The Dissertation Committee for Tori Marquis Strong Certifies that this is the approved version of the following dissertation:

SYNTHESIS TOWARDS SALVINORIN SCAFFOLD TO DEVELOP A POTENTIAL PAIN THERAPEUTIC AND SELECTIVE, POTENT KAPPA OPIOID LIGANDS

Committee:

Scott R. Gilbertson, PhD, Supervisor

Karin Westlund-High, PhD, Co-Supervisor

Kathryn A. Cunningham, PhD

Joel P. Gallagher, PhD

Mahmoud S. Ahmed, PhD

Dean, Graduate School

SYNTHESIS TOWARDS SALVINORIN SCAFFOLD TO DEVELOP A POTENTIAL PAIN THERAPEUTIC AND SELECTIVE, POTENT KAPPA OPIOID LIGANDS

by

Tori Marquis Strong, B.A.

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas Medical Branch

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas Medical Branch

<May, 2012>

Dedication

I would like to dedicate this to two very important people in my life; my great grandmother, the late Gladys Clayton Lockhart, who planted a seed for loving education as much as loving life itself and most of all, my mother Marecia D. Strong, who has been my support my entire life. Her continuing love not only motivated me to be a better scientist; it motivated me to be an even better human being.

“Throughout history, it has been the inaction of those who could have acted; the indifference of those who should have known better, the silence of the voice of justice when it mattered most; that has made it possible for evil to triumph.”

By Haile Selassie

Acknowledgements

I would like to acknowledge my supervisor Dr. Scott R. Gilbertson for providing the platform that allowed me to discover both scientifically and personally. I would also like to acknowledge Dr. Karin Westlund-High and her lab for providing direction and training in the biological application of this project. I would like to thank my dissertation committee for the invaluable insight that helped to frame my research and this document. I truly want to thank the Gilbertson lab members for all their help in my research project. I particularly would like to acknowledge both Bridget Hawkins and Tomas Vasques for their collaboration.

SYNTHESIS TOWARDS SALVINORIN MOTIF TO DEVELOP A POTENTIAL PAIN THERAPEUTIC AND SELECTIVE, POTENT KAPPA OPIOID LIGANDS

Publication No._____

Tori Marquis Strong, Ph.D.

The University of Texas Medical Branch, 2012

Supervisor: Scott R. Gilbertson

Effective chronic pain therapy has posed a challenge for patients who have sought after traditional therapy, opioid analgesics. Particularly in patients with chronic pancreatitis, traditional therapy has failed to assist with pain management causing daily activity to be an even bigger challenge. Drugs like morphine or codeine, mu opioid agonists, have been utilized for pain management although these agonists have non-selective, opioid receptor subtype activity and side effects that ensue with chronic use that include constipation, addiction and respiratory depression. With the pancreas having a high density of kappa opioid receptors, a kappa subtype selective agonist could serve as a target molecule to develop as a pain therapeutic for patients with chronic pancreatitis. Where most alkaloids isolated over the years share characteristics like being plant derived, having similar structural functionalities and having cross affinity to opioid receptor subtypes, Salvinorin A **1** has emerged as a very unique alkaloid. Salvinorin A **1** is a non-nitrogenous alkaloid that selectively binds to the kappa opioid receptor subtype,

implicating it as a potential target for development as a pain therapeutic for chronic pancreatitis as well as a motif for selective kappa opioid ligands. Synthesis of the Salvinorin scaffold is the basis of this body of work to not only build on the profile established in previous work but characterize through modifications that only synthesis can provide. This dissertation describes the synthesis of the key molecule **66** as the pivotal molecule for generating analogues for the Salvinorin scaffold. Likewise, this work demonstrates the nociception response of Salvinorin A in the persistent pancreatitis model. Salvinorin A serves as a unique target for development both chemically and biologically; and this work establishes the foundation to bridge the gap between the two.

Table of Contents

List of Figures

List of Schemes

Chapter 1: General Introduction

I. OPIOID PHARMACOLOGY

Opioid pharmacology has been a well studied field that has led to both neurological discoveries and therapeutics. Often associated with pain treatment and addiction, opioid pharmacology has given unique insight on the central nervous system (CNS) as well as the peripheral nervous system (PNS).[1] Classical opioid pharmacology derives from the isolation and characterization of alkaloids of the opium poppy plant *Papaver somniferum*.[1] In 1803, Serturner isolated the pure alkaloid morphine, named after the Greek god of dreams, Morpheus, demonstrating an early understanding of the effects of opiates. More importantly, isolated alkaloids led to the discovery of opioid receptors, found both in the CNS and PNS.[1] This discovery began to show the connection of the effects of opiates to neurological responses. Opioid receptors are G-protein coupled receptors that are subdivided on the basis of different physiological responses and selectivity to different agonists. Three main types of opioid receptors are mu, delta and kappa (μ, δ and κ respectively) which are all found in the CNS and the periphery and are so delineated because of the selective agonists that led to their discovery.[2]

The opioid receptors subtypes not only have similar function, all being metabotropic, seven-membered transmembrane G-protein coupled receptors (GPCR), but have similar structural homology.[1] Cloning of the genes encoding for μ, δ and κ

receptors revealed the receptor type and their endogenous ligands: endomorphin, enkephalin and dynorphin peptides respectively.[2] However due to their similarities, many exogenous ligands have cross affinity to all the subtypes of the opioid receptors.[2]

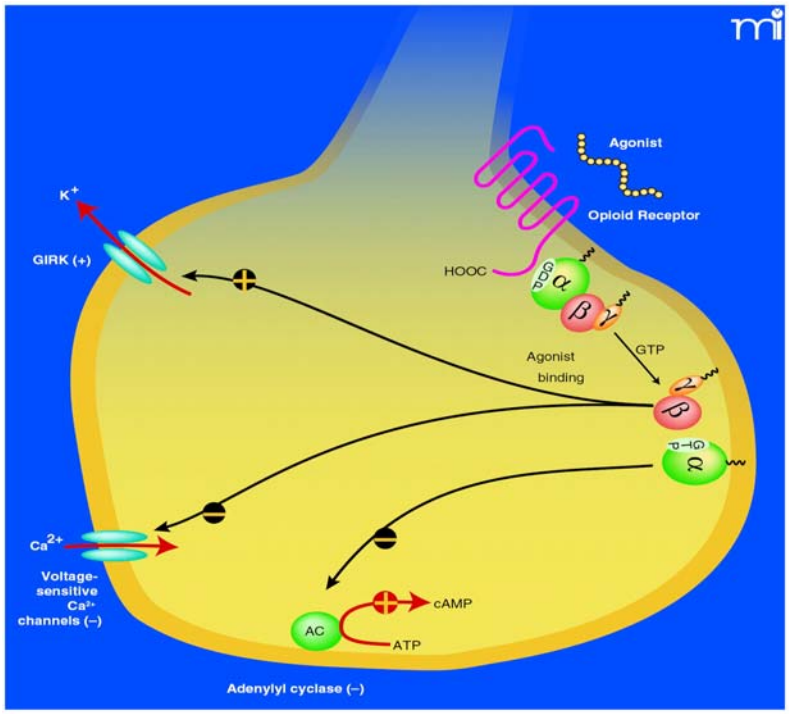


Figure 1.1: Activation of mu opioid receptor. Adopted from ref 2 with permission.

As demonstrated in Figure 1.1, opioid receptors are coupled with the G_i/G_o superfamily where activation of this trimeric protein causes dissociation of the α subunit. GDP, associated with the α subunit during resting conditions, is first replaced with GTP prior to dissociation.[2] Now a conformational change with the receptor takes place, releasing the opiate along with the GTP bound α subunit from the β and γ complex. The release

causes activation of the effector. The effectors lead to activation of inwardly rectifying K^+ channels while inhibiting voltage operated Ca^{2+} conductance, shown in Figure 1.1 where the plus symbol indicates activation and the minus indicates inhibition. There is an inhibition of adenylyl cyclase leading to direct inhibition of transmitter release with an overall decrease in neuronal excitability. GTP is converted back to GDP by the intrinsic enzymatic activity of the α subunit which allows reassociation to the β and γ subunits rendering the complex back to its resting conditions.[2] What is unique is the ability for the subtypes to mediate activation of a number of members of the G_i, G_o and even G_q classes of G protein where these G proteins serve diverse functions in distinct cell types.

As previously mentioned, the pharmacological effects of opioid analgesics are derived from the complex interactions of agonists with the three receptor subtypes; μ, δ, and κ. Opioid receptors are found in the periphery; at both presynaptic and postsynaptic terminals in the spinal cord dorsal horn; and in the brain stem, thalamus and cortex, forming the ascending pain transmission system.[3] These structures also comprise the descending inhibitory system that modulates pain at the level of the spinal cord through a decrease in presynaptic transmitter release, hyperpolarization of postsynaptic elements and disinhibition. Endogenous pain modulation includes the interaction of endogenous opioid peptides through a distinct mechanism.[3] Figure 1.2 gives a depiction of endomorphin-2 (EM-2), along with Dynorphin A (DYN A), interacting with the presynaptic afferent neuron terminal. These endogenous ligands binds to opioid receptors, EM-2 to μ receptors and DYN A to κ receptors, to reduce pain sensation. Figure 1.2 depicts the inhibition of excitatory transmitters; glutamate (Glu), substance P (SP) and calcitonin gene-related peptide (CGRP).

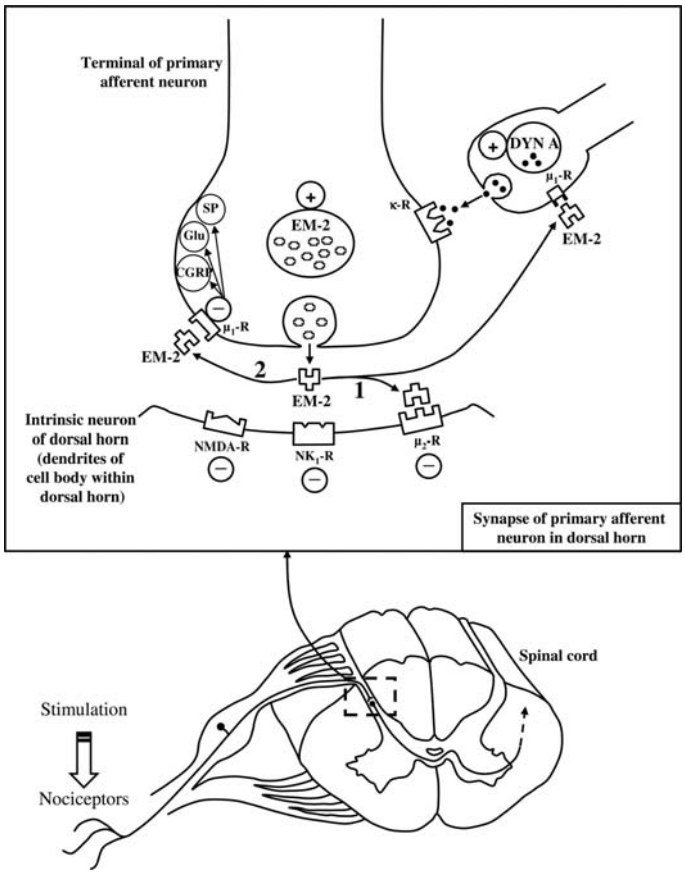


Figure 1.2: Synapse of sensory neuron in Dorsal Horn of Spinal Cord. Adopted from ref 3 with permission.

One well studied mechanism of pain inhibition is the interaction of immune cell-derived opioid peptides with opioid receptors on sensory neurons; primary afferent neurons on the peripheral terminals. Three types of opioid peptides are well characterized within both the central nervous system and the endocrine system; β-endorphin, met-enkephalin and dynorphin.[2] These three interact with μ, δ and κ opioid receptor subtypes respectively to confer analgesia. Once there is peripheral inflamed tissue, these opioid

peptides are produced and then released from immune cells to activate opioid receptors on sensory nerve terminals to modulate inflammatory pain. Inflammation also increases opioid receptor expression in DRG neurons while enhancing transport and accumulation of opioid receptors on the peripheral terminals of sensory neurons. This process is orchestrated by up-regulation of adhesion molecules on endothelial vessels and co-expression by opioid-containing immunocytes. Peptides are then secreted by stressors, CRF and cytokines with the corresponding receptors present on opioid-expressing leukocytes. The peptides bind to the localized receptor on the peripheral sensory nerves thus inhibiting pain.[2,3]

Opioid receptors are often linked and well studied in the areas of addiction, dependence, tolerance and withdrawal.[2,3] When exogenous opiates are used as a therapeutic, the use involves pain management, with morphine still being the standard against which all opiates as well as non-opiates that have strong analgesic action are compared. The mechanism of action for analgesics occurs both in the periphery and the CNS, having cross affinity for all the subtypes of receptors. It is the CNS where side effects such as sedation, respiratory depression, tolerance, dependence and addiction can readily occur.[2] For acute issues, where there is low dose, short-term usage; those CNS side effects pose little to no threat, however for many patients who battle with chronic pain, management therapy becomes more of a challenge. With chronic pain, repeated dosage could lead to tolerance, causing a need for increasing doses over time. With repeated use and increasing doses, the chance of dependence is greatly enhanced as well.[33] Patients with chronic illnesses just wanting to participate in normal daily function have to overcome the challenges the pain the disease causes without becoming dependent

on that very same drug that aids in the pain management. The challenge now becomes whether one can discover an opiate that is peripherally acting and/or target a receptor subtype to see if analgesia can be conferred for chronic pain without the side effects of long term use.

Three unique findings in the field of study regarding peripheral opioid receptors include the peripheral restriction of tetrapeptides, the exclusive functional localization of opioid receptors to primary afferent neurons, and under inflammatory conditions the relative lack of tolerance.[2,3] These findings began to address the previously stated challenges with treating chronic pain. With the advent of these findings, clinical studies have redirected their focus to include the area of chronic arthritis pain.[3] One of the long term goals in all research involving opiates as a therapeutic is the ability to activate exclusively peripheral opioid receptors on sensory neurons, devoid of centrally mediated side effects.[3] As will be discussed further in this dissertation, opioid therapeutics for chronic pain can now be re-examined, with the aims of eliminating side-effects that previous methods of therapy included. Likewise, knowing that inflammatory conditions deter tolerance helps to maintain the potency of the therapy over a long period, particularly in the case of chronic inflammatory conditions.

II. DISCOVERY OF SALVINORIN A

Near the Alemin Reservoir in the Mexican state of Oaxaca, about 100 kilometers from the port of Veracruz, reside the Mazatec Indians. Like many tribal or traditional

cultures in this region, they have included a concomitant relationship between healing and religion. The curandero, or the healer, serves as the shaman for the community performing divination ceremonies for various needs of that community.[4] Psychotropic plants are intimately associated with various ceremonies. One ceremony in particular, loosely translated as "the Way to Heaven", includes the use of the leaves of the mint plant *Salvia divinorum*, a member of the sage genus, in successive increasing doses.[4] The use of this plant, as also described by recreational users, has a dissociative hallucinogenic effect causing the user to have an experience similar to psychedelic drugs.[5] In small doses the effects are perceived as mind clearing and coordination impairing, making it attractive for use during meditation.[4,5] The first specimen cultivated for study was done at the University of California Los Angeles in 1962 by R. Gordon Wasson and given to Carl Epling for further investigation. Cuttings from this original clone have been given to other botanical collections over years, it being the primary source for most of the US's plant cultivations.[5]

The active component of the mint plant has been isolated via solvent extraction and gas chromatography-mass spectrometry. That component has been identified as Salvinorin A, $C_{23}H_{28}O_8$.[6] Along with having similar motifs, one key structural feature of all opiate compounds is a tertiary amine until the discovery of Salvinorin A. Salvinorin A is a non-nitrogenous alkaloid. Structurally, Salvinorin A has no similarities to other opiates.[6] Opiates have cross affinity to various subtypes of the opioid receptors. Morphine is primarily a μ opioid agonist, however it has binding affinity to both κ and δ. Salvinorin A is a unique ligand, it binds to the κ opioid subtype only.[6] Further

investigation to profile the characteristics and action of Salvinorin A helped to setup the framework for this dissertation study.

Bryan Roth's group has investigated both Salvinorin A's potency and selectivity for the κ-opioid receptor (KOR).[7] First the group screens Salvinorin A on a large scale with human cloned GPCR's to demonstrate its selectivity. Because of Salvinorin A's hallucinogenic effect it was tested against LSD, a known serotonergic hallucinogen.

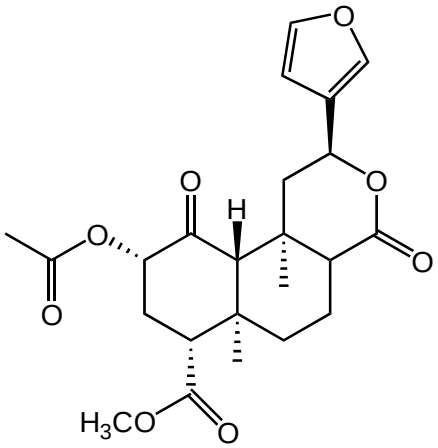


Figure 1.3: Salvinorin A, the active component of Salvia divinorum mint plant.

Both ligands were tested via mean percent inhibition of radiolabeled ligand for 50 receptors and transporters, but required functional assays for metabotropic glutamate receptor screening. As shown in Figure 1.4, Salvinorin A was shown to have affinity to only one receptor, the KOR. With this finding being unique, questions of how hallucinations are receptor mediated are now being raised.[7]

To further demonstrate the selectivity and potency of Salvinorin A with the KOR, two experiments were utilized, as shown in Figure 1.5. Salvinorin A's ability to fully agonize the KOR was shown first through inhibition of ^{3}H-bremazocine versus naloxone

as a control on cloned KOR and second through inhibition of forskolin stimulated cAMP with a known kappa agonist U69593 as a control. Salvinorin A demonstrated competitive binding through inhibition of the ^{3}H-bremazocine binding to KOR. Likewise Salvinorin A displayed a higher potency than naloxone, a known antagonist, for KOR binding. This concept was further measured for Salvinorin with an EC_{50} for forskolin stimulated cAMP inhibition of 1 ± 0.5 nM compared to a 1.2 ± 0.6 nM EC_{50} value for U69593, making Salvinorin A very competitive to a known agonist.[7]

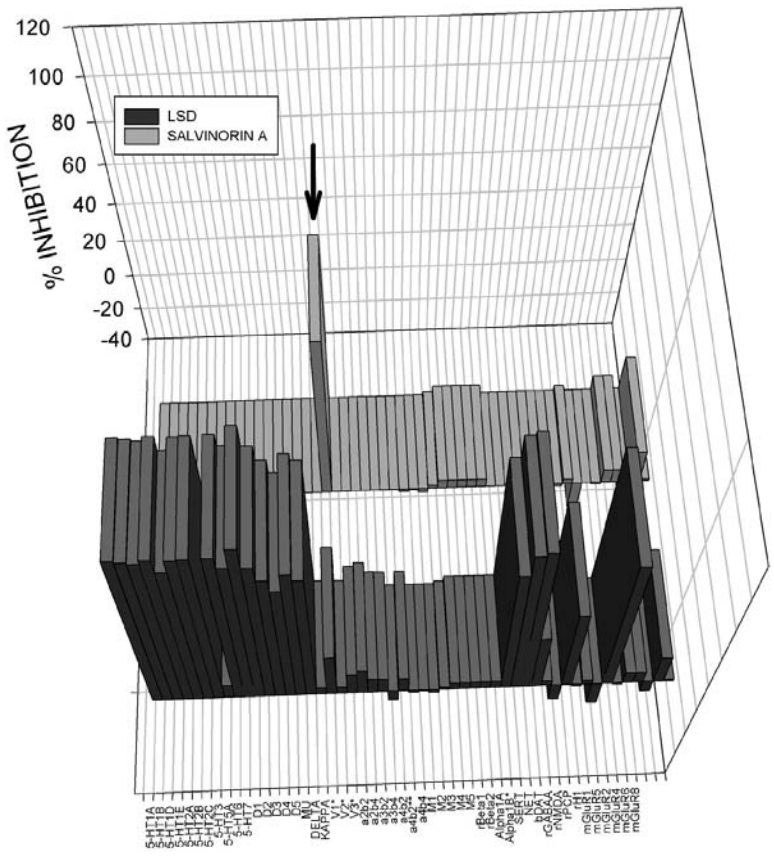


Figure 1.4: Salvinorin A screening demonstrating binding to KOR selectively. Adopted and modified from ref 7 with permission.

The finding of selectivity of Salvinorin A to the KOR is further shown where the effects of Salvinorin A in mouse hippocampal, striatal and prefrontal cortex synaptosomes, isolated from homogenized brain tissue, was investigated by Grilli *et al*.[8] This work focused on exploring whether Salvinorin A could presynaptically control the release of central amines from nerve terminals isolated from different brain regions and if that modulatory effect is mediated by the KOR. Likewise these results are compared to the known agonist U69593. First they demonstrated that Salvinorin A facilitated the modulation of K^+-induced release of tritiated neurotransmitters; noradrenaline (NA), serotonin (5-HT) and dopamine (DA). Basal levels of neurotransmitter were established in superfusion assays of synaptosomes. Along with depolarizing stimulus, Salvinorin A was introduced to the synaptososme tissue and was found to presynaptically modulate the neurotransmitters by increasing exocytosis of NA and reducing both 5-HT and DA. Second they demonstrated the effects of KOR coupling with pertussis toxin-sensitive GPCR's, where synaptosomes were enriched with pertussis toxin and then treated with Salvinorin A. Modification of facilitation of NA nor inhibition of 5-HT and DA were not shown. Salvinorin A seem to have no significant effect on pertussis toxin entrapped synaptosomes. Third they showed the effects of the known KOR antagonist norbinaltorphimine (norBNI). NorBNI was able to block the previous events induced by Salvinorin A, further supporting that Salvinorin A modulation effects on neurotransmitter release is through KOR agonism.[8]

Chavkin *et al* explore Salvinorin A even further by not only investigating Salvinorin A's potency but also its efficacy.[9] A detailed profile begins to be established through comparison of Salvinorin A to select derivatives and to known KOR agonists.

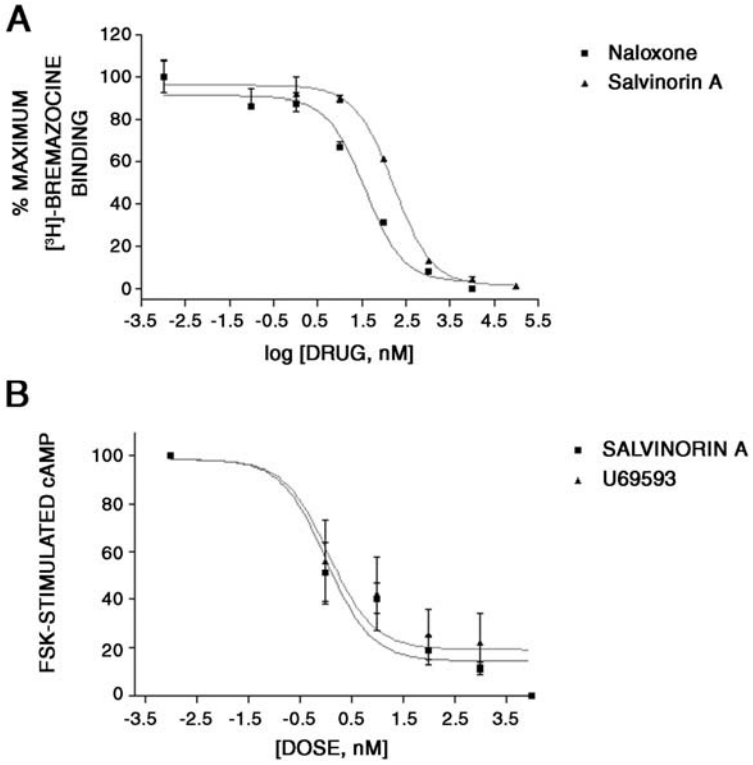


Figure 1.5: A. Salvinorin A inhibition of 3H-bremazocine binding; B. Salvninorin A inhibition of fsk-stimulated cAMP. Both assays showing potency and selectivity. Adopted from ref 7 with permission.

First, the group established the activity that Salvinorin A and derivatives, with substituent modifications at the 2-position, has on human KOR versus other receptor types, including serotonergic, dopaminergic, muscarinic, adrenergic, cannabinoid and σ receptors. Like the previous study, Salvinorin A binds to KOR with no activity at the other receptors. Although Salvinorinyl-2-heptanoate had KOR activity, Salvinorinyl-2-proprionate was the only derivative to have submicromolar affinity to KOR with no significant activity at other receptors, like Salvinorin A. The multi-receptor screening for Salvinorin A and derivatives was followed by measuring activity through inhibition of forskolin stimulated

cAMP with direct comparison to U69593 activation. This assay demonstrated the potency and full agonism of Salvinorin A and the proprionyl derivative against U69593.[9]

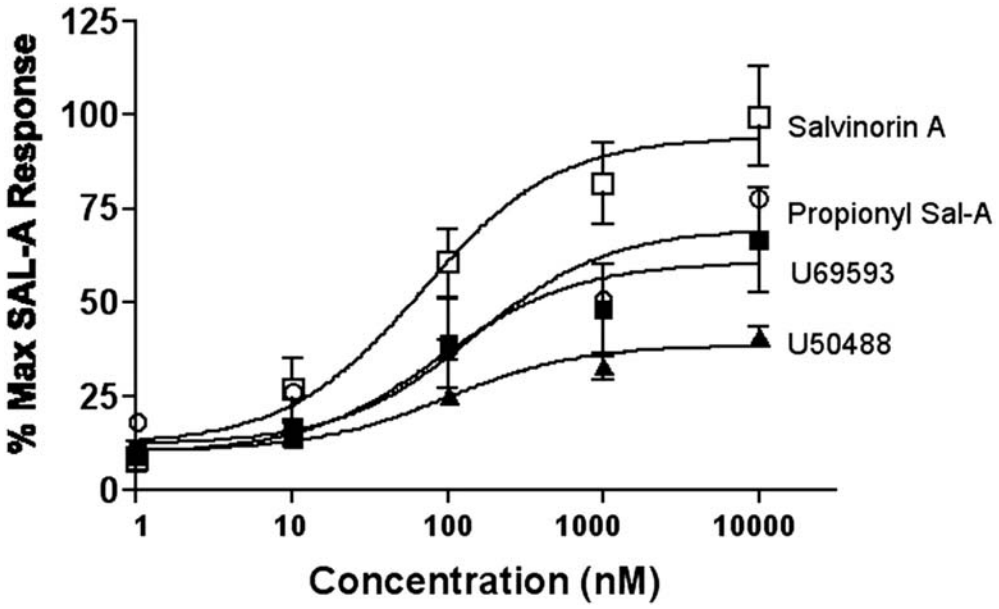


Figure 1.6: Salvinorin A E_{max} compared to other agonists through activation of Kir3 currents in *Xenopus* oocytes. Adopted from ref 9 with permission.

To demonstrate that Salvinorin A is efficacious, this group first establishes K^+ channel activation in *Xenopus* oocytes, in comparison to U69593, and reversal by nor-BNI. Once activation was established, to examine the efficacy, κ-receptor mediated activation of Kir3 currents in oocytes were measured as compared to U50488, U69593 and dynorphin-A, all known κ-opioid receptor agonists, along with its proprionyl derivative. At saturating concentrations, Salvinorin A evoked a larger Kir3 current over the other agonists. Figure 1.6 shows the Salvinorin A eliciting the highest response

amongst the agonists except dynorphin-A; they had relatively the same E_{max} but Salvinorin A had a lower EC_{50} making it more potent but the same efficacy.[9]

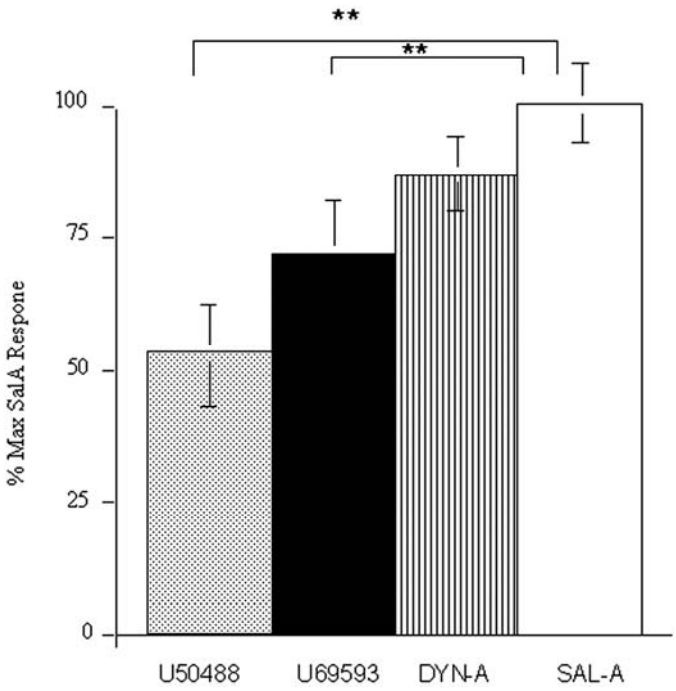


Figure 1.7: Salvinorin A E_{max} compared to other agonists at 10 μM. Adopted from ref 9 with permission.

Salvinorin A further demonstrated a higher efficacy than reference KOR agonists through examination of maximal responses to 10 μM of each agonist. With a lack of spare receptors, Salvinorin A showed a significantly higher maximal response compared to most of the agonist but a slightly greater response compared to dynorphin-A. So Salvinorin A is selective, potent and efficacious making it a great scaffold to develop a library of modified derivatives.[9]

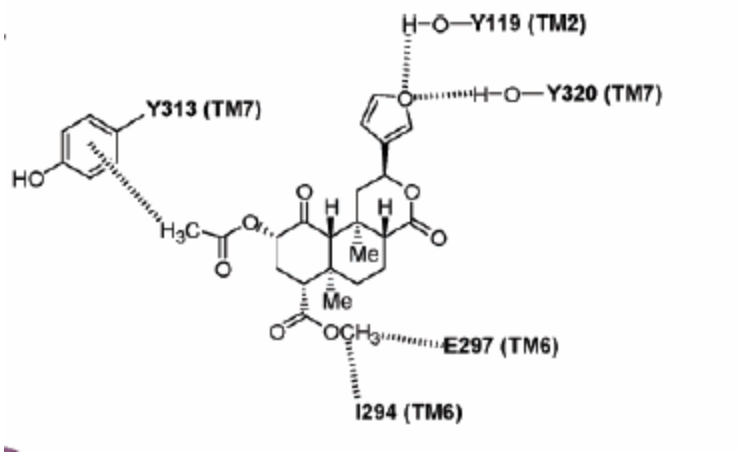


Figure 1.8: Model of Salvinorin A binding uniquely to KOR. Adopted and modified from ref 10 with permission.

The structure of the KOR-ligand complex has not been completely solved, so knowing the binding pocket with certainty is not available. This lack of structural information gave rise to the utilization of combined molecular modeling/mutagenesis studies to investigate ligand interactions along with mechanisms of activation. Typically the focus for ligand receptor interaction has been ionic and hydrogen-bond type interactions with highly conserved charged residues and residues that form hydrogen bonds. This focal point allows for starting the process for anchoring and orienting the ligand in a binding pocket of the receptor. Salvinorin A however, has no ionizable groups, so hydrogen-bonding is the most conceivable starting point.[10] An original 3D molecular modeling of Salvinorin A in the KOR indicated that the molecule interacts with Tyr313 and Tyr312 in transmembrane helix 7, Gln115 in transmembrane helix 3, and Tyr139 in transmembrane helix 2.[7] However, after further investigation, through looking at the effects of Salvinorin A on KOR with various point mutations, Salvinorin A

was found to interact quite differently in the receptor. The new model, shown in Figure 1.8, indicates that there is interaction with Tyr313 in transmembrane helix 7 (as previously noted) and also with Tyr320 of that same helix.[10] Likewise, there are interactions with Tyr 119 of transmembrane helix 2 and with both Glu297 and Ile294 of transmembrane helix 6.[10] Salvinorin A required interactions are in stark contrast to prototypical nitrogenous KOR ligands, where U69593 was used in this study, and endogenous peptidergic KOR ligands, where dynorphin A was utilized in this study.[9] Site directed mutagenesis was used to incorporate Cys residues substitutions on the KOR. A comparison of activation between the three ligands; Salvinorin A, U69593 and dynorphin A; was obtained, demonstrating that Salvinorin A required different residues of interaction for activation of the receptor.[10] This model gives a reference point to begin schematically determining positions for substituent modifications to a scaffold, potentially enhancing its agonist ability or generating a selective antagonist. One can also begin to delineate the pharmacophore for better understanding the structure activity of the Salvinorin A with KOR.

III. OBJECTIVES

The KOR has been implicated in several disease processes making agents with selective affinity worth investigating. Diseases implicated include mood disorders, depression, schizophrenia, cognition enhancement and dementia.[11] The KOR is also involved in drug abuse, alcohol addiction, chronic pain conditions, congestive heart failure, diuresis and renal function augmentation.[11] The KOR becomes a great

pharmacological target for developing ligands to address a variety of diseases. Salvinorin A emerges as a selective, potent and efficacious KOR ligand. It is the first non-nitrogenous molecule to have affinity to an opioid receptor subtype, without having virtually any affinity to any other receptor. Previous groups have established the merit for investigation of Salvinorin A, through isolation, analysis, semi-synthetic preparation and various assay studies demonstrating not only probative value but potential financial value with further development.[11,12,13] Our investigation takes a closer look at the structure of Salvinorin A to begin establishing a scaffold and develop ways to modify around that scaffold, allowing for accomplishments towards therapeutic development.

Salvinorin A serves as a unique target for synthesis. Salvinorin A is a *trans*-neoclerodane diterpene structure containing concentric trans decalins.[5,6] This unique structure positions the substituents in equatorial positions around the three ring system. Salvinorin A is the first natural opiate to not contain nitrogen making it a unique scaffold to make derivatives for binding to the κ opioid receptor.[5,6] A positively charged nitrogen incorporated in opioid compounds was initially thought to be a vital requirement for interaction with opioid receptors in general however, Salvinorin A allows for a new perspective.[10] Figure 1.9 shows Salvinorin A structure with ring and position delineations as well as the structures geometry. Modification of this structure has been limited, in part due to not having a route of synthesis.[7,9] As previously discussed, Salvinorin A uniquely binds to the κ opioid receptor, making Salvinorin a great scaffold to exploit in generating ligands. Based on our knowledge of Salvinorin A, three

objectives emerged for establishing Salvinorin A as a target for both a therapy and a motif for generating selective ligands.

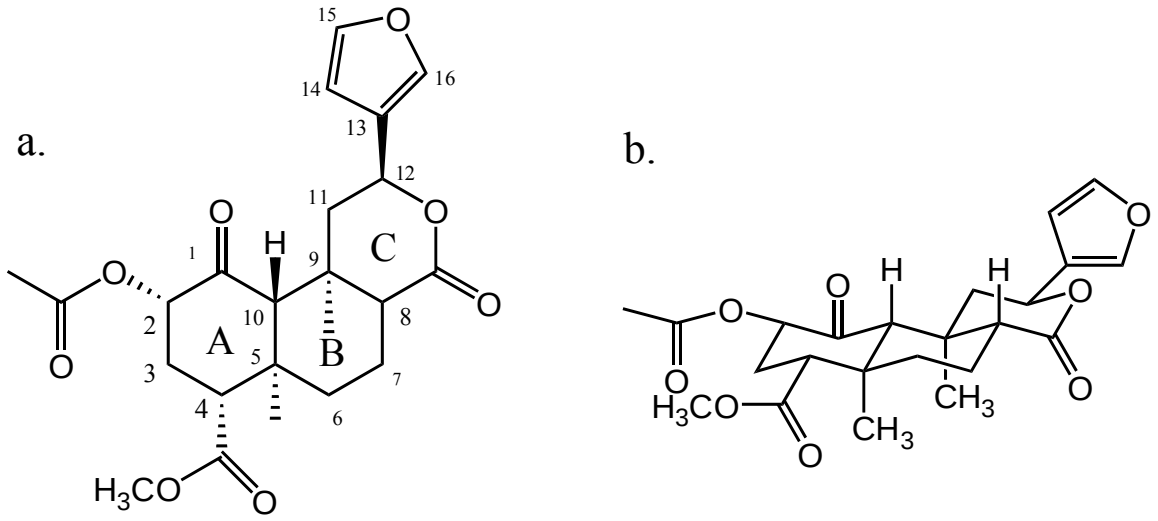


Figure 1.9: Salvinorin A structure; a. labeled to delineate rings and position; b. demonstrate geometry of structure, methyls are axial while other substituents are equatorial.

The first objective is to develop a route for total synthesis of Salvinorin A. When this project began, no synthesis of Salvinorin A had been recorded in the literature. The only reported syntheses were semi-syntheses and minimal modifications to help determine the pharmacophore of Salvinorin's activity.[7,9] With Salvinorin A being so unique, the only method of acquiring the molecule is through isolation from the mint leaf. A total synthesis would serve as an advantageous way to generating an abundance of material for further examination. Likewise, developing a route that allows for facile

modification will aid in generating compounds for investigating structure- activity relationships.

The second objective was to develop a scaffold for generating Salvinorin ligands. Many opiates have cross affinity to the other subtype opioid receptors, however, Salvinorin A only seems to bind to the kappa subtype.[7,9] This scaffold could serve to generate ligands, possibly agonists, antagonists or even inverse agonists, that predominately bind to the kappa subtype. The value of KOR agonism will be further demonstrated in a chronic pain model, however there is value in KOR antagonism particularly in treating mood disorders and depression.[14,15] Even more so, KOR antagonism could be used as a biological tool for investigating other concepts in pharmacology; where blocking the effects of the KOR would render information about things like mechanisms or effectiveness of other molecules. The utility of these ligands could be enormous to not only better understand opioid pharmacology but neuropharmacology in general. Many mechanisms can be explored or revisited to discover new ideas or redefine previous ones.

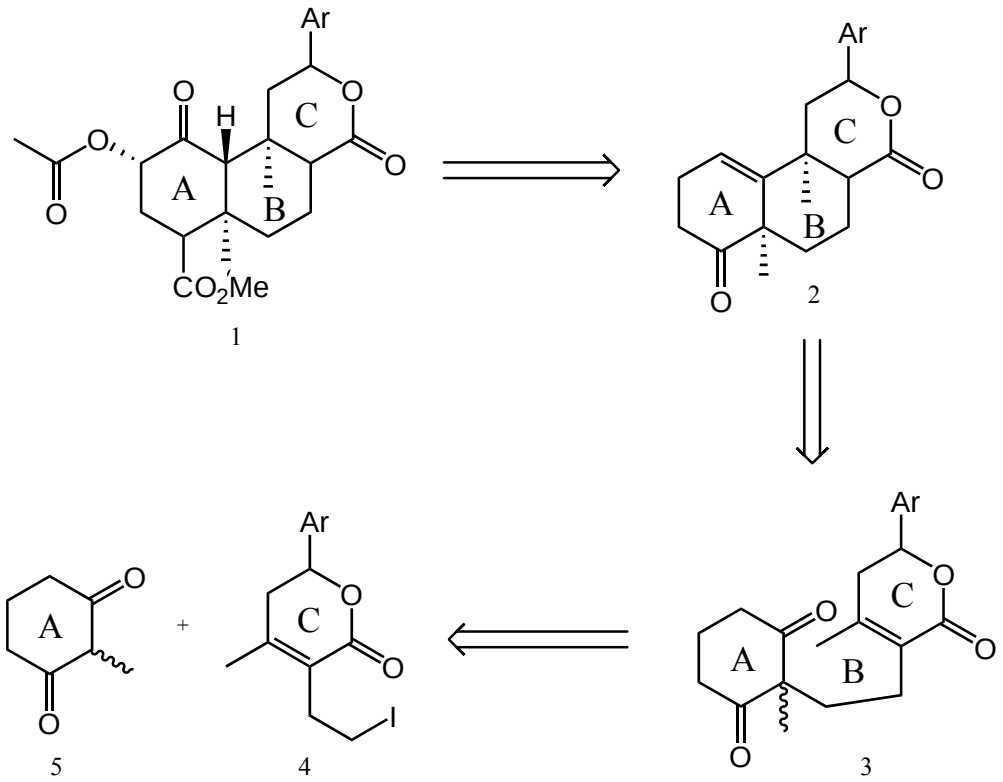
As previously mentioned, KOR is implicated in chronic pain conditions, particularly in pain modulation. The current state of the art of chronic pain therapy still focuses on mu opioid receptor agonism where side effects have been documented.[16] Developing a therapy void of the side effects could enhance the field of chronic pain therapy tremendously, but even more so improve the quality of life of those patients suffering from chronic pain. The third objective is to establish Salvinorin A as a potential target for developing as an analgesic. Salvinorin A may serve as a therapeutic for treating pain in pancreatitis, acute and chronic, as well as pancreatic cancer.[17] Where

traditional pain therapy has failed patients with chronic pancreatitis, this study begins to investigate the utility of Salvinorin A in a persistent pancreatitis model to determine if it could become an excellent candidate for further development. The pancreas having a high density of kappa opioid receptors lends this model to being a great way to begin delving into whether selectively targeting an opioid receptor subtype could prove to be more advantageous as a therapeutic.[17] Using the persistent pancreatitis model we attempted to demonstrate the ability of Salvinorin A to do two things, increase both activity and anti-nociception; two functions altered in patients with chronic pancreatitis that limit their quality of life.[18,19]

Chapter 2: Synthesis of Salvinorin A Scaffold

I. SYNTHETIC ROUTE 1

The first approach for synthesis was to generate Salvinorin A, the natural product, in a fashion that lent itself to facile modification both to completely determine the pharmacophore and to investigate the structure-activity relationship; where previous work had only detailed semi-synthetic methods with limited substituent modification. As shown in Figure 1.9, Salvinorin A is a tricyclic structure labeled A, B and C for the purpose of following throughout the varying attempts of synthesis. When developing a method for forward synthesis, first one performs a retrosynthetic analysis. E. J. Corey defines retrosynthetic analysis as, "…a problem solving technique for transforming the structure of *synthetic target molecule* (*TM*) to a sequence of progressively simpler structures along the pathway which ultimately leads to simple or commercially available starting materials..."[20,21] Essentially, this process allows one to start from the target molecule and work backwards to come up with viable starting material to perform the forward synthesis. This type of analysis allows a chemist to be both creative and disciplined. The discipline comes from learning and understanding the precedent that allows for one to propose viable transformations while the creativity comes to play in creating a pathway that is unique from the recorded literature. It is a fine art to begin to culminate knowledge, experience and imagination to develop a route for synthesis for any natural chemical product.[20,21]



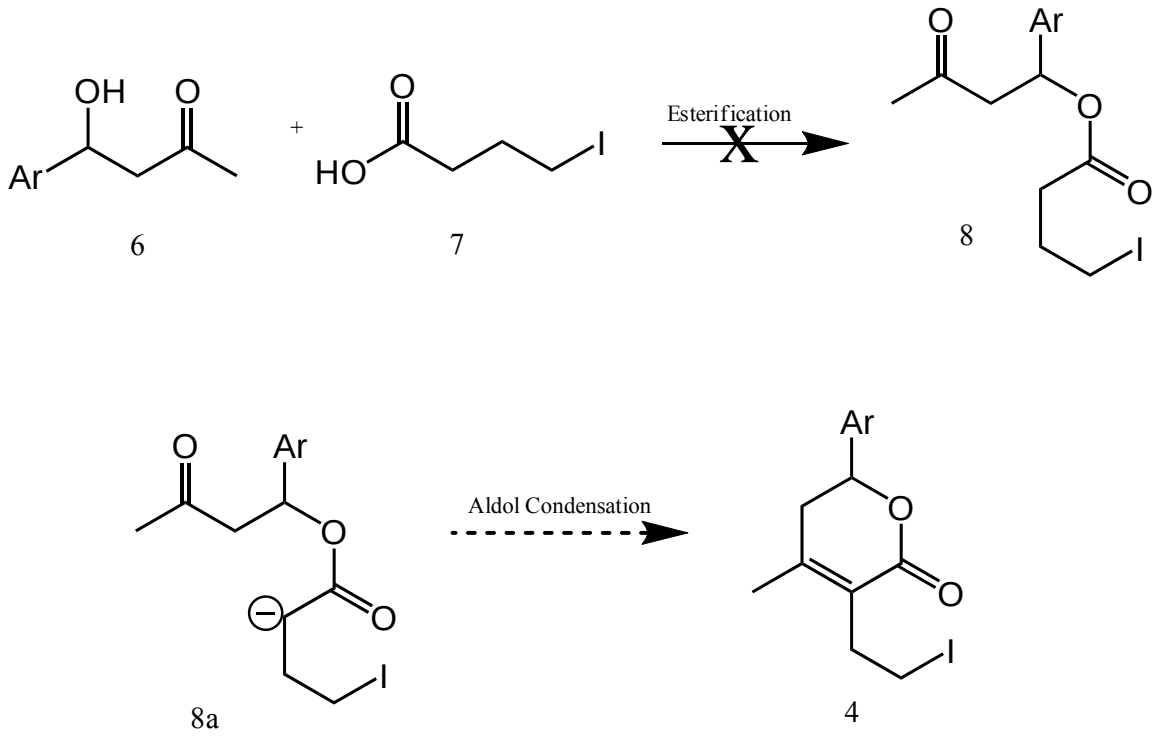
Scheme 2.1: Retrosynthetic Analysis of Synthetic Route 1.

As shown in Scheme 2.1, the retrosynthesis of the first route begins with Salvinorin A, **1**, deriving from a less substituted tricyclic system **2**. The less substituted tricyclic system **2** has two key functionalities in the A ring that lend itself to various approaches for transformation, the unsaturation (double bond) and the carbonyl. The next break is from the formation of the B-ring, which is one of the key reactions in the forward synthetic approach, deriving from compound **3**. This proposed ring formation is by a palladium (Pd) catalyzed Heck reaction. This approach is where creativity and discipline comes into play, by utilizing precedence for intermolecular Heck reactions we demonstrate discipline, while incorporating the same conditions in an intramolecular

format demonstrates creativity.[22] This species **3** is further broken down into two rings, A and C, **5** and **4** respectively. Ring A, **5**, is commercially available, but ring C, **4**, requires synthesis from a simpler molecule. This analysis gives one a starting point to begin the synthesis of the target molecule.

The process of generating the target molecule began with the synthesis of the C ring. While the C ring is a seemingly simple molecule to generate, the formation of this ring proved to be an arduous task, as demonstrated in this body of work. The system investigated for the C ring is an α, β unsaturated lactone. This system has two alkyl substituents along with an aryl substituent in the position that we later would like to modify for analogues. The key to this system is formation of the ester with the substituents in the appropriate position prior to forming the lactone. Although the substituents, particularly the aryl group, is set early in the synthesis, modifications would still be viable later in the synthesis for developing analogues.

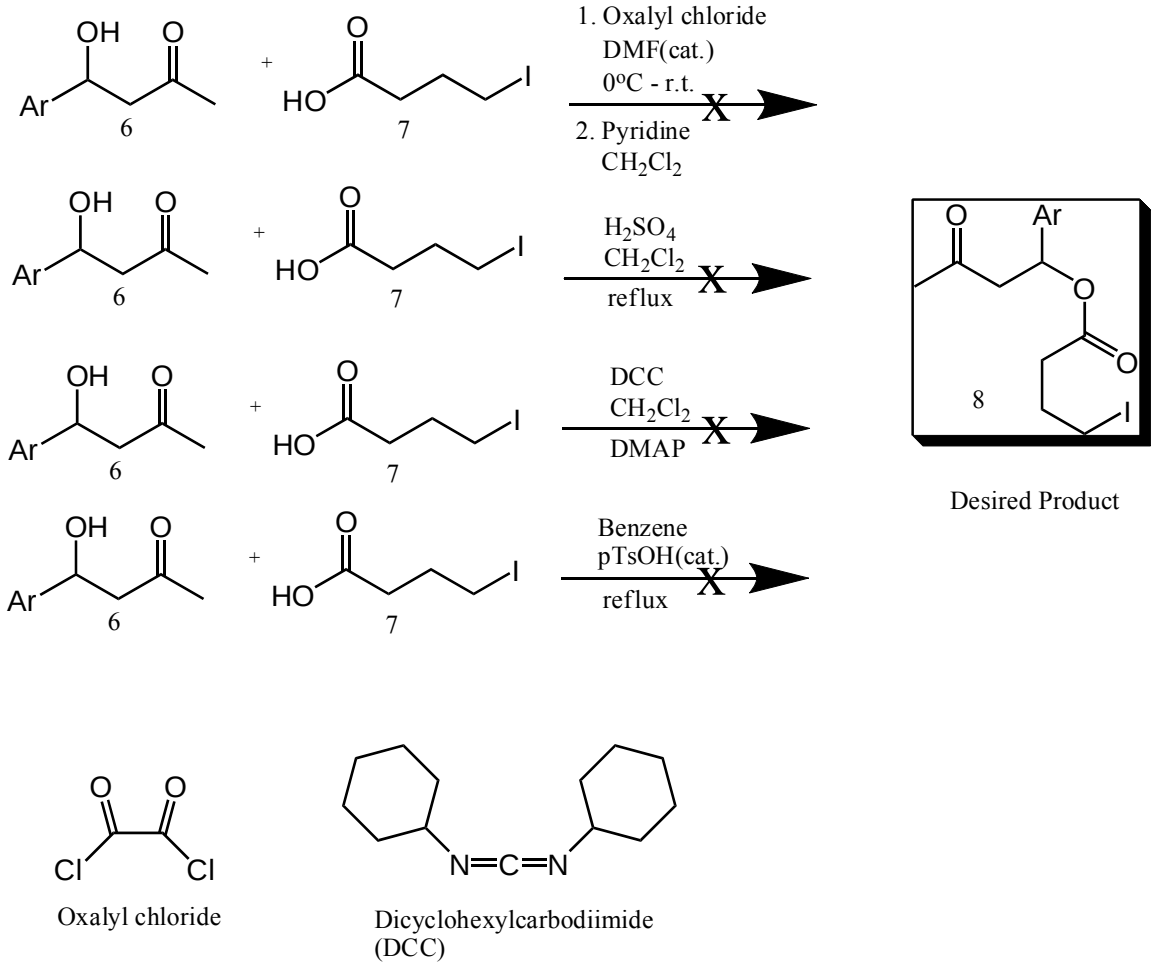
The first attempt at forming the C ring, (Scheme 2.2), began with esterification of an aryl substituted keto alcohol **6**,[23] with iodobutryic acid **7**. For ester **8** formation, various conditions are available. However, formation of the ester **8** proved to be a challenge possibly due to the steric challenge presented by the aryl substituent adjacent to the alcohol, the site of esterification. What is recognizable as a potential challenge is the requirement of selective deprotonation of an α-proton, **8a**, to undergo further condensation to generate the desired lactone **4**, particularlay since other acidic protons are available. Various conditions based on literature precedent were utilized, (Scheme 2.3),



Scheme 2.2: Initial attempt at C ring Formation.

ranging from the formation of an acyl chloride species as an electrophile for the addition of the alcohol to the direct coupling condition using DCC. Kreipi *et al* characterize the formation of an acyl chloride then addition of the alcohol as a generally effective method of ester formation.[24] Typically if ester formation does not occur, it is a result of lack of formation of the acyl chloride.[24] In this synthesis, not shown, there was formation of the acyl chloride through addition of methanol and isopropanol to generate both the methyl and isopropyl ester. Since those products were formed with success, it is more likely that the problem arises with addition of the alcohol. Fischer esterification, using sulfuric acid (H_2SO_4) and para-toluenesulfonic acid (pTsOH) as catalysts, was attempted.[25] Ester

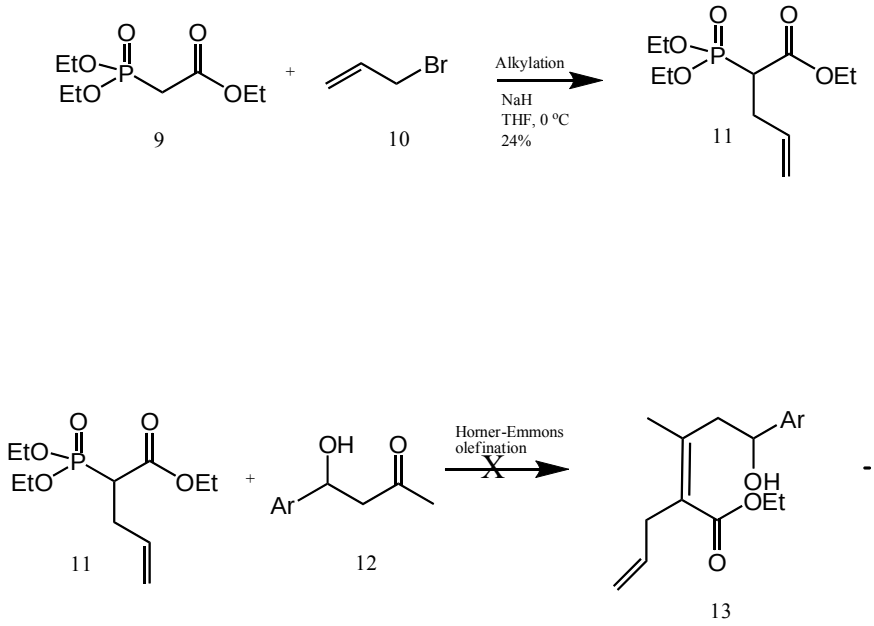
formation through direct coupling using dicyclohexylcarbodiimide, DCC, was also attempted and met with no success.[26] In comparison to these literature precedents, where it should be noted that less substituted species are used, each set of conditions generally prove to be effective methods of ester formation, however in this case these conditions were ineffective. The proximity of both the phenyl ring and the ketone on **6** may have caused the formation of the ester to be sterically less favored.



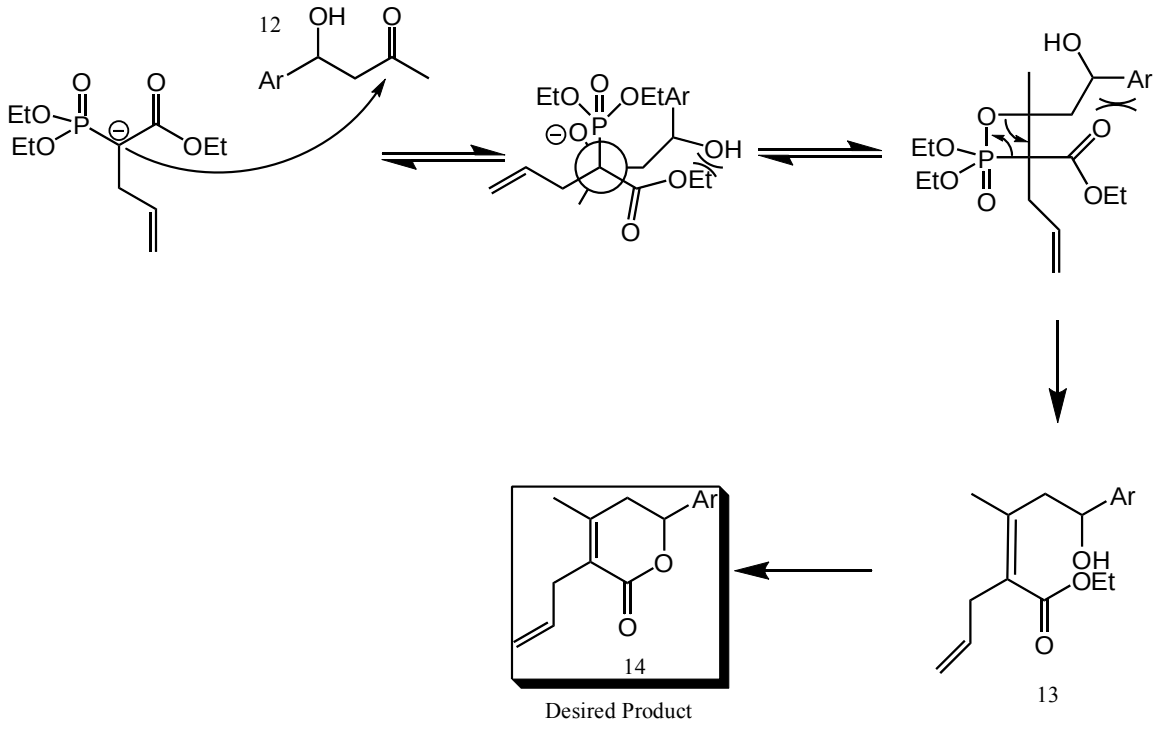
Scheme 2.3: Conditions for Esterification for Precursor of C Ring Lactone.

When developing another approach for C ring formation, once again we re-examined the system to see if one could approach the formation through focusing on a different functionality. As previously mentioned, a double bond is a key functionality in the system. One can focus on the key reaction being the olefination with lactonization being a bi-product of the substituents attached. This focus led to the idea of utilizing a Horner-Emmons Olefination[27,28], to provide the double bond compound that would further condense to the lactone. As shown in Scheme 2.4, this C ring formation starts by following the procedure for the same substrates of Minami *et al*, with direct alkylation of phosphonate **9** with allyl bromide **10** to form the alkylated product **11**.[29] This reaction is followed by the addition of the aryl substituted keto alcohol **12** via a Horner-Emmons reaction which would generate a species, **13**, with both an ester and an alcohol that can condense to form the desired lactone **14**. With the Horner-Emmons reaction, one projected challenge is the formation of the olefin. The ester and alcohol need to be in proximity of each other, **13**, in order for further condensation to be achieved.

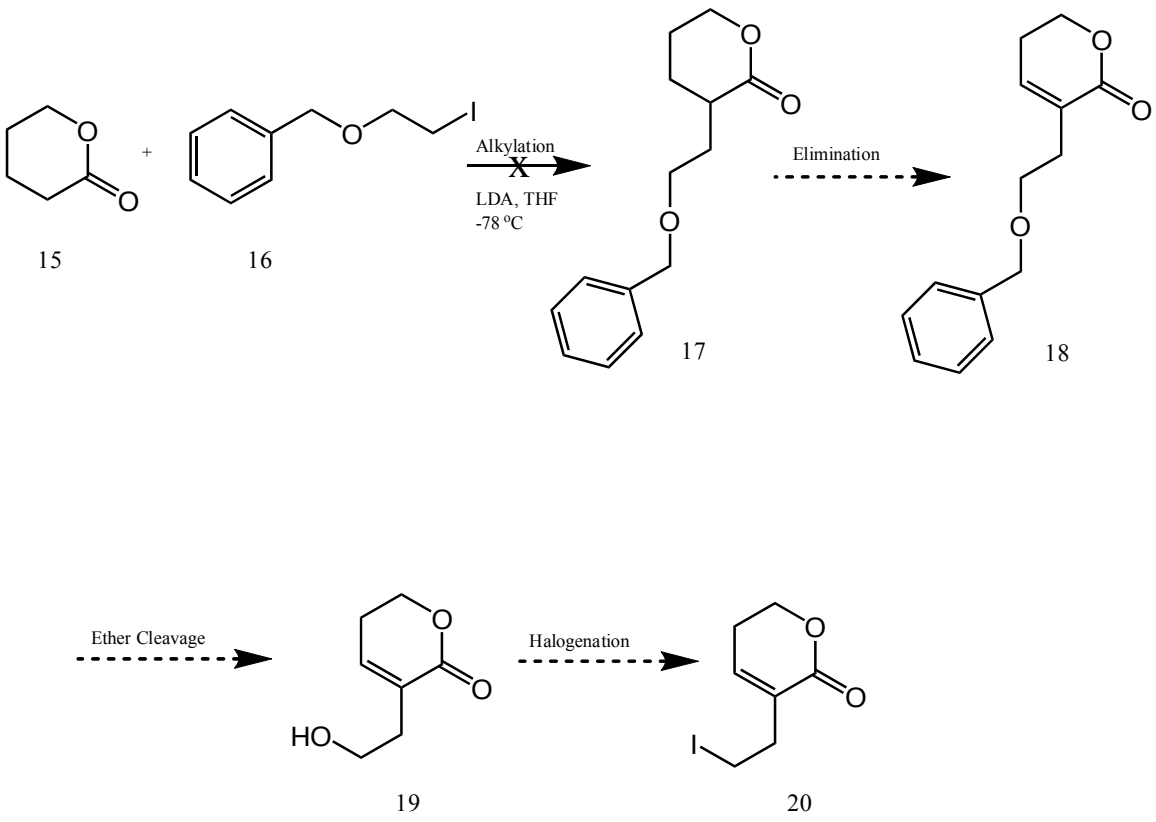
In this scheme, the alkylated phosphonate **11** was afforded however generating the olefin proved to be a challenge. When taking a closer look at the system, formation of the betaine intermediate, may have been difficult due to steric hindrance of the alcohol and ester prior to the prospect of condensation. Scheme 2.5 shows how formation of the anion of phosphonate **11** is required for addition to the carbonyl of **12** to form the precursor to the betaine intermediate. As shown in the scheme, steric congestion in the molecule is present possibly preventing this formation. As the chemical process carries on to formation of the betaine, four-membered ring system, intermediate, there still seems to be steric challenges. Overcoming that challenge proved to be much greater than initially hoped.



Scheme 2.4: Second Approach for C Ring Formation.



Scheme 2.5: Formation of Betaine Intermediate in Second Approach.



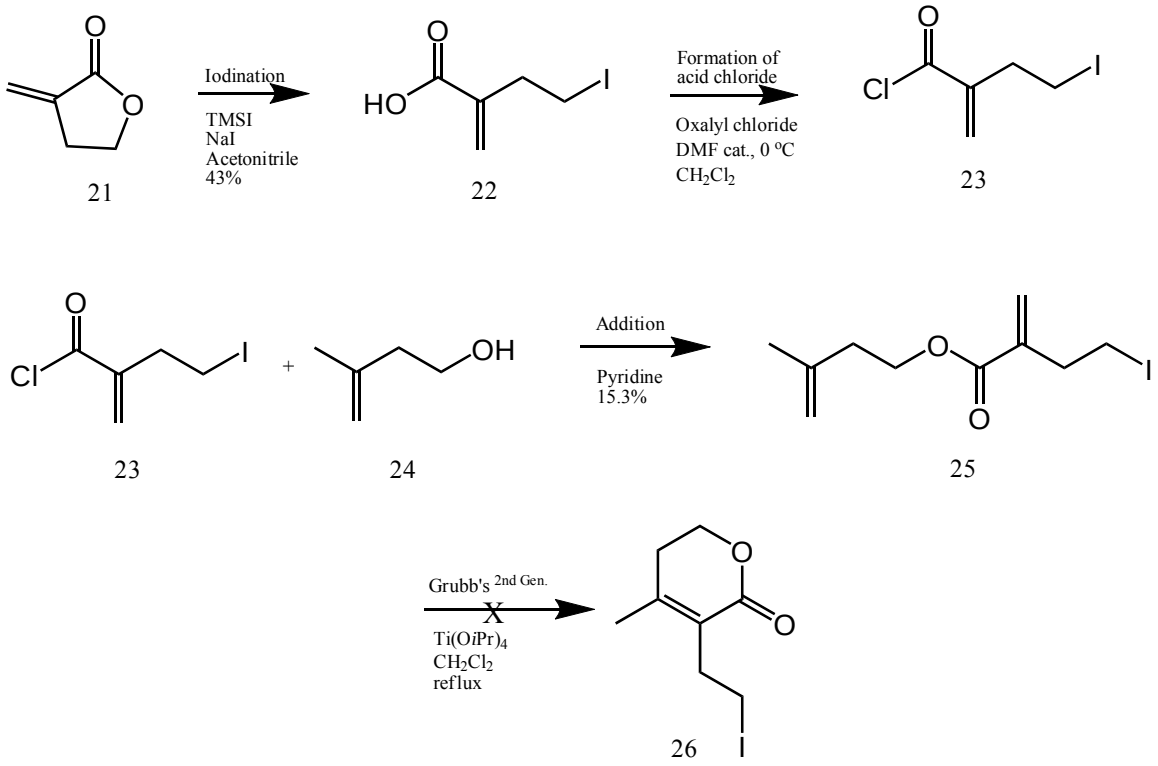
Scheme 2.6: Third Approach at C Ring Formation.

After the second attempt at the C ring formation, the approach began by refocusing on the lactone portion of the system and potentially adding the appropriate substituents around the lactone itself. Instead of attempting to form the lactone with substituents in place, the idea now was to start with commercially available lactone and attempt to modify the system to generate the desired C ring. This approach to C ring formation, as shown in Scheme 2.6, requires alkylation of valerolactone **15** with

iodoethyl benzyl ether **16**, generated from the alcohol following the procedure of Clive *et al*, through the formation of the enolate and addition to the iodinated electrophile to form product **17**.[30] Next is an elimination to add an unsaturation to the lactone ring **18**. This reaction would be followed by deprotection of the primary alcohol **19** which would be converted to a primary halide **20**. The road block with this method occurs with the first reaction, in an attempt to perform the alkylation. Following the procedure of Fuji *et al*, formation of the enolate of lactone **15** with lithium diisopropylamine is required before alkylation with iodoethyl benzyl ether **16**.[31] Formation of the elimination product of the iodoethyl benzyl ether **16** to form the vinyl benzyl ether occurs in this synthesis. This type of problem is well characterized in the literature where attempts at direct alkylation to an alkyl halide can generate the base induced elimination product.[32]

Although previous methods were met without success, continued resolve redirected the focus, after re-examination of the system, to building the desired lactone system from a more simple lactone. This approach takes the cyclic ester butyrylactone and manipulates the system to convert it to the substituted valerolactone through opening the cyclic system, adding the appropriate modifications and closing the modified cyclic system. Opening the cyclic system through halogenation positions the desired alkyl substituent in place while preparing the system for expansion. After the expansion of the system, the key reaction is reformation of the cyclic system while incorporating the double bond. This process can be accomplished through a known reaction, ring-closing olefin metathesis, where through a catalytic cycle, transalkylidenation occurs intramolecularly.[33] Various catalysts have been developed for olefin metathesis; Grubbs' catalyst was first used due to its ability to tolerate varying functionalities in varying

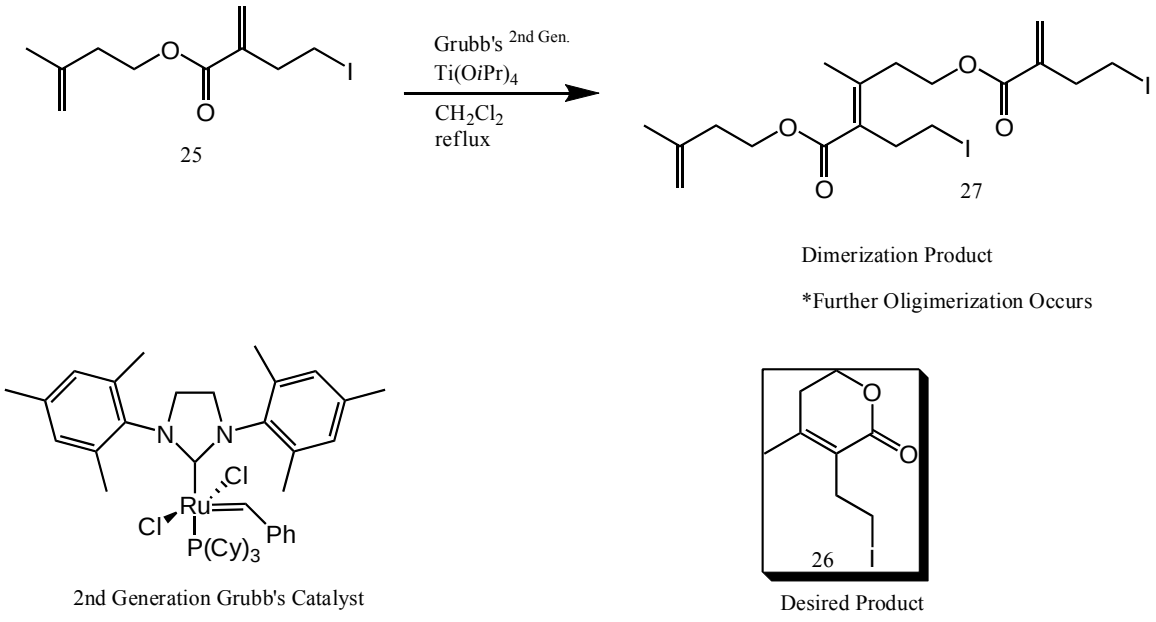
solvent systems.[32] Of the two generations of Grubbs' catalysts developed, the 2nd generation was selected because it is established in the literature to have higher activity.[33]



Scheme 2.7: Fourth Approach of C Ring Formation.

As shown in Scheme 2.7, this C ring formation attempt began with the iodination of a methylene butyrylactone **21** to generate the iodobutyric acid species **22**, following the procedure of Narayana *et al*.[34,35] Formation of the acyl chloride **23** and esterification with olefinated alcohol **24** to form the compound **25** that has an olefin on the two ends of the structure. This product is ready to undergo the Grubb's olefin

metathesis to form the desired lactone ring **26**. The steps in this sequence followed the literature precedent for identical substrates leading up to the olefin metathesis reaction. Opening the butyrylactone **21** to the acid **22** followed by the formation of the acyl chloride **23** were generated with data comparative to the literature precedence.[34,35] Likewise esterification proceeded in low yield, 15.3%, much like precedence for similar substrates.[34,35,36]

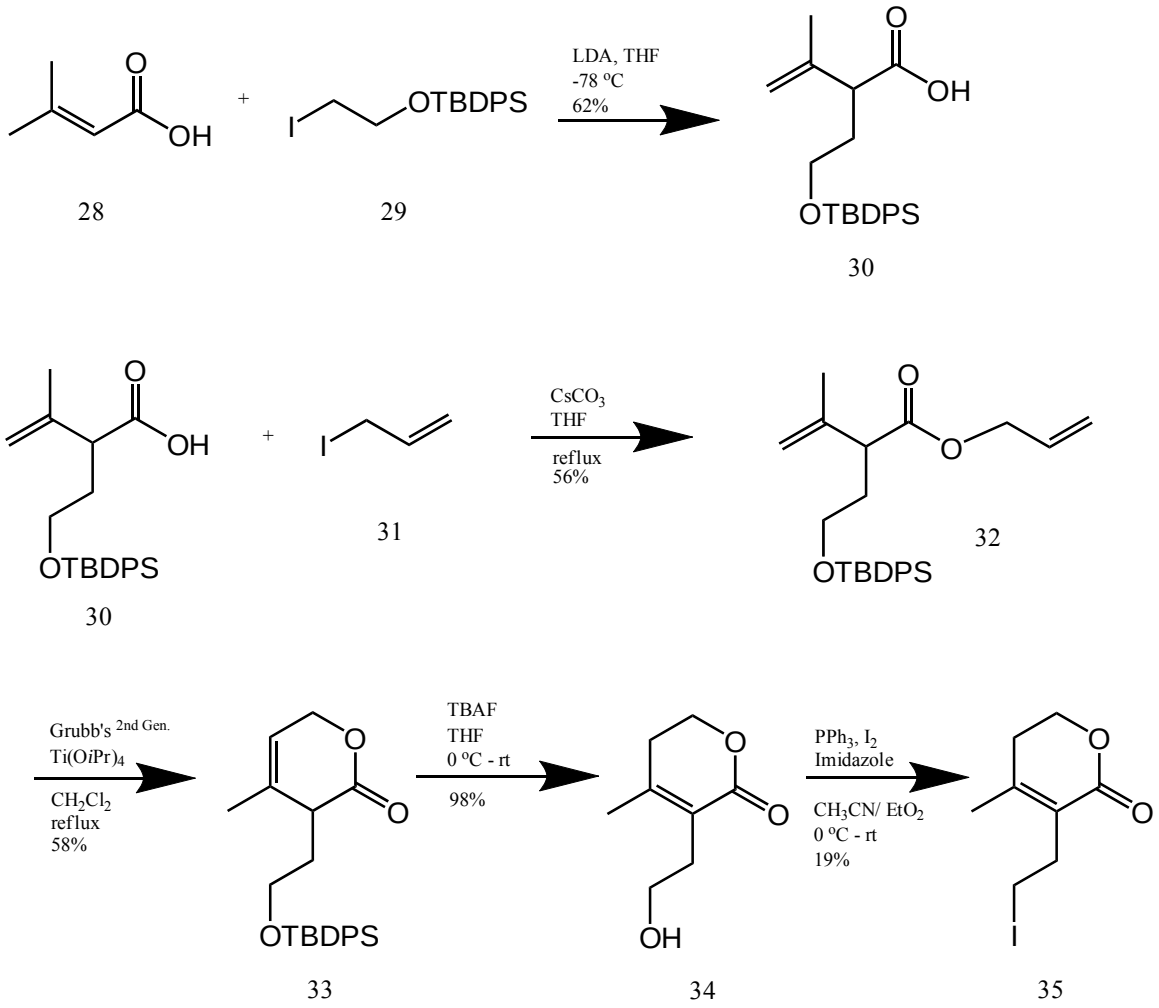


Scheme 2.8: Olefin Metathesis Intermolecular Product Formation.

The challenge with this scheme is formation of the intramolecular olefin metathesis product. The intermolecular product appears to be favored over the intramolecular product.[33] Proximity of the double bonds in the system plays an important role, where a concentrated sample of the substrate will allow for double bonds from

opposing molecules to be in closer proximity than from within the same molecule, where rotation is required for the double bonds to be in the appropriate proximity.[33] Dilution of the reaction mixture is the immediate response to this possibility, however, even after subsequent varying dilutions, the intermolecular product formed over the desired intramolecular product **26**. Scheme 2.8 illustrates the intermolecular dimerization **27** that occurs over formation of the desired product. It is important to note that further oligimerization can occur with continued implementation of reaction conditions.

Sometimes in synthetic chemistry, accomplishing the formation of a molecule does not require total re-examination of the system but just an adjustment in perspective. This final approach to C ring formation, much like the previous method, first required the formation of the alkylated ester with terminal olefins, **32**, in preparation for the ring closing olefin metathesis reaction. This method was met with success generating the desired intramolecular product. However, the metathesis product differs from the previous method by placing the double bond in another position, compound **33**. This repositioning of the double bond may seem to pose a problem however, when looking ahead at the full synthetic scheme, Scheme 2.10, particularly at the portion where the double bond will be required for the key Heck reaction, conversion of molecule **36** to molecule **2**, the double bond in either position could potentially generate the desired product. More interestingly, serendipitously the double bond shifts to the originally desired position after deprotection of **33** generating the alcohol **34**, allowing the double bond to be conjugated to the system.

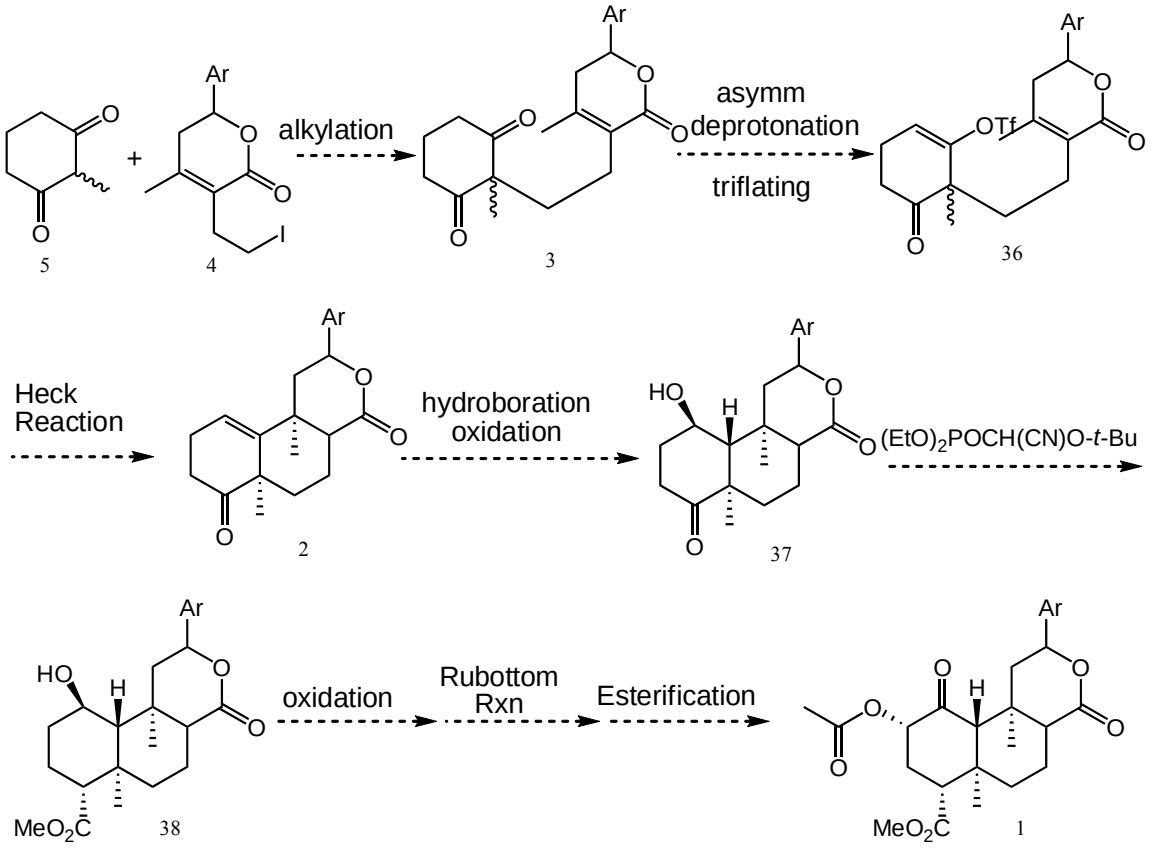


Scheme 2.9: C Ring Formation.

The formation of the C ring, shown in Scheme 2.9, was accomplished by the direct alkylation of dimethyl acrylic acid **28** with protected iodoethanol **29**. Then further alkylation of the hydroxyl group on the acid **30** with allyl iodide **31** was achieved through formation of the allyl ester species **32**. This reaction is followed by the Grubb's ring closing olefin metathesis to generate the lactone **33**. The protected alcohol **33** is then

deprotected causing a shift in the unsaturation allowing it to be conjugated with the carbonyl of the lactone **34**. That alcohol **34** was then converted to the desired iodide **35**, ready for the beginning of the synthesis of Salvinorin A. Although not represented, more attempts to form the C ring with an aryl substituent attached were met with no success. The direction of the synthesis was slightly altered by continuing without the aryl substituent [which may have proved to be advantageous] allowing for substituent modification on the C ring later in the synthesis.

With the C ring formed and the A ring being a commercially available compound, we now possessed the tools to begin the synthesis. As shown in Scheme 2.10, the synthesis would begin with direct alkylation of **5**, the A ring, with **4**, the halogenated alkyl C ring. Once the alkylated product **3** is generated, triflating the enolate generated through asymmetric deprotonation follows to get **36**. This vinyl triflate **36** will now be ready for undergoing the key reaction in this synthesis. Uniquely, the design of this synthesis is utilizing the precedent of carbon coupling through a Pd catalyzed Heck reaction.[37] This method exploits the use of oxidative addition of Pd inserting in the vinyl triflate bond, then directing the coupling to an olefin.[37] If the reaction proceeds as desired, it would proceed through an intermediate with palladium alpha to the ester carbonyl. The generated species, and its tautomer, would be protonated to give the desired product, a trans decalin system.[22] This method has the advantage of the intermediate alkyl palladium not undergoing β-hydride elimination and therefore not inserting a double bond that would have to be removed later.[22]



Scheme 2.10: Forward Synthesis of Salvinorin A Route 1.

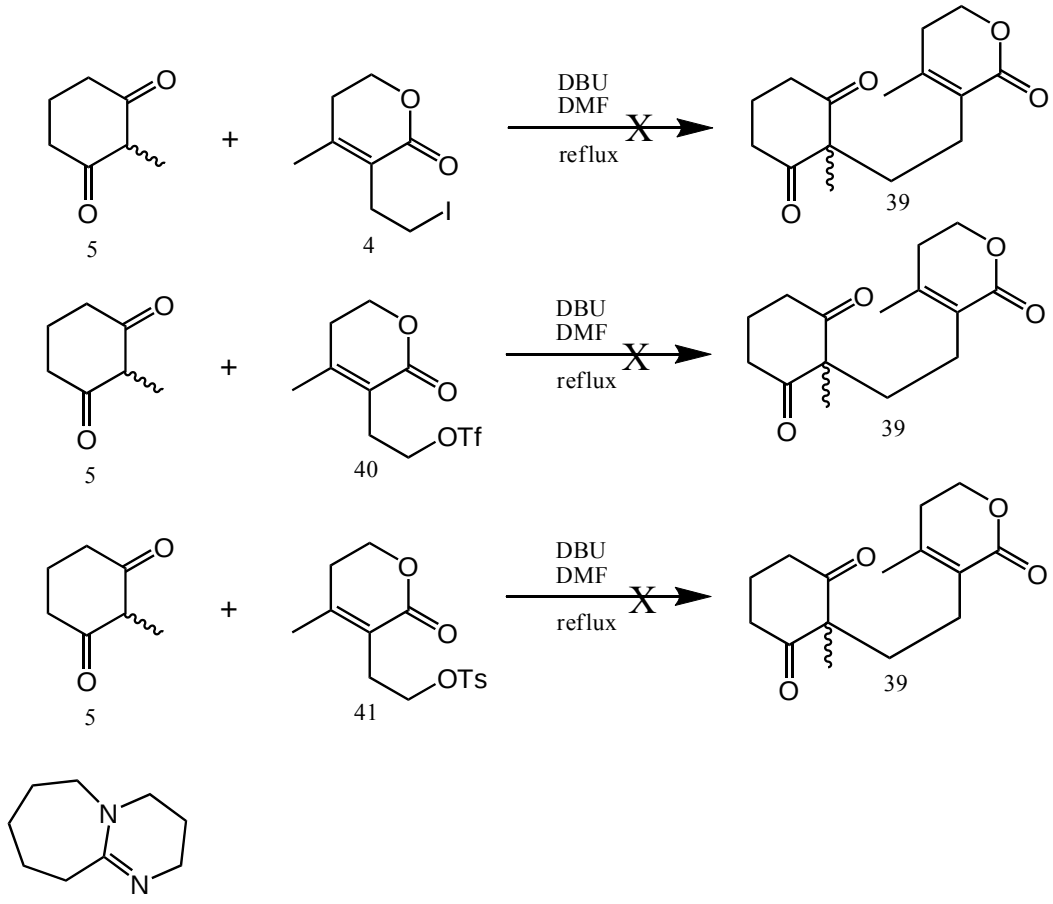
Customarily, this reaction proves to be useful for intermolecular coupling of two compounds, so employing this reaction in an intramolecular fashion makes for a creative use of known tools.[37] The literature precedent established by Overman for intramolecular coupling has shown that coupling to the more substituted end of the alkene is favored thus, in this system, would generate the five-membered ring spirocyclic species instead of the desired product.[37] Our belief for this system, however, is that the steric hindrance of generating the spirocyclic compound and the electronics of the α,β unsaturated system

would prove too difficult to overcome thus allowing the typically less favored coupling to form **2**.

After the tricyclic system is generated, the focus would turn toward substituent modification. The next reaction would be an oxidation to form compound **37** followed by a one carbon homologation of a ketone to the ester through the Horner-Emmons modification of the Wittig reaction using diethyl *tert*-butoxy(cyano)methyl phosphonate to generate compound **38**. Further modification involves the oxidation of the hydroxy group to have a carbonyl in place to now undergo the Rubottom reaction, which introduces a hydroxy group α to the carbonyl, achieved through the reaction of the silyl enol ether with mCPBA and subsequent rearrangement; the desilylation is accomplished with TBAF in aqueous work up.[38] Salvinorin A, **1**, is accomplished through esterification of the newly introduced hydroxy group.

Although formation of the halogenated C ring was a challenge, the roadblock in the synthesis arose when attempting to alkylate the C ring to the A ring. The sequence was first attempted with the C ring without an aryl group, represented differently in Scheme 2.10; incorporation of the aryl substituent later would follow the same procedure. However, as previously mentioned, challenges arose in the first step. What proved to be the challenge was not deprotonation in formation of the enolate on the A ring, demonstrated through silyl trapping of the enolate, but the direct alkylation to the system.[39] In this system, the iodide serves as the leaving group in this substitution reaction, however other leaving groups were explored, as shown in Scheme 2.11. Both triflate and tosylate groups, which were converted directly from the alcohol species **34**, were implemented in an attempt to generate the alkylation product. Neither leaving

group afforded the desired alkylated product. After further investigation of the alkylation reaction, performed by Tomas Vasques, compound **5** demonstrated a preference for O-alkylation over C-alkylation, causing a redirection in the approach for making this synthetic route viable.[39]



1,8 Diazabicyclo[5.4.0]undec-7-ene (DBU)

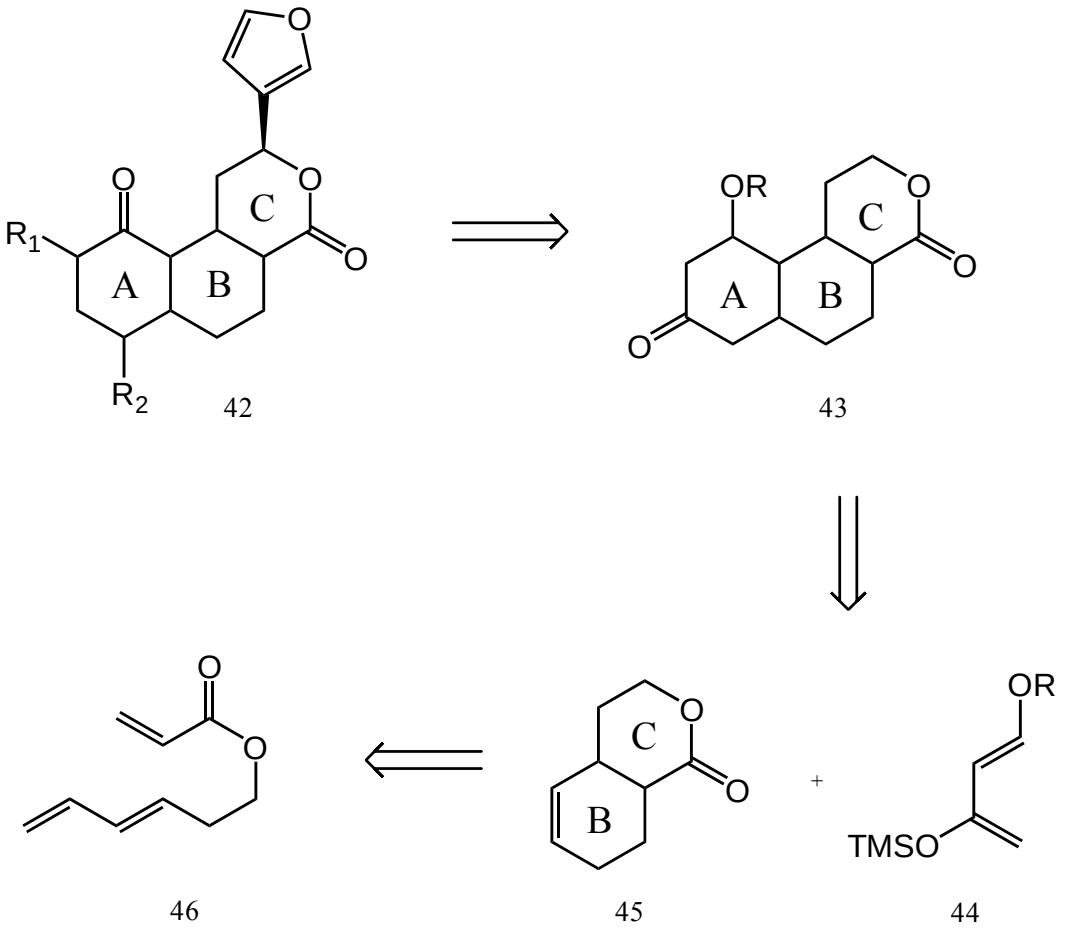
Scheme 2.11: Alkylation Reactions with Various Leaving Groups.

II. SYNTHETIC ROUTE 2 – DIELS ALDER

Like in many total synthesis undertakings, challenges bring about perspective changes regarding not only the methodology but the route of synthesis to reach the target molecule. The originally proposed route proved to be more challenging than anticipated so in turn, a new approach was attempted. Whereas the original approach called for the two outside rings, the A ring and C ring, to be joined for the formation of the B ring; the new approach calls for the formation of adjacent rings followed by the formation of the third ring. A Diels-Alder approach was attempted.

Retrosynthetic analysis demonstrates this new route by first simplifying Salvinorin A, **42**, to a minimally substituted tricyclic structure **43**, shown in Scheme 2.12. The next disconnection comes at the A ring formation joining **45**, the dienophile with the diene, **44**. The bicyclic structure **45**, is further simplified to an acrylic ester **46**, that contains both a diene and dienophile to undergo an intramolecular Diels-Alder reaction.[40] This unique approach potentially allows for a facile method of generating the tricyclic scaffold for analogue development of the Salvinorin system. It is important to note that this approach directs formation of the tricyclic system without the axial methyl groups, allowing for investigation of desmethyl analogues for determination of the utility of the methyl substituents with regard to structure activity relationship.

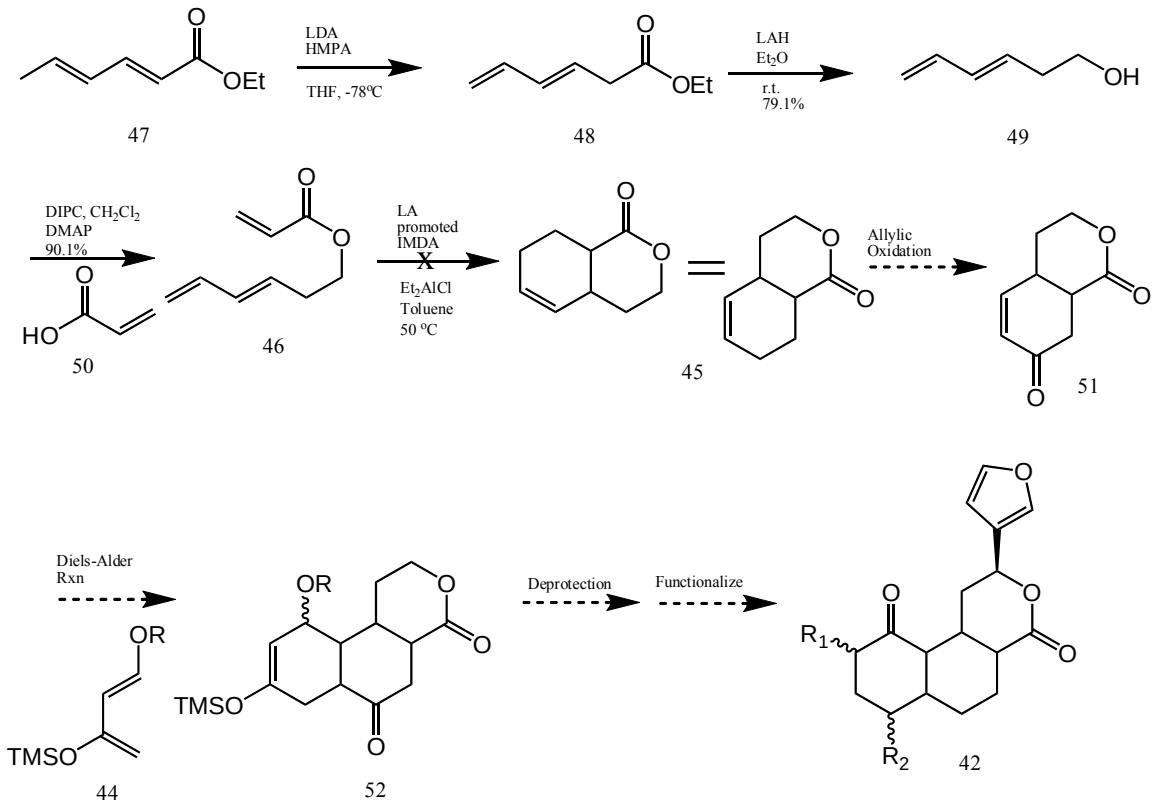
As shown in Scheme 2.13, the approach begins with the commercially available ethyl sorbate **47** being unconjugated through enolate formation and reprotonation, shifting the double bonds to the end of the system to form the unconjugated ester **48**, following Miller *et al*[41], literature precedent for the same substrate.



Scheme 2.12: Retrosynthetic Analysis of Synthetic Route 2.

The sorbate ester is prepared for reduction to the alcohol **49** using lithium aluminum hydride.[42] After formation of the alcohol, addition of acrylic acid to form the acrylic ester **46**, following the previously mentioned coupling procedure, now generates the species to undergo an intramolecular Diels-Alder reaction. Although both the diene and dienophile are in the system, it still requires Lewis acid promotion to undergo the reaction.[40] The goal, met with no success, was to undergo the intramolecular Diels-Alder

reaction, noting in Scheme 2.13 that re-orientation of the rings **45** show how this reaction would have formed the B and C-ring.



Scheme 2.13: Forward Synthesis of Salvinorin A Route 2.

The next step would have been to prepare **45** for an intermolecular Diels-Alder reaction with a highly electron rich Danishefsky diene **44**, through an allylic oxidation to incorporate a carbonyl on **50**.[43] If the intermolecular Diels-Alder had taken place, all three rings **52** would be in place to set up the Salvinorin scaffold. The versatility of this route is that functionality is introduced at the end of the route allowing for a variety of analogues to be generated. Scheme 2.13 demonstrates a forward synthesis towards a desmethyl Salvinorin A. The goals of this route were to both introduce the methyl functionalities late in the synthesis and to discover if the methyl groups are vital to the

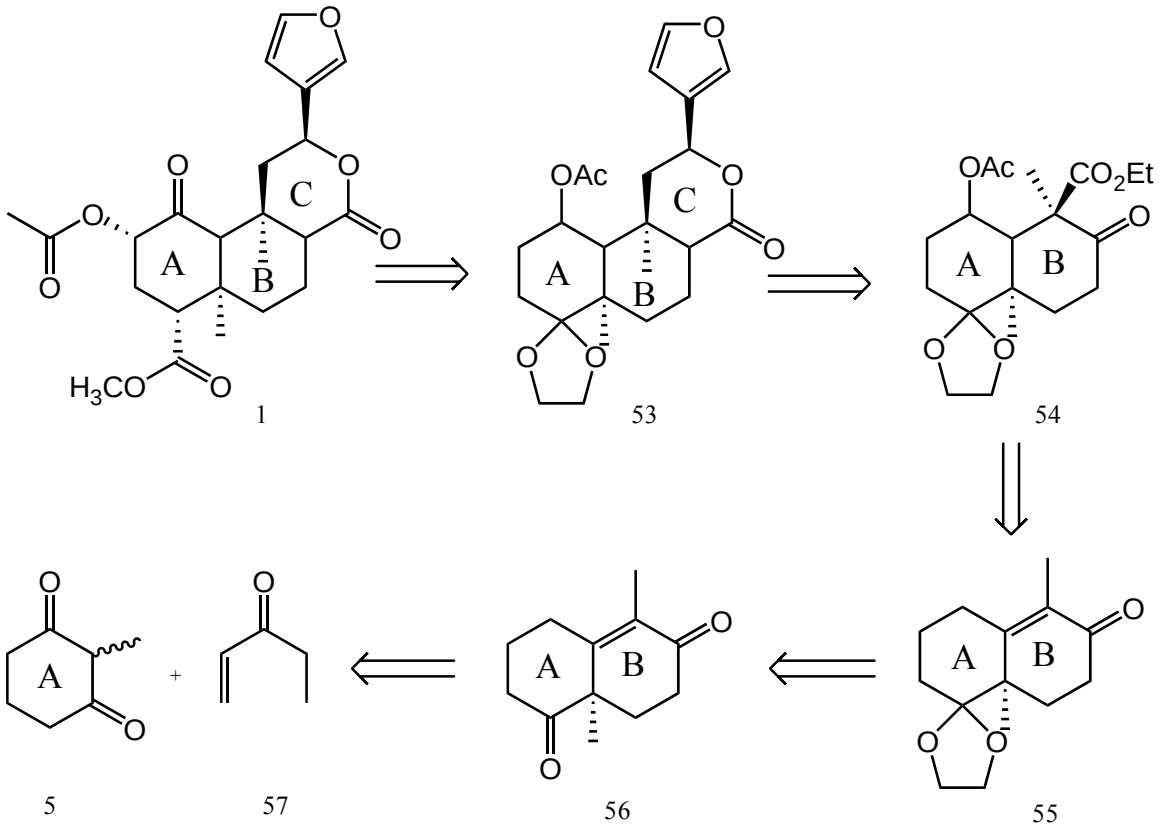
pharmacophore. Either case, this route was designed to provide a facile route to generating the A, B and C-ring system for the Salvinorin scaffold.

However, this route proved to be challenging towards generating the scaffold. The unconjugating conversion of ethyl sorbate **47** to **48** was a challenge but was overcome through creating the driest possible environment and conditions to run the reaction; where Galveston, TX often made that a challenge. Reduction followed by esterification to the acrylic ester were both performed and afforded products **49** and **46** in good yield, 80% and 90% respectively. The biggest challenge of this route is the intramolecular Diels-Alder reaction. This challenge may have been due to the diene and the dienophile not being in proximity with each other even though there is assistance with a Lewis acid. Scheme 2.13 shows **46** in the geometry that is sterically and energetically favored for the reaction, however, more likely, the favored geometry is linear, placing the diene and dienophile on opposite ends of the molecule and not in proximity to undergo the reaction.[40]

III. SYNTHETIC ROUTE 3 – WIELAND-MIESCHER KETONE

Much like the change in perspective that produced the Diels-Alder approach, a third approach was developed to overcome the challenges of the previous routes. Whereas the previous route made use of forming the C and B rings first, followed by the addition of the A ring, this approach starts by formation of the A and B rings followed by the formation of the C ring, exploiting the formation of the Wieland-Miescher ketone. The Wieland-Miescher approach is more advantageous with regard to generating

analogues, due to a desired site of modification being on the C ring allowing the formation to come at the end of the synthesis and not locked in at the beginning.



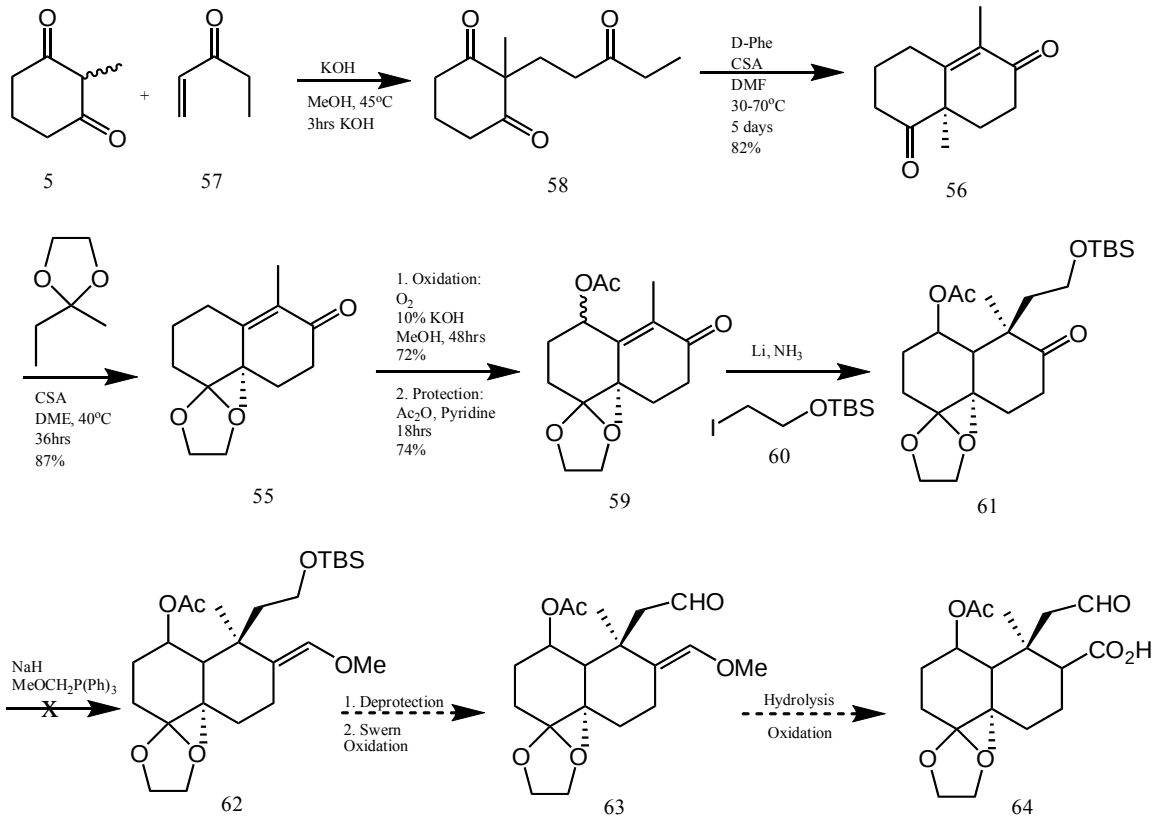
Scheme 2.14: Retrosynthetic Analysis of Synthetic Route 3.

Retrosynthesis begins, as shown in Scheme 2.14, with removal of substituents from the A ring of **1** to give an acetate protected alcohol and a ketal protected carbonyl **53**. This product leads into the next disconnection which occurs at the C ring, with opening of the lactone system along with removal of the electrophilic aromatic

substituent **54**. Further removal of the ester functionality and the acetate protected alcohol, leaves a simple ketal protected species **55**. This compound is derived from a well known Wieland-Miescher diketone **56**.

Synthesis begins with the formation of the Wieland-Miescher diketone by first directly alkylating 2-methyl 1,3 hexadione with ethyl vinyl ketone to form **58**, Scheme 2.15. This product undergoes a Robinson annulation that is stereo controlled with both D-CSA and L-proline to give the desired Wieland-Miescher diketone **56**.[44] Precedent for the stereospecific formation of the diketone is well established in the literature.[44] Absolute verification of the stereochemistry was not performed, with the understanding that if the opposite species is generated, one would simply utilize the opposite CSA and amino acid for the formation of the desired product. The next step is selective ketal protection of the carbonyl **55**. The protection reaction is followed by an allylic oxidation to generate the secondary alcohol which is further protected by an acetate **59**.[44] Now what takes place is one of the key reactions to this route, the reductive alkylation. This reaction adds protected iodoethanol **60**, the beginning part of the C-ring formation and sets the stereochemistry for the methyl group at the B/C-ring juncture to obtain the product **61**. The Wittig reaction to generate the vinyl methyl ether **62**, a synthon for aldehyde, begins the formation of the C-ring. Next is the deprotection of the silyl protected primary alcohol followed by a Swern oxidation to generate the aldehyde species **63**. Then acid hydrolysis of the methyl vinyl ether and oxidation to the carboxylic acid follows to form **64**. At this point, the scaffold is in position to add various aryl substituents to render derivatives with unique functionality. As shown in Scheme 2.16, Addition to this carbonyl would convert the aldehyde over to alcohol which

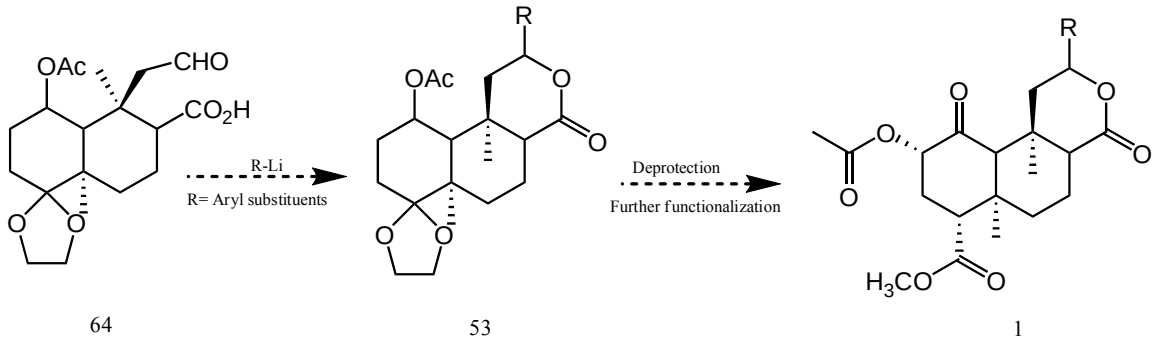
would further condense with the carboxylic acid to form the lactone **53**. Further functionalization leads to Salvinorin A **1** or possible derivatives.



Scheme 2.15: Forward Synthesis of Salvinorin A Route 3, part 1.

In attempt to accomplish this route, Hagiwara's group completed Salvinorin A total synthesis in a similar fashion.[45] Hagiwara has long exploited the use of Wieland-Miescher ketones to produce natural product targets.[45,46] The route his group developed starts with the ketal protected diketone and performs the allylic oxidation. However, this group uses a different hydroxy protection followed by a different alkylating agent with the reductive alkylation reaction. Modification of the C ring was similar in approach by

olefination, but with a terminal methylene, and oxidation to generate a species similar to **53** in Scheme 2.16 prior to condensation. Hagiwara's route afforded Salvinorin A in modest yield.[45]



64 53 1

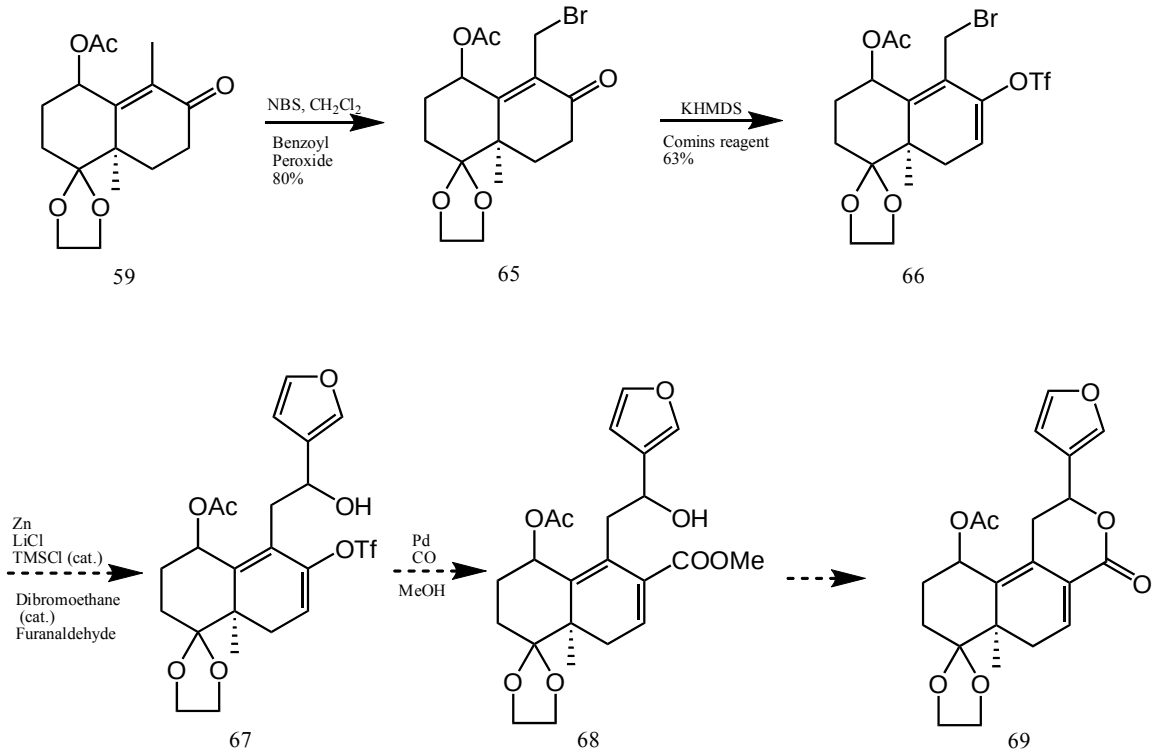
Scheme 2.16: Forward Synthesis of Salvinorin A Route 3, part 2.

IV. SYNTHETIC ROUTE 4 – SALVINORIN SCAFFOLD

It is important to not only develop a synthetic route that leads to a facile method of generating the target molecule, but also to develop a scaffold that lends itself to readily generate analogues. Since Hagiwara developed the total synthesis[45], the focus switched to developing a viable scaffold for generating Salvinorin analogues. My approach led me to developing a key intermediate that would lend itself to variable transformations to generate several ligands.

Much like Scheme 2.15, the scaffold synthesis begins with formation of the Wieland-Miescher ketone, followed by the selective ketal protection of the carbonyl.[46] Then the allylic oxidation along with acetal protection, as previously demonstrated,

follows. The route now changes when introducing a key functionality as shown in Scheme 2.17. The acetal protected species **59** undergoes an allylic bromination to generate the bromide **65**. This product is a key intermediate that sets up the scaffold to have flexibility in adding various substituents in the formation of the C-ring. Next the carbonyl is protected with a triflate through trapping the enolate to form the compound **66** that now allows the bromide to react and serve a vital piece to the C-ring formation. As schematically demonstrated in Scheme 2.17, utilizing Knochel chemistry would be the methodology of adding the furanyl substituent leaving the alcohol **67** in place for further condensation.[47]



Scheme 2.17: Salvinorin Scaffold Synthesis.

This reaction would be followed by Pd catalyzed Stille coupling and trapping with MeOH to generate the ester **68**, which would further condense to get a Salvinorin derivative **69**.[48]

Results

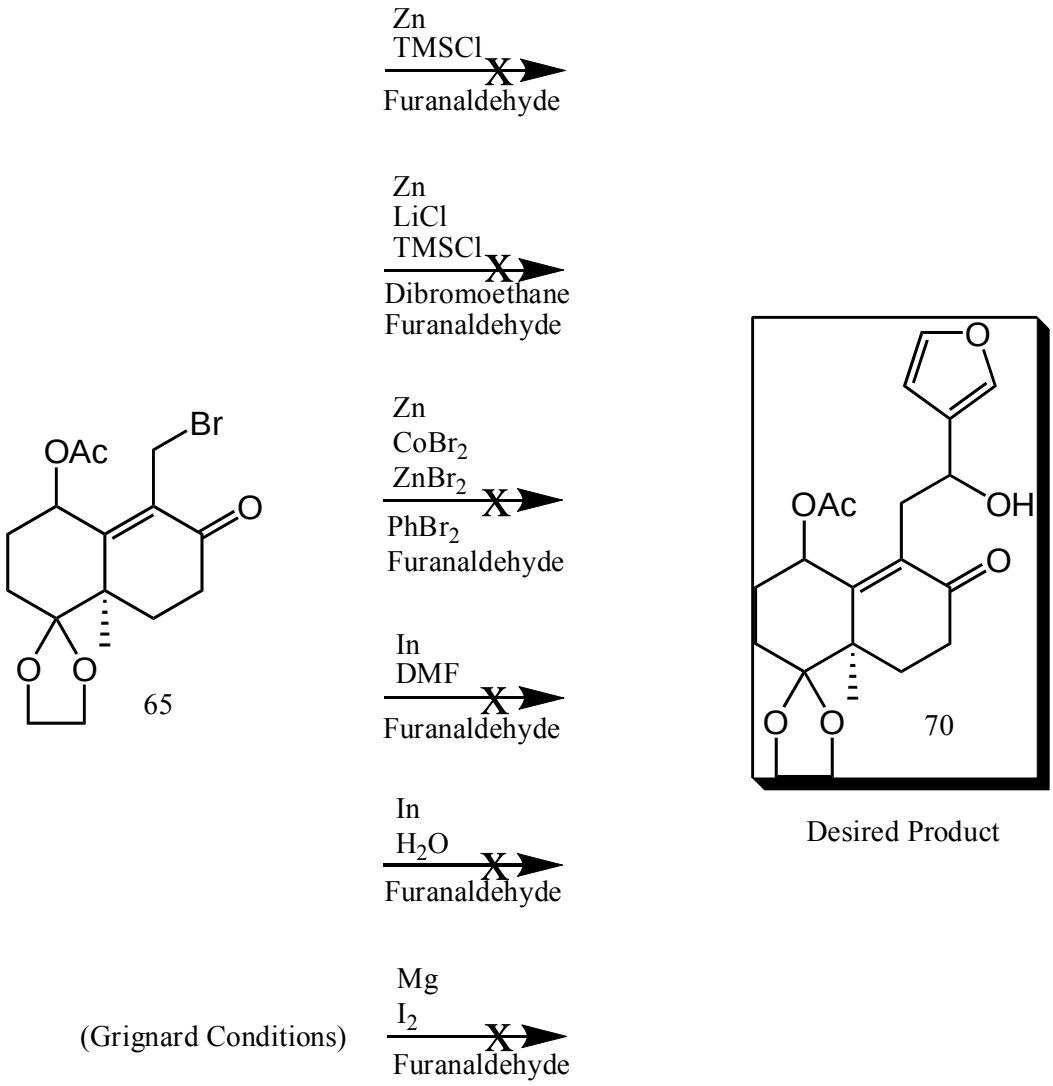
Synthesis begins with formation of the Wieland-Miescher diketone in a selective fashion, placing the methyl group substituent axial. Following the literature precedent, addition of ethyl vinyl ketone **57** to the methyl diketone **5** is afforded in presumably good yield but is carried on without purification to annulation in a stereo controlled fashion.[44] The addition of L-proline directs the annulation to the desired product **56** with a calculated yield of 82% over two steps. After purification, enantiomeric purity is not validated with the understanding that if the wrong enantiomer is generated, simply replacing L-proline with D-proline could direct the appropriate product formation. After looking at both ^{1}H-NMR and ^{13}C-NMR, two products have formed, with the assumption, according to literature precedent, of the major product being the desired enantiomer and the minor product being the other enantiomer. Had the goal of the synthesis been to just generate the natural product, these steps would require optimization.

Selective ketal protection of **56**, also based on literature precedent, afforded the product **55** in 87% yield.[44] Recovery of some starting material **56** was afforded. The following step, allylic oxidation, at first proved to be a challenge. The compound **55** was dissolved in methanolic KOH and either left open to air or streamed with bubbling O_2 over a three day period to generate the alcohol product. At first this method proved to

render very modest yields, so heat was added to the procedure over a twenty-four hour period driving product formation in 72% yield.[43] ^{1}H-NMR indicates diasterotopic products, seeing that the introduction of the hydroxyl group adds another sterogenic center. The necessity to isolate a particular diasteromer is mute do to future conversion of that hydroxyl group to carbonyl later in the synthesis. The hydroxyl product is further protected with an acetate to afford the compound **59** in 74% yield. Now the synthetic route had reached a pivotal point where formation of the C-ring was about to begin. The addition of TBS protected iodoethyl alcohol was accomplished through reductive alkylation to generate the product **61**, after multiple attempts. Product formation afforded a very low yield that was detectable and characterized by ^{1}H-NMR but not by ^{13}C-NMR. Further confirmation of the product **61** is demonstrated with high resolution mass spectrometry, where the accurate mass of 454.2850 was measured. During the period where attempts to increase the yield of this reaction were met with no success, the Hagiwara group developed the complete synthesis of Salvinorin A, utilizing a similar scheme building off the Wieland-Miescher ketone moiety.

Now the synthetic route attempts to set up a scaffold that contains functionalities that lend themselves to modification readily. This route still utilizes the Wieland-Miescher ketone moiety. As previously mentioned, the predominant site for modification is on the C-ring. To address this challenge, incorporation of a functionality with high utility becomes the goal in this synthesis. This route begins like the previous one through formation of the Wieland-Miescher diketone **56**, selective ketal protection **55**, allylic oxidation and acetylated protection **59**. Now the new portion starts with incorporating a

bromide through free radical halogenation (Scheme 2.17). Initially to generate **65**, N-bromosuccinimide (NBS) was the bromine source with



Scheme 2.18: Knochel Conditions (including Grignard Conditions).

azobisisobutyronitrile (AIBN) as the radical initiator, however benzoyl peroxide proved to be a better initiator affording the product in 78% yield. ^{1}H-NMR continues to indicate diasteromers, but even more so the protons adjacent to the bromide in the system

demonstrate vicinal coupling by forming a doublet of doublets with the AB-type coupling. With the incorporation of bromide, the system was thought to be ready for C-ring formation with varying substituent modification. Using Knochel chemistry, metallobromide addition to a carbonyl, was to provide the best method of ring formation along with adding varying functionalities.[47] Multiple attempts, including using Grignard conditions, proved to be problematic.

Further investigation and inquiry with the Knochel group's chemistry revealed that α-protons to the carbonyl of the B-ring disrupt the interaction of the metal, Zn, with the bromide. So this concept shifted the implementation of the Knochel reaction one step later, after modification of the carbonyl of the B-ring. Conversion of the carbonyl to a vinyl triflate was the most advantageous approach. This conversion eliminated the acidic α-protons by placing an unsaturation in the system, but more importantly sets up the system for incorporation of the lactone portion of the C-ring. This incorporation would be accomplished by palladium, Pd, catalyzed Stille like coupling where Pd adds across the vinyl triflate allowing for carbon monoxide, CO, insertion.[48] This insertion would also begin the extension of the C-ring lactone portion. Instead of completing the coupling cycle, like in Stille couplings, one could trap this step with methanol leaving a methyl ester as the product.[48] This methyl ester is one piece of the condensation that will undergo to form the lactone. Conversion of the carbonyl **65** to the triflate **66** was met with success and afforded a product in 63% yield; with an overall yield of 19.2% from the beginning of the synthesis.

Compound **66** now becomes the pivotal molecule, the scaffold, that sets up the framework for making analogues while forming the C-ring. Although the Hagiwara group completed the synthesis for Salvinorin A, their strategy still does not lend itself to facile modification for generating a library of derivatives. It is our contention that this scaffold will serve as a unique strategy for making Salvinorin libraries.

Materials and Methods

General Methods

All reactions were performed under nitrogen atmosphere unless noted differently. Commercially available materials were used without further purification or distillation unless noted otherwise. Tetrahydrofuran was freshly distilled from benzophenone ketyl radical under nitrogen and dichloromethane was freshly distilled from calcium hydride under nitrogen prior to use. All glassware was placed in 50 ^{o}C incubator for a minimum of 24 hours prior to use. ^{1}H-NMR and ^{13}C-NMR spectra were obtained in solutions of deuterochloroform with Varian 360, JOEL 400 and JOEL 500.

Experimental

1-Iodoethyl-2-benzyl ether, 16. To a solution of 1-hydroxyethyl benzyl ether (0.47 mL, 3.29 mmol) in 10mL of THF was added triphenylphosphine, PPh_3, (2.41 g, 9.20 mmol) and imidazole (0.67 g, 9.86 mmol) and cooled to 0^{o}C. After cooling and addition of I_2 (1.92 g, 7.56 mmol), the mixture was allowed to stir for 2.5 hours. While stirring, the mixture was allowed to warm to room temperature. The reaction mixture

was quenched with H_2O and product extracted with EtOAc. The product was subsequently washed with aqueous Na_2SO_3 and 5% HCl; then dried over anhydrous $MgSO_4$. Filtration and evaporation of EtOAc affords 0.71 g (84%) of the halogenated product. Spectral data agreed with reported data, Clive *et al*.[65]

1-Iodoethyl-2-*t*-butyl-dimethylsilane, 29. To a solution of iodoethanol (3.17 mL, 0.04 mol) in 40 mL of CH_2Cl_2 at 0 ^oC was added Et_3N (6.30 mL, 0.04 mol) and catalytic amounts of 4-dimethylaminopyridine, DMAP. After stirring 10 minutes, chloro-*t*-butyl-dimethylsilane (6.75 g, 0.04 mol) was added and stirred allowing mixture to warm to room temperature. The reaction mixture was quenched with H_2O and product extracted with EtOAc. The product was subsequently washed with brine and dried over anhydrous $MgSO_4$. Evaporation of EtOAc is followed by column chromatography with eluent solvents being hexanes and EtOAc (9:1 respectively) of the residue afforded 10.76 g (92%) of the protected alcohol as a viscous oil.

2-Methyl-3-(ethoxy-*t*-butyl-dimethylsilane)-but-1-enoic acid, 30. A solution of diisopropylamine (11.69 mL, 0.08 mol) in THF was cooled to 0 ^oC. To that solution was added n-BuLi (33.35 mL, 0.08 mol) dropwise and cooled to -78 ^oC. Then was added 3,3-dimethylacrylic acid (3.98 g, 0.04 mol) to that mixture and allowed to stir 15 minutes. In a separate flask a solution of the 1-iodoethyl-2-*t*-butyl-dimethylsilane **30** (11.36 g, 0.04 mol) in THF is prepared. The activated acrylic acid is added to the prepared solution via cannula and allowed to stir while warming to room temperature. The THF is evaporated

from the reaction mixture. The reaction mixture is partitioned between H_2O and Et_2O. The organic mixture is subsequently washed with HCl and brine followed by drying over anhydrous $MgSO_4$. Evaporation of ether is followed by column chromatography with eluent solvents being hexanes and EtOAc (3:2 respectively) of the product that afforded 6.36 g (62%) of the alkylated acrylic acid as an oil.

2-Methyl-3-(ethoxy-*t*-butyl-dimethylsilane)-*O*-allyl-but-1-enoate, 32. A solution of the alkylated acrylic acid **31** (0.49 g, 1.88 mmol) and $CsCO_3$ (0.67 g, 2.07 mmol) in THF was heated and stirred at 85 ^oC. After approximately 15 minutes, add allyl iodide (0.19 mL, 2.07 mmol) and reflux. After completion of the reaction, allow to cool and filter insoluble $CsCO_3$. Concentration of the mixture followed by column chromatography with eluent solvents being hexanes and EtOAc (9:1 respectively) of the product generated 0.31 g (56%).

2-(Ethoxy-*t*-butyl-dimethylsilane)-3-methyl-cyclohex-3-enoate, 33. A solution of the allyl ester **33** (0.31 g, 1.05 mmol) in CH_2Cl_2 is diluted to 0.001 M. To that solution is added $Ti(OiPr)_4$. After 1.5 hours of refluxing is added 2nd Generation Grubb's catalyst (0.03 g, 4 mol%) and refluxed for an additional 3 hours. The mixture after cooling is concentrated and filtered through celite. Evaporation of filtering solvent is followed by column chromatography with eluent solvents being hexanes and EtOAc (9:1 respectively) of the product that afforded 0.16 g (58%) of the lactone.

3,5-Hexadien-1-yl ethenoate, 46. Dissolve acrylic acid (0.05 mL, 0.73 mmol) in dry CH_2Cl_2 at room temperature. Add the alcohol **43** (0.09 g, 0.87 mmol) to the suspension followed by addition of diisopropyl carbodiimide, DIC (0.14 mL, 0.87 mmol). Next add catalytic amounts of 4-dimethylaminopyridine, DMAP. After completion of reaction remove solvent *in vacuo* and partition between H_2O and ether. Extract product with more ether and subsequently wash with aqueous $NaHCO_3$, 2 M HCl and brine. Dry organic over anhydrous $MgSO_4$. Evaporation of solvent is followed by column chromatography with eluent solvents being hexanes and EtOAc (9:1 respectively) to afford 0.11 g (90.1%) of the acrylic ester. ^{1}H-NMR (360 MHz, TMS, $CDCl_3$) δ (ppm) 1.21 (dd, 1H), 1.43 (d, *J*= 7.2 Hz, 2H), 3.47 (q, *J*= 10.8 Hz, 1H), 4.00 (m, 2H), 4.15 (m, 1H), 4.43 (m, 1H), 4.63 (d, *J*= 7.2 Hz, 2H), 5.30 (s, 1H), 5.75 (m, 2H), 6.30 (m, 2H).

3,5-Hexadiene-1-yl ethanoate, 48. Dissolve diisopropylamine (1.97 mL, 14.0 mmol) in dry THF and cool to -78 ^{o}C and add dropwise *t*-butyllithium (8.21 mL, 14.0 mmol) allowing 30 minutes to stir. Maintaining mixture at -78 ^{o}C, add hexamethylphosphoramide, HMPA (2.86 mL, 16.4 mmol) allowing to stir 20 minutes. Add ethyl sorbate in THF dropwise and stir for 1 hour, until solution turns dark red. The solution is then poured into a ice cold solvent mixture of H_2O:HOAc (10:1.3 respectively). The product is extracted with pentane and subsequently washed with aqueous $NaHCO_3$ and brine. The product is dried over anhydrous $MgSO_4$. Excess solvent is removed in vacuo and product is carried on to next reaction without purification. ^{1}H-NMR (360 MHz, TMS, $CDCl_3$) δ (ppm) 1.25 (m, 2H), 1.84 (dd, *J*= 7.2

Hz, 2H), 3.46 (q, *J*= 7.2 Hz, 1H), 3.47 (q, *J*= 7.2 Hz, 3H), 4.17 (q, *J*= 7.2 Hz, 2H), 4.18 (q, *J*= 7.2 Hz, 2H), 5.74 (d, *J*= 18 Hz, 1H), 5.75 (d, *J*= 18 Hz,1 H), 6.14 (m, 2H)

3,5-Hexadien-1-ol, 49. Suspend **48** (1.06 g, 7.55 mmol) in ether and slowly add it to a stirred suspension of lithium aluminum hydride (0.40 g, 10.57 mmol) in ether. Stir mixture at room temperature. Quench reaction with sequential addition of 2mL of H_2O, 6 mL of 2 M NaOH and 2 mL of more H_2O. Extract with more ether and filter organic mixture. Wash organic with brine and dry over anhydrous $MgSO_4$. Evaporation of ether is followed by column chromatography with eluent solvent being CH_2Cl_2 affording 0.56 g (79.1%) of the alcohol product. ^{1}H-NMR (360 MHz, TMS, $CDCl_3$) δ (ppm) 0.91 (m, 1H), 1.35 (m, H), 1.55 (m, 1H), 1.85 (d, *J*= 7.2 Hz, 2H), 3.40 (t, *J*= 10.8 Hz, 1H), 4.18 (q, *J*= 7.2 Hz, 2H), 5.75 (d, *J*= 18 Hz, 1H), 6.15 (m, 2H).

(4a*R*)-(-)-1,4a-Dimethyl-4,4a,7,8-tetrahydronaphthalene-2,5(3*H*)(6*H*)-dione-5-ethylene acetal, **55**. A solution of the enone (3.85 g, 20.03 mmol) and D-camphorsulfonic acid (0.37 g, 1.59 mmol) in ethylene acetal of 2-butanone (21.26 mL, 170.00 mmol) and ethylene glycol (13 mL) was heated for 36 hours at 40 ^{o}C. The reaction was allowed to cool and subsequently placed in an ice bath. The solution was then poured into aqueous $NaHCO_3$, and extracted with ether (50 mL x 2). The combined ethereal extracts were washed with water, brine and dried over anhydrous $MgSO_4$. Evaporation of the solvent followed by column chromatography with eluent solvents being hexanes and EtOAc (3:2 respectively) of the residue provided 4.11 g of the acetal (87%) and remaining enone starting material. ^{1}H-NMR (400 MHz, TMS, $CDCl_3$) δ

(ppm) 1.42 (s, 3H), 1.46 (s, 3H), 1.78-1.79 (m, 4H), 1.79 (s, 3H), 1.81 (s, 3H), 2.06-2.16 (m, 2H), 2.39-2.54 (m, 2H), 2.64-2.72 (m, 2H), 2.85 (t, *J*= 4.96 Hz, 2H), 2.89 (t, *J*= 5.76 Hz, 2H), 3.91-4.01 (m, 4H); ^{13}C-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 11.03, 11.16, 11.38, 20.59, 20.69, 21.13, 21.26, 23.12, 23.59, 25.22, 26.17, 26.26, 27.03, 29.36, 29.41, 32.30, 33.05, 33.38, 37.08, 45.02, 48.54, 50.40, 64.78, 65.05, 73.01, 112.48, 128.09, 129.70, 129.74, 130.46, 132.95, 158.12, 160.15, 169.04, 197.44, 198.50, 206.03, 211.66, 211.83; HRMS (TOF MS ES+) for $C_{14}H_{20}O_3$: calcd. 236.1310, found 236.1317.

(4a*R*)-(-)-1,4a-Dimethyl-4,4a,7,8-tetrahydronaphthalene-2,5(3*H*)(6*H*)-dione, **56**. A solution of the triketone carried over from previous reaction, D-*β*-phenylalanine (13.09 g, 0.08 mol) and D-camphorsulfonic acid (9.21 g, 0.04 mol) in DMF (250 mL) is vigorously stirred at room temperature overnight. The mixture is then heated at 30 ^{o}C for 24 hours followed by an increase of 10 ^{o}C intervals for 24 hours within 4 days. After mixture is stirred at 70 ^{o}C for 24 hours, the heat is removed and mixture is poured in to cold aqueous $NaHCO_3$. The product is extracted by ether then subsequently washed with brine and dried over anhydrous $MgSO_4$. Evaporation of ether is followed by column chromatography with eluent solvents being hexanes and EtOAc (3:2 respectively) of the residue afforded 12.61 g (82%) of the enone as a viscous oil. ^{1}H-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 1.42 (s, 3H), 1.64-1.79 (m, 4H), 1.81 (s, 3H), 2.06-2.17 (m, 2H), 2.41-2.53 (m, 2H), 2.64-2.70 (m, 2H), 2.85 (t, *J*= 5.20 Hz, 2H), 2.89 (t, *J*= 4.92 Hz, 2H); ^{13}C-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 6.57, 11.26, 16.41, 18.57, 19.35, 21.46, 21.99, 23.33, 29.59, 29.78, 30.81, 31.33, 32.17, 33.27, 35.82, 36.93, 37.31, 38.11, 38.69, 48.65,

50.62, 53.96, 54.88, 80.62, 81.47,128.36, 130.03, 130.78, 133.38, 158.20, 169.82, 197.71, 211.34, 212.12, 212.82; HRMS (TOF MS ES+) for $C_{12}H_{16}O_2$: calcd. 192.1048, found 192.1045.

2-Methyl-2-(3-oxopentyl)-1,3-cyclohexanedione, **58**. A mixture of ethyl vinyl ketone (11.78 mL, 0.12 mol), 2-methyl-1,3-cyclohexanedione (10.00 g, 0.08 mol) and KOH (0.06 g, 0.99 mmol) is combined in 100 mL of MeOH and refluxed for 3 hours in a 500 mL round bottom flask flushed with nitrogen. The reaction is then cooled followed by the removal *in vacuo* of excess solvent and excess ethyl vinyl ketone. Mixture is carried on to next reaction without purification.

(13*R,S*, 4a*R*)-13-Hydroxy-6,4a-dimethylspiro[1,3-dioxolane-2,5'-3',4',5',6',7',8',4a'-heptahydronaphthalene]-7-one, **71**. A solution of enone (1.26 g, 5.30 mmol) in 30 mL of a methanolic KOH solution (3:1) is heated at 60 ^{o}C and stirred vigorously to open air for 24 hours. Neutralize mixture dropwise with HCl then evaporate excess methanol. Then add aqueous NH_4Cl to the solution and extract with EtOAc. Combine organic extracts and wash with water and brine, then dry over anhydrous MgSO4. Evaporation of the solvent followed by column chromatography of the residue with eluent solvents being hexanes and EtOAc (3:2 respectively) afforded 0.96 g of the alcohol (72%) as an oil. ^{1}H-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 1.42 (s, 3H), 1.53 (s, 3H), 1.58 (s, 3H), 1.81 (s, 3H), 1.67 (t, *J*= 3.72 Hz, 1H), 1.70 (t, *J*= 4.40 Hz, 1H), 1.88 (s, 3H), 1.92 (s, 3H), 1.95-2.12 (m, 2H), 2.20-2.36 (m, 2H), 2.45-2.59 (m,

2H), 2.62-2.72 (m, 2H), 2.84-2.89 (m, 2H), 2.95-3.04 (m, 2H), 3.87-4.02 (m, 4H), 4.93 (t, *J*= 3.32 Hz, 1H), 5.11 (t, *J*= 3.88 Hz, 1H); ^{13}C-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 0.02, 10.96, 11.16, 11.31, 21.51, 22.50, 23.96, 24.61, 25.14, 27.31, 28.96, 29.43, 29.65, 30.87, 32.15, 33.34, 33.38, 33.65, 37.37, 44.27, 49.28, 65.00, 65.35, 66.06, 66.29, 112.41, 128.46, 130.13, 132.97, 149.61; HRMS (TOF MS ES+) for $C_{14}H_{20}O_4$: calcd. 252.1259, found 252.1263.

(13*R,S*, 4a*R*)-13-Acetoxy-6,4a-dimethylspiro[1,3-dioxolane-2,5'-3',4',5',6',7',8',4a'-heptahydronaphthalene]-7-one, **59**. A solution of the alcohol (0.25 g, 1.00 mmol) and acetic anhydride (6.33 mL, 65.23 mmol) in pyridine (3.10 mL, 38.13 mmol) is stirred at room temperature for 18 hours. The mixture is then poured on water and stirred for 15 minutes. The product is extracted with ether (10 mL x 3) and the combined extracts are washed with 1 N HCl, aqueous $NaHCO_3$ and brine. The extract is then dried over anhydrous $MgSO_4$ followed by evaporation of solvent. The residue undergoes purification by column chromatography with eluent solvents being hexanes and EtOAc (4:1 respectively) affording 0.22 g of acetylated product (74%). ^{1}H-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 1.42 (s, 3H), 1.46 (s, 3H), 1.51 (s, 3H) 1.55-1.62 (m, 3H), 1.67 (t, *J*= 3.88 Hz, 1H), 1.70 (t, *J*= 4.40 Hz, 1H), 1.81, (s, 3H), 1.87 (s, 3H), 1.91 (s, 3H), 1.94-1.97 (m, 2H), 2.05 (s, 3H), 2.11 (s, 3H), 2.24-2.31 (m, 2H), 2.39-2.58 (m, 2H), 2.64-2.72 (m, 2H), 2.83-2.91 (m, 2H), 3.92-4.02 (m, 4H), 6.01 (t, *J*= 2.76 Hz, 1H), 6.10 (t, *J*= 4.40 Hz, 1H); ^{13}C-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 11.29, 11.38, 11.45, 21.02, 21.27, 21.53, 23.37, 24.24, 25.18, 27.05, 27.20, 27.26, 27.40, 29.61, 31.12,

32.65, 33.30, 33.63, 37.35, 44.60, 49.40, 50.65, 65.06, 65.36, 68.30, 112.03, 128.30, 128.42, 129.63, 130.09, 133.52, 134.60, 135.04, 152.00, 152.99, 158.18, 169.59, 169.65, 197.72, 198.10, 199.40, 211.12; HRMS (TOF MS ES+) for $C_{16}H_{22}O_5$: calcd. 294.1365, found 294.1355.

Ethoxy 2-((15*R*,*S*, 7*R*, 11*R*)-15-acetoxy-7,11-dimethyl-8-oxospiro[1,3-dioxolane-2,7'-bicyclo[4.4.0]decane]-7-yl) *t*-butyl-dimethylsilane, **61**. Solvate lithium (0.70 mg, 0.08 mmol) in NH_3 and reflux under a cold finger apparatus. To that solution was added acetylated enone (5.4 mg, 0.02 mmol) dropwise and allowed to reflux 2 hours. Rapid addition of TBS protected iodoethanol (55.1 mg, 0.19 mmol) to the mixture follows and reflux 1 hour. Quench reaction with NH_4Cl allowing NH_3 to evaporate. Pour residue in water and extract product with ether. Combine extracts and wash with brine and dry over anhydrous $MgSO_4$. Purification by column chromatography with eluent solvents being hexanes and EtOAc (7:3 respectively) afforded the alkylated product in very low yield. ^{1}H-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 0.01 (s, 9H), 0.14 (s, 6H), 1.42 (s, 3H), 1.46 (s, 3H), 1.55 (s, 3H), 1.58 (s, 3H), 1.68-1.79 (m, 3H), 1.81 (s, 3H), 1.88 (s, 3H), 1.91 (s, 3H), 1.94 (s, 3H), 1.98-2.17 (m, 2H), 2.22-2.72 (m, 2H), 2.85 (t, *J*= 6.32 Hz, 2H), 2.89 (t, *J*= 4.12 Hz, 2H), 2.96-3.04 (m, 2H), 3.89 (t, *J*= 4.96 Hz, 2H), 4.29 (t, *J*= 6.60 Hz, 1H), 4.50 (t, *J*= 4.68 Hz, 2H), 5.11 (t, *J*= 4.12 Hz, 1H); HRMS (TOF MS ES+) for $C_{24}H_{42}O_6Si$: calcd. 454.2829, found 454.2850.

(13*R,S*, 4a*R*)-13-Acetoxy-6-methyl bromide-4a-methylspiro[1,3-dioxolane-2,5'-3',4',5',6',7',8',4a'-heptahydronaphthalene]-7-one, **65**. A solution of acetylated enone (27.30 mg, 0.11 mmol), N-bromosuccinimide (23.30 mg, 0.13 mmol) and catalytic benzoyl peroxide in methylene chloride is refluxed at 80 ^{o}C for 16 hours. Upon completion of reaction, the reaction is allowed to cool to room temperature and then filtered prior to adding aqueous $NaHCO_3$ to residue. Extract product with methylene chloride. Combine extracts, wash with brine and dry over anhydrous $MgSO_4$. After evaporation of solvent the residue undergoes purification by column chromatography with eluent solvents being hexanes and EtOAc (1:1) affording 28.30 mg of allylic bromide (78.2%). ^{1}H-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 1.42 (s, 3H), 1.46 (s, 3H), 1.49 (s, 3H), 1.56-1.66 (m, 3H), 1.81 (s, 3H), 1.96 (s, 3H), 1.99-2.05 (m, 2H), 2.07 (s, 3H), 2.13 (s, 3H), 2.31-2.36 (m, 2H), 2.49-2.61 (m, 2H), 2.87-2.97 (m, 2H), 3.96-4.01 (m, 4H), 4.36 (dd, *J*= 29.44 Hz, AB-type, 2H), 4.43 (dd, *J*= 10.16 Hz, AB-type, 2H), 6.00 (t, *J*= 6.76 Hz, 1H), 6.10 (t, *J*= 3.28 Hz, 1H); ^{13}C-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 11.29, 21.01, 21.29, 21.49, 21.91, 22.00, 22.35, 23.36, 24.45, 25.19, 26.56, 27.12, 27.25, 29.60, 31.04, 31.98, 33.15, 33.30, 33.65, 37.35, 45.18, 49.84, 65.11, 65.35, 67.86, 128.42, 130.08, 134.75, 135.58, 156.68, 157.63, 169.67, 195.21, 210.03; HRMS (TOF MS ES+) for $C_{16}H_{21}BrO_5$: calcd. 372.0407, found 372.0405.

(13*R,S*, 4a*R*)-13-Acetoxy-6-methyl bromide-4a-methylspiro[1,3-dioxolane-2,5'-3',4',5',6',7',8',4a'-hexahydronaphthalene]-7-triflouromethanesulfonate, **66**. A solution of the allylic bromide enone (0.61 g, 1.64 mmol) in THF is cooled to -78 ^{o}C

followed by dropwise addition of 1.0 M lithium bis(trimethylsilyl)amide, LiHMDS, (1.97 mL, 1.97 mmol) in THF and stirred for 30 minutes. A solution of N-phenyl-bis(triflouromethylsulfonyl)imide (1.76 g, 4.92 mmol) in THF is added to the reaction mixture and stirred allowing to warm to room temperature. The reaction is quenched with aqueous NH_4Cl and product extraction with EtOAc. Combine extracts, wash with brine and dry over anhydrous $MgSO_4$. After evaporation of solvent further purification of residue by column chromatography with eleunt solvents being hexanes and EtOAc (4:1 respectively) afforded 0.52 g of the triflated product (63%). ^{1}H-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 1.42 (s, 3H), 1.46 (s, 3H), 1.49 (s, 3H), 1.56 (s, 3H), 1.57 (s, 3H), 1.62-1.70 (m, 3H), 1.81 (s, 3H), 1.98-2.03 (m, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 2.28-2.36 (m, 1H), 2.45-2.61 (m, 2H), 2.87-2.97 (m, 2H), 3.95-4.03 (m, 4H), 4.34 (dd, *J*= 10.16 Hz, AB-type, 2H), 4.40 (dd, *J*= 9.96 Hz, AB-type, 2H), 6.00 (t, *J*= 3.04 Hz, 1H), 6.10 (t, *J*= 3.56 Hz, 1H); ^{13}C-NMR (400 MHz, TMS, $CDCl_3$) δ (ppm) 11.21, 20.93, 21.22, 21.41, 21.88, 21.92, 22.31, 23.28, 24.38, 25.10, 26.47, 27.03, 27.16, 29.49, 30.92, 31.90, 33.06, 33.20, 33.57, 37.26, 45.09, 49.76, 65.02, 65.28, 67.75, 112.07, 128.33, 129.31, 129.97, 133.44, 134.61, 135.43, 156.67, 157.69, 169.64, 170.21, 195.17, 196.63, 209.95.

2-Ethanol-3-methyl-cyclohex-2-enoate, 34. Solvate the protected alkylated lactone **34** (0.13 g, 0.47 mmol) in THF and cool to 0 ^{o}C under N_2. Add tetra-n-butylammonium fluoride (TBAF) (0.94 mL, 0.94 mmol) and stir allowing mixture to warm to room temperature. Evaporate the THF and partition mixture between H_2O and EtOAc. Wash organic with brine and dry over anhydrous $MgSO_4$. Evaporation of

EtOAc is followed by column chromatography with eluent solvents being hexanes and EtOAc (3:2 respectively) affording 0.07 g (98%) of the deprotected alcohol product.

2-Ethyl iodo-3-methyl-cyclohex-2-enoate, 35. The alcohol **63** (0.05 g, 0.32 mmol) is dissolved in CH_3CN:Et_2O (3:5) and cooled to 0 ^oC. Sequential addition of triphenylphosphine (0.11 g, 0.42 mmol), iodine (0.11 g, 0.45 mmol) and imidazole (0.03 g, 0.48 mmol) to mixture is allowed to stir 2 hours then allowed to warm to room temperature. Evaporate solvent mixture and partition between H_2O and EtOAc. Wash organic with brine and dry over anhydrous $MgSO_4$. Evaporation of EtOAc is followed by column chromatography with eluent solvents being hexanes and EtOAc (1:1 respectively) affording 0.01 g (19%) of the iodinated product.

Chapter 3: Salvinorin A in Persistent Pancreatitis Model

I. PANCREATITIS

Pain, albeit both a part of life and vital defense function, is a difficult entity to manage especially for patients with a chronic illness. Certain chronic illnesses bring about recurring pain that makes daily activity virtually impossible, diminishing patient quality of life. Many patients seek traditional therapy, which involves an opioid analgesic, to manage their pain. Classical opiate analgesia involves treatment that binds to the opioid receptor. Drugs like morphine or codeine have been utilized as a pain treatment for patients with a high level of pain, during extensive/invasive surgery, or with chronic pain due to a chronic illness. However, side effects due to the non-selectivity of these drugs ensue with chronic use; constipation, respiratory depression and addiction. These drugs have their highest affinity to the μ opioid receptor.[49] The chronic illness pancreatitis is an example where traditional therapy has failed to suppress and manage effectively the chronic pain that is brought about by obstruction of the pancreaticobiliary tract.[49] While there remains the challenge of developing pain therapy for pancreatitis, previous work has shown that targeting the κ opioid receptor can confer analgesia in patients with pancreatitis. The question is whether using a highly selective, efficacious κ opioid receptor agonist, like Salvinorin A, could confer analgesia for pancreatitis.

Pancreatitis is a disease process in the pancreas that is characterized in two forms, acute and chronic. While both processes similarly cause abdominal pain, the only

sensation elicited from the pancreas, their differences are in treatability and duration. The two most common causes of pancreatitis are gallstones and alcohol abuse, however, other causes are implicated such as hyperlipidemia, trauma/scarring of the pancreas due to postoperative or endoscopic procedures, infectious/congenital diseases and drugs.

The pathogenesis of pancreatitis involves premature activation of peri-pancreatic enzymes leading to autodigestion of the pancreas and surrounding tissues. The mechanism of the intracellular activation of the digestive enzymes is not resolved. Further characterization of pancreatitis is often described in two phases. The initial phase is seen as a result of systemic inflammation leading to organ failures, while the second phase is characterized by the peri-pancreatic auto-digestion, leading to edema, fibrosis and necrosis, a major cause of death in patients.

Acute pancreatitis, although severe in some patients, can easily be treated by eliminating the cause of the disease along with pain therapy. Challenges arise in treatment of patients with chronic or persistent pancreatitis. Patients struggle with day to day activity when managing the pain of chronic pancreatitis. Traditional therapy, as previously mentioned, involves mu opioid agonists, exposing patients to the possibility of constipation, nausea, sedation, dependence or even respiratory depression. It is notable that the pancreas has a high density of kappa opioid receptors. This fact opens the possibility of introducing a selective kappa opiate agonist as a possible agent for conferring analgesia to patients with pancreatitis.

Salvinorin A has been previously implicated in antinociception by McCurdy *et al*.[50] In their study, Salvinorin A was given an antinociceptive profile in both thermal and

chemo-nociceptive assays. This study sets out to establish the peak effect time for antinociceptive activity of purified Salvinorin A as well as demonstrate selectivity through a reversal study with norbinaltorphimine (norBNI), a known antagonist, *in vivo*. To explore Salvinorin A's profile, with male Swiss mice, three types of experiments are utilized; tail-flick study, hotplate study and acetic acid abdominal constriction study. The tail flick assay was used to illuminate two concepts; first it characterized both a dose-response and time-course effect of Salvinorin A on thermal nociception and second it characterized the antagonist effects of norBNI on Salvinorin A antinociception. The experiments started with establishing a baseline 10 minutes prior to administration of Salvinorin A. In the dose-response/time-course experiment, mice received either vehicle or Salvinorin A (0.5, 1.0, 2.0 or 4.0 mg/kg) and tested 10, 20 and 30 minutes after injection. The results exhibited both a dose and time dependent antinociceptive effect that was demonstrated by increased latencies 10 to 15 minutes post drug administration with a return to baseline 20 to 30 minutes post administration. In the norBNI challenge experiment, 10 mg/kg of norBNI was given 1 hour prior to 2.0 mg/kg of Salvinorin A administration. As a result, pretreatment with norBNI reversed the antinociceptive effect of Salvinorin A, where mice with norBNI had a significantly lower latency than mice with saline, the control group.

The hotplate study was utilized to further characterize the dose-response effect of Salvinorin A on thermal nociception.[50] Typically the hotplate study is less sensitive to κ opiates, but was utilized here to illustrate the involvement of spinal and supraspinal nociceptive processing, the mechanisms associated with peripheral neuropathy. In this experiment, the mice were given an apparatus habituation trial on a 40 ^{o}C maintained hotplate prior to administration of either vehicle or Salvinorin A (0.5, 1.0, or 2.0 mg/kg). The results from testing showed that mice with 1.0 mg/kg of Salvinorin A showed a

significant increase in latency while the other doses had marginal changes. The third type of study, the acetic acid abdominal constriction study, functions to show both a dose-response and time-course effects of Salvinorin A. The mice are treated with acetic acid to produce a writhing response and given Salvinorin A to examine a reduction in that writhing response. Mice given vehicle had an intense writhing response within ten minutes that stabilizes in fifteen minutes. A robust antinociceptive effect was exhibited with a reduction in writhing in a dose-dependent fashion in the initial phase of the test, demonstrating a short duration of action. The overall work justifies the need to further explore the possibility of developing Salvinorin A as an analgesic, however, this work recognizes the limitations when Salvinorin A is centrally acting because of its psychoactive effects.

A second group investigated the anticociceptive profile of Salvinorin A *in vivo*, John *et al*.[51] This animal model uses adult male CD-1 mice and utilizes the tail flick assay to display the antinociceptive profile of Salvinorin A. This group focuses on establishing first that Salvinorin A has an effect in thermal nociception and then confirming the unique binding to the κ opioid receptor by exploring Salvinorin's effect after pretreatment with μ, δ, and κ opioid antagonists; β-funaltrexamine (β-FNI), naltrindole (NTI), and nor-binaltorphimine (norBNI) respectively. To examine thermal nociception, the mice were divided into two groups, vehicle and Salvinorin A. The mice given Salvinorin A were injected with various doses, ranging from 11.6 to 23.1 nmol. After a pretreatment baseline is established, the mice were given an intrathecal injection in between the L5 and L6 region of the spinal cord and the tail flick assay was performed. The results demonstrated a dose dependent and time dependent response, corresponding to previous findings. Next the mice were pretreated with the selective antagonists twenty-four hours prior to intrathecal administration to determine which antagonist would

block the effects of Salvinorin A. Tail flick latency was not affected by β-FNA or NTI. However, nor-BNI completely eliminated the inhibition of tail flicking by Salvinorin A. This experiment further demonstrated the selectivity of Salvinorin A in regards to its binding selectively to the κ opioid receptor subtype.

Interestingly, Salvinorin A has also shown to have limited addictive potential based on its effects with the reward pathway of mice, Zhang *et al*.[52] This investigation focuses on Salvinorin A's effect of basal dopamine levels in the caudate putamen and the nucleus accumbens. They also determine whether Salvinorin A induces conditioned place preference or aversion. The first experiment, where measuring changes in the striatal and accumbal dopamine levels, employs the use of microdialysis for dopamine collection on male C57BL/6J mice with varied doses of Salvinorin A (0, 0.32, 1.0, 3.2 mg/kg) administered intraperitoneally; a separate group of mice were pretreated with norBNI to determine whether the action of the changes in dopamine levels is mediated through the κ opioid receptor. As a result, in the caudate putamen there was a decrease in basal dopamine levels is a dose dependent fashion with the effect being reversed with pretreatment with norBNI. In the nucleus accumbens, very little change, a marginal decrease, in the basal dopamine levels occurred. The second experiment looks at the potential positive or negative hedonic properties of Salvinorin A by placing the mice in a mouse place preference chamber, that has three distinct compartments, after one of the following combinations of two injections were given; vehicle + vehicle, vehicle + Salvinorin A (1.0, 3.2 mg/kg), vehicle + norBNI, and Salvinorin A (1.0, 3.2 mg/kg) + norBNI. As a result, the mice displayed conditioned place aversion to the drug paired compartments. The norBNI blocked the induction of the conditioned place aversion by Salvinorin A. With a decrease in striatal dopamine levels, a marginal decrease in accumbal dopamine levels and an induction of place aversion, the data suggests

Salvinorin A would possibly be aversive, or non-addictive, to humans. However, cannabinoids also induce conditioned place aversion in mice suggesting a species difference.

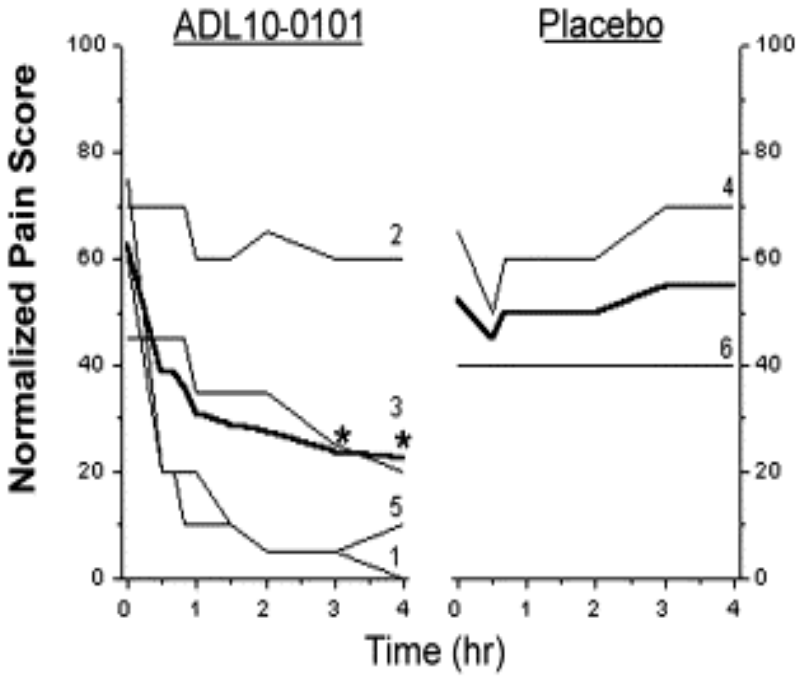


Figure 3.1: Pain assessment of κ opioid agonist in patients with pancreatitis. Adopted from ref 53.

Previous work by Eisenach *et al.*[53], indicates that analgesia can be conferred by a peripherally acting synthetic κ opioid agonist, ADL-10-0101, for patients with chronic pancreatitis. This placebo study included six patients, three men and three women, with chronic pancreatitis, induced from various causes ranging from a bile tumor to dye infiltration used in radial endoscopic procedures. For this study the minimum duration of having the chronic illness was three years, with one patient having had pancreatitis for

thirteen years. Each patient was assessed and given a normalized pain score. The agonist, ADL-10-0101, and saline, the placebo, was administered by intravenous infusion and their pain was re-assessed thirty minutes post administration up to four hours. As shown in Figure 3.1, patients given ADL-10-0101 individually and on average had a reduction in pain score, while the placebo effectively had no change in pain. The data shows patient 2 having minimal change in his or her pain score post administration of ADL10-0101. Although this data is preliminary with a small sample size, the data indicates the promise of developing a pain therapy for chronic pancreatitis by targeting κ opioid receptor. Further examination of a selective kappa opioid agonist like Salvinorin A could prove to be more advantageous and therefore should be investigated.

Salvinorin A is a potential target for a pain therapeutic being a selective, potent and efficacious κ opioid agonist particularly with pancreatitis. Before introducing Salvinorin A to human patients with chronic pancreatitis, it is worth investigating its effects in a persistent pancreatitis model to begin to assess whether a selective molecule that uniquely binds to the κ opioid receptor is effective. This model should not only lend itself to assessing nociception but also activity. In chronic pancreatitis, not only is there an increase in pain but there is a decrease in activity. Visceral pain dramatically reduces the patient's ability to accomplish daily function thus diminishing their quality of life. Exploiting a model to evaluate both characteristics of nociception and activity would be most advantageous to properly determine if Salvinorin A is a good candidate for drug therapy.

II. PERSISTENT PANCREATITIS MODEL

Persistent pancreatitis has long been investigated in Dr. Karin Westlund-High's lab. A model has been established to address, investigate and understand visceral pain caused by damage to the pancreas.[54,55] This model involves inducing pancreatitis in male Lewis rats through tail vein injection of dibutyltin dichloride (DBTC) and maintaining a naïve vehicle group for comparison. The model allows for development of pancreatitis through toxin damage, along with exacerbating that damage through incorporation of ethanol, a common cause of pancreatitis in human patients, in the rats' diet. The rats are baseline tested for both nociception and activity. The rats are then administered the therapeutic compound, originally morphine alone and co-administered with other compounds. For our study Salvinorin A is the only therapeutic component. Then an evaluation of both nociception and activity is performed. Pain testing is performed through thermal nociception with a hotplate while the activity testing is performed with a photobeam activity box for open field testing. The key to the success of this experiment is to create a consistent environment for the rats, in order to not introduce undue stress to the rats. Also, the tests are to be performed in a double blind fashion, where the person inducing pancreatitis, administering Salvinorin A and evaluating the pain and activity are three different people, to prevent bias from any particular person. The identity of the rats given DBTC versus vehicle is only revealed after evaluating thermal nociception and open field activity. This persistent pancreatitis model lends itself to various modifications to examine dose-response, time course and response reversal.

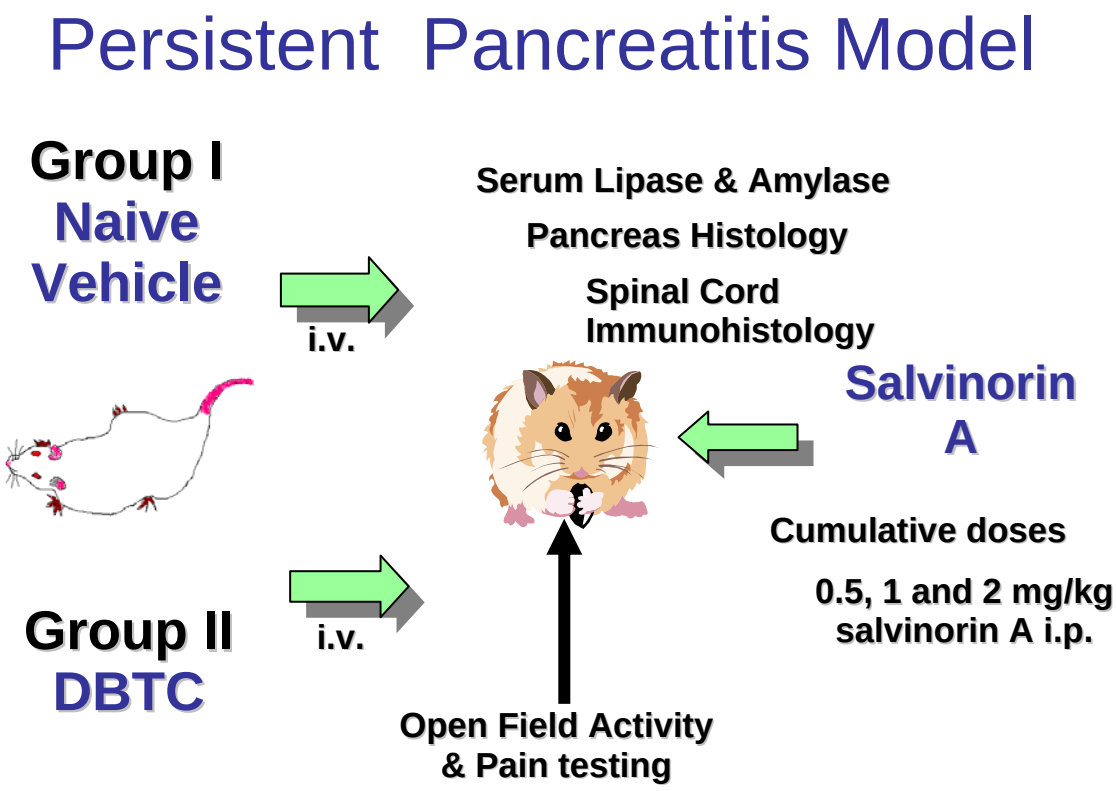


Figure 3.2: Persistent Pancreatitis Model developed by Westlund-High *et al*.

The preliminary experiment included 7 rats, to test the model using Salvinorin A. The small sample size was chosen to ensure, despite previous data, Salvinorin A was not toxic to the animals, particularly in this model. Following the protocol described in materials and methods, four rats were given DBTC while the remaining four were given the vehicle. Persistent pancreatitis was allowed to develop in the rats and the initial day of testing, day six, is to establish a baseline for comparison of post administration of Salvinorin A. It is noted that included in the diet of the rats is ethanol to exacerbate the

damage to the pancreas by DBTC. On day seven all the rats are given an intraperitoneal (i.p.) injection of Salvinorin A, 1mg/kg dose based on previous data in the literature, and are hotplate tested by measuring the latency of response in five minute intervals. After the first fifteen minutes one rat expired and further data was acquired with six rats instead of seven.

Since this was the first time Salvinorin A was used in this model, our approach was cautious, noting data even after a short duration; searching for a trend within the first set of data points, zeroing in on the first fifteen minutes. As shown in Figure 3.3, the data shows an increase of latency for the DBTC rats over the fifteen minute period while the vehicle group shows little change in latency. Immediately one could interpret this as an effect however a longer duration was initially determined to be investigated prior to drawing conclusions. As shown in Figure 3.4, the data was further measured, however with one rat removed from the sample, to determine the trend of latency over a forty-five minute period for the DBTC rats, noting that no rats were on the hotplate a maximum of twenty seconds to prevent foot sensitivity.

The data after that forty-five minute period showed an increase in latency for the DBTC rats, while again very little change in latency in the vehicle rats. This preliminary data indicates a need to further investigate by extending the time duration of the experiment as well as look at various doses of Salvinorin A. Overall the increase in latency with the DBTC rats remains throughout the 45 minutes; leaving invaluable data following that time duration to see how long or short acting the effects of Salvinorin A are. Also, knowing whether the action of Salvinorin A occurs is a dose-response fashion could prove to be pertinent data. Because pancreatitis causes visceral pain that is difficult to manage, daily activity is greatly reduced. The goal of pain therapy is to increase that diminished activity along with conferral of analgesia.

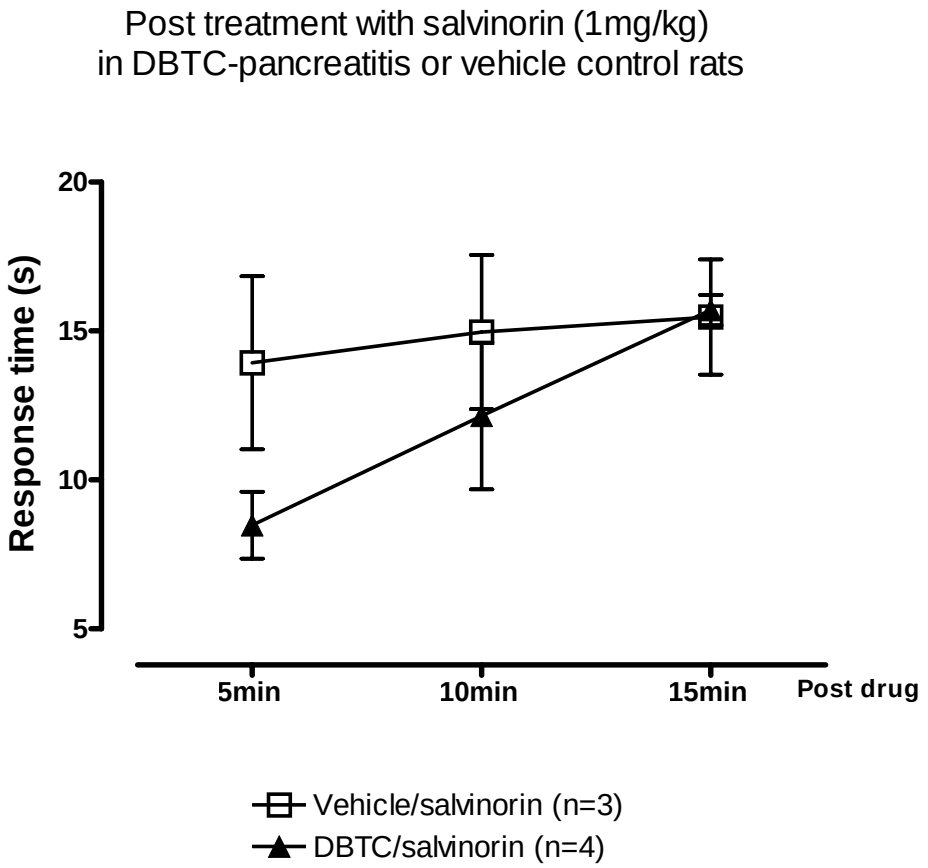


Figure 3.3: Preliminary data of response 15 min post Salvinorin A administration of DBTC vs vehicle.

In this model, activity of rats decreases when persistent pancreatitis is induced. Prior to DBTC administration, the rats are quite active, as seen during the acclimation period. After DBTC administration, the rats are recoiled and less active. To investigate the effects of Salvinorin A on activity, a baseline of activity is first established in a photobeam activity system (PAS) that three dimensionally divides the box in multiple quadrants of photobeams.

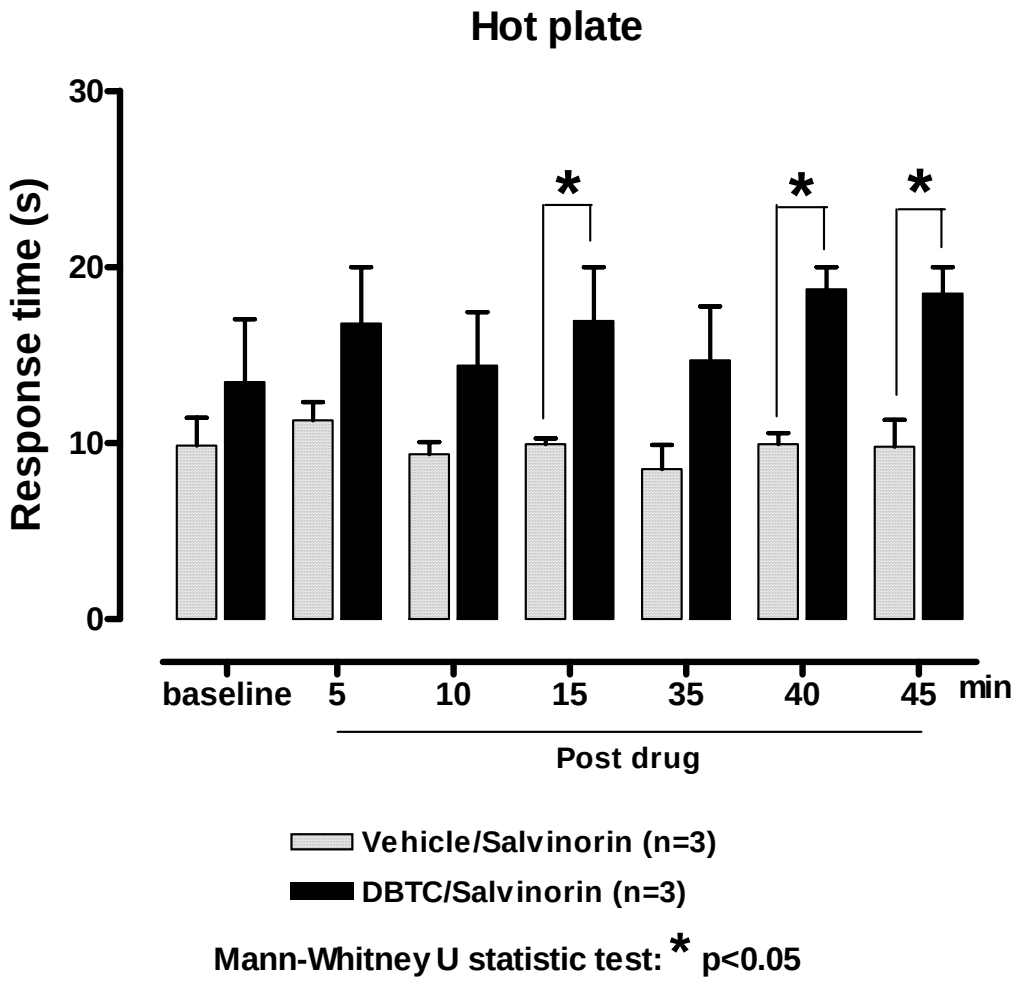


Figure 3.4: Preliminary data of responses 45 min post Salvinorin A administration in animals with either DBTC induced pancreatitis or uninflamed vehicle treated.

Activity is measured by the number of beam breaks the rat performs in a given time period. The box accounts for rearing or vertical motion, not just horizontal movement. Prior to administration of Salvinorin A, both the DBTC and vehicle rats' activity is measured for fifteen minutes. Fifteen and thirty-five minutes post Salvinorin A administration, activity is measured.

As shown in Figure 3.5, the data from the PAS demonstrates no changes in activity. Interpretation of this data is based on understanding that rats get acclimated to the environment and typically will settle to a region of the activity box. The pattern of habituation shown is the same for both DBTC and vehicle rats, indicating that Salvinorin A does not enhance activity. Similar data was reported by Zhang *et al*.[52]

The preliminary data demonstrated a further need for investigation but with improvements on the protocol. One of the changes in protocol included a longer post administration period to determine the duration of the effects of Salvinorin A. An increase in sample size to give a better assessment with statistical relevance was required. Finally the addition of varying doses of Salvinorin A to further determine dose response data. With these changes the protocol follows as delineated in materials and methods. Due to preliminary PAS activity data not demonstrating enhancement, no further testing was performed.

After incorporation of the adjustments to the protocol, results from the larger sample size experiment as shown in Figure 3.6 demonstrated an effect by Salvinorin A in a dose-response fashion for the hotplate test. Vehicle rats showed very little change in latency while the DBTC rats seemed to have an effect over a 75 minute period post Salvinorin A administration. Salvinorin A is short acting but still shows promise requiring further study to demonstrate if it could serve as an excellent candidate for drug therapy.

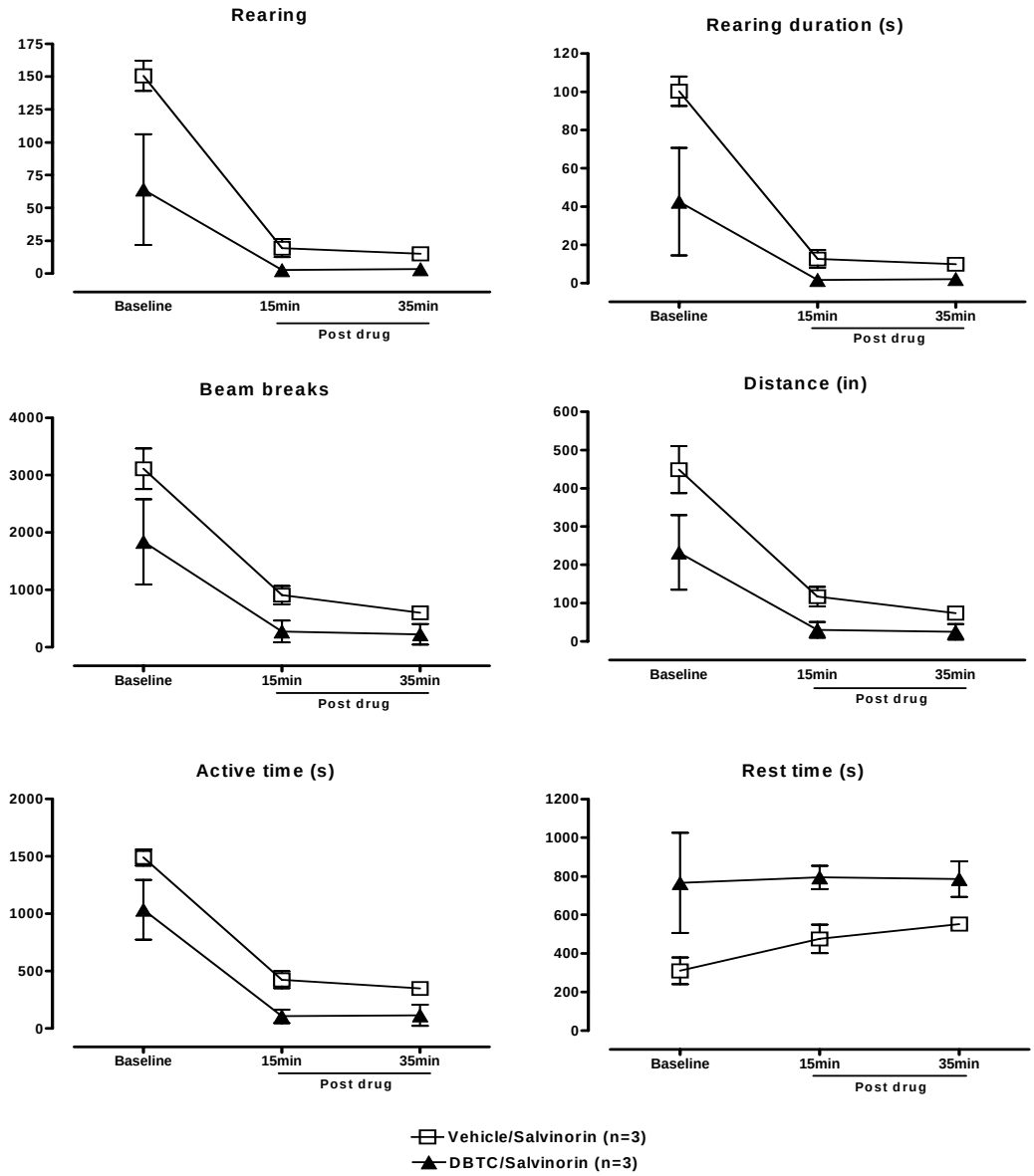


Figure 3.5: Preliminary PAS activity data post Salvinorin A administration of DBTC vs vehicle.

Discussion

The data presented indicates the potential Salvinorin A could have with chronic pancreatitis. Salvinorin A's ability to selectively and potently bind to the kappa opioid receptor has been well established in previous work. From the previous work, we were able to determine the range of dosing for this model to begin investigating Salvinorin A's effects in persistent pancreatitis. Induction of pancreatitis with DBTC was effective in establishing the persistent pancreatitis, following the procedure established in lab. A preliminary group of rats were used to first establish the viability of using Salvinorin A in the model. Once established, monitoring both the effect on thermal nociception along with open field activity of the rats post drug administration were essential to get a preliminary base for continuing investigation. While the thermal nociception assay exhibited an increase in latency for rats with Salvinorin A, the open field activity assay showed a pattern for habituation. Because of the activity data, we only continued with the thermal nociception assay. However, revisiting the activity assay with a comparison of drug administered versus non-drug administered or varying doses may determine whether Salvinorin A has any measurable effect on activity.

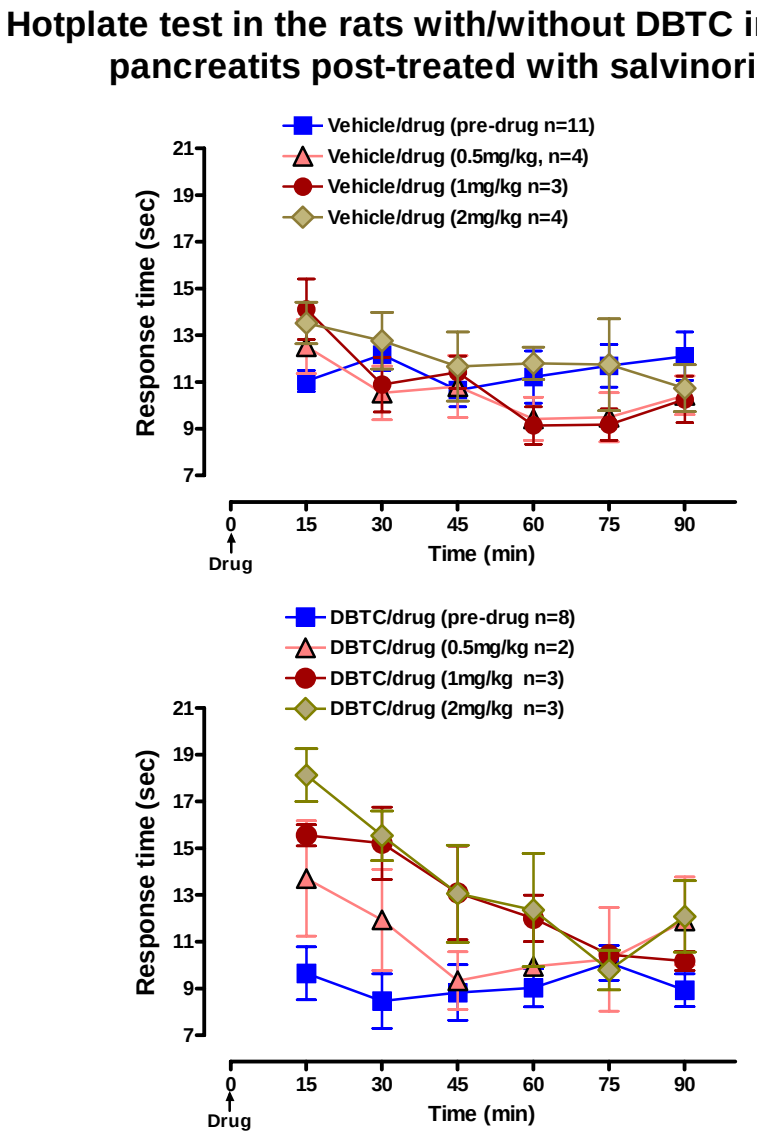


Figure 3.6: Data of response post Salvinorin A administration of DBTC vs vehicle; data demonstrate dose-response trend.

After seeing an increase in latency in the thermal assay, the focus now became seeing if there was any statistical relevance or dose dependence with Salvinorin A. The first thing was to increase sample size from 6 to 19, to allow for more statistical correlation. The next thing was to increase the time duration of the assay along with

varying doses to see if dose dependence could be established. As shown in Figure 3.6, the data demonstrated an effect in a dose dependence fashion with the DBTC drug administered group. The two highest doses significantly reversed the decrease in hotplate latency present after induction of pancreatitis at the 15 and 30 minute time points. What is also interesting to note is that the vehicle group showed no change in latency post administration of Salvinorin A, further indicative of locomotor activity not being altered by Salvinorin A. Although the grand leap to being a therapeutic cannot be assessed at this point, this data show preliminary promise of Salvinorin A being an effective target for treating pancreas pain. More experiments will be needed to further establish the utility of Salvinorin A, such as pancreas histology to examine the direct interaction of Salvinorin A with the organ, continuous versus bolus administration of Salvinorin A to see if the effect of a steady-state or spiking matters with regard to administration, or a comparison to other kappa agonists in the persistent pancreatitis model to see if the same effect can be garnished by other synthetic ligands. Once Salvinorin A derivatives are generated, even more experiments will follow to examine potency and efficacy in this model, possibly producing an even better candidate for being a pain analgesic.

Materials and Methods

Experiments were approved by the University of Texas Medical Branch Animal Care and Use Committee and were in direct accordance with the guidelines on the Ethical Treatment of Research Animals published by the International Association for the Study

of Pain. Male, Lewis inbred rats weighing between 150 g to 200 g were maintained under the standard conditions of two rats per cage and Teklab diet 8626 administered *ad libitum*; a diet low in soybean oil since data has shown that the soybean isolectin has demonstrated antinociceptive effects.

Preparation of dibutyltin dichloride, DBTC, includes generating a solution of DBTC in 90% propylene glycol and 10% DMSO. First dissolve the DBTC in DMSO and add the propylene glycol and vortex mixture to generate what appears to be a homogenous solution. Preparation of Salvinorin A, based on 2 mg/kg of solution, includes mixing 2 mg of Salvinorin A in 250 μL of DMSO and 2250 μL of propylene glycol. The proper amounts are removed when needed for injection.

Behavioral tests include hotplate test for nociception at 50 ^{o}C with a cutoff time of 20 seconds and photobeam activity system for open field testing. The experimental procedure is as follows. Day 0 – Rats are divided into two groups, DBTC and vehicle. All rats are anesthetized with halothane and administered either DBTC or just vehicle pending on the group the rat belongs. Both DBTC and vehicle are administered via tail vein injections at dose of 8 mg/kg, for DBTC, with a syringe pump at a rate of 25 μL/min for 10 minutes. Days 1-4 – Incorporation of 10% EtOH and 5% apple juice into the diet of the rats to exacerbate the damage to pancreas by DBTC. Day 5 – Removal of EtOH from diet at approximately 2:00 pm that evening. Day 6 – Behavior before Salvinorin A administration. A baseline is established via hotplate test with three trials at 5 minute intervals for 2 hrs. A baseline is also established via PAS box where open field testing of two trials over a 15 minute interval. Day 7 – Administration of Salvinorin A is given via

i.p. injection. Hotplate test begins 5 minutes post injection with three trials in 15 minute intervals (preliminary test stopped after 45 minutes, extended experiment stopped after 120 minutes). The PAS test begins 15 minutes post injection with two trials in 15 minute intervals. Animals are euthanized with i.p. injection of a solution of 0.1 mL sodium pentobarbital in 7.8% isopropyl alcohol and 0.4 mL of 0.9% saline.

Chapter 4: Conclusion and Future Directions

I. SUMMARY

Salvinorin A is a unique target chemically and biologically. Previous work has established Salvinorin A to bind uniquely to the κ opioid receptor both selectively and with efficacy.[7,9] Structurally it is unique being touted as a non nitrogenous alkaloid.[6,10] The culmination of these factors makes Salvinorin A a prime target for investigation. This work has even further demonstrated that Salvinorin A serves as a motif to discover new findings in biological science. Traditional opioid therapy non-selectively agonizes the mu subtype, conferring analgesia for acute pain.[2,3,50] However, that same traditional therapy has failed to be effective with chronic pain, particularly in chronic pancreatitis. Salvinorin A has emerged as a target for developing as an analgesic, in chronic pancreatitis, due to the pancreas having a high density of k opioid receptors coupled with Salvinorin A's ability to selectively target the κ opioid receptor only.

Along with Salvinorin A being structurally unique, it uniquely binds to the κ opioid receptor. Modeling studies demonstrate the points of interaction of Salvinorin A with specific residues of the κ opioid receptor.[10,56] Examining that interaction allowed for the determination of the substituents of Salvinorin A that are structurally vital for activity. Likewise, that interaction sets up the framework for also planning modification to Salvinorin A. Early structure activity relationship studies have given pivotal insight[9,57], however to fully investigate Salvinorin A, the ability to modify the structure

and in turn test whether that modification can modulate the system would prove to be invaluable.

Three main objectives to investigate Salvinorin A was the focus of this body of work; develop a route for the total synthesis Salvinorin A, develop a synthetic route for generating analogues with the Salvinorin scaffold, and incorporating Salvinorin A in an established persistent pancreatitis animal model. These objectives serve to extend the profile of Salvinorin A by demonstrating that kappa ligands based on the Salvinorin scaffold can be developed which will further serve as the platform for obtaining future discoveries.

II. SALVINORIN A SCAFFOLD SYNTHESIS

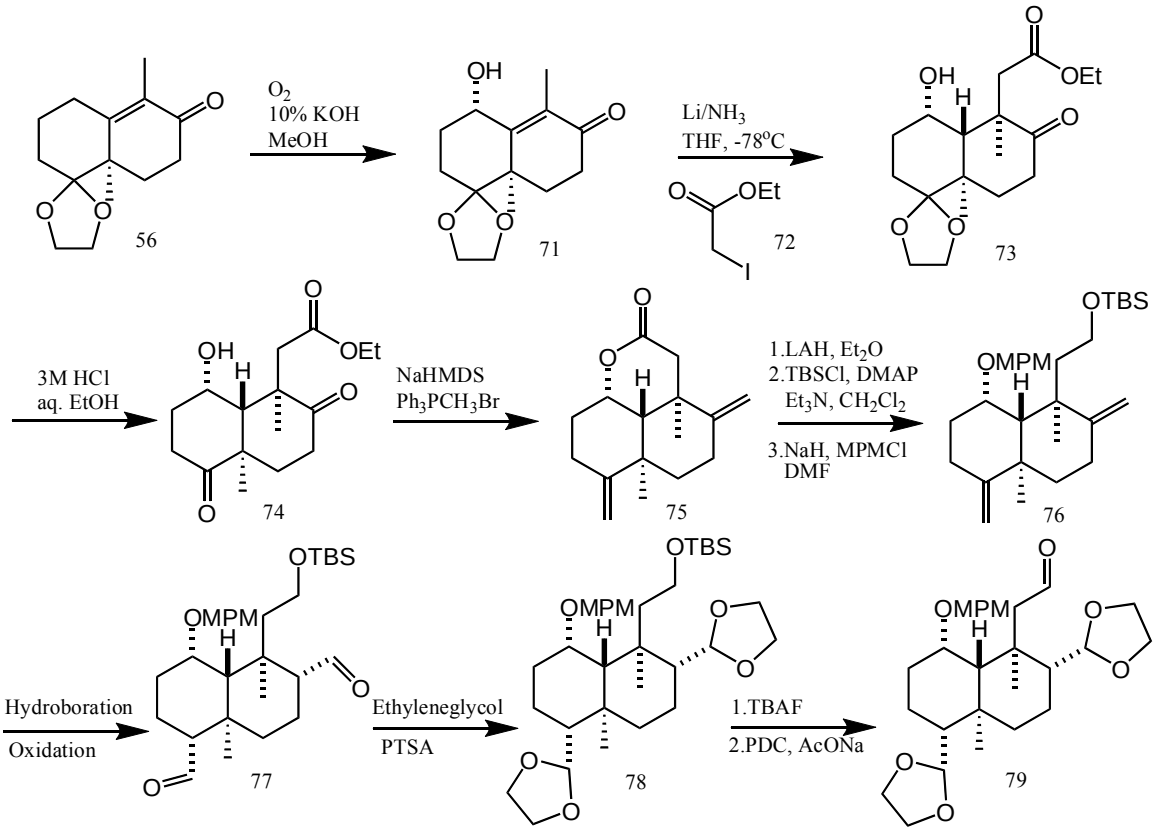
We began by developing a route for total synthesis, particularly with the perspective of also generating a facile method of producing analogues. The approach of forming the A-ring and C-ring, then joining them to form the B-ring had several road blocks causing a shift in perspective with the synthesis. C-ring formation was the initial challenge but after its formation the even bigger challenge became alkylation of the C-ring to the A-ring. This challenge was further addressed by co-worker but still was met with challenges when attempts at formation of the B-ring occurred.

In lieu of the synthetic challenges, the approach shifted from formation of the two outside rings followed by the formation of the inner ring to trying to building off two adjacent rings to form the third ring. The first attempt with this perspective was developed in order to have a facile method of formation of the three ring system through

exploiting both an intramolecular and intermolecular Diels-Alder reaction.[43] While reaching the three ring target was designed to be accomplished in few steps, overcoming the spatial arrangement of the molecule undergoing the Lewis acid promoted Diels-Alder was more challenging than initially perceived. Instead an alternative route emerged and proved to have more success with developing both a route for total synthesis and scaffold for generating analogues. As shown in Schemes 2.15 and 2.16, the route employs the use of the Wieland-Miescher ketone system to begin developing the Salvinorin scaffold. Although total synthesis was accomplished by the Hagiwara group first[45], the viability of the route proposed is bolstered by their groups accomplishing the total synthesis in similar fashion.

When comparing the two routes, they both exploit the formation of the diketone followed by the oxidation to form the hydroxylated compound **71** demonstrated in Scheme 4.1. In this body of work, the same steps are demonstrated but carried on to acetal protection **59**, as shown in Scheme 2.15. The next series of steps in Scheme 4.1 include reductive alkylation with the iodoethyl ester **72** to generate the product **73**. This key step in the synthesis not only begins with formation of the C-ring but also positions the methyl group down for the appropriate geometry of Salvinorin A. Their synthesis continues by removal of the ketal protection converting **73** back to the carbonyl form **74**. This step is followed by the Wittig reaction to convert both carbonyls to olefins **75**. It is important to note that this conversion is assisted by the condensation of the ester and secondary alcohol to form a lactone, allowing the carbonyl to be more accessible by the Wittig reagent. Reduction after opening of the lactone is followed by silyl protection of the primary alcohol and ethereal protection of the secondary alcohol **76**. Compound **76** is

a key molecule, comparable to **62** of the proposed route in Scheme 2.15. Their precursors, compounds **75** and **59**, functionally are the same. Although our proposed route in Scheme 2.15 accomplishes the formation of **61**, earlier in the synthesis, the low yield prevented further development of our route. Hagiwara et al. detail their challenge with their attempt at also introducing the alkyl primary alcohol early in the synthesis.[45]

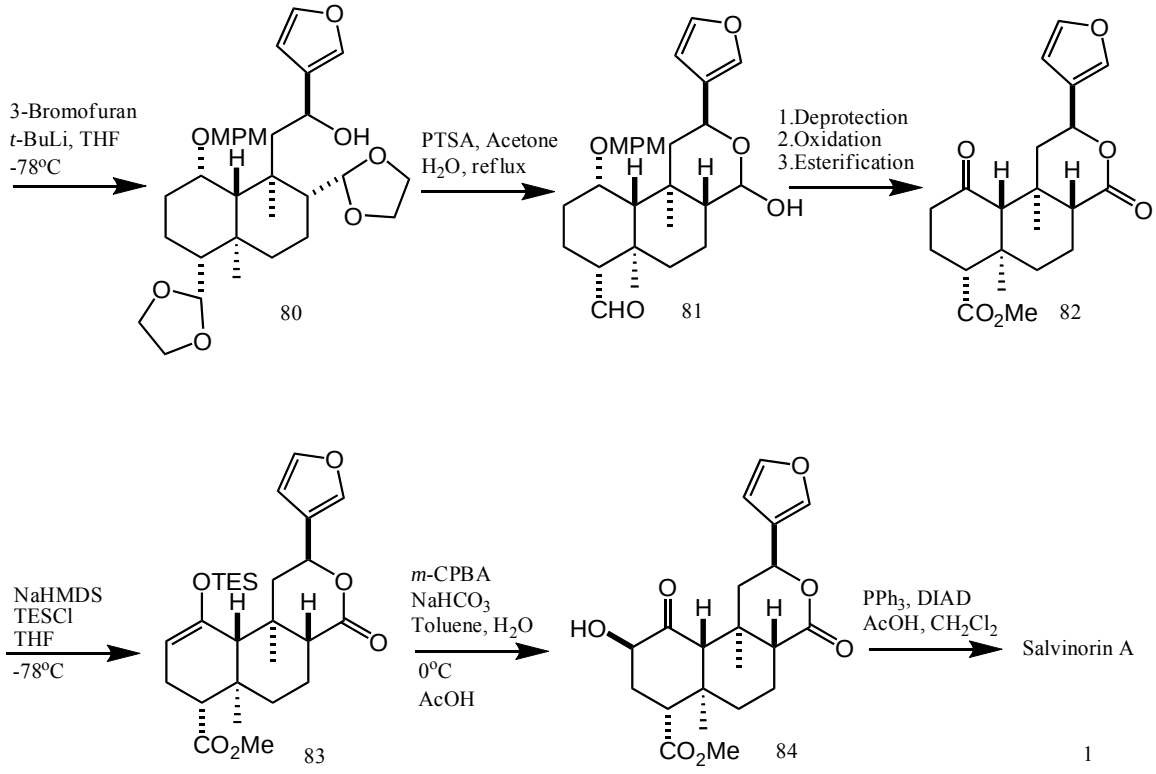


Scheme 4.1: Hagiwara's Salvinorin A Synthesis, part 1.

Much like Hagiwara's discovery of their challenge to introduce the alkyl primary alcohol early in the synthesis prompted a change in direction with the synthetic route for

Salvinorin A, the scale up challenge of **61** prompted a change in direction with our synthetic route. Even more so, the completion of the synthesis Salvinorin A, as demonstrated in Schemes 4.1 and 4.2, caused a refocusing of the development of our synthesis of Salvinorin A. In order to not duplicate the work of this group, we began to look at the features the Hagiwara synthesis does not exploit. One important concept to note is that a route that lends itself to facile modification has been the underlying goal of our synthesis. The Hagiwara synthesis affords Salvinorin A through an efficient process. However their route does not substantiate a key intermediate that would allow for variable modification in the positions that previous work had not been able to modify. Now our focus became developing a synthetic scaffold that would allow facile modifications in those key positions to generate analogues.

Beyond developing a route for total synthesis, the route discussed in this body of work will prove to be a method for generating several unique analogues that may serve as important ligands in general. In Scheme 2.17, the route has a pivotal point where various derivatives can be generated toward the end of the synthesis. This implementation was intended to allow for variability in the C-ring functionality, where previous synthetic routes, including the Hagiwara synthesis[45], have not been able to accomplish. Previous work has demonstrated that the acetyl ester is important for activity, as shown in Figure 4.1. However, the furan substituent serves as the initial position for modification. As delineated in Figure 4.1, the methyl ester can be modified, but more interestingly the methyl groups, in our route, can be incorporated or left out.



Scheme 4.2: Hagiwara's Salvinorin A Synthesis, part 2.

Our synthetic route will allow Salvinorin to become a functional scaffold to begin developing a library of compounds. This scaffold could prove to generate a class of selective kappa ligands allowing exploration to delineate mechanisms and to find new discoveries in neuropharmacology. Understanding further the structure activity relationship of Salvinorin A and derivatives, could also lead to important discoveries in the field of opioid pharmacology in general.

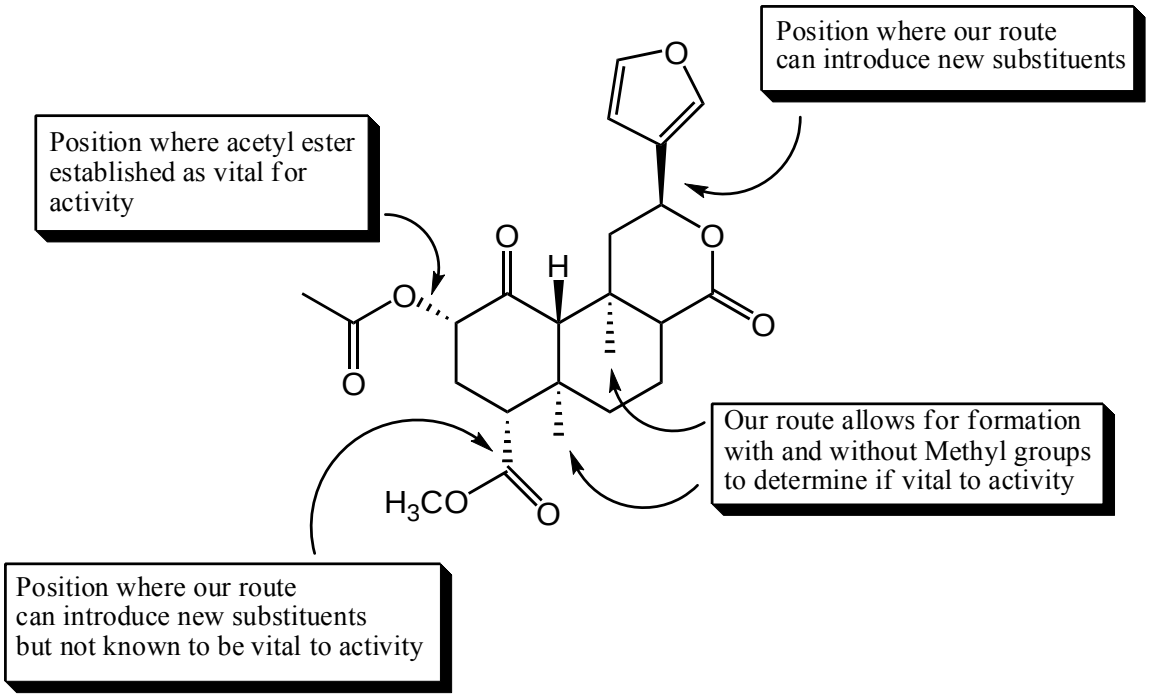
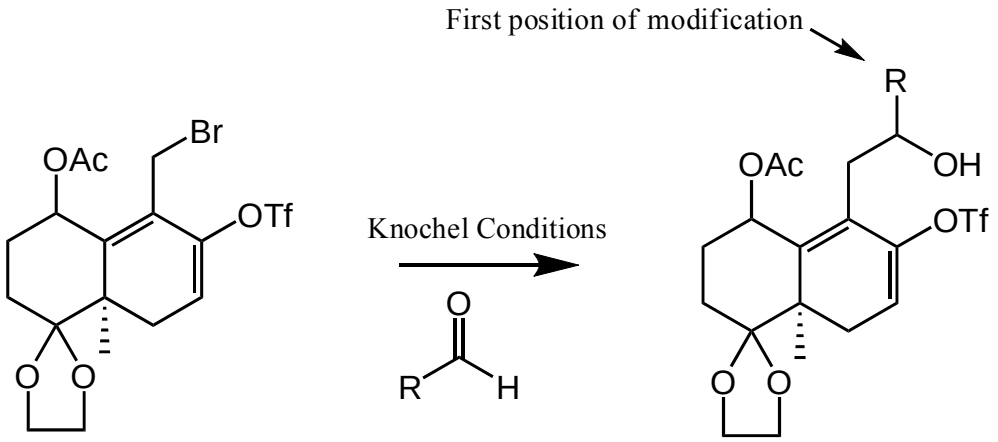
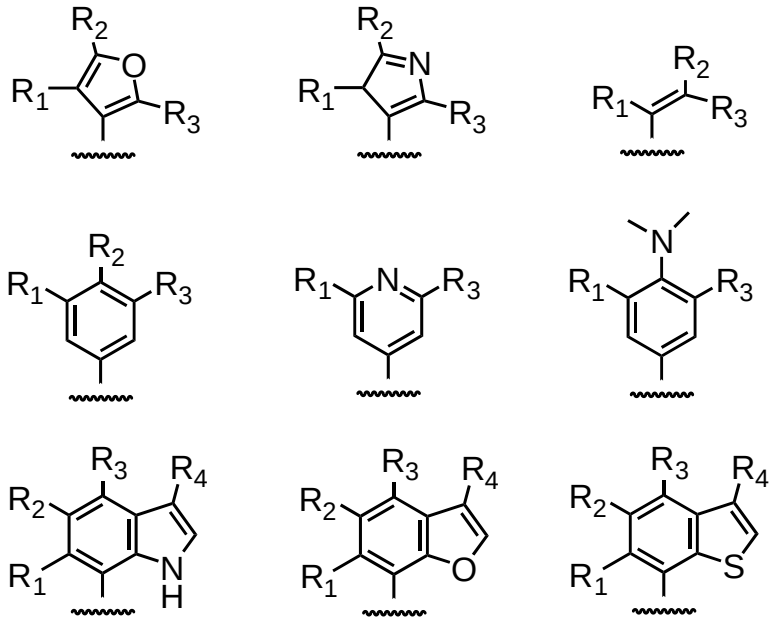


Figure 4.1: Salvinorin A positions for modification.

One future direction still involves completing the total synthesis of Salvinorin A, utilizing the current scaffold route. One of the challenges involved attempting Knochel chemistry[47], metal incorporation into the bromide **65** of Scheme 2.18. The acidic α-proton of the unprotected carbonyl disrupts the metal incorporation leading to first forming the triflate protected enolate prior to attempting the Zn incorporation. The next step would be now to employ the addition with not only furanaldehyde, but other aromatic functionalities to begin developing analogues (Scheme 4.3).



Potential R groups



Scheme 4.3: Potential Aryl Substituent Modifications. (*may require further modification to achieve substituent)

After addition of aromatic functionalities (or any viable functionality), we would continue on to total synthesis. One particular goal with regard to total synthesis is generating Salvinorin A in fewer steps than the reported synthesis. The Hagiwara synthesis affords the tricyclic structure in 10 steps followed by modification to achieve Salvinorin A while this scaffold route will afford the tricyclic structure in 8 steps followed by further modification. While saving two steps may seem miniscule, it potentially could save on cost and improve overall yield of the final product.

However, as previously mentioned, the synthetic focus is demonstrating the variability of functionalities that can be added. One way to begin to approach varying functionality is by looking at the work of Prisinzano and Rothman, where modifications to Salvinorin A was accomplished through semi-synthetic and biosynthetic methods in order to demonstrate that key functionalities are vital to activity.[58] Their work included investigating the structure activity relationship of Salvinorin A with opioid receptors; particularly with the activity and selectivity for kappa opioid receptors as well as its action as an allosteric modulator of mu opioid receptors (despite its low affinity to the mu receptor).[58] Characterizations of effects with C2 position modification of the acetoxy group is reiterated, however detailing the effects of modification of the furanyl substituent along with the carbonyl of the lactone (C-ring) has shown interesting results. Reduction of the carbonyl to form a lactol both reduced the affinity and activity of the kappa receptor while removal of the carbonyl altogether had little effect on affinity; although activity was altered.[58] Modification to the furanyl substituent was well tolerated, have some reduction in affinity but maintaining comparable affinity to that of Salvinorin A. Most of the changes occur with regard to activity, have up to 17-fold

decreases in activity. The modifications included reduction of the furan, bromination of the furan and replacement oxazolines and pyrroles, five-membered ring systems.

While modification was made to the Salvinorin A system, our scaffold route will allow for even more robust modifications. We plan to see if other aromatic substituents are well tolerated with regard to both affinity and activity. Where Prisinzano's group demonstrate that five-membered rings are well tolerated, we intend to incorporate a six-membered system as well as bicyclic systems. Our synthesis will give a wider range of variability to profile the structure activity relationship.

III. SALVINORIN A IN PERSISTENT PANCREATITIS MODEL

Pancreatitis, along with terminal pancreatic cancer, renders a great deal of pain to patients causing a disruption in daily activity. Many who endure this visceral pain are left with few options to manage the pain. Pain management with traditional therapy renders two problems, potential for abuse particularly in non cancer chronic pain patients and development of tolerance. Developing Salvinorin A and analogues as a possible alternative to treating persistent pancreatic pain is a worthwhile challenge. Much like previous work, both our preliminary data and extended data show that targeting the kappa opioid receptor selectively could illicit a reduction of nociception. Further investigation with persistent pancreatitis and Salvinorin A is required to fully profile the effects of

Salvinorin A. Demonstrating a dose-dependence corresponds to previous reports of the effects of Salvinorin A.

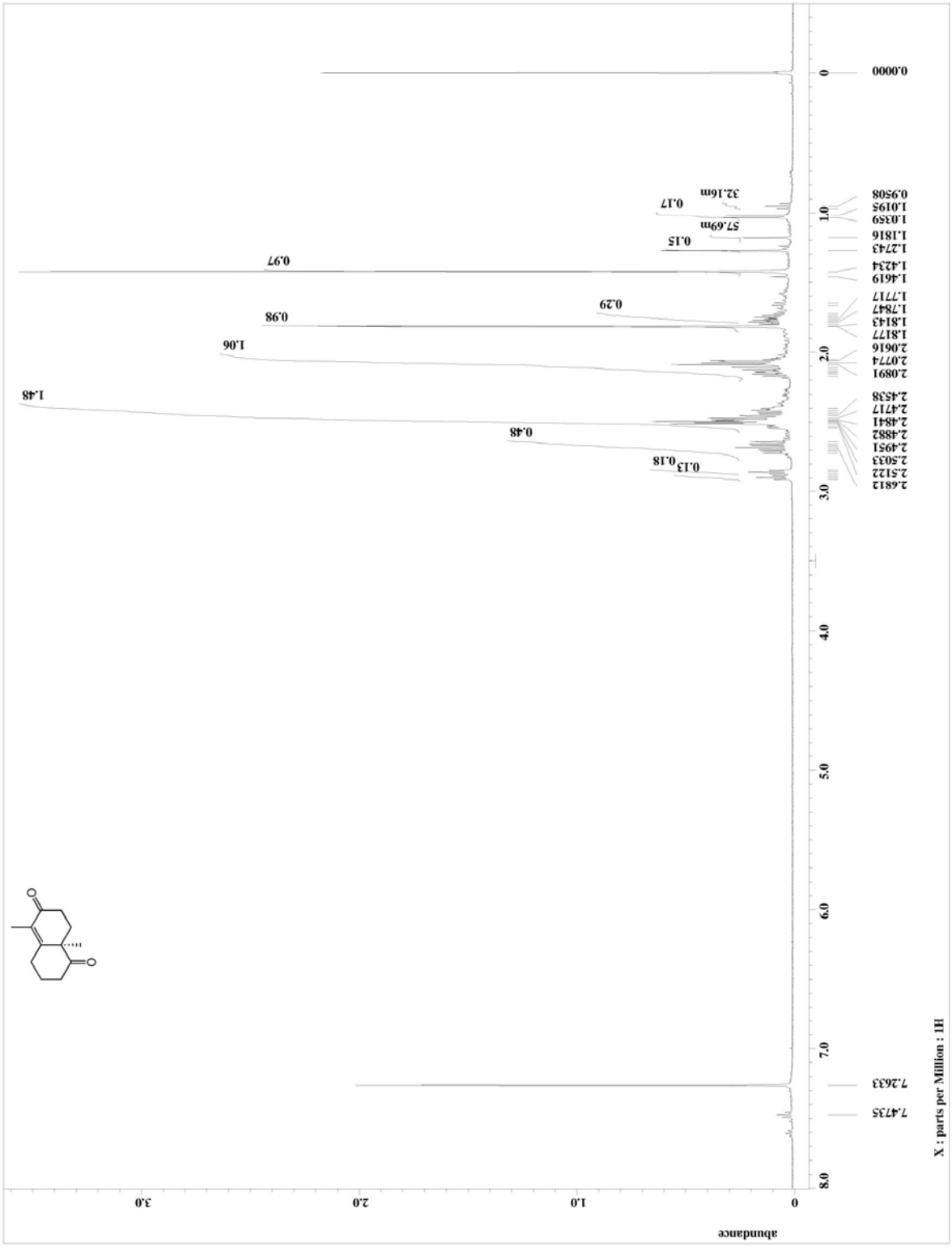
The data presented from the persistent pancreatitis model although it showed desired results, it also generated more questions for future directions. Experiments to further determine whether antinociception is occurring with kappa ligands in general and whether Salvinorin A is most efficacious in comparison to the other ligands would begin to validate Salvinorin A as a drug candidate. One way to determine if the action seen is mediated through kappa opioid agonism is to introduce a known KOR antagonist to see if the Salvinorin A antinociceptive activity is reversed. Then follow that assay with comparison of various known kappa agonists; lending itself to measuring potency and efficacy. Analogues of Salvinorin A should also be investigated in this model. The short comings of Salvinorin A; being short acting, causes hallucinations and may not stay peripherally acting; may be non existent with a Salvinorin analog. Although our data is preliminary, there is a viable chance that Salvinorin A could serve as the moiety to develop pain therapeutics for chronic pancreatitis.

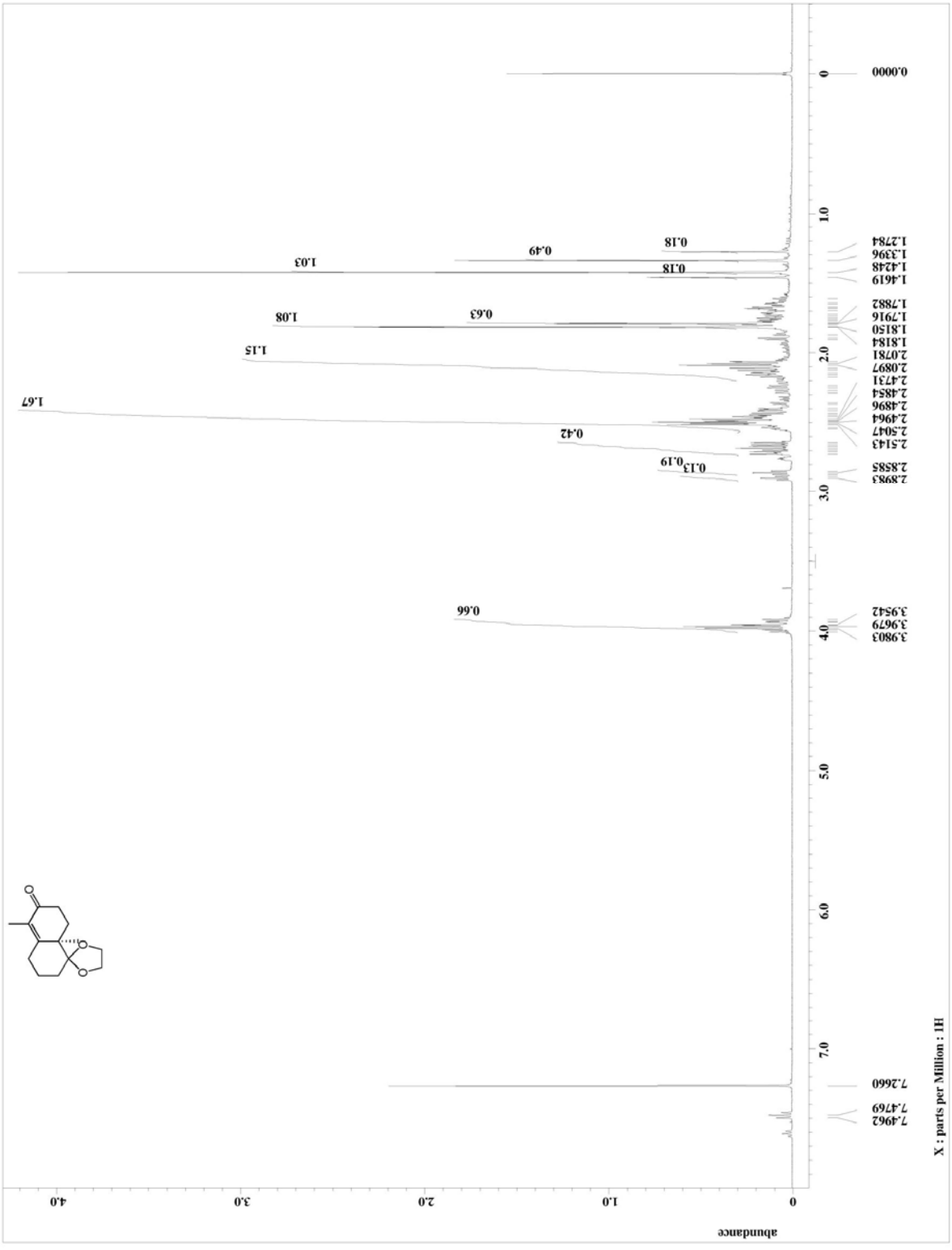
Amongst the Salvinorin analogues generated, not all are intended to serve as agonists or lead to drug therapy. The development of selective kappa antagonists may have advantages as well. Antagonists serve as great biological tools for investigating and developing profiles of drugs. Comparative analysis of a drug's ability to activate a receptor has proven to be one of the most valuable tools in pharmacology.[59] Even more interestingly, kappa antagonists have demonstrated their potential as drug therapy themselves. Mague *et al* show antidepressant effects of a kappa antagonists; nor-binaltorphimine, 5'-guanidinonaltrindole and 5'-acetamidinoethylnaltrindole; in a rat

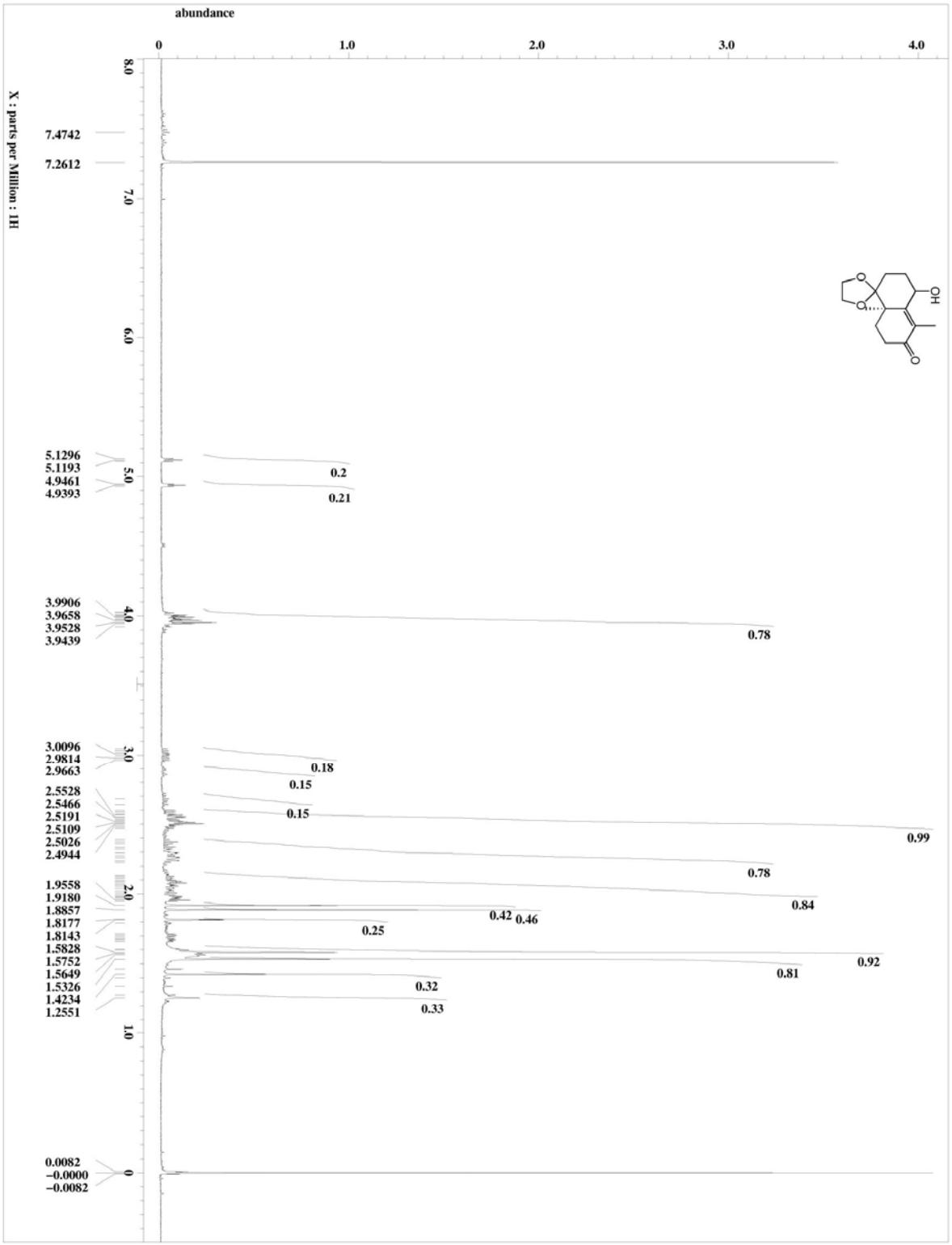
forces swim test model that examines the effects of cAMP response element-binding protein (CREB) within the nucleus accumbens regulating immobility.[60] Designing more antagonists will allow for the exploration of therapy through antagonism of the kappa opioid receptor.

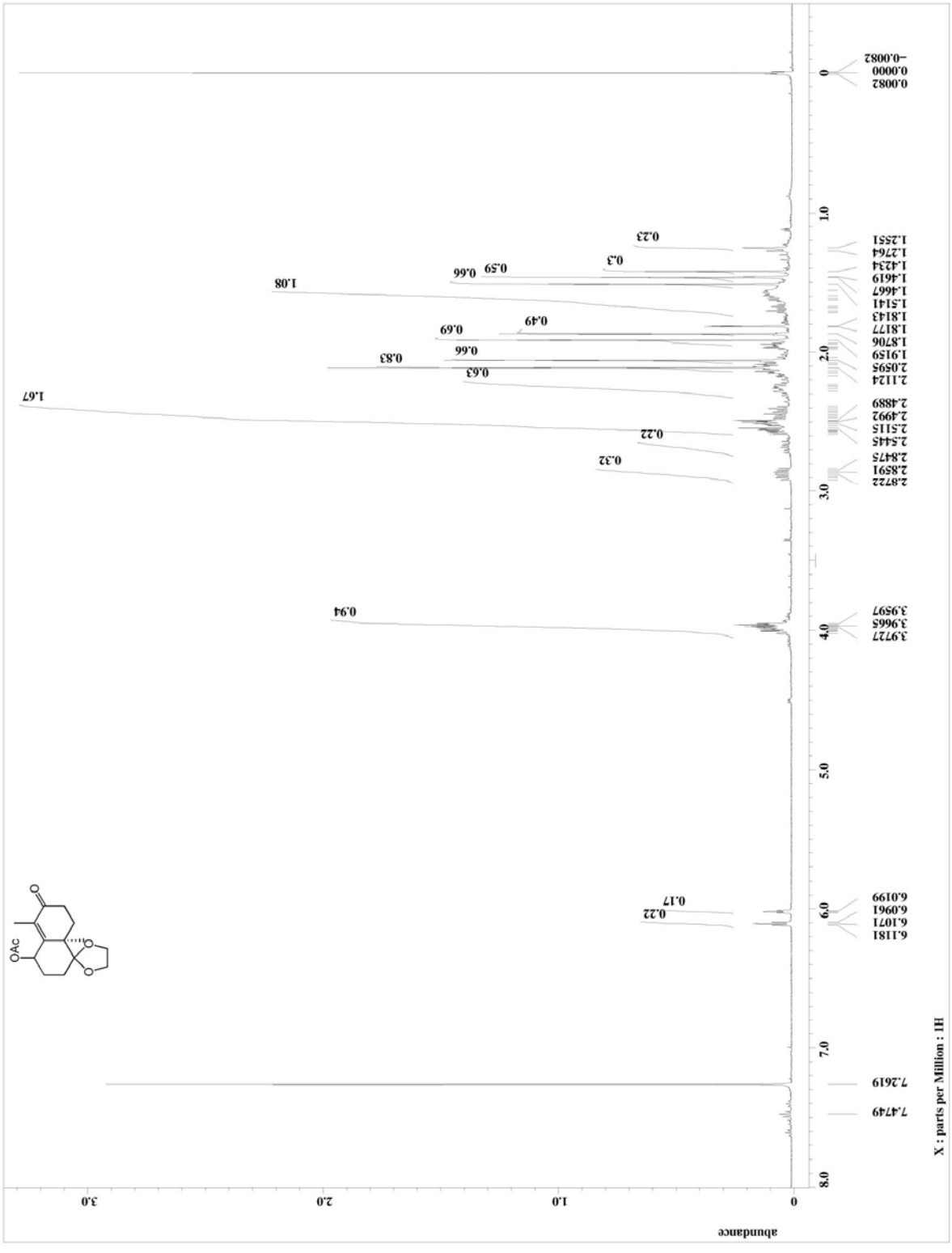
Appendix A

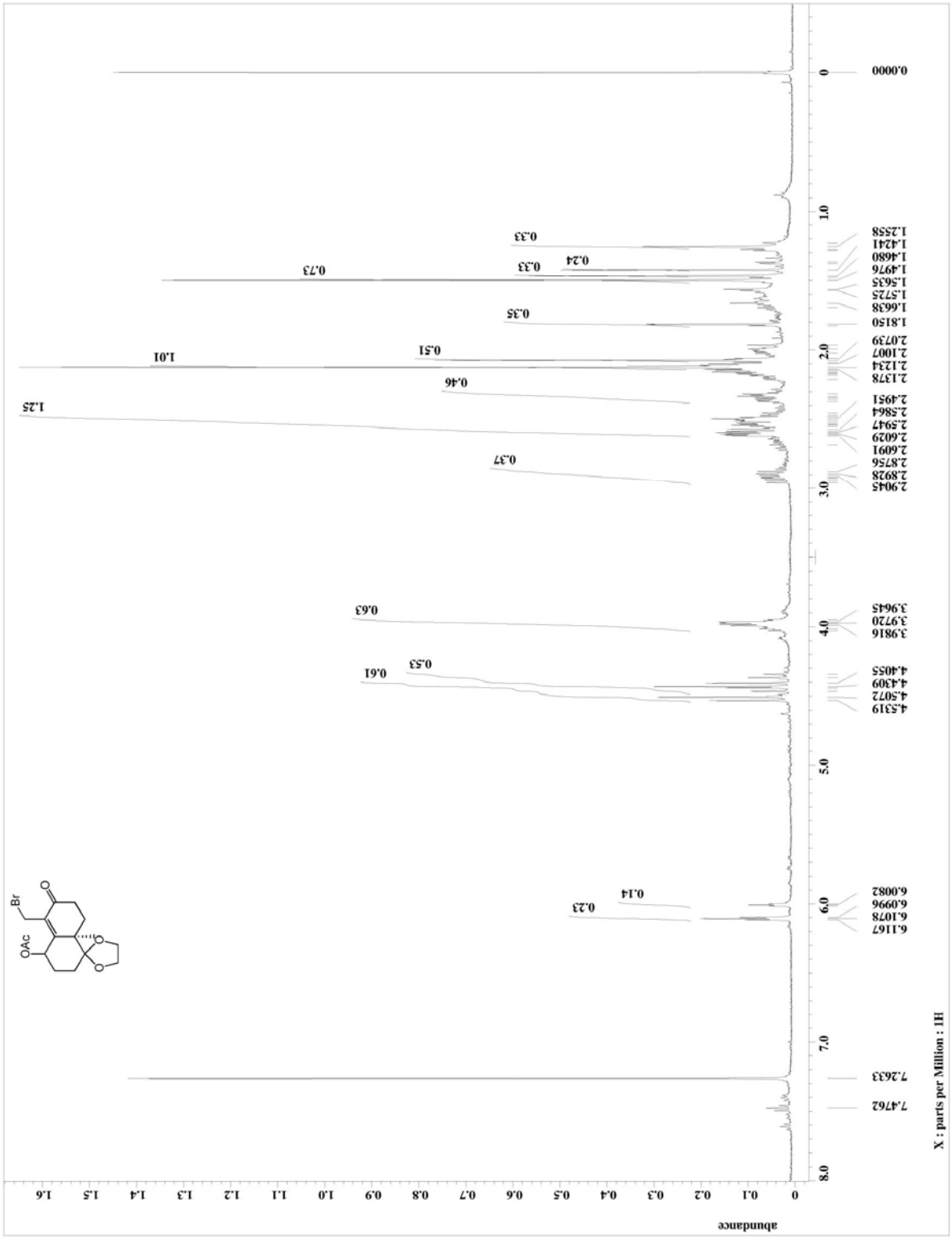
Spectral Data: ^{1}H-NMR and ^{13}C-NMR

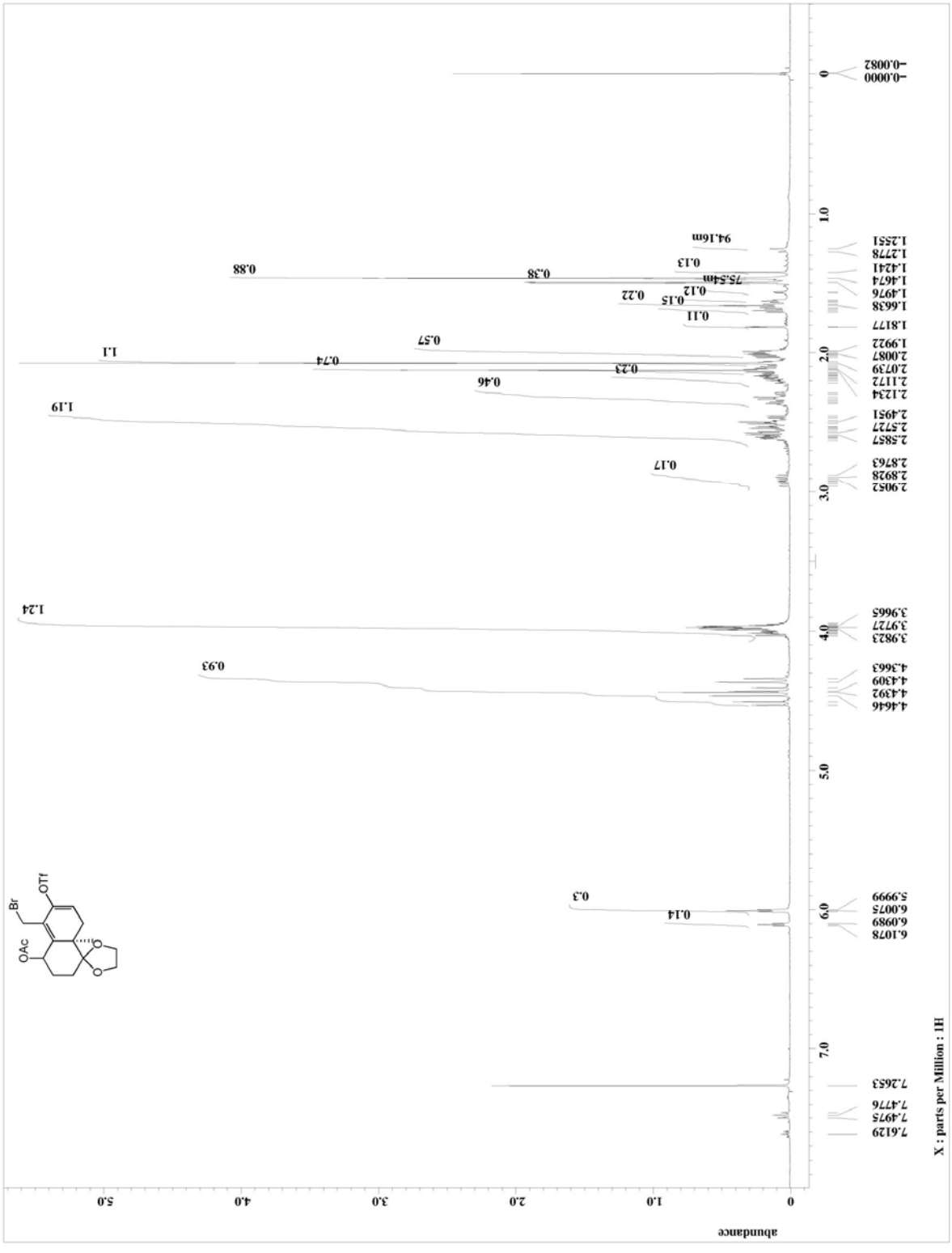

X : parts per Million : 1H
7.4735
7.2633
2.6812
2.5122
2.5033
2.4951
2.4882
2.4841
2.4717
2.4538
2.0891
2.0774
2.0616
1.8177
1.8143
1.7847
1.7717
1.4619
1.4234
1.2743
1.1816
1.0359
1.0195
0.9508
0.0000
32.16m
0.17
57.69m
0.15
0.97
0.29
0.98
1.06
0.13
0.18
0.48
1.48
abundance

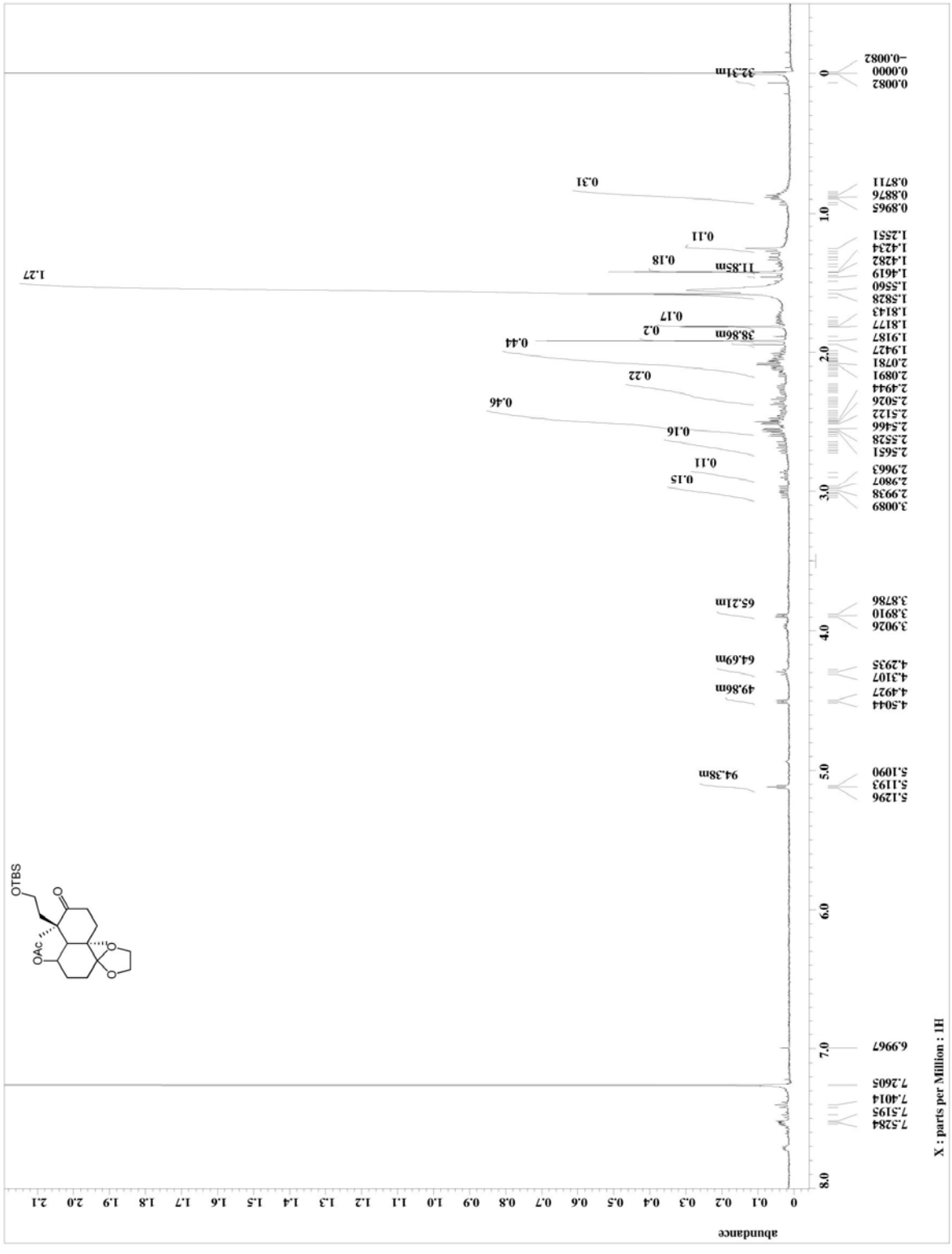

abundance
0
1.0
2.0
3.0
4.0
X : parts per Million : 1H
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.4962
7.4769
7.2660
3.9803
3.9679
3.9542
2.8983
2.8585
2.5143
2.5047
2.4964
2.4896
2.4854
2.4731
2.0781
2.0897
1.8184
1.8150
1.7916
1.7882
1.4619
1.4248
1.3396
1.2784
0.0000
0.66
0.13
0.19
0.42
1.67
1.15
0.63
1.08
0.18
0.49
1.03
0.18

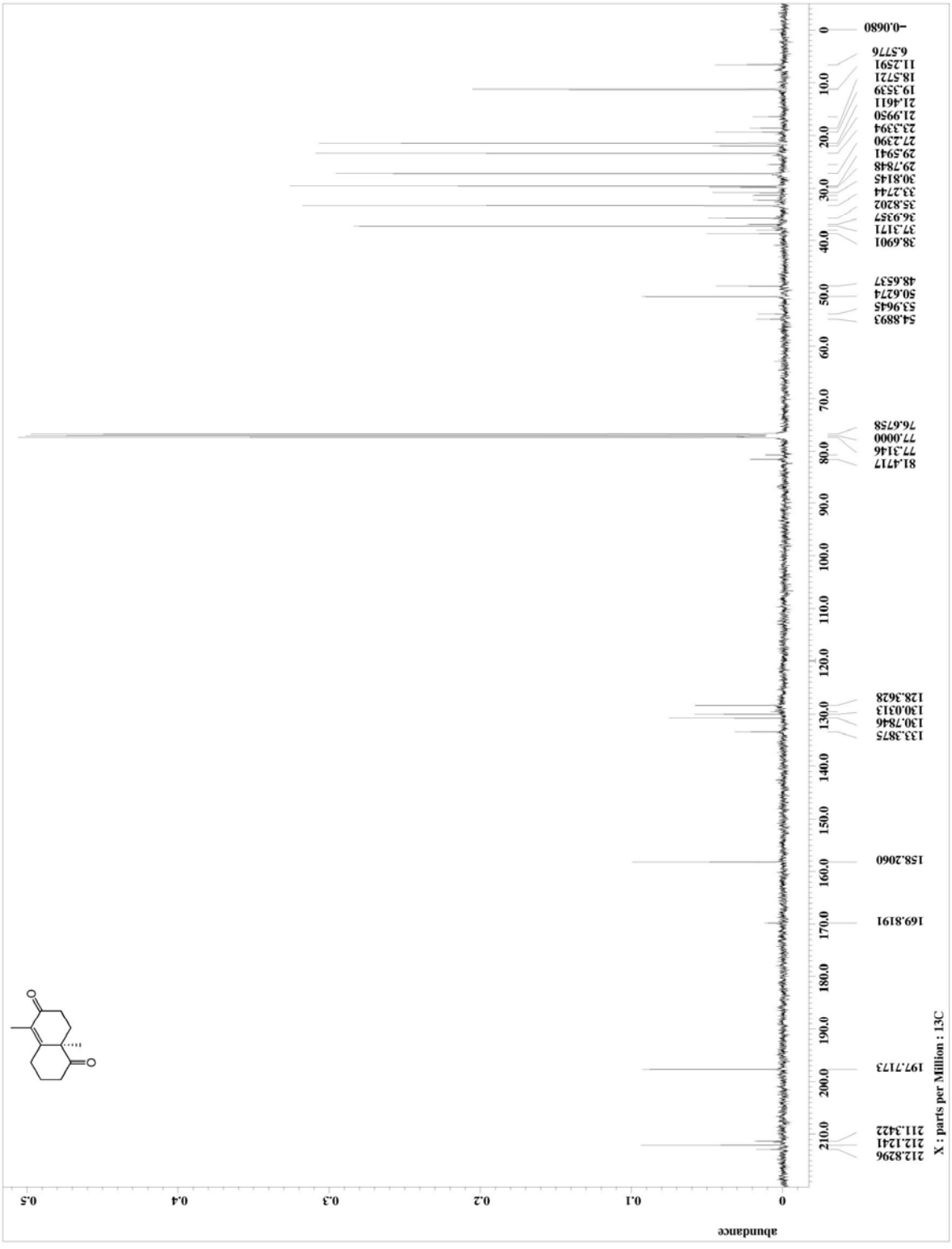

abundance
0
1.0
2.0
3.0
4.0
X : parts per Million : 1H
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
7.4742
7.2612
5.1296
5.1193
4.9461
4.9393
0.2
0.21
3.9906
3.9658
3.9528
3.9439
0.78
3.0096
2.9814
2.9663
2.5528
2.5466
2.5191
2.5109
2.5026
2.4944
1.9558
1.9180
1.8857
1.8177
1.8143
1.5828
1.5752
1.5649
1.5326
1.4234
1.2551
0.18
0.15
0.15
0.99
0.78
0.84
0.42
0.46
0.25
0.92
0.81
0.32
0.33
0.0082
−0.0000
−0.0082
OH
O
O
O

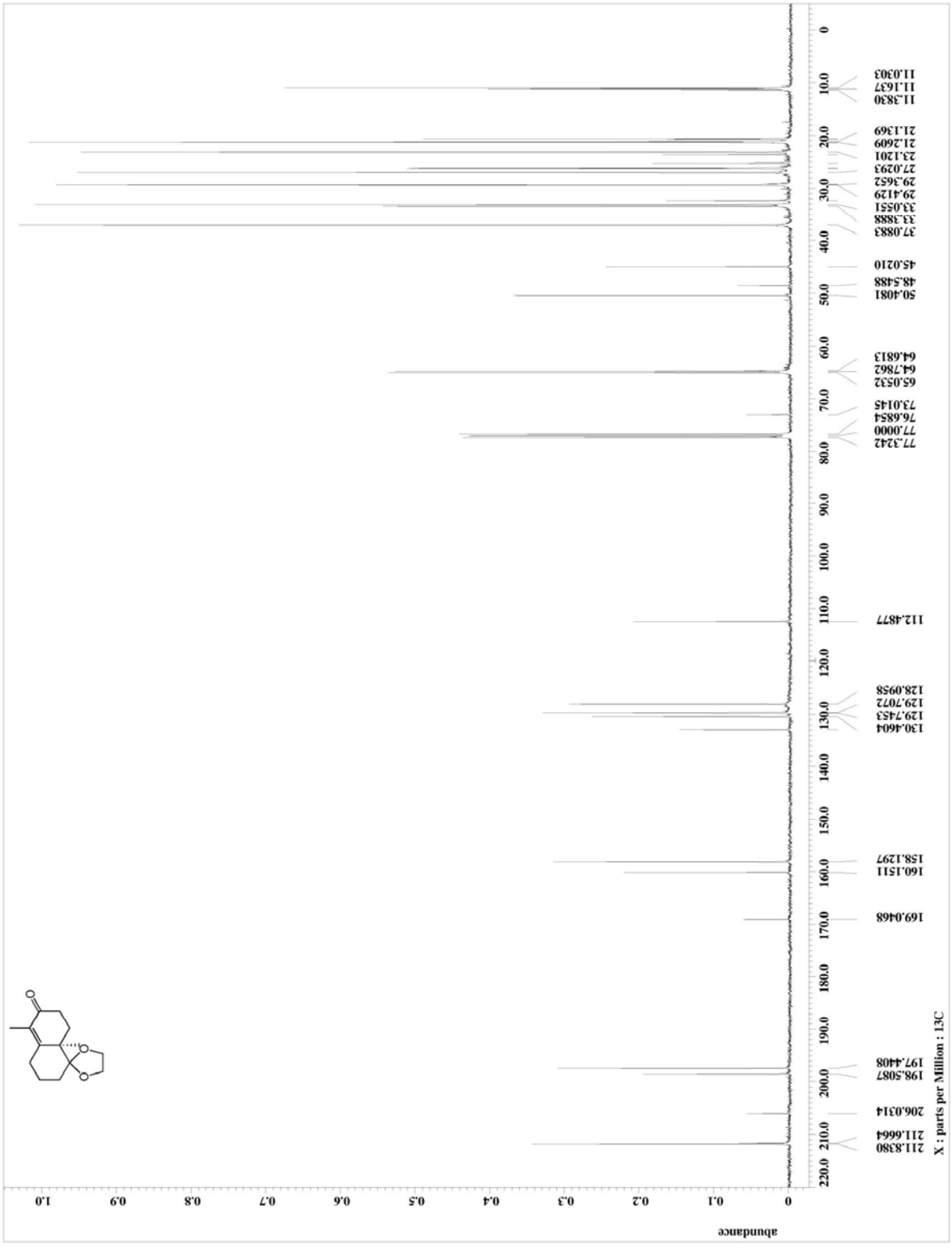

abundance
0
1.0
2.0
3.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
X : parts per Million : 1H
7.4749
7.2619
6.1181
6.1071
6.0961
6.0199
3.9727
3.9665
3.9597
2.8722
2.8591
2.8475
2.5445
2.5115
2.4992
2.4889
2.1124
2.0595
1.9159
1.8706
1.8177
1.8143
1.5141
1.4667
1.4619
1.4234
1.2764
1.2551
0.0082
0.0000
−0.0082
0.23
0.3
0.59
0.66
1.08
0.49
0.69
0.66
0.83
0.63
1.67
0.22
0.32
0.94
0.22
0.17
OAc

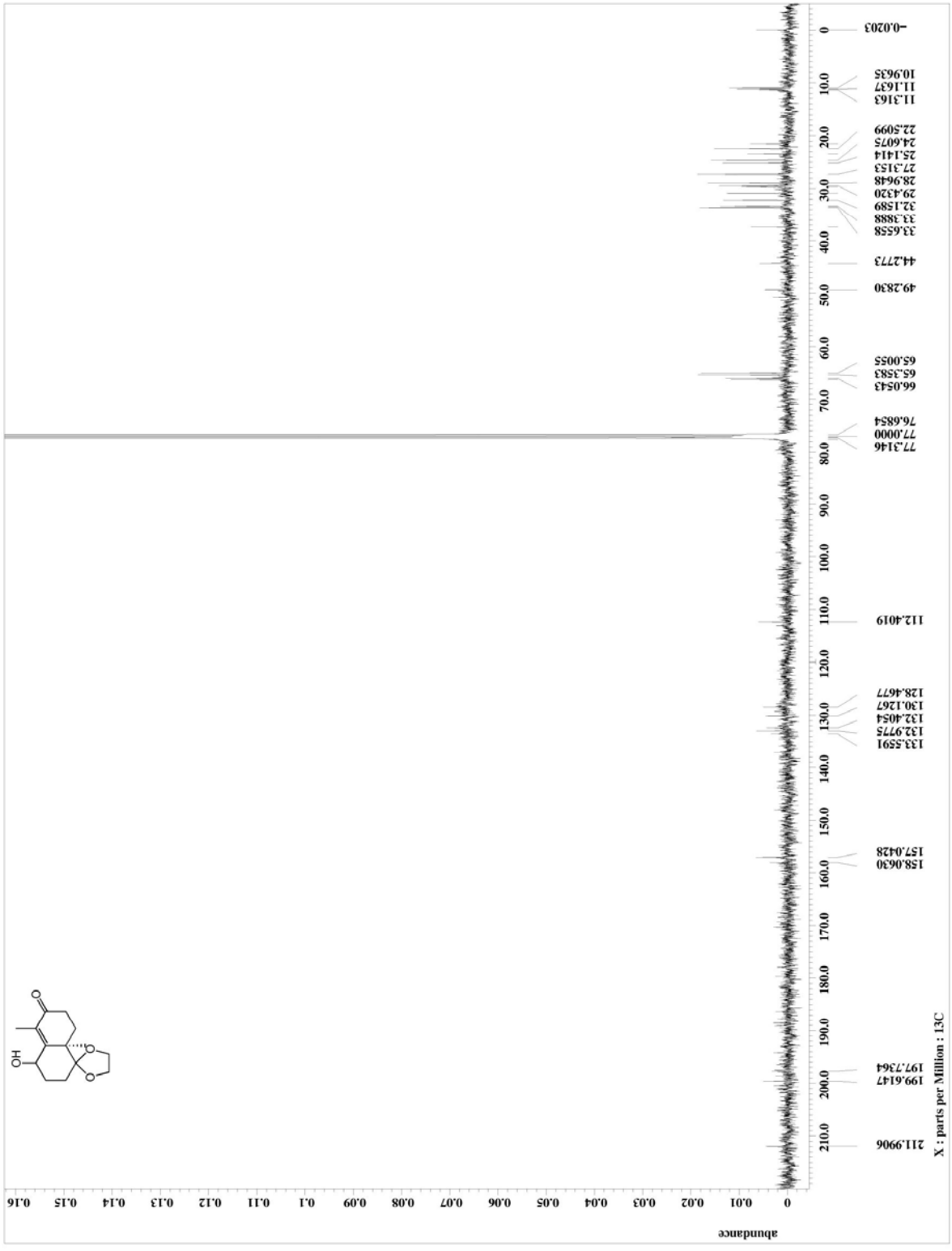

abundance
X : parts per Million : 1H
Br
OAc
0.0000
1.2558
1.4241
1.4680
1.4976
1.5635
1.5725
1.6638
1.8150
2.0739
2.1007
2.1234
2.1378
2.9045
2.8928
2.8756
2.6091
2.6029
2.5947
2.5864
2.4951
3.9645
3.9720
3.9816
4.4055
4.4309
4.5072
4.5319
6.0082
6.0996
6.1078
6.1167
7.2633
7.4762
0.14
0.23
0.53
0.61
0.63
0.37
1.25
0.46
1.01
0.51
0.35
0.24
0.33
0.73
0.33

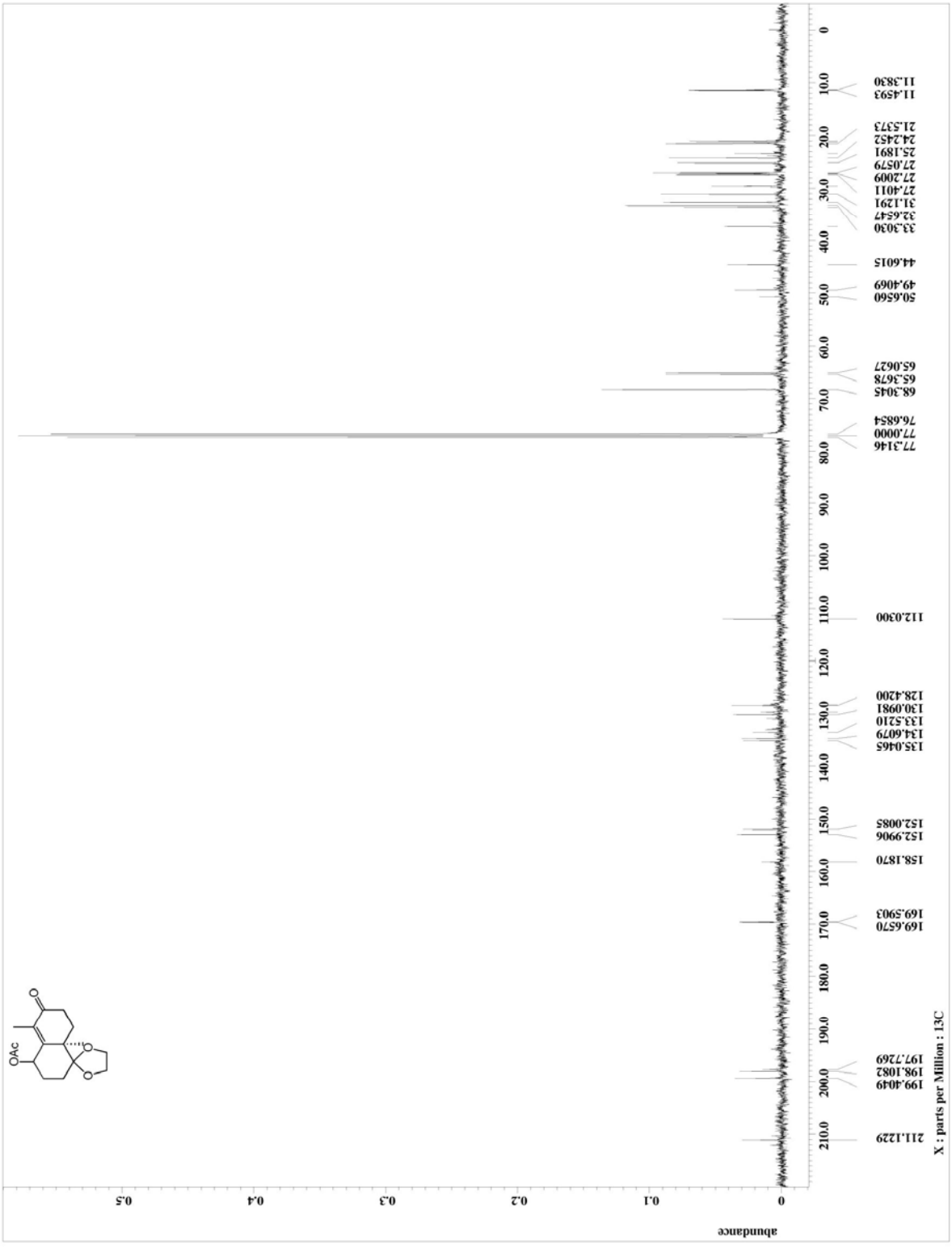

abundance
0
1.0
2.0
3.0
4.0
5.0
X : parts per Million : 1H
7.6129
7.4975
7.4776
7.2653
6.1078
6.0989
6.0075
5.9999
4.4646
4.4392
4.4309
4.3663
3.9823
3.9727
3.9665
2.9052
2.8928
2.8763
2.5857
2.5727
2.4951
2.1234
2.1172
2.0739
2.0087
1.9922
1.8177
1.6638
1.4976
1.4674
1.4241
1.2778
1.2551
0.0000
-0.0082
94.16m
75.54m
1.24
0.93
0.3
0.14
1.19
0.17
0.46
0.23
0.74
1.1
0.57
0.11
0.15
0.12
0.22
0.38
0.88
0.13
OTf
Br
OAc
O
O
O

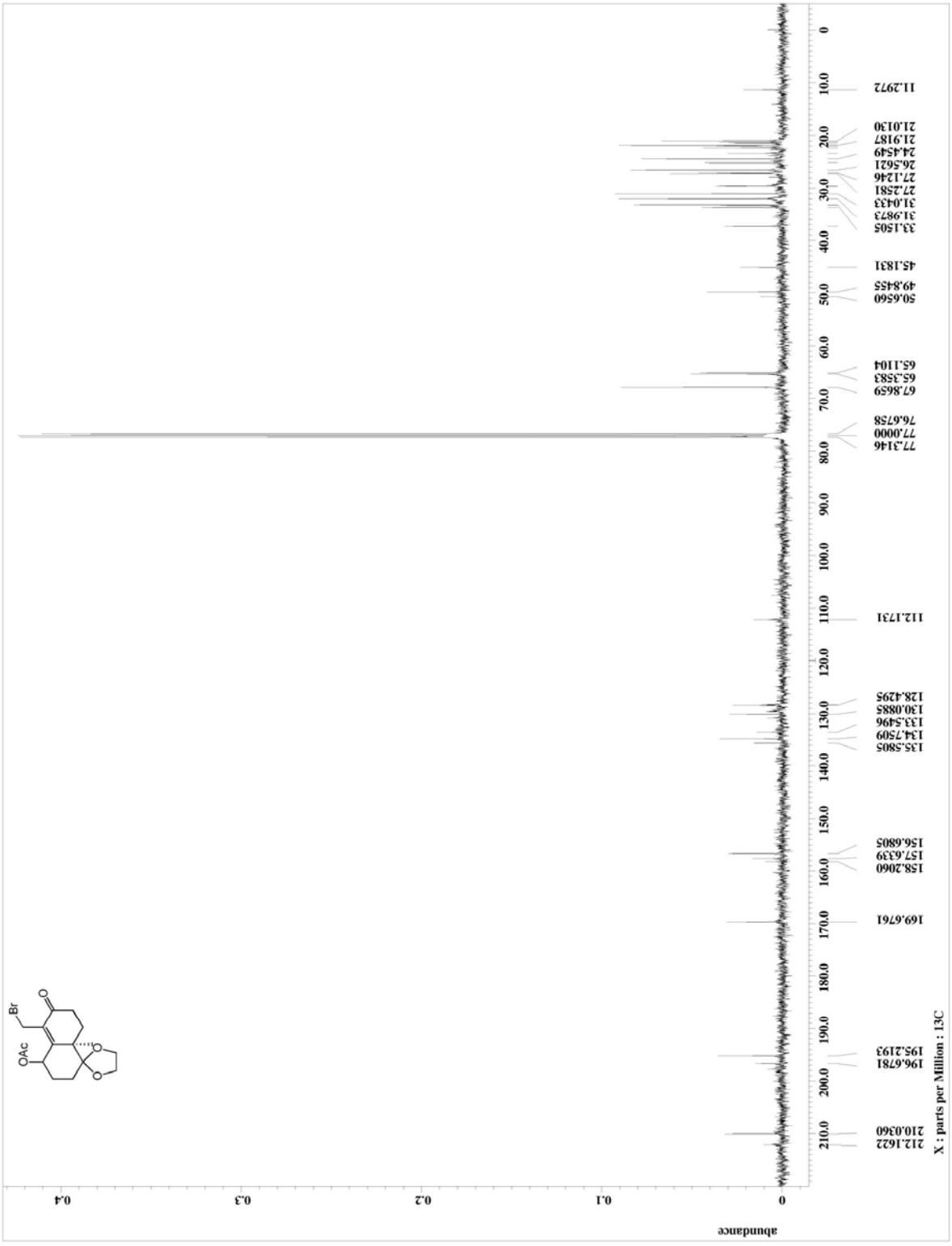

OTBS
OAc
O
O
O
abundance
X : parts per Million : 1H
7.5284
7.5195
7.4014
7.2605
6.9967
5.1296
5.1193
5.1090
4.5044
4.4927
4.3107
4.2935
3.9026
3.8910
3.8786
3.0089
2.9938
2.9807
2.9663
2.5651
2.5528
2.5466
2.5122
2.5026
2.4944
2.0891
2.0781
1.9427
1.9187
1.8177
1.8143
1.5828
1.5560
1.4619
1.4282
1.4234
1.2551
0.8965
0.8876
0.8711
0.0082
0.0000
−0.0082
32.31m
94.38m
49.86m
64.69m
65.21m
38.86m
11.85m
1.27
0.46
0.44
0.31
0.22
0.18
0.17
0.16
0.15
0.11
0.11
0.2

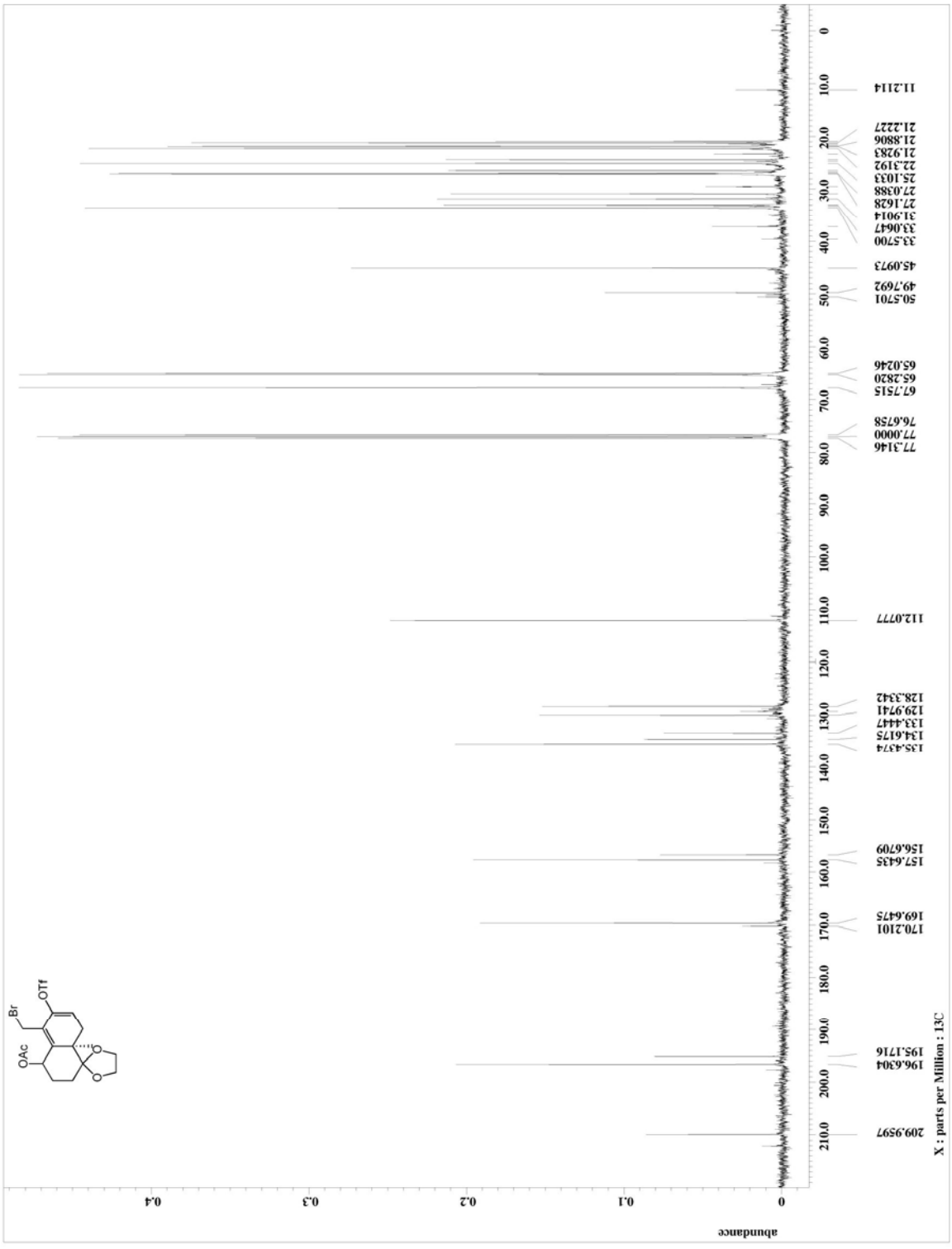

abundance
0
0.1
0.2
0.3
0.4
0.5
X : parts per Million : 13C
212.8296
212.1241
211.3422
197.7173
169.8191
158.2060
133.3875
130.7846
130.0313
128.3628
81.4717
77.3146
77.0000
76.6758
54.8893
53.9645
50.6274
48.6537
38.6901
37.3171
36.9357
35.8202
33.2744
30.8145
29.7848
29.5941
27.2390
23.3394
21.9950
21.4611
19.3539
18.5721
11.2591
6.5776
-0.0680

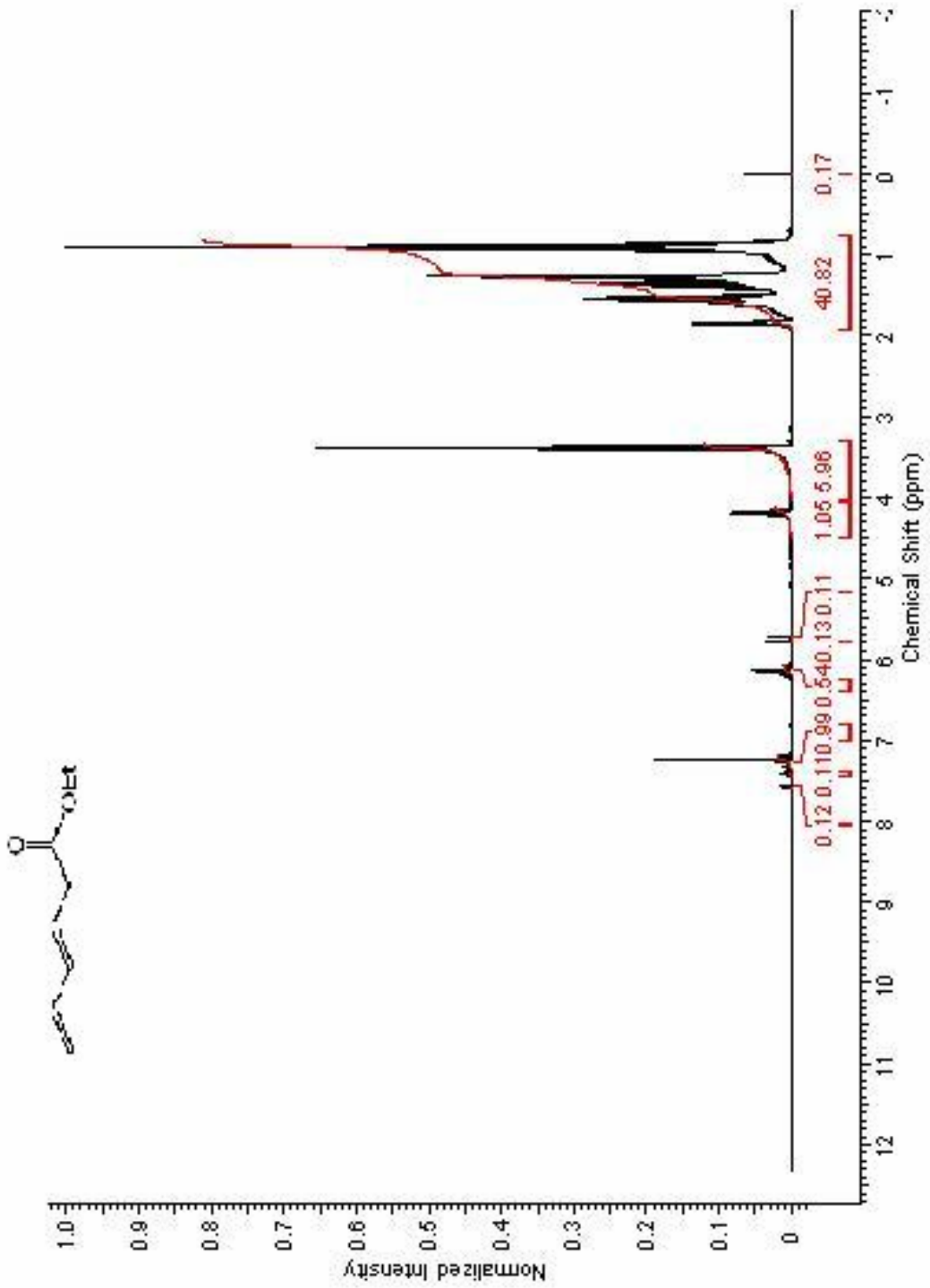

abundance
X : parts per Million : 13C
211.8380
211.6664
206.0314
198.5087
197.4408
169.0468
160.1511
158.1297
130.4604
129.7453
129.7072
128.0958
112.4877
77.3242
77.0000
76.6854
73.0145
65.0532
64.7862
64.6813
50.4081
48.5488
45.0210
37.0883
33.3888
33.0551
29.4129
29.3652
27.0293
23.1201
21.2609
21.1369
11.3830
11.1637
11.0303

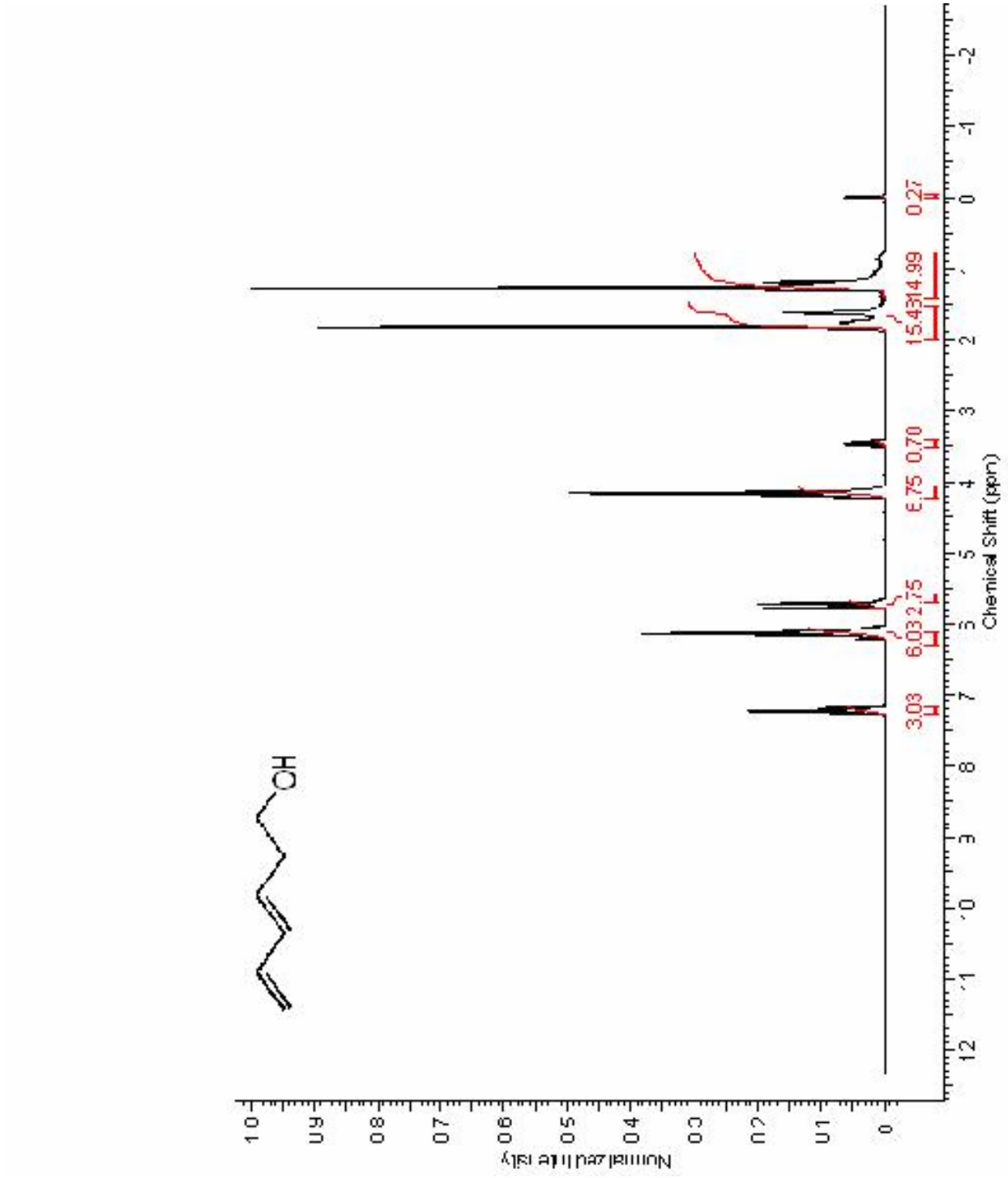

X : parts per Million : 13C
abundance
-0.0203
10.9635
11.1637
11.3163
22.5099
24.6075
25.1414
27.3153
28.9648
29.4320
32.1589
33.3888
33.6558
44.2773
49.2830
65.0055
65.3583
66.0543
76.6854
77.0000
77.3146
112.4019
128.4677
130.1267
132.4054
132.9775
133.5591
157.0428
158.0630
197.7364
199.6147
211.9906
OH
O
O
O

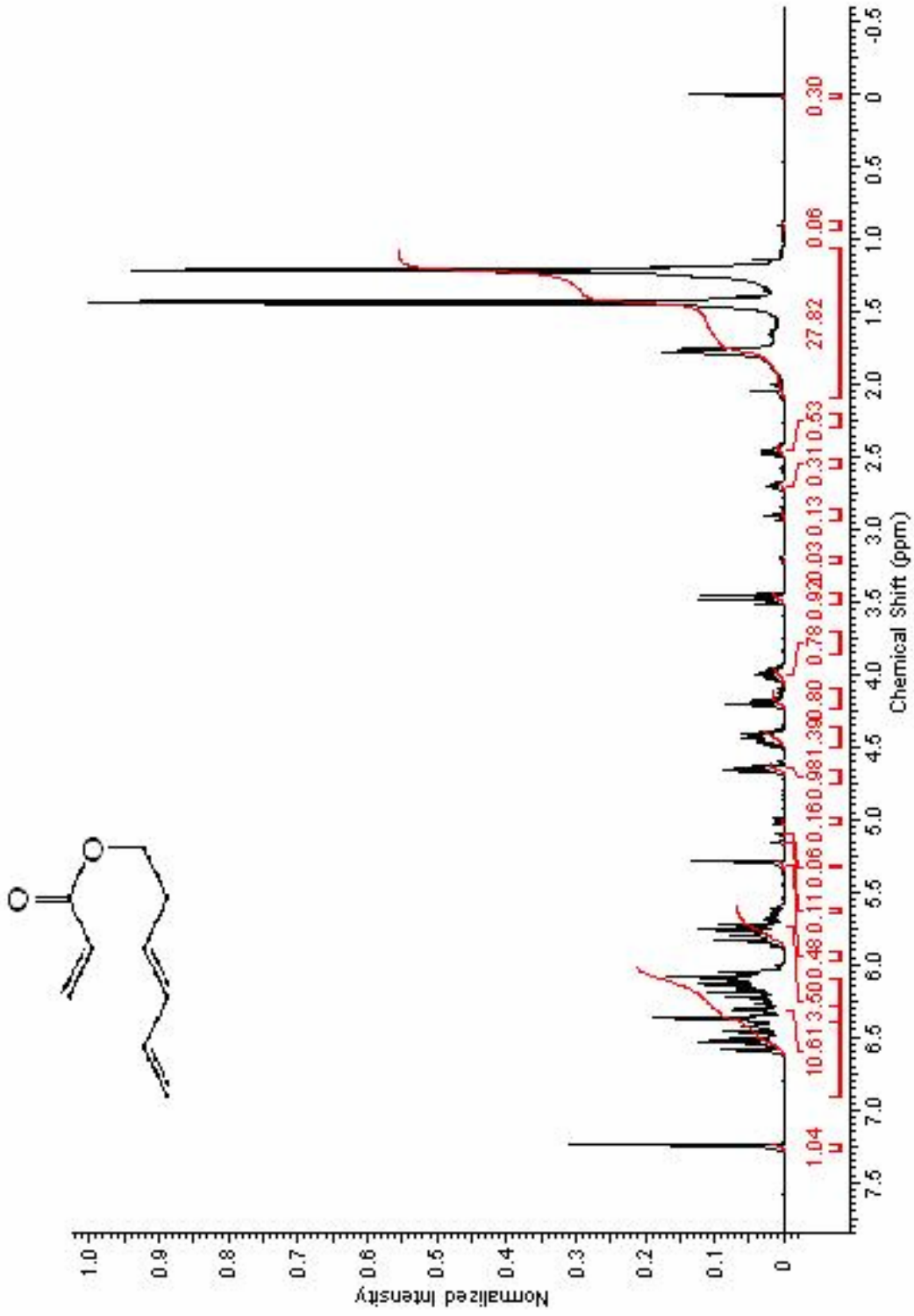

OAc
abundance
X : parts per Million : 13C
211.1229
199.4049
198.1082
197.7269
169.6570
169.5903
158.1870
152.9906
152.0085
135.0465
134.6079
133.5210
130.0981
128.4200
112.0300
77.3146
77.0000
76.6854
68.3045
65.3678
65.0627
50.6560
49.4069
44.6015
33.3030
32.6547
31.1291
27.4011
27.2009
27.0579
25.1891
24.2452
21.5373
11.4593
11.3830


abundance
0
0.1
0.2
0.3
0.4
X : parts per Million : 13C
212.1622
210.0360
196.6781
195.2193
169.6761
158.2060
157.6339
156.6805
135.5805
134.7509
133.5496
130.0885
128.4295
112.1731
77.3146
77.0000
76.6758
67.8659
65.3583
65.1104
50.6560
49.8455
45.1831
33.1505
31.9873
31.0433
27.2581
27.1246
26.5621
24.4549
21.9187
21.0130
11.2972
Br
OAc
O
O
O


abundance
0
0.1
0.2
0.3
0.4
X : parts per Million : 13C
209.9597
196.6304
195.1716
170.2101
169.6475
157.6435
156.6709
135.4374
134.6175
133.4447
129.9741
128.3342
112.0777
77.3146
77.0000
76.6758
67.7515
65.2820
65.0246
50.5701
49.7692
45.0973
33.5700
33.0647
31.9014
27.1628
27.0388
25.1033
22.3192
21.9283
21.8806
21.2227
11.2114
OTf
Br
OAc
O
O
O


Normalized Intensity
Chemical Shift (ppm)
0.12 0.11 0.99 0.54 0.13 0.11
1.05 5.96
40.82
0.17


OH
3.03
6.03
2.75
6.75
0.70
15.43
14.99
0.27
Chemical Shift (ppm)
Normalized Intensity


Normalized Intensity
Chemical Shift (ppm)
1.04
10.61
3.50
0.48
0.11
0.06
0.16
0.98
1.39
0.80
0.78
0.92
0.03
0.13
0.31
0.53
27.82
0.06
0.30

References

1. Snyder, S.H., and Pasternak, G.W. Historical Review: Opioid Receptors. *TRENDS in Pharmacological Sciences.* **24**: 198-205 (2003).

2. Kreek, MJ., LaForge, KS. Stress Responsivity, Addiction, and a Functional Variant of the Human Mu-Opioid Receptor Gene. *Viewpoint Molecular Interventions*. **7**: 74-78 (2007).

3. Fichna, J., Janecka, A., Costentin, J., Do Rego, JC. The Endomorphin System and Its Evolving Neurophysiological Role. *Pharmacol Rev*. **59**: 88-123 (2007).

4. Valdes, L.J., Diaz, J.L., and Paul, A.G. Ethnopharmacology of Ska Maria Pastora (*Salvia Divinorum*, Epling and Jativa-M.). *Journal of Ethnopharmacology*. **7**: 287-312 (1983).

5. Giroud, C., Felber, F., Augsbuger, M., Horisberger, B., River, and L., Mangin, P. *Salvia divinorum*: An Hallucinogenic Mint Which Might Become a New Recreational Drug in Switzerland. *Forensic Science International*. **112**: 143-150 (2000).

6. Sheffler, D.J., and Roth, B.L. Salvinorin A: The 'Magic Mint' Hallucinogen Finds a Molecular Target in the Kappa Opioid Receptor. *TRENDS in Pharmacological Sciences*. **24**: 107-109 (2003).

7. Roth, B., Baner, K., Westkaemper, R., Siebert, D., Rice, K., Steinberg, S., Ernsberger, P., Rothman, R. Salvinorin A: A Potent Naturally Occurring Nonnitrogenous κ Opioid Selective Agonist. *PNAS*. **99**: 11934-11939 (2002).

8. Grilli, M., Neri, E., Zappettini, S., Massa, F., Bisio, A., Romussi, G., Marchi, M., and Pittaluga, A. Salvinorin A Exerts Opposite Presynaptic Controls on Neurotransmitter Exocytosis from Mouse Brain Nerve Terminals. *Neruropharmacology*. **57**: 523-530 (2009).

9. Chavkin, C., Sud, S., Jin, W., Stewart, J., Zjawiony, J.K., Siebert, D.J., Toth, B.A., Hufeisen, S.J., and Roth, B.L. Salvinorin A, an Active Component of the Hallucinogenic Sage *Salvia divinorum* Is a Highly Efficacious κ-Opioid Receptor

Agonist: Structural and Functional Considerations. *The Journal of Pharmacology and Experimental Therapeutics*. **308**: 1197-1203 (2004).

10. Yan, F., Mosier, P.D., Westkaemper, R.B., Stewart, J., Zjawiony, J.K., Vortherms, T.A., Sheffler, D.J., and Roth, B.L. Identification of the Molecular Mechanisms by Which the Diterpenoid Salvinorin A Binds to κ-Opioid Receptors. *Biochemistry*. **44**: 8643-8651 (2005).

11. Zjawiony, J., Fahmy, H., Stewart, D., Roth, B. Agents With Selective κ-Opioid Affinity. *US Patent Application Publication*. US 7,687,538 B2 (2010).

12. Prisinzano, T. Opioid Receptor Ligands and Methods for Their Preparation. *US Patent Application Publication*. US 2006/0058264 A1 (2006).

13. Beguin, C., Carlezon, W., Cohen, B., He, M., Lee, D., Richards, M., Liu-Chen, L. Salvinorin Derivatives and Uses Thereof. *US Patent Application Publication*. US 2007/0213394 A1 (2007).

14. Carey, G.J. and Bergman, J. Enadoline Discrimination in Squirrel Monkeys: Effects of Opioid Agonists and Antagonists. *The Journal of Pharmacology and Experimental Therapeutics*. **297**:215-223 (2001).

15. Picker, M., Heise, J.W., and Dykstra, L.A. Evaluation of the Effects of Opioid Agonists and Antagonists Under a Fixed-Consecutive-Number Schedule in Rats. *Pharmacology Biochemistry & Behavior*. **27**: 73-80 (1987).

16. McConalogue, K., Grady, E.F., Minnis, J., Balestra, B., Tonini, M., Brecha, N.C., Bunnett, N.W., and Sternini, C. Activation And Internalization Of The μ-Opioid Receptor By The Newly Discovered Endogenous Agonists, Endomorphin-1 And Endomorphin-2. *Neroscience*. **90**: 1051-1059 (1999).

17. Riviere, PJ. Review: Peripheral Kappa-Opioid Agonists for Visceral Pain. *British Journal of Pharmacology*. **141**: 1331-1334 (2004).

18. Smiley, MM., Lu, Y., Vera-Portocarrero, LP., Zidan, A., Westlund, K. Intrathecal Gabapentin Enhances the Analgesic Effects of Subtherapeutic Dose Morphine in a Rat Experimental Pancreatitis Model. *Anesthesiology*. **101**: 759-765 (2004).

19. Lu, Y., Vera-Portocarrero, LP., Westlund, K. Intrathecal Coadministration of D-APV and Morphine is Maximally Effective in a Rat Experimental Pancreatitis Model. *Anesthesiology*. **98**: 734-740 (2003).

20. Corey, E. Retrosynthetic Thinking – Essentials and Examples. *Chem Soc Rev*. **17**: 111-133 (1988).

21. Corey, E., Cheng, X-M. The Logic of Chemical Synthesis. New York: Wiley (1995).

22. Tietze, L. F., Stewart, S. G., and Polomska, M. E. Intramolecular Heck Reactions for the Synthesis of the Novel Antibiotic Mensacarcin: Investigation of Catalytic, Electronic and Conjugative Effects in the Preparation of the Hexahydroanthracene Core. *Journal of European Chemistry*. 1752-1759 (2005).

23. Tang, Z., Jiang, F., Yu, L., Cui, X., Gong, L., Mi, A., Jiang, Y., and Wu, Y. Novel Small Organic Molecules for Highly Enantioselective Direct Aldol Reaction. *Journal of the American Chemical Society*. **125**: 5262-5263 (2003).

24. Kreipl, A.T., Reid, C., and Steglich, W. Total Syntheses of Marine Pyrrole Alkaloids Polycitone A and B. *Organic Letters*. **4**: 3287-3288 (2002).

25. Hoye, T.R., Eklov, B.M., Ryba, T.D., Voloshin, M., and Yao, L.J. No-D NMR (No-Deuterium Proton NMR) Spectroscopy: A Simple Yet Powerful Method for Analyzing Reaction and Reagent Solutions. *Organic Letters* **6**: 953-956 (2004).

26. Franklin, C., Li, H., and Martin, S.F. Design, Synthesis, and Evaluation of Water-Soluble Phospholipid Analogues as Inhibitors of Phospholipase C from *Bacillus cereus*. *J. Org. Chem*. **68**: 7298-7307 (2003).

27. Minami, T., Hirakawa, K., Koyanagi, S., Nakamura, S., and Yamaguchi, M. A New Synthesis of α-Methylene Lactones. *J. Chem. Soc. Pekin Trans. 1*. 2385-2390 (1990).

28. Hsiao, Y., Rivera, N. R., Yasuda, N., Hughes, D. L., and Reider, P. J. Highly Regioselective Friedlander Reaction. *Organic Letters*. **3**: 1101-1103 (2001).

29. Yamaguchi, M., Okuma, T., Nakamura, S. and Minami, T. Synthesis of Semivioxanthin *Via* a Polyketide. *J.Chem.Soc. Perkins Trans. 1*. 183-185 (1990).

30. Clive, D.L.J., Fletcher, S.P., and Liu, D. Formal Radical Cyclization onto Benzene Rings: A General Method and Its Use in the Synthesis of *ent*-Nocardione A. *J. Org. Chem*. **69**: 3282-3293 (2004).

31. Fuji, K., Node, M., Nagasawa, H., Naniwa, Y., Taga, T., Machida, K., and Snatzke, G. Direct Asymmetric Synthesis of Quaternary Carbon Centers by the Addition Elimination Process: Nitroolefination of .Alpha.-Substituted.Delta.-Lactones. *Journal of the American Chemical Society*. **111**: 7921-7925 (1989).

32. Baughman, T.W., Sworen, J.C., and Wagener, K.B. The Facile Preparation of Alkenyl Metathesis Synthons. *Tetrahedron*. **60**: 10943-10948 (2004).

33. Hong, S.H., Sander, D.P., Lee, C.W., and Grubbs, R.H. Prevention of Undesirable Isomerization During Olefin Metathesis. *Journal of the American Chemical Society*. **127**: 17160-17161 (2005).

34. Narayana, C., Padmanabhan, S., and Kabalka, G.W. Cleavage of Ethers and Geminal Diacetates Using the Boron Triiodide-N,N-diethylaniline Complex. *Tetrahedron Letters*. **31**: 6977-6978 (1990).

35. Narayana, C., Reddy, N.K., and Kabalka, G.W. An Efficient Cleavage of Lactones with Boron Triiodide-N,N-diethylaniline Complex. *Tetrahedron Letters*. **32**: 6855-6856 (1991).

36. Alanine, A., Fishwick, C., Jones, A., and Mitchell, M. Preparation of Methyl-2-(ω-iodoalkyl)propenoates and a Facile Route to Carbomethoxy-1,3-butadiene. *Tetrahedron Letters*. **30**: 5653-5654 (1989).

37. Dounay, A., Overman, L. Asymmetric Intramolecular Heck Reaction in Natural Product Total Synthesis. *Chem Rev*. **103**: 2945-2964 (2003).

38. Rubottom, G.M., Vasquez, M.A., Pelegrina, D.R. Peracid Oxidation of Trimethylsilyl Enol Ethers: A Facile a-Hydroxylation Procedure. *Tetrahedron Letters*. **15**: 4319-4322 (1974).

39. White, J.D., Theramongkol, P., Kuroda, C., and Engelbrecht, J.R. Enantioselective Total Synthesis of (-)-Monic Acid C Via Carbosulfenylation of a Dihydropyran. *J. Org. Chem*. **53**: 5909-5921 (1988).

40. Burns, A., Forsyth, C. Intramolecular Diels-Alder/Tsuji Allylation Assembly of the Functionalized *trans*-Decalin of Salvinorin A. *Organic Letters*. **10**: 97-100 (2008).

41. Miller, C. and Batey, R. Hetero Diels-Alder Reactions of Nitrosoamidines: An Efficient Method for the Synthesis of Functionalized Guanidines. *Organic Letters*. **6**: 699-702 (2004).

42. DeBouef, B., Counts, W.R., and Gilberston, S.R. Rhodium-Catalyzed Synthesis of Eight-Membered Rings. *J. Org. Chem*. **72**: 799-804 (2007).

43. Aladro, F., Guerra, F., Moreno-Dorado, F., Bustamante, J., Jorge, Z., Massanet, G. Enantioselective Synthesis of (+)-decipienin A. *Tetrahedron*. **57**: 2171-2178 (2001).

44. Hagiwara, H., Uda, H. Optically Pure (4a*S*)-(+)- or (4a*R*)-(-)-1,4a-Dimethyl-4,4a,7,8-tetrathydronaphthalene-2,5(3*H*,6*H*)-dione and Its Use in the Synthesis of an Inhibitor of Steroid Biosynthesis. *J. Org. Chem*. **53**: 2308-2311 (1988).

45. Nozawa, M., Suka, Y., Hoshi, T., Suzuki, T., Hagiwara, H. Total Synthesis of the Hallucinogenic Neoclerodane Diterpenoid Salvinorin A. *Organic Letters*. **10**: 1365-1368 (2008).

46. Hagiwara, H., Suka, Y., Nojima, T., Hoshi, T., Suzuki, T. Second-Generation Synthesis of Salvinorin A. *Tetrahedron*. **65**: 4820-4825 (2009).

47. Knochel, P., Singer, R. Preparation and Reactions of Polyfunctional Organozinc Reagents in Organic Synthesis. *Chem. Rev*. **93**: 2117-2188 (1993).

48. Casado, A.L., Espinet, P., and Gallego, A.M. Mechanism of the Stille Reaction. 2. Coupling of Aryl Triflates with Vinyltributyltin. Observation of Intermediates. A More Comprehensive Scheme. *Journal of the American Chemical Society*. **122**: 11771-11782 (2000).

49. He, L., Fong, J., von Zastrow, M., and Whistler, J.L. Regulation of Opioid Receptor Trafficking and Morphine Tolerance by Receptor Oligomerization. *Cell*. **108**: 271-282 (2002).

50. McCurdy, CR., Sufka, KJ., Smith, GH., Warnick, JE., Nieto, MJ. Antinociceptive Profile of Salvinorin A, a Structurally Unique Kappa Opioid

Receptor Agonist. *Pharmacology, Biochemistry and Behavior*. **83**: 109-113 (2006).

51. John, TF., French, LG., Erlichman, JS. The Antinociceptive Effect of Salvinorin A in Mice. *European Journal of Pharmacology*. **545**: 129-133 (2006).

52. Zhang, Y., Butelman, E., Schlussman, S., Ho, A., Kreek, MJ. Effects of Plant-Derived Hallucinogen Salvinorin A on Basal Dopamine Levels in the Caudate Putamen and in a Conditioned Placed Aversion Assay in Mice: Agonist Actions at Kappa Opioid Receptors. *Psychopharmacology*. **179**: 551-558 (2005).

53. Eisenach, J.C., Carpenter, R., and Curry, R. Analgesia from a Peripherally Active κ-Opioid Receptor Agonist in Patients with Chronic Pancreatitis. *Pain*. **101**:89-95 (2003).

54. Vera-Portocarrero, L.P., Lu, L., Westlund, K.N. Nociception in Persistent Pancreatitis in Rats: Effects of Morphine and Neuropeptide Alterations. *Anesthesiology*. **98**: 474-484 (2003).

55. Lu, Y., Vera-Portocarrero, L.P., Westlund, K.N. Intrathecal Coadministration of D-APV and Morphine is Maximally Effective in a Rat Experimental Pancreatitis Model. *Anesthesiology*. **98**: 734-740 (2003).

56. Filizola, M., Laakkonen, L., and Loew, G.H. 3D Modeling, Ligand Binding and Activation Studies of the Cloned Mouse δ, μ, κ Opioid Receptors. *Protein Engineering*. **12**: 927-942 (1999).

57. Munro, T., Rizzicasa, M., Roth, B., Toth, B., and Yan, F. Studies Toward the Pharmacophore of Salvinorin A, a Potent κ Opioid Receptor Agonist. *J. Med. Chem.* **48**: 345-348 (2005).

58. Prisinzano, T. and Rothman, R. Salvinorin A Analogues as Probes in Opioid Pharmacology. *Chem Rev*. **108**: 1732-1743 (2008).

59. Metcalf, M., Coop, A. Kappa Opioid Antagonists: Past Success and Future Prospects. *AAPS Journal*. **7**: E704-E722 (2005).

60. Mague, S., Pliakas, A., Todtenkopf, M., Tomasiewicz, H., Zhang, Y., Stevens, W., Jones, R., Portoghese, P., Carlezon, W. Antidepressant-Like Effects of

Kappa-Opioid Receptor Antagonists in the Forced Swim Test in Rats. *J Pharmacol Exp Ther*. **305**: 323-330 (2003).

61. Butelman, E.R., Harris, T.J., and Kreek, M.J. The Plant-Derived Hallucinogen, Salvinorin A, Produces κ-Opioid Agonist-Like Discriminative Effects in Rhesus Monkeys. *Psychopharmacology*. **172**: 220-224 (2004).

62. Simonin, F., Valverde, O., Smadja, C., Slowe, S., Kitchen, I., Dierich, A., LeMeur, M., Roques, B.P., Maldonado, R., and Kieffer, B.L. Disruption of the κ-Opioid Receptor Gene in Mice Enhances Sensitivity to Chemical Visceral Pain, Impairs Pharmacological Actions of the Selective κ-Agonist U-50,488H and Attenuates Morphine Withdrawal. *The EMBO Journal*. **17**: 886-897 (1998).

63. Valdes, L.J., Chang, H., Visger, D., and Koreeda, M. Salvinorin C, a New Neoclerodane Diterpene from a Bioactive Fraction of the Hallucinogenic Mexican Mint *Salvia divinorum*. *Organic Letters*. **3**: 3935-3937 (2001).

64. Lavecchia, A., Greco, G., Novellino, E., Vittorio, F., and Ronsisvalle, G. Modeling of κ-Opioid Receptor/Agonists Interactions Using Pharmacophore-Based and Docking Simulations. *J. Med. Chem.* **43**: 2124-2134 (2000).

65. Holzgrabe, U., and Brandt, W. Mechanism of Action of the Diazabicyclononanone-type κ-Agonists. *J. Med. Chem.* **46**: 1383-1389 (2003).

66. Neumeyer, J.L., Mello, N.K., Negus, S.S., and Bidlack, J.M. Kappa opioid agonists as targets for pharmacotherapies in cocaine abuse. *Pharmaceutica Acta Helvetiae*. **74**: 337-344 (2000).

67. Bruins Slot, L.A. and Colpaet, F.C. Opiates States of Memory: Receptor Mechanisms. *The Journal of Neuroscience*. **19**: 10520-10529 (1999).

68. Breslin, H.J., Miskowski, T.A., Rafferty, B.M., Coutinho, S.V., Palmer, J.M., Wallace, N.H., Schneider, C.R., Kimball, E.S., Zhang, S., Li, J., Colburn, R.W., Stone, D.J., Martinez, R.P., and He, W. Rationale, Design, and Synthesis of Novel Phenyl Imidazoles as Opioid Receptor Agonists for Gastrointestinal Disorders. *J. Med. Chem*. **47**: 5009-5020 (2004).

69. Wentland, M.P., Ye, Y., Cioffi, C.L., Lou, R., Zhou, Q., Xu, G., Duan, W., Dehnhardt, C.M., Sun, X., Cohen, D.J., and Bidlack, J.M. Syntheses and Opioid

Receptor Binding Affinities of 8-Amino-2,6,-methano-3-benzazocines. *J. Med. Chem*. **46**: 838-849 (2003).

70. Narayana, C., Reddy, N. K., and Kabalka, G. W. An Efficient Cleavage of Lactones with Boron Triiodide-*N,N*-diethylaniline Complex. *Tetrahedron Letters*. **32**: 6855-6856 (1991).

71. Smith, III, A. B., Freeze, S., LaMarche, M. J., Hirose, T., Brouard, I., Rucker, P.V., Xian, M., Sunderman, K. F., Shaw, S. J., Burlingame, M. A., Horwitz, S. B., and Myles, D.C. Design, Synthesis, and Evaluation of Carbamate-Substituted Analogues of (+)-Discodermolide. *Organic Letters*. **7**: 311-314 (2005).

72. Knopff, O., Alexakis, A. Tandem Asymmetric Conjugate Addition-Silylation of Enantiomerically Enriched Zinc Enolates. Synthetic Importance and Mechanistic Implications. *Organic Letters*. **4**: 3835-3837 (2002).

73. Wysocka, W. The mechanism of the heterogenous catalysis of α and γ-cyclic six-membered amnioketones. *Tetrahedron*. **41**: 681-692 (1985).

74. Jauch, J. Stereochemistry of the Rubottom Oxidation with Bicyclic Silyl Enol Ethers; Synthesis and Dimerization Reactions of Bicyclic α-Hydroxy Ketones. *Tetrahedron*. **50**: 12903-12912 (1994).

75. Genarri, C. Colombo, L., Bertolini, G., and Shimperna, G. $TiCl_4$-Mediated Reactions of the Silyl Ketene Acetals Derived from N-Methylephedrine Esters: An Asymmetric Variant of the Mukaiyama Reaction. *J. Org. Chem.* **53**: 2754-2760 (1987).

76. Stacy, C., Kaplan, A., Williams, G. The Fight Against Pain. Consumer Report Books. 1992 Consumers Union of U.S., Inc., Yonkers, NY 10703.

77. Katzung, B. Basic & Clinical Pharmacology 9th edition. Lange Medical Books/ McGraw-Hill (2004).

78. Prisinzano, T. Minireview: Psychopharmacology of the Hallucinogenic Sage *Salvia divinorum*. *Life Sciences*. **78**: 527-531 (2005).

79. Willmore-Fordham, C., Krall, D., McCurdy, C., Kinder, D. The Hallucinogen Derived from *Salvia divinorum*, Salvinorin A, Has κ-Opioid Agonist

Discriminative Stimulus Effects in Rats. *Neuropharmacology*. **53**: 481-486 (2007).

80. Wang, Y., Tang, K., Inan, S., Siebert, D., Holzgrabe, U., Lee, D., Huang, P., Li, J., Cowan, A., Liu-Chen, L. Comparison of Pharmacological Activities of Three Distinct κ Ligands (Salvinorin A, TRK-820 and 3FLB) on κ Opioid Receptors In Vitro and Their Antipruritic and Antinociceptive Activities In Vivo. *The Journal of Pharmacology and Experimental Therapeutics*. **312**: 220-230 (2005).

81. Scheerer, J., Lawrence, J., Wang, G., Evans, D. Asymmetric Synthesis of Salvinorin A, A Potent κ-Opioid Receptor Agonist. *J Am Chem. Soc.* **129**: 8968-8969 (2007).

82. Zorn, N., Lett, R. Enol Triflates Derived from the Wieland-Miescher Ketone and an Analog Bearing an Angular Acetoxymethyl Group: Their Highly Regioselective Synthesis and Stille Coupling with Vinyl(tributyl)tin. *Tetrahedron Letters*. **47**: 4331-4335 (2006).

83. Vera-Portocarrero, L.P., Westlund, K.N. Attenuation of Nociception in a Model of Acute Pancreatitis by an NK-1 Antagonist. *Pharmacology Biochemistry and Behavior*. **77**: 631-640 (2004).

84. KO Studios Medical Illustrations. www.kostudios.com

85. Silverthorn, D.U. *Human Physiology: An Integrated Approach* 3rd edition. (2004).

86. Westlund, K.N. Chapter 3 Animal Models of Visceral Pain. *Animal Models of Pain*, Neuromethods, Chao Ma and Jun-Ming Zhang eds. **49**: 41-68 (2011).

Vita

Tori Strong was born in Houston, Texas on March 21, 1977. He majored in Biochemistry and received a Bachelor of Arts degree from Baylor University in Waco, Texas. After graduation Tori joined Dr. Kevin Pinney's lab, under the Pinney/OxiGene Inc. Grant, as a Research Technician conducting research involving the synthesis of tubulin binding ligands that served as tubulin depolymerizers as well as tumor vasculature targeting agents. Tori continued on to the University of Texas Medical Branch to study Pharmacology under the supervision of Dr. Scott Gilbertson where his researched focused on the synthesis and development of Salvinorin A as a motif for generating selective kappa opioid ligands. His dissertation demonstrates the synthesis of the precursor for developing Salvinorin A analogues.

Education

B.A., May 1999, Baylor University, Waco, Texas

Teaching Experience

1999-2001, Teaching Assistant, Baylor University, Waco, Texas

2004-2006, Biochemistry Instructor, UTMB Joint Admissions Medical Program, Galveston, Texas

Publication

Kevin G. Pinney, Zhi Chen, Vani P. Mocharla, Nandeo Choony and **Tori Strong**, Synthesis of a Benzo[b]thiophene-Based Vascular Targeting Prodrug and Related Anti-Tubulin Ligands, 220th American Chemical Society, National Meeting, Division of Organic Chemistry, Abstract No. 196, Washington, D.C., August 20-24, 2000.

Summary of Dissertation

Effective chronic pain therapy has posed a challenge for patients who have sought after traditional therapy, opioid analgesics. Particularly in patients with chronic pancreatitis, traditional therapy has failed to assist with pain management causing daily activity to be an even bigger challenge. Drugs like morphine or codeine, mu opioid agonists, have been utilized for pain management although these agonists have non-selective, opioid receptor subtype activity and side effects that ensue with chronic use that include constipation, addiction and respiratory depression. With the pancreas having a high density of kappa opioid receptors, a kappa subtype selective agonist could serve as a target molecule to develop as a pain therapeutic for patients with chronic pancreatitis. Where most alkaloids isolated over the years share characteristics like being plant derived, having similar structural functionalities and having cross affinity to opioid receptor subtypes, Salvinorin A **1** has emerged as a very unique alkaloid. Salvinorin A **1** is a non-nitrogenous alkaloid that selectively binds to the kappa opioid receptor subtype, implicating it as a potential target for development as a pain therapeutic for chronic pancreatitis as well as a motif for selective kappa opioid ligands. Synthesis of the Salvinorin motif is the basis of this body of work to not only build on the profile established in previous work but characterize through modifications that only synthesis can provide. This dissertation describes the synthesis of the key molecule **66** as the

pivotal molecule for generating analogues for the Salvinorin motif. Likewise, this work demonstrates the nociception response of Salvinorin A in the persistent pancreatitis model. Salvinorin A serves as a unique target for development both chemically and biologically; and this work establishes the foundation to bridge the gap between the two.

Permanent address: 2197 FM 1252 E, Kilgore, TX 75662

This dissertation was typed by Tori Marquis Strong.