpart with defined (*R*) stereochemistry at C-13 (**57**) (Figure 23).[25] This compound had similar affinity for κ opioid receptors as **1**, but was found to be less active. Isosteric replacement of the furan ring with a 2-oxazoline ring (**58**) decreased affinity for κ opioid receptors.[25] This occurrence was also seen with the 4-carbomethoxyoxazole (**59**).[355] Several sulfonyl pyrrole derivatives of **1** (**60-62**) were found to have reduced affinity at the κ opioid receptor.[355] However, these compounds were found to be partial κ opioid agonists.

Replacement of the furan ring with a 4-methyl-1, 3,5-oxadiazole (**63**) reduced affinity for the κ opioid receptor 30-fold. This compound was found to be an antagonist at both the μ and δ opioid receptors.[25]

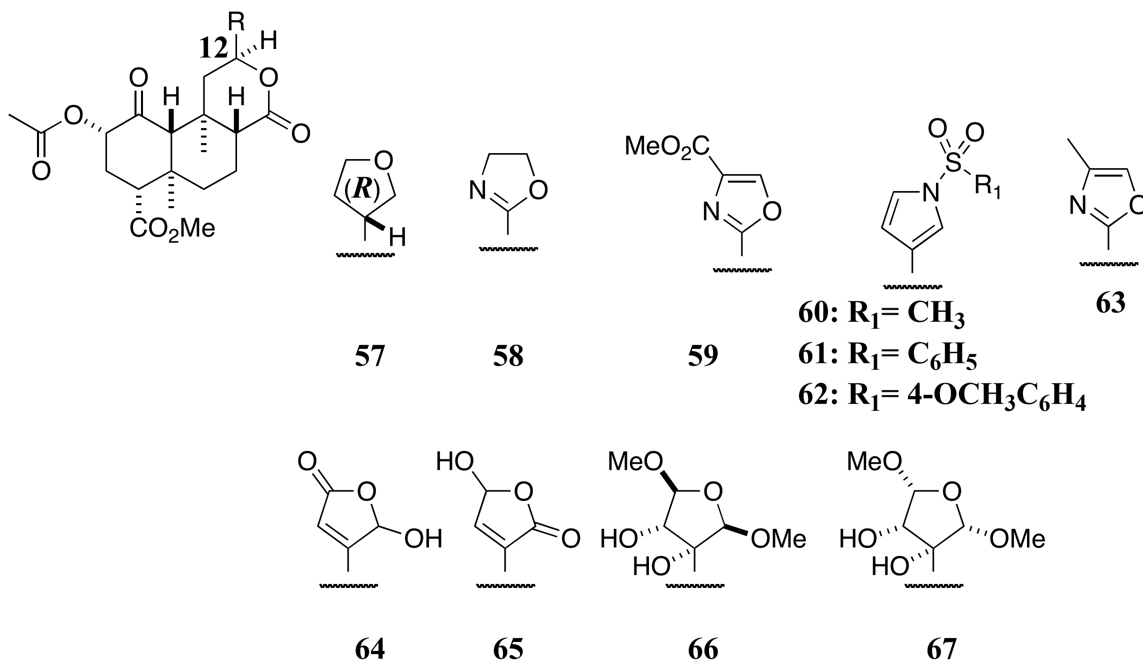


Figure 23: Selected C-12 analogues of salvinorin A.

S. divinorum produces over 20 neoclerodanes and several of these possess the core structure of salvinorin A save for modified furan rings.[25, 336, 337] These compounds were also investigated in the effort to further elucidate interactions at opioid receptors. Salvidivin A (**64**) and B (**65**) were both isolated from *S. divinorum*[336] and possess a γ-hydroxy butenolide ring. Since their isolation, these compounds have been synthesized

in our laboratory and it was found that **64** had antagonist activity at κ opioid receptors.[25] Our laboratory was also able to isolate salvinicin A (**66**) and B (**67**) from *S. divinorum* as described previously.[337] These compounds possessed a 3,4-dihydroxy-2, 5-dimethoxytetrahydrofuran ring which has been found in other clerodanes but had not been seen to that point in any isolated from the *Salvia* species of plant. Both of these altered furan ring compounds were screened for opioid activity and compound **66** was found to be a partial agonist at κ opioid receptors while **67** showed antagonist activity at μ opioid receptors. This was the first reported example of a neoclerodane acting as an μ opioid antagonist.

Chemical Methodology Towards Analogues

The key to SAR studies is the synthesis of analogues of the parent compound. On a complex natural product this is not trivial. Salvinorin A is a highly functional molecule with seven chiral centers. Compounding matters are the fact that several of these centers are readily epimerized including C-8. Epimerization at this center has shown to abolish affinity at opioid receptors; illustrating the influence that stereochemistry has in the interaction of salvinorin A at opioid receptors.

C-2 Methodology

Initial forays into SAR studies of salvinorin A involved the removal of the C-2 acetate. Heating of **1** with strong base led to the formation of a rearranged product (**68**) (Figure 24) in 69% yield.[354] Using KOH in CH_3OH caused oxidation and gave products **69a** and **70** in 53% and 37% yield, respectively.[356] Replacing KOH with $Ba(OH_2)$ gave **69b** in 75% yield.[27] Another attempt to remove the acetate using KCN in a refluxing mixture of CH_3OH and tetrahydrofuran was successful in cleaving the acetate but it also

caused C-8 epimerization as the major product in 51% yield.[303] Ammonolysis of **1** with NH_3 and CH_3OH at 0 ˚C gave **2** in 15% yield, along with the C-8 epimer in a 1:1ratio.[26] Selective removal of the C-2 acetate was achieved with Na_2CO_3 in CH_3OH to afford **2** in 77% yield.[357] With **2** in hand, the coupling of various acid chlorides, carboxylic acids and alkyl chlorides were able to proceed to yield many of the esters, carbonates, ethers, sulfonates and carbamates previously mentioned. Conversion of the C-2 hydroxyl of **2** to its bromo counterpart (**71**) proceeded using a mixture $CBr_4/PPh_3/CH_2Cl_2$ in 59% yield.[28] This transformation allowed for displacement of the bromo by thiosulfides to yield the thioesters. The bromo at C-2 of **71** was also displaced with an azide and reduced to the corresponding amine **72** in 36% yield.[28] The C-2 amine of **72** was then acetylated to afford the amide analogues. The C-2 hydroxyl of **2** was also converted to the triflate in 26%, which was subsequently displayed by various primary and secondary amines to yield the amine analogues.[352]

8-*epi*-1 **2** **68** **69a: R_1= OH** **69b: R_1= H**

70 **71** **72**

Figure 24: Structures derived from C-2 methodology.

C-1 Methodology

The reactivity of the C-1 carbonyl as been investigated by several research groups. Treatment of **1** with $NaBH_4$ at 35 °C gives a 1:1 mixture of the α– and β–alcohols at this position in 40% yield.[303] This reaction has a caveat, however, as it causes C-8 epimerization in 40% yield to afford 8-*epi*-1 (Figure 24). The reduction was improved through the use of an aqueous solution of $NaBH_4$ in tetrahydrofuran.[358] This strategy gave the α-alcohol **73** (Figure 25) at C-1 in 77% yield while avoiding epimerization at C-8 and minimal reduction at the C-17 lactone.[358] The α-alcohol was

further used as an intermediate to generate the 1,10-alkene seen in compound **74** in 58% yield.[358]

C-17 Lactone Methodology

The C-17 lactone was converted to the corresponding lactol **75** (Figure 25) with an excess of DIBAL-H in tetrahydrofuran as a 1:1 mixture in 65% yield.[359] This transformation was conducted without affecting the other functionalities of **1**. Formation of **75** allowed for the synthesis of the C-17 ethers. Lactol **75** itself is not stable however as it undergoes elimination overnight.[359]

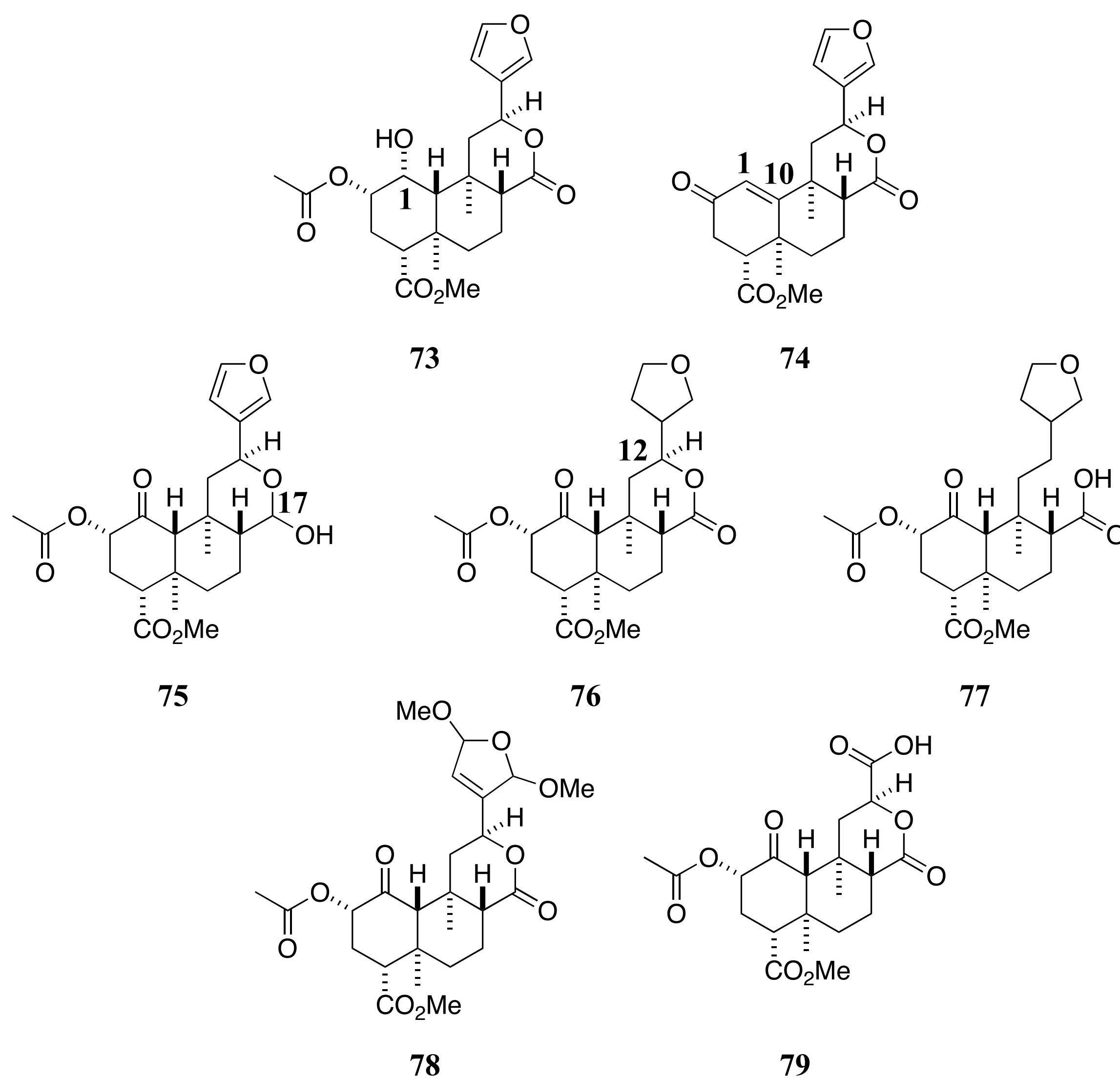


Figure 25: Structures derived from C-1, C-17 & C-12 methodology.

Furan Methodology

The furan ring of **1** was hydrogenated to the tetrahydrofuran **76** (Figure 25) as a mixture of C-13 epimers with the use of 5% rhodium on carbon in 59% yield.[25] This technique also resulted in hydrogenolysis of **1** to give **77** in 28% yield.[25, 303] Replacement of rhodium with 10% palladium on carbon saw a favoring of the hydrogenolysis product. Treatment of **1** with Br_2 and CH_3OH in CH_2Cl_2 affords the dimethoxy, dihydrofuran **78** as a mixture of isomers in 61% yield.[25, 355] The alkene of this compound can then be reduced using 5% rhodium on carbon to give the dimethoxy, tetrahydrofuran intermediate in 92% yield, which serves as the intermediate to synthesize the sulfonyl pyrrole compounds **60-62**. Oxidative degradation of the furan ring employing $NaIO_4$, a catalytic amount of $RuCl_3 \cdot 3H_2O$ in a mixture of $CH_3CN/H_2O/CCl_4$ gave the carboxylic acid **79** in 93% yield.[355] This intermediate was used to generate compounds **58**,**59** and **63**. Also, while **64** and **65** can be isolated in small amounts from *S. divinorum*, this compound can also be synthesized from the photo-oxidation of **1** to give a 1:3 (**64**:**65**) mixture of these compounds in 25% yield.[25]

While **1** has been able to be modified at several sites on the molecule, further investigation of its pharmacophore must be conducted in order to delineate how it interacts at the opioid receptors. Due to its complex structure, some analogues cannot or would be very difficult to obtain through semi-synthesis. This raises the need for the development of a total synthesis of **1** that would be amenable towards analogue development.

Total Synthesis Efforts Towards Salvinorin A

The unique pharmacological profile of salvinorin A along with its interesting chemical structure has made it an attractive target for total synthesis. This is further compounded by the need for further analogues that may not be accessible with semi-synthesis. Also, total synthesis of salvinorin A may lead to the development of new chemical methodology that can be applicable towards other neoclerodanes amongst other natural products.

To date, there have been 3 successful total syntheses of salvinorin A. Evans and co-workers reported the first successful total synthesis of salvinorin A in 2007. They were able to achieve salvinorin A in 33 steps with 4.5% overall yield.[360] In 2008, Hagiwara and co-workers reported the total synthesis of salvinorin A in 20 steps with 0.95% overall yield, employing a similar strategy previously utilized for the total synthesis of (-)-methyl barbascoate.[361] Recently, Hagiwara published a revised total synthesis of salvinorin A, starting with an intermediate that was synthesized in the 1st generation. This newer route achieved salvinorin A in 13 steps with 2.8% overall yield.[362] Several other approaches to syntheses of salvinorin A have been published and these include efforts from the laboratories of Forsyth[363], Lingham[364] and Perlmutter[365].

Summary

The pharmacological profile of salvinorin A is unique as it is active as a hallucinogen despite not having any interaction at the 5-HT_{2A} receptor. Along with this, salvinorin A has been found to be a full agonist at κ opioid receptors with high affinity. Remarkably, despite these characteristics, salvinorin A bears no resemblance to the typical ligands that interact at these receptors. This quality has resulted in the investigation of

salvinorin A by pharmacologists to explore its pharmacological effects both *in vitro* and *in vivo*. Along with the interest in its pharmacological properties, the complex structure of salvinorin A has also generated great interest from both organic and medicinal chemists. A natural product like salvinorin A, which is highly functionalized and has multiple chiral centers, presents a challenging target for total synthesis. These qualities also present a challenge to medicinal chemists as they try investigating the pharmacology of salvinorin A through SAR studies with the goal of pharmacophore elucidation. Attempts at the total synthesis of salvinorin A has provided new avenues towards the synthesis of other neoclerodanes as well as to analogues of salvinorin A that may help establish its pharmacophore. Analogues derived from chemical modification of salvinorin A have helped to probe receptors and perhaps more importantly, have aided in establishing neoclerodanes as a novel scaffold for opioid ligands. This novel scaffold may provide a platform for the development of novel opioid based therapeutics that do not have the deleterious consequences associated with other opioid ligands. Furthermore, chemical modification of salvinorin A has helped create chemical methodology for altering other neoclerodanes for the purpose of SAR study. The ability to modify other biologically active neoclerodanes greatly aids the ability for themselves to become useful biological probes or therapeutics.

While numerous analogues of salvinorin A have been synthesized, the true pharmacophore has yet to be elucidated which warrants further investigation of its structure including the importance of several structural features such as the C-2 acetate and the furan ring. This thesis will present the work done on the chemical modification of salvinorin A in the effort to create biological probes for the opioid receptors that will aid in determining its pharmacophore. Data will be presented on a novel series of analogues and the chemistry employed to synthesize them as well as the related pharmacological results.

CHAPTER III. RATIONALE & SPECIFIC AIMS

Salvinorin A is a hallucinogenic neoclerodane diterpene, which does not display any activity at the serotonin 5-HT_{2A} receptor, the typical target receptor for classical hallucinogens. While hallucinogenic, salvinorin A actually interacts at opioid receptors without having any structural similarity to other opioid ligands. Initial investigations into the SAR of salvinorin A showed that certain chemical modifications were tolerated at opioid receptors. This is significant as salvinorin A and potentially neoclerodanes, represent a novel scaffold to explore opioid receptors. Opioid receptors themselves have been linked to a variety of physiological functions and conditions such as pain as well as gastrointestinal and mood disorders. Further investigation into the SAR of salvinorin A will create novel opioid ligands with altered pharmacological profiles that will serve as probes for the opioid receptors. The information gained from these probes would be instrumental in the development of novel therapeutics that will address different phenomenon associated with opioid receptors such as though mentioned above.

Specific Aim #1: Prepare and evaluate C-2 analogues to explore further the structure-activity relationship of salvinorin A at this position

Investigations into the role of the C-2 acetate of salvinorin A have shown that modifications made at this position can alter affinity, selectivity and efficacy at opioid receptors.[346] A prime example of this can be seen with the incorporation of an aromatic functionality into analogues. Previous work with esters (**14**), carbamates (**30**) and amides (**50**) has shown that incorporation of a phenyl ring will alter selectivity from κ to μ opioid receptors.[346] However, this was not seen with the corresponding sulfonate (**55**). Incorporation of a 4-methyl group (**56**) bestowed affinity for μ opioid receptors but did

not alter affinity for κ opioid receptors compared to its unsubstituted counterpart **55**. To further explore and understand this phenomenon, a series of substituted benzene sulfonates were synthesized. Along with examining the effects of substitution on the phenyl ring of the sulfonate, these compounds will also infer more information into the effects of the type of linkage at C-2.

Specific Aim#2: Prepare and evaluate furan analogues/isosteres of salvinorin A to explore the structure-activity relationship at this position

Studies have suggested that the furan ring of salvinorin A influences activity at opioid receptors. Furan rings themselves have been linked with hepatotoxicity.[366] Furthermore, while their have been no definitive studies linking salvinorin A to toxicity, a computational toxicology study (MC4PC, Multi-CASE, Inc.) involving salvinorin A and three similarly structured compounds (columbin, diosbulbin G, salvinorin B) predicted salvinorin A to be a reproductive toxicant in mammals.[21]

To investigate further the role of the furan ring of salvinorin A and its influence in binding and activity at opioid receptors, as well as attempting to circumvent the potential hepatotoxicity associated with furan rings, a series of analogues containing modified furan rings were synthesized. These analogues include the structural replacements of the furan ring as a means to circumvent this potential toxicity as well as an attempt to likely improve pharmacokinetic properties of salvinorin A such as water solubility. Also the incorporation of steric bulk, was examined as a way to avoid potential toxicity and give insight into whether salvinorin A has a tight binding pocket at opioid receptors which can be affected by potential hydrophobic interactions.

These specific aims are intended to prove that salvinorin A can be altered and these derivatives will serve as novel opioid probes. The ability to synthesize novel opioid analogues from salvinorin A further demonstrates its utility as a lead molecule and furthermore, it provides evidence that neoclerodane diterpenes can be employed as structural scaffolds for the construction of biologically relevant compounds that interact at opioid receptors. The results of these studies will be presented and discussed.

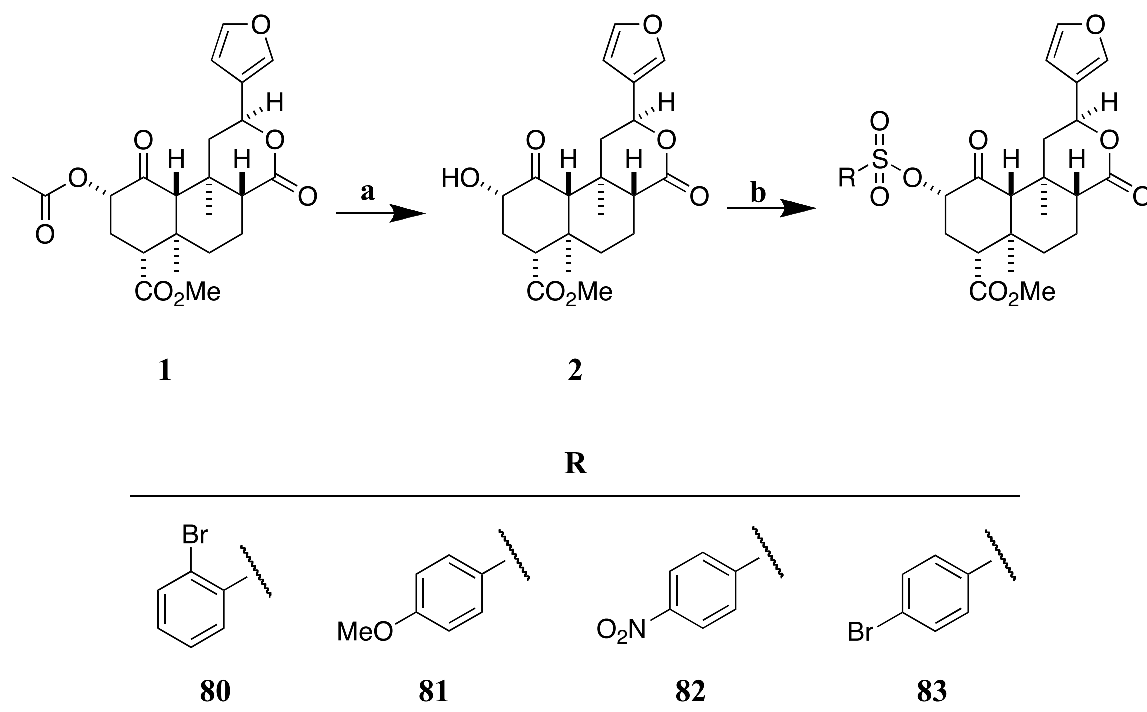
CHAPTER IV. RESULTS AND DISCUSSION

Introduction

The pharmacophore of salvinorin A has yet to be fully elucidated. In an effort to enhance our knowledge and understanding of the structure-activity relationship of salvinorin A, a series of salvinorin A analogues were synthesized and evaluated for the ability to bind at opioid receptors. Several compounds that displayed high affinity ($K_i \leq$ 150 nM) for opioid receptors were further evaluated for efficacy at opioid receptors. The data presented here will first discuss the synthesis of these analogues and design rationale behind analogues followed by the pharmacological results obtained from testing.

C-2 Investigation Chemistry

An investigation into how the C-2 sulfonate linker atom affected activity at opioid receptors, led to the synthesis of a series of substituted phenyl sulfonates (**80-83**). The synthesis of these benzyl sulfonates is described in Scheme 1. The reaction of **1** with Na_2CO_3 in CH_3OH for four hours afforded **2** in 77% yield. Once **2** was achieved, it was subsequently reacted with the appropriate sulfonyl chloride in CH_2Cl_2 at room temperature with NEt_3 and a catalytic amount of DMAP to generate compounds **80-83**.



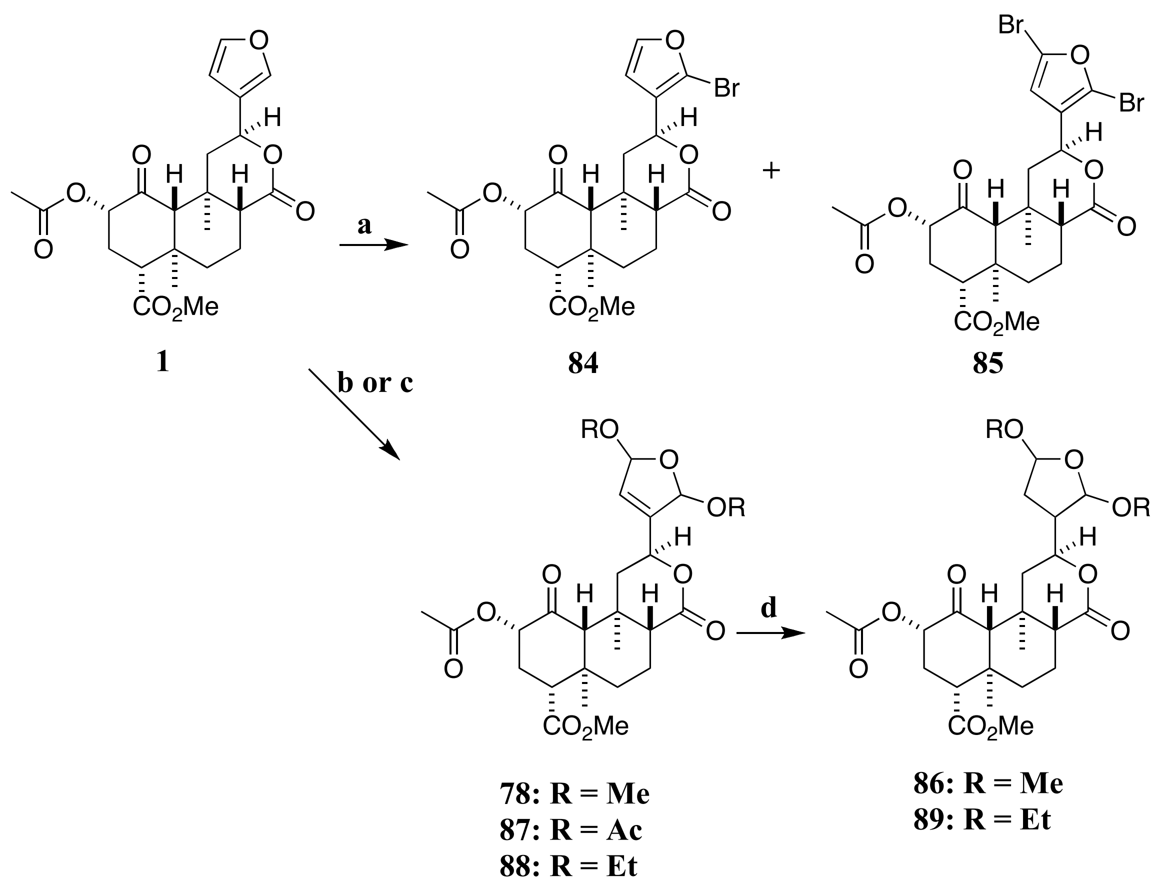
Scheme 1: Synthesis of Sulfonate Analogues. *Reagents and Conditions*: (a) Na_2CO_3, CH_3OH, r.t. (b) Appropriate sulfonyl chloride, NEt_3, DMAP, CH_2Cl_2, r.t., 25-48% yield.

Furan Ring Investigation Chemistry

Alteration of The Furan Ring

Substitution on the furan ring and its effects on activity was initially explored through the synthesis of the mono and di-brominated salvinorin A analogues **84** and **85**. These compounds were achieved through the reaction of salvinorin A and *N*-bromosuccinimide in CH_2Cl_2 for three hours (Scheme 2). Further studies looked to investigate the SAR of compounds **66** and **67** (Figure 23), naturally occurring

neoclerodanes from *S. divinorum* with modified furan rings who themselves had opioid activity. This prompted the synthesis of compounds **78** and **86-89**. Initial attempts to synthesize compound **78** employing a reaction of salvinorin A with 2 equivalents of Br_2 in CH_3OH and CH_3CN at 0 °C was not successful, as only degradation occurred. Reactions using lower temperatures were attempted which eventually required the switching of solvent from CH_3CN to CH_2Cl_2 which was found to be better suited for solubility. Reaction temperatures of -20 and -30 °C produced modest reaction yields with degradation remaining an issue. The use of less Br_2 was explored and this seemed to limit the amount of degradation observed. Eventually, the reaction was optimized and **78** was synthesized as mixture of isomers through the reaction of salvinorin A with CH_3OH and 1 equivalent of Br_2 in solution of a 1:1 CH_3OH/CH_2Cl_2 at -30 °C. Compound **88** was achieved in similar fashion by substituting CH_3OH with CH_3CH_2OH. The synthesis of compound **87** could also be synthesized in this manner; however, it requires the replacement of CH_3OH/CH_3CH_2OH with CH_3CO_2H and the addition of NaOAc. Reduction of **78** and **88** with 5% rhodium on carbon in CH_3CH_2OH at room temperature afforded the *trans* isomers of **78**(**86**) and **88**(**89**) respectively. Attempts to perform this transformation with 5% Pd/C resulted in the hydrogenolysis of the lactone ring along with the incomplete reduction of **78** and **88**.

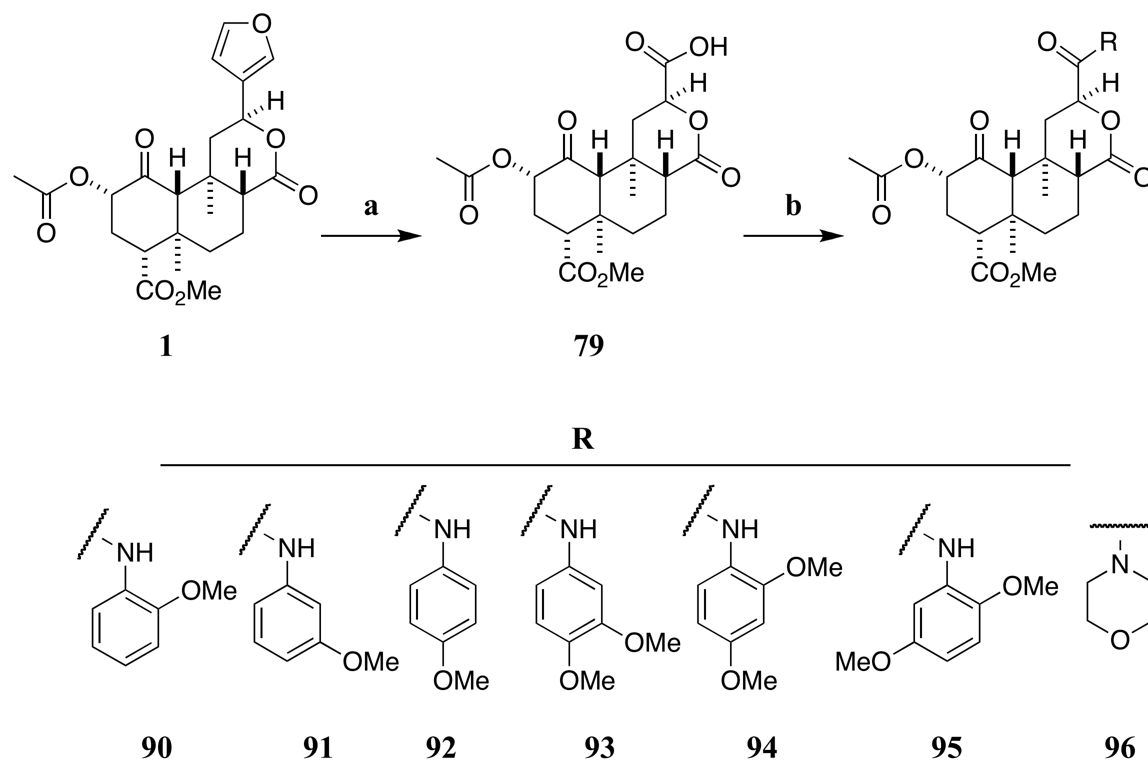


Scheme 2: Synthesis of Altered Furan Analogues. *Reagents and Conditions*: (a) NBS, CH_2Cl_2, rt; 22-48% yield. (b) Br_2, CH_3OH or CH_3CH_2OH, CH_2Cl_2, -30 ºC; 45-62% yield. (c) Br_2, CH_3CO_2H, NaOAc, CH_2Cl_2, -30 ºC; 37% yield. (d) 5% Rh/C, CH_3CH_2OH, rt.; 90% yield.

Replacement of The Furan Ring

A series of cyclic and aromatic amides (**90-96**) was synthesized as structural replacements for the furan ring of salvinorin A (Scheme 3). These compounds were created in part because of their probable reduced toxicity as well as potentially improved

water solubility. The reaction of salvinorin A with $NaIO_4$ and catalytic $RuCl_3$•$3H_2O$ in a mixture of $CH_3CN/H_2O/CCl_4$ for 2 hours produced **79**. Reaction of **79** with the appropriate amine, EDCI and HOBT in CH_2Cl_2 at room temperature afforded amides **90-96** in 18 – 75% yield. The use of these water soluble coupling agents was found to be far superior to using DCC as HOBT and EDCI improved overall reaction yields and made purification more facile (DCC yields 15-52%).



Scheme 3: Synthesis of Furan Ring Replacement Analogues. *Reagents and Conditions*: (a) $NaIO_4$, $RuCl_3$•$3H_2O$, $CH_3CN/CCl_4/H_2O$, rt.; (b) Appropriate amine, EDCI, HOBt, CH_2Cl_2, rt.; 18-75% yield.

Salvinorin A Diels-Alder Chemistry

While there are numerous examples of furan rings undergoing Diels-Alder reactions to form cycloadducts, there are synthetic challenges that have to be overcome. Historically, furan rings are poor dienes and examples of furan rings that are substituted at the three-position participating in Diels-Alder reactions are relatively rare.[367] Traditionally, Lewis acids such as $AlCl_3$ or $TiCl_4$ have been used to improve yields.[367] In this regard, Diels-Alder reactions with the furan ring of **1** must overcome multiple synthetic challenges. There are multiple oxygens in **1** that have the ability to coordinate with Lewis acids. Due to these characteristics, conditions had to be developed for the Diels-Alder reaction to proceed.

Initially, maleic anhydride and maleimide were reacted with **1** in an attempt to form cycloadducts. These dieneophiles were chosen in part because of literature precedent[368, 369] as they were shown to react with furan rings in the Diels-Alder reaction with relative ease. Several different reaction conditions were attempted in order to synthesize these compounds including varying solvent and atmosphere (Table 1). Unfortunately, the reaction of **1** with these compounds was unsuccessful. This may be due in part to the nature of salvinorin A as a diene. Typical Diels-Alder reactions involve the reaction of an electron poor dieneophile with an electron rich diene. While maleic anhydride and maleimide are electron poor, the furan ring of **1** is more electron neutral than electron rich. This characteristic may contribute to its inability form the orbital overlaps necessary to facilitate the reaction with these particular dieneophiles.

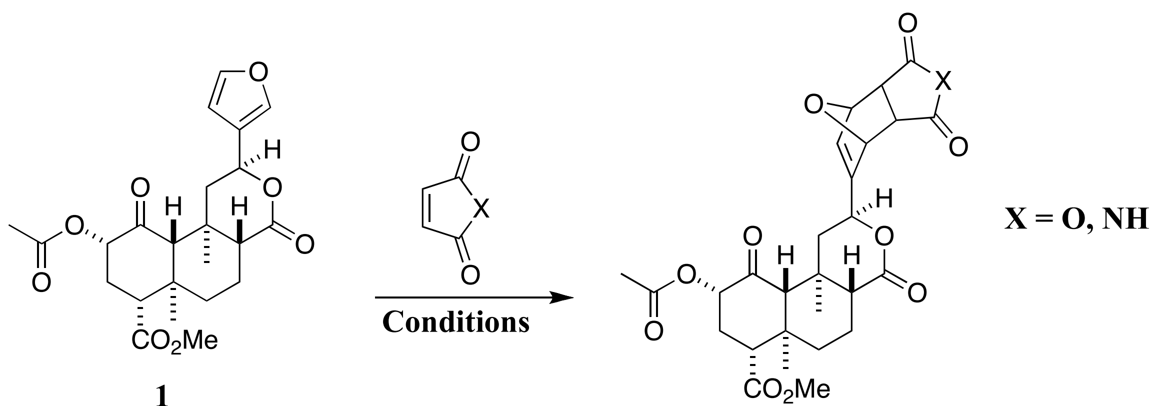


Table 1: Reaction conditions attempted for salvinorin A & maleic anhydride/maleimide.

Time/Hrs	Temperature	Solvent	Atmosphere	Yield %
12	25 °C	THF	Air	NR[a]
12	25 °C	THF	Argon	NR[a]
12	25 °C	Ether	Air	NR[a]
12	25 °C	Ether	Argon	NR[a]
12	25 °C	CH_2Cl_2	Air	NR[a]
12	25 °C	CH_2Cl_2	Argon	NR[a]
12	25 °C	Toluene	Air	NR[a]
12	25 °C	Toluene	Argon	NR[a]

Note: [a] No reaction observed.

Additionally, several other electron poor dieneophiles including benzoquinone, dimethyl maleate, dimethyl fumarate, diethyl maleate, diethyl fumarate, methyl vinyl ketone and several diazo-dicarboxylates were treated with compound **1** in an attempt to form cycloadducts in part because of literature precedent showing that these readily formed cycloadducts with furan rings.[368, 369] Several reaction conditions were employed

including reactions at - 20 ºC, 0 °C, room temperature, 40 ºC and reflux in different solvents (CH_2Cl_2, THF, toluene, ether and benzene). However, the Diels-Alder reaction with **1** did not proceed (Table 2). The Lewis acids $AlCl_3$ and $TiCl_4$ were utilized in an attempt to catalyze the reaction but these efforts failed to produce cycloadducts as well. Another Lewis acid, $HfCl_4$, was used in an attempt to catalyze the Diels-Alder reaction with **1**. $HfCl_4$ is not as moisture/air sensitive as other Lewis acids.[370] Furthermore, it is not as reactive as $AlCl_3$ and $TiCl_4$ as it was thought that the failure of **1** to form cycloadducts with the aforementioned Lewis acids were due to their propensity to complex with the oxygens present in **1**, as well as the dieneophile. $HfCl_4$ was used in order to potentially circumvent these issues, thus, in theory, allowing for a more facile reaction. $HfCl_4$ did succeed in catalyzing the reaction of **1** with dimethyl maleate; unfortunately, it was in trace amounts and as a mixture of products including **1**. Lewis acids were not employed for reactions proceeding at higher temperatures due to their heat sensitive natures. In addition to possible complexing of the Lewis acids with the multiple oxygens of **1**, the failure of the Lewis acids to catalyze the Diels-Alder reaction may have arisen from their inability to adequately decrease the lowest unoccupied molecular orbital (LUMO) of the dieneophiles; thus not making them electron poor enough to overcome the electron neutral nature of the furan ring in **1**, thereby making the necessary electronic interactions needed for the Diels-Alder unable to proceed.

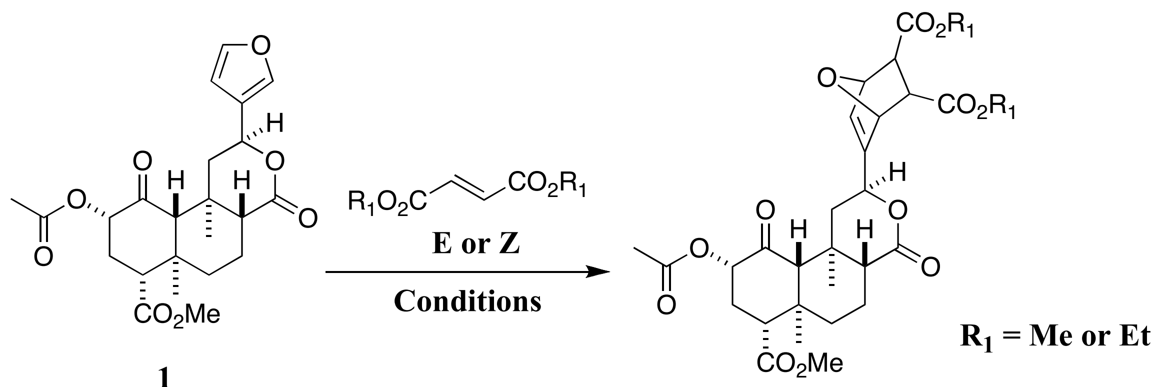


Table 2: Reaction conditions attempted for Diels-Alder reaction[a,b] with salvinorin A.

Time/Hrs	Temperature	Lewis Acid[c]	Yield %
12	-20 °C	$TiCl_4$ or $AlCl_3$	NR[d]
12	-20 °C	$HfCl_4$	NR[d]
12	0 °C	$TiCl_4$ or $AlCl_3$	NR[d]
12	0 °C	$HfCl_4$	≥ 1% (R_1= Me, Z)
12	25 °C	$TiCl_4$ or $AlCl_3$	NR[d]
12	25 °C	$HfCl_4$	NR[d]
12	40 °C	None	NR[d]
12	Reflux	None	NR[d]

Note: [a]Other dieneophiles tried: benzoquinone, dimethyl maleate, dimethyl fumarate, diethyl maleate, diethyl fumarate, methyl vinyl ketone, dibenzyl azodicarboxylate, diethyl azodicarboxylate, di-tert-butyl azodicarboxylate.

[b]Solvents used: CH_2Cl_2, THF, toluene, ether, benzene.

[C]Amount of Lewis Acid used: 10 mol %.

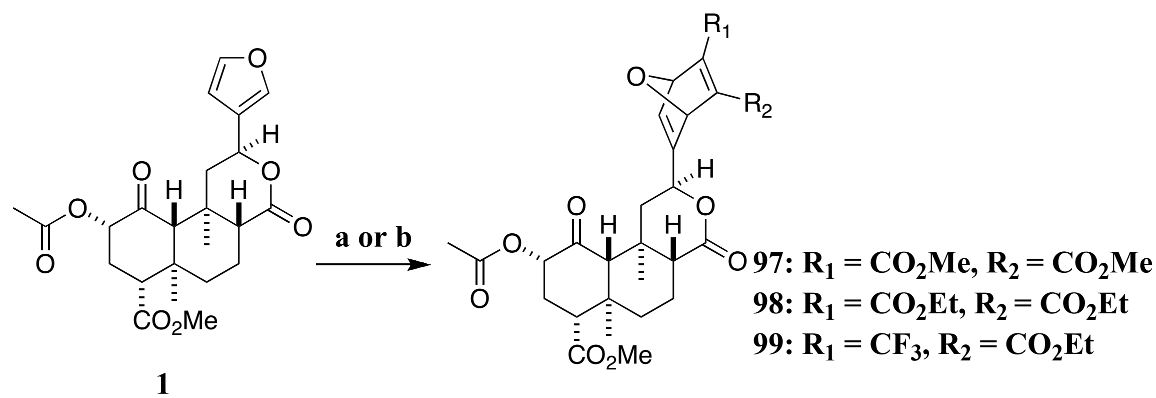
[d]No observed reaction.

A second set of dieneophiles including dimethyl and diethyl acetylene dicarboxylate were reacted with **1**. These were chosen due to literature precedent as well.[371] Additionally, the alkyne dieneophiles were chosen due to their inability to form

endo vs. *exo* isomers in regards to their electron withdrawing substituents due to the alkynes symmetrical nature. Multiple reaction conditions were employed to try to spur the Diels-Alder reaction between these dieneophiles and **1** including those mentioned previously. It was found that refluxing **1** with dimethyl or diethylacetylene dicarboxylate in toluene for 2 days yielded cycloadducts **97** and **98** (Scheme 4). To the best of our knowledge, this was one of the first examples of a successful Diels-Alder reaction with the furan ring of a neoclerodane. Attempts to decrease the reaction times was sought after as prolonged exposure to reflux conditions resulted in the retro Diels-Alder reaction, resulting in the reformation of **1** and the alkyne. This occurrence was observed for reactions allowed to go for more than 2.5 days, which made the synthesis of cyclo-adducts **97** and **98** problematic as they needed extended reaction times to form.

In order to optimize reactions times, microwave irradiation was investigated as a means for synthesis. Several solvents were screened for use in the microwave including xylenes, benzene, toluene, and dioxane. Furthermore, different time lengths of exposure and absorbance levels were also investigated. Eventually optimal conditions were found to involve using either dry and degassed toluene or dioxane in the microwave at 100 ºC for 30 minutes (Table 3). Microwave and standard reflux conditions also afforded cycloadduct **99** from the reaction of **1** and ethyl 4, 4, 4-trifluoro-2-butynoate. Upon the determination of optimal reaction conditions for the Diels-Alder with the alkyne dieneophiles, and in order to further probe the reactivity of the furan ring, additional alkynes were employed in this reaction. These dieneophiles included methyl propiolate, methyl 2-butynoate, methyl phenyl propiolate and acetylene dicarboxylic acid. Additionally, the original dieneophiles initially attempted (maleic anhydride, maleimide,

benzoquinone, dimethyl maleate, dimethyl fumarate, diethyl fumarate, methyl vinyl ketone and diazocarboxylates) were tried using microwave conditions, but again, cycloaddition failed to take place with these and the other alkynes tried, indicating that the presence of an alkyne flanked by electron withdrawing groups on both sp carbons are more suitable for Diels-Alder reaction with the furan ring of **1**. As a consequence of this, the ability of the alkynes with dual electron withdrawing groups to form cycloadducts with **1** may be attributed to the lowering of the energy of activation, allowing reaction to proceed. This favorable energy swing may not be able to be achieved by the other dieneophiles, resulting in no reaction. Further testing, including energy calculations need to be carried out in order to test this hypothesis.



Scheme 4: Synthesis of Cycloadduct Analogues. *Reagents and Conditions*: (a) Appropriate alkyne, toluene, reflux; (b) Appropriate alkyne, toluene/dioxane, microwave irradiation 100 ºC; 24-70% yield.

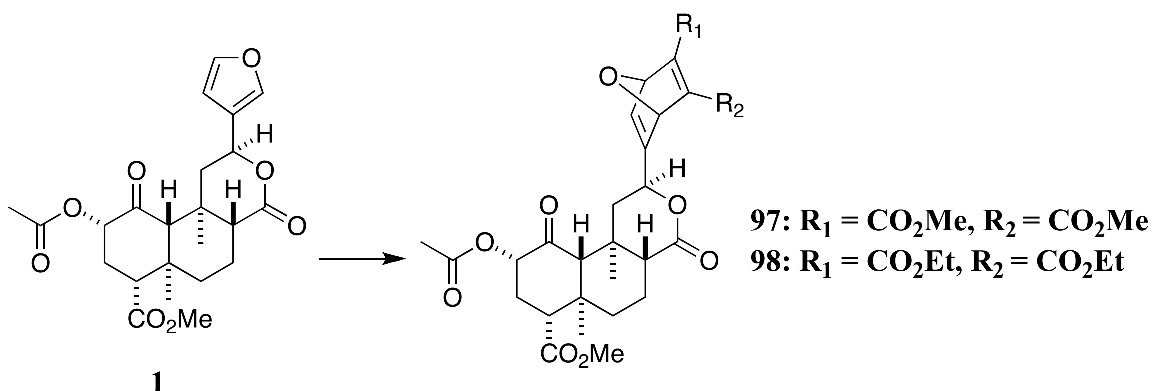


Table 3: Microwave reaction conditions for the synthesis of cycloadducts **97** & **98**.

Time/minutes	Absorbance[a]	Temperature	Solvent	Compound	Yield %
30	Normal	50 °C	Dioxane	97/98	21
60	Normal	50 °C	Dioxane	97/98	23
30	Low	100 °C	Dioxane	97/98	62
60	Low	100 °C	Dioxane	97/98	64
30	Normal	100 °C	Dioxane	97/98	70
60	Normal	100 °C	Dioxane	97/98	70
30	Normal	50 °C	Toluene	97/98	24/15
60	Normal	50 °C	Toluene	97/98	30
30	Low	100 °C	Toluene	97/98	55
60	Low	100 °C	Toluene	97/98	55
30	Normal	100 °C	Toluene	97/98	67
60	Normal	100 °C	Toluene	97/98	67

Note: [a]Refers to rate of heating, slow, normal or fast.

Elucidation of the structure of compound **98** began with high-resolution mass spectrometry (HRESIMS). Compound **98** was isolated as a white powder and the HRESIMS of **98** showed a [M + Na] ion peak at *m*/*z* 625.1553, which corresponds to a

molecular formula of $C_{31}H_{38}O_{12}$, the predicted chemical formula of **98**. Inspection of the ^{1}H spectra displayed that the proton signals, which correspond to C-15 (triplet at 7.390) and C-16 (singlet at 7.408) of **1**, were not present (Figure 26). This finding was verified in the ^{13}C spectra, as the corresponding signals (C-15, 143.71 and C-16, 139.41) were no longer present. Furthermore, the proton at C-14, which exists as singlet in **1** at 6.373, now appeared as a multiplet at 6.92. Further examination of the ^{1}H spectra displayed a multiplet at 1.32, which integrated for 6 protons and another multiplet at 4.28, which integrated for 4 protons. These particular shifts are indicative for $–OCH_2-$ bonds, which are connected to $-CH_3$ groups. Combining these data, the assumption was made that two additional $-OCH_2CH_3$ groups were incorporated into the molecule. This was verified by the ^{13}C spectra, as additional signals at 61.78 and 61.76 corresponded with the $-OCH_2-$ groups, and signals at 18.08 and 18.12 represented the new $-CH_3$ groups. While the signals that are associated with the C-15 and C-16 furanyl protons were not present in the spectra of **98**, two new signals that both integrated to 1 proton were found at 5.66 (triplet) and 5.60 (doublet). These shifts seemed to be in agreement with protons that would be expected on the oxygen bridge of **98**. Examination of the ^{13}C spectra, found two signals in the region of C-O bond linkage at 85.55 and 85.46. These data are conducive with the presence of an oxygen bridge that would form at C-14 and C-15 via the Diels-Alder mechanism. The remaining signals in the ^{13}C spectra were found to be indicative of two carbonyls, and four sp^2 carbon bonds. Using the dieneophile structure as a guideline, the carbonyls were believed to belong to the newly introduced esters. The signals in the ^{13}C spectra at 157.73 and 152.59 pointed to the presence of α-β unsaturated esters. These would be in agreement with the proposed structure. The remaining two sp^2 ^{13}C signals were attributed to C-13 and C-14, as they correspond to the other alkene carbons of the proposed structure. These general techniques were also employed to propose the structures of the previously mentioned **97** and **99** as well as the yet to be mentioned **102**, **103** and **104**. NMR analysis has aided in proposing the general structure of these

cycloadducts including the orientation of the oxygen bridge in regards to its position (*endo* vs. *exo*). Using coupling correlations observed from NMR spectra, the drawn structures are those that have been proposed. However, to aid further in the determination of the absolute configuration of the cycloadducts, crystals have been obtained for compounds **97**, **98**, **99**, **103** and **104** and have been submitted for x-ray analysis to aid in the goal to determine absolute configuration of these compounds.

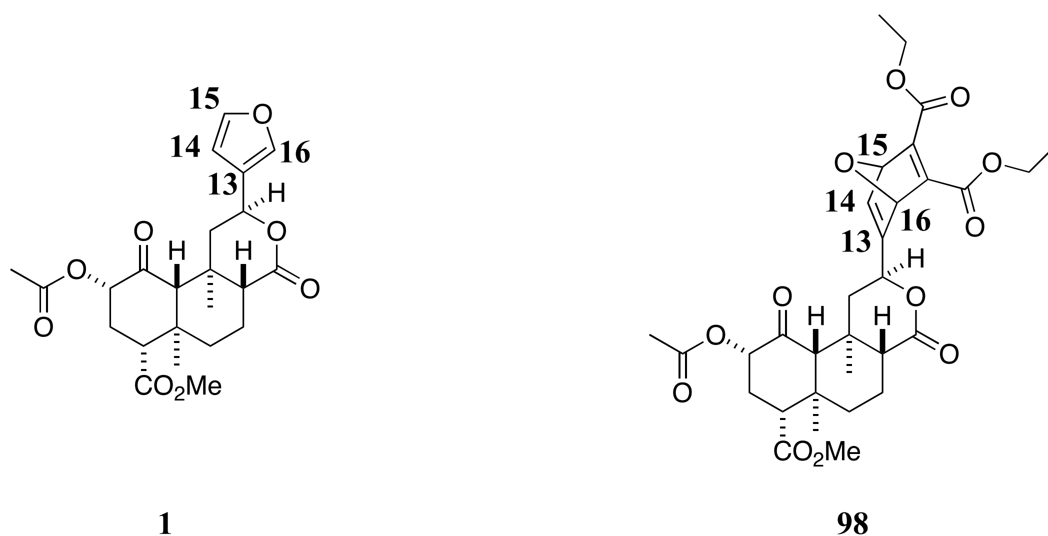
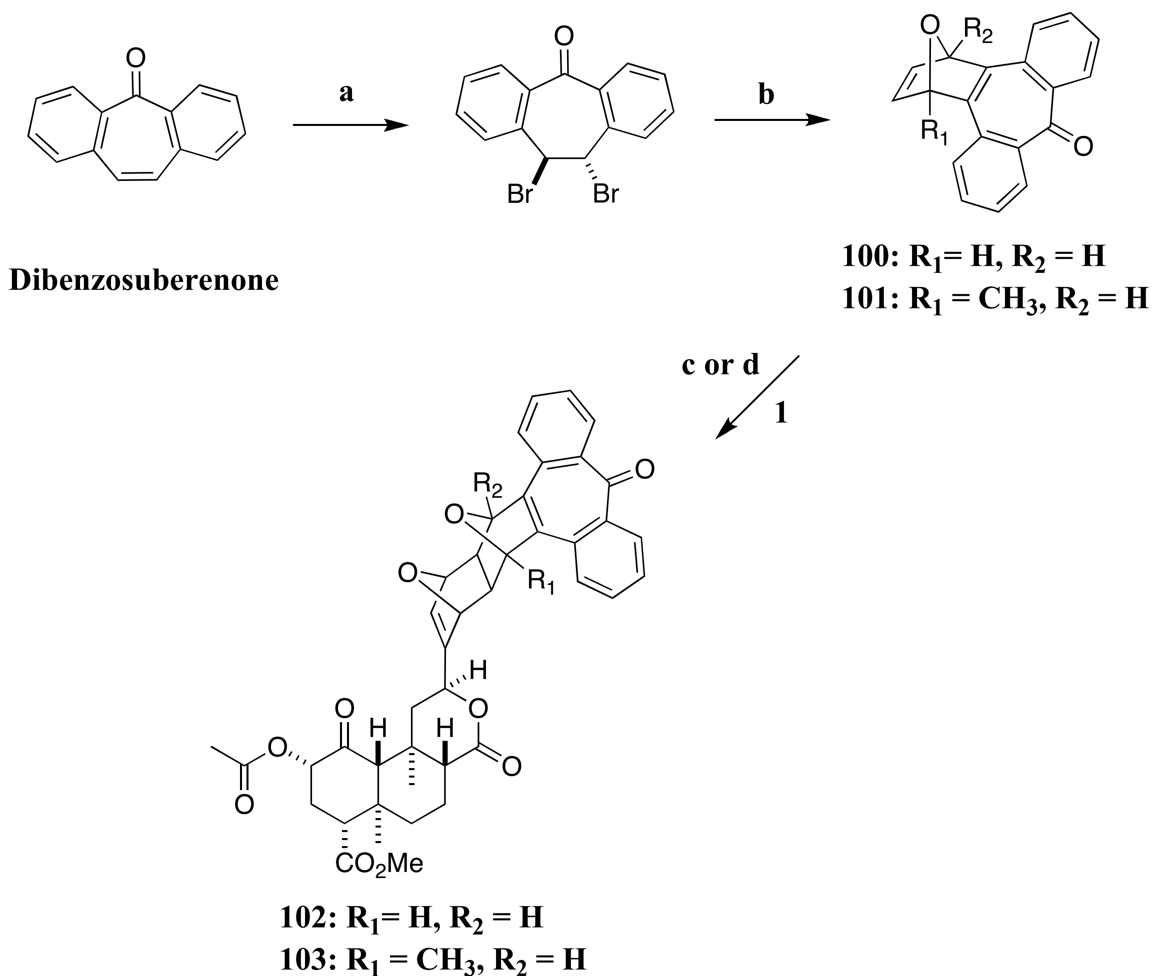


Figure 26: Partial numbering system for structure elucidation of **98**.

Reactions of Fluorescent Dieneophiles

Using the synthetic strategy employed previously, fluorescent dieneophiles could be incorporated into the structure of **1**. These dieneophiles were themselves derived from the reaction of dibenzosuberenone with Br_2 in CH_2Cl_2 at 0 ºC to yield the di-bromo intermediate, which was treated with KOtBu and either furan or 2-methyl furan in tetrahydrofuran to produce compounds **100** and **101**(Me) (Scheme 5). These compounds were fluorescent under uv light (254 and 365 nM) and there was literature precedent for them behaving as dieneophiles.[372] It was decided to try to take advantage of this reactivity and incorporate them into **1** to form potential fluorescent ligands for opioid receptors that also would add a significant amount of steric bulk. Compounds **100** and **101** were reacted with **1** in refluxing toluene for 3.5 days to afford cyclo-adducts **102** as a mixture of *endo* and *exo* products and **103**. While these reactions proceeded under the same microwave conditions as the alkynes, it was found that refluxing was more effective for their synthesis.



Scheme 5: Synthesis of Fluorescent Cycloadduct Analogues. *Reagents and Conditions*: (a) Br_2, CH_2Cl_2, 0 ºC, 98% yield; (b) KOtBu, THF, furan or 2-methyl furan, 0º C, 55-60% yield; (c) **100**/**101**, toluene, reflux, 45-60% yield; (d) **100**/**101**, microwave irradiation, 100 ºC, 42-56% yield.

A similar strategy to what was employed for determining the structure of **97**, **98**, **99** and **104** was used to determine the lower half of the molecule. To determine the

location of the methyl group located on the oxygen bridge, as in principle could be on either side (C-25 or C-41) (Figure 27), extensive NMR experiments were conducted.

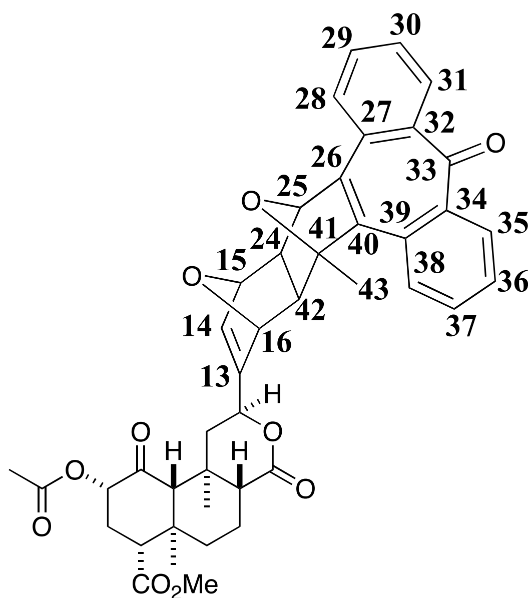


Figure 27: Partial numbering system for structural elucidation of compound **103**.

The first step involved defining the two separate aromatic AMNX spin systems for C-28 through C-31 and C-35 through C-38. ^{1}H NMR spectrum and ^{1}H-^{1}H COSY spectra established two overlapping yet distinct AMNX spin systems. Spin system one (C-28 through C-31) displayed signals at 7.43, 7.52, 7.65 and 7.97. Spin system two (C-31 through C-35) displayed signals at 7.97, 7.59 and two overlapping signals at 7.52.

With the establishment of the spin systems, it was now possible to establish which spin system was close to either the $–CH_3$ group at C-43 and/or the lone -CH group at C-25. The ROESY spectrum was able to establish through space correlations between C-43 (-CH_3 group) and spin system two. With the determination of which spin system the $–CH_3$ group is correlated with, the last step was to determine whether C-43 was proximal or distal (*cis* or *trans*) to the core of **1**. HMBC experiments were able to show ^{1}H and ^{13}C couplings to trace the carbon framework of **103** showing that C-43 is in fact proximal or *cis* to the salvinorin core (Figure 28) along with helping to establish the carbon framework of **103** which was fond to be in agreement with the proposed product of the Diels-Alder reaction. Establishing the location of the methyl group at the bridgehead of **103** was also instrumental in establishing the orientation of the oxygen bridges. Coupling correlations that could be established from the methyl group and surrounding carbons, provided evidence for the existence of the proposed structure.

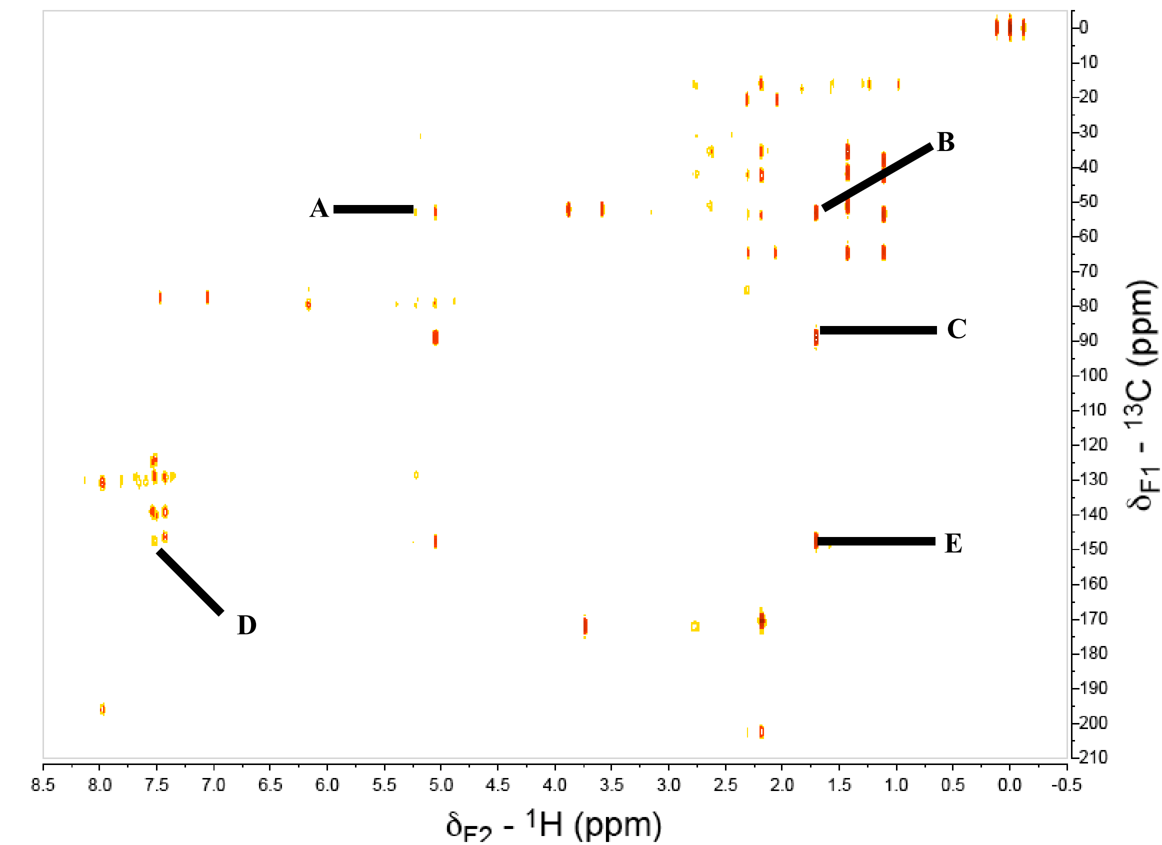
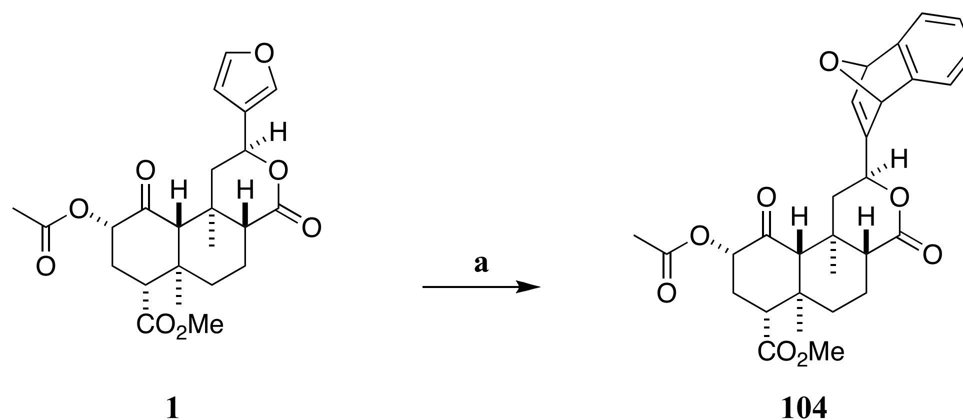


Figure 28: HMBC of **103**. Correlations that establish the regiochemistry of **103**. Specific 2 or 3 bond ^{1}H-^{13}C coupling is in are represented by bold lines where **A** shows correlations between C-14 and C- 42. **B** shows correlations between C- 41, C-42 and C- 43. **C** shows correlations between C- 43 and the oxygen bridge between C- 25 and C- 41. **D** shows correlations between C-38, C- 39 and C- 40. **E** shows correlations between C- 40, C- 41, and C- 43.

Benzyne Chemistry

While the furan ring of salvinorin A is not an especially reactive diene, it can participate in the Diels-Alder reaction with several reactive dieneophiles, including the

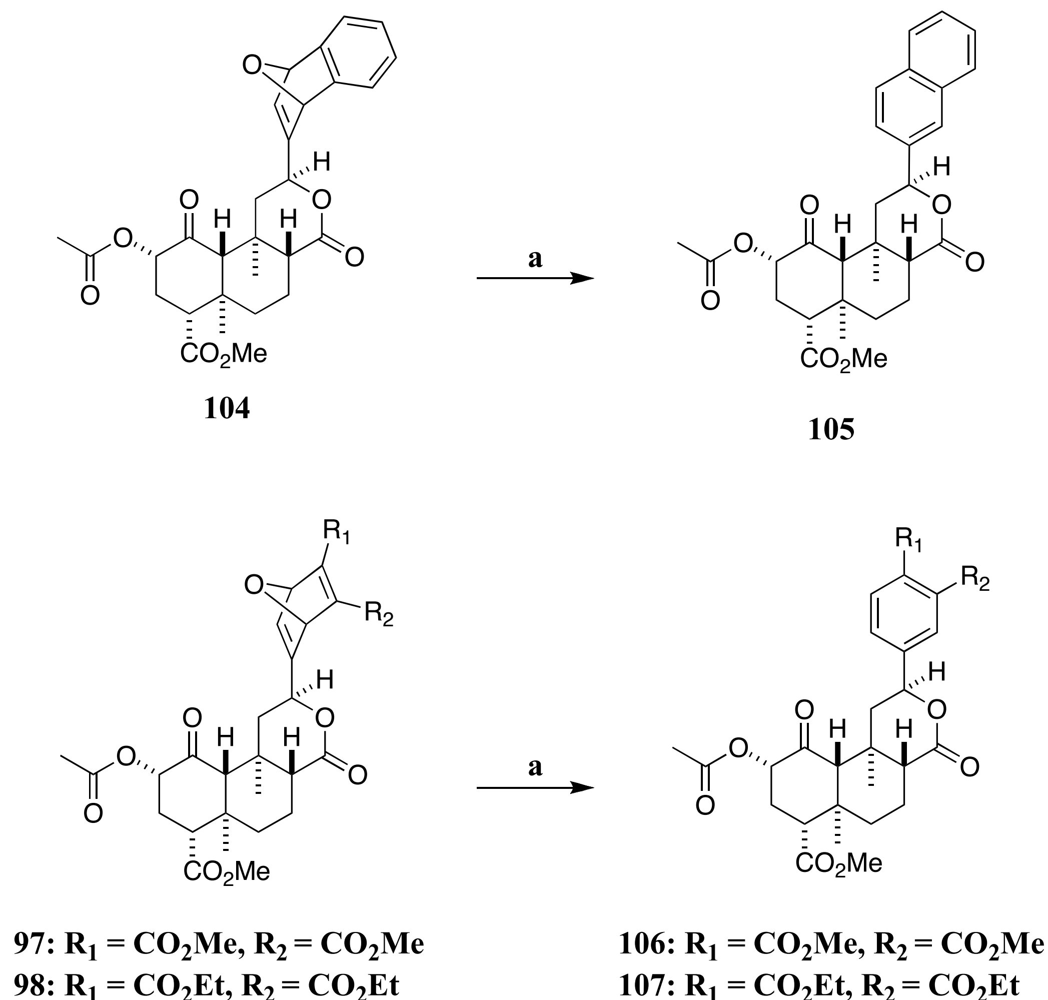
electron poor alkynes previously mentioned. A very reactive dieneophile in Diels-Alder reactions is benzyne. There is literature precedent that furan rings can trap reactive benzyne intermediates to form the corresponding cyclo-adducts[373, 374] so in the effort to modify the furan ring and increase the amount of steric bulk, **1** was used to trap benzyne. Initially, anthranilic acid was chosen as the benzyne precursor. Treatment with isoamyl nitrite to generate the benzyne and subsequent trapping with the furan proved to be unsuccessful despite numerous tries with altered temperatures (room temperature, 50 °C, reflux) and solvents (Toluene, CH_3OH, CH_3CN). An explanation for failure of cycloadduct formation could be attributed to the probable failure of benzyne formation *in situ*. Trimethyl silyl phenyl trifluoromethane sulfonate was then employed as the benzyne precursor and upon reaction with CsF in CH_3CN at room temperature; the benzyne intermediate was formed and trapped by the furan ring of **1** to form **104** in 49% yield (Scheme 6).



Scheme 6: Synthesis of Benzyne Cycloadduct Analogue. *Reagents and Conditions*: (a) Trimethylsilyl phenyl trifluoromethane sulfonate, CsF, acetonitrile, r.t.; 49% yield.

Formation of Non-Heterocyclic Aromatic Rings

Previous work, including that presented here has modified the furan ring through reduction or alkylation, or replaced it with both aromatic and non-aromatic heterocycles, such as oxazoles, oxadiazoles and pyrroles. Replacement of the furan ring introduced non-heterocyclic aromatic moieties through amide linkage at C-12. However, to date, there has not been a salvinorin A analogue that contained a non-heterocyclic aromatic ring coming off the core of the molecule directly at C-12. In the efforts to explore the effects of non-heterocyclic aromatics at this position as well as further probe the role of steric bulk and potential hydrophobic interactions, a series of substituted phenyl rings at C-12 was synthesized. Deoxygenation of Diels-Alder cycloadduct **104** with $Fe_2(CO)_9$ in toluene at reflux[375] afforded the napthyl derivative **105** in 70% yield (Scheme 13). It was perceived that the iron first complexed with the alkene at C-13, C-14 and aromatization was achieved through the exothermic elimination of water. These conditions were used to transform cycloadduct **97** and **98** to **106** and **107** respectively in 77% yield. To our knowledge, this is the first reported conversion of the furan ring of a neoclerodane to a phenyl ring.



Scheme 7: Synthesis of Non-Heterocyclic Aromatic Analogues. *Reagents and Conditions*: (a) $Fe_2(CO)_9$, toluene, reflux, 70-77% yield.

Affinity and Activity Studies

Each of the synthesized compounds were evaluated for affinity at opioid receptors. Several compounds were also evaluated for efficacy at opioid receptors.

Sulfonate Analogues

Replacement of the C-2 acetoxy of **1** with a 2-bromobenzenesulfonate resulted in analogue **80**. Previously, substitution at the 4-position on the phenyl ring of **55**(**56**) seemed to bestow affinity for the μ opioid receptor.[26] Compounds **80** - **83** were synthesized to examine if substitution at another position would be tolerated for opioid receptors and whether this change would alter selectivity. Sulfonate **80** had no affinity at any of the opioid receptors, which indicates that the 2-position of the benzene sulfonates may not be amenable to substitution (Table 4). Further investigation into the effects of substitution at this position will help determine if this is indeed the case. Furthermore, **80** also differs from its ester counterpart, **20**. The ester analogue **20** had a K_i of 110 nM at μ opioid receptors while having 45-fold less affinity at κ than **1** (K_i = 90 nM vs. K_i = 1.9 nM).[28] The effects of different substituents at the 4-position of the benzene sulfonate were then examined. Placing the bromo group at the 4-position of the benzene sulfonate (**83**) allowed us to explore the role of size and electronics at this position. Compound **83** was found to have a 24-fold increase in affinity at μ opioid receptors compared to **80**. This is similar to the trend seen with **56** as substitution at the 4-position of the benzene ring with a methyl group increased affinity for μ opioid receptors 45-fold over **55**. Compound **83** was also found to have approximately 3-fold higher affinity for κ opioid receptors than **55** (**83** K_i = 22 nM vs. **55** K_i = 60 nM) however **83** had affinity at κ opioid receptors 10-fold lower than **1** (**83** K_i = 22 nM vs. 1 K_i = 1.9 nM). This relatively high binding affinity demonstrates that size/bulk is somewhat tolerated at this position. When compared to its ester counterpart **26**, **83** had 40-fold less affinity for μ opioid receptors (**83** K_i = 410 vs. **26** K_i = 10 nM).

Further exploration at the 4-position of **55** led to examining the effects of a strong electron-withdrawing group. Placement of the 4-nitro group in the benzene ring resulted in sulfonate **82** and this modification resulted in no affinity at μ or δ opioid receptors. When compared to **55** and **56**, **82** was found to have roughly 9-fold less affinity for κ opioid receptors as well, indicating that electron withdrawing groups are not favorable at the 4-position of the benzene sulfonates as they decrease affinity at opioid receptors. The ester counterpart of **82**, **25** also had no affinity at δ opioid receptors but did display some affinity at μ opioid receptors (**25** K_i = 260 nM). Compounds, **82** and **25** had decreased affinity at κ opioid receptors in comparison to **1** with **82** having slightly more affinity than its ester derivative (**82** K_i = 430 nM vs. **25** K_i = 570 nM).

With both size (bromo) and electron withdrawing (nitro) effects explored at the 4-position, it was decided to examine the effects of an electron donating group (methoxy) at this position. Along with the electron donating properties of the methoxy group, the oxygen could potentially contribute to hydrogen bonding interactions, which may be favorable for affinity/activity at opioid receptors. Sulfonate **81** possessed a methoxy group at the 4-position of the benzene sulfonate and this compound was found to have affinity at κ, μ and δ opioid receptors with preference for κ; a phenomenon that was also seen in **56**. Furthermore, **81** was found to have similar affinity at κ opioid receptors as **55** and **56** (**81** K_i = 76 nM vs. **55** K_i = 60 nM vs. **56** K_i =50 nM. This demonstrates that the 4-position of the benzene sulfonates is amenable to groups that can donate electrons into the ring as both the 4-methyl (**56**) and 4-methoxy (**81**) derivatives have affinity at opioid receptors with a preference for κ receptors. The ester counterpart of **81**, **24** also had affinity at all three opioid receptors however; it was preferential towards μ as sulfonate

81 had 26-fold less affinity for μ than ester **24** (**24** K_i = 70 nM vs. **81** K_i = 1,800 nM).

Overall, substitution at the 4-position of the benzene sulfonate analogues seems to be tolerated as these compounds all displayed affinity for opioid receptors. While the presence of a nitro group at this position decreases affinity for κ opioid receptors in comparison to other analogues, it does enhance selectivity. An electron donating group at this position is tolerated at receptors and it also bestows affinity for μ opioid receptors as this was seen with the 4-methyl (**56**) and 4-methoxy (**81**) analogues, additionally, larger sized groups seemed to be tolerated at this position as was seen with the 4-bromo (**83**). Interestingly, the ester counterparts of **81**-**83** are opposite in this regard as they favor μ over κ opioid receptors which provides additional evidence that the substituent at C-2 influences how the molecule interacts at opioid receptors.

Table 4: [^{125}I]-IOXY binding affinity of sulfonate analogues and ester counterparts.

Compound	μ K_i ± SD (nM)	δ K_i ± SD (nM)	κ K_i ± SD (nM)	μ/κ	δ/κ
1	*	**5,800 ± 980**	**1.9 ± 0.1**	**ND**[a]	**3,052**
20	**110 ± 1**	**> 10,000**	**90 ± 10**	**1**	**> 111**
24	**70 ± 4**	**1,860 ± 140**	**540 ± 40**	**0.13**	**3**
25	**260 ± 210**	**> 10,000**	**570 ± 40**	**0.5**	**18**
26	**10 ± 1**	**1,410 ± 80**	**740 ± 40**	**0.01**	**1.9**
55	**> 10,000**	**> 10,000**	**60 ± 6**	**> 167**	**> 167**
56	**220 ± 20**	**3,720 ± 400**	**50 ± 5**	**4**	**74**
80	**> 10,000**	**> 10,000**	**> 10,000**	**> 1**	**> 1**
81	**1,800 ± 150**	*	**76 ± 4**	**24**	**ND**[a]
82	**> 10,000**	**> 10,000**	**430 ± 50**	**> 23**	**> 23**
83	**410 ± 40**	*	**22 ± 2**	**19**	**ND**[a]

Note: * Denotes partial inhibitor.
[a] Value not determined.
[b][^{3}H] was used as radioligand where ^{3}H-DAMGO was used for μ, ^{3}H-DADLE for δ and ^{3}H-U69,593 for κ opioid receptors.

Altered Furan Ring Analogues

The furan ring was mono-brominated at C-16 (**84**) and di-brominated at the C-16 and C-15 positions (**85**). Bromination at the C-16 was well tolerated as **84** had similar affinity as **1** at κ (K_i = 3 nM vs. K_i = 1.9 nM) (Table 5). Compound **84** also had similar selectivity for κ opioid receptors as **1**. Di-bromination of the furan ring was similarly well tolerated as this compound (**85**) had almost equal affinity and selectivity for κ opioid receptors as **1** (K_i = 2 nM vs. K_i = 1.9 nM).[346]

Investigation into the SAR of the salvinicins A and B (**66**,**67**), led to the synthesis of **78**. This compound lacked the hydroxyl at C-13 and C-14 seen in **66** and **67** and

instead had an alkene in this position. Also, due to the relative ease of synthesis, **78**, along with its ethyl (**88**) and acetyl (**87**) counterparts were initially evaluated as a mixture of stereoisomers. Compound **78** had no appreciable affinity at μ or δ opioid receptors but was found to have similar affinity as **66** at κ opioid receptors (**78** K_i = 440 nM vs. **66** K_i = 390 nM) (Table 3). Extension to the diethoxy compound **88**, diminished affinity at κ opioid receptors (K_i = 3,360 nM) and this trend was also seen with the diacetoxy compound **87** (K_i = 1,750 nM). Further investigation into how stereochemistry at C-15 and C-16 should also be conducted as the separation of isomers may yield valuable information on how stereochemistry influences biological activity. The observation that **78** had similar affinity as **66** indicates that the hydroxyl groups found at C-13 and C-14 in this compound were not needed for affinity at κ opioid receptors. This initial hypothesis was further investigated by the reduction of the alkene found in **78** to give **86**. Reduction of the alkene increased affinity for κ opioid receptors 11-fold over **78** (**86** K_i = 40 nM vs. **78** K_i = 440 nM). This modification also resulted in a 2-fold increase in affinity at μ opioid receptors in comparison to **78** (K_i = 4,810 nM vs. > 10,000 nM). Similarly, reduction of the C-13, C-14 alkene in compound **88**(**89**) increased affinity at κ opioid receptors 6-fold (**89** K_i = 550 nM vs. **88** K_i = 3,360 nM). This observation provides additional evidence that hydroxy functionalities at the C-13 and C-14 position are not required for affinity at opioid receptors. Future studies examining the influence of C-13 stereochemistry should yield information on how stereochemistry also contributes to affinity/activity at opioid receptors. In addition to exploring the need for the C-13 and C-14 hydroxyls, compound **86** and **89** investigated the effects of substitution at the C-15 and C-16 positions of the tetrahydrofuran ring found in compound **77**. Substitution at

these positions is somewhat tolerated as **86** has diminished but still appreciable affinity for κ opioid receptors compared to **77** (K_i = 40 nM vs. K_i = 14 nM). Extension of the alkoxy group decreases affinity at opioid receptors. This observation shows that some alteration may be tolerated at these positions but size along with potential hydrophobic interactions of substituents may factor into opioid binding and activity.

Table 5: ^{3}H binding affinity of altered furan analogues[a]

Compound	μ K_i ± SD (nM)	δ K_i ± SD (nM)	κ K_i ± SD (nM)	μ/κ	δ/κ
1[b]	*	**5,800 ± 980**	**1.9 ± 0.1**	**ND[c]**	**3,052**
78[b]	**> 10,000**	**> 10,000**	**440 ± 30**	**> 23**	**> 23**
84[b]	**1,450 ± 60**	**7,620 ± 180**	**3.0 ± 0.2**	**483**	**2,540**
85[b]	**970 ± 70**	**5,270 ± 250**	**2.0 ± 0.1**	**485**	**2,635**
86[b]	**4,810 ± 420**	**> 10,000**	**40 ± 1**	**120**	**> 250**
87	**> 10,000**	**> 10,000**	**1,750 ± 190**	**> 6**	**> 6**
88	**> 2,500**	**> 10,000**	**3,360 ± 680**	**>.75**	**> 3**
89	**> 2,500**	**> 10,000**	**590 ± 50**	**> 4**	**> 17**

Note: [a][^{3}H] was used as radioligand where ^{3}H-DAMGO was used for μ, ^{3}H-DADLE for δ and ^{3}H-U69,593 for κ opioid receptors.

[b][^{125}I]-IOXY binding study conducted.

[c] Value not determined.

* Denotes partial inhibitor

Prompted by the similar affinity for κ opioid receptors as **1**, compound **84** was evaluated in the [^{35}S]-GTP-γ-S assay in order to determine efficacy (Table 6).

Table 6: [^{35}S]-GTP-γ-S activity assay of altered furan ring analogue.

Compound	κ EC_{50} ± SD, nM	κ E_{Max}[a] ± SD, nM
1	**40 ± 10**	**120 ± 2**
84	**50 ± 10**	**104 ± 4**

Note: [a]E_{max} is % at which compound stimulates in comparison to (-)-U50,488 (500 nM) at κ opioid receptors.

Compound **84** was found to be a full agonist at κ opioid receptors with similar potency of **1**, (EC_{50} = 50 nM vs. EC_{50} = 40 nM) suggesting that substitution may be tolerated at this position for both affinity and efficacy. Furthermore, with the furan ring seemingly tolerable to bromination, a radiolabelled ^{76}Br may be incorporated into the structure thus giving a potential imaging agent for κ opioid receptors.

Furan Ring Replacements

Furan ring replacements were synthesized in the efforts to reduce potential hepatotoxicity that has been associated with furan rings, as well as probe the binding site of salvinorin A and determining if the furan ring does indeed play a role in binding. A series of amides were synthesized for several reasons. Besides an increase in water solubility, it was though that the carbonyl of the amide may interact in similar fashion as the oxygen of the furan ring and the appendage off of the amide may influence affinity and activity at opioid receptors. Initially, the furan ring was replaced with an anilido group as it was hypothesized that the benzene ring could have hydrophobic interactions similar to the furan ring along with hydrogen bonding of the amide group. Unfortunately, this compound had no affinity at opioid receptors. The addition of substituents (methoxy

groups) to the ring was explored in efforts to enhance affinity at opioid receptors. Compounds **90** and **91**, which had a methoxy group at the 2 and 3-position respectively, had no affinity at opioid receptors (Table 7). Placing the methoxy group in the 4-position (**92**) did not enhance affinity for κ opioid receptors yet interestingly, did alter selectivity from κ by bestowing weak binding affinity for μ and δ opioid receptors (μ K_i = 2,490 nM and δ K_i = 3,690 nM).

Further exploration into this phenomenon led to the incorporation of another methoxy group with the thought that it may participate in hydrogen bonding that could potentially enhance affinity. Also, perhaps its electron donating nature may improve the overall electronics of the aromatic ring, which may make it more favorable for binding. Unfortunately, compounds **93** (3,4 dimethoxy), **94** (2,4 dimethoxy) and **95** (2,5 dimethoxy) had no affinity at opioid receptors.

Due to the overall lack of affinity for opioid receptors of the aromatic replacements, non-aromatic groups were incorporated into the structure of **1** through similar amide linkage at C-12. Initially, a cyclohexylamine moiety was incorporated into the structure and was found to have weak affinity at κ opioid receptors (K_i = 1,930 nM) so efforts to increase the affinity were undertaken. To explore the role of size, the cylohexamine ring was contracted and oxygen introduced for potential hydrogen bonding to give morpholine derivative **96**. These changes were tolerated as they did indeed improve binding at κ opioid receptors compared to the cyclohexamine predecessor by 8-fold (**96** K_i = 230 nM vs. K_i = 1,930 nM). Additional investigation into this occurrence needs to be conducted to further determine the role of ring size along with substitution of non-aromatic amide analogues of **1** and how they interact at opioid receptors.

Table 7: ^{3}H binding affinity of furan replacements.[a]

Compound	μ K_i ± SD (nM)	δ K_i ± SD (nM)	κ K_i ± SD (nM)	μ/κ	δ/κ
90	**> 10,000**	**> 10,000**	**> 10,000**	**> 1**	**> 1**
91	**> 10,000**	**> 10,000**	**> 10,000**	**> 1**	**> 1**
92	**2,490 ± 80**	**3,690 ± 330**	**> 10,000**	**> 0.25**	**> 0.4**
93	**> 10,000**	**> 10,000**	**> 10,000**	**> 1**	**> 1**
94	**> 10,000**	**> 10,000**	**> 10,000**	**> 1**	**> 1**
95	**> 10,000**	**> 10,000**	**> 10,000**	**> 1**	**> 1**
96	**> 10,000**	**> 10,000**	**230 ± 20**	**> 43**	**> 43**

Note: [a][^{3}H] was used as radioligand where ^{3}H-DAMGO was used for μ, ^{3}H-DADLE for δ and ^{3}H-U69,593 for κ opioid receptors.

Diels-Alder Cycloadduct Analogues

Cycloadducts may aid in giving insight into the position of the oxygen bond and its ability to participate in hydrogen bonding. Additionally, these compounds would give us some measure of the amount of steric bulk that was tolerated at opioid receptors. Cyclo-adduct **97** had decreased but still appreciable affinity for κ opioid receptors in comparison to **1** (K_i = 60 nM vs. K_i = 1.9 nM) (Table 8). Extension of the esters of the cyclo-adduct from methyl to ethyl (**98**), decreased affinity at κ opioid receptors by 2-fold in comparison to **97** (K_i = 120 nM vs. K_i = 60 nM), however, this change increased affinity for δ opioid receptors nearly 3-fold in comparison to **1** (**98** K_i = 2,260 nM vs. **1** K_i = 5,800 nM). Along with the bridgehead oxygen, it was thought that the carbonyls of the additional esters might be interacting in hydrogen bonding at opioid receptors. Investigation of this hypothesis along with further probing the reactivity of the furan ring in the Diels-Alder reaction, reaction of **1** with the corresponding alkyne, led to one of the

ethyl esters of **98** being replaced with a CF_3 group (**99**). This change caused a 16-fold decrease in affinity at κ opioid receptors in comparison to **98** (K_i = 1,970 nM vs. 120 nM). While these initial results indicate that an additional carbonyl in this position assists in binding, a clear explanation has yet to be determined which is in part due to **99** being tested as one of the possible two regioisomers. A possible explanation for this observance could be that the orientation of the CF_3 group in the dieneophile prefers one conformation to the other, leading to one product formation. Continued investigation into this phenomenon needs to be conducted to further explain this occurrence. Furthermore, synthesis and evaluation of the other possible regioisomer will be necessary to further investigate the impact of the missing carbonyl as well as the position of the CF_3. This will aid in the investigation of our initial hypothesis pertaining to the hydrogen bonding capabilities of the carbonyl esters and their role in binding at opioid receptors.

Cycloadducts **102-103** examined the impact of steric bulk by adding large appendages to the core structure of **1**. These compounds also proved to be fluorescent so along with exploring steric bulk, it was the hope that potential fluorescent analogues of **1** could be used as visualizing agents for opioid receptors. Unfortunately, cycloadduct **102** had no affinity at opioid receptors as it had a K_i of > 13,000 nM at k opioid receptors and negligible affinity at μ and δ opioid receptors. It is important to note that **102** was tested as a mixture of *endo* and *exo* cycloadducts. This may contribute to the overall lack of affinity as one of the isomers may be antagonizing the interaction of the other at opioid receptors, therefore possibly negating affinity. The successful separation of these compounds will aid in proving this hypothesis. Interestingly, the inclusion of a methyl group into the bridgehead of **102**(**103**) increased affinity for κ opioid receptors 44-fold

(K_i = 290 nM vs. K_i = > 13,000 nM). In an effort to provide some explanation in the difference in binding affinities between **102** and **103** besides **102** being tested as a mixture of *exo* and *endo* products, molecular dynamic simulations were conducted to observe the differences between the compounds in an energy minimized state (Figure 29).

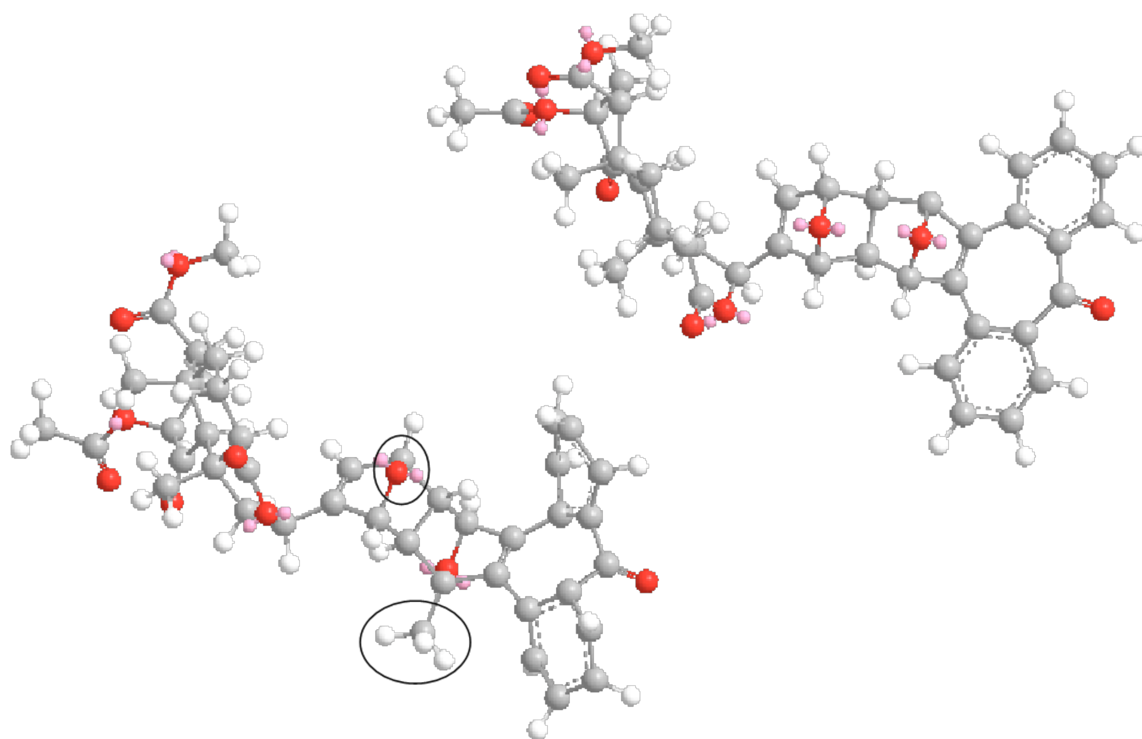


Figure 29: Molecular dynamics model. Note: Top structure (**102**), bottom (**103**). Circled are the methyl group and the oxygen bridge of **103**.

The results from these studies suggest that the methyl group on the bridgehead of **103** (C-43) causes a puckering of the dibenzosuberenone section portion of the molecule, causing the oxygen of the oxygen bridge in **103** to point away from the core of **1** perhaps

allowing it to partake in hydrogen bonding that aids affinity. Additionally, the methyl group seems to be causing the dibenzosuberenone moiety to pucker, and that may aid binding from a hydrophobic interaction standpoint. These subtle differences seem to be allowing **103** to take on a more favorable conformation for binding at κ opioid receptors. While synthetically, compounds **102** and **103** are achieved from **1** with considerable effort and difficulty, further studies to examine the effects of larger alkyl groups in the bridgehead positions of these compounds are necessary to further test this hypothesis. If these compounds retain the fluorescence seen in **102** and **103**, but improve affinity, they may provide valuable visualizing ligands for opioid ligands.

While not as large as **102** and **103**, cycloadduct **104** also explored the role of steric bulk in activity at opioid receptors along with increasing the lipophilic/hydrophobic characteristics of **1** allowing us to investigate how these characteristics factor into activity at opioid receptors. Cycloadduct **104** was found to have decreased affinity at κ opioid receptors (K_i = 790 nM) as compared to **1**. This decreased affinity may be a by-product of **104** not having the ability to form hydrogen bonds like cyclo-adducts **97** and **98**. The installation of substituents that may partake in hydrogen bonding on the benzene ring of **104** may improve affinity at opioid receptors and warrants further investigation.

Table 8: ^{3}H binding affinity of cycloadduct analogues.[a]

Compound	μ K_i ± SD (nM)	δ K_i ± SD (nM)	κ K_i ± SD (nM)	μ/k	δ/k
1	*	**5,800± 980**	**1.9 ± 0.1**	**ND[a]**	**3,046**
97	**> 4,800**	**> 5,000**	**60 ± 10**	**> 80**	**> 83**
98	**> 3,200**	**2,260 ± 280**	**120 ± 10**	**> 27**	**> 19**
99	**1,670 ± 150**	**> 5,000**	**1,970 ± 80**	**0.85**	**> 2**
100	**> 2,700**	**> 5,200**	**>13,000**	**> 0.2**	**> 0.4**
101	**> 1,700**	**> 5,000**	**300 ± 20**	**> 6**	**> 17**
104	**> 2,500**	**> 10,000**	**790 ± 200**	**> 3**	**> 13**

Note: [a][^{3}H] was used as radioligand where ^{3}H-DAMGO was used for μ, ^{3}H-DADLE for δ and ^{3}H-U69,593 for κ opioid receptors.

*Denotes partial inhibitor.

Cycloadducts **97** and **98** were evaluated for efficacy at opioid receptors in the [^{35}S]-GTP-γ-S assay due to their relatively high affinity at κ opioid receptors. Cyclo-adduct **97** was found to be a full agonist at κ opioid receptors compared to the known κ agonist (-)U50,483 (E_{max} = 100 ± 10) (Table 9). Cycloadduct **98** was also found to have high efficacy in this assay (E_{max} % = 90 ± 10), however both compounds were less potent than **1** (**97** ED_{50} = 980 nM vs. **98** ED_{50} = 2,150 vs. **1** ED_{50} = 40 nM). These compounds show that extension of the cyclo-adduct esters has more of an effect on potency than overall efficacy and both compounds further illustrate that the furan ring **1** of is not essential for binding or efficacy at opioid receptors and some steric bulk is tolerated at this position.

Table 9: [^{35}S]GTP-γ-S activity assay of cycloadducts.

Compound	κ EC_{50} ± SD, nM	κ E_{Max}[a] ± SD, nM
1	**40 ± 10**	**120 ± 2**
97	**980 ± 200**	**100 ± 10**
98	**2,150 ± 500**	**90 ± 10**

[a]E_{max} is % at which compound stimulates in comparison to (-)-U50,488 (500 nM) at κ receptors.

Non-Heterocyclic Aromatic Analogues

A series of phenyl ring analogues was synthesized to explore steric bulk and the effects of non-heterocyclic aromatics at the C-12 position. The napthyl derivative, compound **105**, had no affinity at opioid receptors (μ, δ, κ K_i = > 10,000 nM), indicating that the lack of any groups that may interact in hydrogen bonding is detrimental for affinity at opioid receptors. This possible explanation seems to be corroborated by compounds **106** and **107** as they retained some affinity for κ opioid receptors though less than **1** (**106** K_i = 286 nm & **107** K_i = 228 nm vs. 1 κ K_i = 1.9 nM).

Table 10: Preliminary ^{3}H binding affinity of non-heterocyclic aromatic analogues[a]

Compound	μ K_i ± SD (nM)	δ K_i ± SD (nM)	κ K_i ± SD (nM)	μ/k	δ/k
105	**> 3,000**	**> 5,000**	**> 8,000**	**> 0.4**	**> 0.6**
106	**> 3,000**	**> 5,000**	**286 ± 19**	**> 8**	**> 17**
107	**> 3,000**	**> 5,000**	**228 ± 12**	**> 13**	**> 22**

Note: [a][^{3}H] was used as radioligand where ^{3}H-DAMGO was used for μ, ^{3}H-DADLE for δ and ^{3}H-U69,593 for κ opioid receptors.

A series of salvinorin A analogues was synthesized and evaluated for affinity and efficacy at opioid receptors. These analogues sought to probe the role of substitution on benzene sulfonate analogues of salvinorin A. Furthermore, these analogues also explored the SAR at the C-2 position of salvinorin A. Analogues of the furan ring sought to further probe and explore its influence in opioid activity. The results of these experiments have granted the ability to form certain conclusions about salvinorin A itself.

CHAPTER V. CONCLUSIONS

Probing the chemical reactivity and SAR of natural products has aided our understanding on how these unique compounds interact biologically. In the same vein, this study was conducted to further probe the chemical reactivity and SAR of the neoclerodane diterpene, salvinorin A. A series of analogues was created and several of these compounds were found to have affinity and efficacy at opioid receptors despite fundamental structural differences to salvinorin A (see figure 30 for a brief summary of salvinorin A SAR derived from this study).

Substitution on the phenyl ring of the C-2 benzene sulfonate salvinorin A analogues was investigated through a series of designed and synthesized analogues. These compounds not only provided insight into the role of ring substitution but into the role of the C-2 substituent as well. A series of modified furan rings and furan ring replacements was designed and synthesized to further probe the role of the furan ring in activity and in effort to circumvent potential hepatotoxicity that has been associated with furan ring containing compounds. Exploration of the chemical reactivity of the furan ring established that under suitable conditions, it may act as a diene and partake in Diels-Alder reactions. This led to the synthesis of a series of cycloadducts that were further able to probe salvinorin A SAR by investigating the impact of steric bulk at this position. Additionally, these Diels-Alder cycloadducts provided a means for the synthesis of a series of C-12 phenyl compound that was used to probe the need for heterocyclic moieties at this position. To our knowledge, these compounds mark the first conversion of a furan ring-containing neoclerodane to a phenyl ring analogue, which may have synthetic utility down the line, along with being the first salvinorin A analogues to have non-heterocyclic aromatic rings directly attached to its core.

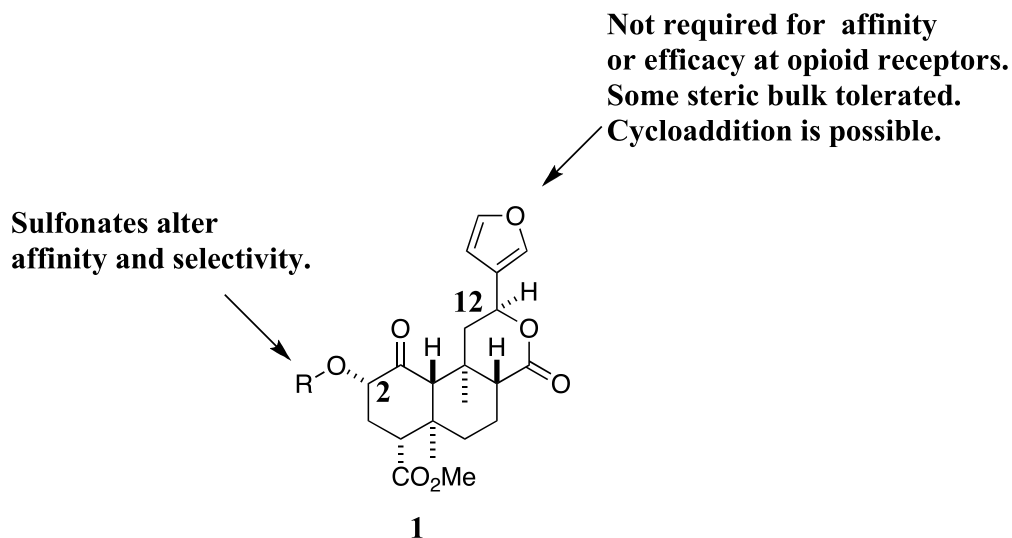


Figure 30: Synopsis of salvinorin A SAR established from study.

The series of substituted benzenesulfonate analogues that was synthesized demonstrated that electronic effects influence affinity and selectivity at opioid receptors, specifically κ opioid receptors. Furthermore, when compared to their ester counterparts, the sulfonates showed marked differences in their affinity and selectivity for opioid receptors as the previously synthesized ester analogues altered selectivity from κ to μ opioid receptors, while the benzenesulfonates remained selective for κ receptors. Furthermore, unlike their carboxylate counterparts, substitution at the 2-position of the aromatic ring was not tolerated, as all affinity was lost. These results indicate that the C-2 sulfonate analogues are interacting at opioid receptors in a different manner than their ester derivatives and demonstrates that the C-2 substituent can have a marked impact on the pharmacological profile of salvinorin A.

Investigation of the furan ring led to the synthesis of a variety of modified furan rings and furan ring replacements. This work showed that the furan ring could tolerate

substitution at the C-15 and C-16 position as brominated analogues were not only found to have high affinity at κ opioid receptors but one of these analogues (**84**) was found to be fully efficacious at κ opioid receptors with comparable potency to salvinorin A. Additionally, steric bulk seems to be tolerated at this position as bicyclic cycloadducts were found to have affinity for κ opioid receptors and several were found to be full agonist at κ opioid receptors (**97**, **98**), which indicates that in addition to hydrogen bonding, hydrophobic interactions may influence how salvinorin A interacts at opioid receptors. Furthermore, several analogues that did not contain a furan ring still retained affinity for opioid receptors and demonstrated some selectivity for κ opioid receptors (**78**, **86**, **96**). Overall, the results obtained from these compounds demonstrate that the furan ring is not required for affinity or efficacy at opioid receptors. This is significant as the absence of a furan ring may decrease the potential for toxicity in salvinorin A, enhancing its potential ability to be a lead molecule for therapeutic development.

While this study of salvinorin A has generated additional information about its interactions at opioid receptors, the complete pharmacophore remains unknown. Therefore, further investigation needs to be conducted in order to fully elucidate how salvinorin A interacts at opioid receptors. The information inferred from these studies will facilitate the development of novel opioid probes derived from salvinorin A, which may themselves lead to the development of new and improved opioid therapeutics.

CHAPTER VI. EXPERIMENTAL

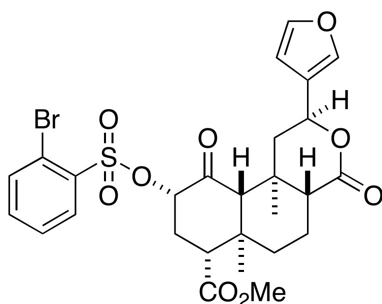
Unless otherwise indicated, all reagents were purchased from commercial suppliers and were used without further purification. Melting points were determined on a Thomas-Hoover capillary melting apparatus. NMR spectra were recorded on either a Bruker Advance-300 spectrometer, Bruker DRX-400 with qnp probe or a Bruker AV-500 with cryoprobe using δ values in ppm (TMS as internal standard) and *J* (Hz) assignments of ^{1}H resonance coupling. High resolution mass spectrometry data was collected on either a LCT Premier (Waters Corp., Milford, MA) time of flight mass spectrometer or an Agilent 6890 N gas chromatograph in conjunction with a quarto Micro GC mass spectrometer (Micromass Ltd, Manchester UK). Thin-layer chromatography (TLC) was performed on 0.25 mm plates Analtech GHLF silica gel plates using ethyl acetate/*n*-hexanes, in 1:1 ratio as the solvent system unless otherwise noted. Spots on TLC were visualized by uv (254 or 365 nm), phosphomolybdic acid in CH_3CH_2OH or vanillin/H_2SO_4 in CH_3CH_2OH. Column chromatography was performed with Silica Gel (32 – 63 μ particle size) from Bodman Industries (Atlanta, GA). Analytical HPLC was carried out on an Agilent 1100 Series Capillary HPLC system with diode array detection at 254.8 nm on an Agilent Eclipse XDB-C18 column (4.6 × 150 mm, 5 mm) with isocratic elution in 60% CH_3CN/40% H_2O) at a flow rate of 5.0 mL/min unless otherwise noted. The systematic name for salvinorin A (**1**) is **(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate**.

<u>Isolation of Salvinorin A from Plant Material</u>

Dried *Salvia divinorum* leaves (5 kg) (Ethnogens.com) were ground into a fine powder in an industrial blender. The powder was placed into a 20 L glass column and steeped with 13 L of xylenes at room temperature overnight. The leaves were filtered

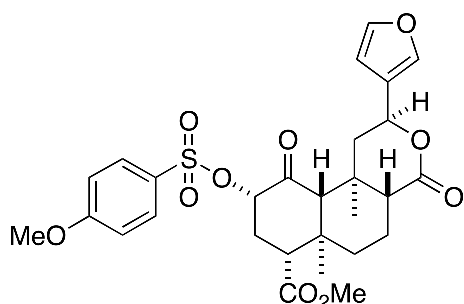
and the xylene run-off was collected and removed under reduced pressure to produce a thick green tar. This process was repeated two additional times. The resulting green tar was dissolved in CH_3OH and this mixture was placed in a 0 °C freezer for 24 hours. The resulting precipitate was collected by filtration and recrystallized from a mixture of ethyl acetate/hexanes to afford 25 g of salvinorin A (**1**) (0.5%) as a light green powder with a melting point of 238-240 °C (lit.[301, 305] 240-242 °C).t_R = 5.847 min; purity = 96.52%.

Synthesis

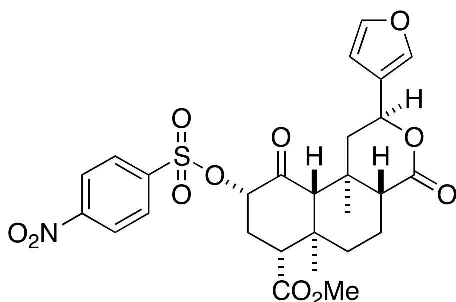


(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-(2-bromophenylsulfonyloxy)-2-(furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (80). A solution of **2**[357] (100 mg, 0.2561 mmol), 2-bromobenzyl sulfonate (65 mg, 0.2561), NEt_3 (26 mg, 0.2561), and a catalytic amount of DMAP in CH_2Cl_2 was stirred at room temperature overnight. Upon completion, the solution was washed with 2N HCl (3 × 20 mL), saturated aqueous $NaHCO_3$ (3 × 20 mL) and a solution of NaCl (3 × 20 mL) and dried (Na_2SO_4). Removal of the solvent under reduced pressure. The compound was purified by column chromatography (eluent: ethyl acetate/*n*-hexanes, 2:3) to afford **80** as a white solid, mp 162 – 163 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 – 8.15 (m, 1H), 7.81 – 7.78 (m, 1H), 7.53 – 7.47 (m, 2H), 7.45 – 7.43 (m, 2H), 6.41 (s, 1H), 5.55 (dd, *J* = 5.1, 11.6, 1H), 5.21 (dd, *J* = 7.6, 12.3, 1H), 3.74 (s, 3H), 2.74 (d, *J* = 3.5, 1H), 2.58 – 2.37 (m, 3H), 2.19

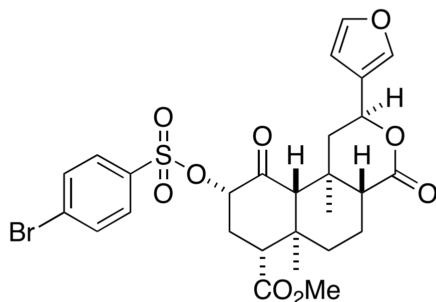
(d, J = 10.8, 2H), 2.08 (d, J = 10.0, 1H), 1.81 (d, J = 13.0, 1H), 1.72 – 1.59 (m, 2H), 1.54 (s, 1H), 1.47 (s, 3H), 1.13 (s, 3H). ^{13}C NMR δ 198.60, 169.61, 169.55, 142.40, 138.06, 135.48, 134.19, 133.40, 130.50, 126.36, 123.79, 119.23, 106.98, 80.28, 70.62, 62.86, 52.11, 50.75, 50.00, 41.91, 40.62, 36.70, 34.15, 30.39, 16.70, 15.09, 13.73. Elemental analysis calculated for $C_{27}H_{29}O_9BrS$•0.25 H_2O; C 52.82%, H 4.84%,O 24.10%; observed: C 52.73%, H 4.84, O 23.26.



(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 2-(furan-3-yl)-9-(4-methoxyphenylsulfonyloxy)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (81). Compound **81** was synthesized as described for **80** from **2** using 4-methoxybenzenesulfonyl chloride to afford 69 mg of **81** (48%) as a white solid, mp 163 – 165 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 – 7.85 (m, 2H), 7.42 (d, J = 1.5, 2H), 7.02 – 6.95 (m, 2H), 6.38 (t, J = 1.4, 1H), 5.52 (dd, J = 5.1, 11.7, 1H), 4.94 (dd, J = 7.8, 12.2, 1H), 3.85 (s, 3H), 3.71 (s, 3H), 2.69 (d, J = 3.7, 1H), 2.44 – 2.37 (m, 2H), 2.35 – 2.29 (m, 1H), 2.17 (d, J = 3.5, 1H), 2.14 (d, J = 3.2, 1H), 2.07 (s, 1H), 2.04 (dd, J = 3.1, 11.7, 1H), 1.78 (d, J = 13.2, 1H), 1.51 (dd, J = 8.8, 21.2, 2H), 1.43 (s, 3H), 1.08 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.18, 169.18, 169.09, 162.13, 141.94, 137.60, 128.35, 125.90, 125.45, 123.32, 112.52, 106.52, 77.58, 70.06, 62.47, 53.84, 51.65, 50.24, 49.47, 41.35, 40.14, 36.20, 33.64, 30.41, 18.17, 16.22, 14.56, 13.27. Elemental analysis calculated for $C_{28}H_{32}O_{10}S$• 0.1 H_2O; C 59.97%, H 5.79%,O 28.53%; observed: C 59.48%, H 5.79, O 28.97.

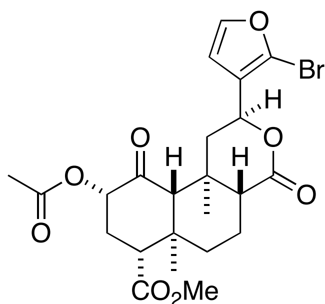


(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 2-(furan-3-yl)-6a,10b-dimethyl-9-(4-nitrophenylsulfonyloxy)-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (82). Compound **82** was synthesized as described for **80** from **2** using 4-nitro benzene sulfonyl chloride to afford 37 mg of **82** (25%) as a white powder, mp 168 – 170 °C (dec.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (d, J = 8.7, 2H), 8.19 (d, J = 8.6, 2H), 7.45 (s, 2H), 6.40 (s, 1H), 5.55 (dd, J = 5.0, 11.6, 1H), 5.12 (dd, J = 7.9, 12.0, 1H), 3.74 (s, 3H), 2.80 – 2.70 (m, 1H), 2.44 (dd, J = 8.3, 20.7, 3H), 2.30 - 2.16 (m, 1H), 2.08 (d, J = 10.1, 1H), 1.81 (d, J = 12.9, 1H), 1.66 (d, J = 14.2, 1H), 1.60 (s, 1H), 1.54 (m, 2H), 1.45 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.45, 170.40, 170.36, 150.41, 143.50, 141.97, 139.12, 128.84, 124.67 (2C), 123.95, 107.86, 99.59, 80.46, 71.44, 63.94, 52.96, 51.80, 50.88, 42.93, 41.58, 35.15, 31.71, 29.56, 17.64, 16.07, 14.73. HRESIMS (*m/z*): [M+Na] calculated for $C_{27}H_{29}NO_{11}SNa$ 598.1359; found 598.1328. HPLC t_R = 6.070 min; purity = 95.69 %.



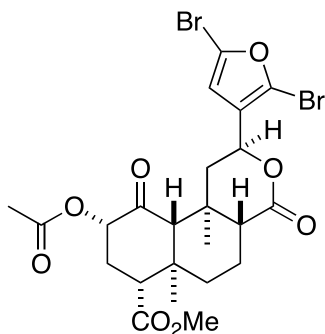
(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-(4-bromophenylsulfonyloxy)-2-(furan-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (83). Compound **83** was synthesized as

described for **80** from **2** using 4-bromo benzene sulfonyl chloride to afford 44 mg **83** (28%) as a white solid, mp 165 – 166 °C (dec.); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, *J* = 8.8, 2H), 7.69 (d, *J* = 8.8, 2H), 7.45 – 7.41 (m, 2H), 6.38 (s, 1H), 5.52 (s, 1H), 4.98 (s, 2H), 3.72 (s, 3H), 2.70 (s, 2H), 2.41 (d, *J* = 13.3, 3H), 2.20 – 2.13 (m, 2H), 2.05 (s, 1H), 1.78 (s, 2H), 1.43 (s, 3H), 1.09 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.82, 170.90, 170.84, 143.84, 139.48, 135.56 (2C), 132.54 (2C), 129.37, 129.35, 125.11, 108.31, 80.04, 71.90, 64.38, 53.45, 52.15, 51.32, 43.31, 41.99, 38.05, 35.52, 32.18, 18.05, 16.43, 15.13. Elemental analysis calculated for $C_{27}H_{29}O_9BrS{\bullet}0.05\ H_2O$; C 53.12%, H 4.95%, O 23.59%; observed: C 53.11%, H 4.94, O 23.41.

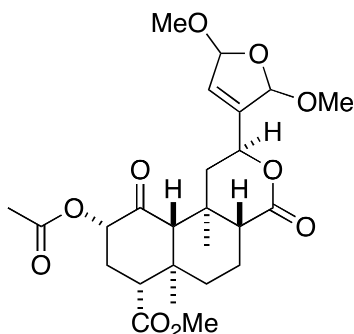


(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(2-bromofuran-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (84). A solution of **1** (100 mg, 0.2312 mmol), NBS (46 mg, 0.2584 mmol), and CH_2Cl_2 (30 mL) was stirred at room temperature while exposed to sunlight. Upon completion, solvent was removed under reduced pressure. The compound was purified by column chromatography (eluent: ethyl acetate/*n*-hexanes, 2:3) to afford 26 mg of **84** (22%) as a white powder, mp 171 – 174 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 2.4, 1H), 6.40 (d, *J* =2.1, 1H), 5.45 (dd, *J* = 5.4, 12.0, 1H), 5.14 (dd, *J* = 9.6, 10.5, 1H), 5.14 (dd, *J* = 9.6, 10.5, 1H), 3.75 (s, 3H), 2.78 (dd, *J* = 8.4, 8.4, 1H), 2.31 (m, 3H), 2.18 (m, 2H), 2.17 (s, 3H), 1.82 (dd, *J* = 3.5, 10.6, 1H), 1.62 (m, 3H), 1.49 (s, 3H), 1.14 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.80,

171.51, 170.94, 169.91, 144.62, 122.70, 121.31, 110.75, 75.00, 71.90, 63.90, 53.62, 52.00, 51.60, 42.80, 42.20, 38.20, 35.51, 30.70, 20.50, 18.10, 16.40, 15.01. HRESIMS (*m*/*z*): [M+H] calculated for $C_{23}H_{28}O_8Br$, 511.0947; found, 511.0968. HPLC t_R = 11.39 min; purity = 96.52 %.

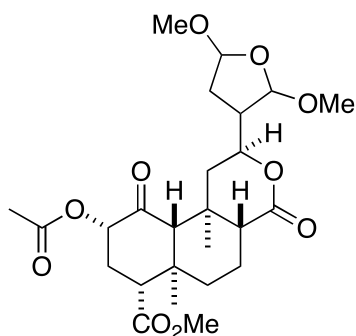


(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(2,5-dibromofuran-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (85). Compound **85** was synthesized as described for **84** from **1** to afford 66 mg of **85** (48%) as white powder, mp 130-133 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.35 (s, 1H), 5.38 (dd, *J* = 5.1, 12.1, 1H), 5.14 – 5.09 (m, 1H), 3.74 (s, 3H), 2.77 – 2.73 (m, 1H), 2.39 (dd, *J* = 5.2, 13.6, 1H), 2.30 (dd, *J* = 7.8, 13.5, 2H), 2.17 (s, 3H), 2.08 (d, *J* = 11.0, 6.0, 2H), 1.81 (d, *J* = 13.0, 7.1, 1H), 1.65 (d, *J* = 14.2, 8.0, 2H), 1.53 (d, *J* = 3.0, 12.8, 2H), 1.46 (s, 3H), 1.12 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.49, 169.07, 168.19, 167.56, 123.44, 120.62, 118.50, 110.11, 72.59, 69.10, 61.43, 51.16, 49.65, 49.16, 40.25, 39.69, 35.69, 33.07, 28.27, 18.17, 15.66, 14.00, 12.55. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{23}H_{26}O_8Br_2Na$, 610.9892; found, 610.9883; HPLC t_R = 12.86 min; purity = 96.29%.



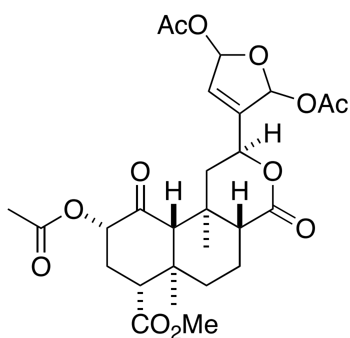
(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(2,5-dimethoxy-2,5-dihydrofuran-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (78). A solution of **1** (200 mg, 0.4625 mmol), Br_2 (74 mg, 0.4625 mmol), CH_2Cl_2 (30 mL) and CH_3OH (6 mL) was stirred at -30 °C temperature for 2 hours. Upon completion, the solution was washed with $NaHCO_3$ (3 × 30 mL) and saturated NaCl (3 × 30 mL) and dried with Na_2SO_4. Solvent was removed under reduced pressure. Compound was purified by column chromatography (eluent: ethyl acetate/*n*-hexanes, 1:1) to afford 137 mg of **78** (60%) as a white solid, mp 135 – 140 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.94 – 5.74 (m, 2H), 5.63 – 5.55 (m, 1H), 5.21 – 5.08 (m, 2H), 3.73 (s, 3H), 3.41 (ddd, *J* = 6.7, 7.8, 9.4, 5H), 2.78 – 2.69 (m, 1H), 2.52 – 2.42 (m, 1H), 2.34 – 2.24 (m, 2H), 2.17 (d, *J* = 5.9, 4H), 2.07 – 1.98 (m, 1H), 1.78 (d, *J* = 12.8, 1H), 1.69 – 1.48 (m, 4H), 1.40 (s, 3H), 1.26 (d, *J* = 7.0, 1H), 1.10 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.98, 199.95, 199.90, 169.55, 168.88, 168.69, 168.60, 167.93, 167.87, 167.85, 142.48, 142.00, 141.83, 141.78, 125.26, 124.54, 123.41, 123.27, 106.23, 105.96, 105.24, 104.93, 104.81, 104.24, 104.19, 74.78, 72.99, 72.95, 72.90, 71.48, 71.11, 69.99, 69.92, 62.07, 62.03, 61.90, 53.30, 53.17, 53.13, 52.63, 52.48, 52.38, 52.37, 52.03, 51.57, 51.55, 51.50, 50.00, 49.29, 49.25, 49.05, 40.05, 39.98, 39.58, 38.74, 38.40, 36.10, 36.09, 36.05, 33.38, 33.28, 33.25, 28.77, 27.70, 27.06, 20.66, 18.59, 16.12,

16.06, 14.36, 13.30, 13.26, 12.91, 12.88, 12.13. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{25}H_{34}O_{10}Na$, 517.2050; found, 517.2042.



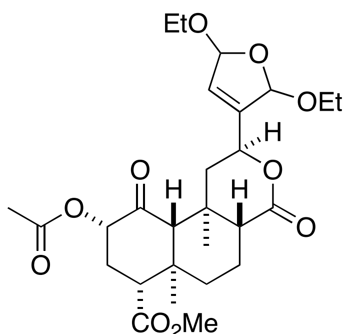
(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(2,5-dimethoxytetrahydrofuran-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (86). A solution of **78** (150 mg, 0.3033 mmol), 5% Rh/C (catalytic), and CH_3OH (30 mL) was stirred at room temperature under a hydrogen atmosphere for 3 hours. Upon completion, solution was filtered through a pad of celite. Solvent was removed under reduced pressure and a mixture of ethyl acetate and hexanes was added to the residue. The resulting solid was collected by vacuum filtration and dried to yield 136 mg of **86** (90%) as a white powder, mp 122-125 °C (dec.); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.42 (s, 1H), 5.19 (s, 2H), 5.13 – 5.02 (m, 1H), 4.87 (d, *J* = 20.1, 1H), 3.82 (d, *J* = 18.5, 1H), 3.73 (s, 1H), 3.72 (s, 3H), 3.71 (m, 2H), 3.31 (t, *J* = 5.0, 1H), 2.29 (s, 3H), 2.18 (s, 3H), 2.16 (d, *J* = 4.2, 3H), 1.77 (s, 2H), 1.62 (s, 2H), 1.43 (dd, *J* = 12.5, 21.9, 4H), 1.26 (t, *J* = 7.1, 3H), 1.10 (d, *J* = 2.7, 3H). ^{13}C NMR (126, MHz, $CDCl_3$) δ 200.67, 200.34, 170.l7, 169.64, 169.61, 168.28, 167.94, 167.92, 163.60, 145.74, 145.01, 135.37, 135.23, 130.99, 97.35, 91.14, 75.87, 75.30, 75.05, 74.80, 72.98, 69.83, 69.05, 61.96, 51.51, 50.02, 40.13, 40.07, 39.97, 39.78, 36.15, 36.07, 35.98, 32.69, 29.62, 28.95, 28.78, 27.08, 20.68, 19.41, 18.73, 18.63, 16.12, 14.33,

14.15, 13.97, 13.04, 12.95, 12.90, 12.16. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{25}H_{36}O_{10}Na$, 519.2206; found, 519.2238.



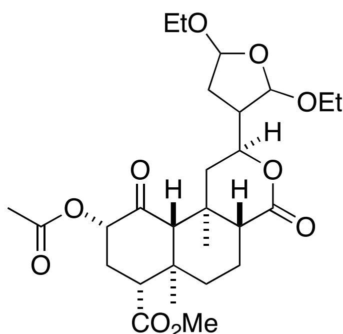
3-((2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-acetoxy-7-(methoxycarbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromen-2-yl)-2,5-dihydrofuran-2,5-diyl diacetate (87). A solution of **1** (200 mg, 0.4625 mmol), Br_2 (74 mg, 0.4625 mmol), NaOAc (470 mg, 5.780 mmol), CH_3CO_2H (175 mg, 2.310 mmol) and CH_2Cl_2 (40 mL) was stirred at -30 °C temperature for 2 hours. Upon completion, the solution was washed with 2N HCl (3 30 mL), $NaHCO_3$ (3 × 30 mL) and saturated NaCl (3 × 30 mL) and dried with Na_2SO_4. The solvent was removed under reduced pressure and the crude residue was purified by column chromatography (eluent: ethyl acetate/*n*-hexanes, 1:1) to afford **87** as a white solid, mp 112- 116 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.17 – 6.07 (m, 1H), 5.27 – 5.08 (m, 2H), 3.73 (d, *J* = 1.9, 3H), 2.75 (dd, *J* = 7.8, 13.9, 1H), 2.55 – 2.36 (m, 1H), 2.35 – 2.22 (m, 2H), 2.21 – 1.92 (m, 11H), 1.83 – 1.74 (m, 1H), 1.58 (dt, *J* = 8.6, 17.9, 3H), 1.48 – 1.42 (m, 1H), 1.40 (s, 3H), 1.32 – 1.17 (m, 2H), 1.10 (d, *J* = 2.5, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.42, 200.75, 200.69, 200.58, 170.49, 170.25, 170.23, 168.99, 168.96, 168.92, 168.71, 168.63, 168.54, 168.47, 168.39, 168.35, 145.29, 142.31, 142.20, 136.79, 128.07, 126.27, 125.67, 124.68, 124.58, 104.45, 99.65, 99.56, 98.52, 98.43, 98.40, 98.10, 97.08, 96.93, 73.85, 73.77, 73.70, 73.63, 71.16, 70.92, 70.83, 70.65, 70.54,

68.94, 62.70, 62.55, 62.53, 62.44, 58.36, 52.29, 52.18, 51.75, 50.78, 50.74, 50.67, 50.18, 50.09, 49.93, 49.87, 49.42, 47.88, 41.03, 40.84, 40.79, 40.76, 40.49, 39.67, 39.54, 38.87, 38.63, 38.49, 36.79, 36.10, 34.21, 34.02, 34.00, 29.68, 29.51, 29.40, 19.97, 19.85, 19.83, 19.76, 19.72, 19.51, 19.36, 19.33, 19.30, 19.18, 17.99, 17.97, 16.89, 16.86, 16.79, 15.17, 15.15, 15.03, 14.69, 14.66, 14.10, 13.82, 13.80, 13.76, 13.67, 13.62, 12.95, 12.89. HRESIMS (*m/z*): [M+Na] calculated for $C_{27}H_{34}O_{12}Na$, 573.1948; found, 573.2004.



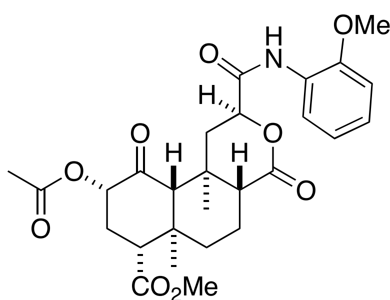
(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(2,5-diethoxy-2,5-dihydrofuran-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (88). A solution of **1** (200 mg, 0.4625 mmol), Br_2 (74 mg, 0.4625), CH_2Cl_2 (30 mL) and CH_3CH_2OH (6 mL) was stirred at -30 °C temperature for 2 hours. Upon completion, the solution was washed with $NaHCO_3$ (3 x 30 mL) and saturated NaCl (3 x 30 mL) and dried with Na_2SO_4. Solvent was removed under reduced pressure and the resulting residue was purified by column chromatography (eluent: ethyl acetate/*n*-hexanes, 1:1) to afford **88** as a white powder, mp 87 – 89 °C; ^{1}H NMR (500 NMR, $CDCl_3$) δ 5.93, (s, 1H), 5.90 (s, 1H), 5.66 – 5.62 (m, 1H), 5.19 – 5.09 (m, 2H), 3.83 – 3.74 (m, 1H), 3.73 (s, 3H), 3.70 – 3.51 (m, 2H), 2.78 – 2.68 (m, 1H), 2.56 – 2.44 (m, 1H), 2.30 (t, *J* = 10.6, 2H), 2.20 – 2.10 (m, 5H), 2.06 – 1.97 (m, 1H), 1.78 (d, *J* = 13.0, 1H), 1.57 (dt, *J* = 12.3, 19.3, 4H), 1.38 (d, *J* = 19.0, 3H), 1.29 – 1.15 (m, 6H), 1.10 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) first isomer: δ

200.33, 200.27, 169.96, 169.31, 169.16 169.03, 168.23, 168.16, 142.27, 142.18, 125.58, 125.00, 105.33, 104.84, 104.08, 103.78, 75.68, 75.43, 73.35, 73.31, 71.93, 70.59, 62.57, 62.55, 62.40, 62.36, 61.65, 61.29, 52.00, 51.99, 51.95, 50.39, 49.74, 49.67, 40.59, 40.44, 39.25, 38.88, 33.78, 33.66, 30.00, 29.16, 28.11, 21.07, 18.98, 18.97, 16.52, 14.78, 14.70, 13.89, 13.67, 13.65, 13.36, 13.31. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{27}H_{38}O_{10}Na$, 545.2363; found, 545.2117.



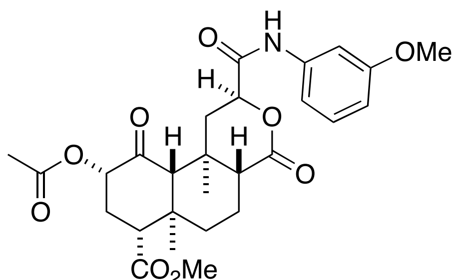
(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(2,5-diethoxytetrahydrofuran-3-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (89). A solution of **89** (200 mg, 0.3827 mmol), 5% Rh/C (catalytic) and CH_3OH (35 mL) was stirred at room temperature under a hydrogen atmosphere for 3 hours. Upon completion, solution was filtered through a pad of celite. Solvent was removed under reduced pressure and a mixture of ethyl acetate and hexanes was added to the residue. The resulting solid was collected by vacuum filtration and dried to yield 185 mg **89** (90%) as a white solid mp 91 – 94 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.91 (d, *J* = 11.5, 1H), 5.13 (d, *J* = 11.3, 2H), 4.91 (s, 1H), 3.75 – 3.71 (m, 3H), 3.70 (s, 1H), 3.29 (s, 1H), 2.73 (s, 2H), 2.30 (s, 4H), 2.20 – 2.16 (m, 3H), 2.14 (s, 3H) 2.02 – 1.88 (m, 2H), 1.76 (s, 1H), 1.72 – 1.66 (m, 1H), 1.55 – 1.46 (m, 2H), 1.40 (s, 1H), 1.35 (d, *J* = 9.5, 3H), 1.27 – 1.14 (m, 6H), 1.09 (d, *J* = 4.6, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 200.76, 200.31, 170.05, 170.03, 170.00, 168.26, 125.62, 105.57,

104.88, 103.91, 103.03, 102.38, 101.79, 100.21, 76.95, 76.79, 76.07, 76.00, 75.96, 75.86, 75.71, 75.37, 75.35, 75.33, 75.21, 74.59, 70.21, 62.67, 62.65, 62.61, 62.59, 62.52, 62.41, 61.69, 61.42, 52.11, 52.02, 52.01, 50.43, 50.41, 50.30, 50.04, 49.93, 49.80, 49.71, 40.53, 40.48, 40.45, 38.92, 36.73, 36.70, 36.60, 36.57, 33.67, 33.53, 29.27, 29.26, 29.23, 29.19, 19.06, 19.02, 16.63, 16.60, 16.57, 14.83, 14.79, 14.76, 14.43, 13.73, 13.70, 13.68, 13.18. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{27}H_{40}O_{10}Na$, 547.2519; found, 547.2488.

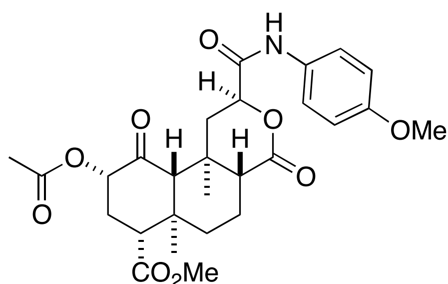


(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(2-methoxyphenylcarbamoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**90**). A solution of **4**[355] (150 mg, 0.3654 mmol), *o*-anisidine (0.3564 mmol), EDCI (0.5481 mmol) and HOBt (0.5481 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature overnight. The solution was washed with 3N HCl (3 x 30 mL), saturated $NaHCO_3$ (3 x 30 mL) and a solution of NaCl (3 x 30 mL) and dried with Na_2SO_4. Solvent was removed under reduced pressure and a mixture of ethyl acetate and hexanes was added to the residue. The resulting solid was collected by vacuum filtration and dried to yield 71.54 mg (38.4%) of **90** as a white solid, mp 127 – 128 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.30 (dd, *J* = 1.6, 8.0, 1H), 7.12 – 7.06 (m, 1H), 6.96 (dd, *J* = 4.5, 11.1, 1H), 6.89 (dd, *J* = 1.2, 8.2, 1H), 5.17 (dd, *J* = 8.2, 11.8, 1H), 5.03 (dd, *J* = 6.5, 10.3, 1H), 3.88 (s, 3H), 3.72 (s, 3H), 2.81 – 2.71 (m, 2H), 2.34 – 2.28 (m, 2H), 2.17 (s, 3H), 2.15 – 2.07 (m, 2H), 1.82 –1.71 (m, 2H), 1.71 – 1.62

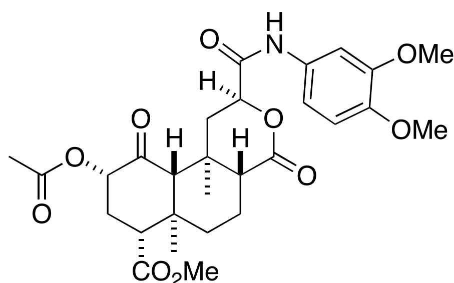
(m, 1H), 1.61 – 1.51 (m, 2H), 1.42 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.69, 171.77, 170.37, 169.88, 167.35, 148.58, 126.64, 124.84, 121.19, 120.19, 110.22, 77.48, 76.20, 74.95, 64.35, 55.96, 53.64, 52.19, 51.05, 42.13, 39.28, 38.02, 35.69, 20.79, 18.37, 16.48, 16.01. HRESIMS (*m/z*): [M+Na] calculated for $C_{27}H_{33}$NO9Na, 538.2055; found, 538.2053. HPLC t_R = 6.932 min; purity = 98.38%.



(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(3-methoxyphenylcarbamoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**91**). Compound **91** was synthesized as described for **90** from **4** using *m*-anisidine to afford 140.5 mg (75%) of **91** as a white solid, mp 122 – 124 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 –8.65 (m, 1H), 8.32 – 8.28 (m, 1H), 7.11 7.07 (m, 1H), 6.99 – 6.95 (m, 2H), 5.19 – 5.13 (m, 2H), 5.05 – 5.01 (m, 1H), 3.88 (s, 3H), 3.72 (s, 3H), 2.85 – 2.67 (m, 5H), 2.38 – 2.30 (m, 2H), 2.17 (s, 3H), 1.72 – 1.62 (m, 3H), 1.43 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.35, 171.85, 171.02, 168.98, 167.31, 148.72, 126.84, 124.62, 121.19, 120.97, 119.97, 110.00, 77.48, 75.97, 74.73, 64.13, 55.74, 53.42, 51.97, 50.83, 41.91, 39.06, 35.47, 20.57, 18.15, 16.25, 15.79. HRESIMS (*m/z*): [M+H] calculated for $C_{27}H_{34}NO_9$, 516.2216; found, 516.2234. HPLC t_R = 6.003 min; purity = 95.39%.

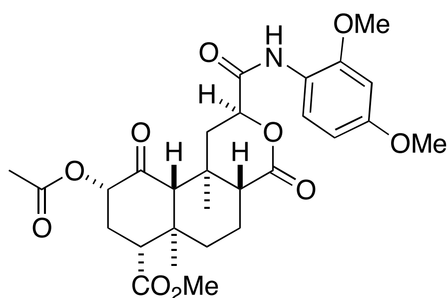


(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(4-methoxyphenylcarbamoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (92). Compound **92** was synthesized as described for **90** from **4** using *p*-anisidine to afford 81.3 mg (43.2%) of **92** as a white solid, mp 147 – 150 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.22 (s, 1H), 7.60 (d, J = 9.1, 2H), 6.88 (d, J = 9.1, 2H), 5.27 (dd, J = 12.5, 7.5, 1H), 5.05 (dd, J = 10.8,6.4, 1H), 3.76 (s, 3H), 3.68 (s, 3H), 3.03 (dd, J = 13.2, 3.5, 1H), 2.52 (dd, J = 13.5, 6.4, 1H), 2.32 (ddd, J = 10.9, 7.7, 3.7, 2H), 2.25 – 2.13 (m, 1H), 2.09 (s, 3H), 2.07 – 1.99 (m, 2H), 1.66 (dtd, J = 18.3, 11.4, 5.0, 4H), 1.36 (s, 3H), 1.07 (s, 3H). ^{13}C NMR (126 MHz, $CHCl_3$) δ 203.50, 172.70, 170.90, 169.91, 168.71, 157.35, 132.41, 122.40 (2C), 114.72 (2C), 76.65, 76.02, 63.39, 55.71, 53.50, 52.02, 50.82, 42.62, 39.97, 38.46, 36.02, 31.63, 20.60, 19.10, 16.55, 15.77. HRESIMS (*m/z*): [M+H] calculated for $C_{27}H_{34}NO_9$, 516.2155; found, 516.2227. HPLC t_R = 13.203 min; purity = 98.80%.



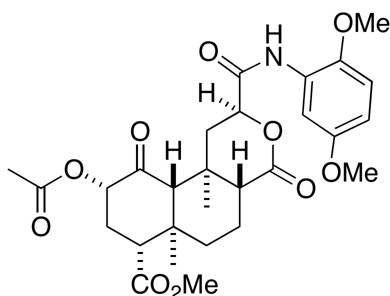
(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(3,4-dimethoxyphenylcarbamoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**93**). Compound **93** was synthesized as described for **90** from **4** using 4-amino veratrole to afford 36.8 mg (18.5%) of **93** as a white solid,

mp 124 – 126 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 6.98 (dd, *J* = 2.4, 8.6, 1H), 6.84 (d, *J* = 8.7, 1H), 5.19 (dd, *J* = 8.4, 11.7, 1H), 5.04 (dd, *J* = 6.1, 11.0, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.74 (s, 3H), 2.80 (d, *J* = 6.1, 1H), 2.74 (d, *J* = 5.0, 1H), 2.32 (dd, *J* = 4.3, 7.6, 2H), 2.19 (s, 3H), 2.17 – 2.05 (m, 2H), 1.81 (d, *J* = 13.0, 1H), 1.75 – 1.59 (m, 4H), 1.56 (d, *J* = 10.0, 1H), 1.46 (d, *J* = 12.3, 3H), 1.18 – 1.09 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.42, 171.52, 170.15, 169.70, 166.98, 149.07, 146.29, 122.79, 112.01, 111.24, 104.73, 76.71, 76.64, 74.74, 63.94, 56.08, 55.97, 52.00, 51.17, 50.38, 41.91, 39.17, 37.85, 35.42, 20.57, 18.11, 16.32, 15.47. HRESIMS (*m/z*): [M+Na] calculated for $C_{28}H_{35}NO_{10}Na$, 568.2161; found, 568.2154. HPLC t_R = 4.645 min; purity = 98.44%.

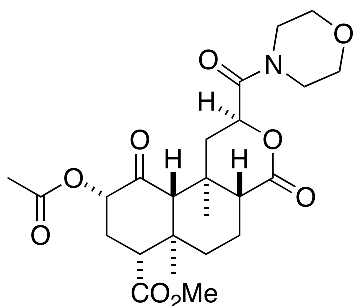


(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(2,4-dimethoxyphenylcarbamoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzoisochromene-7-carboxylate (**94**). Compound **94** was synthesized as described for **90** from **4** using 2,4-dimethoxy aniline to afford 36.7 mg (18.4%) of **94** as a white solid, mp 121 – 123 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 8.17 (d, *J* = 9.6, 1H), 6.49 (d, *J* = 6.8, 2H), 5.18 (dd, *J* = 8.4, 11.6, 1H), 5.03 (dd, *J* = 6.5, 10.1, 1H), 3.87 (s, 3H), 3.82 (s, 3H), 3.73 (s, 3H), 2.84 – 2.70 (m, 2H), 2.32 (dd, *J* = 4.4, 7.8, 2H), 2.19 (s, 3H), 2.12 (d, *J* = 9.5, 2H), 1.85 – 1.66 (m, 3H), 1.63 – 1.53 (m, 2H), 1.44 (s, 3H), 1.12 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.63, 174.11, 171.43, 169.99, 168.79, 160.07, 156.40, 141.81, 121.26, 120.01, 103.92, 98.68, 75.96, 74.70, 64.23, 55.76, 55.54, 53.35, 51.97, 50.81, 41.87, 39.16, 37.98, 35.46, 20.57, 18.09, 16.26, 15.80. HRESIMS (*m/z*): [M+Na]

calculated for $C_{28}H_{35}NO_{10}Na$, 568.2161; found, 568.2175. HPLC t_R =6.468 min; purity = 98.92%.

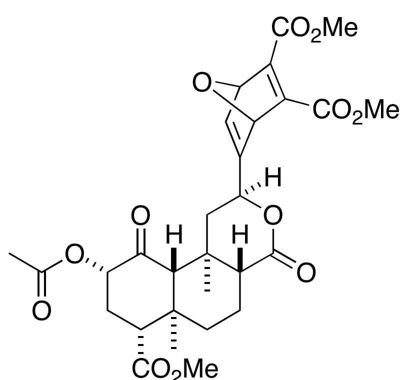


(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(2,5-dimethoxyphenylcarbamoyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**95**). Compound **95** was synthesized as described for **90** from **4** using 2,5-dimethoxy aniline to afford 53 mg (26.8%) of **95** as a white solid, mp 119 – 121 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.04 (d, *J* = 3.0, 1H), 6.62 (dd, *J* = 3.0, 8.9, 1H), 5.16 (dd, *J* = 8.2, 11.8, 1H), 5.02 (dd, *J* = 6.4, 10.3, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.72 (s, 3H), 2.82 – 2.71 (m, 3H), 2.37 – 2.28 (m, 2H), 2.18 (d, *J* =9.6, 4H), 2.15 – 2.04 (m, 3H), 1.81 – 1.69 (m, 3H), 1.42 (s, 3H), 1.11 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.47, 171.55, 170.06, 169.68, 167.19, 153.70, 142.54, 127.01, 110.78, 109.58, 106.03, 77.22, 75.94, 74.74, 64.12, 56.22, 55.80, 53.44, 51.98, 50.87, 41.92, 39.08, 37.82, 35.47, 20.57, 18.14, 16.26, 15.74. HRESIMS (*m/z*): [M+Na] calculated for $C_{28}H_{35}NO_{10}Na$, 568.2161; found, 568.2164. HPLC t_R = 7.896 min; purity = 99.38%.



(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-6a,10b-dimethyl-2-(morpholine-4-carbonyl)-4,10-dioxododecahydro-1*H*-

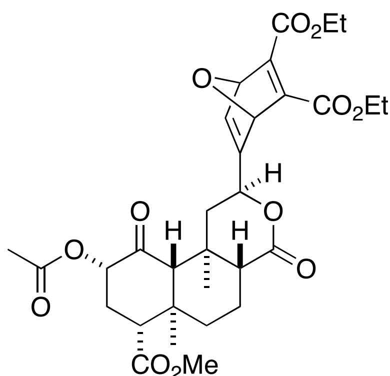
benzo[*f*]isochromene-7-carboxylate (**96**). Compound **96** was synthesized as described for **90** from **4** using morpholine to afford 71.0 mg **96** (41%) as a white powder, mp 128-130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.25- 5.14 (m, 2H), 3.82 – 3.72 (m, 5H), 3.71 – 3.60 (m, 4H), 3.50 (t, *J* = 16.0 2H), 2.79 (dd, *J* = 5.8, 10.9, 1H), 2.37 – 2.24 (m, 4H), 2.19 (s, 3H), 2.09 (d, *J* = 16.5, 1H), 1.95 (dd, *J* =7.5, 13.6, 1H), 1.77 (d, *J* = 12.4, 1H), 1.71- 1.52 (m, 3H), 1.39 (s, 3H), 1.09 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.23, 171.64, 170.98, 169.79, 167.28, 74.98, 71.27, 66.67, 64.53, 53.26, 51.95, 49.14, 46.20, 42.86, 42.00, 37.72, 73.54, 35.11, 35.10, 30.73, 20.59, 18.17, 17.01, 16.03. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{24}H_{33}NO_9Na$, 502.2055; found, 502.2045. HPLC t_R = 3.490 min; purity = 98.69%.



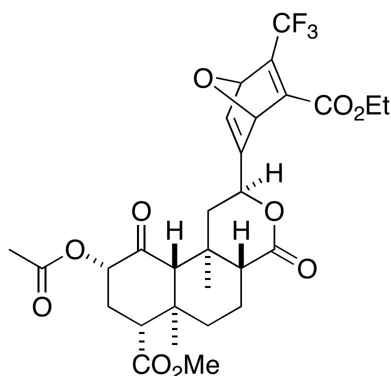
Dimethyl 5-((2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-acetoxy-7-(methoxycarbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromen-2-yl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate (**97**). A solution of **1** (200 mg, 0.462 mmol), dimethyl acetylene dicarboxylate (71 mg, 0.500 mmol) and toluene (20 mL) were allowed to stir at room temperature and gradually heated to reflux over 45 minutes. Solution was allowed to stir at reflux for 2 days. Upon completion, solvent was removed under reduced pressure and the resulting residue was purified by column chromatography (eluent: ethyl acetate/*n*-hexanes, 2:3) to afford 152 mg (70%) of **97** as a white solid, mp 107 – 110 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ

6.91 (d, J = 12.2, 1H), 5.71 (d, J = 1.6, 1H), 5.67 (s, 1H), 5.63 (d, J = 1.6, 1H), 5.40 – 5.35 (m, 1H), 5.32 – 5.26 (m, 1H), 3.85 (s, 3H), 3.82 (d, J = 1.4, 3H), 3.73 (s, 3H), 2.74 (s, 1H), 2.42 (s, 1H), 2.31 (d, J = 7.4, 2H), 2.17 (d, J = 3.7, 4H), 2.05 (s, 2H), 1.80 – 1.75 (m, 1H), 1.47 (s, 2H), 1.40 (s, 3H), 1.10 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 200.79, 171.53, 170.57, 169.95, 162.82, 157.79, 137.94, 136.52, 84.25, 84.19, 84.10, 73.64, 72.53, 71.72, 62.73, 62.68, 52.16, 51.41, 51.21, 50.77, 49.66, 40.71, 39.84, 36.69, 34.14, 19.36, 16.78, 15.08, 13.99. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{29}H_{34}O_{12}Na$, 597.1948; found, 597.1772. HPLC t_R = 4.757 min; purity = 97.11%.

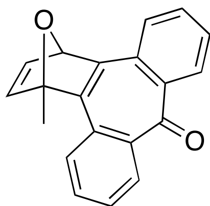
Dimethyl 5-((2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-acetoxy-7-(methoxycarbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromen-2-yl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate (**97**). A solution of **1** (100 mg, 0.231 mmol), dimethyl acetylene dicarboxylate (35 mg, 0.250 mmol) and toluene (25 mL) were placed in a sealed 25 mL quartz tube and irradiated in a microwave reactor (Biotage Initiator™) at 100 °C for 30 minutes with normal absorbance levels. Solvent was removed under reduced pressure and the resulting residue was purified using column chromatography (ethyl acetate/*n*-hexanes, 2:3) to afford 69 mg (63%) of the same material described above.



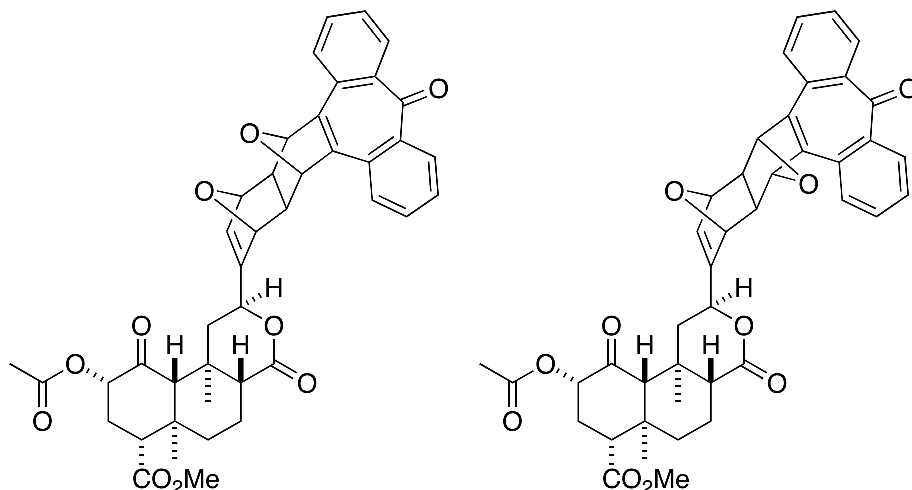
Diethyl 5-((2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-acetoxy-7-(methoxycarbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromen-2-yl)-7-oxabicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylate (**98**). Compound **98** was synthesized as described for **97** from **1** using diethyl acetylene dicarboxylate to afford 262 mg of **98** (70%) as a white powder, mp 84 – 86 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.92 (dt, *J* = 1.9, 3.6, 1H), 5.66 (t, *J* = 1.8, 1H), 5.60 (d, *J* = 1.6, 1H), 5.39 (dd, *J* = 3.8, 11.7, 1H), 5.29 (d, *J* = 7.7, 1H), 5.22 – 5.08 (m, 2H), 4.33 – 4.23 (m, 4H), 3.73 (s, 3H), 2.78 – 2.72 (m, 1H), 2.43 (d, *J* =5.6, 1H), 2.41 (d, *J* = 5.5, 1H), 2.31 (dd, *J* = 3.2, 11.0, 2H), 2.17 (d, *J* = 3.1, 4H), 1.80 -1.75 (m, 1H), 1.50 (dd, *J* = 11.3, 23.9, 2H), 1.40 (d, *J* = 1.6, 3H), 1.32 (ddd, *J* = 2.8, 6.3, 10.1, 6H), 1.10 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 202.03, 171.55, 170.66, 169.91, 163.14, 157.75, 152.81, 151.89, 138.01, 136.44, 85.55, 85.26, 74.92, 73.94, 64.05, 61.76, 61.55, 53.50, 52.00, 51.15, 42.03, 41.16, 40.29, 38.03, 35.44, 30.75, 20.60, 18.12, 16.34, 15.30, 14.10. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{31}H_{38}O_{12}Na$, 625.2261; found, 625.1553. HPLC t_R = 6.802 min; purity = 96.20%.



(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-2-(5-(ethoxycarbonyl)-6-(trifluoromethyl)-7-oxabicyclo[2.2.1]hepta-2,5-dien-2-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**99**). Compound **99** was synthesized as described for **90** from **1** using ethyl 4,4,4-trifluoro-2 butynoate to afford 44 mg of **99** (24%) as a white powder, mp 119 – 121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (dd, *J* = 1.9, 3.8, 1H), 5.64 (dt, *J* = 1.8, 5.5, 1H), 5.39 (dd, *J* = 3.8, 11.8, 1H), 5.30 (dd, *J* = 3.7, 11.6, 1H), 5.19 – 5.12 (m, 1H), 4.36 – 4.22 (m, 2H), 3.73 (s, 3H), 2.80 – 2.71 (m, 1H), 2.43 (td, *J* = 5.5, 13.4, 1H), 2.35 – 2.26 (m, 2H), 2.17 (d, *J* = 2.5, 3H), 2.13 (d, J = 3.3, 1H), 1.82 – 1.76 (m, 2H), 1.59 (s, 3H), 1.50 (td, *J* = 4.9, 12.6, 1H), 1.41 (s, 3H), 1.33 (dt, *J* = 7.1, 14.3, 3H), 1.11 (s, 3H). (500 MHz, $CDCl_3$) δ 202.04, 169.94, 169.06, 168.35, 160.37, 156.91, 149.26, 135.80, 134.19, 121.43, 83.44, 82.86, 73.29, 72.31, 62.42, 60.58, 51.90, 51.86, 49.63, 49.41, 40.44, 39.50, 38.76, 36.36, 33.68, 29.12, 18.97, 16.52, 14.76. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{29}H_{33}F_3O_{10}Na$, 621.1924; found, 621.1986. HPLC t_R = 10.305 min; purity = 98.48%.

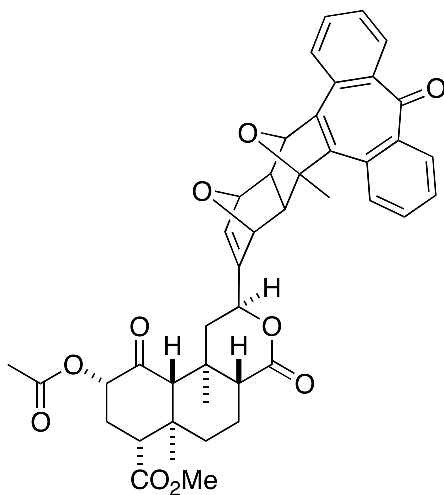


3,6-Epoxy-3-methyl-3,6-dihydrotribenzocycloheptatrienone (101) A solution of dibenzosuberenone (500 mg, 2.423 mmol) and Br_2 (774 mg, 4.848 mmol) in CH_2Cl_2 (100 mL) was allowed to stir at -30 °C for 2 hours. The solvent was removed under reduced pressure to afford a crude resiude. THF (100 mL) was added to the residue and the resulting mixture was treated with t-BuOK (815 mg, 7.269 mmol) and 2-methyl furan (200 mg, 2.423 mmol at 0 °C overnight. The solvent was removed under reduced pressure and CH_3OH was added to the residue resulting in an off-white precipitate. The precipitate was filtered to afford 416 mg of **101**(60%) as an off-white powder, mp 130 -132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (ddd, *J* = 1.1, 4.6, 7.9, 2H), 7.84 – 7.76 (m, 2H), 7.73 – 7.63 (m, 3H), 7.60 – 7.53 (m, 2H), 7.43 (s, 1H), 6.02 (d, *J* = 1.8, 1H), 2.22 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.02, 149.76, 148.44, 144.12, 141.95, 138.10, 136.95, 130.26, 130.20, 130.15, 129.38, 128.30, 128.03, 127.51, 127.12, 121.84, 121.56, 92.46, 82.54, 27.69. HRESIMS (*m*/*z*): [M+H] calculated for $C_{20}H_{15}O_2$, 287.1072; found, 287.1055. HPLC t_R = 32.15; purity = 97.80.



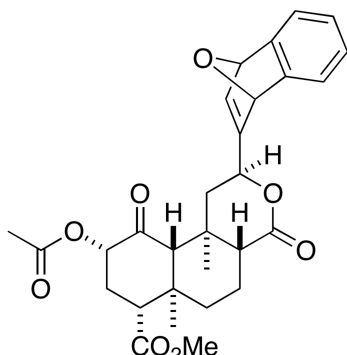
(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-2-(7-oxabicyclo[2.2.1]hepta-2,5-dien-2-yl)- 3,6-epoxy-3,6-dihydrotribenzocycloheptatrienone-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**102**). Compound **102** was synthesized as described for **97** from **1** using **100**[375] to afford 43.2 mg of the *exo* and *endo* isomers of **102** (32%) as a white powder, mp 211 – 214 °C; ^{1}H NMR (500 NMR, $CDCl_3$) δ 8.12 (ddd, *J* = 1.2, 6.8, 12.3, 5H), 7.74 – 7.64 (m, 6H), 7.55 (d, *J* = 7.5, 4H), 7.51 – 7.44 (m, 3H), 7.40 (d, *J* = 7.9, 3H), 6.18 (d, *J* = 2.1, 1H), 6.11 (t, *J* = 2.0, 2H), 5.32 (s, 2H), 5.23 – 5.21 (m, 3H), 5.14 (s, 2H), 3.73 (s, 5H), 3.69 (s, 3H), 3.06 – 2.97 (m, 5H), 2.82 – 2.57 (m, 7H), 2.31 (dd, *J* = 7.5, 14.8, 5H), 2.20 (d, *J* = 2.5, 4H), 2.18 (s, 4H), 2.14 – 2.08 (m, 3H), 2.05 – 2.00 (m, 2H), 1.45 (s, 3H), 1.43 (s, 4H), 1.11 (s, 2H), 1.10 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.85, 199.56, 191.69, 191.61, 169.20, 169.19, 168.93, 168.71, 167.69, 145.89, 145.58, 142.72, 142.69, 142.17, 136.21 (2C), 136.04, 136.01, 129.72 (2C), 129.60, 129.44, 129.38, 128.24, 128.22, 128.10, 128.01, 127.72, 127.64(2C), 126.72, 126.70, 126.65, 126.59, 125.55, 125.15, 122.72, 122.63, 122.27, 122.19, 78.48, 78.28, 78.10, 77.76, 77.20, 76.94, 76.53, 74.83, 74.63, 74.37, 72.67, 72.64, 72.60, 70.82, 61.93, 61.56, 51.13, 50.96, 49.61, 49.56, 48.70, 48.69, 47.85, 47.83, 47.43, 47.18, 39.71, 39.64, 38.95, 38.25, 35.67, 35.45, 32.98, 32.72, 28.37, 18.25,

18.23, 15.79, 15.71, 13.95, 13.91, 13.32, 12.94. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{42}H_{40}O_{10}Na$, 727.2519; found, 727.2719. HPLC t_R = 5.657 min; purity = > 99.35%.



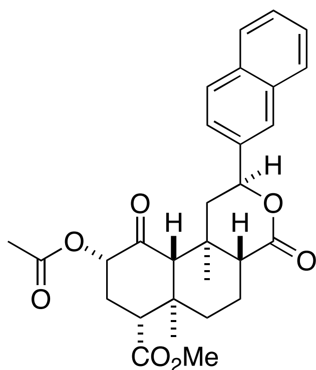
(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-2-(7-oxabicyclo[2.2.1]hepta-2,5-dien-2-yl)- 3,6-epoxy-3-methyl-3,6-dihydrotribenzocycloheptatrienone-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**103**). Compound **103** was synthesized as described for **97** from **1** using **101** to afford 14.2 mg of **103** (26%) as a white powder, mp 198 – 200 °C (dec.); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.8, 2H), 7.65 (dt, *J* = 1.4, 7.5, 1H), 7.59 (m, 1H), 7.52 (d, *J* = 8.7, 3H), 7.43 (d, *J* = 7.32, 1H), 6.17 (t, *J* = 1.9, 2H), 5.21 (m, 3H), 5.05 (s, 2H), 3.73 (s, 3H), 3.17 (m, 3H), 2.76 (dd, *J* = 10.8, 6.1, 2H), 2.63 (dd, 13.3, 5.8, 1H), 2.31 (m, 2H), 2.18 (s, 3H), 1.81 (s, 1H), 1.70 (s, 3H), 1.59 (m, 1H), 1.42 (s, 3H), 1.26 (s, 2H), 1.11 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.36, 194.90, 171.60, 170.74, 169.48, 148.00, 147.20, 146.20, 138.80, 131.70, 130.80, 129.81, 129.55, 129.32, 129.08, 129.01, 128.50, 128.44, 124.70, 123.91, 88.80, 79.48, 79.31, 78.49, 77.59, 75.01, 74.98, 64.39, 53.53, 53.04, 52.51, 52.01, 51.04, 42.10, 41.49, 38.05, 35.42, 30.79, 20.64, 18.18, 17.53, 16.30, 15.73. HRESIMS (*m*/*z*): [M+Na]

calculated for $C_{43}H_{42}O_{10}Na$, 741.2676; found, 741.2693. HPLC t_R = 10.938 min; purity = 98.93%.

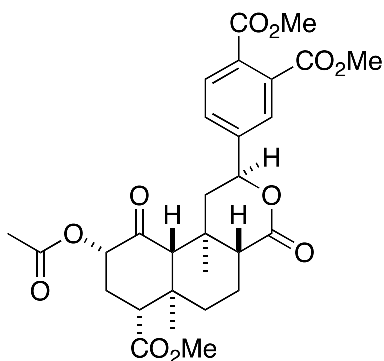


(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-Methyl 9-acetoxy-2-(7-benzo-oxabicyclo[2.2.1]hepta-2,5-dien-2-yl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**104**) To a solution of **1** (500 mg, 1.156 mmol), trimethyl silyl phenyl trifluoromethane (1.05 g, 3.519), and acetonitrile (20 mL), CsF (1.0 g, 6.995 mmol) was added and the solution was allowed to stir at room temperature overnight. Upon completion, the reaction was diluted with water (25 mL) and ether (25 mL). The organic layer was extracted with ether and dried with Na_2SO_4. Compound was purified by column chromatography (eluent: ethyl acetate/*n*-hexanes, 2:3) to afford 42.4 mg of **104** (49%) as a white powder, mp 242 – 245 °C (dec.); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 – 7.31 (m, 1H), 7.25 – 7.22 (m, 1H), 7.01 (dd, *J* = 3.0, 5.1, 2H), 6.69 (t, *J* = 2.0, 1H), 5.72 (d, *J* = 11.9, 2H), 5.17 – 5,09 (m, 2H), 3.72 (s, 3H), 2.73 - 2.68 (m, 1H), 2.37 – 2.33 (m, 1H), 2.31 – 2.26 (m, 2H), 2.17 (s, 3H), 2.11 (s, 1H), 1.95 (d, *J* = 11.9, 1H), 1.76 (d, *J* = 13.4, 1H), 1.66 – 1.58 (m, 1H), 1.53 – 1.45 (m, 2H), 1.37 (s, 3H), 1.31 (s, 1H), 1.07 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.67, 169.08, 168.49, 167.55, 153.93, 146.17, 145.69, 134.12, 123.09, 122.89, 118.25, 117.76, 80.53, 79.82, 72.67, 71.44, 61.80, 51.14, 49.56, 48.46, 39.84, 37.61, 35.62,

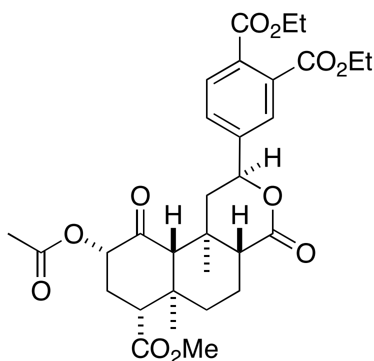
32.84, 28.29, 18.16, 15.62, 13.87, 13.16. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{29}H_{32}O_8Na$, 531.1995; found, 531.1961. HPLC t_R = 13.703 min; purity = 99.06%.



(2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-methyl 9-acetoxy-6a,10b-dimethyl-2-(naphthalen-2-yl)-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromene-7-carboxylate (**105**). A solution of **104**, (85 mg, 0.167 mmol) $Fe_2(CO)_9$, (150 mg, 0.412 mmol) and toluene (15 mL) was allowed to stir at 40 °C for 20 minutes. Once the solution turned black, it was gradually heated to reflux and allowed to stir for 2 hours. The solution was filtered through a pad of celite and solvent was removed under reduced pressure. The residue was purified by column chromatography (eluent: 50% ethyl acetate/50% *n*-hexanes) to afford 41 mg **105** (70%) as a white powder, mp 240 – 242 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (dd, *J* = 7.2, 12.6, 3H), 7.75 (s, 1H), 7.51 – 7.46 (m, 2H), 7.38 (d, *J* = 1.8, 1H), 5.72 (dd, *J* = 5.2, 11.8, 1H), 5.11 – 5.06 (m, 1H), 3.73 (s, 3H), 2.76 -2.70 (m, 1H), 2.62 (dd, *J* = 5.3, 13.6, 1H), 2.30 (dd, *J* = 5.8, 13.1, 2H), 2.22 (s, 3H), 2.16 (d, *J* = 4.2, 2H), 2.15 (s, 3H), 1.81 (s, 1H), 1.63 (s, 1H), 1.55 (s, 3H), 1.14 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 200.23, 169.74, 169.62, 168.06, 135.63, 131.27, 131.22, 126.80, 126.19, 125.88, 124.63, 124.46, 122.58, 121.33, 74.94, 73.17, 62.22, 51.77, 50.18, 49.80, 43.62, 40.30, 36.38, 33.97, 28.93, 27.88, 18.74, 16.39, 14.61. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{29}H_{32}O_7Na$, 515.1945; found, 515.1949. HPLC t_R = 9.175 min; purity = 99.54%.



Dimethyl 4-((2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-acetoxy-7-(methoxycarbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromen-2-yl)phthalate (**106**). Compound **106** was synthesized as described for **105** from **97** to afford 55.2 mg of **106** (77%) as a white powder, mp 116 – 119 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.0, 1H), 7.64 (s, 1H), 7.45 (d, *J* = 7.9, 1H), 5.60 (d, *J* = 6.9, 1H), 5.13 – 5.06 (m, 1H), 3.91 (s, 3H), 3.91 (s, 3H), 3.73 (s, 3H), 2.72 (d, *J* = 6.3, 1H), 2.55 (d, *J* = 8.5, 1H), 2.31 (d, *J* = 9.8, 2H), 2.22 (s, 1H), 2.19 (s, 1H), 2.16 (s, 3H), 2.12 (s, 1H), 1.81 (d, *J* = 13.2, 1H), 1.66 (s, 1H), 1.59 (s, 2H), 1.51 (s, 3H), 1.13 (s, 3H). ^{13}C NMR δ 200.11, 169.60, 168.96, 167.98, 165.81, 165.53, 141.86, 130.86, 129.51, 127.58, 125.95, 123.80, 75.88, 73.06, 61.95, 51.65, 50.88, 50.84, 50.13, 49.70, 43.18, 40.18, 36.18, 33.92, 28.84, 18.67, 16.22, 14.54, 13.28. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{29}H_{34}O_{11}Na$, 581.1999; found, 581.1994. HPLC t_R = 4.103 min; purity = 96.04%.



Diethyl 4-((2*S*,4a*R*,6a*R*,7*R*,9*S*,10a*S*,10b*R*)-9-acetoxy-7-(methoxycarbonyl)-6a,10b-dimethyl-4,10-dioxododecahydro-1*H*-benzo[*f*]isochromen-2-yl)phthalate (**107**). Compound **107** was synthesized as described for **105** from **98** to afford 55 mg of **107** (75%) as a white powder, mp 110 - 112° C; ^{1}H NMR (500 NMR MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.0, 1H), 7.62 (d, *J* = 1.8, 1H), 7.43 (dd, *J* = 1.7, 8.1, 1 H), 5.60 (dd, *J* = 5.1, 11.9, 1H), 5.12 - 5.06 (m, 1H), 4.40 - 4.33 (m, 4H), 3.73 (s, 3H), 2.73 (dd, *J* = 6.3, 10.5, 1H), 2.55 (dd, *J* = 5.1, 13.6, 1H), 2.33 - 2.26 (m, 2H), 2.19 (s, 1H), 2.16 (s, 3H), 2.14 (s, 1H), 2.12 (d, *J* = 3.1, 1H), 1.81 (d, *J* = 13.2, 1H), 1.72 – 1.62 (m, 1H), 1.58 (s, 1H), 1.50 (d, *J* = 8.0, 3H), 1.47 – 1.42 (m, 1H), 1.37 (q, *J* = 7.1, 6H), 1.13 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 201.73, 171.19, 170.60, 169.53, 167.06, 166.66, 143.25, 132.84, 131.35, 129.16, 127.32, 125.30, 77.54, 74.64, 63.54, 61.54, 61.40, 53.23, 51.70, 51.28, 44.82, 41.77, 37.77, 35.50, 30.43, 22.35, 20.24, 17.81, 16.12, 13.80, 13.78. HRESIMS (*m*/*z*): [M+Na] calculated for $C_{31}H_{38}O_{11}Na$, 609.2314; found, 609.2332. HPLC t_R = 11.978 min; purity = > 99.95%.

Opioid Binding Affinity Studies

The binding assays employed in this study were conducted in collaboration with Christina M. Dersch in the laboratory of Dr. Richard B. Rothman at the NIDA in Bethesda, MD. The opioid binding sites for μ receptors were labeled using [^{3}H]D-Ala^{2}-MePhe^{4},Gly-ol^{5}]enkephalin-([^{3}H]DAMGO) 2.0 nM, SA = 45.5 Ci/mmol) using rat brain

membranes as the tissue sample. The δ opioid receptor binding sites were labeled using $[^3H]$[D-Ala^2,D-Leu^5]enkephalin (2.0 nM, SA = 47.5 Ci/mmol) also utilizing rat brain membranes. The κ opioid receptor binding sites were labeled using $[^3H]$U69,593 (2.0 nM, SA = 45.5 Ci/mmol) and guinea pig brain membranes that were pretreated to deplete any residual μ and δ opioid binding sites.

Additionally, binding assays were conducted using $[^{125}I]$-IOXY (6β-iodo-3, 14-dihydroxy-17-cyclopropylmethyl- 4,5α-epoxymorphinan) as the radioligand. The assays were ran in 50 mM Tris-HCl, pH 7.4, with 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 0.1% BSA, 100 μM GDP and 50 pM GTP-γ-S. Brandel Cell Harvesters were used to filter triplicate samples over Whatman GF/B filters after an incubation period of 2-3 hours at 25 °C and washed twice with 5 mL ice-cold 10 mM Tris-HCl, pH 7.4. The filters themselves were punched into 12 x 75 mm glass test tubes and counted at 80% efficiency in a Micromedic gamma counter. Nonspecific binding was determined with 10 μM naloxone. The data obtained from the $[^{125}I]$-IOXY binding studies were grouped into the two-parameter logistic equation, employing the nonlinear least squares curve program, MLAB-PC, for best-fit estimates of IC_{50} data and slope factor. The following equation calculated the K_i values of the test compounds: $K_i = IC_{50}/(1 + [L]/K_d)$, where L stood for the concentration of $[^{125}I]$-IOXY and K_d was the dissociation constant of $[^{125}I]$-IOXY for opioid receptors.

Opioid Functional Activity (Efficacy) Studies

As were the binding affinity studies, all functional activity studies were conducted in collaboration with Christina M. Dersch in the laboratory of Dr. Richard B. Rothman at NIDA in Bethesda, MD. The $[^{35}S]$-GTP-γ-S assay was administered to determine

efficacy of compounds. These studies were carried out as follows: test tubes were filled with 50 μL of buffer A (50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA), 50 μL of GDP in buffer A (final concentration = 50 μM), 50 μL of drug in buffer A/0.1% bovine serum albumin, 50 μL of [^{35}S]-GTP-γ-S in buffer A (final concentration = 50 pM), and 300 μL of cell membranes (50 μg of protein). The final concentration of reagents in the [^{35}S]-GTP-γ-S assay were: 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, and 0.1% bovine serum albumin. The reagents were incubated at 55 °C for 2 hours. GTP-γ-S (40 μM) was used to determine non-specific binding while bound and free [^{35}S]-GTP-γ-S was separated out by vacuum filtration with Whatman GF/B filters. The filters were punched into 24-well plates, which had 0.6 mL of liquid scintillation mixture (Cytoscint; MP Biomedicals, Irvine, CA) added. After an overnight extraction, samples were counted in a Trilux liquid scintillation counter at an efficiency of 60%.

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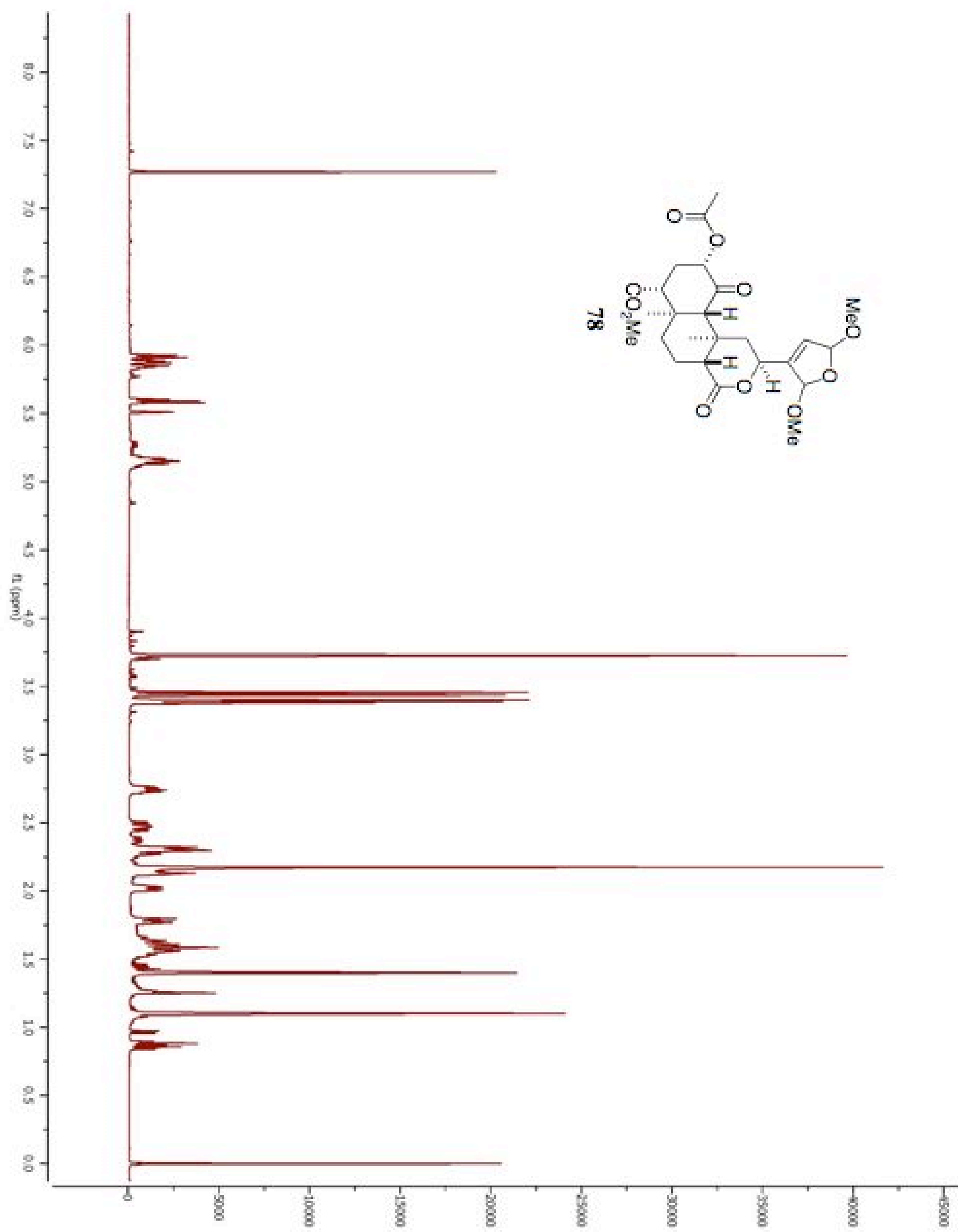
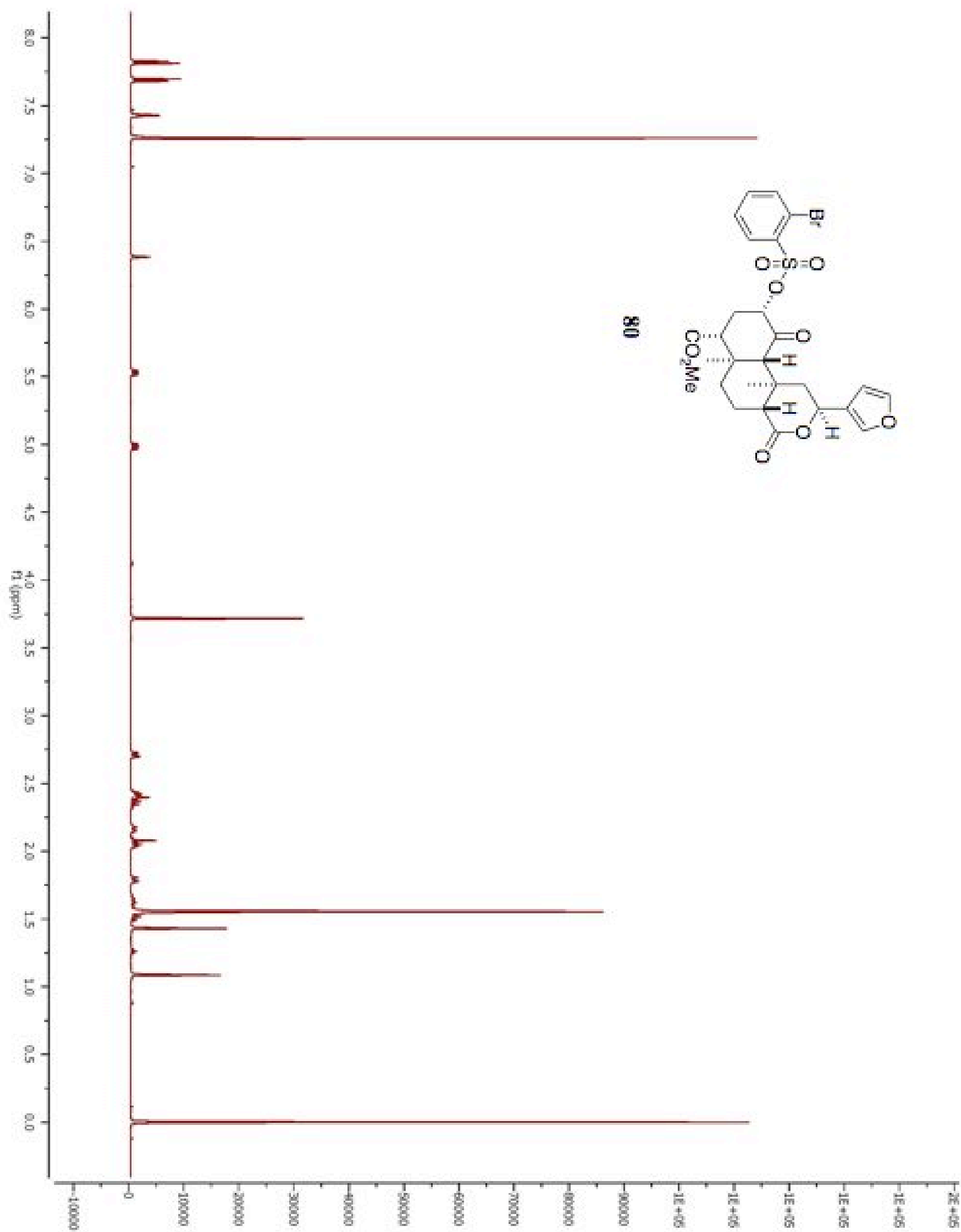
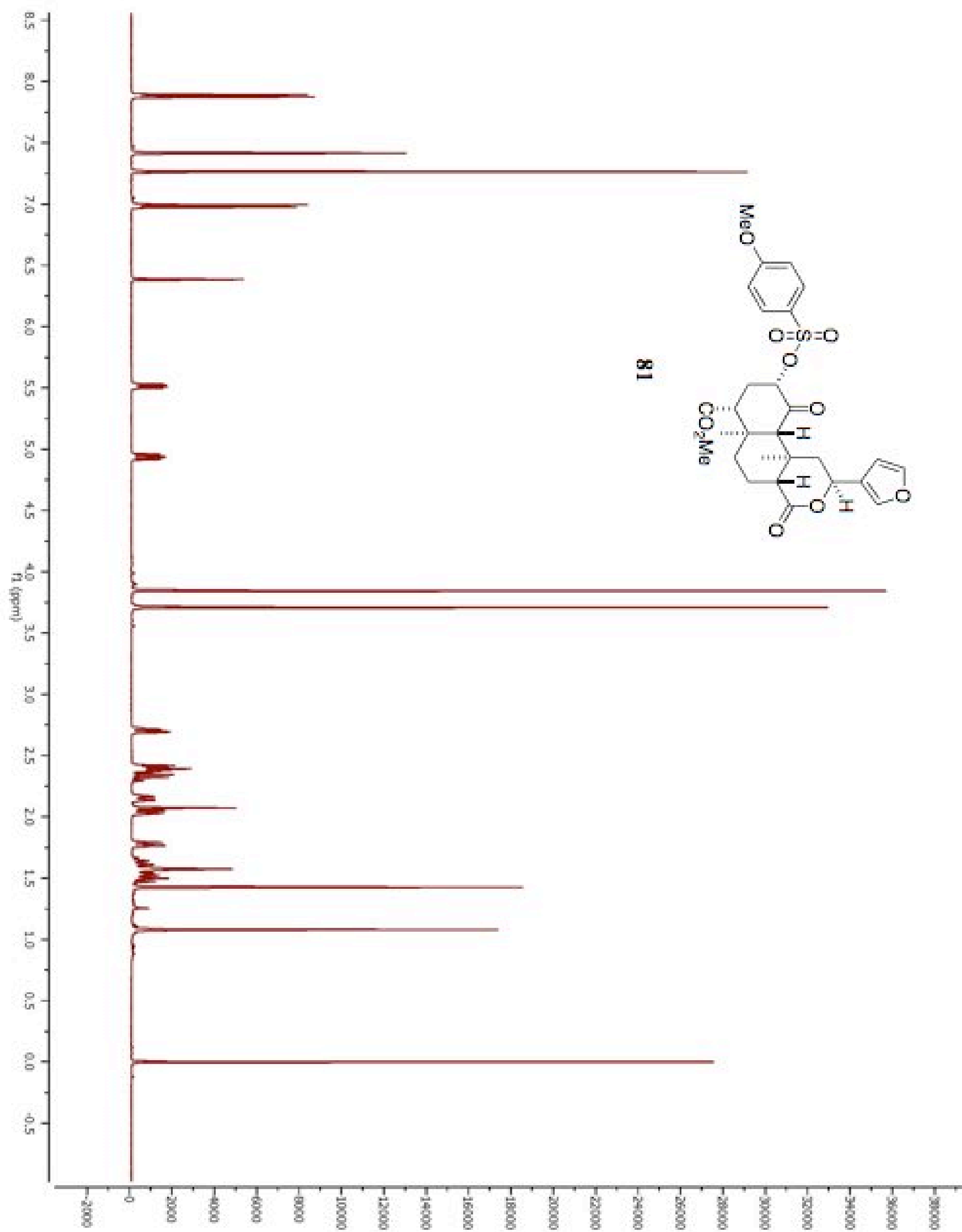
APPENDIX A: ^{1}H NMR SPECTRA

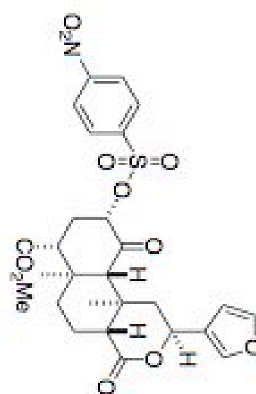


MeO
OMe
H
H
H
O
CO_2Me
78
f1 (ppm)

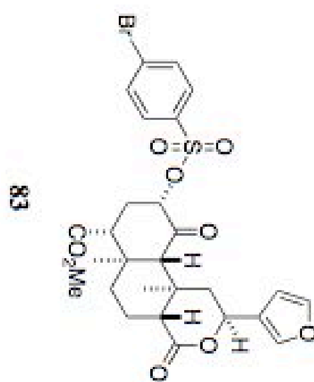


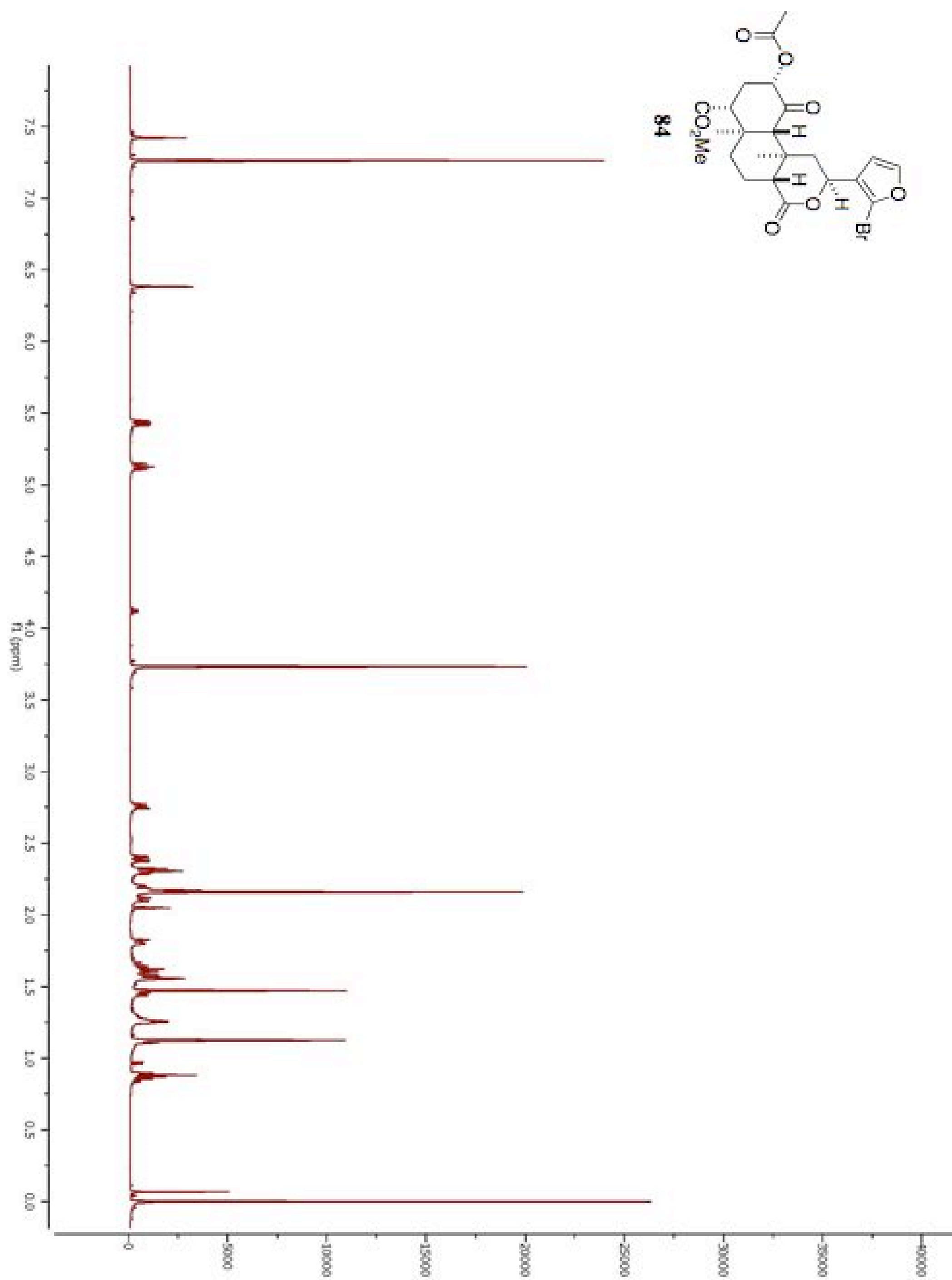
80
CO2Me
Br
f1 (ppm)

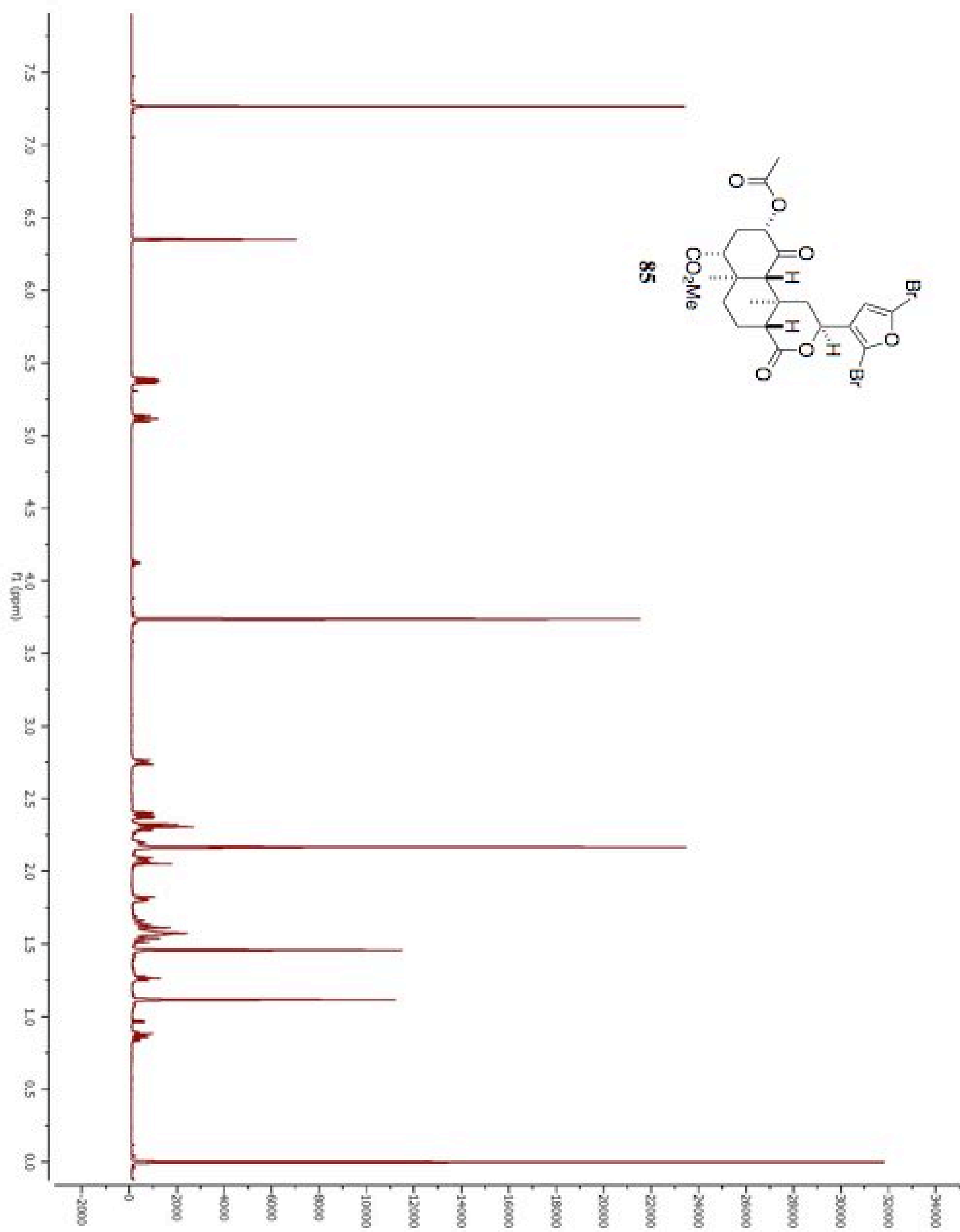

81
MeO
CO2Me
f1 (ppm)


O2N
O=S=O
O
H
O
H
H
O
O
CO2Me
82

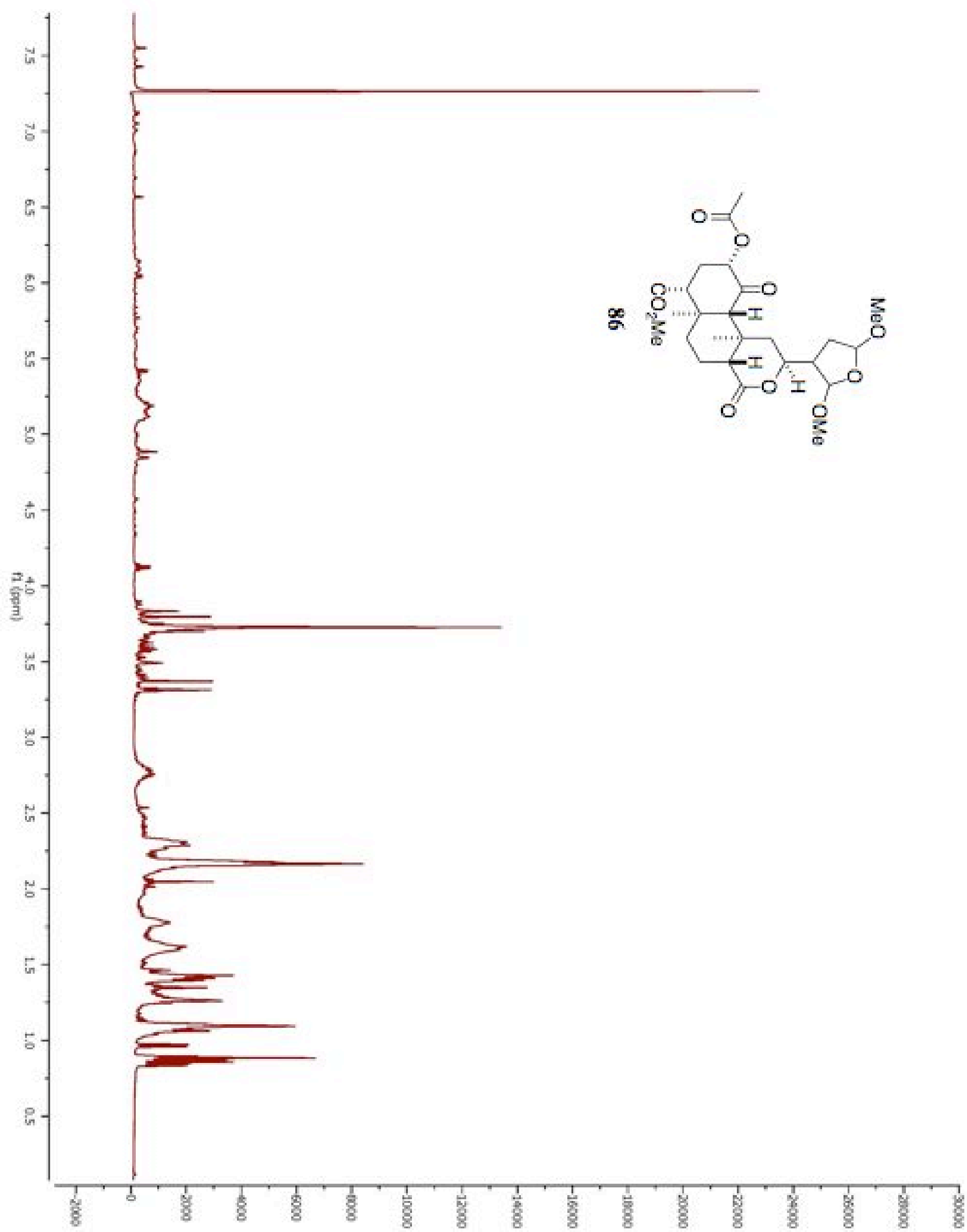
f1 (ppm)

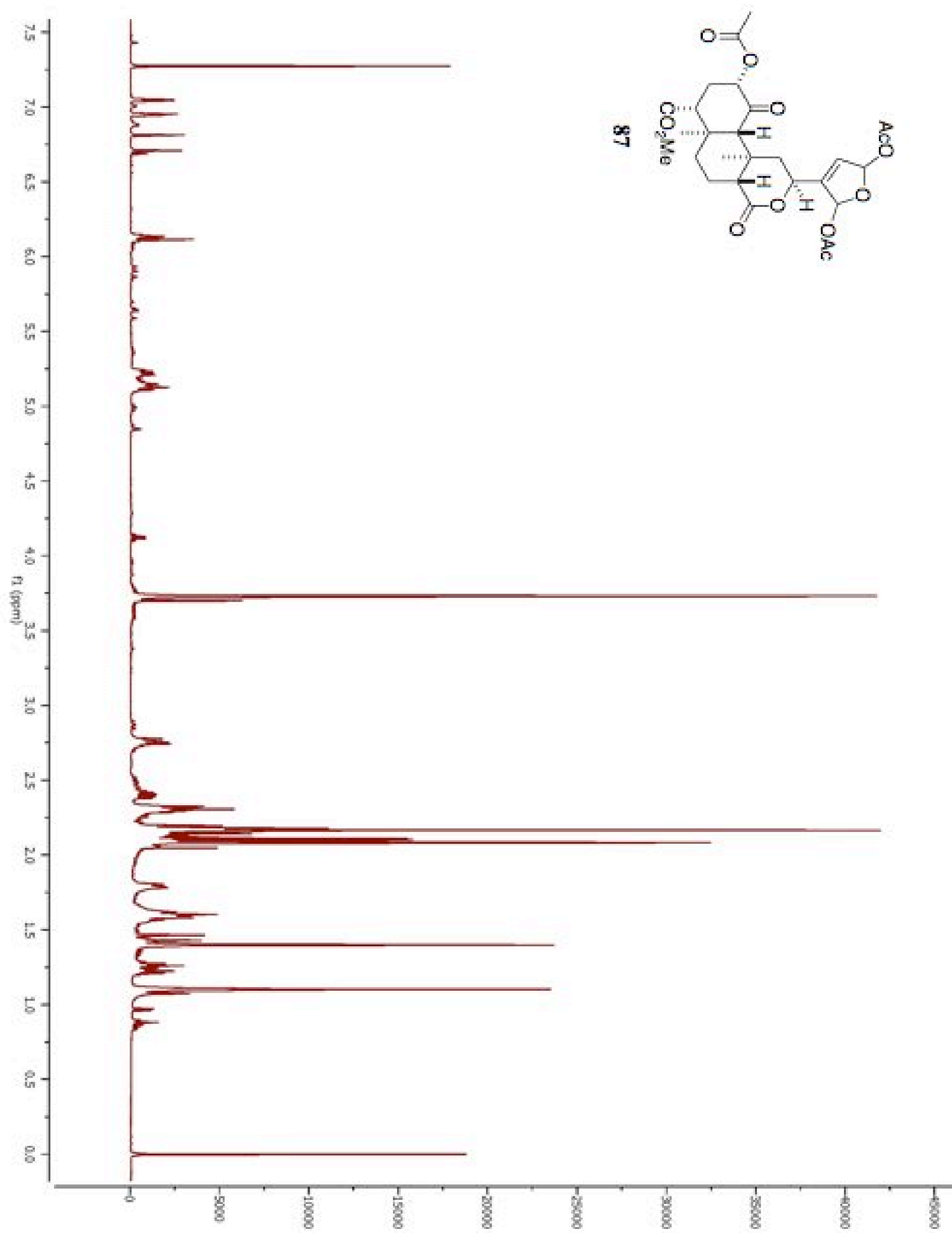

83
CO2Me
Br

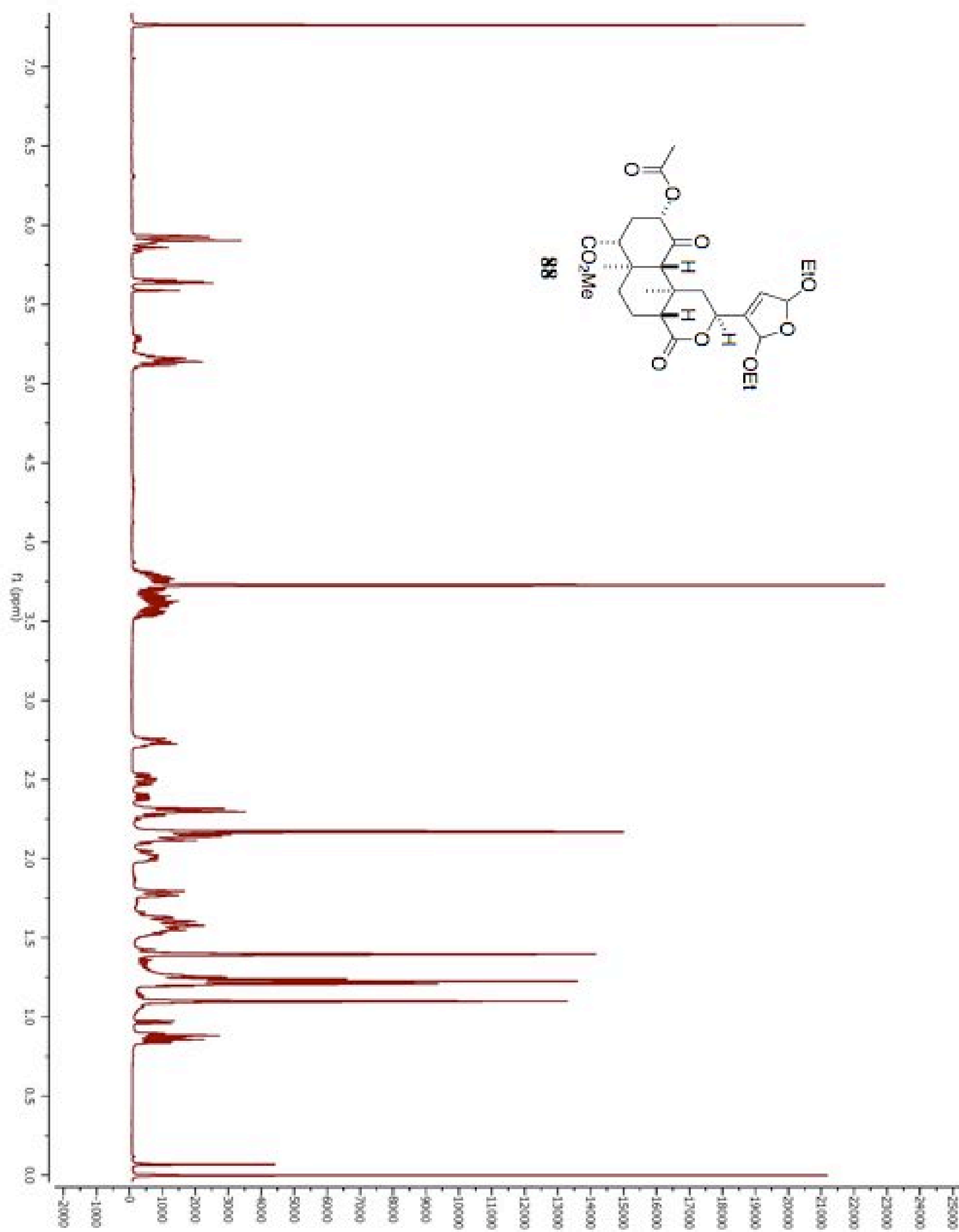

84
CO2Me
Br
f1 (ppm)

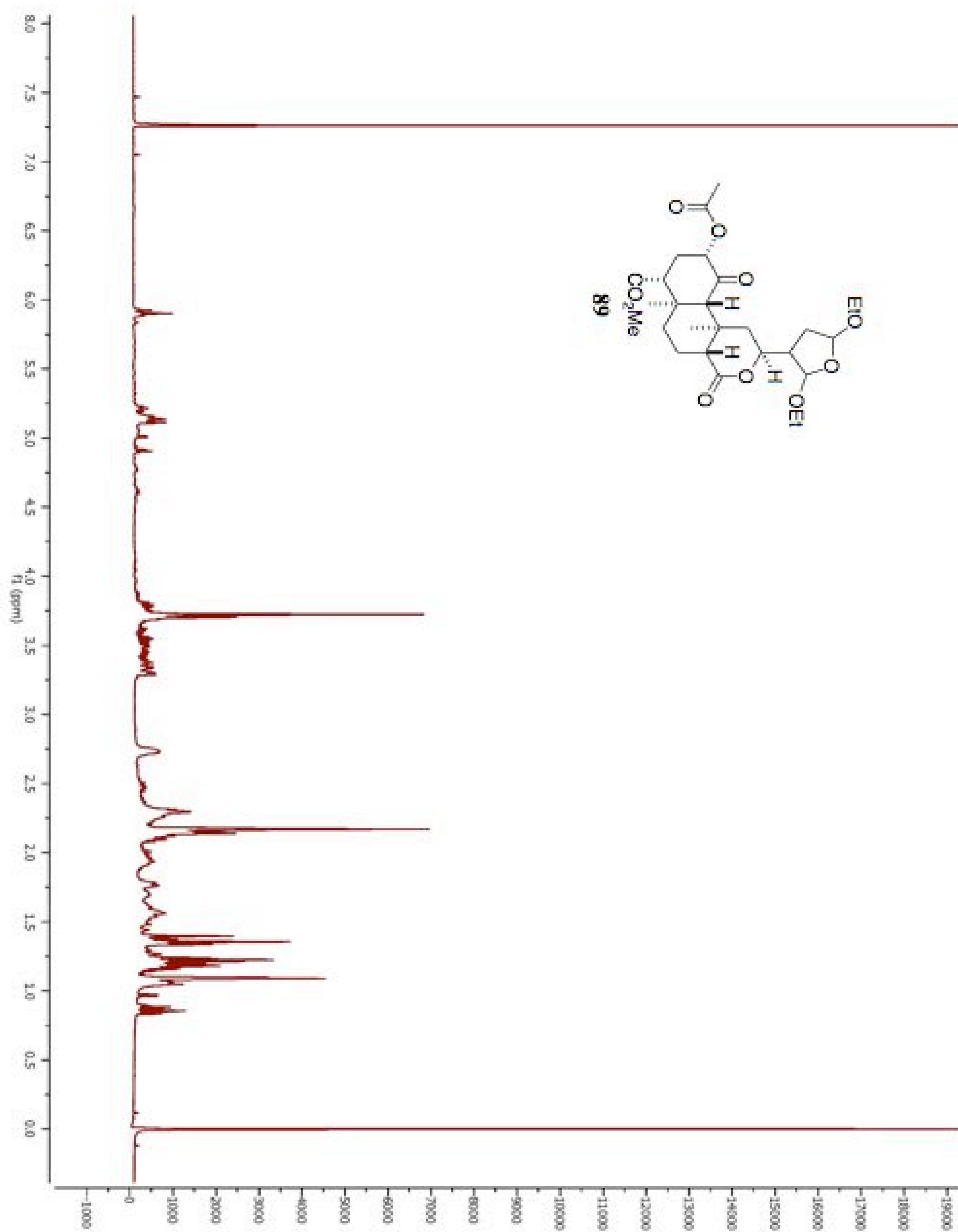


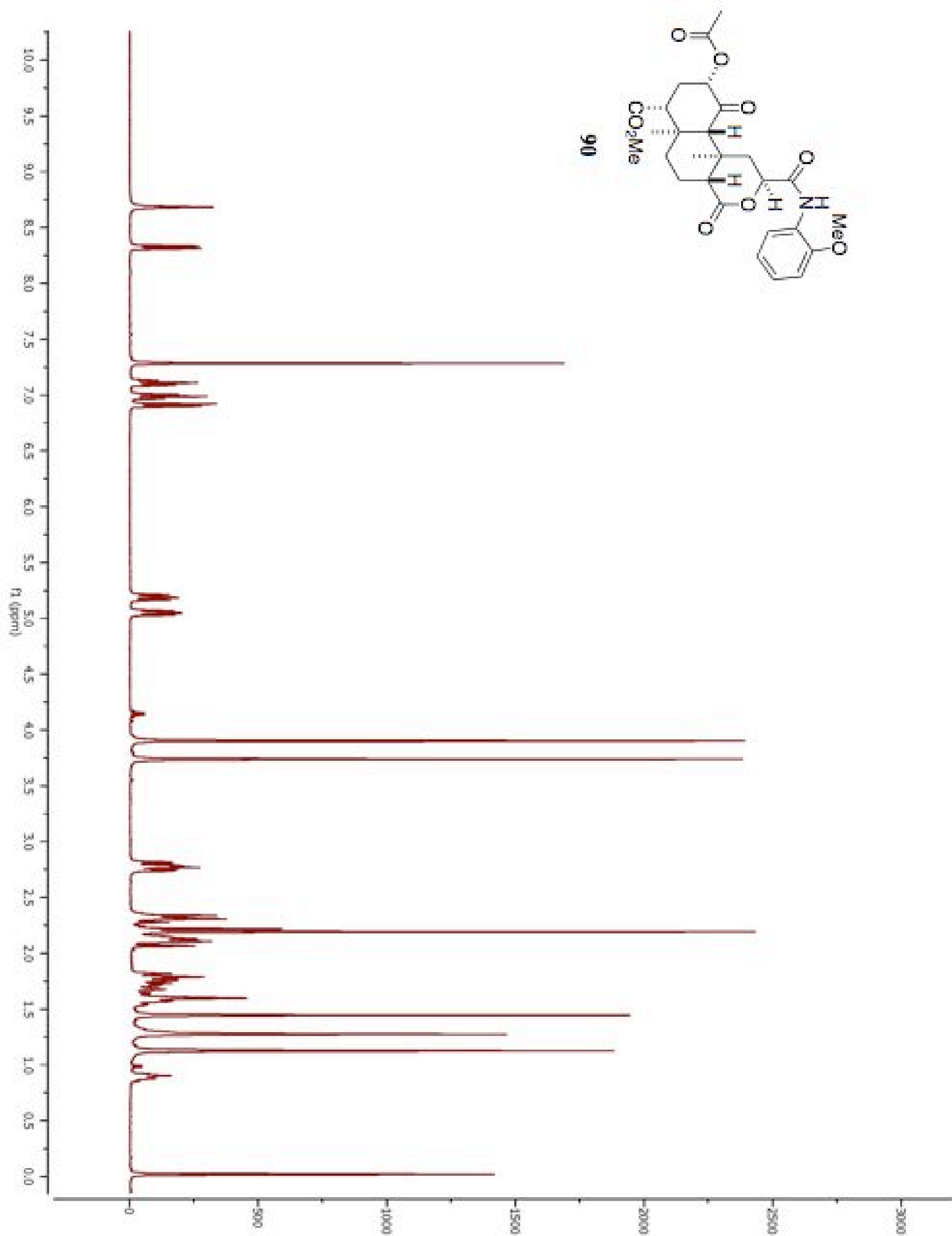
85
CO2Me
Br
Br
f1 (ppm)

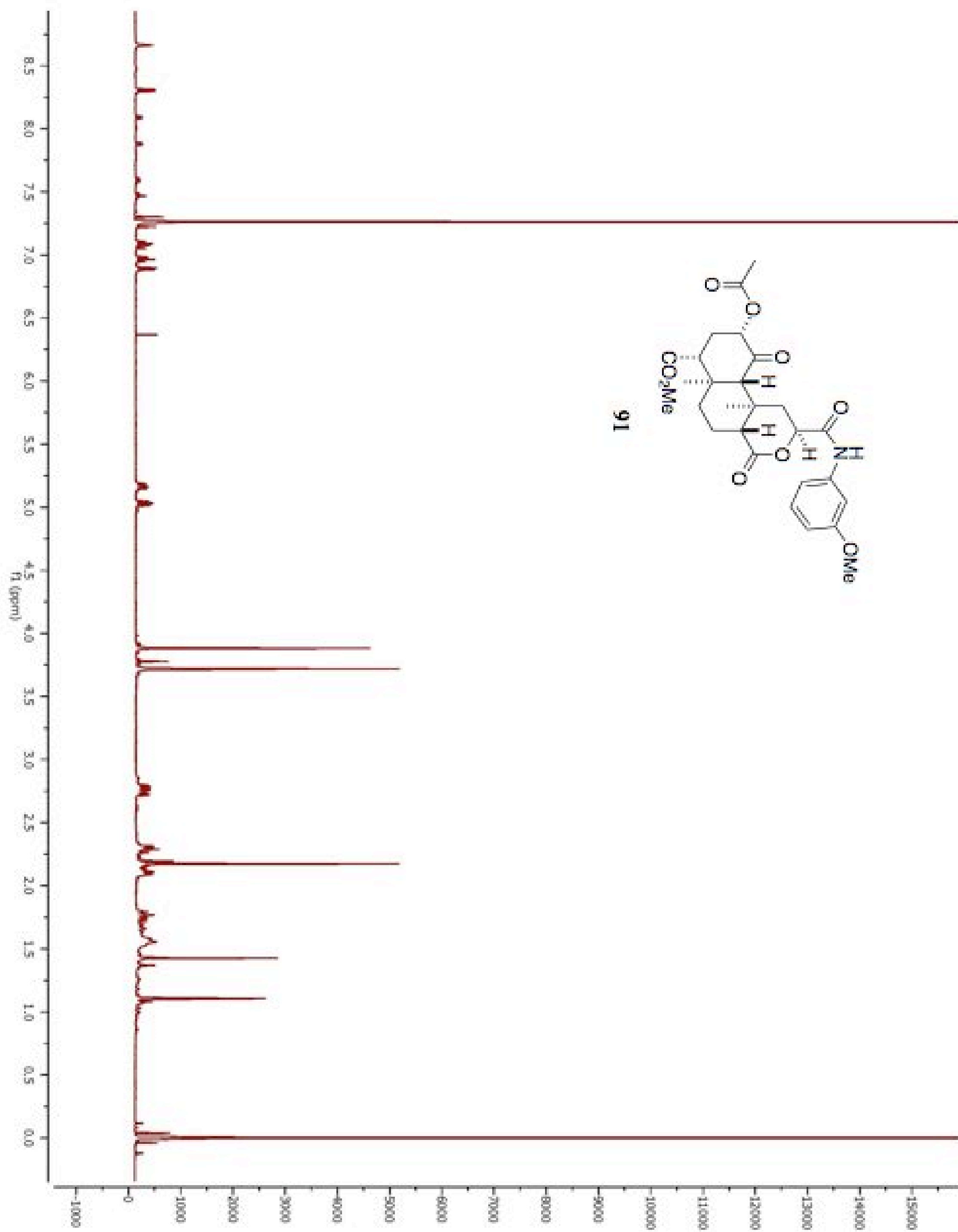

86
CO2Me
MeO
OMe
f1 (ppm)


87
AcO
OAc
CO2Me
f1 (ppm)

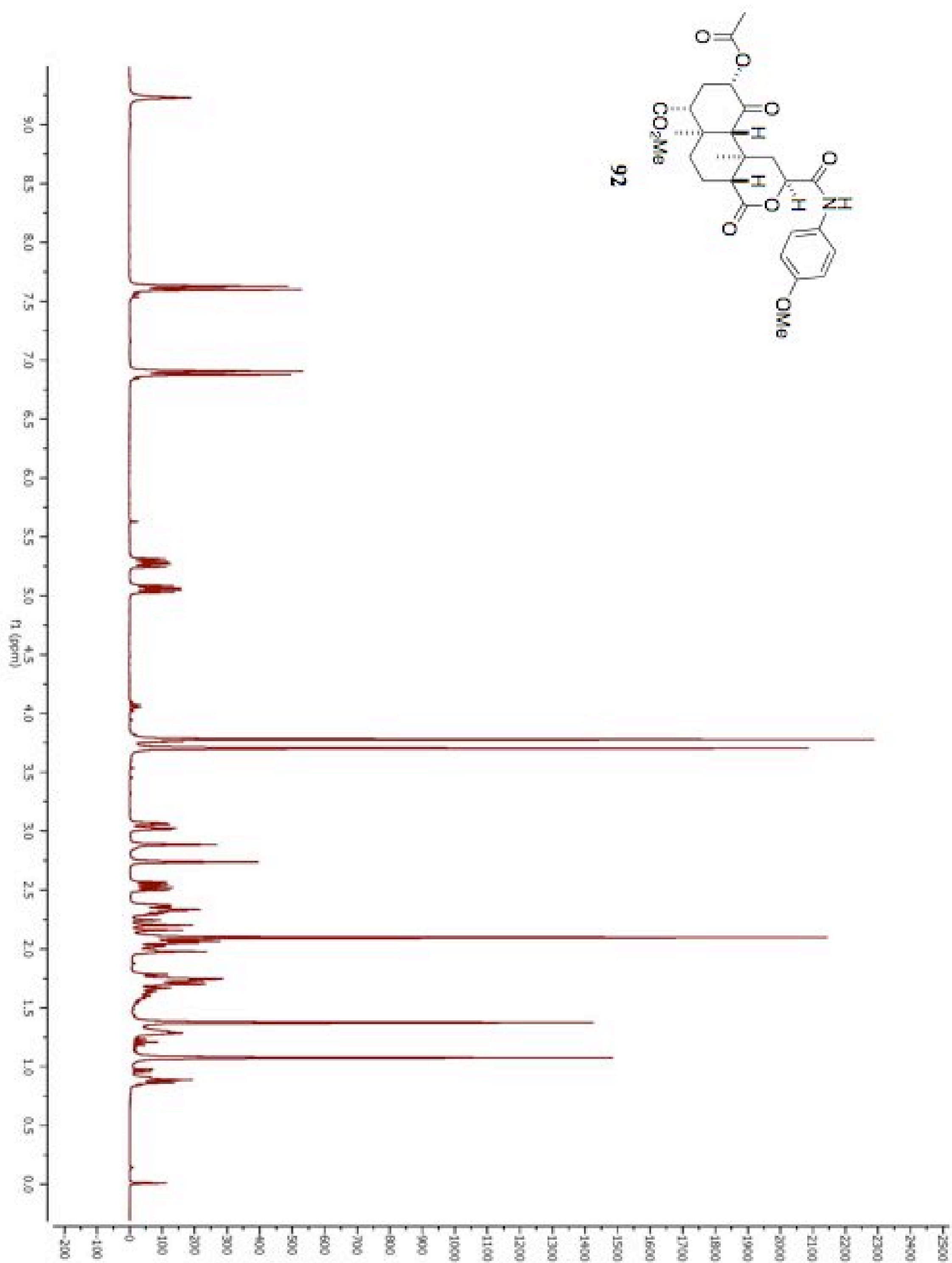


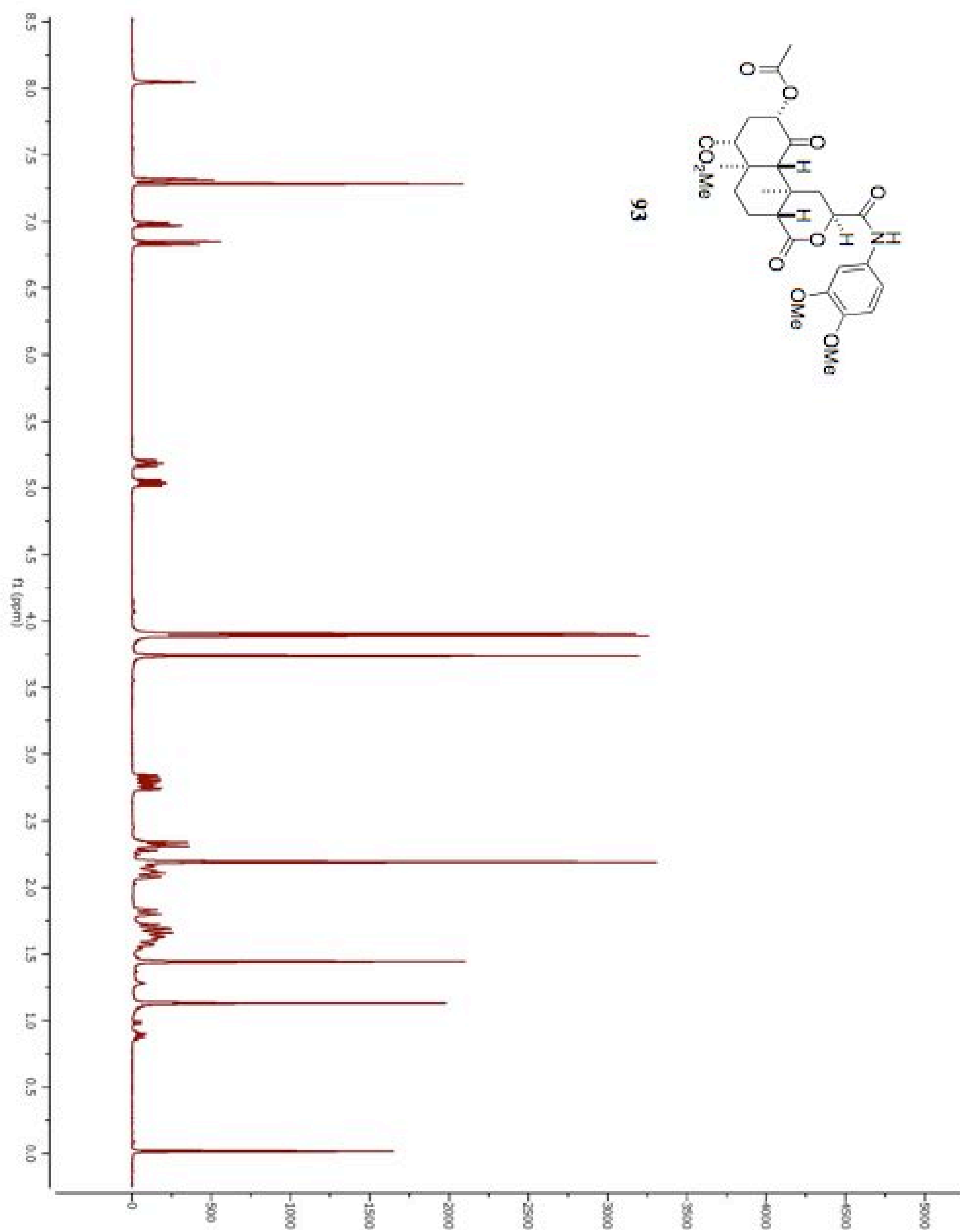

89
CO2Me
EtO
OEt
f1 (ppm)

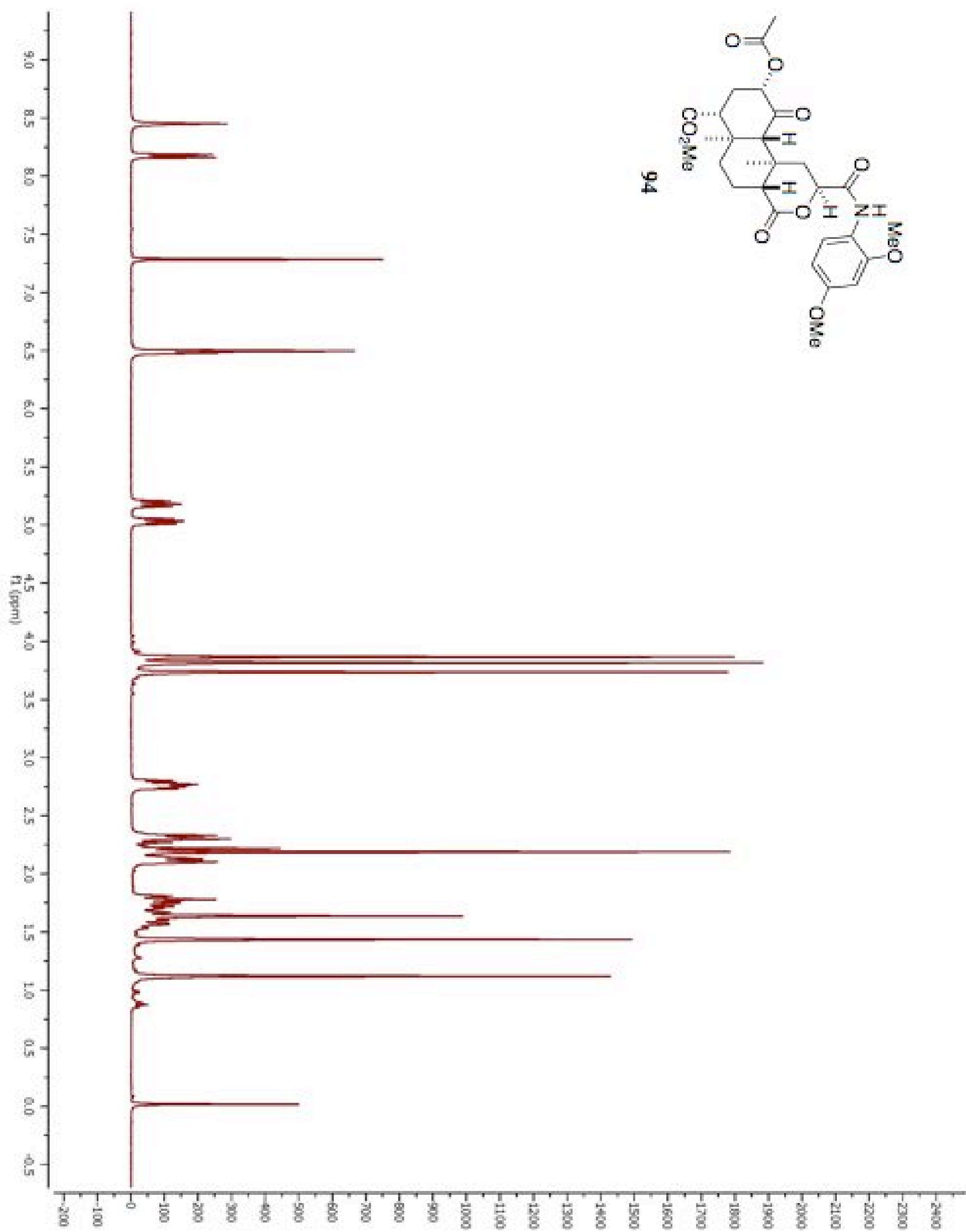

90
CO2Me
MeO
f1 (ppm)



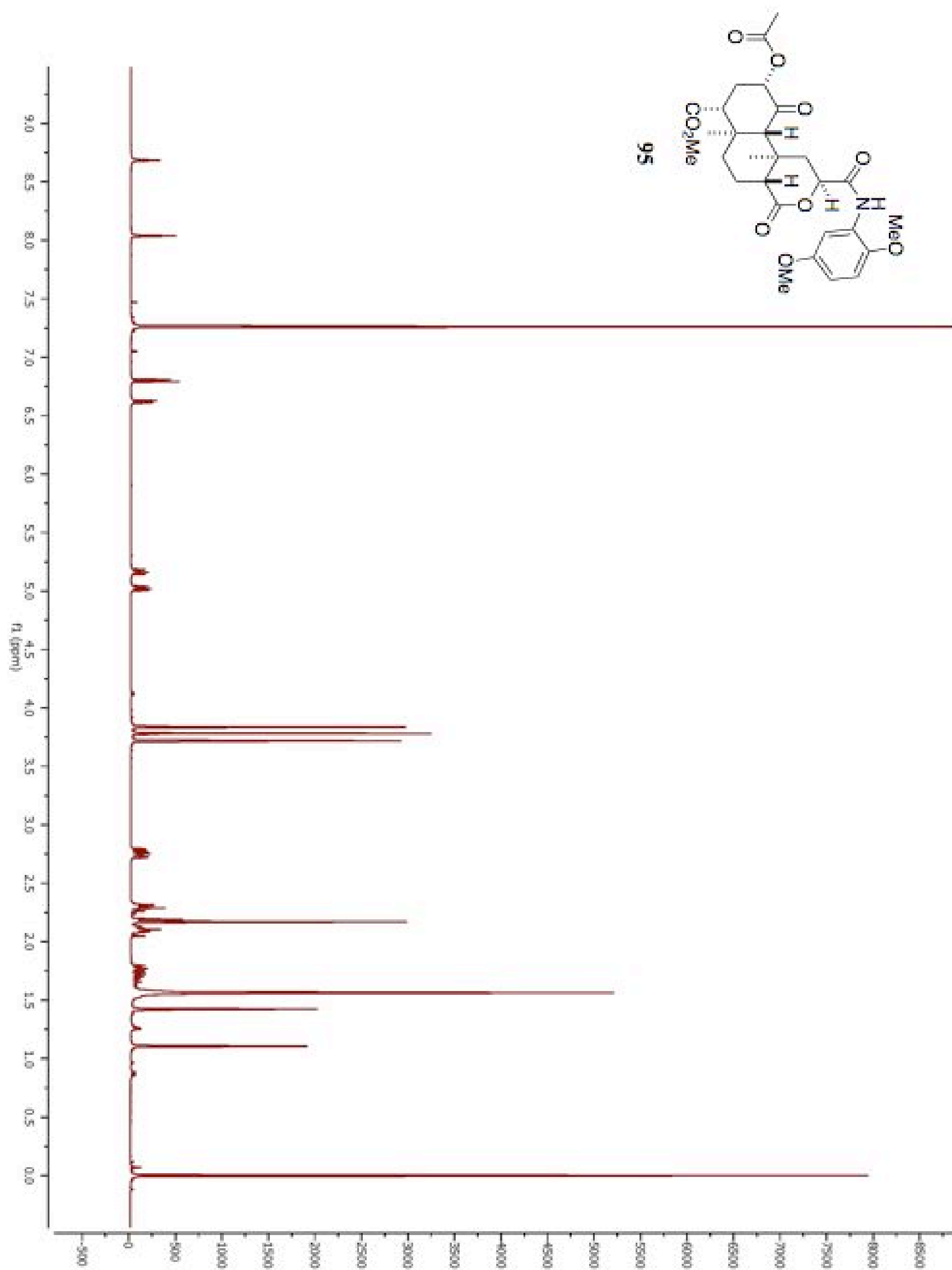
91
CO2Me
OMe
f1 (ppm)

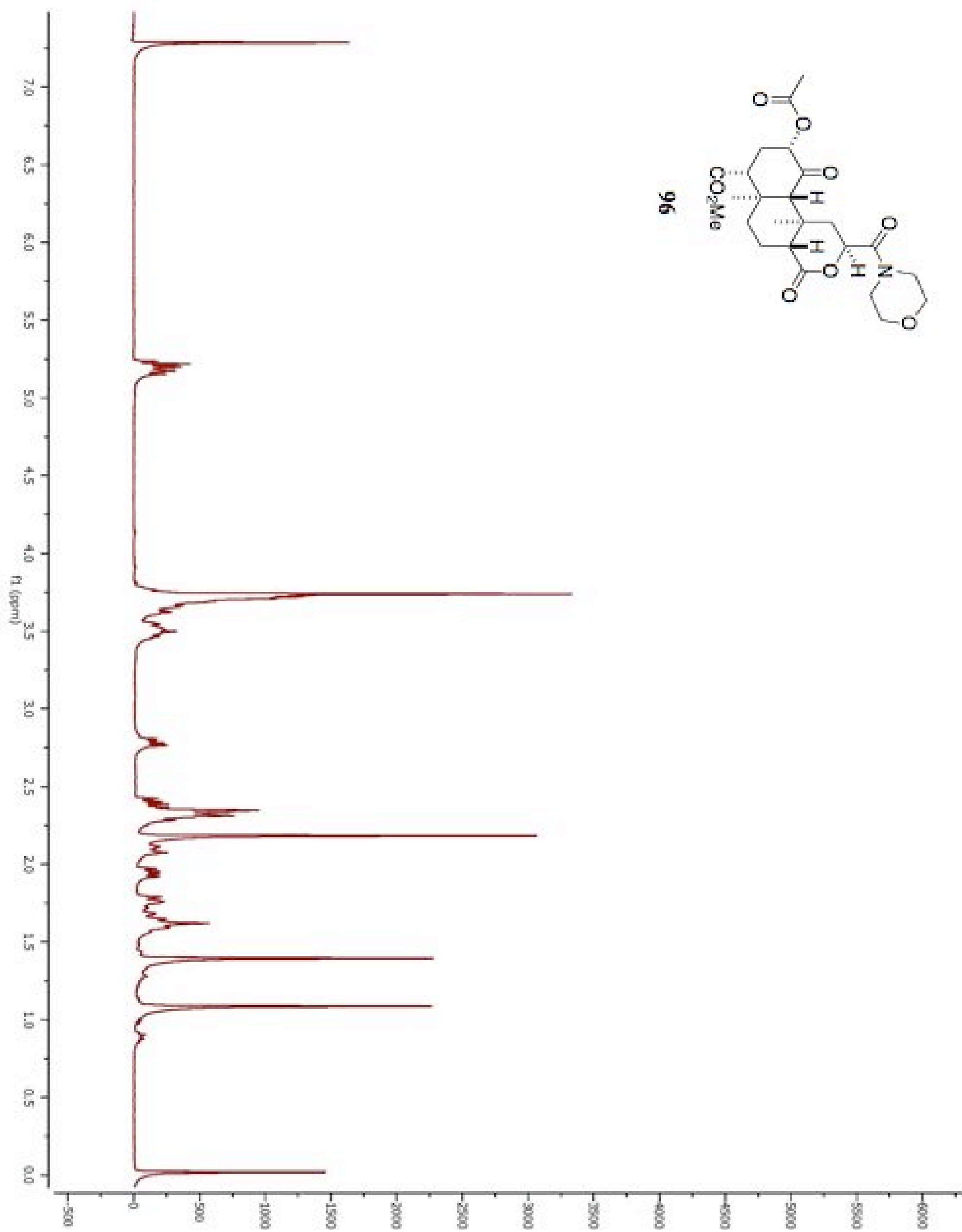

OMe
CO$_2$Me
92
f1 (ppm)


93
OMe
OMe
CO_2Me
f1 (ppm)

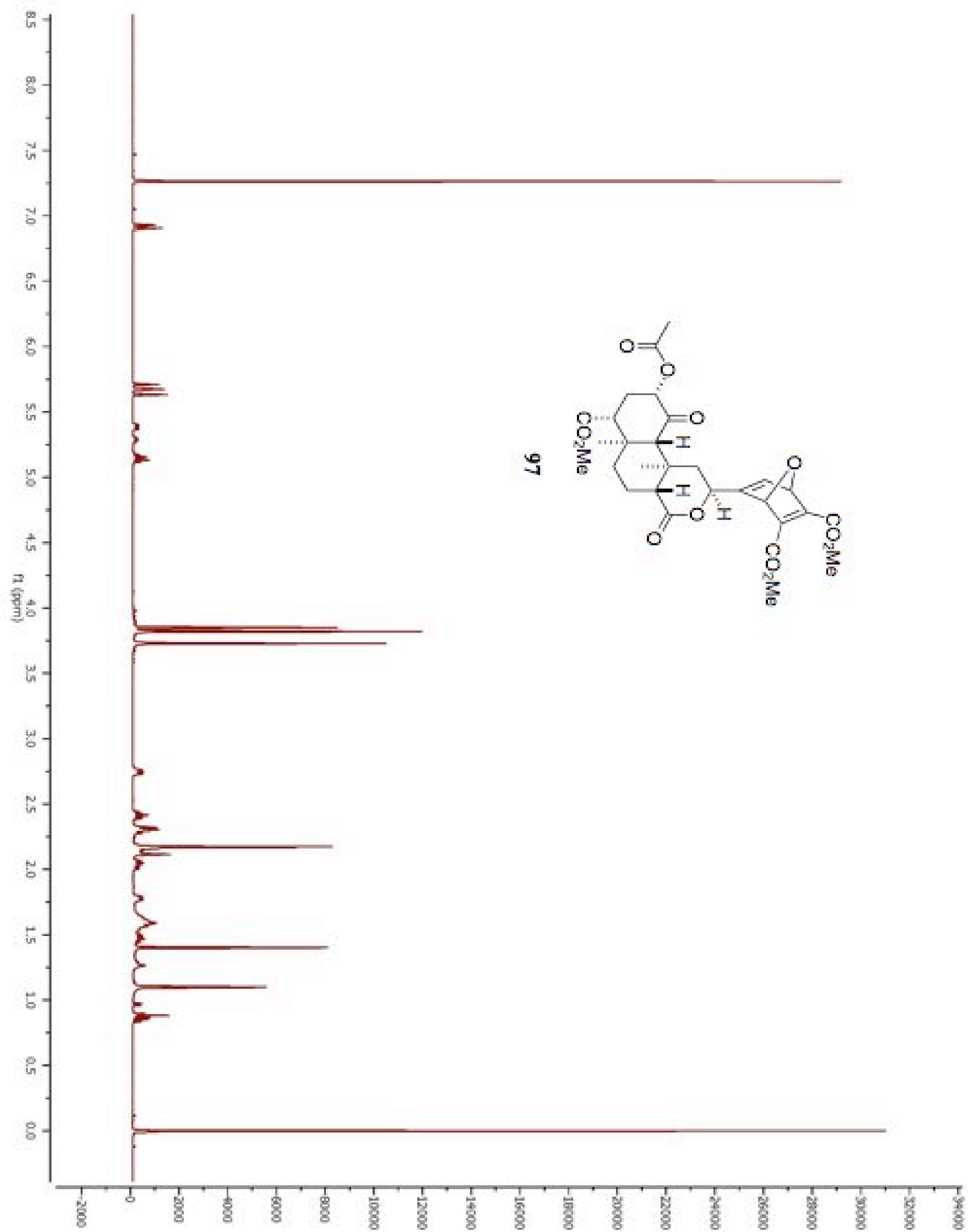


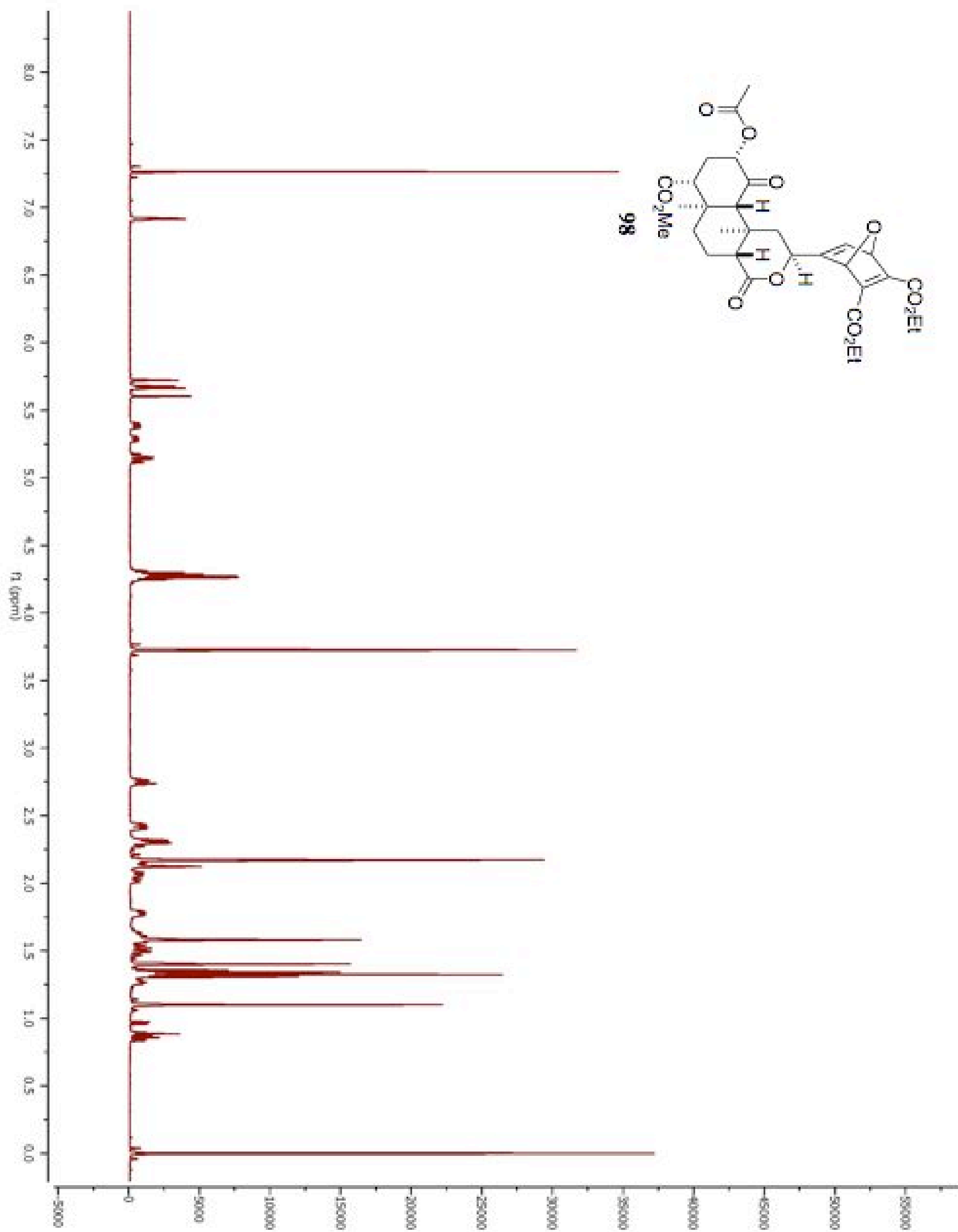
94
MeO
OMe
CO_2Me
f1 (ppm)

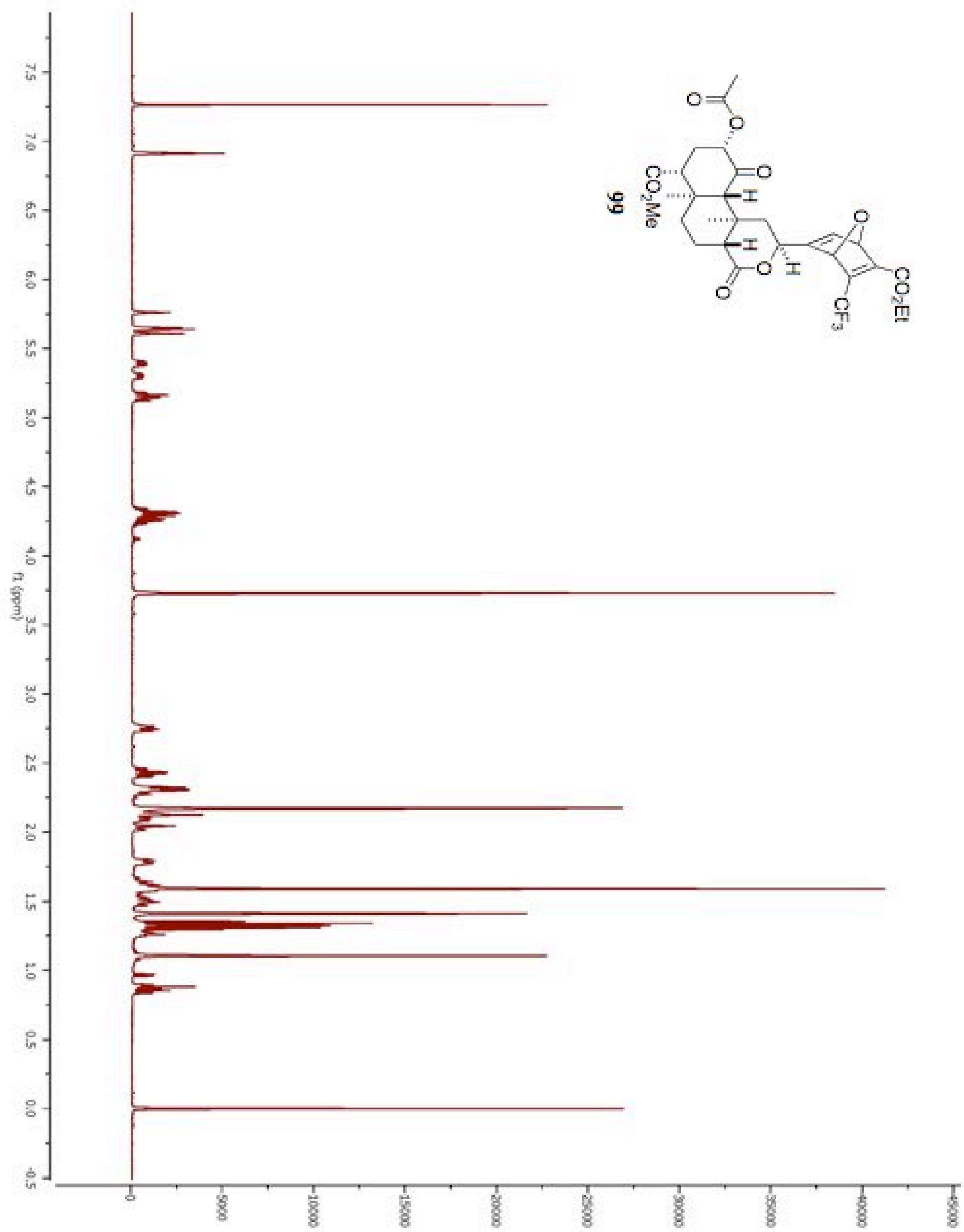

95
CO2Me
MeO
OMe
f1 (ppm)

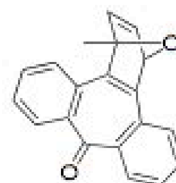


96
CO2Me
f1 (ppm)

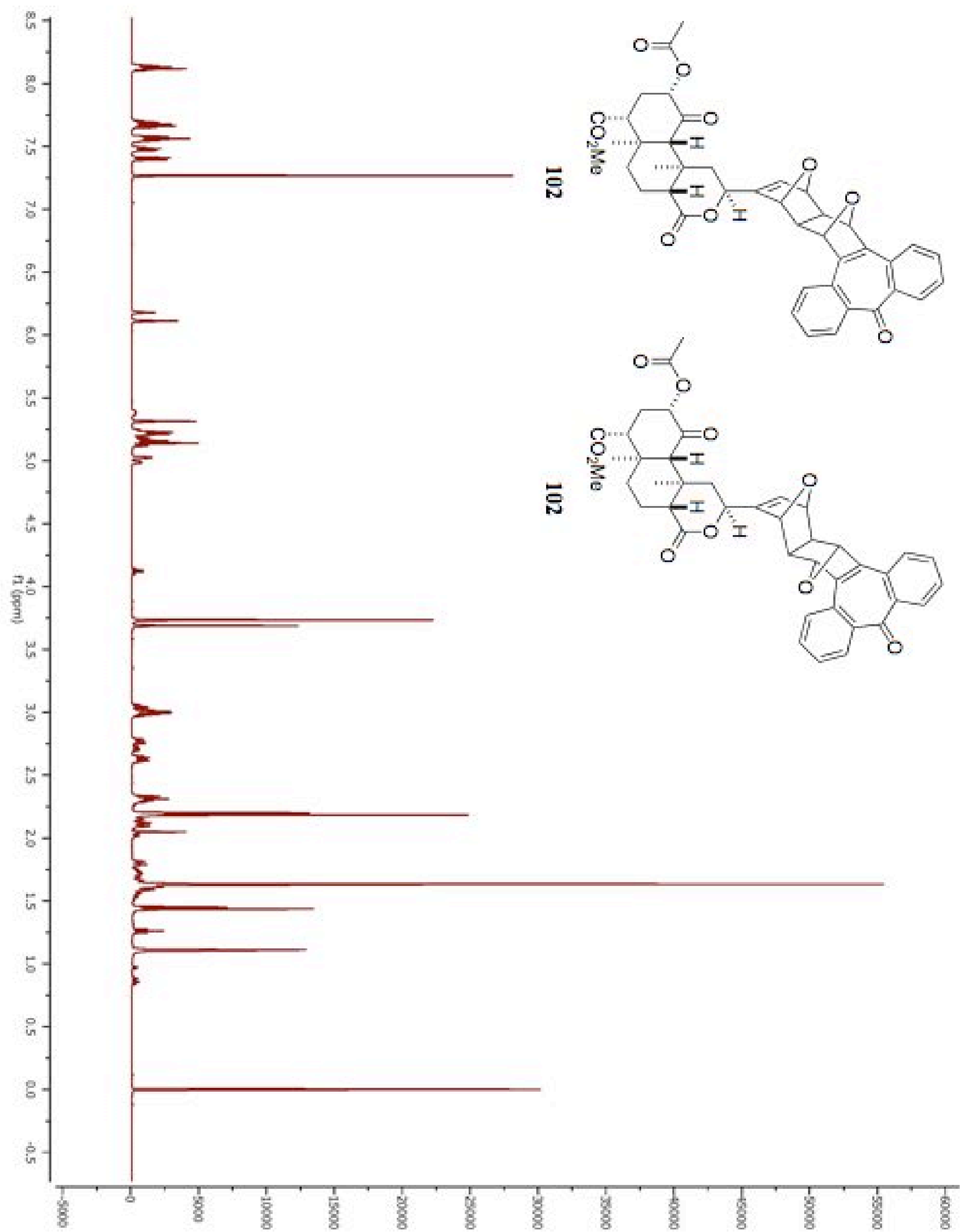

97
CO2Me
CO2Me
CO2Me
f1 (ppm)


98
CO2Me
CO2Et
CO2Et
f1 (ppm)

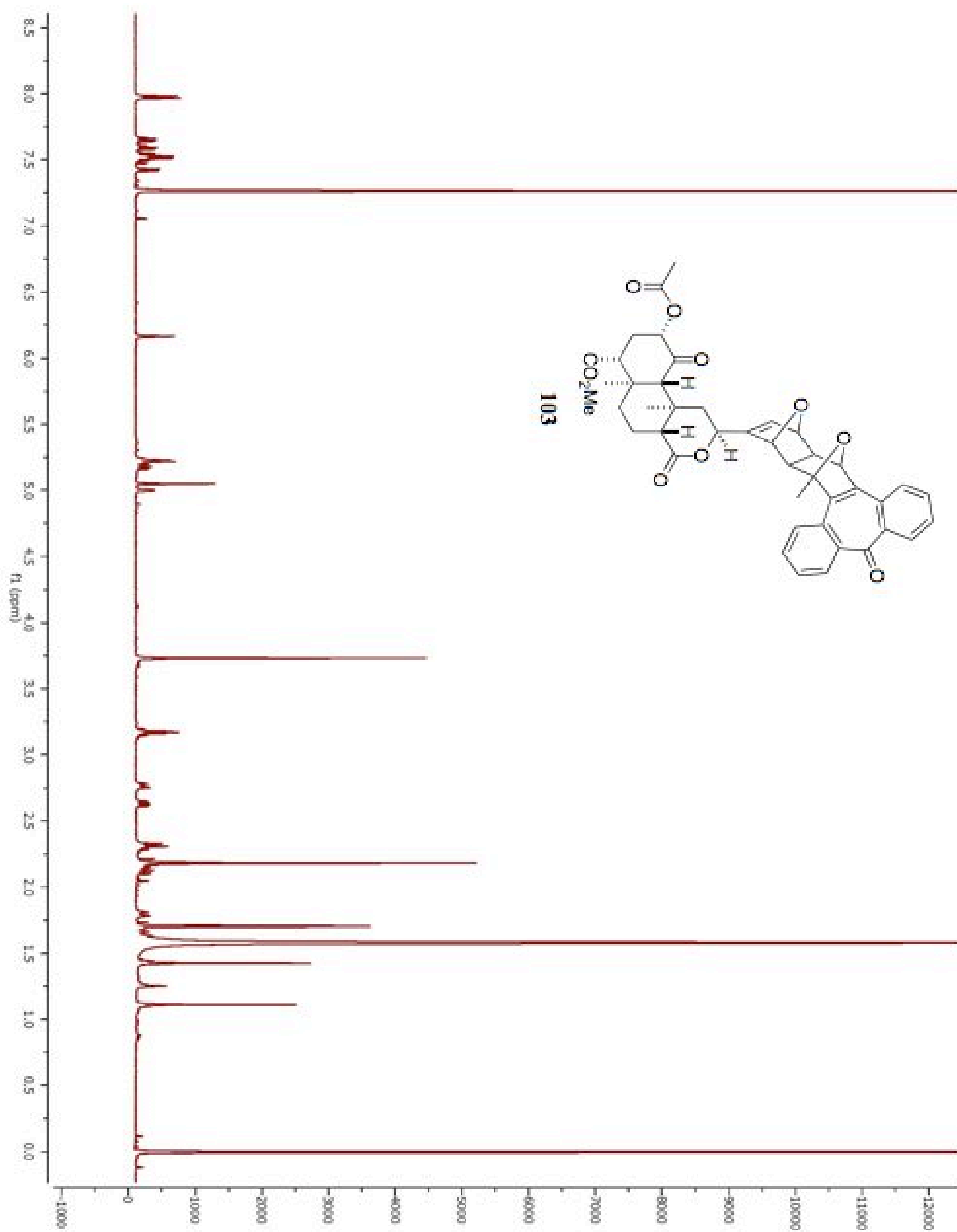

CO2Et
CF3
O
H
O
O
H
H
O
CO2Me
O
O
99
f1 (ppm)

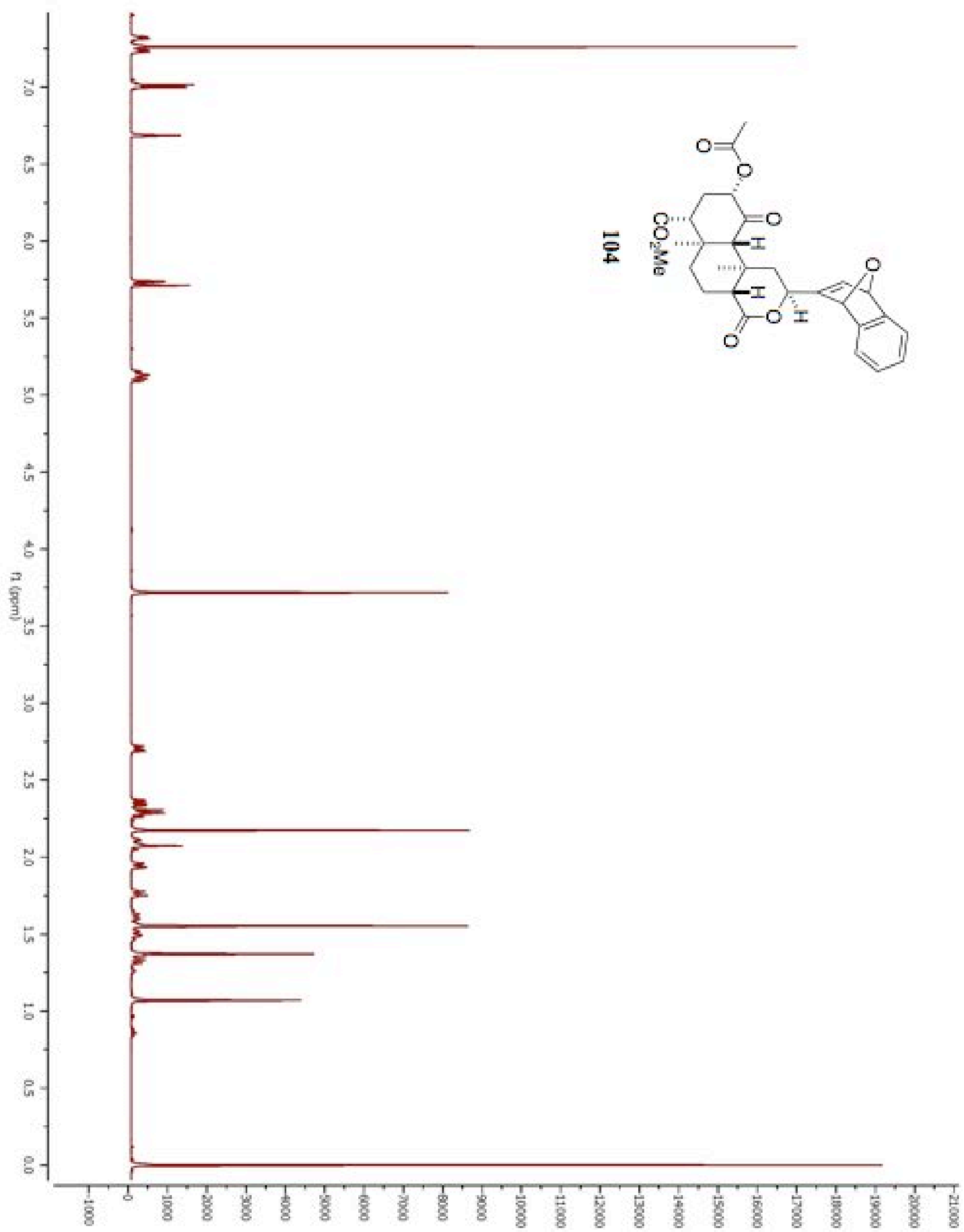


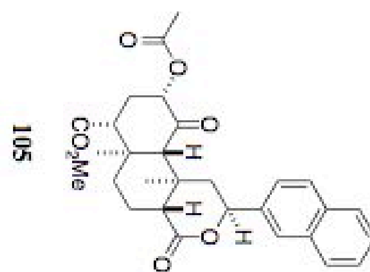
101

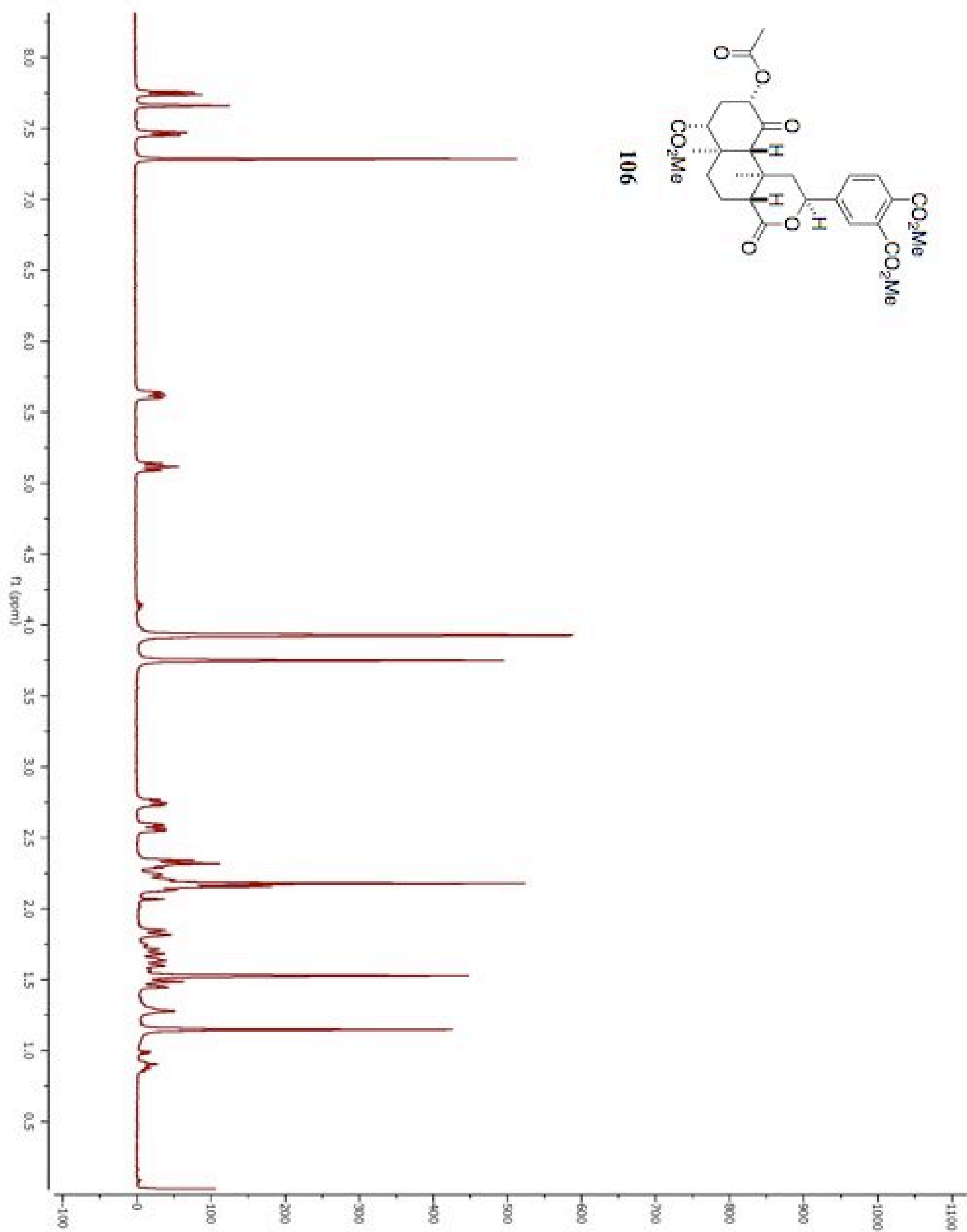


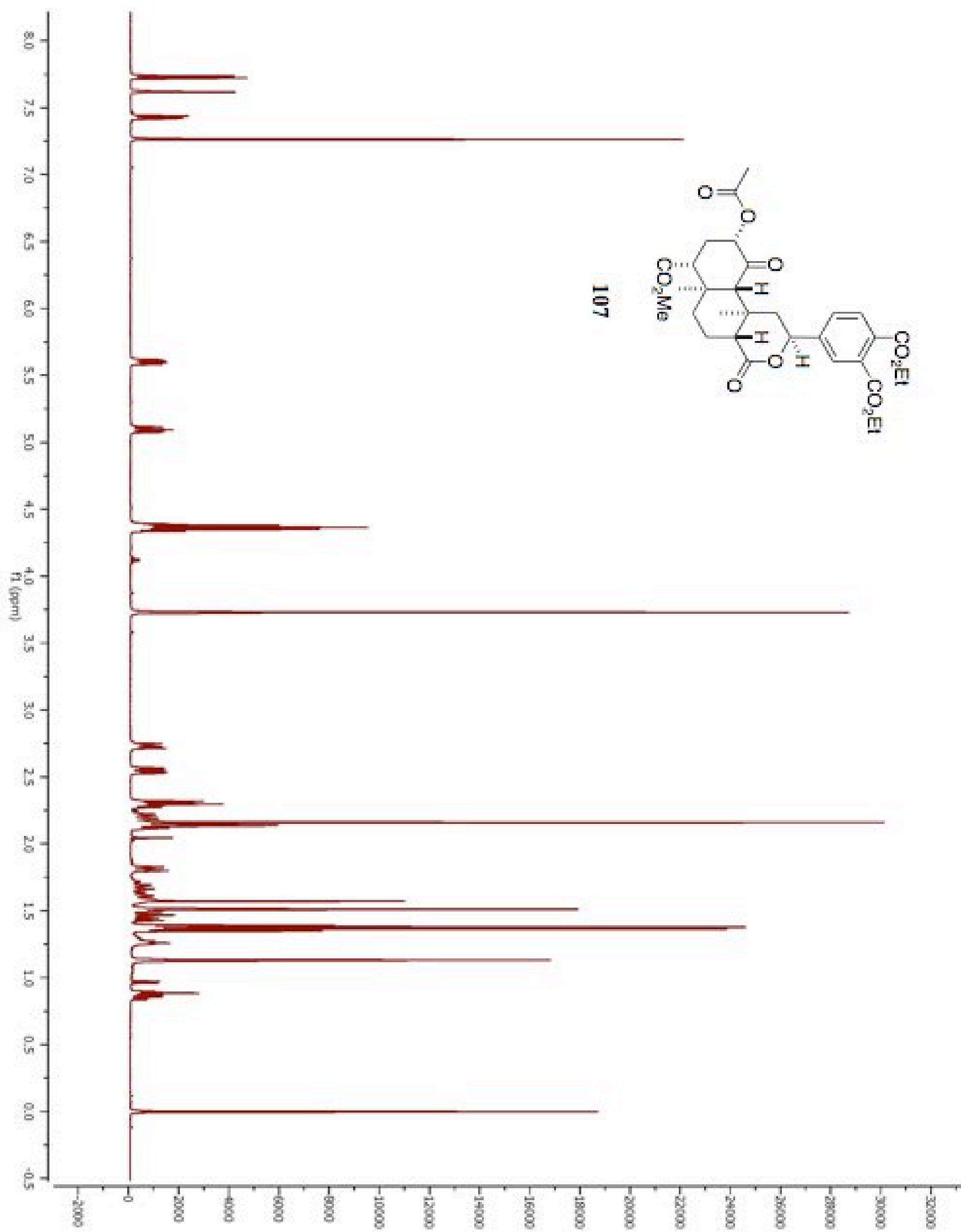
102
CO2Me
102
CO2Me
f1 (ppm)


103
CO2Me
f1 (ppm)

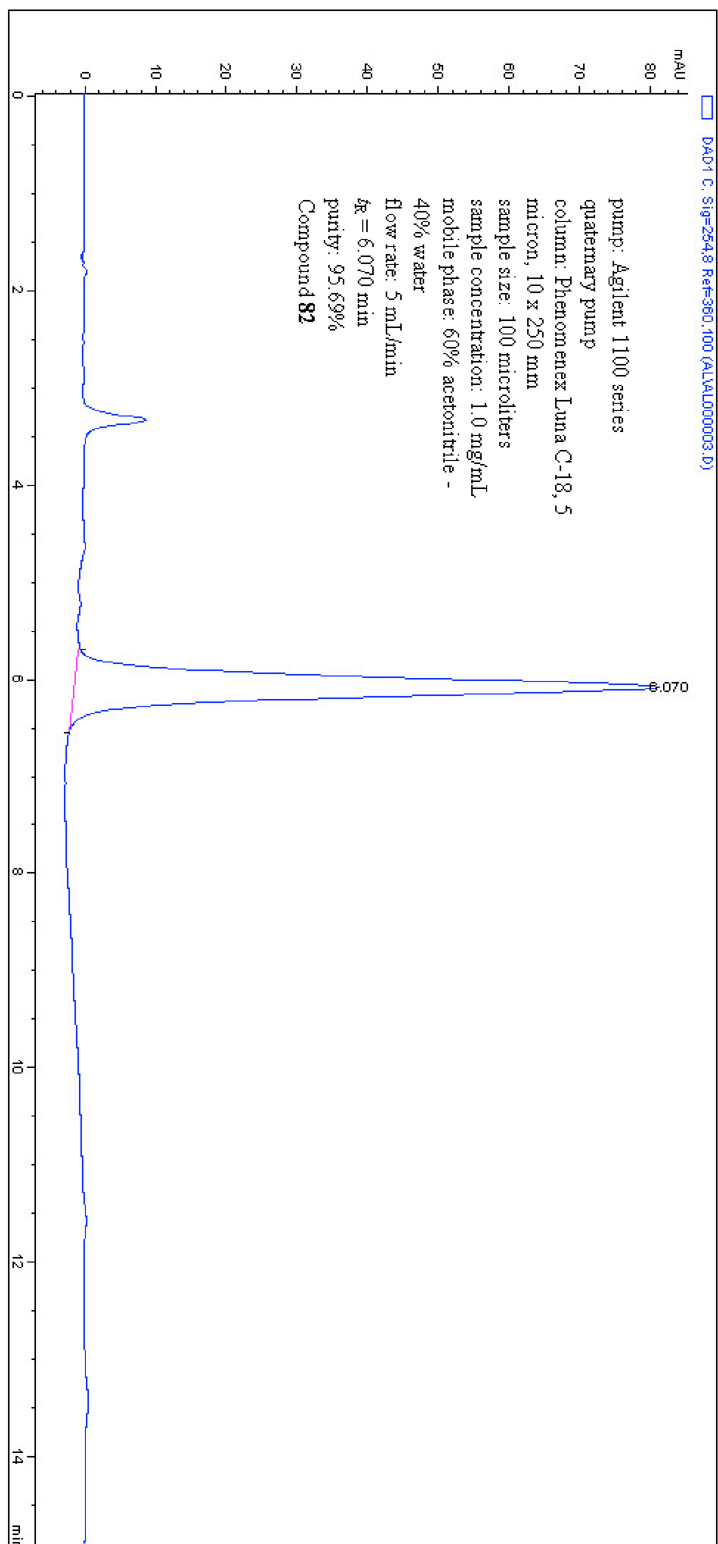

104
CO2Me
f1 (ppm)

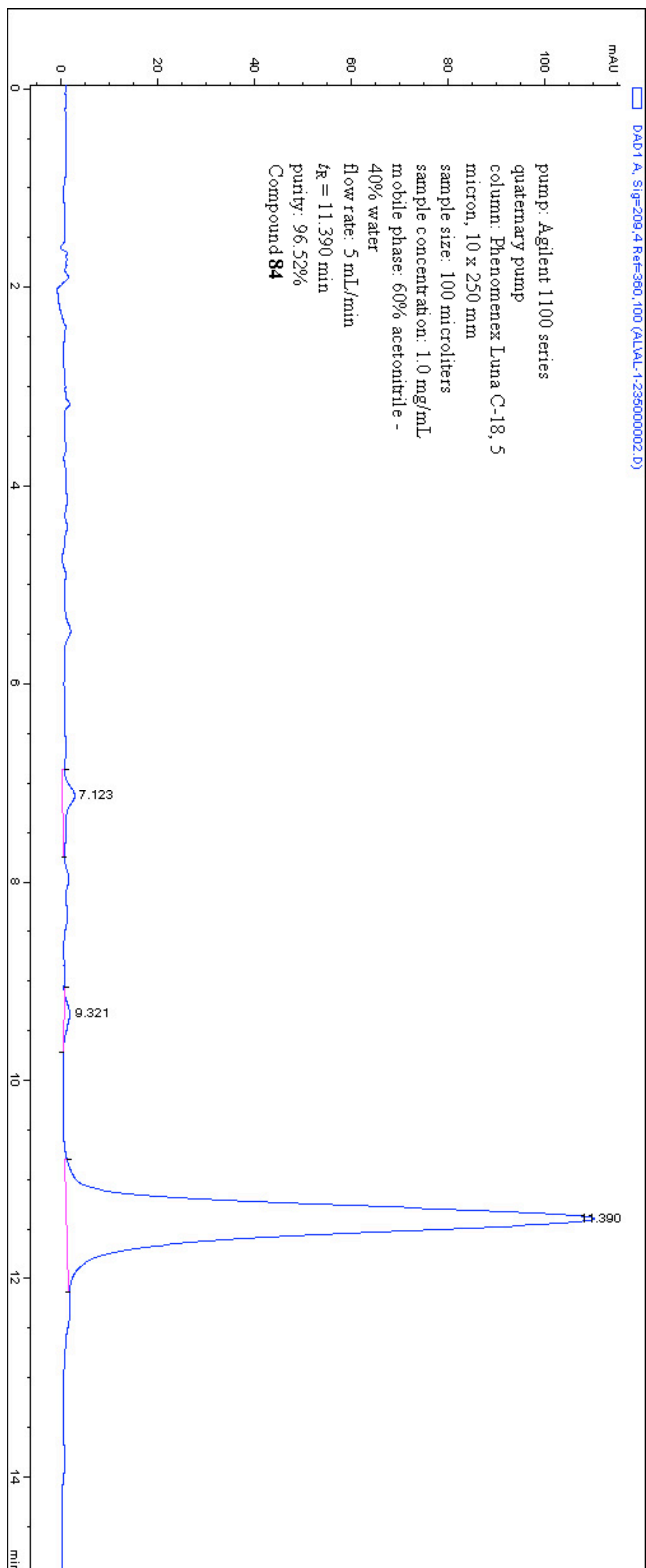

105
CO2Me

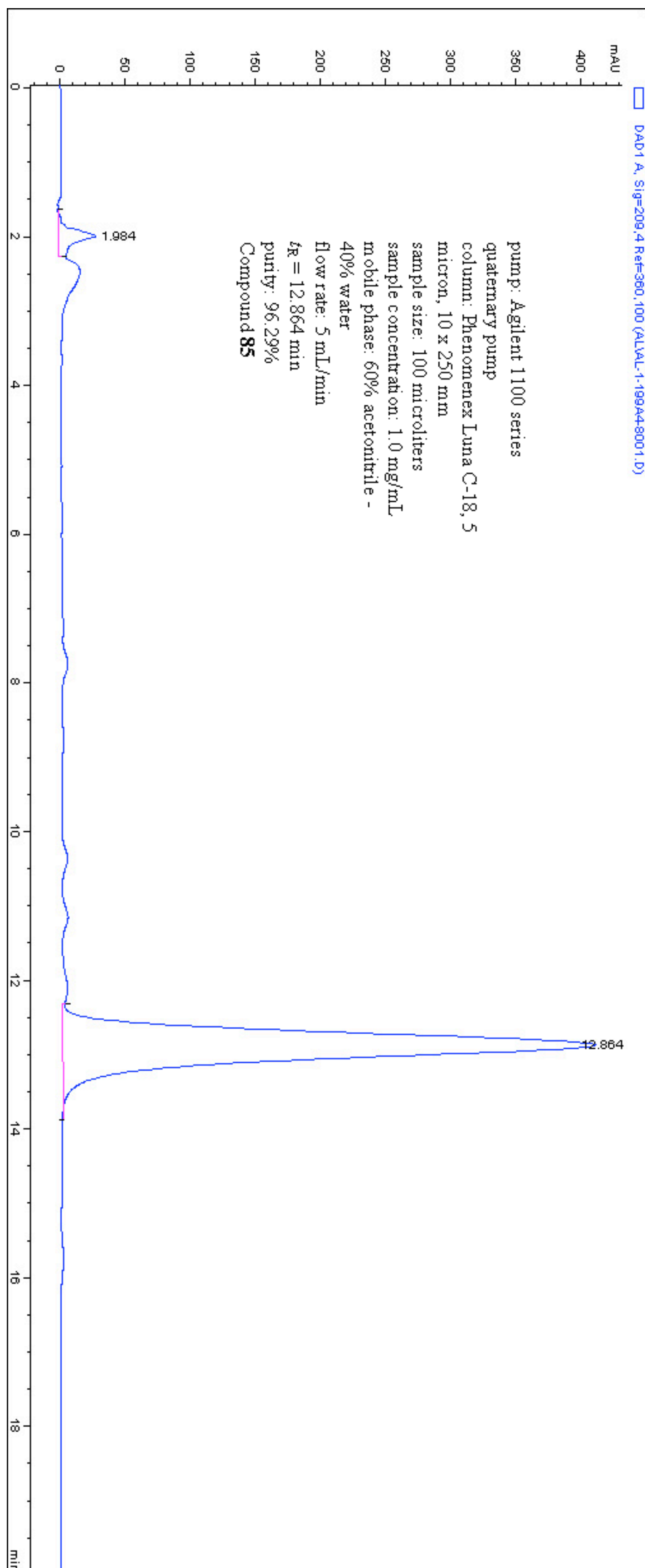

106
CO2Me
CO2Me
CO2Me
f1 (ppm)

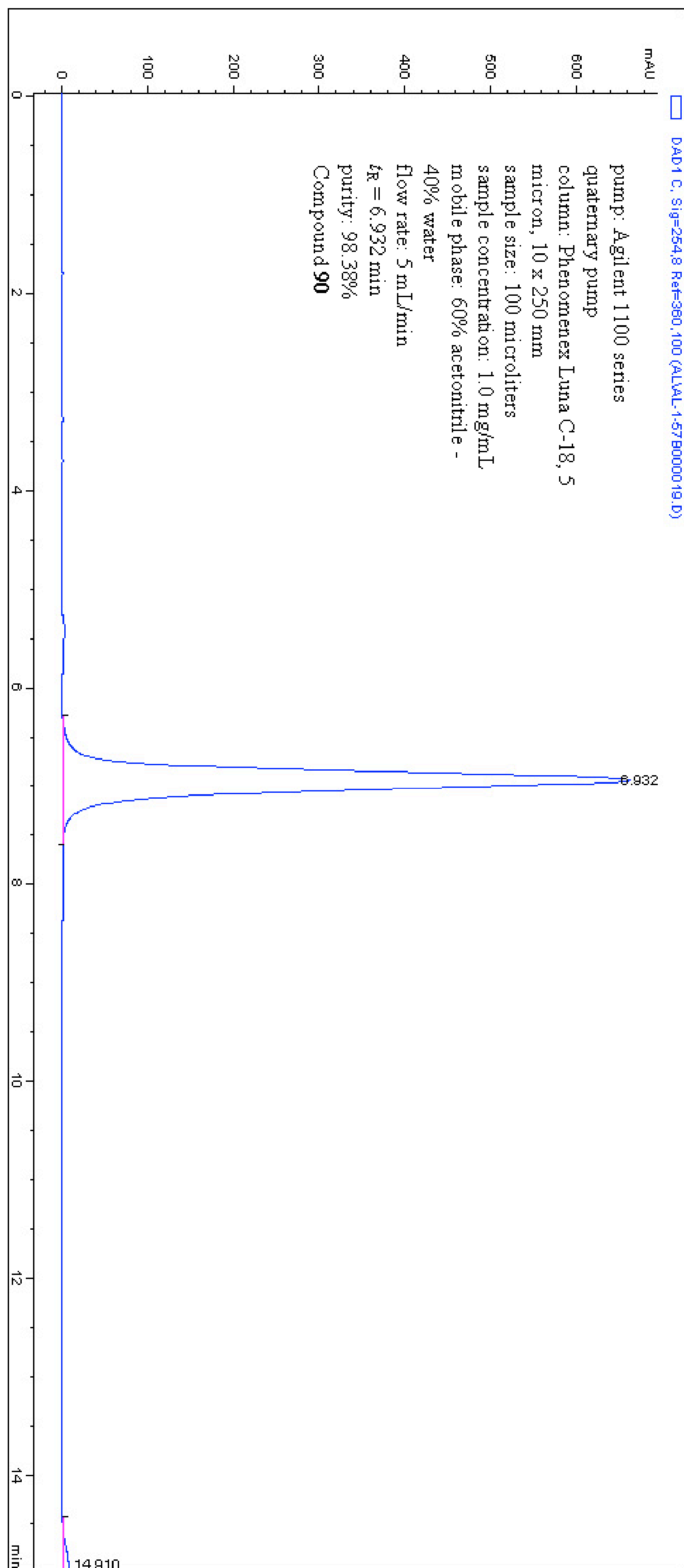

107
CO2Me
CO2Et
CO2Et
f1 (ppm)

APPENDIX B: HPLC CHROMATOGRAMS

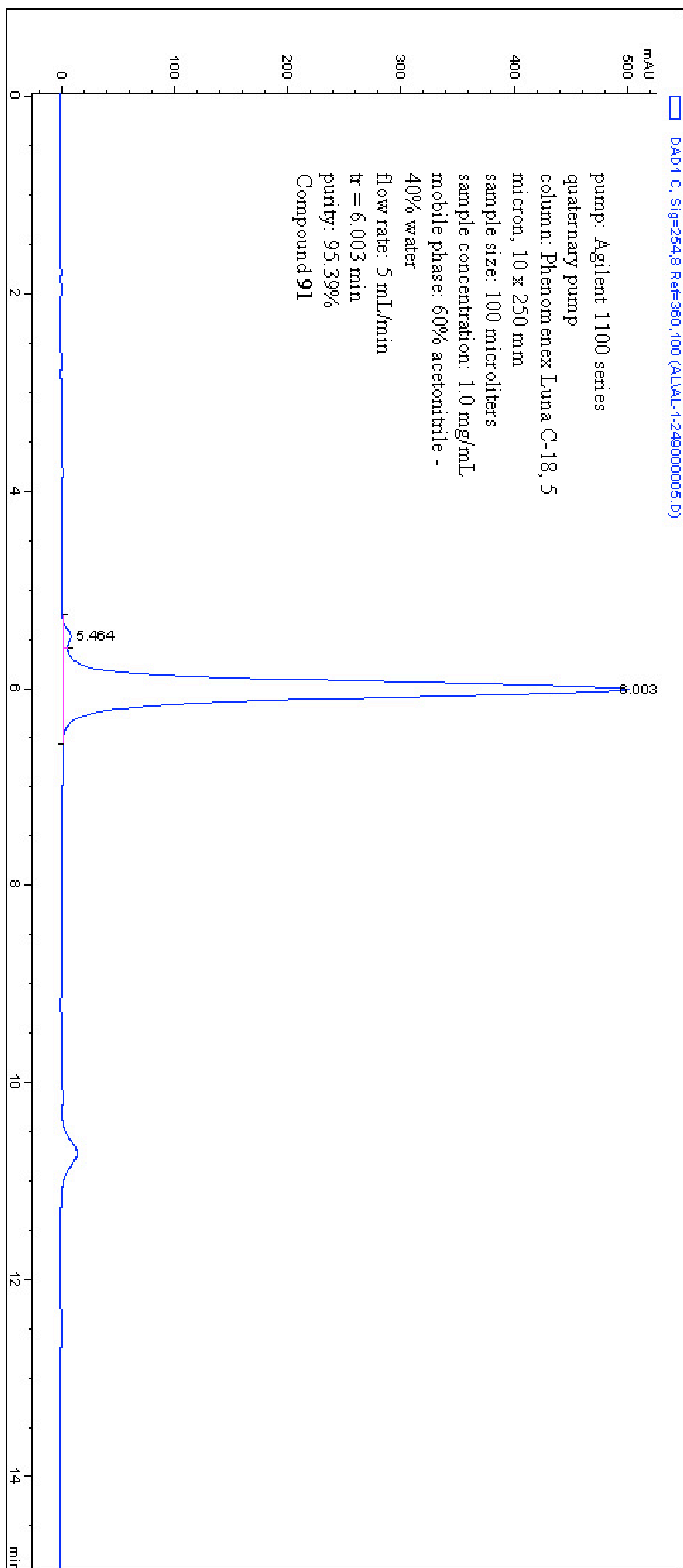

DAD1 C, Sig=254,8 Ref=360,100 (ALAL000003.D)
mAU
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
tR = 6.070 min
purity: 95.69%
Compound 82
6.070
min


DAD1 A, Sig=209,4 Ref=360,100 (AL\AL-1-235000002.D)
mAU
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
tR = 11.390 min
purity: 96.52%
Compound 84
7.123
9.321
11.390
min

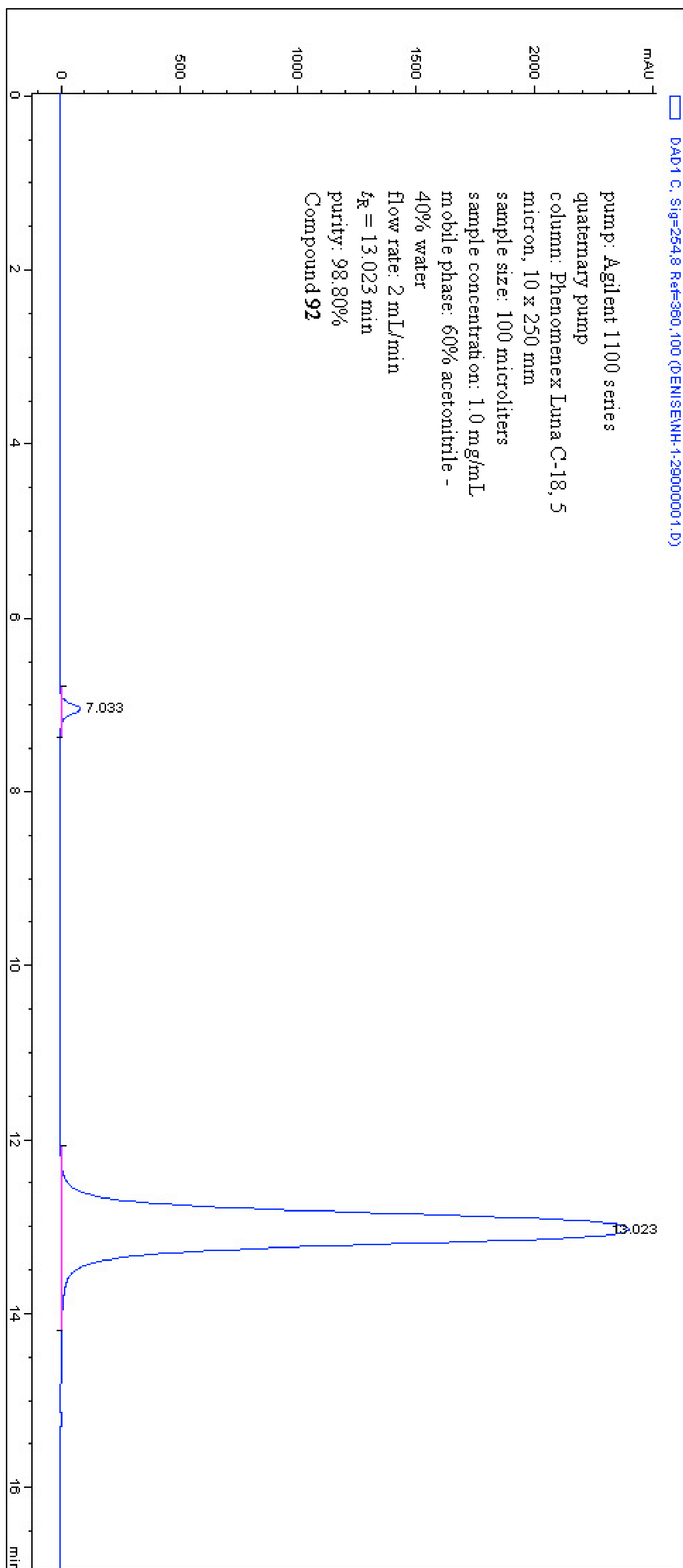

DAD1 A, Sig=209,4 Ref=360,100 (AL\AL-1-199A4-8001.D)
mAU
400
350
300
250
200
150
100
50
0
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
t_R = 12.864 min
purity: 96.29%
Compound **85**
1.984
12.864
0
2
4
6
8
10
12
14
16
18
min



DAD1 C, Sig=254,8 Ref=360,100 (ALVAL-1-57B00019.D)
mAU
600
500
400
300
200
100
0
pump: Agilent 1100 series quaternary pump
column: Phenomenex Luna C-18, 5 micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile - 40% water
flow rate: 5 mL/min
t_R = 6.932 min
purity: 98.38%
Compound **90**
6.932
14.910
0
2
4
6
8
10
12
14
min



DAD1 C, Sig=254,8 Ref=360,100 (AL\AL-1-249000005.D)
mAU
500
400
300
200
100
0
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
tr = 6.003 min
purity: 95.39%
Compound 91
6.003
5.464
0
2
4
6
8
10
12
14
min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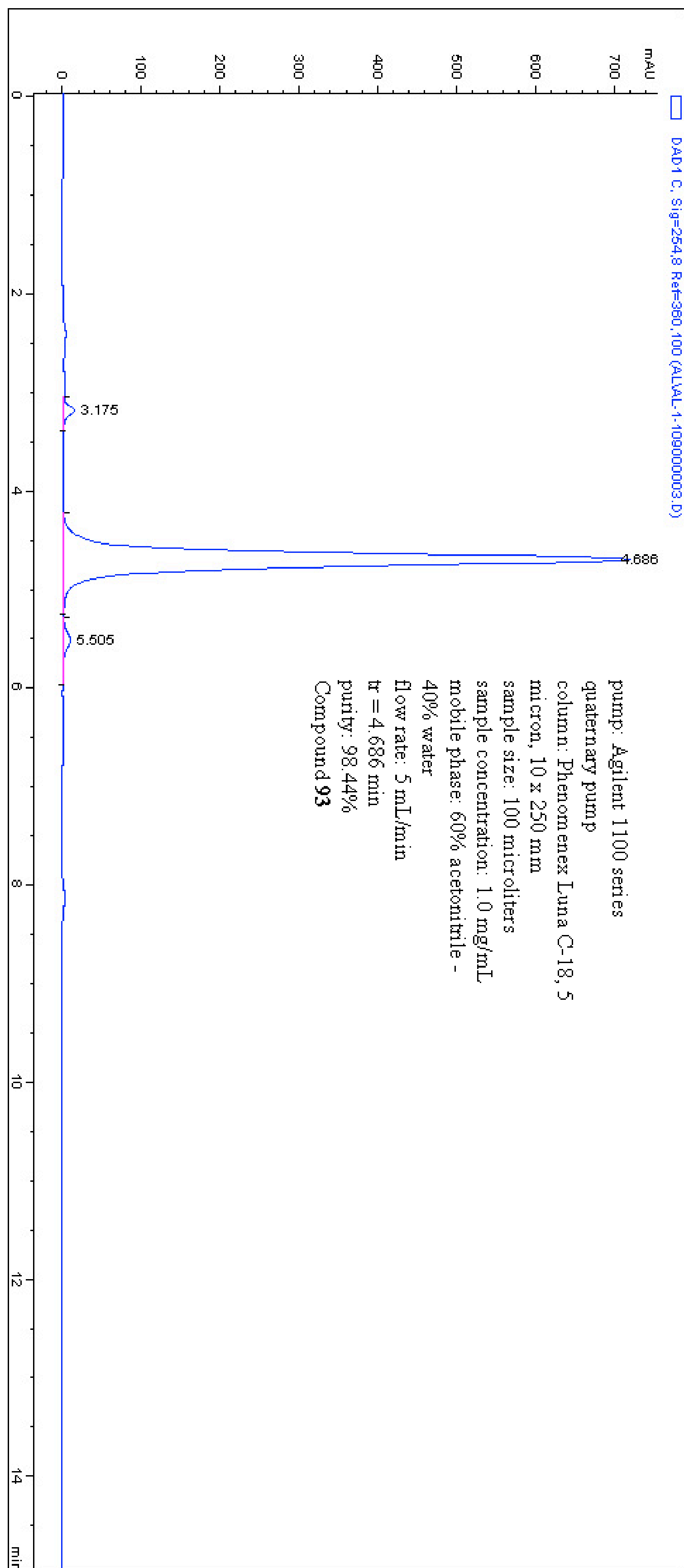
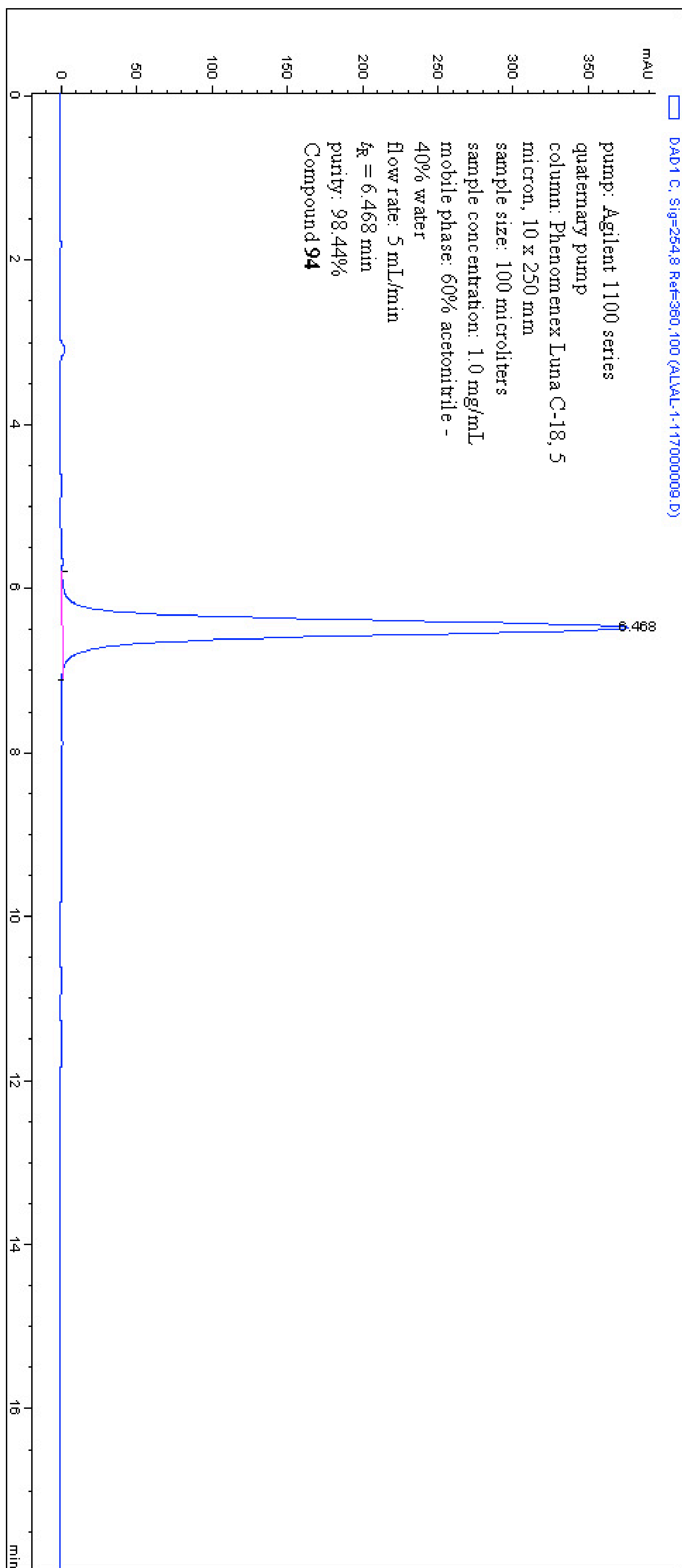
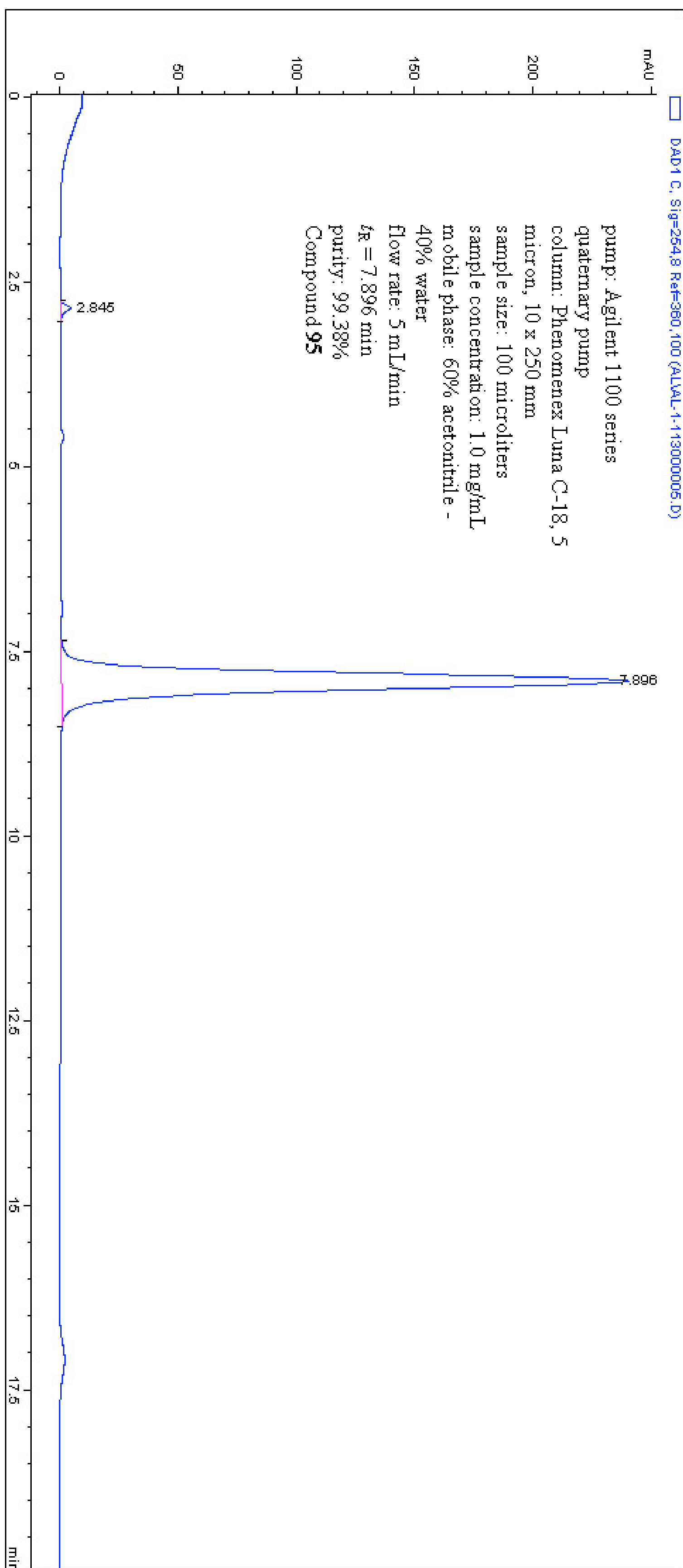
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 2 mL/min
t_R = 13.023 min
purity: 98.80%
Compound **92**
mAU
2000
1500
1000
500
0
7.033
13.023
0
2
4
6
8
10
12
14
16
min


DAD1 C, Sig=254,8 Ref=360,100 (AL\AL-1-10900003.D)
mAU
700
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
min
3.175
4.686
5.505
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
tr = 4.686 min
purity: 98.44%
Compound **93**


DAD1 C, Sig=254,8 Ref=360,100
mAU
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
t_R = 6.468 min
purity: 98.44%
Compound **94**
6.468
min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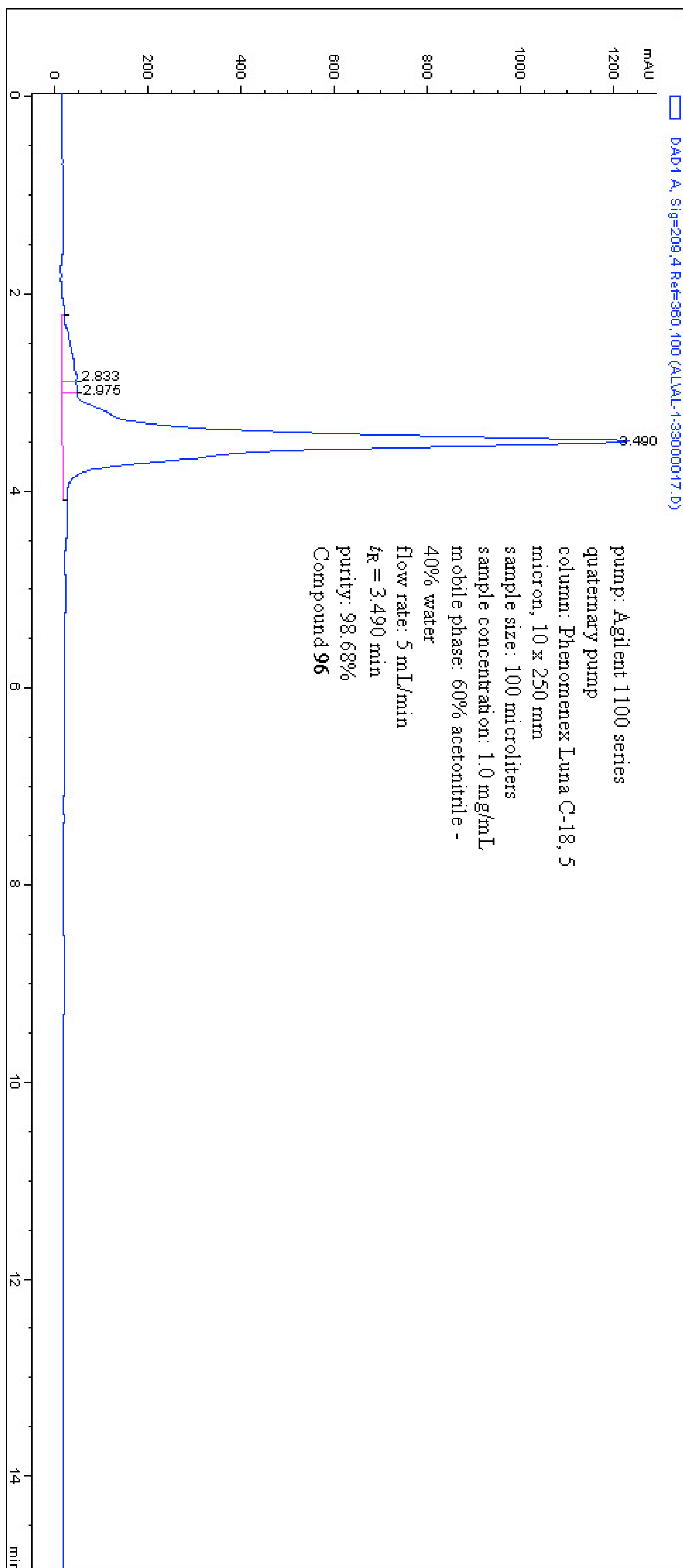
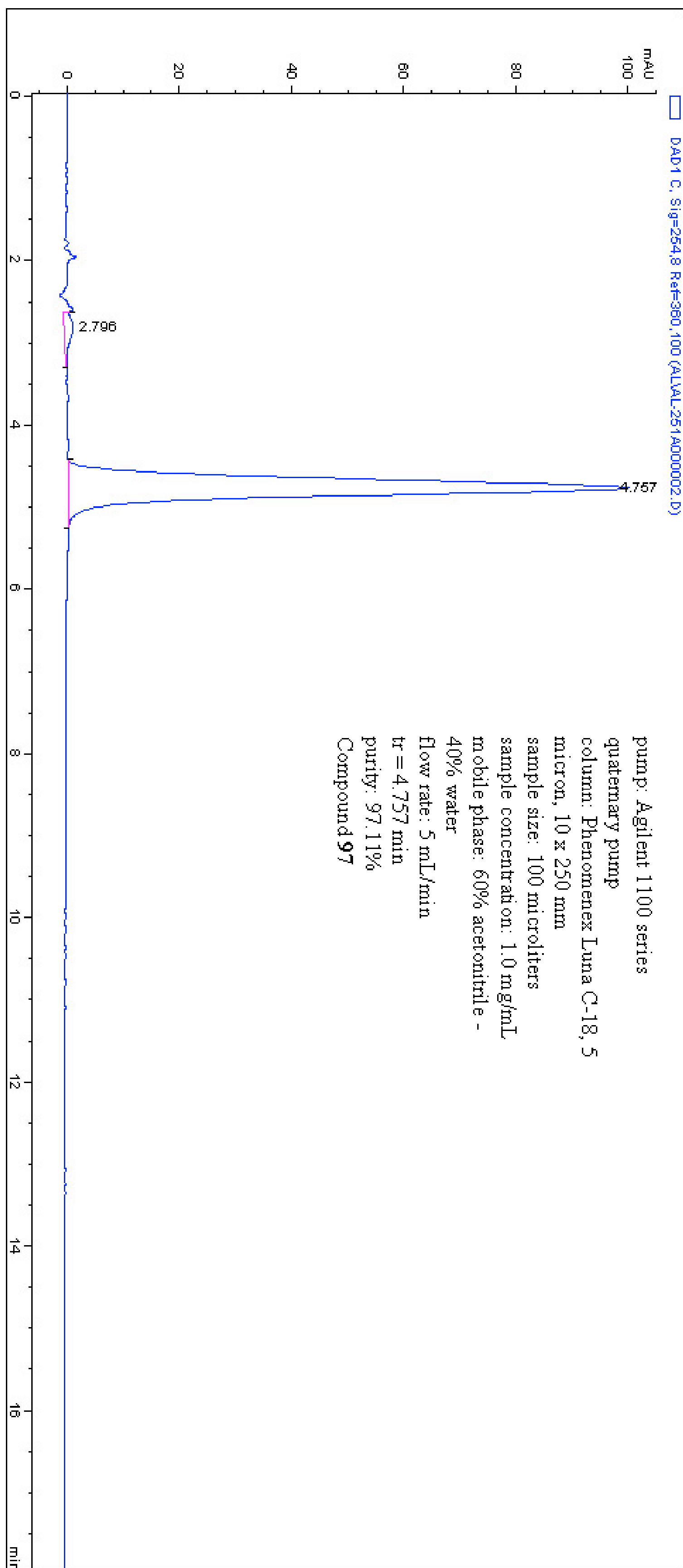
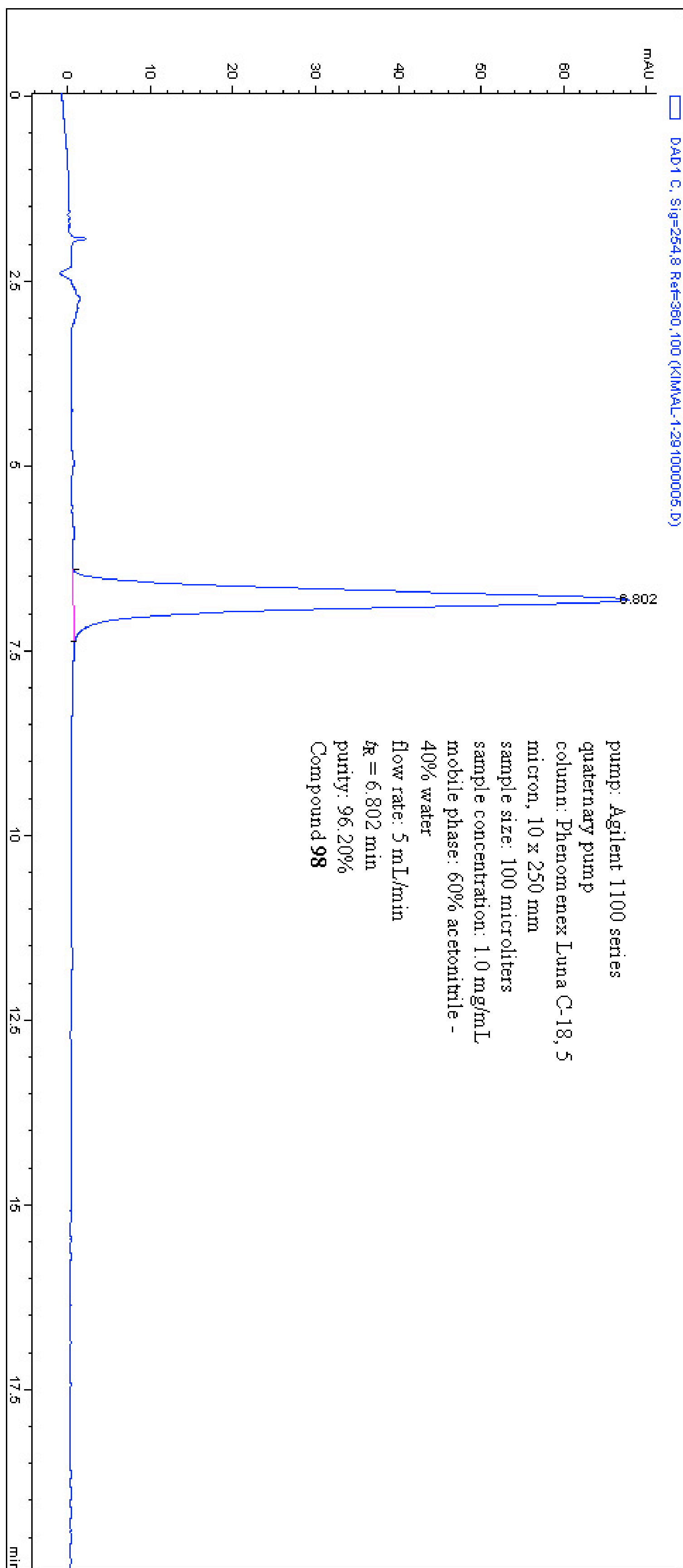
mAU
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
tR = 7.896 min
purity: 99.38%
Compound 95
2.845
7.896
min



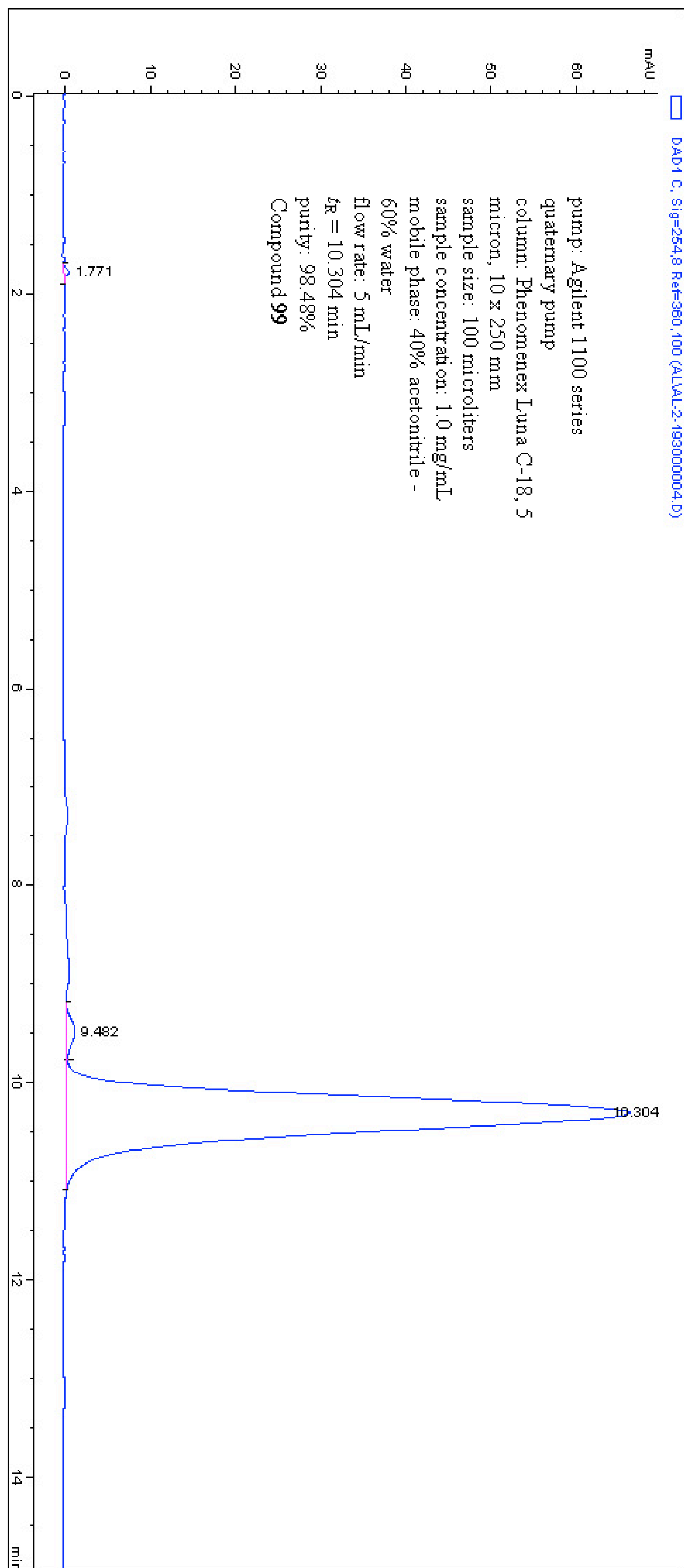
DAD1 A, Sig=209,4 Ref=360,100 (ALVAL-1-33000017.D)
mAU
1200
1000
800
600
400
200
0
2.833
2.975
3.490
0
2
4
6
8
10
12
14
min
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
tR = 3.490 min
purity: 98.68%
Compound 96

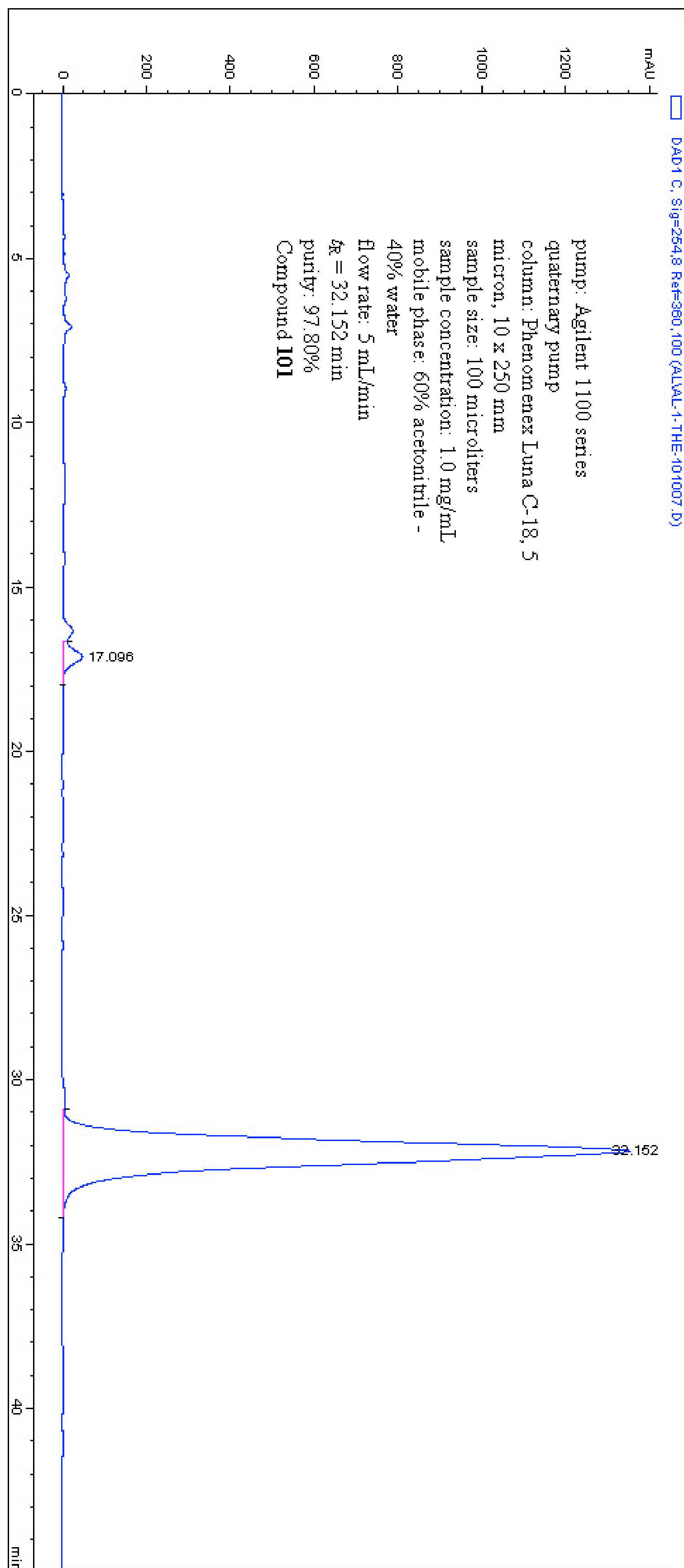


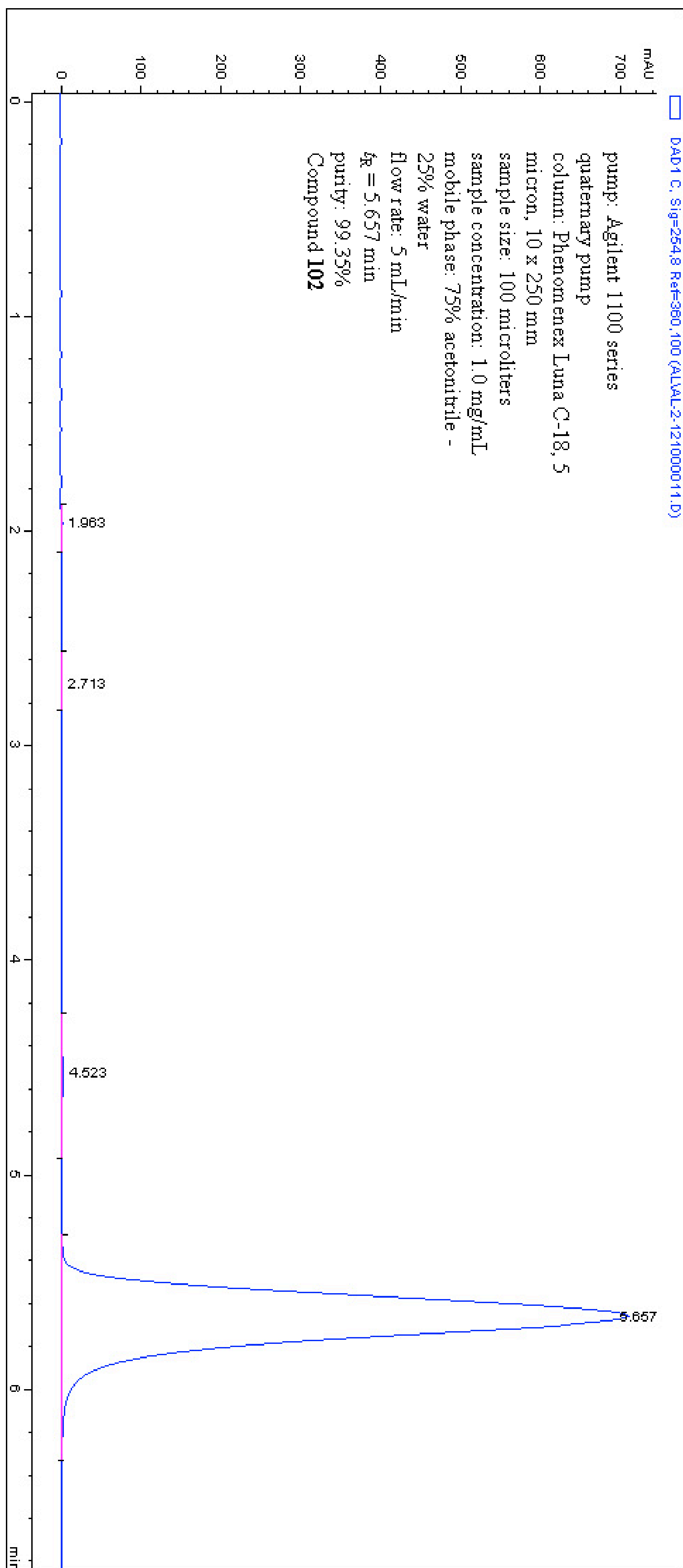
DAD1 C, Sig=254,8 Ref=360,100 (ALVAL-251A000002.D)
mAU
100
80
60
40
20
0
2.796
4.757
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
tr = 4.757 min
purity: 97.11%
Compound **97**
0
2
4
6
8
10
12
14
16
min



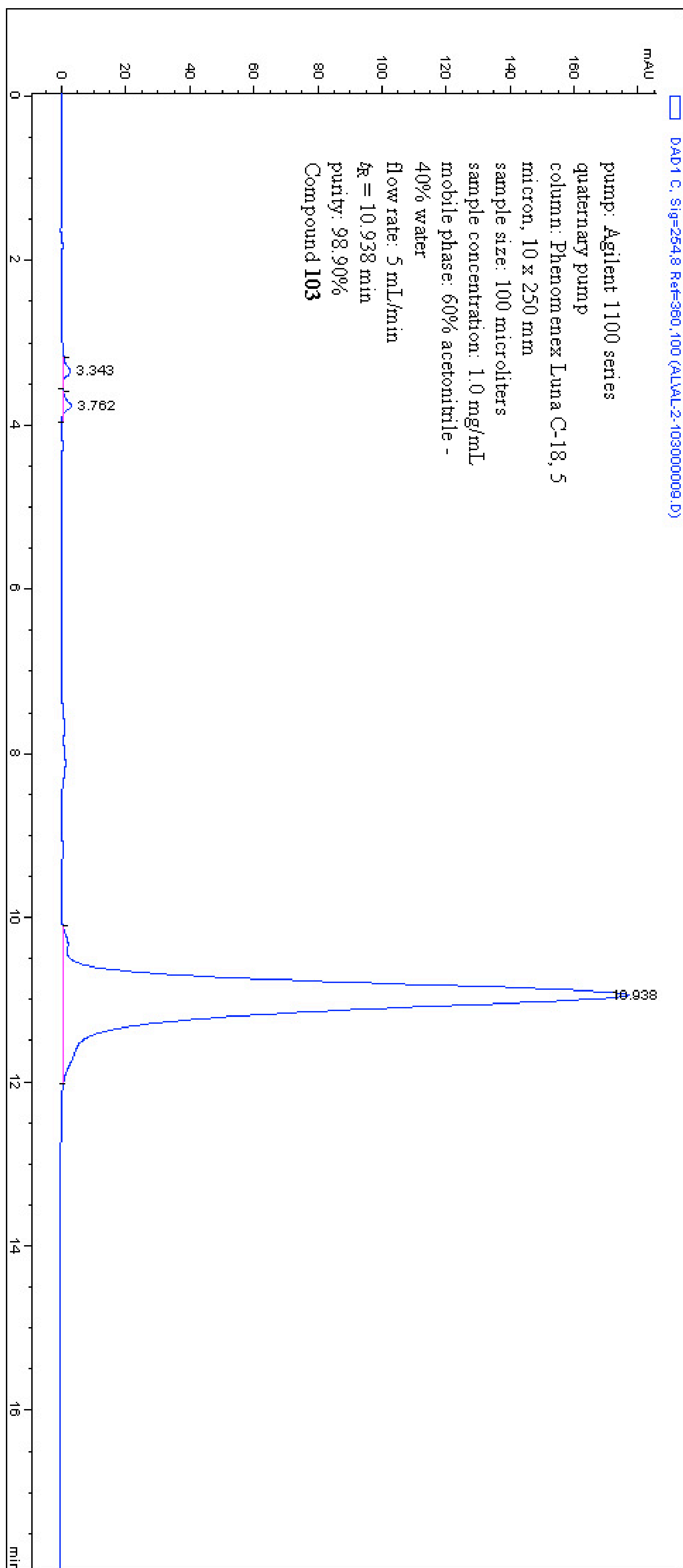
DAD1 C, Sig=254,8 Ref=360,100 (KIMAL-1-291000005.D)
mAU
0
10
20
30
40
50
60
6.802
0
2.5
5
7.5
10
12.5
15
17.5
min
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
tR = 6.802 min
purity: 96.20%
Compound 98


DAD1 C, Sig=254,8 Ref=360,100 (AL\AL-2-19300004.D)
mAU
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
min
1.771
9.482
10.304
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 40% acetonitrile -
60% water
flow rate: 5 mL/min
tR = 10.304 min
purity: 98.48%
Compound 99

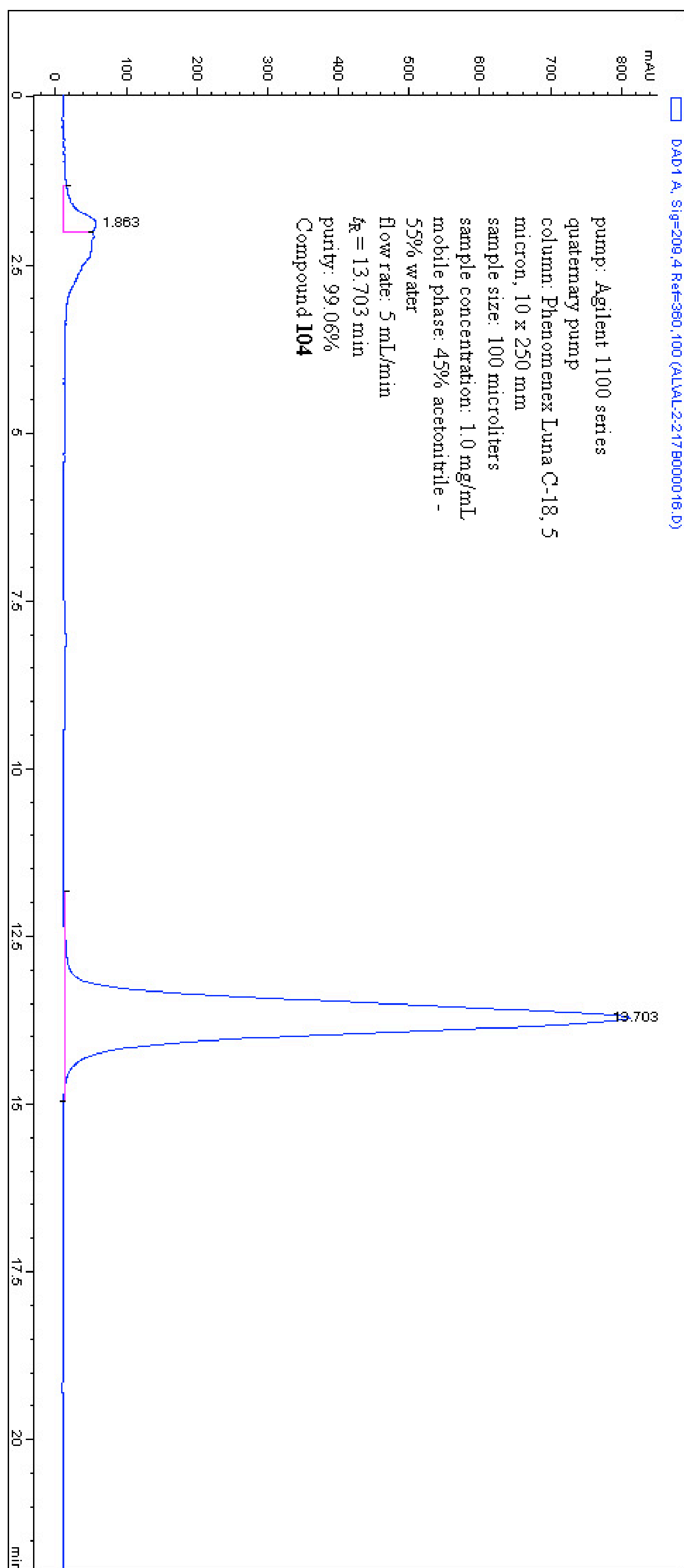

DAD1 C, Sig=254,8 Ref=360,100 (ALVAL-1-THE-101007.D)
mAU
1200
1000
800
600
400
200
0
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
t_R = 32.152 min
purity: 97.80%
Compound **101**
17.096
32.152
0
5
10
15
20
25
30
35
40
min



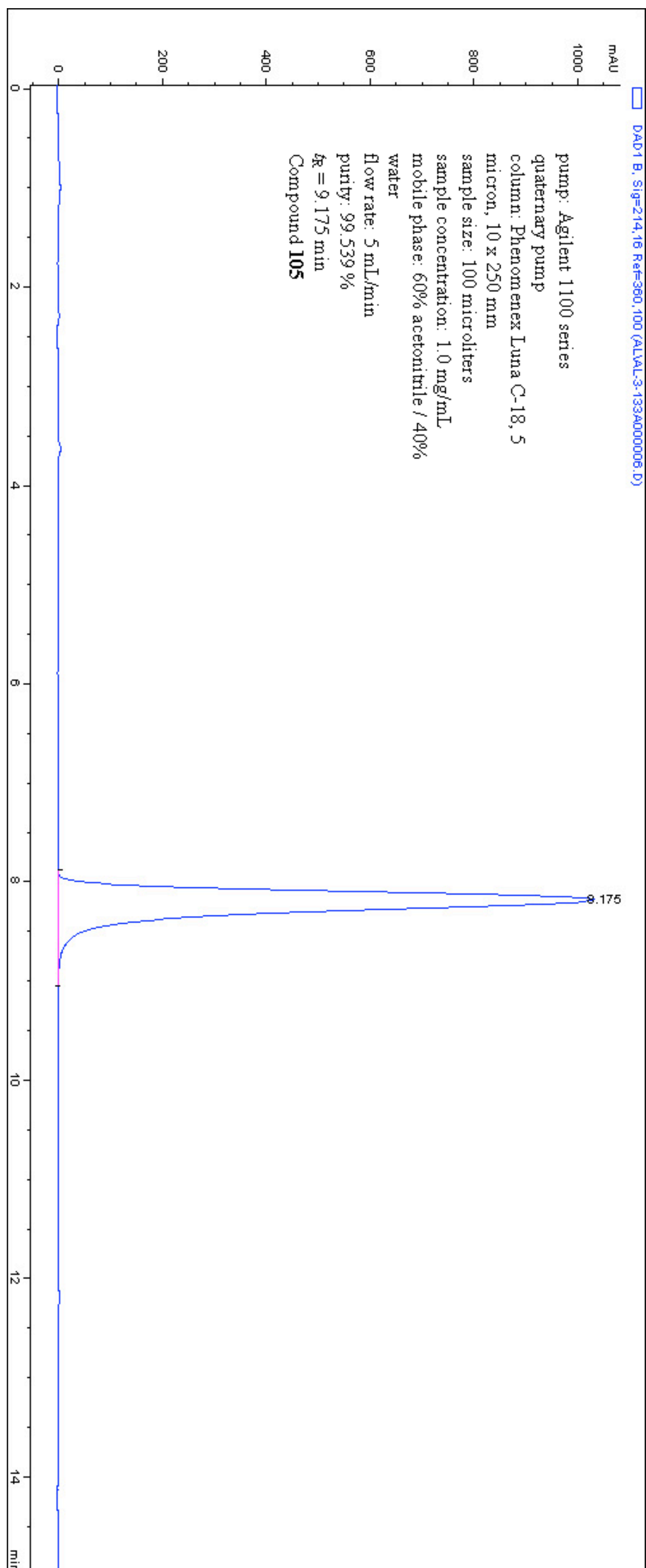
DAD1 C, Sig=254,8 Ref=360,100 (ALVAL-2-121000011.D)
mAU
700
600
500
400
300
200
100
0
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 75% acetonitrile -
25% water
flow rate: 5 mL/min
t_R = 5.657 min
purity: 99.35%
Compound **102**
1.963
2.713
4.523
5.657
0
1
2
3
4
5
6
min



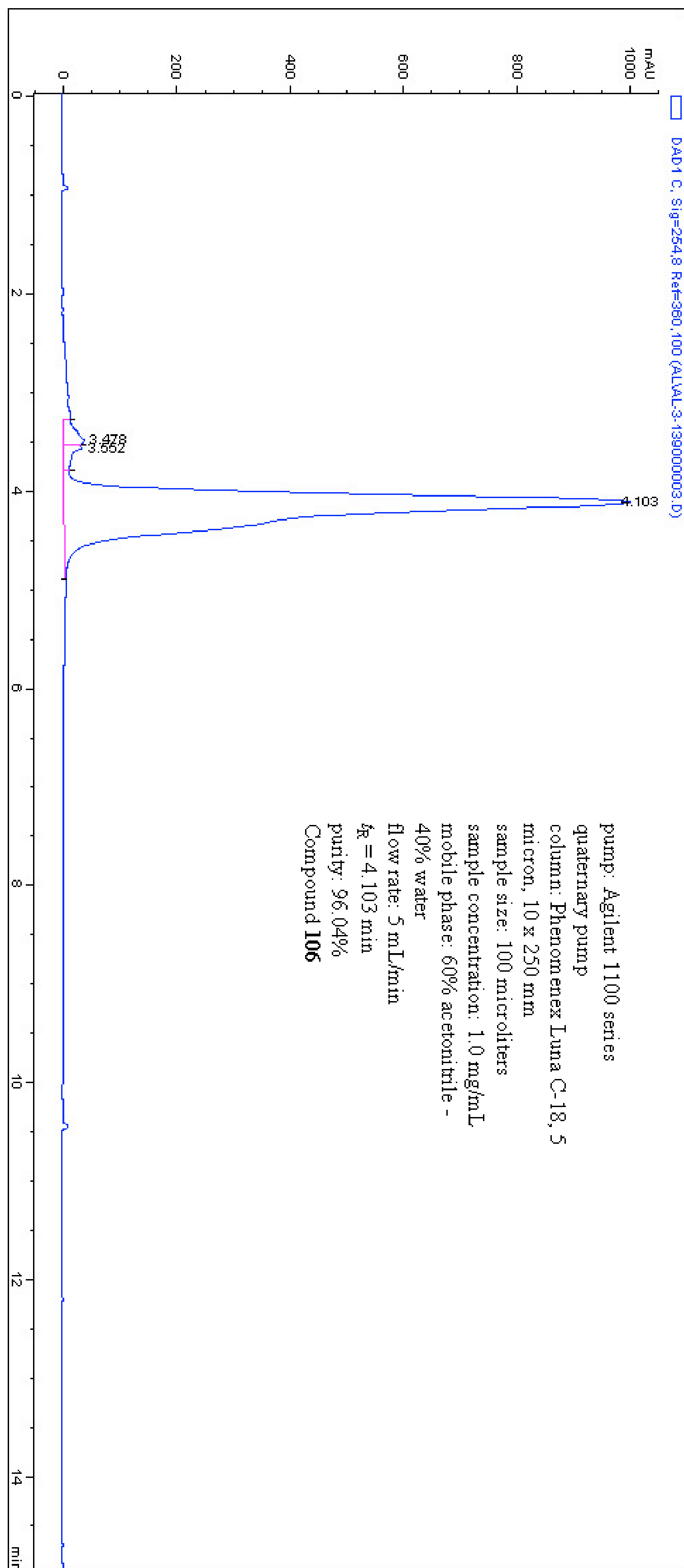
DAD1 C, Sig=254,8 Ref=360,100 (AL\AL-2-10300009.D)
mAU
0
20
40
60
80
100
120
140
160
min
0
2
4
6
8
10
12
14
16
3.343
3.762
10.938
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
tR = 10.938 min
purity: 98.90%
Compound 103



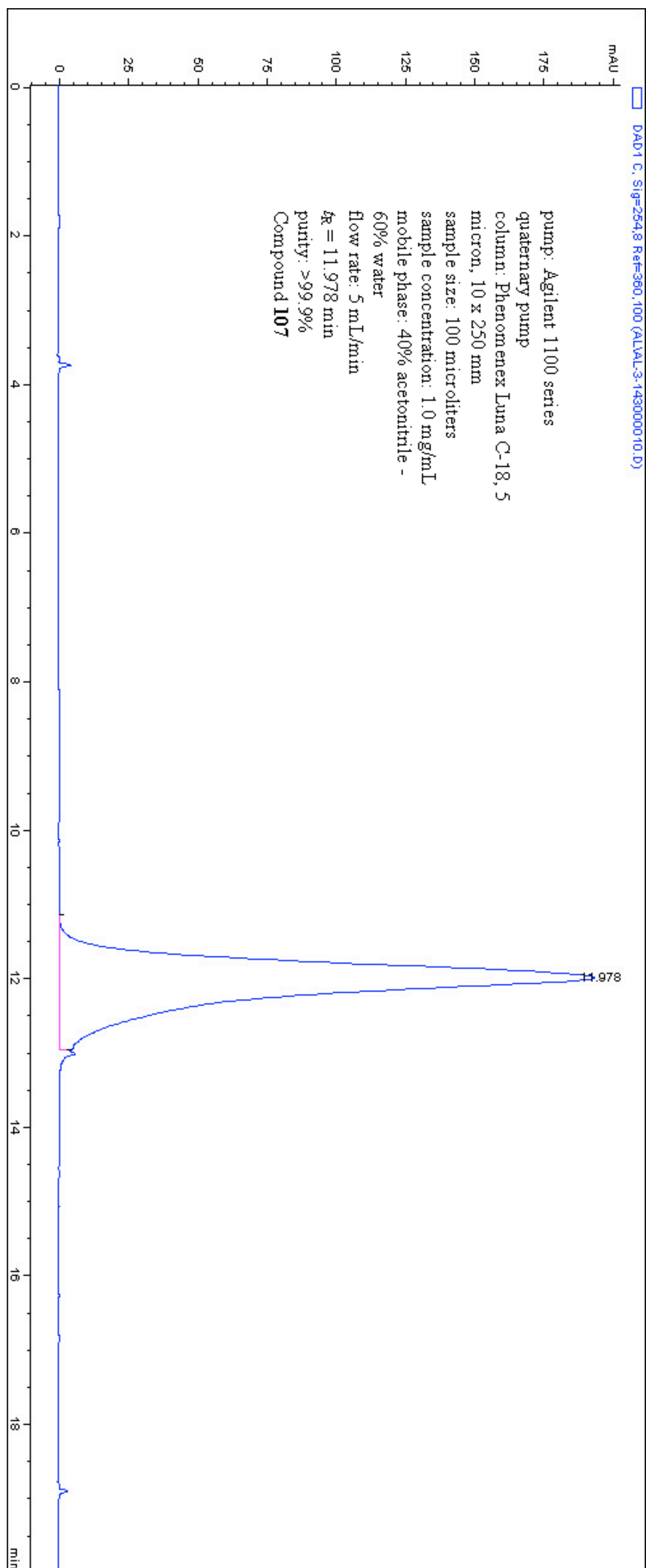
DAD1 A, Sig=209,4 Ref=360,100 (ALVAL-2-217B00016.D)
mAU
min
1.863
13.703
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 45% acetonitrile -
55% water
flow rate: 5 mL/min
t_R = 13.703 min
purity: 99.06%
Compound **104**



DAD1 B, Sig=214,16 Ref=360,100 (ALVAL-3-133A000006.D)
mAU
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
min
9.175
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile / 40%
water
flow rate: 5 mL/min
purity: 99.539 %
tR = 9.175 min
Compound 105



DAD1 C, Sig=254,8 Ref=360,100
mAU
1000
800
600
400
200
0
4.103
3.478
3.552
0
2
4
6
8
10
12
14
min
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 60% acetonitrile -
40% water
flow rate: 5 mL/min
t_R = 4.103 min
purity: 96.04%
Compound **106**


DAD1 C, Sig=254,8 Ref=360,100 (AL\AL-3-143000010.D)
mAU
175
150
125
100
75
50
25
0
pump: Agilent 1100 series
quaternary pump
column: Phenomenex Luna C-18, 5
micron, 10 x 250 mm
sample size: 100 microliters
sample concentration: 1.0 mg/mL
mobile phase: 40% acetonitrile -
60% water
flow rate: 5 mL/min
t_R = 11.978 min
purity: >99.9%
Compound **107**
11.978
0
2
4
6
8
10
12
14
16
18
min

APPENDIX C: ELEMENTAL ANALYSIS

Comp #	Molecular Formula	Calculated	Found
80	**$C_{27}H_{29}O_9BrS$•0.25 H_2O**	**C, 52.73; H, 4.84; O, 23.26**	**C, 52.73; H. 4.84; O, 23.26**
81	**$C_{28}H_{32}O_{10}S$•0.25 H_2O**	**C, 59.51; H, 5.80; O, 29.02**	**C, 59.48; 5.79; O, 28.97**
83	**$C_{27}H_{29}O_9BrS$**	**C, 53.21; H, 4.80 O, 23.63**	**C, 53.11; H, 4.94; O, 23.41**