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Stephen J. Cutler, Chair and Professor of Medicinal Chemistry

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Synthesis of Analogues Based on the Opioid Alkaloid Mitragynine

A Dissertation

Presented for the Doctor of Philosophy Degree

The University of Mississippi

Marco Arribas

DEDICATION

This work is dedicated to my beloved wife, Leila

and to my parents, Aurelio and Palmira Arribas.

ACKNOWLEDMENTS

I would like first to thank my professor, Dr. Christopher McCurdy, who has encouraged me for all these years, saying that I was capable of a such achievement. I thank also my committee members, Dr. Stephen Cutler, Dr. John Rimoldi and Dr. Samir Ross, who not only provided me with valuable insights in order to complete this work, but also made me think beyond. My special thanks goes to my colleague, Dr. Christophe Mesangeau, whose knowledge in chemistry was so important that this work would not be accomplished without him. Last, but not least, I would like to thank Dr. McCurdy's group, past and present, and my friends Robert, Velvet and Tammy. And for those who are not mentioned here, I apologize, but keep in mind that people from this and other departments played a crucial role in the completion of this work.

ABSTRACT

Opioid analgesics such as morphine and its derivatives are the most frequently prescribed narcotics for the treatment of severe and chronic pain. Among other side effects caused by the administration of these opioid analgesics, physical dependence and addiction are the most undesirable ones. Currently, pharmacological approaches to treat opioid dependence include mainly methadone and LAAM (μ-agonists), buprenorphine (μ-partial agonist) and clonidine (α2-adrenergic agonist). Considering the negative aspects of the relapsing nature correlated to opioid dependence, it is important to search for new ways to overcome this problem. The scope of this work is to study novel compounds based on the alkaloids present in *Mitragynina speciosa* (Korth), a tree indigenous to Southeast Asia. Consumption of leave extracts of this plant have been linked to the attenuation of the withdrawal syndrome associate with opioid dependence. Taking into consideration the structure of the major alkaloid, mitragynine, two classes of compounds were designed and synthesized: phenylpiperidines, including phenylaminopiperidines and phenylamidopiperidines, and tetrahydro-β-carbolines. They were further submitted to *in vitro* evaluation on μ-, δ- and κ-opioid receptors for their binding affinities. Out of six piperidines submitted to biological evaluation, four revealed significant affinity with *Ki* values in the micromolar range on either μ- and δ-receptors or κ-opioid receptors. On the other hand, out of five tetrahydro-β-carbolines tested, two showed κ-receptors affinities in the nanomolar range. Although none of the compounds displayed binding affinities as high as mitragynine, it is possible to say that distinct moieties attached to the piperidines had different selectivities among μ-, δ- and κ-opioid

receptors. Tetrahydro-β-carbolines, which mimics the four-ringed structure of mitragynine, showed only relevant affinity towards κ-receptors. The important factor for the higher affinity of tetrahydro-β-carbolines, when compared to piperidines, is that their less flexible structure is responsible for their greater affinity. More specifically, the methyl acetyl moiety at the C15 position had higher affinity than the methyl methoxyacrylate on κ-receptors. Unfortunately, this work did not focus on stereoselective syntheses or chiral separations, which in the case of the chiral tetrahydro-β-carbolines could have given valuable insights about the spatial requirements for affinity among the three main opioid receptors.

Table of contents

List of figures

List of tables

List of schemes

1. Pain

The perception of pain is an intrinsic part of life that is only perceived during consciousness. Although uncomfortable to the individual, it is essential to protect the body from harm. Hereditary insensitivity to pain, a rare syndrome, can lead to multiple injuries such as biting ones tongue, burning or bone fractures without the person realizing them. It is evident that not only human beings are capable of perceiving pain, but other vertebrates can also experience it. Theories seeking explanations of the origin of pain have followed mankind. It is not difficult to explain the pain caused by an accidental injury; however pain sensation caused by an internal disease was not easy to understand. Ancient cultures such as in Egypt usually tried to explain the causes of pain that was not derived from an injury through the acts of gods and spirits of death[1]. Several medical treatises written in Greek between 430 and 380 BC known as the Hippocratic Collection gave attention to the causes of pain. There was some disagreement about the distinction of humors, but the School of Cos considered that pain was caused by an imbalance of four humors: phlegm, blood, yellow and black bile[2]. In the 17th and 18th centuries based on the discoveries of natural sciences, medicine and physics, physicians began to treat the parts of the human body as parts in a machine. One of the earliest concepts of modern physiology proposed by René Descartes (1596 - 1650) depicted a touch that produces a painful stimulus traveling from the peripheral endings all the way to the brain, where it brings about an image of the stimulus in the soul[1].

The development of modern physiology led not only to a better understanding of the mechanisms that cause pain, but also to pathological states, such as increased sensation of pain (hyperalgesia). Melzack and Wall proposed in the 1960's a gate control

theory of pain[3]. The cells in the substantia gelatinosa (a functional unit that extends the length of the spinal cord) connect with one another by small and long fibers, but do not project to other parts of the brain. Even in the absence of stimulus, the spinal cord is bombarded by incoming nerve impulses carried predominantly by small fibers. As the stimulus increase, more fibers are recruited to fire at a higher frequency, which produces a change in the ratio between long and small fibers. The inhibitory effect of the substantia gelatinosa is increased by the activity of long fibers and decreased by small fibers. A gentle pressure in the skin leads not only to the firing of T cells (cells that connect to other parts of the brain), but also close the presynaptic gate. If the stimulus is increased, the gate opens further and the output of the T cells rises. The perception of pain is thus marked by the actions of the T cells that interacts with the gate control system[3]. Although the gate control theory of pain is probably wrong in various details, the authors believed that their most important contribution was the emphasis on central nervous system (CNS) mechanisms instead of explaining pain exclusively in terms of peripheral factors[1].

From the periphery, painful stimuli trigger neurons to send signals to the dorsal horn ganglia in the spinal cord, forming along with the brainstem and thalamus, the spinothalamic tract. There are basically two types of connections: a direct spinothalamic system, which carries sensory signals right to the thalamus, and a more diffuse system called spinoreticulothalamic pathway that terminates in the brainstem and reticular nuclei[4]. The direct spinothalamic pathway ends in the thalamus and it is responsible for the discriminatory aspects of pain, such as location, intensity and nature. Cells in the more dispersed spinoreticulothalamic system are probably involved in the arousal for painful stimulus and reflex. The ascending pathway depicted above can be suppressed by

a descending analgesic pathway that is, in part, mediated in the spinal cord, rich in opioid receptors and endogenous opioid peptide agonists. Other areas that are involved in the suppression of nociception (perception of pain) include periaqueductal gray, medullary raphe and thalamus, which also contain large amounts of opioid receptors and endogenous opioid peptides[4,32]. A more detailed discussion of the effects of opioid receptors and endogenous opioid peptides will be given in subsequent sections.

Pain can be classified according to its nature: somatic pain and neuropathic pain. Somatic pain can be described as well-localized, when the location is easily pointed by the patient, and visceral pain, when pain is diffused, e.g. myocardial pain which is usually referred to the left arm and shoulder. Neuropathic pain arises from injury or changes in sensory pathways in the periphery or in more central structures that include exaggerated response to noxious stimulus (hyperalgesia) or touch (hyperesthesia), spontaneous burning or aching sensation (dysesthesia) and painful sensation from non-painful stimulus (allodynia)[4].

As old as attempts to explain the origins of pain are the methods to alleviate it. Egyptian papyri describe ceremonies and rituals as well as vomiting, sneezing and urinating as therapeutic strategies aimed to expel the bad spirits that caused pain. The Roman physician Galen in the second century recommended theriac, a mixture that included opium, saffron, cinnamon, rhubarb, pepper and ginger mixed with wine and honey[2]. In the Middle Ages ointments containing opium and mandrake as well as theriac were used to treat pain. Willow bark extracts have also been used since antiquity as source of salicylates for the treatment of pain caused by inflammation. In the 19th Century, the discovery of cocaine and its industrial production gave rise to a widespread

use of the drug as a local anesthetic and stimulant. Electricity produced by electric fish was used by Greeks and Romans for treatment of rheumatoid arthritis, headaches and other types of chronic pain. After the development of techniques to produce and accumulate electricity in the 18th and 19th centuries, a better understanding of the relationship between electricity and the neurophysiology of pain gave opportunities to modern explanations of electrotherapy to alleviate pain such as TENS (transcutaneous electric nerve stimulation), SCS (spinal cord stimulation) and DBS (deep brain stimulation)[1].

Different causes of pain require distinct remedies. Alleviation of mild and moderate pain can generally be achieved with non-narcotic analgesics such as aspirin, acetaminophen or ibuprofen. Nevertheless, one of the most efficacious ways to treat intense and chronic pain is the use of narcotic analgesics such as morphine and its derivatives[32].

2. Poppy, opium and morphine

The use of opium for its pain relief and euphoric properties has been known for centuries. Ancient Babylonians knew over 6000 years ago the psychological effects of the extracts of the poppy plant (*Papaver somniferum* L.) and related species of *Papaver* genera. Sumerian clay tablets dated about 3000 BC described the cultivation and opium extraction from poppy plants, which they called "joy plant". Since the third century BC opium has been used to assuage pain and promote sleep. Gastrointestinal problems such as dysentery and diarrhea were also targeted by opium[5]. The word opium is derived from "opos" and "opion", Greek names for juice and poppy juice respectively, whereas morphine comes from Morpheus, the Greek god of dreams and sleep. Opium found its way to Europe as a part of several formulations like laudanum, popularly useful for treating plague. Historically associated with China, where by the end of the 17th century 25 percent of the population was smoking opium, the plant was widely cultivated in India and smuggled into China. Although prohibited in China, in the 19th century British opium traders had the monopoly of transportation, which led to two wars between these nations. In order to control opium production, the International Opium Commission was created in 1909 and by 1924, sixty-two countries were participating in agreements to decrease opium production. Laws regulating importation, exportation, sales and distribution for medical and scientific purposes were passed afterwards by signatory countries of the League of Nations. Nowadays, international opium regulation is carried out by the International Narcotics Control Board of the United Nations, with India being the larger supplier of world demands. In the United States opium is considered a pharmaceutical necessity and it is designated as Schedule II by the Drug Enforcement Agency[6].

The dried latex exuded from immature poppy capsules is composed of about 12% morphine and other alkaloids such as codeine (0.7 - 5%) , thebaine (0.1 - 2.5%) (Figure 1), papaverine (0.5 - 1.5%) and noscapine (1 - 10%)[7], but some crops can produce morphine in concentrations as low as 0.03%. More than 40 alkaloids have been identified which are normally classified into 12 main groups: benzylisoquinolines, benzyltetrahydroisoquinolines, proaporphines, aporphines, promorphinanes, morphinanes, protopines, protoberberines, berberines, phtalideisoquinolines, rhoeadine/papaver-rubines and benzophenanthridines[8]. Morphine was first isolated in 1804 by Sertüner, a pharmacist in Germany, but its structure was first elucidated more than a century later in 1925[9]. Albeit the complicated pentacyclic skeleton of morphinanes, the first laboratory synthesis of morphine was achieved by Gates *et al.* in 1952[10]. Although a number of synthetic studies and total syntheses of have been reported to date[11], industrial scale production of morphine and its derivatives are not considered economically advantageous and therefore the plant is still the major source of opium alkaloids[7].

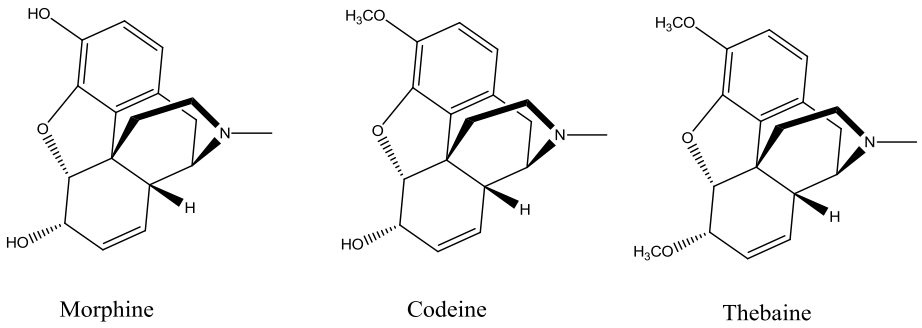


Figure 1 - Morphinan alkaloids.

Benzylisoquinoline alkaloids are a structurally diverse group of nitrogen-containing secondary metabolites comprised of approximately 2500 identified substances, including morphinan alkaloids[12]. Opium alkaloids are biosynthetically

derived from the aromatic amino acids phenylalanine, tyrosine and 3,4-dihydroxyphenylalanine, which in turn are obtained via the shikimic acid pathway in the plant[8] (Figure 2).

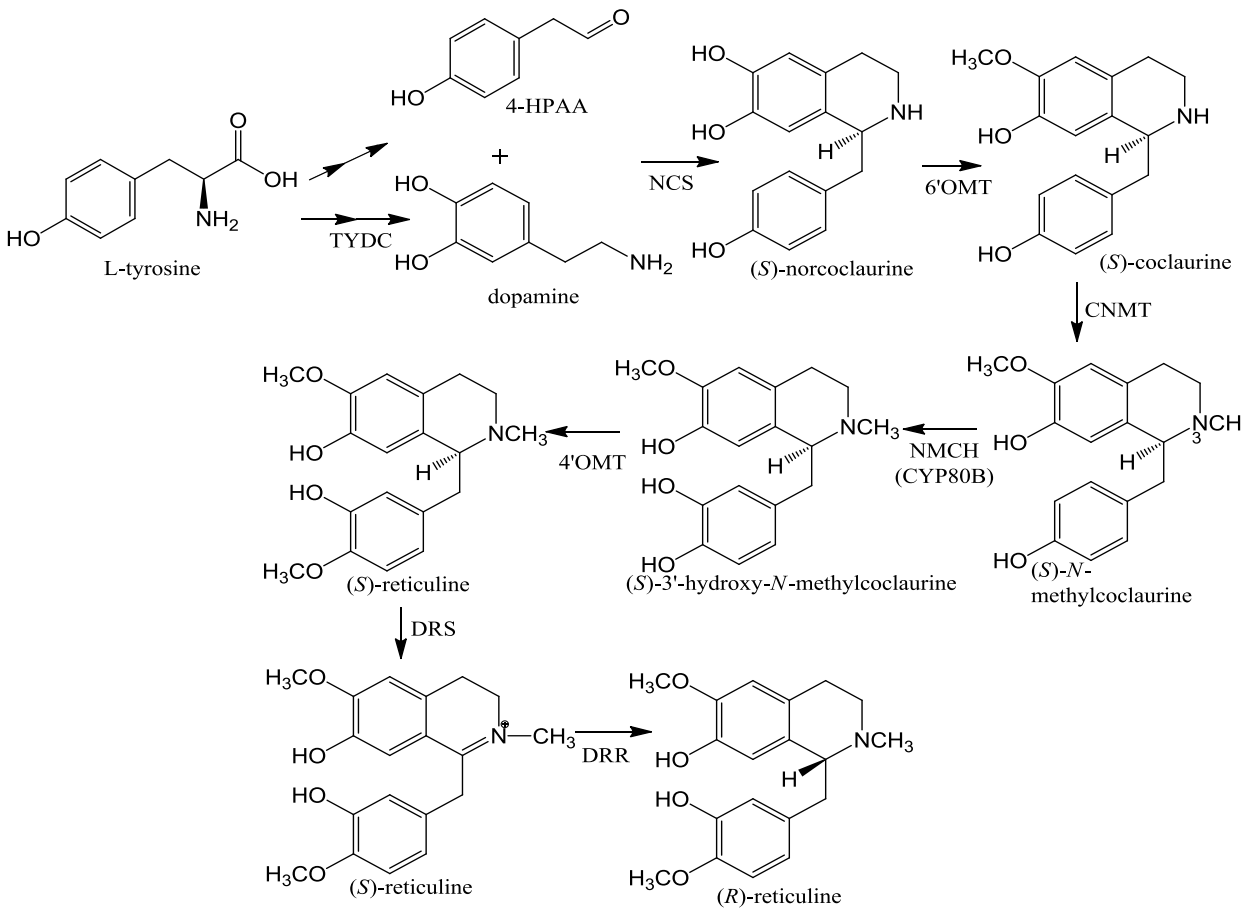


Figure 2 - Benzylisoquinoline alkaloid biosynthesis. 4-HPAA, *p*-hydroxyphenyl acetaldehyde; TYDC, tyrosine decarboxylase; NCS, (*S*)-norcoclaurine synthase; 6'OMT, (S)-norcoclaurine 6-O-methyltransferase; CNMT, (S)-coclaurine N-methyltransferase; NMCH, (*S*)-N-methylcoclaurine 3'-hydroxylase (CYP80B subfamily); 40OMT, (S)30-hydroxy N-methylcoclaurine 4'-O-methyltransferase; DRS, 1,2-dehydroreticuline synthase; DRR, 1,2-dehydroreticuline reductase. (Adapted from ref. 12)

Despite the fact that poppy-derived opiates are major drugs of abuse with an illicit market totaling around 4.8 million kilograms annually[7], production of morphine derivatives is only economically feasible by obtaining morphinan compounds through

plant cultivation. The majority of morphine naturally obtained from the poppy plant is used in the production of codeine, which is present in the opium extract in low quantities and is medically employed as a mild analgesic and cough suppressant. However, simple *O,O*-diacetylation of morphine affords the highly addictive and illegal heroin. Although illicit poppy production might be difficult, if not impossible to eradicate, genetically engineered or mutagenized plants that give low morphine levels and are rich in codeine, thebaine and oripavine content might be useful to circumvent the problem of heroin manufacturing. Indeed, thebaine and oripavine are the starting materials for the synthesis of valuable and powerful drugs in the market such as oxycodone (OxyContin), buprenorphine (Subutex), naloxone (Narcan) and natrexone (ReVia)[13]. One of the key enzymes necessary to biosynthesize morphinan alkaloids is salutaridine reductase (SalR), which is a member of a class of short chain dehydrogenase/reductases overexpressed in *Papaver* species (Figure 3)[12]. Studies have demonstrated that gene knockout of codeinone reductase (COR1 - enzyme that converts condeinone to codeine and morphinone to morphine) may be used for metabolic engineering of the opium poppy, circumventing the biosynthesis of morphine and codeine, but maintaining the levels of other useful morphinanes such like thebaine[7].

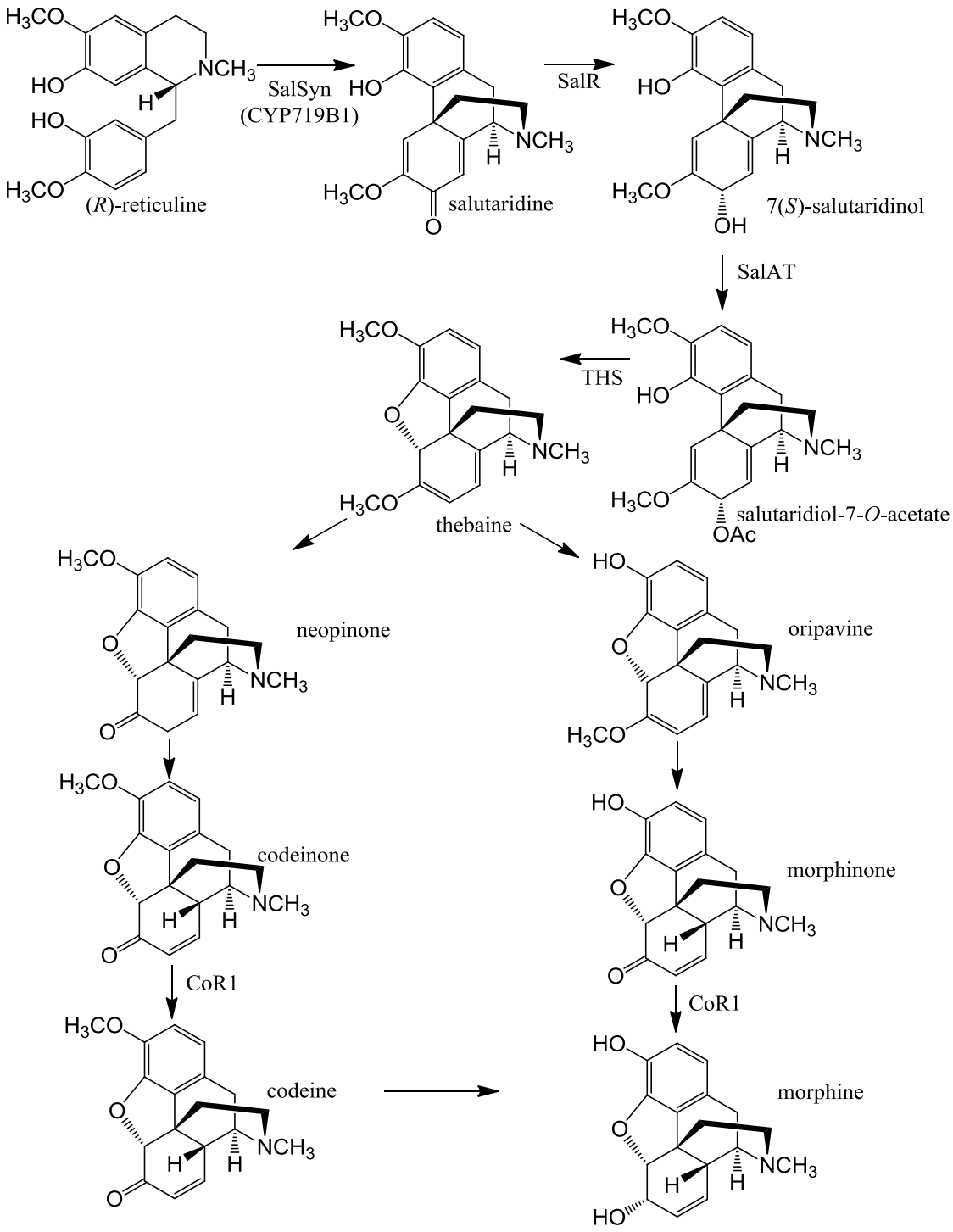


Figure 3 - Morphinan alkaloids biosynthesis. SalSyn, salutaridine synthase; SalR, salutaridine reductase; SalAT, 7(S)-salutaridinol 7-O-acetyltransferase; THS, thebaine synthase; CoR1, codeinone reductase 1 (Adapted from ref. 12).

3. Opioid receptors and their actions

The concept that drugs, hormones and neurotransmitters perform their functions by binding to highly specific sites called receptors was developed in the twentieth century. Specific receptors that mediate opioid actions were postulated based on the stereospecificity of analgesic compounds and on the observation that minor structural changes resulted in substances that can antagonize the action of opioids. By the 1970's, the first endogenous opioid peptides were isolated and sequenced. These peptides were named enkephalins, dynorphins, β-neoendorphin, β-endorphin, dermophins and deltorphins. In 1973, Simon *et al.* described the highly specific binding of radiolabeled etorphine (an opioid with a molar potency of 3200 greater than morphine[14]) in rat brain homogenate supporting the existence of opioid receptors[15]. In same year, other groups corroborated independently these findings in mammalian brain (Pert and Snyder; Terenius)[18]. The concept of multiple opioid receptors rose after observations of the actions of opioid agonists, antagonists and mixed agonist-antagonists. Neurophysiologic observations in the dog spinal cord led to the proposal of three opioid receptors, named in Greek letters after the drugs used in the studies: μ (for morphine, which induces analgesia, miosis, bradycardia, hypothermia), κ (for ketocyclazocine, which causes miosis, general sedation, depression of flexor reflexes) and σ (for SKF 10,047 or *N*-allylnormetazocine, which induces mydriasis, increased respiration, tachycardia and delirium). A fourth type of opioid receptor was proposed after observing the effects of opioid peptides in the mouse vas deferens and named δ (for deferens). After observations that the σ-receptor is non-opioid in nature, there are thus three main types of pharmacologically defined receptors: μ, δ and κ. Molecular biology approaches have also

confirmed their existence mid-1990's by cloning, with binding and functional properties compatible with their identities. In addition to the well-established three types of opioid receptors, an orphan opioid receptor-like (ORL1) receptor was cloned. Although the use of Greek letters is generally accepted by pharmacologists, molecular biologists usually employ DOR, KOR, MOR, meaning delta, kappa and mu opioid receptor respectively. To complicate the matter, the International Union of Pharmacology has proposed that the receptors should be numbered after the chronological order of their cloning and sequencing: OP_1, OP_2 and OP_3 for δ-, κ- and μ-receptor respectively[17]. The three classes of receptors can be further subdivided into their subtypes and their analgesic action localization. Spinal and supraspinal antinociception is mediated by δ_2 and κ_1; spinal analgesia is produced by activation of μ_2; supraspinal analgesia is caused by μ_1 and κ_3[16].

Opioid receptors are not only present in the central nervous system, but also at the periphery: preparations of isolated guinea pig ileum and the vas deferens from mouse, rat, rabbit and hamster are routinely used for pharmacological assays of opioid receptors[17]. In the central nervous system, there are regional variations: binding of [^{3}H]naloxone (a compound that blocks opioid action) is almost negligible in the cerebellum, but it is very high in the corpus striatum. The dissection of monkey brain revealed even more drastic variations that could explain the pharmacological actions of opioids. Receptors are present in the periaqueductal gray, where electrical stimulation produces analgesia that is antagonized by naloxone. The density of receptors in the medial thalamus, which takes the 'emotional' components of pain to the cerebral cortex, is almost four times higher than in the lateral thalamus, which conveys the 'pin prick' pain sensation that is not influenced by opiates[18].

Opioid receptors belong to the large family of rhodopsin-like G-protein (guanine nucleotide binding protein) coupled receptors (GPCR), with an overall amino acid sequence identity of 60% for all three types of receptors. GPCRs are the most widespread of several family of receptors, controlling virtually all known physiological functions in mammals[19]. It is estimated that half of the drugs in the market act on GPCRs. Interesting noting is that this receptor superfamily can bind to a broad range of substances, including monoamines, nucleotides, amino acids, peptides, proteins and pherormones. This class of receptors are also referred to as seven-transmembrane receptors (7TM), since their amino acid sequence crosses the membrane seven times to make the connection between the intracellular and extracellular medium[20]. The odd number of transmembrane spanning domains place the N-terminal and C-terminal portions of the receptor protein on opposite sides of the membrane, allowing ligand binding and glycosylation at the extracellular N-terminal and phosphorylation and palmitoylation at the intracellular C-terminal for desensitization and internalization. The versatility of functions may be explained by the fact that the seven transmembranes form six loops, offering the core sufficient size for contact sites, specificities and regulatory mechanisms[21]. Upon ligand binding to the receptor, the signal is transduced by guanine nucleotide binding proteins (G-proteins) that are coupled to the receptor. The G-protein is a trimeric protein, consisting of α-, β- and γ-subunits. Conformational changes caused by ligand binding catalyzes the hydrolyzation of GTP to GDP in the α-subunit, which leads to its dissociation from the β- and γ-subunits. This process modulates downstream effectors such as adenylate cyclase inhibition that occur during opioid activity[22]. Other common actions include activation of a potassium conductance, inhibition of calcium conductance and inhibition of

neurotransmitter release (Figure 4). More recent observations also include activation of protein kinase C, release of calcium from extracellular stores and activation of the mitogen-activated kinase cascade[23]. All three types of opioid receptors conserve sequence similarity in rat mainly in the TM2 (transmembrane 2), TM3, TM7, the first extracellular loop, the second and third as well as the fourth intracellular loops, whereas TM1, TM4, TM5, TM6, the second and third extracellular loops are less conserved[24]. An interesting property of GPCRs is that they can combine to form new functional structures such as homodimers (two receptors of the same type) and heterodimers (two receptors of different types). It has been observed that heterodimerization of κ-δ-opioid receptors modulates their function in a way that is distinct from the properties of single receptors. This heterodimer has decreased affinities for their selective ligands, however synergistic functional responses can be elicited by selective agonists acting cooperatively[25].

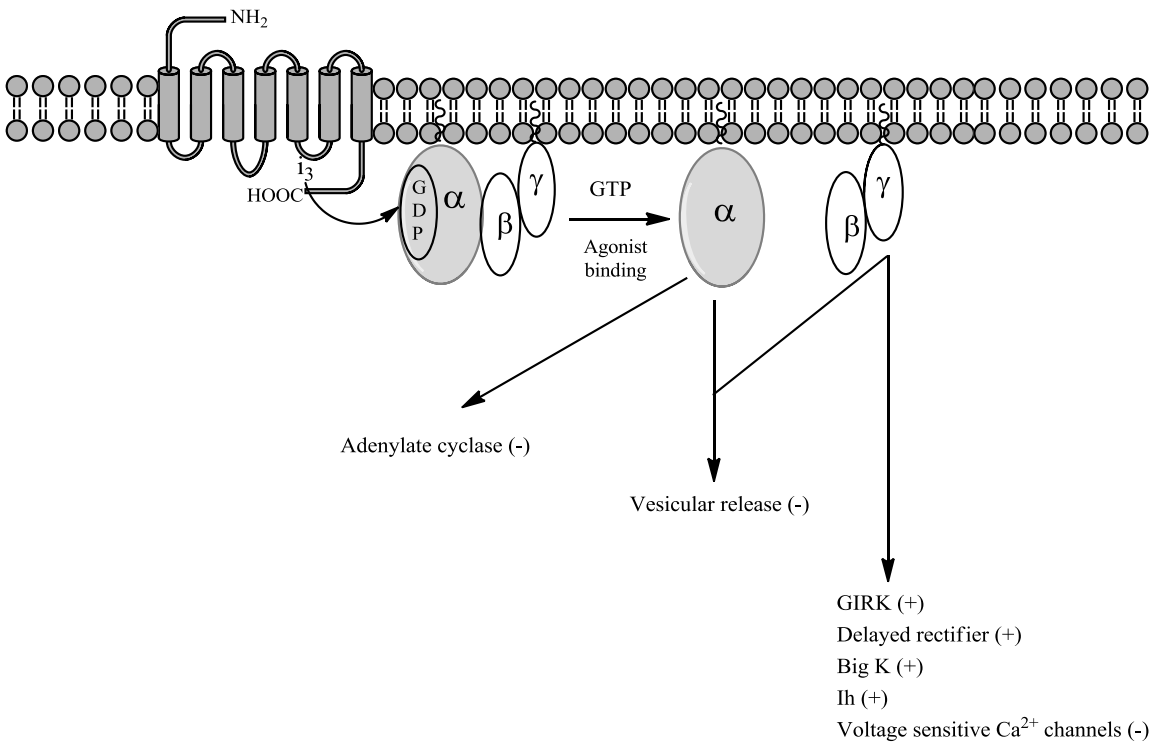


Figure 4 - Illustration of the effects that occur after opioid activation. Three primary classes of effectors include inhibition of adenylyl cyclase, inhibition of vesicular release and interactions of different ion channels (adapted from reference 23).

Most opioids have moderate selectivity among different types of opioid receptors, eliciting similar pharmacological responses upon their stimulation. Nonetheless, in the past 20 years, techniques such as receptor cloning, knockout animals and antisense models have helped to investigate their actions separately. Generally, activation of all three major classes of opioid receptors produces analgesia, but other agonist actions vary depending on the receptor type. Euphoria, slow gastrointestinal motility, respiratory depression (in volume), immune suppression and emesis are related to activation of μ-opioid receptors, whereas activation of δ-opioid receptors brings about respiratory depression (rate) and immune stimulation. While μ-opioid receptor stimulation is rewarding, inducing euphoria, the effects upon κ-opioid receptors are sometimes the

opposite. Indeed, besides producing sedation, miosis and diuresis, κ-agonists cause dysphoria and avoidance in animals and humans[32,34]. Increased food intake is also affected by administration of μ-, δ- and κ-opioid receptors agonists, whereas antagonists such as naltrexone and naloxone are able to diminish feeding response[26]. Other important actions of opioids include antitussive properties as well as undesirable tolerance and dependency.

4. Opioid ligands

Opioid ligands, whether they elicit or block pharmacological action, can be basically divided into three groups. The first group contains natural products and semi-synthetic derivatives such as morphine and heroin respectively. Totally synthetic compounds are part of the second group, which may have little or no resemblance to the natural and semi-synthetic structures. The last group comprises naturally occurring (or endogenous) and synthetic peptides[36].

4.1. Non-peptide ligands

The first and second group of opioids can be aggregated into the non-peptide opioids. The prototypical opioid agent is the natural product morphine, from which most of the clinically available opioid analgesics are derived by either semi-synthesis or simplification of the natural product template. The first synthetic opioid obtained was the heroin (3,6-diacetylmorphine) in 1874 through semi-synthesis from morphine. Heroin was one of the first examples of a prodrug aimed to reduce the inconveniences of respiratory depression and dependency of morphine, but it soon became apparent that these claims were unfounded. Synthetic opioids can be broadly grouped into 4,5α-epoxymorphinans, morphinans, benzomorphinans, phenylpiperidines and phenylpropylamines. The progressive simplification from the morphine scaffold to morphinans and benzomorphinans as well as phenylpiperidines to phenylpropylamines illustrates how other classes of opioids were obtained (Figure 5)[27].

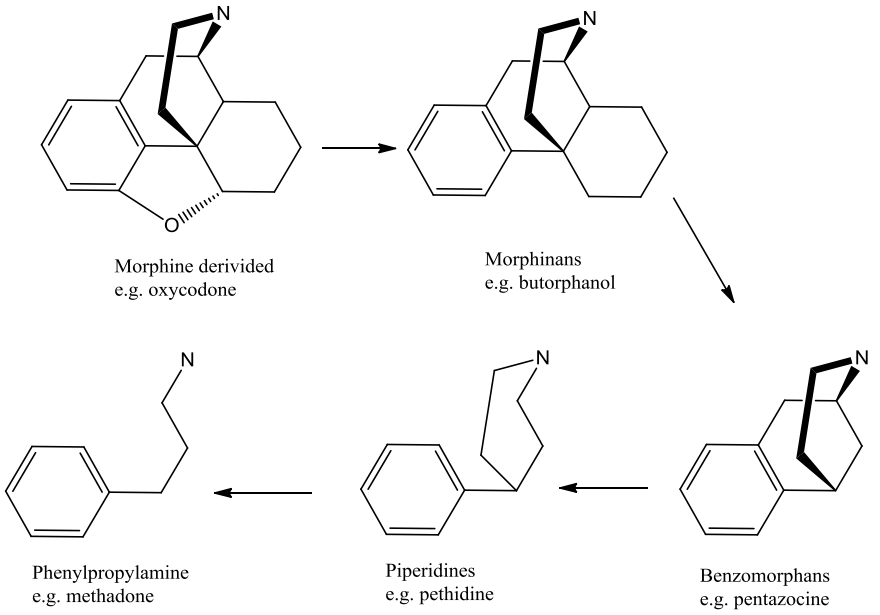


Figure 5 - Illustration of the progressive simplification of morphine-related drugs (Adapted from reference 27)

It is beyond the scope of this work to summarize structure-activity of all classes of opioid analgesics. However the basic requirements for opioid activity of morphine and its derivatives proposed by Beckett and Casy can be briefly described in Figure 6. At physiological pH, the nitrogen atom is protonated and binds to the receptor anionic site. The rigid piperidine ring is accommodated in the cavity on the receptor binding site, whereas the phenolic ring adhere to the flat surface of the binding site. On the other hand, the synthetic (+)-morphine is devoid of opioid activity since it cannot bind to the receptor in the same way the natural (-)-morphine does[32,36].

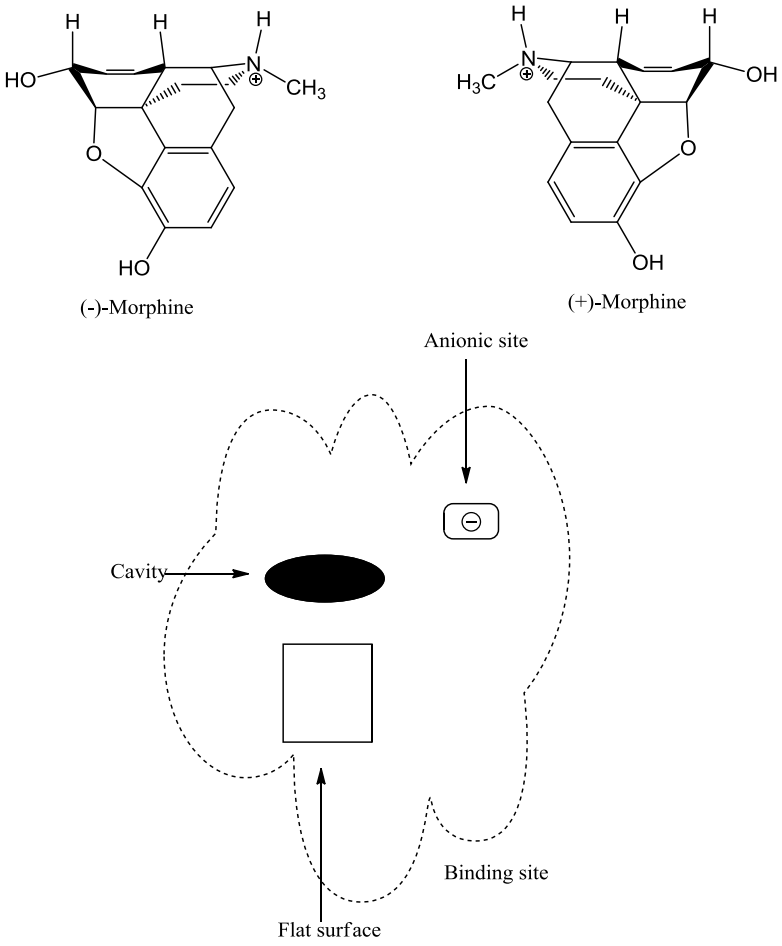


Figure 6 - The opioid binding site model. (Adapted from references 32 and 36)

Since the synthesis of heroin, the first semi-synthetic opioid, numerous structural modifications have been made to several portions of the morphine molecule. However, alterations have been concentrated in three regions: the phenol at position 3, the C ring and the nitrogen (Figure 7). Changes in the phenolic hydroxyl usually decrease opioid activity. Either methylation (codeine) or acetylation (heroin) diminishes opioid receptor activity; however codeine retains around 10% of morphine potency due to *in vivo* demethylation to morphine, whereas heroin is twice as potent as morphine since its fast penetration into the blood-brain barrier and further hydrolysis to morphine. Oxidation or etherification at the 6-position as well as saturation of the C ring (7-8 position) increase

activity. Finally, demethylation of the basic nitrogen decreases activity, while introduction of bulky groups at this position leads to antagonists[28].

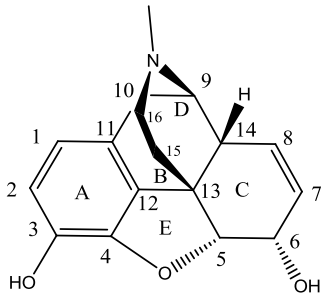


Figure 7 - Structure of morphine**.**

The basic three-point requirements for opioid binding as well as a brief structure-active relationship of the morphine analogues have been previously mentioned. Another important aspect worth to be brought up is how different compounds bind preferably to distinct opioid receptor types. While common structural features of compounds interact with conserved residues among the three main classes of opioid receptors, uncommon moieties interact with amino acid residues that are dissimilar. This can be explained by the "message-address" concept, which in a nutshell states that the common portion of ligands represents the "message", whereas the variable moiety acts as the "address", thus conferring selectivity. This concept can be illustrated by comparing the high affinity nonspecific opioid antagonist naltrexone against the κ-selective 5-guanidinylnaltrindole (GNTI) and the δ-selective naltrindole (NTI) in Figure 8. In the case of nonspecific opioids naltrexone and naloxone, both satisfy the 3-point requirement model (Figure 6) "message", but the "address" locus is lacking. The obvious difference regarding the δ-selective opioids naltrindole and 7-spiroindanyloxmorphone (SIOM) is the presence of a hydrophobic group, indole and spiroindane respectively, which acts as the "address" portion, therefore giving binding selectivity. Addition of a second basic moiety in the

"address" site of 5-guanidinylnaltrindole (GNTI) and norbinaltorphimine (nor-BNI) provides selectivity toward κ receptors, implicating the formation of an ionic bond with a glutamate residue (Glu VI:23) unique to this receptor. Unfortunately, the message-address concept cannot be verified with μ-selective compounds such as morphine and the irreversible antagonist β-funaltrexamine (β-FNA) because they lack a common "address" site[29].

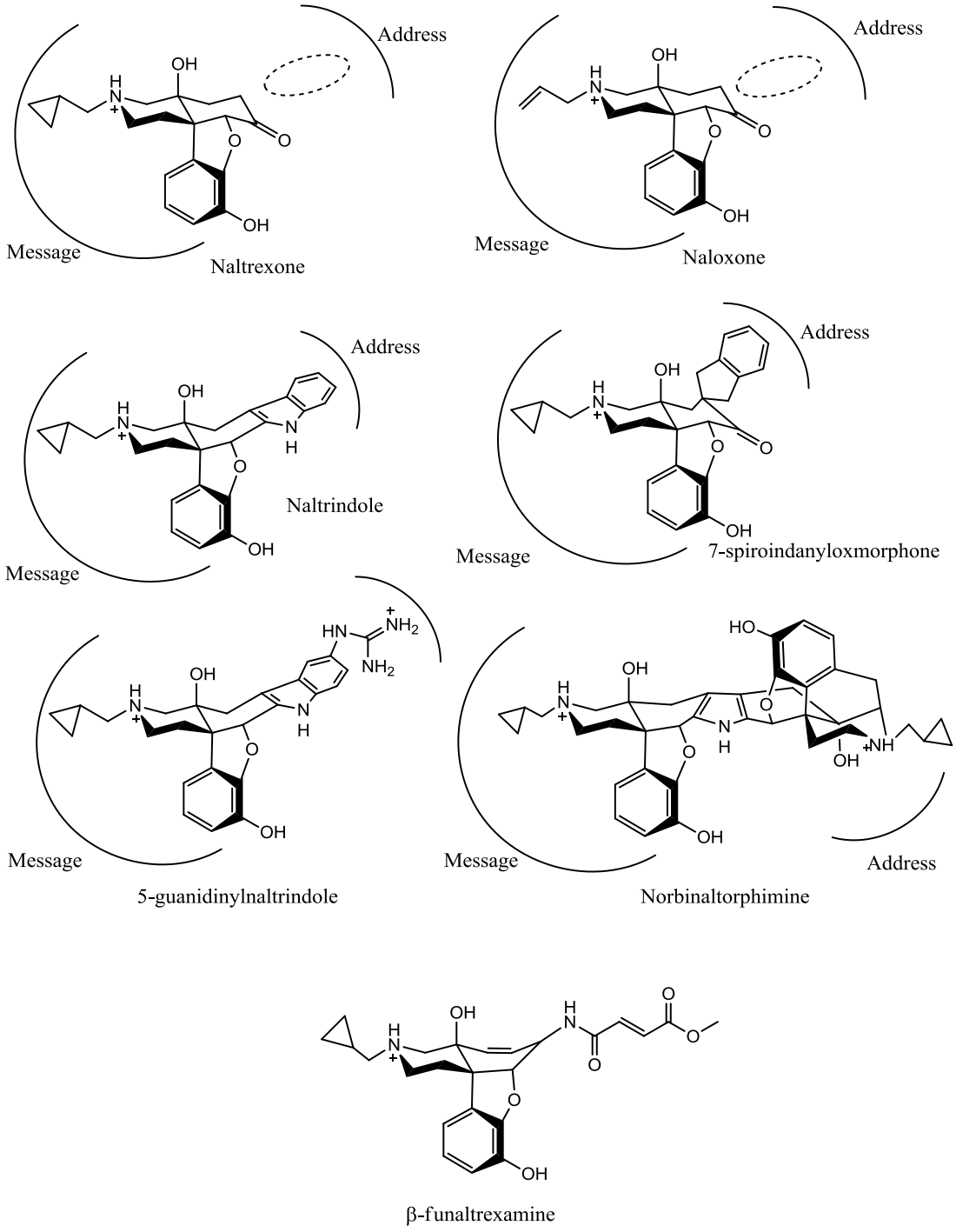


Figure 8 - Message-address moieties of nonspecific (naltrexone and naloxone), δ-selective (NTI and SIOM), κ-selective (GNTI and nor-BNI) and μ-selective (β-FNA) opioids.

Pain is the major reason for a patient to seek medical advice, hence analgesics represent a huge therapeutic market of over $70 billion in the United States. Opioid sales in 2007 were $8.4 billion in the US, which constitutes 70% of the global market for this drug class. Sales of opioids have been growing 13% on average since 2001[30]. There are about 20 opioid drugs available on the US market that are sold with various trade names and routes of administration, ranging from patches to intravenous injections. Besides the naturally occurring morphine and the cough suppressant codeine, commercially available drugs that represent the five main classes (Figure 5) of non-peptide opioids are depicted in Figure 9[31].

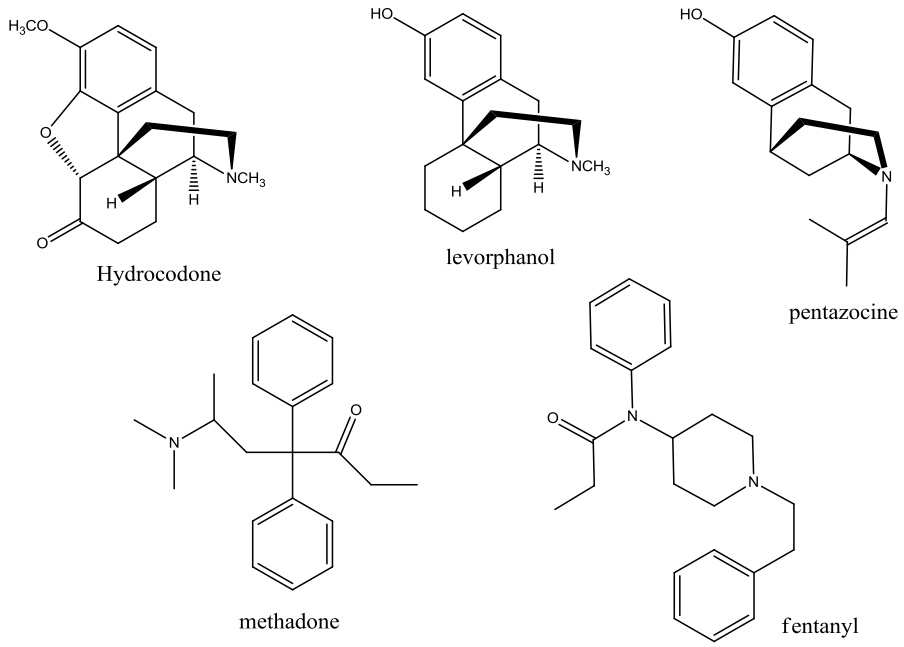


Figure 9 - Commercially available analgesics representing the five main opioid classes.

4.2. Peptide ligands

Scientists had postulated that morphine and other synthetic opioids were not the natural ligands for opioid receptors and that other analgesic substances must exist in the

brain. In the mid-1970's, the first endogenous opioid peptides were discovered and collectively named as endorphins, which is a combination of the words endogenous and morphine. Endogenous opioid peptides are produced from parts of large precursor proteins and the three major types of opioid peptides have their own precursor protein. Proopoimelanocortin is the precursor for β-endorphin, whereas proenkephalin A is the precursor for met-enkephalin and leu-enkephalin. The pro-opioid peptide proenkephalin B is the precursor for dynorphin and α-neoendorphin. All pro-opioid peptides are synthesized in the cell nucleus and transported to the nerve terminals where they are released. Proteases hydrolyze the pro-opioid peptides into their active form by recognition of a double basic amino acid before and after the opioid peptide sequences[32]. The analgesic action of peptides is short, since they are rapidly degraded by several peptidases such as aminopeptidases and dipeptidyl peptidases[33]. Although selectivity towards distinct receptor types is generally considered weak, peptides in the enkephalin and β-endorphin groups bind preferably to μ- and δ-receptors, whereas dynorphin is more selective towards κ-receptors[34]. The precursors, amino acid sequences and affinities of these endogenous peptides are shown in Table 1.

Table 1. Mammalian endogenous ligands of opioid receptors[35]

Precursor	Endogenous peptide	Amino acid sequence	Affinity
Pro-enkephalin	[Met]-enkephalin [Leu]-enkephalin Metorphamide	Tyr-Gly-Gly-Phe-Met Tyr-Gly-Gly-Phe-Leu Tyr-Gly-Gly-Phe-Met-Arg-Phe Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-NH2	δ, μ (δ>> μ)
Pro-opiomelanocortin	β-endorphin	Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu	μ= δ
Pro-dynorphin	Dynorphin A Dynorphin A(1-8) Dynorphin B α-neoendorphin β-neoendorphin [Leu]-enkephalin	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Gln-Phe-Lys-Val-Val-Thr Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro Tyr-Gly-Gly-Phe-Leu	κ, μ, δ (κ>> μ, δ)
Unknown	Endomorphin-1 Endomorphin-2	Tyr-Pro-Trp-Phe-NH2 Tyr-Pro-Phe-Phe-NH2	μ

Although opioid peptides never became marketed drugs due to their poor pharmacokinetic properties (very short half life and inability to cross blood-brain barrier), several strategies to improve stability have been attempted such as incorporation of unnatural D-amino acids, methylated amino acids and conversion of the carboxylic acid terminal to amide as well reduction to its alcohol derivatives. Inclusion of β-amino acids and cyclic peptides as well as formation of peptide dimers and oligomers have also been tried. While *in vivo* stability of opioid peptides is the concerning issue, peptide analogues have been widely employed as pharmacological tools in opioid receptor research. For example, radiolabeled DAMGO (Tyr-D-Ala-Gly-*N*-MePhe-Gly-OH) is used as high affinity μ-agonist in binding studies, whereas the cyclic pentapeptide DPDPE (Tyr-D-Penicillamine-Gly-Phe-D-Penicillamine [2,5-dissulfide bridge]) is considered the prototypical δ-selective opioid peptide[36].

5. Opioid tolerance and dependence

In addition to respiratory depression, the most serious adverse effect of opioid agonists administration is the development of tolerance and dependence. For a long time, it was hypothesized that repeated administration of certain drugs can provoke long-lasting changes in the brain, leading to abuse. In order to investigate the neurophysiologic causes of addiction, the National Institute on Drug Abuse (NIDA) was created in 1974, which then became part of National Institutes of Health (NIH) in 1992[37]. Tolerance refers to a state of diminished responsiveness to a drug, whereas physical dependence arises from the cessation of drug administration that leads to withdrawal syndrome; administration of the drug is therefore necessary in order to reverse these effects[38]. Besides being a devastating disease that can lead to many personal problems and death, addiction also puts a huge burden on public health. The American Psychiatric Association defines substance abuse in the *Diagnostics and Statistical Manual of Mental Disorders* (DSM-IV) as: "[A] maladaptive pattern of substance use with physiological addiction, impaired control of substance taking, and/or adverse consequences (e.g., problems in social or occupational functioning)."[39].

The potential factors connected to addiction or abuse are related to dose, route of administration, co-administration with other drugs, context of use and expectations. Particularly, pain relievers are the most abused prescribed drugs, increasing from 628,000 initiates in 1990 to 2.4 initiates in 2001[40]. The psychopharmacological elements underlying drug-seeking behavior are as complicated as shown in Figure 10.

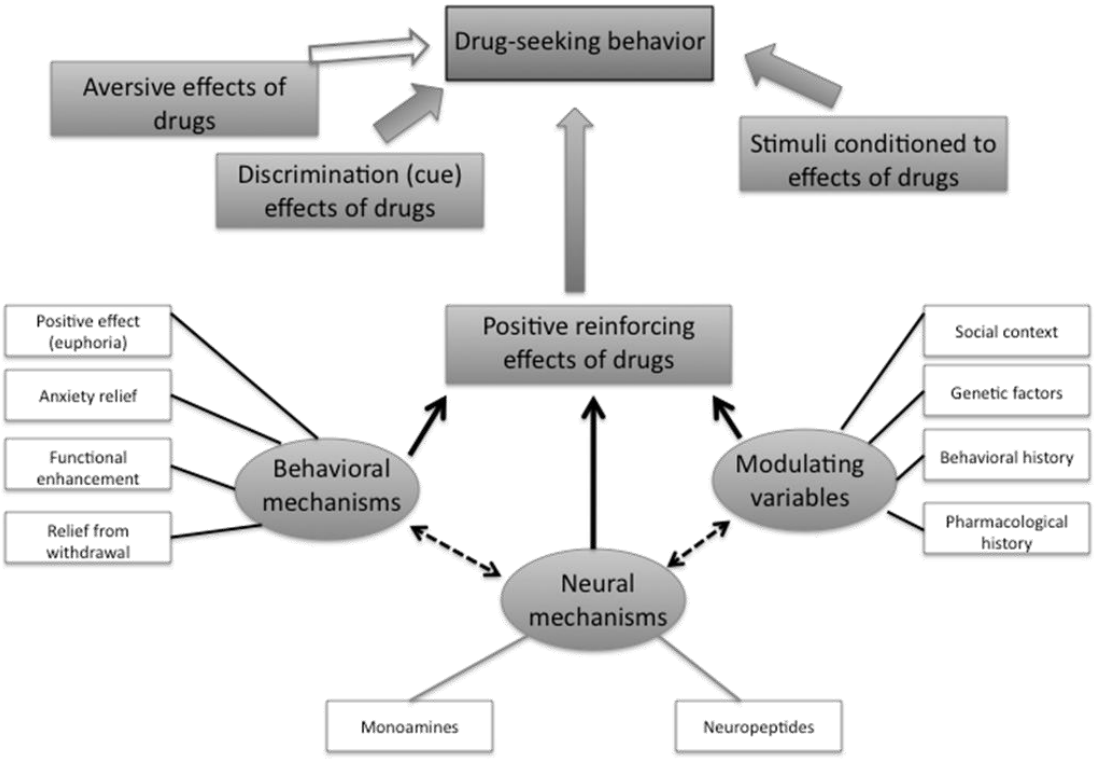


Figure 10 - Factors that influence the psychopharmacological effects of drug abuse.

5.1. Molecular mechanisms of tolerance and dependence

In spite of the fact that almost 40 years have passed since the creation of NIDA, the mechanism of addiction is not yet fully understood, though enormous progress has been achieved. At the biochemical level, the binding to opioid receptors brings about the inhibition of adenylate cyclase, the enzyme responsible for the conversion of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). Acting as a second messenger, cAMP carries the signal from extracellular binding to intracellular effectors. Upon repetitive exposure, the late compensatory increase of adenylate cyclase counteracts opioid inhibition and rises far above the normal levels. Adenylate cyclase induction is responsible for opioid tolerance and withdrawal symptoms that lead dependence[41]. An important consequence of adenylate cyclase up-regulation is the

increase of cAMP-dependent protein kinase (PKA) responsible for the phosphorylation of cAMP response element binding protein (CREB). Functioning as a transcription factor, CREB alters gene expression that regulates numerous cellular processes involved in tolerance and dependence[42]. It is also worth mentioning that a cascade of events upon receptor activation promotes receptor desensitization and endocytosis. Following endocytosis, receptors can be recycled and sent back to the membrane, encapsulated in a vesicle or degraded. For recycled receptors, like μ-opioid receptors, endocytosis is the first step toward resensitization. Tolerance and dependence of chronic morphine treatment come from the fact that this opioid receptor does not induce endocytosis, thus no resensitization takes place[43].

5.2. Neuronal mechanisms of addiction

Basically, there are four brain circuits implicated in addiction: reward, motivation, memory and control. They are linked to the different concepts: reward, internal state (motivation/drive), learned associations (memory) and conflict resolution (control). In the addicted brain, the inhibitory control of the prefrontal cortex is overwhelmed by the augmented value of the drug in the reward, drive and memory circuits. The lack of control participation favors a positive feedback among the three remaining circuits, perpetuating drug consumption (Figure 11)[44].

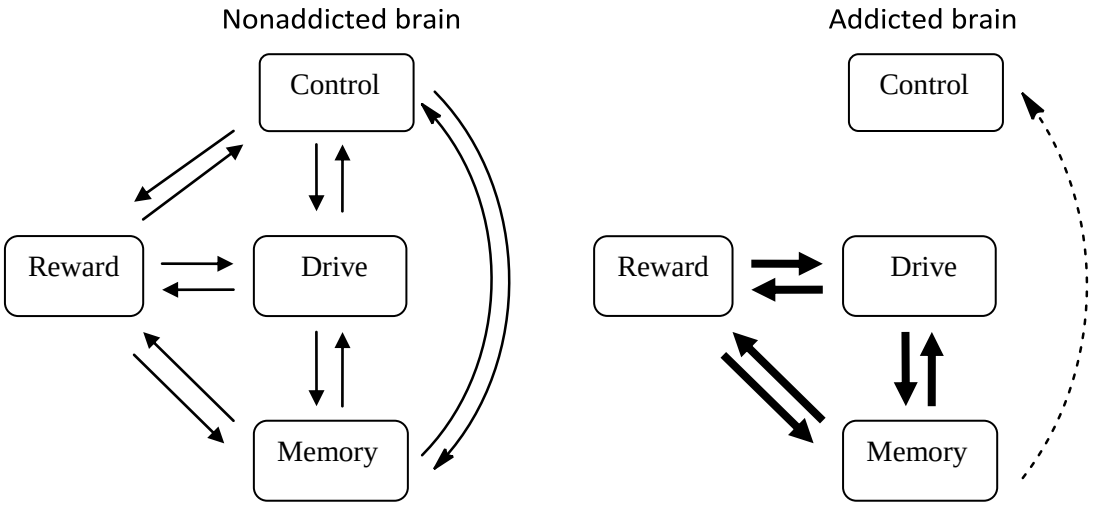


Figure 11 - Proposed model of circuits involved with addiction in nonaddicted and addicted brain[44].

Functional changes in the mesolimbic dopaminergic (DA) neurons are caused by abrupt interruption of opioid administration, which leads to aversive effects like dysphoria and anhedonia; therefore, for the addict, the urge to administer the drug again is necessary to avoid these symptoms. Chronic use of opioids modifies the activity of the nucleus accumbens (NAc), which is targeted by DA neurons and give feelings of pleasure. Neurons from NAc project to the ventral pallidum and the ventral tegmental area (VTA) and contain GABA, opioid peptides enkephalin and dynorphin as well as substance P. It is thought that alterations in these neurons play a role in the negative effects of opioid withdrawal. There are two parts in the NAc: the core may be involved in drug-seeking behavior, while the shell is related to psychostimulant effects of drugs of abuse. Other structure connected to the VTA is the prefrontal cortex, which is responsible for behavioral control. The main neural effect of drugs of abuse is the stimulation of dopaminergic neurons in the VTA that releases dopamine in the NAc, contributing to the

reward response. Besides the NAc and VTA, structures that contain opioid peptides and may be responsible for opioid reward are: locus coerelus, amygdala, substantia nigra, periaqueductal gray and arcuate nucleus[37,42,45].

5.3. Treatment of opioid dependence

The first step in dependence treatment demands identification of the opioid abuser, whether the patient is a street drug user or taking a prescribed opioid. In 2008, there were a total of 1,132 facilities offering opioid treatment programs[46]. Treatment options include counseling and pharmacologic therapies such as maintenance with opioid agonists (e.g. methadone) as well as detoxification with opioid antagonists (e.g. naltrexone and naloxone)[47]. Most medications used to treat opioid addiction interact with μ-receptors, like the abused opioids themselves, but their actions last longer. Methadone and L-alpha acetylmethadol (LAAM) are full μ-agonists with different durations of action, whereas buprenorphine is a partial agonist[48]. Non-opioid therapies include clonidine, an α_2-adrenergic agonist that relieves up to 85% of withdrawal symptoms[49]. Another non-narcotic α_2-adrenergic agonist closely related to clonidine that is currently in phase III clinical trials is lofexidine[50,51]. Along with psychosocial interventions, there are basically two pharmacological approaches used to treat opioid addiction: detoxification and maintenance.

Although not life threatening, abrupt termination of opioid use leads to almost unbearable withdrawal symptoms, including chills, sweating, diarrhea, nausea, anxiety, irritability and insomnia. In order to reduce these symptoms, clonidine and other α_2-adrenergic agonists are employed with fewer side effects than methadone and patient participation, though clonidine has been linked to sedation and hypotension[52]. Besides

being used to avoid death by overdose, opioid antagonists (naloxone and naltrexone) can also be utilized along with deep sedation or anesthesia for rapid recovering from withdrawal side effects[53]. Another method of detoxification is called tapering, which is the process of substituting the abused opioid for another agonist (methadone or buprenorphine) and then slowly decrease the dose[52]. Methadone is the oldest drug used for tapering and it still considered the drug of choice (frequently in conjunction with buprenorphine) in most opioid addiction treatment programs[46].

The main objectives of maintenance treatments are to decrease craving and undesired withdrawal symptoms as well as avoid euphoria by carefully dosing the opioid agonist, therefore reducing drug-seeking behavior linked to overdose risk, HIV infection and criminal activity. Despite the fact that individuals undergoing opioid maintenance treatments are still physically addicted, these programs are aimed to reduce drug-related activities and allow patients to transition into drug-free programs[52]. Since the 1960's, methadone has been used for maintenance treatment and it is the most well studied, first line drug for these therapy. However, LAAM and buprenorphine have two to three times the duration of methadone and they can be administered three times a week. The longer duration, besides providing fewer plasma level fluctuations between doses, may be advantageous over methadone in maintenance treatment programs[54]. Drugs currently used for treatment of opioid dependence are shown in Figure 12.

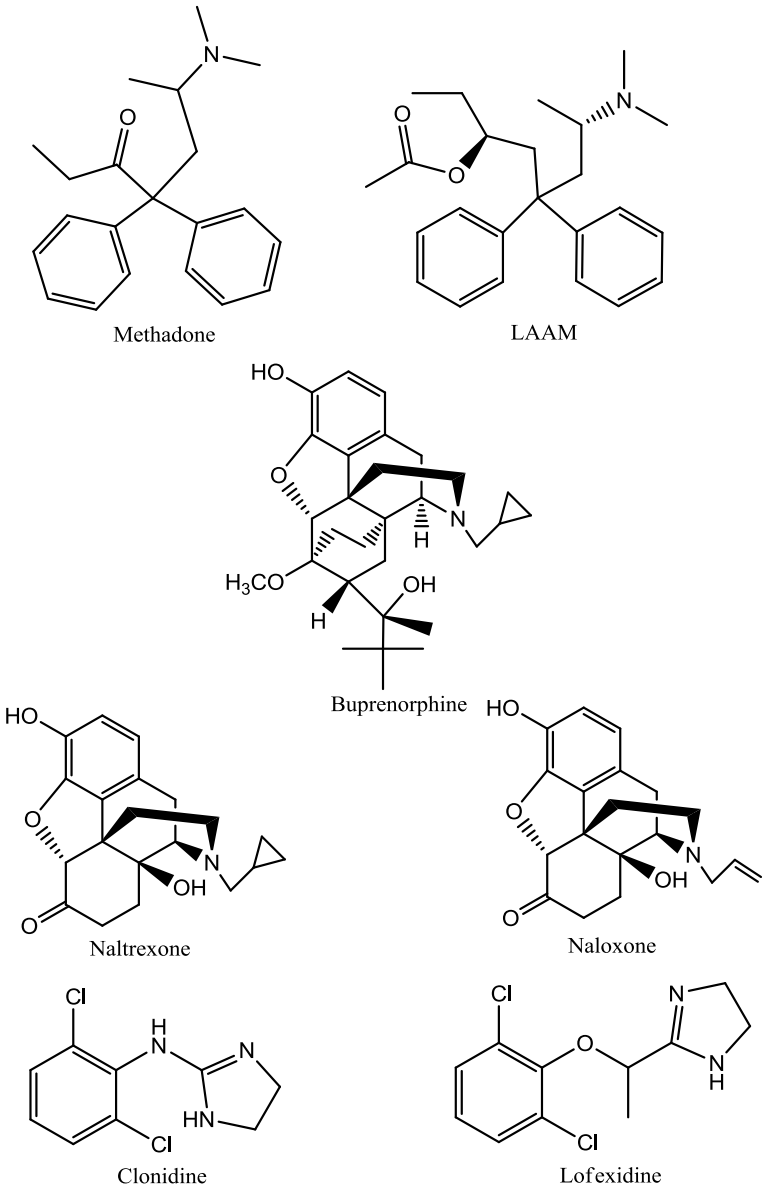


Figure 12 - Substances used for opioid addiction treatment: full opioid agonists (methadone and LAAM), partial agonist (buprenorphine), antagonists (naltrexone and naloxone) and α_2-adrenergic agonists (clonidine and lofexidine).

5.4. Treatment of opioid dependence and *Mitragyna speciosa*

Although several pharmacological and psychological treatment approaches are effective, it is clear that, giving the relapsing nature of opioid addiction, many individuals will return to an opioid-dependent lifestyle. In order to prevent the negative outcomes

related to opioid addiction, it is imperative to search for new ways to help addicted patients to overcome opioid dependence. The scope of this work is to study novel compounds based on the alkaloids present in *Mitragynine speciosa*, also known as kratom. Descriptions dated back to as early as 1897 show that this plant was indicated for treating pain and opium abstinence syndrome. Indeed, a recent case report describes that a patient who abused hydromorphone was able to manage the intensity of opioid withdrawal by ingesting kratom tea[55]. This finding is also corroborated by several individuals depicting modulation of opioid withdrawal by ingesting kratom[56].

6. *Mitragyna speciosa* and its alkaloids

Mitragyna speciosa Korth is a tree member of the *Rubiacea* family that is indigenous to tropical Southeast Asia. Popularly known as kratom in Thailand and Biak-Biak in Malaysia, the leaves have been consumed by natives to induce opium-like effect and coca-like stimulant. The plant has been also used as an opium surrogate to decrease withdrawal syndrome in opioid abusers. Although it has been outlawed in those countries as well as in Australia, people still ingest the leaves by brewing tea, chewing or smoking[57]. In the United States leaves, extracts and powders are legally available through an increasing number of websites. Descriptions of preparations and effects of Kratom are widely available online through a growing number of testimonials[58,59].

Mitragynine is the major constituent of *M. speciosa* leaves, making up to 66% of the total alkaloidal extract. Other Corynanthe-type alkaloids present in the crude extract are paynantheine (8.6%), speciogynine (6.6%) and speciociliantine (0.8%). Another minor plant component (2%) of interest regarding opioid activity is 7α-hydroxy-7*H*-mitragynine[57]. Figure 13 shows the structures of these five alkaloids. Biosynthetically, corynanthe-type alkaloids are derived from a Mannich-like reaction between the ethylamine portion of tryptamine and a keto acid to give the tetrahydro-β-carboline system[60].

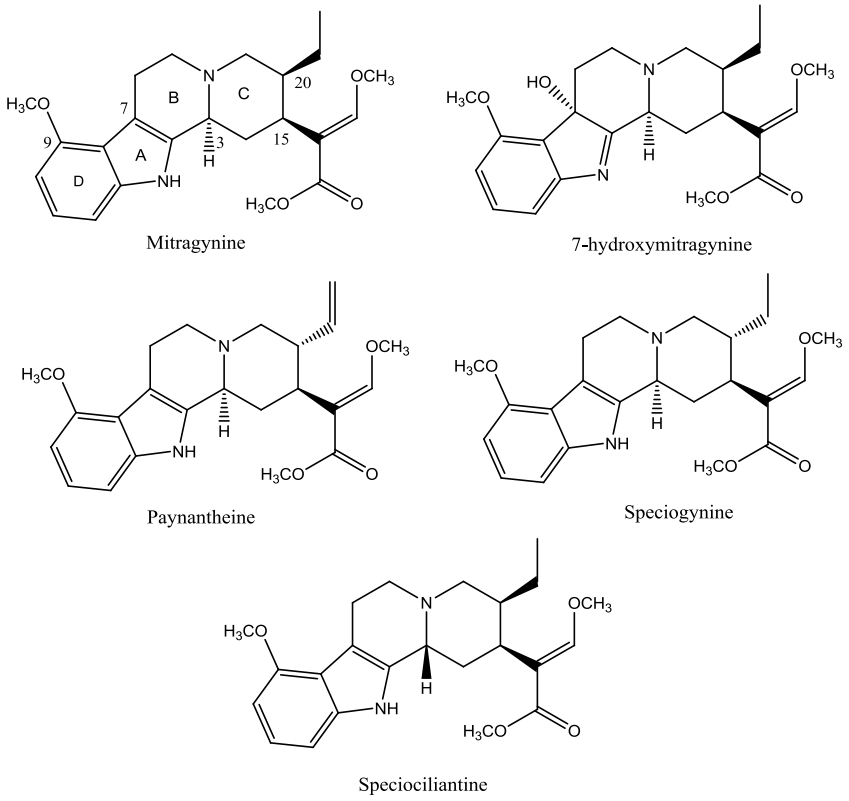


Figure 13 - Structures of alkaloids present in *Mitragyna speciosa.*

Studies have shown that mitragynine has analgesic and antitussive properties comparable to codeine, without producing respiratory depression in comparison to this classic opioid. *In vitro* assays using membranes of guinea-pig brain showed that mitragynine binds preferably to μ-opioid receptors, with a pK_i of 8.14 ± 0.28 as compared to 8.46 ± 0.28 of morphine in saturated radioligand ([^{3}H]DAMGO). Preparations of electrically-stimulated contractions in guinea-pig ileum displayed an inhibitory activity of 95% relative to morphine, however it showed a relative potency of only 26%. Antinociceptive activity in the tail-flick test in mice by intracerebroventricular administration of mitragynine revealed an estimated EC_{50} value of 60 nmol/mouse, whereas the average value for morphine was 3.2 nmol/mouse. On the other hand, 7-hydroxymitragynine showed a relative potency to morphine of over 10-fold, while

maintaining its relative inhibitory activity of 99% in the electrically-stimulated guinea-pig ileum test. Moreover, antinociceptive activity 7-hydroxymitragynine in both tail-flick and hot-plate test in mice after either oral or subcutaneous administration was higher than of morphine. In fact, 7-hydroxymitragynine antinociceptive effect was elicited after oral administration of 5 – 10 mg/kg in the tail-flick test in mice, whereas morphine did not produce any analgesia at 20 mg/kg p.o. in either assay[57,61].

Mitragynine obtained by our group was also subjected to binding assays; however discrepancies were observed when compared to studies described in the previous paragraph. Although there is an agreement that its affinity is higher for μ-opioid receptors, the K_i value obtained was 81.97±5.49 nM, around 10-fold greater than the result observed in the previous study (7.24±0.52 nM). Opioid receptors are not the solely targets of mitragynine; this alkaloid also has affinity for other receptor types (Figure 14). It is worth noting that mitragynine binds to α_2-adrenergic receptors, which might explain its opioid withdrawal attenuation property, since this receptor is agonized by clonidine, an α_2-adrenergic agonist.

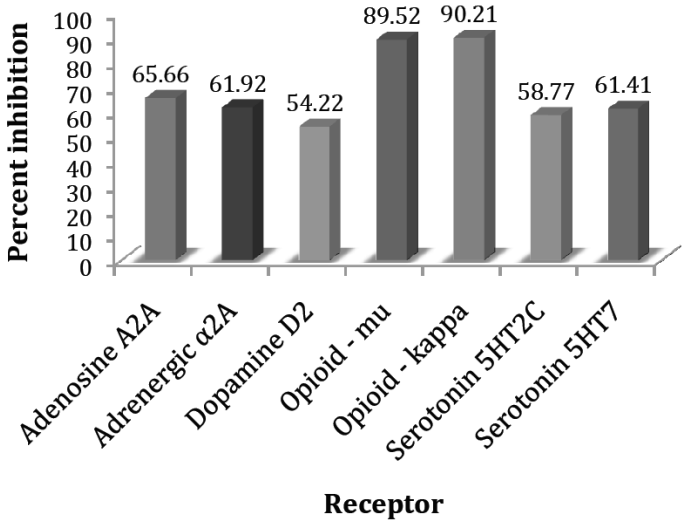


Figure 14 - Percentage of inhibition by mitragynine among different receptors.

6.1. Mitragynine SAR

Some known structure-activity relationships (SAR) in electrically-stimulated guinea-pig ileum preparations are depicted on Figure 15. The 9-methoxy group is optimal to affinity and, as discussed above, 7-hydroxylation substantially increases opioid activity. Basic nitrogen is also required, since *N*-oxidation leads to inactivity. Speciociliantine, a C3 stereoisomer of mitragynine, has very low potency compared to mitragynine (13-fold decrease), which means that the four-ring structure must be on the same plane (*S*-configuration) in order to optimize opioid activity[61].

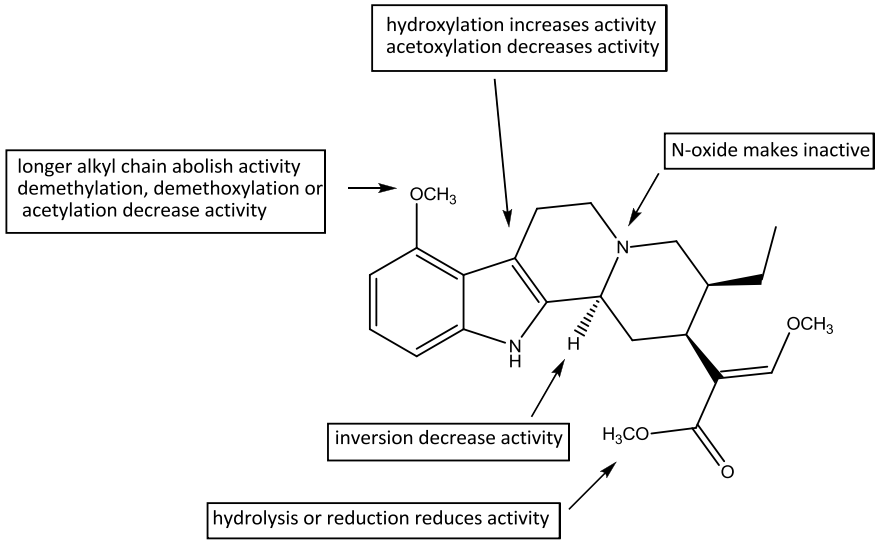


Figure 15 - *In vivo* mitragynine SAR.

7. Rationale

There are basically two different classes of molecules that were envisioned based on mitragynine: phenylpiperdine (including phenylaminopiperidines and phenylamidopiperidines) and tetrahydro-β-carboline derivatives. The idea was to simplify and increase flexibility of molecules in order to create novel opioid-active compounds and potentially comprehend the requisites necessary for opioid receptor affinity and activity. In the field of morphine analogue development, this approach has led to several marketed compounds with potent opioid activity that possess minimal structural requirements for binding and activity[62]. Tetrahydro-β-carboline derivatives are also to be explored for their tetracycle similarity to mitragynine and the ability to synthetically eliminate some functional groups present in this alkaloid.

7.1. Phenylpiperidines, phenylaminopiperidines and phenylamidopiperidines

Initially, in order to investigate the pharmacophore moiety that may be involved in the opioid action of mitragynine, simple compounds **1 - 4** were proposed based on the backbone of mitragynine as show in Figure 16.

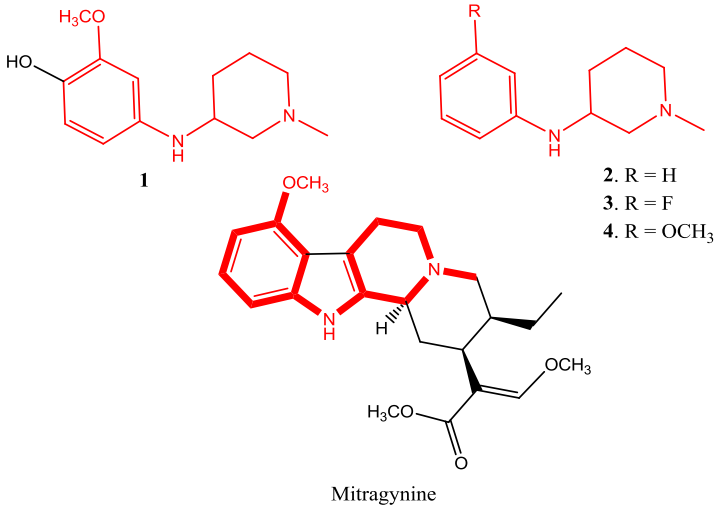


Figure 16 - Simple phenylaminopiperidines. Common features in red.

In order to investigate the pharmacophore responsible for the opioid activity of mitragynine, some moieties were proposed based on the structure of this alkaloid. One of the envisaged structures is **5**, which resembles mitragynine, but it is designed to alleviate the constraints on rings A and C (Figure 13). Since all four rings of mitragynine form a planar and rigid structure, it was thought that a more flexible molecule could have more contact points with opioid receptors. The highlighted scaffold is depicted in blue in Figure 17.

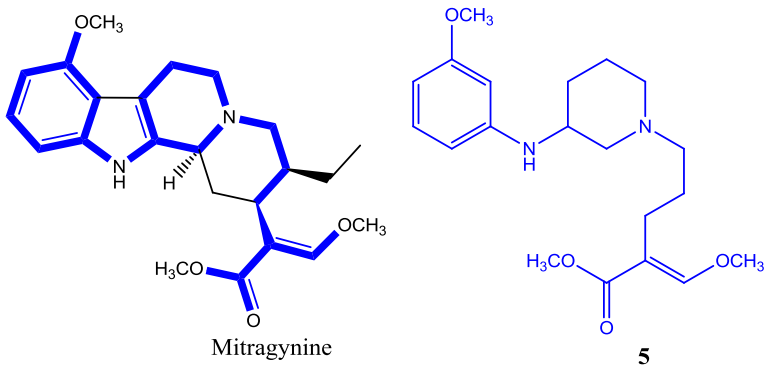


Figure 17 - Mitragynine and proposed phenylaminopiperdine. Common features in blue.

Fentanyl and structurally related analgesics in the phenylpiperidine family such as carfentanyl and sufentanyl as well as meperidine are potent μ-opioid agonists. In fact only 0.12 mg of fentanyl is required to produce the same analgesia as 10 mg of morphine[63]. By combining some structural elements of mitragynine (blue) and fentanyl (orange) along with remifentanil as shown in Figure 18, it was hypothesized that compound **6** may have opioid activity.

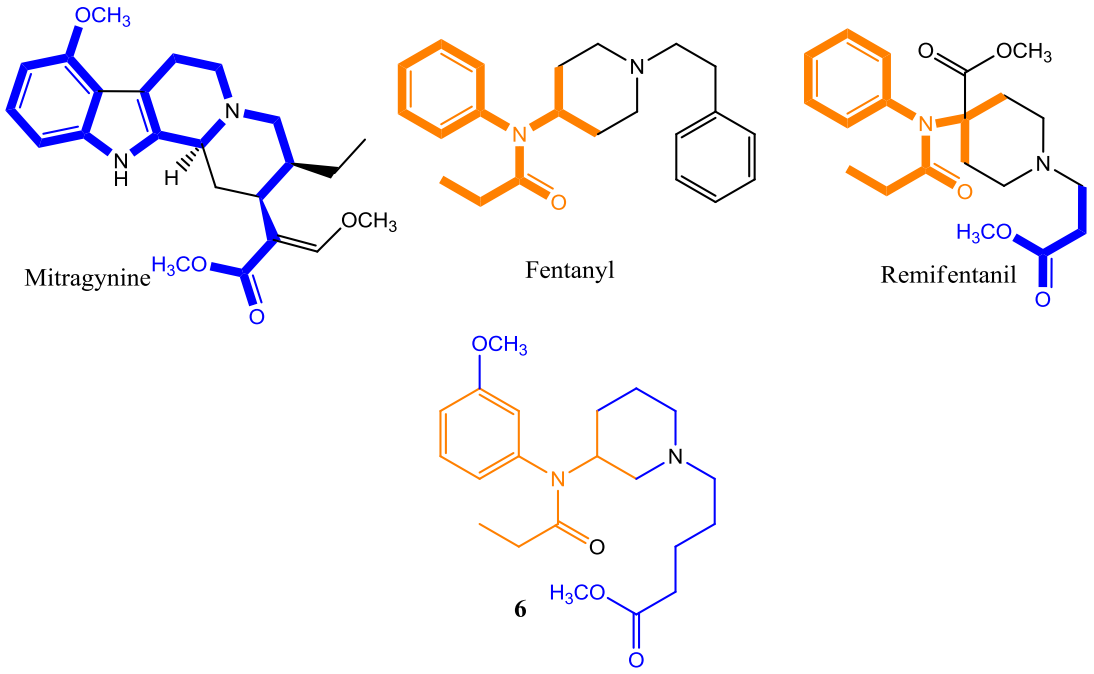


Figure 18 - Proposed phenylamidopiperidine based on mitragynine, fentanyl and remifentanil.

A wide variety of 4-phenylpiperidines have been widely investigated for their affinity for opioid receptors[64]. Taking this into account along with the structures of mitragynine and its 7-hydroxy derivative, other structures were proposed as shown in Figure 19. Compound **7** resembles the backbone of mitragynine (blue), whereas compound **8** is similar to the structure of 7-hydroxymitragynine. Both structures were planned having in mind the alleviation of constraint of rings A and C (Figure 13).

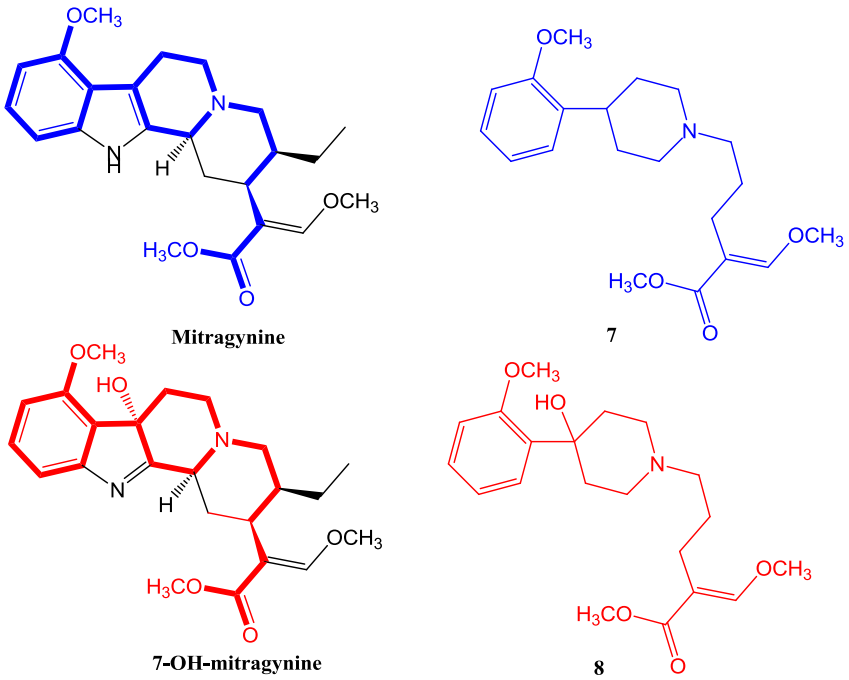


Figure 19 - Mitragynine, 7-hydroxy-mitragynine and phenylpiperidines. Similarities are in blue (mitragynine) and red (7-hydroxy- mitragynine).

7.2. Tetrahydro-β-carbolines

As previously discussed, phenylpiperidines, phenylaminopiperidines and phenylamidopiperidines were planned to mimic mitragynine and its 7-hydroxy derivative, while introducing flexibility on the backbone by removing rings A and C. Nonetheless, it was thought necessary to explore smaller changes on the natural product structure. To this end, tetrahydro-β-carbolines were proposed to closely resemble the four-ring structure of mitragynine and explore smaller changes to elucidate some structure-activity relationships. These modifications were concentrated on the 15-position of mitragynine structure (Figure 13). Another compound of interest is **38**, which is based on hydroxylated 7-hydroxymitragynine, an alkaloid with *in vivo* antinociceptive properties

in both tail-flick and hot-plate test higher than morphine. For the purpose of synthetic simplification, the ethyl group was omitted as illustrated in Figure 20.

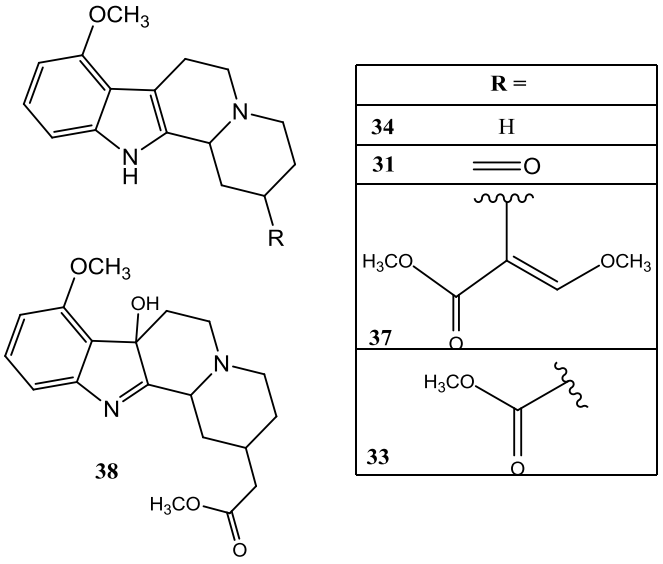


Figure 20 - Tetrahydro-β-carbolines based on mitragynine and 7-hydroxymitragynine.

8. Synthetic strategies and discussion

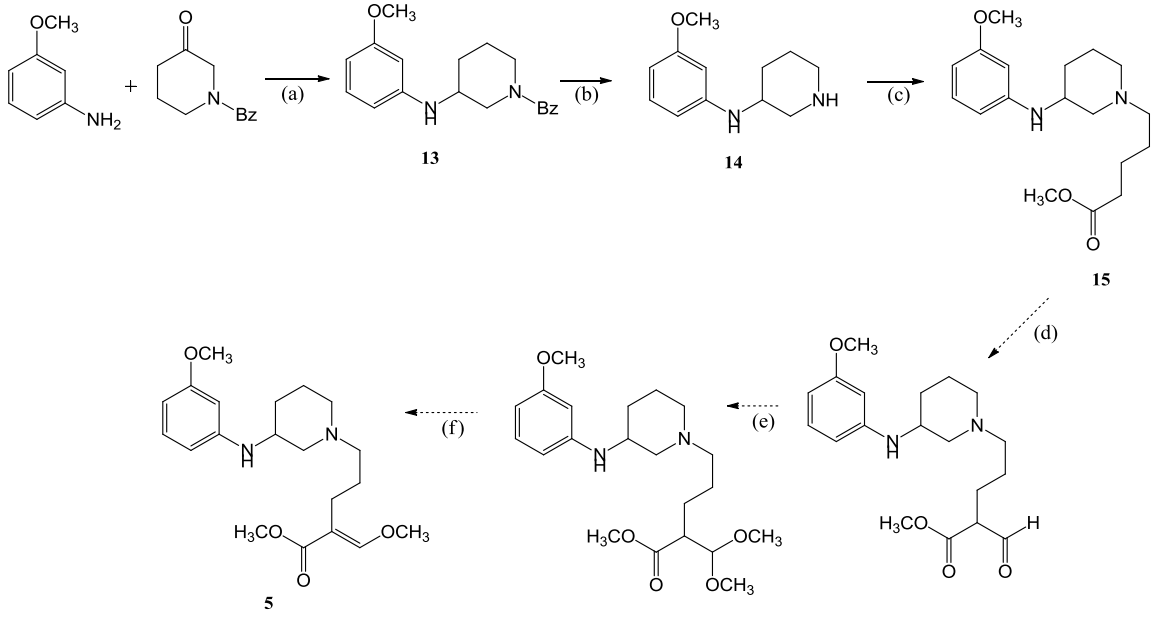
The next section will be subdivided into two classes of compounds: phenylpiperidines (inclunding phenylaminopiperidines and phenylamidopiperidines) and Tetrahydro-β-carbolines. Schemes and reactions conditions as well as problems encountered will be depicted and discussed. Furthermore, stereochemistry considerations will be addressed in the end of this section.

8.1. Phenylpiperidines, phenylaminopiperidines and phenylamidopiperidines

The general strategy to obtain **2** – **4** is to first transform *N*-methyl-3-piperidinol into its methanesulfonate ester and further react with the corresponding aniline derivative. The overall yield for these reactions were very low (<2%) due to the difficulty of sulfonate ester formation. Synthesis of the toluenesulfonyl and trifluoromethanesulfonyl esters[65] were attempted, but they also gave very low yields. To obtain the aniline of compound **1**, first the hydroxyl group of 4-nitroguaiacol was protected with the acetyl group[66], then the nitro group was reduced using palladium on carbon (10%) under pressurized hydrogen atmosphere[67], which was reacted with *N*-methyl-3-piperidinomethanesulfonyl ester.

As previously discussed, overall low yields were obtained with sulfonate esters. In this case, reductive amination between *m*-anisidine and a piperidone was considered as alternative route. First, the oxidation of *N*-methyl-3-hydroxypiperidine to the corresponding ketone was tried through two different approaches: (a) oxidation by aqueous sodium hypochlorite[68], and (b) oxidation by using Dess-Martin periodinane[69], however both methods gave low yields (less than 7%). Reductive amination was primarily carried out using the readily available *N*-methyl-4-piperidone and sodium

cyanoborohydride in acetonitrile at reflux or applying microwave radiation[70]. Formation of the amine bond was only improved from less than 3% to 12% after employing the less polar solvent 1,2-dichloroethane (DCE) at room temperature[71].



Scheme 1. (a) AcOH, $NaBH(OAc)_3$, DCE (b) H_2 (55 psi), Pd/C, MeOH (c) K_2CO_3, r.t. (d) LDA, methylformate, THF -78 ºC (e) *p*-TsOH, trimethyl orthoformate, MeOH, reflux (f) *t*-BuOK, DMSO

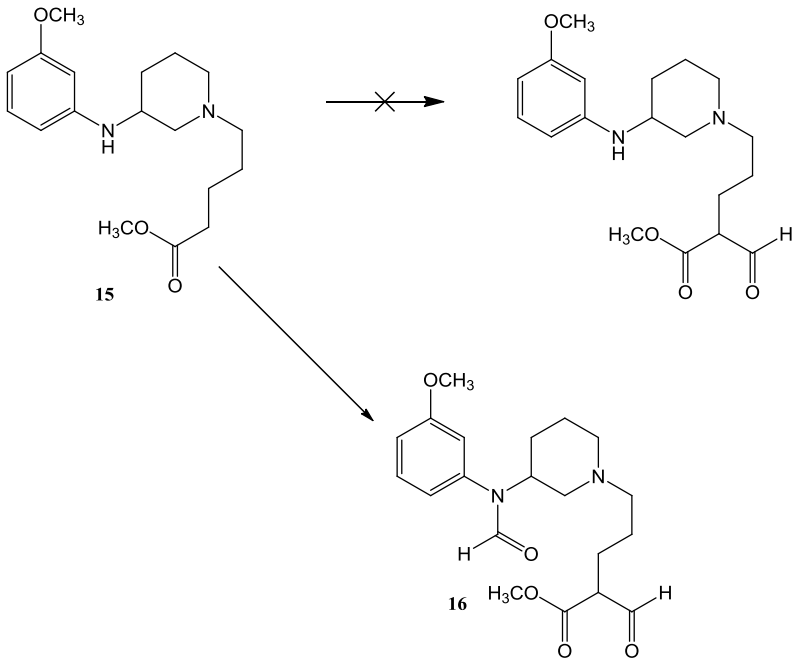
The first step was carried out using *m*-anisidine and the readily available benzyl-protected ketone: *N*-benzyl-4-piperidone[71] (Scheme 1). The yield was considerably higher as compared to the reductive amination described above, from 12% to 70%. Since the desired amine bond is at 3-position relative to the piperidine ring, reductive amination was accomplished using the commercially available *N*-benzyl-3-piperidone hydrochloride, which was extracted to the corresponding base before reacting with *m*-anisidine. Reductive amination was also carried out using sodium triacetoxyborohydride and acetic acid in 1,2-dichloroethane[71] at room temperature for 72h, which after purification gave a cleaner product according to NMR analysis with 50% yield.

The next step consisted in the removal of the benzyl group. Several approaches were used to deprotect the piperidine ring employing palladium on carbon and ammonium formate or formic acid[72] as sources of hydrogen in methanol. Zinc was also used as catalyst along with ammonium formate in methanol at room temperature, but no reaction took place after 45 hours. Even after irradiating with microwave at 80 ºC for one hour, no change in TLC (Thin Layer Chromatography) was observed[73]. Another method for debenzylation employed palladium on carbon and a mixture of ethanol and cyclohexene under reflux for 24 hours[74]. Only after using palladium (10%) on carbon under hydrogen atmosphere at 55 psi suspended in methanol, the reaction time was reduced to 4h. The last approach also provided up to 85% yield after purification through chromatography[75].

Alkylation of the secondary amine formed after the elimination of the benzyl group was attempted by reacting methyl 5-bromovalerate using potassium carbonate and a phase transfer catalyst[76] such as tetrabutylammonium iodide or bromide in DMF. Different reaction temperatures ranging from room temperature to 55 °C were tried; yet concomitant alkylation of the aromatic amine probably took place as a byproduct according to mass spectroscopy. The formation of the this derivative could be circumvented by not employing a phase transfer catalyst and simply using potassium carbonate in DMF at room temperature, giving a yield of 53%.

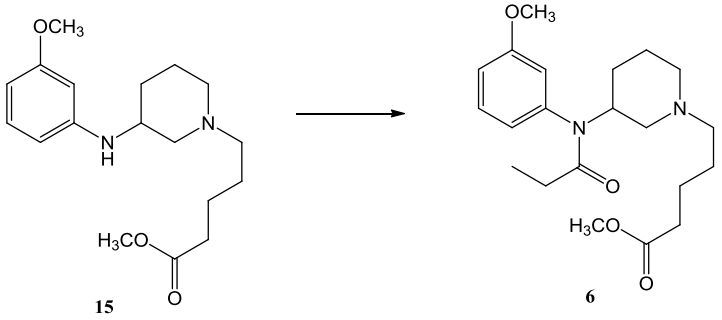
In order to introduce the formyl group alpha to the ester **15**, it was found necessary to first protect the aromatic amine, as lithium diisopropylamine reacts with the amino hydrogen, giving compound **16** according to mass spectroscopy (Scheme 2). This should have been expected, since the aromatic amine is more acidic than the methylene

next to the carbonyl group. The first attempt to benzylate the amino group used benzyl bromide in DMF and different bases (K_2CO_3 or KH)[77]; however it was observed through TLC that formation of a quaternary amine in the piperidine ring took place. Introduction of the benzyl group through reductive amination using benzaldehyde and $NaBH(OAc)_3$ in acidic conditions was not successful. Protection of the aromatic amine was also attempted using di-*tert*-butyl dicarbonate[78], but the product recovered by flash chromatography was the starting material. Carbamate protection using ethyl chloroformate[79] in DCE or DMF at temperatures up to 85 ºC was not successful after several hours. Although some product was identified by mass spectroscopy, it was observed by TLC that the yield was very low, even after addition of up to 15 equivalents of ethyl chloroformate.



Scheme 2. LDA, THF, -70 °C. Unsuccessful introduction of the formyl group alpha to the ester **15**.

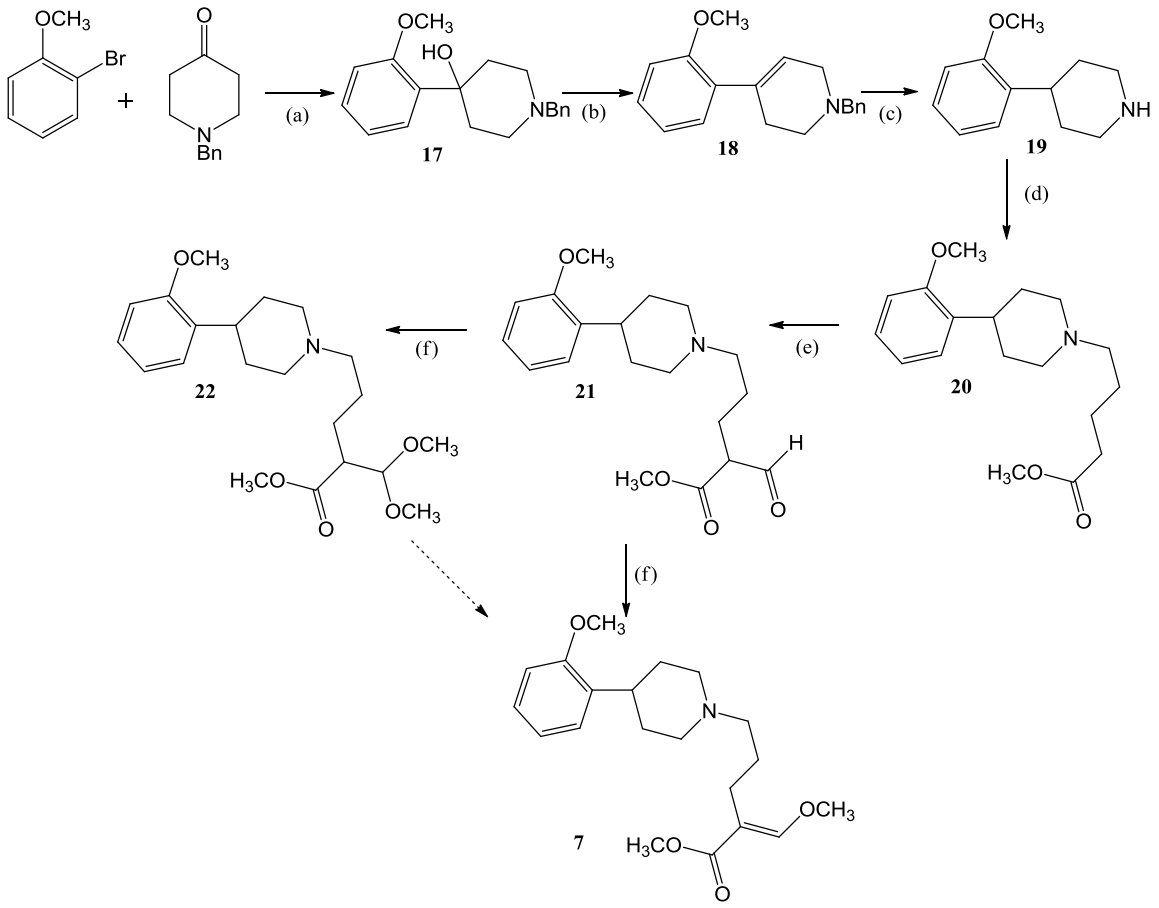
The phenylamidopiperidine **6** based on structural similarities among potent opioids such as fentanyl and remifentanyl as well as the alkaloid mitragynine (Figure 18) was easily obtained as shown on scheme 3. Compound **15** was treated with propionyl chloride and triethylamine in refluxing DCE under argon, yielding 95% after purification by silica gel chromatography[80].



Scheme 3. Propionyl chloride, Et_3N, DCE, reflux.

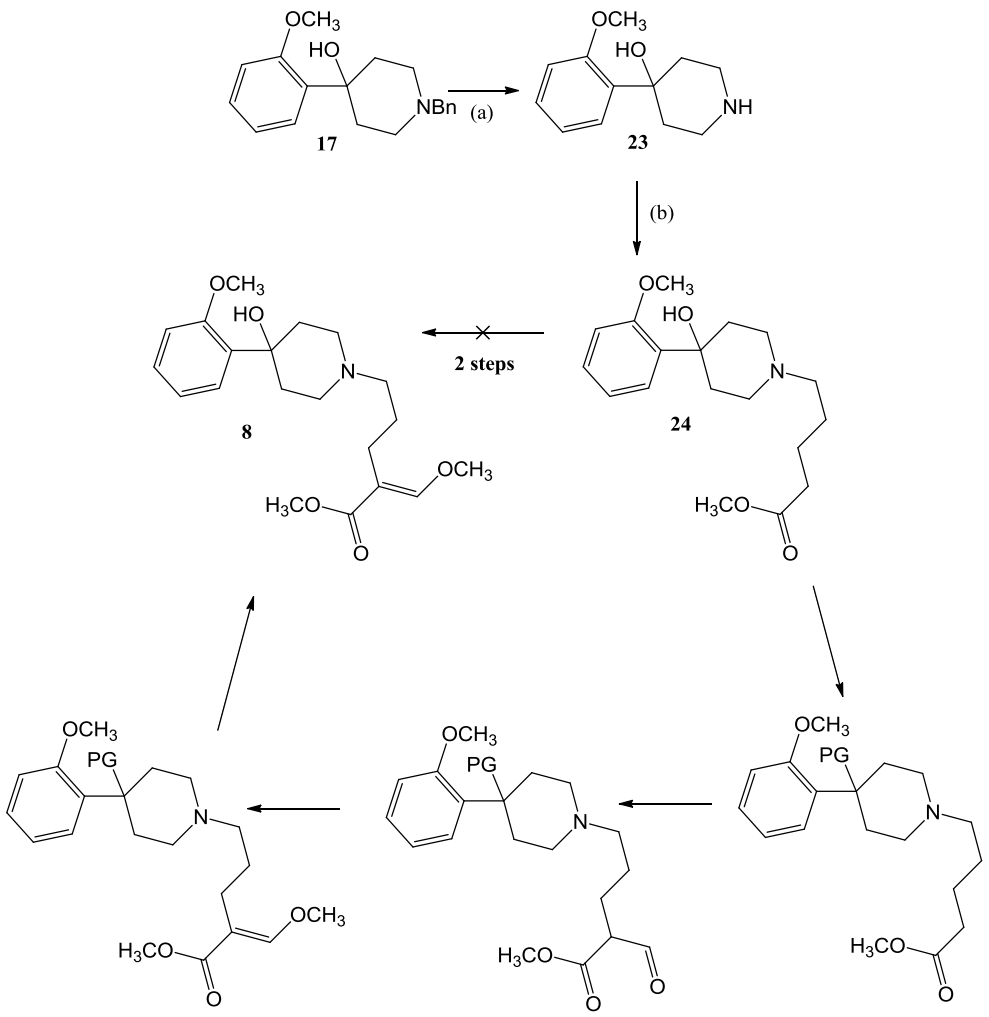
Regarding the rationale depicted in Figure 19, the phenylpiperidine **7** was obtained as shown in scheme 4. The tertiary alcohol **17** was obtained by transforming 2-bromoanisole into the corresponding Grignard reagent and then adding *N*-benzyl-4-piperidone, yielding 27% along with three byproducts[81]. Intramolecular elimination of water using acidic condition in refluxing toluene yielded 56% of pale yellow oil (**18**). Posterior debenzylation and olefin reduction was catalyzed by palladium under hydrogen atmosphere to give the secondary amine **19**. When compared to the conditions used to get **15**, alkylation of the secondary amine **19** was improved both on yield (81%) and time (2 hours) by heating to 60 ºC and using $NaHCO_3$ (**20**). Introduction of the formyl group alpha to the ester was achieved by treating with lithium diisopropylamide in THF at -70 ºC and then adding methyl formate. The product obtained (**21**) with 70% yield was a mixture of aldehyde and enol tautomers, which was verified by both TLC and NMR analysis. The three spots on the TLC are compatible to a mixture of the aldehyde form as

well as two enolic geometric isomers. Taking into account the proton NMR spectrum, the aldehyde form was predominantly present in a 2/3 ratio. As it will be described later in the case of tetrahydro-β-carbolines, it was thought that in order to obtain the methoxyacrylate **7**, compound **21** would have to be converted to the acetal **22** and then submitted to basic treatment with potassium tert-butoxide. Interestingly, the last step could be circumvented by extending the reaction time and adding an excess of tosic acid and trimethyl orthoformate, without affecting the yield (23%)[82]. Regarding the methyl methoxyacrilate isomer, it was expected that the (*E*)-isomer was obtained[83] due to the high repulsion energy of oxygen atoms while in (*Z*)-configuration (4.46 kcal/mol according to ChemBio3D software MM2 energy minimization).



Scheme 4. (a) Mg, THF, I_2 (b) *p*-TsOH, toluene, reflux (c) H_2, Pd/C, EtOH (d) methyl 5-bromovalerate, $NaHCO_3$, DMF, 60 ºC (e) LDA, methyl formate, THF (f) TsOH, trimethyl orthoformate, MeOH, reflux

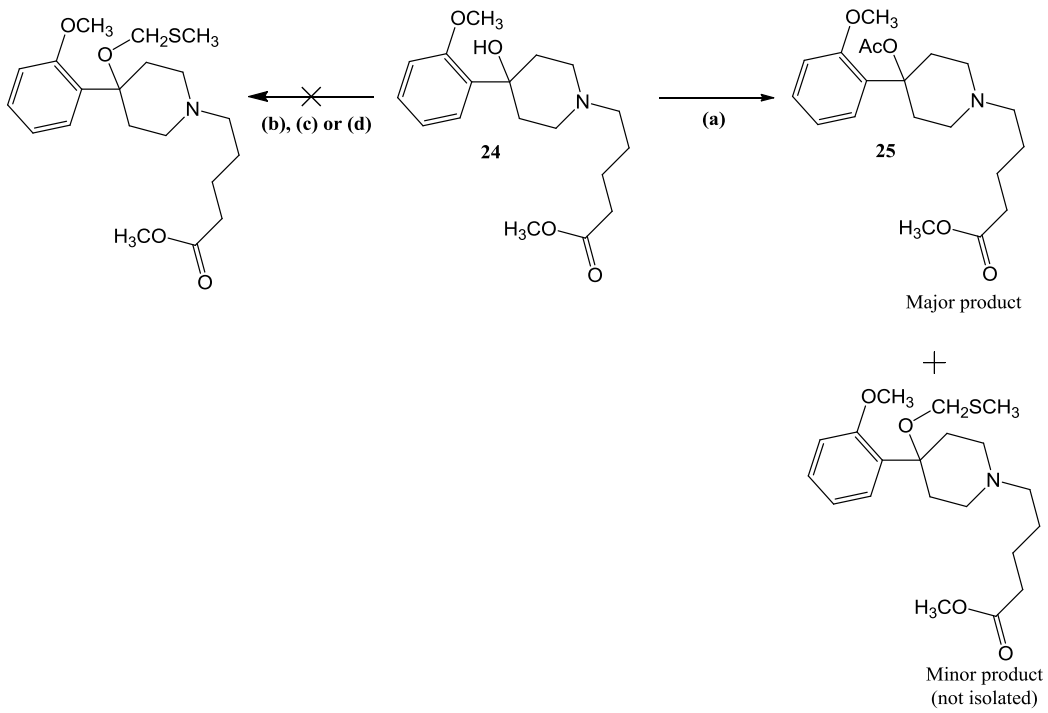
Compounds **17**, **23** and **24** (Scheme 5) where again prepared with similar yields as previously described in Scheme 4. Nevertheless, it was found to be necessary to protect the tertiary alcohol **24** to obtain **8**, as two key steps are incompatible with this hydroxyl group. First, formyl group introduction requires a strong base, lithium diisopropylamide, which easily deprotonates the tertiary alcohol; second, tertiary alcohols readily undergo intramolecular dehydration under acidic conditions, like the one employed to obtain the methoxyvinyl moiety.



Scheme 5. (a) H_2, Pd/C, EtOH (b) methyl 5-bromopentanoate, $NaHCO_3$, DMF, 60 ºC. PG: protective group**.**

Since formation of the methoxyvinyl moiety requires the use of tosic acid, the chosen protecting group would have to survive this condition. Silyl ethers are among the most commonly used protective groups for the alcohol function, but are susceptible toward acid hydrolysis[84]. Protection by the benzyl group was thought as an alternative, as his ether are resistant toward acid hydrolysis. Nevertheless, by submitting compound **7** to the deprotection conditions (H_2, Pd/C in EtOH), it was observed that the methoxyvinyl double bond underwent reduction according to mass spectroscopy. Another possible

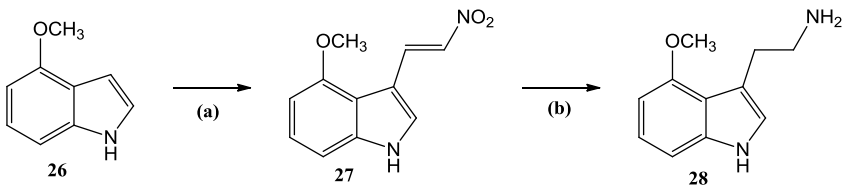
choice to protect the tertiary alcohol explored the advantage of the acidic stability of methylthiomethyl (MTM) ether, which can be removed with the neutral mercuric chloride[84]. The first method to introduce the MTM group in **24** employed DMSO and acetic anhydride at room temperature[85,86], which gave the protected MTM ether in minor quantities along with the acetate ester and intramolecular water elimination product. Besides the evidence by mass spectroscopy and TLC of MTM ether and byproducts formation, the reaction mixture was submitted to deprotection using $HgCl_2$ in water/acetonitrile[87], which gave back the starting material **24** (according to TLC). The difficulty to introduce the MTM group to a tertiary alcohol was evident, as different methods were attempted without success: (a) methylthiomethyl chloride, NaH, NaI, THF[87]; (b) methylthiomethyl chloride, $AgNO_3$, triethylamine, benzene[86]; (c) methylthiomethyl chloride, $AgNO_3$, triethylamine, toluene. As the only method that looked promising used dimethyl sulfoxide and acetic anhydride to form a sulfonium ion, which then reacts with the hydroxyl group to give the MTM ether[88], the rest of starting material **24** was submitted to this procedure. However, the only compound obtained after purification was the acetate ester **25**. Scheme 6 exemplifies the different strategies pursued to obtain the protected hydroxyl group by MTM ether.



Scheme 6. (a) Ac_2O, DMSO (b) Cl-CH_2SCH_3, NaH, NaI, THF (c) Cl-CH_2SCH_3, $AgNO_3$, Et_3N, benzene, 60 ºC (d) Cl-CH_2SCH_3, $AgNO_3$, Et_3N, toluene, 70 ºC.

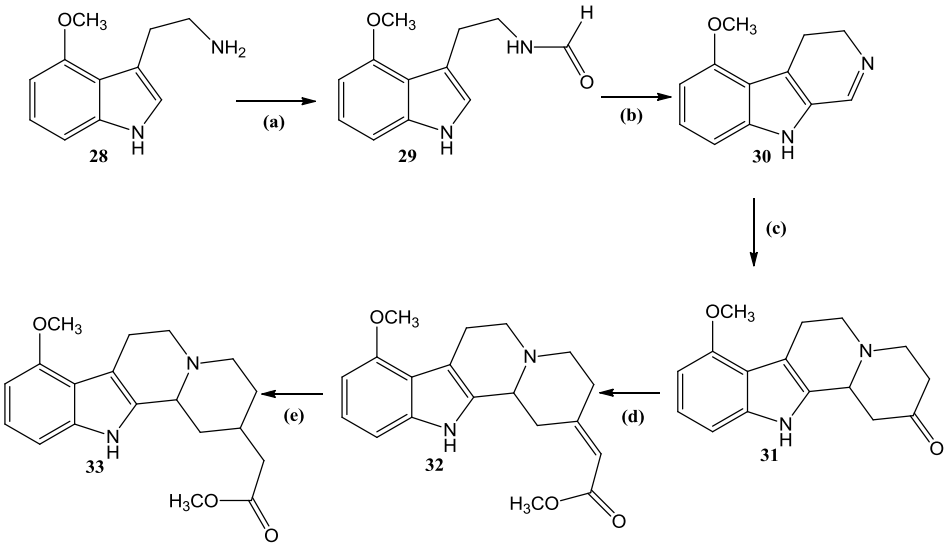
8.2. Tetrahydro-β-carbolines

The tryptamine **28** was obtained as shown in scheme 7. Commercially available 4-methoxyindole **26** underwent nitro olefination at 3-position with *N*,*N*-dimethylamino-2-nitroethylene in trifluoroacetic acid[89]. Reduction of both ethylene and nitro groups in **27** was carried out employing lithium aluminum hydride in THF to give **28** with 15.5% yield (two steps).



Scheme 7. (a) *N*,*N*-dimethylamino-2-nitroethylene, TFA, 0 ºC then r.t. (b) LAH, THF, -78 ºC then r.t.

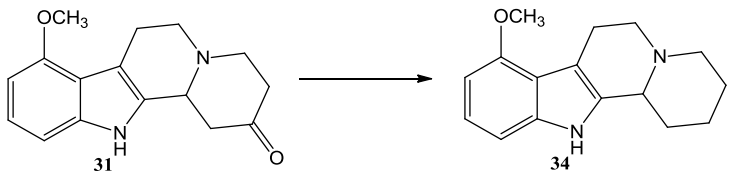
Scheme 8 shows that after obtaining the formamide **29** with a refluxing mixture of ethyl formate in methanol (90% yield), phosphoryl chloride was employed to cyclize ring B to give the imine **30** via Bischler-Naperalski reaction[90,91,92] . Without isolating the dihydro-β-carboline **30**, the fourth ring in **31** was constructed by using methylvinyl ketone and zinc chloride in methanol at reflux temperature (64% yield)[93]. Horner-Wadsworth-Emmons reaction, i.e. replacement of ketone with olefinic methyl ester **32,** was accomplished by using trimethyl phosphonoacetate and sodium hydride in THF, yielding 98%[94,95]. The reduced compound **33** was obtained by simply employing hydrogen and palladium as catalyst with 55% yield.



Scheme 8. (a) ethyl formate, MeOH, reflux (b) $POCl_3$, 50 ºC (c) methylvinyl ketone, $ZnCl_2$, methanol, relux (d) trimethyl phosphonoacetate, NaH, THF, 0 ºC than r.t. (e) H_2, Pd/C, MeOH.

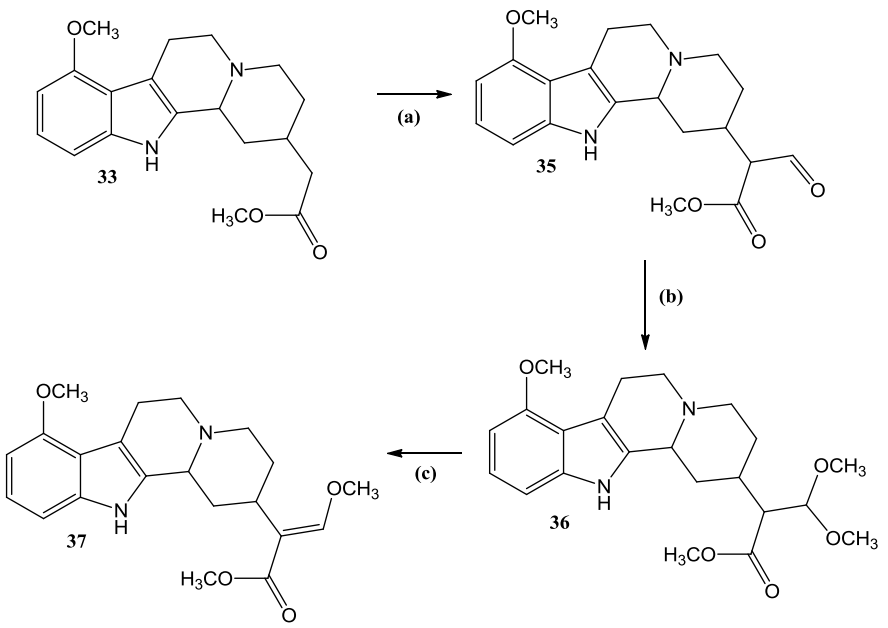
Wolff-Kishner reduction of ketone **31** was first carried out with hydrazine sulfate and potassium hydroxide in ethylene glycol at 190 ºC without success[96]. Only after

substituting hydrazine hydrate for hydrazine sulfate, it was possible to obtain **34** with 86% yield as depicted in Scheme 9.



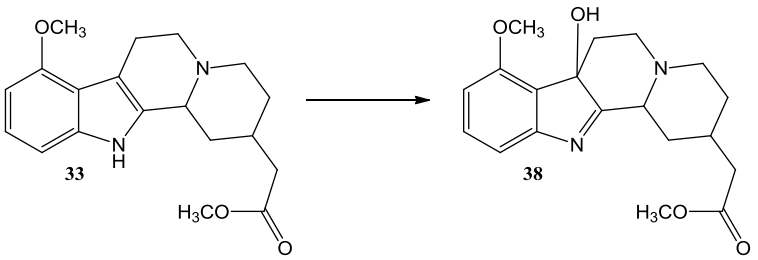
Scheme 9. $NH_2NH_2.H_2O$, KOH, ethylene glycol, 190 ºC.

The final three steps to obtain the tetrahydro-β-carboline **37** are shown in Scheme 10. As described previously in relation with phenylpiperidines, formyl group introduction alpha to the methyl ester **33** was carried out with lithium diisopropylamide and methyl formate in THF; however the yield was significantly low when compared to **21** (10%). After obtaining **35** as a mixture of tautomers, the next step was to form the acetal moiety with timethyl orthoformate and tosic acid in refluxing methanol, which gave **36** with 69% yield[82]. Elimination of one acetal methoxyl group using a strong base, potassium tert-butoxide, in dimethylformamide gave the methoxyvinyl moiety in **37** with 31% yield. As discussed earlier, it is expected that the (*E*)-isomer was obtained[83] due to higher repulsion energy of oxygen atoms while in (*Z*)-configuration.



Scheme 10. (a) LDA, methyl formate, THF, -78 ºC then r.t. (b) trimethylorthoformate, PTSA, MeOH (c) *t*-BuOK, DMF, 60 ºC

Oxidation at 7-position of **33** was carried out by [bis-(trifluoroacetoxy)-iodo]-benzene (PIFA) as the oxidizer in a mixture of acetonitrile and water at 0 ºC under argon[97] (Scheme 11). The presence of the oxidized compound was confirmed by mass spectroscopy and proton NMR, that is, the absence of indolic proton and the appearance of a signal upfield in the ^{13}C spectrum (C2). The reaction was performed four times under similar conditions; however after six attempts to purify **38** by silica gel column using different mobile phases, the compound could not be isolated with sufficient purity for testing. Only after using preparative HPLC to isolate **38**, it was possible to obtain it with reasonable purity according to analytical HPLC (94.3%) at 15% yield.



Scheme 11. PIFA, MeCN, H_2O.

8.3 Sterereochemistry considerations

Previously, the focus on the discussion was based on the synthetic strategies and the problems encountered as well as how to circumvent them. Notwithstanding, besides mentioning which methoxyacrylate geometric isomer was obtained, nothing was said about the absolute configuration of the different stereogenic centers observed during the course of these syntheses, especially regarding the final products submitted for biological evaluation. Synthesis of piperidines **6** and **15** were carried out by reductive amination between *m*-anisidine and 1-benzyl-3-piperidone employing the non-stereoselective reducing agent $NaBH(OAc)_3$, therefore it is expected that they were obtained as racemic mixtures. On the other hand, synthesis of tetrahydro-β-carbolines generated up to three chiral centers, thus they deserve a more detailed discussion.

Compound **31** and its derivative **34** have one chiral center (see position 3 in Figure 21) and they were first analyzed by analytical HPLC using a chiral column with cellulose tris-(4-methoxybenzoate) coated on silica gel. Moreover, the chromatograms showed two distinct peaks, which says that they were obtained as a mixture of enantiomers. Moreover, the specific rotation for **31** was zero, proving that both compounds were obtained as racemic mixtures. In regard to compound **33**, more extended considerations are necessary since it has two stereocenters (see positions 3 and 15 in Figure 21). As in the case of compounds **31** and **34,** it was observed that the chiral

separation of **33** showed two peaks and the specific rotation was zero, which means that this compound was obtained as a racemic mixture. Fact also confirmed by the single TLC spot isolated during the course of the synthesis.

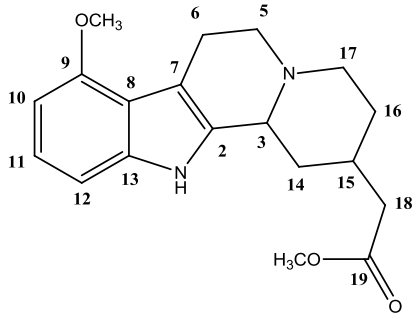


Figure 21 - Compound **33** ring numbering.

As it is displayed in Figure 22, there are four diastereoisomers that could be obtained. Since the optical rotation of the tested substance was zero, they must be a pair of either 3*S*, 15*R* and 3*R*, 15*S* or 3*S*, 15*S* and 3*R*, 15*R* isomers. The question that remains is what pair of enantiomers were submitted to biological testing. In order to answer this question, different NMR techniques were performed, namely DEPT135, COSY, HMQC and NOESY as well as the commonly used proton and carbon ones. After carefully analyzing these spectra, it was possible to draw some conclusions about which pair of enantiomers was produced. Following chemical shifts assignments of each carbon and the hydrogens connected to these carbons, NOESY spectrum showed a correlation between the indolic proton at 1-position and the proton attached to carbon 15, which means that the distance separating these protons must be less than 5 Å in order to this correlation to be observed. By employing molecular mechanics energy minimization (MM2; ChemBioDraw 12.0), it was visible that the only pair of enantiomers that fulfilled the less

than 5 Å requirement was 3*S*, 15*R* and 3*R*, 15*S* isomers, as opposed to the other pair (3*S*, 15*S* and 3*R*, 15*R*), which showed distances greater than 5 Angstroms.

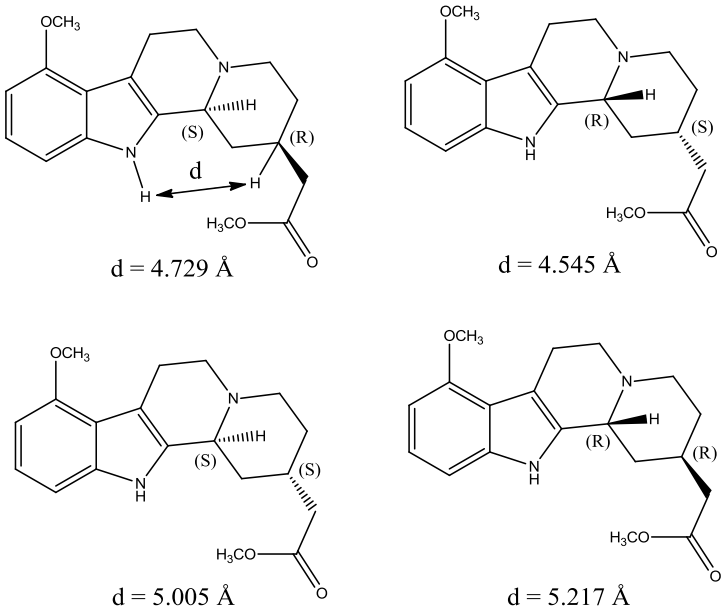


Figure 22 - Four diastereomers of compound **33** and the distances between position 1 and position 15.

Indeed, not only NMR techniques, but also the crystal structure, obtained by solubilizing the hydrochloride salt of compound **37** (a derivative of **33**) in ethanol and then evaporate the solvent, corroborates the conclusion about which pair of enantiomers underwent biological evaluation. Although only one isomer was crystallized, it is evident in Figure 23 that the recovered crystal was the 3*S*, 15*R* isomer of **33**, which belongs to the same pair examined before by NMR techniques. Interestingly, the crystal structure also confirmed that the methoxyacrylate geometric isomer (**37**) acquired was, as discussed above, the trans-compound. Moreover, despite the fact that no crystal structure of **7** was analyzed, it is possible, taking into account the energy minimization differences between cis and trans isomers as previously considered, to infer that the isomer produced was the trans compound.

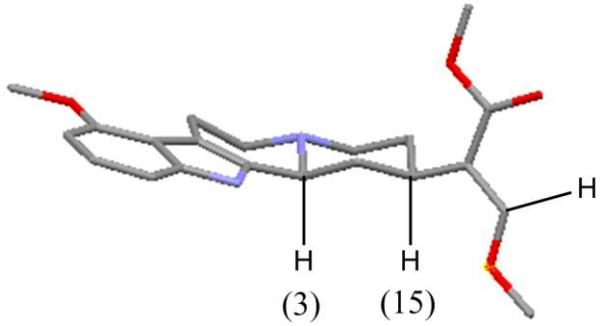
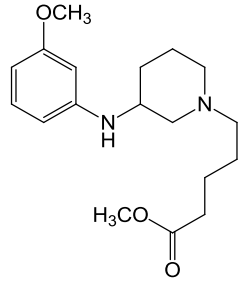
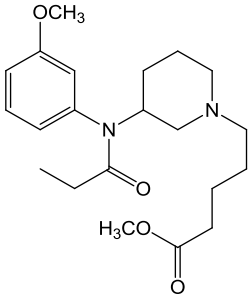


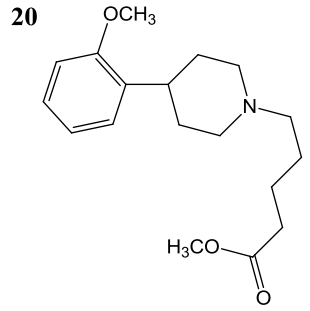
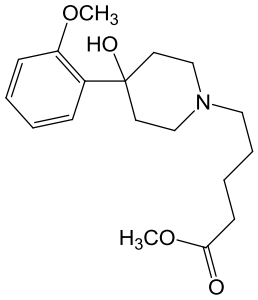
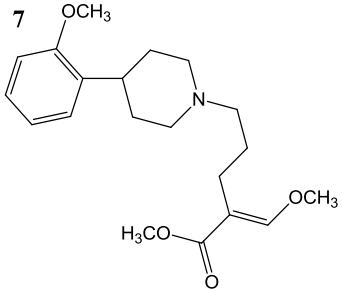
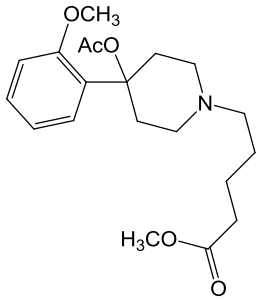
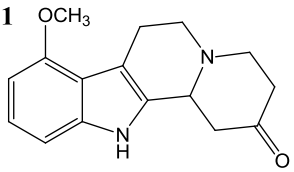
Figure 23 - X-ray crystal structure of compound **37**. Carbons in gray, nitrogens in blue and oxygens in red.

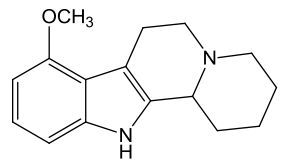
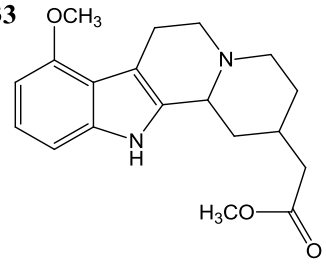
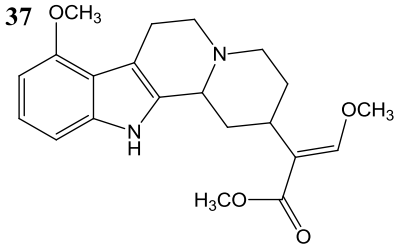
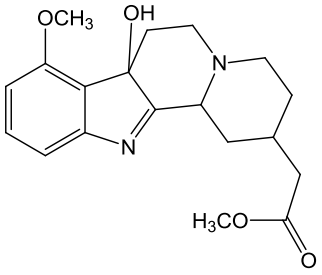
9. Biological results and discussion

Initially, compounds in table 2 were pre-screened to compare their percentage of binding with the reference compound, the opioid receptor antagonist naloxone, for each human opioid receptor. The constant of inhibition (K_i) and the half maximum inhibitory concentration (IC_{50}) where further evaluated for compounds that displayed a percentage greater than 50%, as highlighted in table 2. Most of compounds were tested as either hydrochloride salt (HCL) or monobasic oxalate salt (OXA); the remaining substances were evaluated as free bases.

Table 2- Percentage of binding compared to the reference compounds. OXA: monobasic oxalate salt; HCL: hydrochloride salt.

Compound	μ % binding	δ % binding	κ % binding
Mitragynine	<u>88.16</u>	<u>75.52</u>	<u>90.21</u>
15  MA66 OXA	2.22	-16.97	1.48
6  MA71 OXA	3.73	0.47	<u>52.23</u>

20  MA92 OXA	15.72	-8.32	31.03
24  MA94 OXA	29.58	-7.66	<u>51.35</u>
7  MA114	<u>80.93</u>	<u>87.80</u>	25.95
25  MA127	<u>72.59</u>	<u>58.30</u>	10.79
31  MA103	-7.59 -7.51 (HCL)	46.81 42.01 (HCL)	5.14 7.79 (HCL)

34  MA104 HCL	14.55	8.33	8.64
33  MA91 OXA	0.78	-16.64	<u>90.84</u>
37  MA108 HCL	14.86	11.56	<u>75.58</u>
38  MA116	2.43	48.82	26.48

From the preliminary qualitative binding assays depicted above, two compounds had significant displacement on human μ-receptors, two on δ-receptors and four on κ-receptors. Phenylaminopiperdine **15** and phenylpiperidine **20** lack binding affinity, however the flexible phenylamidopiperdine **6** as well as phenylpiperidines **7**, **24** and **25** had noticeable displacement on at least one type of opioid receptor. One explanation for **6** having κ-opioid affinity, when compared to **15,** is that it possesses a propyl group in the anilinic nitrogen, as it is also the case in fentanyl, which has κ- and μ-opioid affinity. The

methoxyvinyl group alpha to the methyl ester in **7** is important for the affinity for μ- and δ-receptors, since a similar compound **20** does not have affinity for any receptor. Selectivity change from κ-receptor to μ- and δ-receptors was observed by transforming the tertiary hydroxyl group in the piperidine ring of **24** into the acetoxy moiety in **25**. In the case of the more rigid tetrahydro-β-carbolines, only two out of five substances had considerable displacement on κ-receptors. The methyl ester in **33** and **37** seems to be important for κ-receptor affinity, since the tetrahydro-β-carbolines **31** and **34** did not show significant displacement. Surprisingly, compound **38**, which is similar to the efficacious antinociceptive agent 7-hydroxymitragynine (about 10 times the relative potency of morphine), did not display substantial displacement of naloxone in any receptor type.

Compounds that had a percentage of displacement greater than 50% in relation to naloxone were further incubated with radioligands specific for each opioid receptor: [^{3}H]DAMGO (μ), [^{3}H]Enkephlin (δ) and [^{3}H]U-69,593 (κ). Unfortunately, none of the compounds synthesized showed binding affinity as low as mitragynine (determined by our group), as displayed in table 3. With the exception of compounds **33** and **37**, all compounds showed values in the micromolar range. The compound with the highest binding affinity was the tetrahydro-β-carboline **33**, with a *K*i value of 391.4 nM for κ-receptor. Introduction of a methoxyvinyl moiety alpha to the methyl ester in **33** afforded **37**, which caused an almost two-fold decrease in affinity for κ-receptor. On the other hand, installation of the same moiety in the flexible phenylpiperidine **20**, which did not show significant naloxone displacement, provided **7**, with a binding affinity for μ- and δ-receptors of 2.097 μM and 8.836 μM, respectively. Neither the methoxyvinyl in **7**, nor the

acetoxy group in **25** had impact on μ affinity, nevertheless the binding affinity of **25** for the δ-receptor diminished by a factor of four. When comparing κ-receptor affinities, tetrahydro-β-carbolines revealed higher binding affinity than piperidines. Conversely, some piperidines displayed μ- and δ-receptors affinity, but no tetrahydro-β-carboline demonstrated significant affinity for those receptors.

It is also worth bringing into attention the absolute configuration of the compounds tested. Out of the two piperidine derivatives with a stereocenter, the one that had considerable inhibition, compound **6**, was obtained in a racemic fashion. Unfortunately, nothing can be said about which isomer is the major responsible for the activity, since they were not evaluated independently. On the other hand, the more complex tetrahydro-β-carbolines, have up to three stereocenters. As none of the tetrahydro-β-carbolines, **33** and **37**, had significant displacement in μ- and δ-receptors compared to mitragynine, it is possible to say that not only the ethyl group at position 20 in mitragynine (Figure 13) may play an role in the activity on these receptors, but also the absolute configuration of the pair of enantiomers tested. Indeed, though one compound shows identical configuration as mitragynine (see 3*S*,15*R* in Figure 22), the other displays the opposite on both positions. The same can be said about the *K*i values of **33** and **37** on κ-receptors, which were not as low as for mitragynine. Interestingly, the mixture of enantiomers **33** reveals a two-fold decrease in affinity on κ-receptors when compared to mitragynine, which can be explained by the fact that 50% of the composition is composed by the 3*R*,15*S* isomer. In fact, the natural product speciociliantine also has the opposite configuration on position 3 and it has an 13-fold decreased affinity when confronted with mitragynine. Finally, the reason why compound **38**, which was based on

the efficacious antinociceptive substance 7-hydroxymitragynine, did not show significant naloxone displacement is that it was evaluated as a mixture of enantiomers. It can also be added that the hydroxyl group configuration at the 7-position, which in 7-hydroxymitragynine is α, was not determined in **38**, thus there may be a chance that this is another justification for why this compound was not significantly active.

Figures 24 through 27 show the binding curves as well as *K*i and IC_{50} values of compounds that showed significant displacement.

Table 3 - Inhibition constant values for each receptor type. N.D.: Not determined

Compound	μ [^{3}H]DAMGO	δ [^{3}H]Enkephlin	κ [^{3}H]U-69,593
	K_i (μM)	K_i (μM)	K_i (μM)
Mitragynine	0.0820 ± 0.0055	0.3828 ± 0.0580	0.2156 ± 0.0149
6 (MA71 OXA)	N.D.	N.D.	1.885 ± 0.209
7 (MA114)	2.097 ± 0.190	8.836 ± 1.127	N.D.
24 (MA94 OXA)	N.D.	N.D.	8.347 ± 2.607
25 (MA127)	1.982 ± 0.327	38.92 ± 9.19	N.D.
33 (MA91 OXA)	N.D.	N.D.	0.3914 ± 0.0300
37 (MA108 HCL)	N.D.	N.D.	0.7257 ± 0.1548

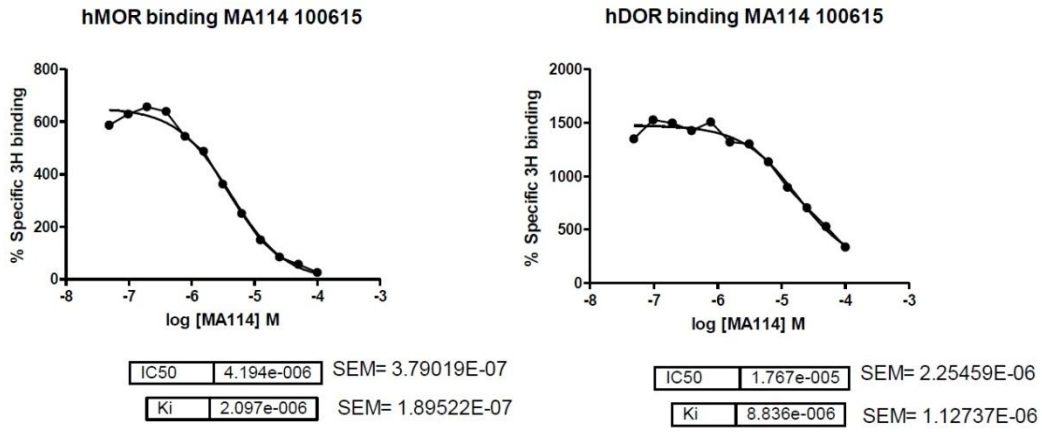


Figure 24 - Binding curves for compound 7 (μ- and δ-receptors).

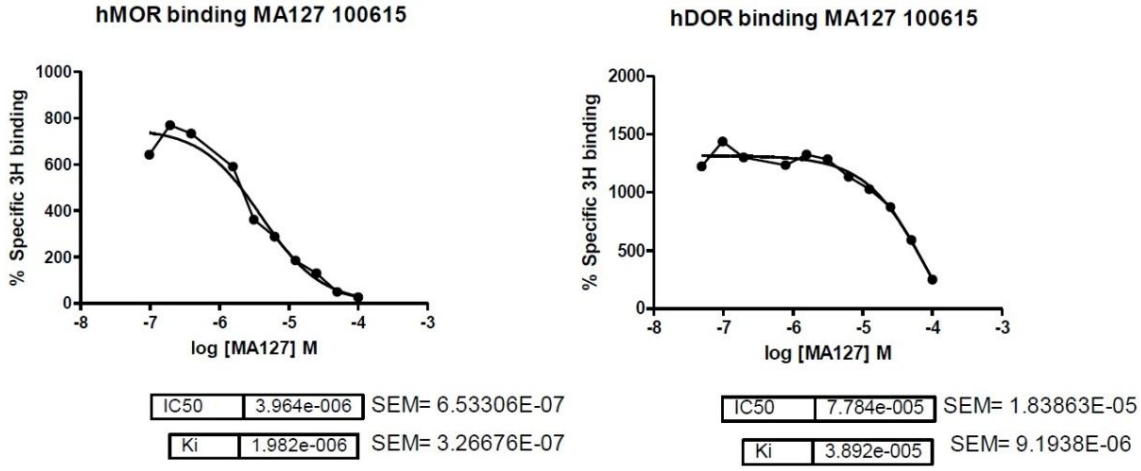


Figure 25 - Binding curves for compound 25 (μ- and δ-receptors).

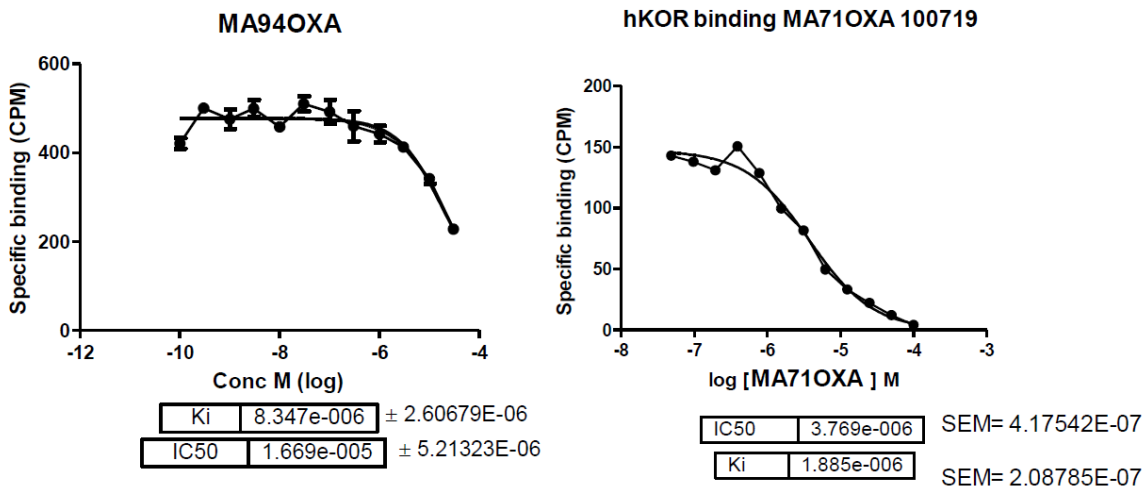


Figure 26 - Binding curves for compound 6 (MA71 OXA) and 24 (MA94 OXA) (κ-receptor).

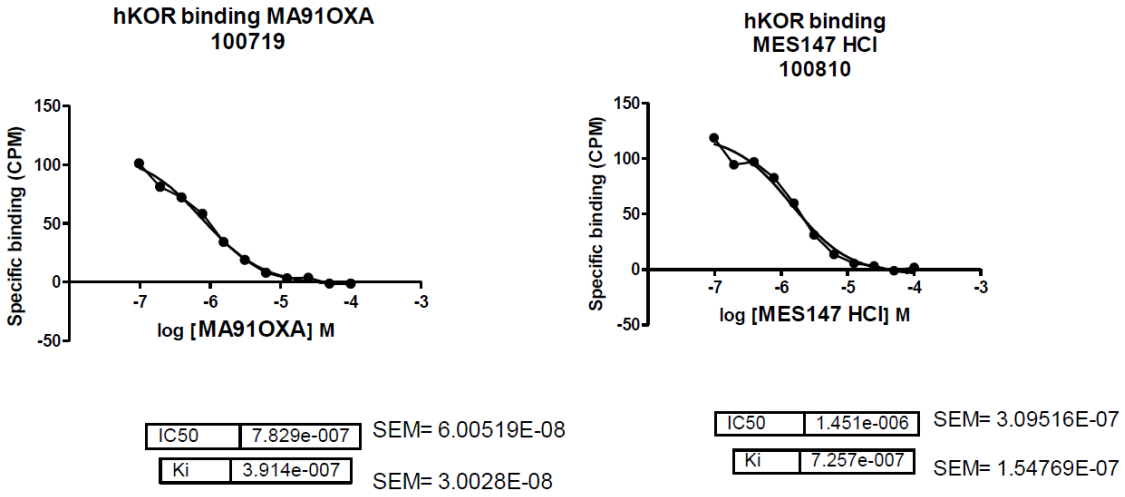


Figure 27 - Binding curves for compounds 33 (MA91 OXA) and 37 (MES147 HCL; obtained by the same method employed for MA108 HCL) (κ-receptor).

10. Final considerations

The biological results discussed above clearly show that piperidines (phenylpiperidines, phenylaminopiperidines and phenylamidopiperidines) displayed binding affinities that are lower than tetrahydro-β-carbolines affinities. Moreover, none of the compounds synthesized exhibited binding affinities lower than mitragynine. However, those binding studies demonstrated that these compounds showed moderate selectivity among μ-, δ- and κ-opioid receptors. Although piperidines had affinities in the micromolar range, two of them had significant inhibition constant on κ-receptors, but no expressive inhibition on μ- and δ-receptors was observed. Conversely, the other two piperidines with significant affinity for μ- and δ-receptors did not show important affinity for κ-receptors. In the case of tetrahydro-β-carbolines, they revealed higher binding affinities for κ-receptors than the piperidines. A general conclusion that can be drawn is that the higher flexibility of the piperidines and less similarity to mitragynine are detrimental to the overall binding affinity for opioid receptors. Indeed, tetrahydro-β-carbolines, which are structurally closer to mitragynine, showed affinities in the nanomolar range for at least one opioid receptor. It is also worth pointing out that the synthetic methods used to obtain these compounds did not take into consideration the stereoisomers produced during the synthesis. This drawback was more evident in the case of tetrahydro-β-carbolines synthesis, which generated multiple diastereoisomers. Isolation of these stereoisomers by preparative chiral chromatography, for instance, could have provided more insights about the requirements for opioid receptor binding.

As it was said before, the flexibility related to piperidines did not produce any significant affinity towards any opioid receptors when compared to mitragynine, since

molecules with more degrees of freedom are supposed to have less affinity. Indeed, the structurally rigid four-ringered terahydro-β-carbolines had the highest affinity on at least one type of opioid receptors, namely κ-receptors. Although no functional tests were performed, the higher affinity on κ-opioid receptors when compared to the other two receptor types may be advantageous in the future, as κ-receptors agonists are linked to the suppression of the rewarding effects induced by morphine[98].

Regarding the tetrahydro-β-carbolines biologically tested, it is worth noting that some portions of the four-ringered molecule play an important part on κ-opioid affinity, that is the methyl ester and the methyl methoxyacrylate moiety. While the methoxy allyl group is detrimental to affinity, the methyl ester at C15 revealed κ-affinity very close to mitragynine. In fact, unsubstituted and ketone substituted compounds at 15-position did not show any significant affinity for κ-receptors. From these observations, it is plausible to say that some portions of the designed compounds might not be necessary for affinity. Therefore, it is possible to address that the indolic portion is not essential, whereas the double-ringed heterocycle, octahydro-1*H*-quinolizine is the common feature between the active and not active tetrahydro-β-carbolines. Moreover, this multi-substituted moiety (mainly phenyl and methyl substitutions) is present in recent studies that show opioid affinity[99]. Consequently, octahydro-1*H*-quinolizine derivatives might be worthwhile to investigate in the future for opioid affinity. Furthermore, this work demonstrated that variations at the 15-position change opioid affinities dramatically, hence different groups at this position could be also explored for general opioid affinity.

11. Experimental section

Chromatography was performed with silica gel 60 (230 x 400 mesh). Mass spectra were obtained on a Waters micromass ZQ detector. NMR spectra were recorded on either a Bruker AVIII 400 spectrometer or Bruker DRX500. HPLC analyses were performed on a reverse phase XTerra R_8 (5 μm) column (4.6 X 100 mm) with 30% water, 10% NH_4OH solution in H_2O and 60% of CH_3CN as the mobile phase for 5 minutes, then the gradient changed to 100% CH_3CN in 5 minutes and the column was washed with CH_3CN for another 5 minutes. The flow rate was maintained at 1 mL/min on a Waters apparatus (Photodiode Array detector 996 and Separation Module 2695). Peaks were monitored at maximum absorbance from 210 to 400 nm. Chiral separations were performed on the same Waters apparatus with a Daicel Chemical Industries Chiracel OJ-H column (250 X 4.6 mm) using isopropanol with 0.5% diethylamine and hexane as eluents. Initial condition was 23% of isopropanol for 5 minutes at 1.45 mL/min, then a gradient to 10% isopropanol from 5 to 10 minutes, and finally maintaining 10% for the rest of chromatography analysis. Optical rotations were measured with Rudolph Research Analytical Autopol IV at 589 nm and concentration of 10% (m/v) in chloroform.

1-benzyl-*N*-(3-methoxyphenyl)piperidin-3-amine (13; MA62): 5.006 g (22.18 mmol) of 1-benzyl-3-piperidone hydrochloride was dissolved in water, basified with K_2CO_3, extracted three times with ethyl acetate and washed with brine. The organic phase was dried over $MgSO_4$ and the solvent was evaporated using reduced pressure, giving 4.0520 g (21.42 mmol) of an oily product (97% yield). In a round-bottom flask was added 75 mL of 1,2-dichloroethane, 2.51 mL (21.42 mmol) of *m*-anisidine and 4.0520 g (21.42 mmol) of 1-benzyl-3-piperidone. To the stirring solution under argon atmosphere was

added 1.22 mL (21.42 mmol) of acetic acid and 6.651 g (31.49 mmol) of $NaBH(OAc)_3$. The reaction mixture was stirred for 72 h at room temperature and then quenched with 1N NaOH, extracted with three portions of DCM, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude extract was purified by a silica column using a gradient of ethyl acetate/hexanes (from 10% to 70% ethyl acetate). It was obtained 3.17 g (10.7 mmol) of a yellowish oily liquid (50% yield). MS (ESI) *m/z* 296 (M + 1).

^{1}H NMR (400MHz, $CDCl_3$): δ ppm 7.36 (d, *J* = 4.5 Hz, 4H ,Ar), δ 7.29 (dd, *J* = 8.5, 4.2, 1H, Ar), δ 7.09 (t, 1H, *J* = 8 Hz, Ar), δ 6.28 – 6.24 (m, 2H, Ar), δ 6.19 (t, 1H, *J* = 4 Hz, Ar), δ 3.78 (s, 3H), δ 3.58 (s, 1H), δ 3.55 (d, 2H, *J* = 4 Hz), δ 2.76 (s, 1H), δ 2.43 (s, 2H), δ 2.31 (s, 1H), δ 1.76 (m, 2H), δ 1.58 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 160.94 (Cq), 148.53 (Cq), 138.32 (Cq), 129.01 (CHar), 128.25 (2 CHar), 128.24 (2 CHar), 127.04 (CHar), 106.38 (CHar), 102.17, 99.04, 63.19 (CH_2), 59.07 (CH_2), 55.08, 53.70, 48.78, 47.70, 22.73 (CH_2).

***N*-(3-methoxyphenyl)piperidin-3-amine (14; MA64):** In a flask designed for a Parr apparatus, approximately 150 mL of methanol was added along with 2.76 g (9.3 mmol) of 1-benzyl-*N*-(3-methoxyphenyl)piperidin-3-amine (**13**) and 690 mg of palladium on carbon (10%). The mixture was submitted to hydrogen atmosphere at 55 psi for 4 hours at room temperature and then the catalyst was filtered off with Celite in a fritted funnel under vacuum and the solvent was evaporated. Purification was performed using flash chromatography with a gradient of dichloromethane and methanol with ammonia (from 5% to 10% methanol). It was obtained a yellow oil with 85% yield (1.83 g; 7.9 mmol). MS (ESI) *m/z* 207.2 (M + 1).

^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.06 (t, *J* = 8.1, 1H), 6.24 (dt, *J* = 8.0, 1.9, 2H), 6.18 (t, *J* = 2.2, 1H), 3.76 (s, 3H), 3.45 (s, 2H), 3.22 (d, *J* = 11.7, 1H), 2.88 – 2.91 (m, 1H), 2.71 (d, *J* = 8.5, 1H), 2.55 (dd, *J* = 11.7, 7.6, 1H), 2.48 (s, 1H), 1.93 (d, *J* = 11.7, 1H), 1.79 – 1.72 (m, 1H), 1.56 – 1.47 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 160.91, 148.43, 130.05, 106.27, 102.28, 99.09, 55.07, 51.91, 50.40(2C) 49.15(2C), 46.44, 30.57, 24.19.

methyl 5-(3-(3-methoxyphenylamino)piperidin-1-yl)pentanoate (15; MA66): To a round-bottom flask equipped with a stirring bar, it was added 1.67 g (8.08 mmol) of *N*-(3-methoxyphenyl)piperidin-3-amine (**14**) dissolved in 20 mL of DMF, 1.39 mL (1.89 g; 9.7 mmol) of methyl 5-bromovalerate and 3.34 g (24.2 mmol) of anhydrous potassium carbonate. The mixture was stirred overnight at room temperature, poured in a sodium carbonate solution (pH = 9), extracted with three 50 mL portions of ethyl acetate, washed with three portions of brine, dried with Na_2SO_4, filtered and evaporated. The product was isolated by flash chromatography starting with dichloromethane and then a gradient of methanol/dichloromethane. It was obtained an yellow syrup with 53% yield (1.39 g; 4.33 mmol). MS (ESI) *m/z* 321.1 (M^+ + 1).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (t, *J* = 8.1, 1H), 6.23 (d, *J* = 8.2, 2H), 6.17 (t, *J* = 2.2, 1H), 4.04 (s, 1H), 3.76 (s, 3H), 3.67 (s, 3H), 3.54 (s, 1H), 2.68 (s, 1H), 2.36 – 2.30 (m, 6H), 2.23 (s, 1H), 1.70 (d, *J* = 10.9, 2H), 1.64 (dd, *J* = 15.1, 7.6, 2H), 1.51 (tt, *J* = 9.0, 4.5, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.00, 160.90, 148.49, 129.99, 106.26, 102.16, 99.98, 99.03, 59.19, 58.19, 55.02, 53.97, 51.46, 48.65, 33.87, 26.29, 22.90, 22.76.

methyl 5-(3-(3-methoxyphenylamino)piperidin-1-yl)pentanoate oxalate (MA66 OXA): A solution of 72 mg (0.225 mmol) of methyl 5-(3-(3-methoxyphenylamino)piperidin-1-yl)pentanoate in approximately 4 mL of THF was put to stir at room temperature in a 20 mL vial when another solution of 62 mg (0.69 mmol) oxalic acid in THF (approximately 4 mL) was added dropwise. It was observed that after each drop a suspension was formed and soon disappeared into the yellowish solution. The precipitate was visible only by addition of diethyl ether. The suspension was stirred for 1 h, filtered under reduced pressure in a fritted funnel, rinsed with approximately 180 mL of ether and dried in high vacuum. A white powder was obtained with quantitative yield. ^{1}H NMR (400 MHz, DMSO) δ 6.96 (t, *J* = 7.9, 1H), 6.22 (d, *J* = 9.2, 2H), 6.13 (d, *J* = 8.3, 1H), 5.74 (br-s, 1H), 3.66 (s, 4H), 3.57 (s, 3H), 3.38 (d, *J* = 10.2, 1H), 3.29 (d, *J* = 8.4, 1H), 2.98 (t, *J* = 8.0, 2H), 2.78 (s, 1H), 2.49 (s, 2H), 2.33 (t, *J* = 7.0, 2H), 1.88 (s, 2H), 1.77 (d, *J* = 10.3, 1H), 1.63 (s, 2H), 1.53 – 1.49 (m, 2H), 1.40 (d, *J* = 10.3, 1H).

methyl 5-(3-(N-(3-methoxyphenyl)propionamido)piperidin-1-yl)pentanoate (6; MA71): methyl 5-(3-(3-methoxyphenylamino)piperidin-1-yl)pentanoate (**15**) (100 mg; 0.312 mmol) was dissolved in 4 mL of 1,2-dichloroethane (DCE) in a round-bottom flask equipped with a condenser and stirring bar. To this solution, it was added 82 μL (0.936 mmol) of propionyl chloride and the mixture was refluxed for 3 hours. After this period 90 μL (0.65 mmol) of triethylamine in 1 mL of DCE was added dropwise over 5 minutes and the reaction mixture was refluxed for another 2 hours. The mixture was poured into K_2CO_3 solution and extracted with 3 portions of DCM, washed with brine, dried over Na_2SO_4, filtered and evaporated. Purification took place by flash chromatography with

silica gel and a gradient of methanol/DCM to give orange oil with 95% yield (111.4 mg; 0.296 mmol). MS (ESI) *m/z* 377.1 [M^+ + H]. HRMS *m/z* 277.2433 (calculated 277.2440). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (t, *J* = 8.0, 1H), 6.87 (dd, *J* = 8.3, 2.1, 1H), 6.62 (d, *J* = 7.7, 1H), 6.57 (s, 1H), 3.77 (s, 3H), 3.61 (s, 3H), 2.77 (d, *J* = 7.1, 1H), 2.32 - 2.22 (m, 5H), 1.90 (dd, *J* = 14.5, 7.1, 2H), 1.76 (t, *J* = 10.4, 2H), 1.59 – 1.56 (m, 2H), 1.57 (dd, *J* = 14.9, 7.4, 3H), 1.47 (dd, *J* = 15.1, 8.0, 2H), 1.06 (t, *J* = 7.5, 2H), 0.96 (t, *J* = 7.4, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.83, 173.35, 160.15, 140.68, 129.76, 122.26, 116.17, 113.30, 57.75, 56.92, 55.34, 52.84, 51.82, 51.29, 33.74, 29.07, 28.26, 26.04, 24.30, 22.81, 9.48.

methyl 5-(3-(N-(3-methoxyphenyl)propionamido)piperidin-1-yl)pentanoate oxalate (MA71 OXA): methyl 5-(3-(N-(3-methoxyphenyl)propionamido)piperidin-1-yl)pentanoate was dissolved in 2 mL of THF and put to stir in a 20 mL vial. Oxalic acid (100 mg) dissolved in 2 mL of THF was added, but no precipitate was observed. A suspension was only formed after adding few milliliters of anhydrous diethyl ether. The precipitate was filtered with a fritted funnel under reduced pressure and the excess of oxalic acid was rinsed with 200 mL of ether. A white solid was obtained (50.4 mg). (Yield not calculated).

^{1}H NMR (400 MHz, DMSO) δ 7.37 (s, 1H), 7.01 (s, 1H), 6.83 (s, 2H), 4.71 (s, 1H), 3.77 (s, 3H), 3.58 (s, 3H), 3.46 (s, 1H), 3.37 (s, 1H), 3.21 (s, 1H), 2.88 (s, 2H), 2.49 (s, 3H), 2.34 (s, 2H), 1.83 (d, *J* = 25.9, 4H), 1.56 (d, *J* = 26.1, 4H), 1.08 (s, 2H), 0.88 (s, 3H).

1-benzyl-4-(2-methoxyphenyl)piperidin-4-ol (17; MA109): To an oven-dried 250 mL two-neck round-bottom flask equipped with a condenser was added 989 mg (40.7 mmol) of magnesium turnings, 50 mL of freshly dried THF, two crystals of iodine and

approximately 5 mL of 1-bromo-2-methoxybenzene dissolved in 125 mL of THF. Using a heat gun and vigorous agitation, the solution was heated until it became colorless. The remaining solution of 1-bromo-2-methoxybenzene was added and the mixture was refluxed for 2.5 hours and brought to room temperature. It was observed that the solution turned into dark green over time and the magnesium disappeared after only one hour. In a 500 mL round-bottom flask, 6.60 mL (37.0 mmol) of *N*-benzyl-4-piperidone was dissolved in 125 mL of THF at 0 °C. The Grignard reagent was then slowly transferred to the piperidone solution and stirred for two hours. A solution of ammonium chloride was added and the crude obtained by extracting with ethyl acetate, washing with brine, drying with anhydrous sodium sulfate and concentrating. After purification using a methanol/dichloromethane gradient in silica, it was obtained 2.97 g (10 mmol; 27% yield). MS (ESI) *m/z* 298.3 [M^+].

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.29 (m, 7H), 6.99 (td, *J* = 7.6, 1.0, 1H), 6.95 (d, *J* = 8.2, 1H), 4.06 (s, 1H, OH), 3.89 (s, 3H), 3.63 (s, 2H), 2.81 (d, *J* = 10.8, 2H), 2.65 (td, *J* = 11.8, 2.6, 2H), 2.17 (td, *J* = 12.8, 4.3, 2H), 2.07 (dd, *J* = 13.9, 2.5, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 157.35, 138.71, 135.24, 129.34, 128.24, 126.98, 125.66, 121.19, 111.42, 71.15,63.38, 63.34, 63.30, 55.36, 55.32, 55.27, 55.23, 55.27, 49.30, 36.25.

1-benzyl-4-(2-methoxyphenyl)-1,2,3,6-tetrahydropyridine (18; MA110): Dehydration of the tertiary alcohol took place by refluxing 40 mL of toluene with 2.97 g (10 mmol) of 1-benzyl-4-(2-methoxyphenyl)piperidin-4-ol (**17**) and 2.28 g (12 mmol) of *p*-toluenesulfonic acid for 27h. The mixture was poured into a potassium carbonate solution, extracted with three portions (60 mL) of ethyl acetate and dried over sodium

sulfate. After purification with methanol/dichloromethane in silica gel, 1.67 g (5.6 mmol) of yellowish viscous oil with 56% yield was obtained. MS (ESI) *m/z* 280.3 [M^+].

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 7.6, 2H), 7.39 (d, *J* = 7.1, 2H), 7.34 – 7.28 (m, 1H), 7.26 – 7.22 (m, 2H), 6.96 (t, *J* = 7.4, 1H), 6.90 (d, *J* = 8.1, 1H), 5.84 (s, 1H), 3.84 (s, 3H), 3.70 (s, 2H), 3.22 (s, 2H), 2.74 (t, *J* = 5.6, 2H), 2.61 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.85, 138.31, 129.45, 129.31, 129.27, 128.45, 128.23,128.13, 127.06, 120.56, 111.13, 110.83, 110.79, 84.72, 62.84, 55.36, 53.27, 49.94, 29.52.

4-(2-methoxyphenyl)-piperidine (19; MA111): To a hydrogenation flask with 130 mL of ethanol was added 1.48 g (5.3 mmol) of 1-benzyl-4-(2-methoxyphenyl)-1,2,3,6-tetrahydropyridine (**18**) and 300 mg of 10% w/w of palladium on carbon. The mixture was shaken in a Parr hydrogenation apparatus under 55 psi of hydrogen for 47 hours. Palladium and carbon were filtered off using Celite under reduced pressure. After purification by chromatography with methanol with ammonia/dichloromethane, it was obtained 726 mg (3.8 mmol) of a pale yellow solid with 72% yield. MS (ESI) *m/z* 192.3 [M^+].

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (dd, *J* = 13.3, 7.0, 2H), 6.94 (t, *J* = 7.4, 1H), 6.86 (d, *J* = 8.0, 1H), 3.83 (s, 3H), 3.22 (d, *J* = 11.3, 2H), 3.13 – 3.07 (m, 2H), 2.80 (t, *J* = 11.6, 2H), 1.82 (d, *J* = 12.1, 2H), 1.66 (qd, *J* = 12.1, 3.1, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 156.70, 134.52, 126.88, 126.62, 120.65, 110.35, 55.31, 47.04 (2C), 35.35, 32.73 (2C).

Methyl 5-(4-(2-methoxyphenyl)piperidin-1-yl)pentanoate (20; MA112): To 10 mL of *N*,*N*-dimethylformamide was added 716 mg (3.74 mmol) 4-(2-methoxyphenyl)-

piperidine (**19**) and 943 mg (11.2 mmol) of anhydrous sodium bicarbonate along with 0.591 mL (4.12 mmol) of methyl 5-bromovalerate. The mixture was stirred for two hours at 60 °C. It was then extracted with ethyl acetated in aqueous potassium carbonate (pH=9), washed with brine and dried over sodium sulfate. After flash column chromatography using methanol/dichloromethane as eluents, 81.6% (930 mg; 3.05 mmol) yield was obtained. MS (ESI) *m/z* 306.3 [M + 1].

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (dd, *J* = 7.6, 1.5, 1H), 7.16 (td, *J* = 7.7, 1.5, 1H), 6.92 (t, *J* = 7.4, 1H), 6.84 (d, *J* = 8.2, 1H), 3.81 (s, 3H), 3.66 (s, 3H), 3.04 (d, *J* = 11.5, 2H), 2.99 - 2.92 (m, 1H), 2.41 -2.33 (m, 4H), 2.08 (td, *J* = 11.2, 3.7, 2H), 1.79 (dd, *J* = 9.8, 3.5, 3H), 1.75 - 1.70 (m, 1H), 1.68 – 1.54 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.01, 156.81, 134.36, 126.81, 126.56, 120.63, 110.28, 58.62, 55.31, 55.28, 54.58, 51.47, 51.45, 35.07, 33.95, 31.99, 26.50, 23.10.

Methyl 2-formyl-5-(4-(2-methoxyphenyl)piperidin-1-yl)pentanoate (21; MA113): It was added 911 mg (2.98 mmol) of methyl 5-(4-(2-methoxyphenyl)piperidin-1-yl)pentanoate (**20**) to an oven dried three-neck round-bottom flask with 40 mL of freshly distilled THF in argon atmosphere. While maintaining the solution stirring at -70 °C, 3.7 mL (7.4 mmol) of lithium diisopropylamide in a 2 M solution was added dropwise. After 40 minutes, 0.922 mL (14.9 mmol) of methyl formate was added and the mixture was agitated for one hour at -70 °C and for two hours at room temperature. The reaction mixture was poured into a solution of ammonium chloride, extracted with ethyl acetate and dried over Na_2SO_4. Purification with flash chromatography using methanol/dichloromethane afforded 694 mg (2.08 mmol; 70%) of an orange sticky syrup. MS (ESI-) *m/z* 332.3 [M -1].

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.68 (s, 1H), 7.99 (s, 1H), 7.19 (dd, *J* = 12.9, 7.4, 2H), 6.92 (t, *J* = 7.5, 1H), 6.85 (d, *J* = 8.1, 1H), 3.82 (s, 3H), 3.67 (s, 3H), 3.14 (d, *J* = 11.7, 2H), 3.09 – 3.01 (m, 1H), 2.46 (t, *J* = 6.2, 2H), 2.40 – 2.37 (m, 2H), 2.28 – 2.21 (m, 2H), 1.93 – 1.89 (m, 4H), 1.76 (dt, *J* = 12.5, 6.3, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.94, 156.70, 132.90, 127.27, 126.55, 120.77, 110.32, 55.26, 54.30, 53.77, 53.19, 51.52, 50.76, 34.66, 30.47, 23.70, 19.64.

(*E*)-methyl 2-(methoxymethylene)-5-(4-(2-methoxyphenyl)piperidin-1-yl)pentanoate (7; MA114): In 125 mL of methanol in a round-bottom flask with a condenser was dissolved 668 mg (2.0 mmol) of methyl 2-formyl-5-(4-(2-methoxyphenyl)piperidin-1-yl)pentanoate (**21**) and added a total of 6.54 mL (30 mmol) of trimethyl orhoformate and 2.28 g (12.0 mmol) of *p*-toluenesulfonic acid. The mixture was refluxed for 12 hours and extracted with three portions of ethyl acetate in aqueous K_2CO_3, washed with brine, dried with Na_2SO_4, filtered and evaporated. After purifying three times with flash chromatography employing methanol/dichloromethane as eluents, 114 mg (0.33 mmol; 16.5%) of pale yellow solid was obtained. MS (ESI^+) *m/z* 348.3 [M + H]. HPLC analysis showed 98% purity.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 1H), 7.22 (dd, *J* = 7.5, 1.5, 1H), 7.23 – 7.14 (m, 1H), 6.92 (t, *J* = 7.5, 1H), 6.85 (d, *J* = 8.2, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.70 (s, 3H), 3.05 (d, *J* = 11.5, 2H), 2.98 – 2.91 (m, 1H), 2.40 – 2.36 (m, 2H), 2.26 (t, *J* = 7.2, 2H), 2.11 – 2.04 (m, 2H), 1.80 – 1.75 (m, 4H), 1.70 – 1.62 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.88, 158.89, 156.80, 134.51, 126.75, 126.58, 120.62, 110.66, 110.27, 61.20, 58.75, 55.31, 54.51, 51.13, 35.09, 32.06, 26.03, 21.99.

(*E*)-methyl 2-(methoxymethylene)-5-(4-(2-methoxyphenyl)piperidin-1-yl)pentanoate hydrochloride (MA114 HCL): (*E*)-methyl 2-(methoxymethylene)-5-(4-(2-methoxyphenyl)piperidin-1-yl)pentanoate (73.7mg; 0.212 mmol) was dissolved in THF and a saturated solution of hydrochloric acid in diethyl ether as added. After stirring for few minutes, the solvent was evaporated under reduced pressure and 57 mg (0.14 mmol) a pale yellow solid was obtained by drying in high vacuum.

^{1}H NMR (400 MHz, DMSO) δ 10.34 (s, 1H), 7.20 (t, *J* = 7.7, 1H), 7.11 (d, *J* = 7.1, 1H), 6.97 (d, *J* = 8.2, 1H), 6.92 (t, *J* = 7.4, 1H), 3.77 (s, 3H), 3.73 – 3.66 (m, 2H), 3.62 – 3.59 (m, 1H), 3.48 (d, *J* = 9.7, 2H), 3.37 (s, 6H), 3.27 – 3.13 (m, 2H), 3.02 (s, 3H), 2.01 (d, *J* = 11.5, 2H), 1.85 (d, *J* = 13.7, 2H), 1.74 (d, *J* = 5.6, 2H).

4-(2-methoxyphenyl)-4-hydroxypiperidine (23; MA118): In a pressure flask for a Parr hydrogenation apparatus was added 1.40 g (4.7 mmol) of 1-benzyl-4-(2-methoxyphenyl)piperidin-4-ol (**17**) dissolved in 100 mL of ethanol. Palladium on carbon (700 mg; 10%) was slowly added to the solution and the flask was shaken for 15 hours under 55 psi of hydrogen at room temperature. The palladium and carbon were filtered off under reduced pressure using Celite as a filter aid. The product was purified by column chromatography using a gradient of methanol/dichloromethane. A white solid was obtained (747.7 mg; 3.61 mmol) with 76.8% yield. MS (ESI) m/z 208.2 [M + 1].

^{1}H NMR (400 MHz, DMSO) δ 7.53 (d, *J* = 7.6, 1H), 7.18 (t, *J* = 7.7, 1H), 6.95 (d, *J* = 8.2, 1H), 6.89 (t, *J* = 7.5, 1H), 4.55 (br s, 1H), 3.78 (s, 3H), 2.94 (t, *J* = 11.7, 3H), 2.69 (d, *J* = 10.4, 2H), 2.28 (td, *J* = 12.8, 4.5, 2H), 1.35 (d, *J* = 12.6, 2H).

^{13}C NMR (101 MHz, DMSO) δ 171.84, 156.85, 137.69, 136.39, 128.12, 126.72, 120.59, 112.20, 71.27, 55.69, 42.28, 36.15.

Methyl 5-(4-hydroxy-4-(2-methoxyphenyl)piperidin-1-yl)pentanoate (24; MA94): In a 35 mL round-bottom flask was put a solution of 612.8 mg (2.95 mmol) of 4-(2-methoxyphenyl)-4-hydroxypiperidine (**23**) in 7 mL *N*,*N*-dimethylformamide, 0.464 mL (3.24 mmol) of methyl 5-bromovalerate and 734 mg (8.85 mmol) of sodium bicarbonate. The reaction mixture was stirred at 60 °C and followed by TLC. After 1:15 h no starting material could be observed. The product was poured in aqueous $NaHCO_3$ and extracted with three portions (35 mL) of ethyl acetate, washed three times with brine, dried over Na_2SO_4, filtered and evaporated. Purification took place by using a silica gel column and methanol/dichloromethane gradient (2 - 20% of methanol). A yellow oil was obtained with 70% yield (664.9 mg; 2.07 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (dd, *J* = 7.8, 1.5, 1H), 7.26 – 7.22 (m, 1H), 6.96 (dd, *J* = 7.6, 1.1, 1H), 6.92 (d, *J* = 7.4, 1H), 4.06 (s, 1H), 3.89 (s, 3H), 3.66 (s, 3H), 2.86 (d, *J* = 11.3, 2H), 2.64 (t, *J* = 10.7, 2H), 2.50 (t, *J* = 7.3, 2H), 2.35 (t, J = 7.0, 2H), 2.20 (td, *J* = 13.5, 4.7, 2H), 2.05 (dd, *J* = 14.1, 2.6, 2H), 1.64 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.94, 157.20, 136.39, 134.57, 128.37, 125.59, 121.23, 111.34, 70.80, 58.13, 55.29, 51.50, 49.15, 35.72, 33.83, 26.08, 22.96.

methyl 5-(4-hydroxy-4-(2-methoxyphenyl)piperidin-1-yl)pentanoate oxalate (MA94 OXA) : A solution of approximately 180 mg (0.56 mmol) of methyl 5-(4-hydroxy-4-(2-methoxyphenyl)piperidin-1-yl)pentanoate in THF was stirring when 178 mg (1.98 mmol) of oxalic acid dissolved in 10 mL of diethyl ether was added. The suspension was stirred for one hour and then filtered under reduced pressure and washed with 200 mL of ether to remove the excess of oxalic acid. A white solid was obtained (244 mg; 0.59 mmol).

^{1}H NMR (400 MHz, DMSO) δ 7.54 (d, *J* = 7.5, 1H), 7.24 (t, *J* = 7.1, 1H), 7.00 (d, *J* = 8.1, 1H), 6.95 (t, *J* = 7.0, 1H), 5.18 (s, 7H), 3.84 (s, 3H), 3.63 (s, 3H), 3.26 (d, *J* = 7.8, 4H), 3.03 (m, 2H), 2.70 (dd, *J* = 19.7, 11.3, 2H), 2.37 (t, *J* = 7.0, 2H), 1.76 (d, *J* = 11.0, 4H), 1.65 (m, 2H).

Methyl 5-(4-acetoxy-4-(2-methoxyphenyl)piperidin-1-yl)pentanoate (25; MA127): In a 10 mL round-bottom flask dried with an heat gun was added 1.3 mL (18 mmol) of dimethylsulfoxide and 1.6 mL of acetic anhydride (17 mmol). The solution was stirred under argon atmosphere for 30 min. After this period, a solution of 249.5 mg (0.776 mmol) of methyl 5-(4-hydroxy-4-(2-methoxyphenyl)piperidin-1-yl)pentanoate (**24**) in 1.0 mL (14 mmol) of dimethylsulfoxide was added and the stirring mixture turn into yellow. The reaction mixture was followed by TLC for 21 hours, but no progress was observed after 5 hours. The solution turned into orange and it was extracted with an aqueous solution of K_2CO_3 (pH=9) and three portions of 25 mL of ethyl acetate, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The product was purified by flash chromatography using a gradient of MeOH (saturated with NH_3)/DCM (2 – 4% of MeOH). A yellow solid was obtained with a yield of 41.4% (116.5 mmol; 0.321 mmol). MS (ESI+) m/z 264.2 $[M + H]^+$. HPLC analysis showed 95.8% purity.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, *J* = 6.8, 1H), 7.25 – 7.21 (m, 1H), 6.93 (t, *J* = 7.6, 1H), 6.88 (d, *J* = 8.2, 1H), 3.81 (s, 3H), 3.67 (s, 3H), 2.87 (d, *J* = 11.4, 2H), 2.67 – 2.60 (m, 2H), 2.45 (dd, *J* = 15.0, 8.7, 3H), 2.37 (dd, *J* = 13.7, 6.8, 3H), 2.27 – 2.20 (m, 2H), 2.04 (s, 3H), 1.71 – 1.58 (m, 4H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.96, 169.42, 156.83, 136.39, 128.65, 126.72, 120.47, 111.61, 99.99, 79.87, 63.60, 58.10, 55.20, 51.51, 49.38, 33.81, 33.57, 26.19, 22.92, 21.71.

(*E*)-4-methoxy-3-(2-nitrovinyl)-1*H*-indole (27; MA81): Trifluoroacetic acid (20 mL) was poured into a round-bottom flask and brought to 0° C under agitation in argon atmosphere when 4.34 g (37.4 mmol) of (*E*)-N,N-dimethyl-2-nitroethenamine was introduced. To this solution, 5.00 g (34.0 mmol) of 4-methoxyindole was added and stirred for 15 minutes. Since 4-methoxyindole did not dissolve, 20 mL of DCM was added, bringing it to solution, which was stirred for another 30 minutes at room temperature. The reaction mixture was poured into ice-water and extracted with three 120 mL portions of ethyl acetate. The organic phase was then put in a conical flask and a saturated solution of $NaHCO_3$ was slowly added under vigorous agitation until no CO_2 formation was observed. The crude extract was washed with brine, dried over Na_2SO_4, filtered and evaporated to obtain a dark orange solid (yield not calculated).

^{1}H NMR (400 MHz, DMSO) δ 12.23 (s, 1H), 8.56 (d, *J* = 13.3, 1H), 8.25 (s, 1H), 8.10 (d, *J* = 13.3, 1H), 7.16 (t, *J* = 7.9, 1H), 7.10 (d, *J* = 8.0, 1H), 6.73 (d, *J* = 7.7, 1H), 3.95 (s, 3H).

^{13}C NMR (101 MHz, DMSO) δ 154.11, 139.26, 135.97, 132.71, 124.72, 115.60, 108.42, 106.44, 102.95, 99.99, 55.79.

4-methoxytryptamine (28; MA83): Freshly distilled THF (250 mL) was put in a round-bottom flask along with 7.41 g (34 mmol) of (*E*)-4-methoxy-3-(2-nitrovinyl)-1*H*-indole. The solution was brought to -78° C with acetone/dry ice bath and stirred under argon atmosphere for 20 minutes. After this period, 28.3 mL (68 mmol) of lithium aluminum hydride in THF (2.4 M) was added dropwise over 20 minutes and agitated for another 30 minutes. The reaction mixture was brought to room temperature, stirred overnight and quenched with 2.7 mL of water, 2.7 mL of aqueous NaOH (15%) and 2.7 mL of water

until no hydrogen formation was observed. The precipitate was filtered off and rinsed with ethyl acetate. The solvents were evaporated and the solid was dissolved in ethyl acetate, dried over Na_2SO_4, filtered and evaporated. The compound was purified with a silica gel column using a gradient of methanol (with dissolved NH_3)/DCM. It was obtained 1.0 g of an orange solid with an overall yield (2 steps) of 15.5 %. MS (ESI) *m/z* 191.1 [M^+ + H].

^{1}H NMR (400 MHz, CDCl3) δ 8.63 (s, 1H), 7.07 (t, *J* = 7.9, 1H), 6.94 (d, *J* = 8.1, 1H), 6.84 (s, 1H), 6.47 (d, *J* = 7.7, 1H), 3.90 (s, 3H), 3.01 (s, 4H), 2.01 (s, 2H).

^{13}C NMR (101 MHz, CDCl3) δ 154.81, 138.30, 122.64, 121.21, 117.32, 113.73, 104.60, 99.25, 55.09, 43.10, 30.82.

***N*-(2-(4-methoxy-1*H*-indol-3-yl)ethyl)formamide (29; MA95):** In a round-bottom flask equipped with a condenser was added 5.03 g (26.4 mmol) of 4-methoxytryptamine (**28**), 30 mL of ethyl formate and 10 mL of methanol (4-methoxytryptamine is not soluble in ethyl formate). The solution was refluxed for 20 hours and solvents evaporated. The product was purified with flash chromatography using silica gel and 3% methanol/DCM as eluent to obtain 5.20 g (24.0 mmol) with 90% yield.

^{1}H NMR (400 MHz, CDCl3) δ 8.59 (s, 1H), 8.04 (s, 1H), 7.10 (t, *J* = 8.0, 1H), 6.96 (dd, *J* = 8.2, 3.1, 1H), 6.84 (d, *J* = 2.3, 1H), 6.51 (d, J = 7.8, 1H), 6.08 (s, 1H), 3.93 (d, J = 1.9, 3H), 3.62 (q, *J* = 6.2, 2H), 3.09 (t, *J* = 6.5, 2H).

^{13}C NMR (101 MHz, CDCl3) δ 161.49, 154.40, 138.29, 122.82, 121.55, 117.17, 112.79, 104.92, 99.44, 55.14, 39.81, 26.29.

5-methoxy-4,9-dihydro-3*H*-pyrido[3,4-b]indole (30; MA97): To a round-bottom flask were added 4.1 g (18.8 mmol) of *N*-(2-(4-methoxy-1*H*-indol-3-yl)ethyl)formamide (**29**)

and 25 mL of phosphorus oxychloride (26.9 mmol). The mixture was stirred at 50 °C for 1:20 h, $POCl_3$ was partially evaporated under reduced pressure (15 minutes) and then quenched with ice/water (caution: very reactive!). The dark green mixture was poured into a conical flask and ammonium hydroxide was added under agitation, making the color change to orange. The product was extracted with 4 portions of DCM, washed with brine, dried over Na_2SO_4, filtered and evaporated.

^{1}H NMR (400 MHz, DMSO) δ 11.29 (s, 1H), 8.30 (s, 1H), 7.07 (t, *J* = 7.9, 1H), 6.97 (d, *J* = 8.2, 1H), 6.47 (d, *J* = 7.7, 1H), 3.83 (s, 3H), 3.72 (t, *J* = 7.6, 2H), 2.92 (m, 2H).

^{13}C NMR (101 MHz, DMSO) δ 155.23, 151.72, 138.31, 127.71, 125.03, 114.03, 110.53, 105.94, 99.87, 55.52, 48.64, 20.97.

8-methoxy-1,3,4,6,7,12b-hexahydroindolo[2,3-a]quinolizin-2(12*H*)-one (31; MA103 [MA88]): To a round-bottom flask equipped with a condenser were added 2.36 g (11.8 mmol) of 5-methoxy-4,9-dihydro-3*H*-pyrido[3,4-b]indole (**30**) dissolved in 400 mL of methanol, 236 mg (1.73 mmol) of zinc chloride and 2.9 mL (35.4 mmol)mg of methylvinylketone. The mixture was refluxed under agitation for 4 hours and then the solvent was evaporated. The crude material was basified with aqueous K_2CO_3, extracted with ethyl acetate, dried over Na_2SO_4, filtered and evaporated. Product isolation was carried out by flash chromatography in silica gel using a gradient of ethyl acetate/hexanes. After evaporating the solvent, 2.73 g (10.1 mmol) of white powder was obtained with a 64% yield. MS (ESI) *m/z* 271 (M + 1). HPLC purity 99%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.06 (t, *J* = 8.0, 1H), 6.93 (d, *J* = 8.1, 1H), 6.50 (d, *J* = 7.7, 1H), 3.90 (s, 3H), 3.59 (d, *J* = 10.9, 1H), 3.34 – 3.30 (m, 1H), 3.32 – 3.15

(m, 2H), 3.12 – 3.17 (m, 1H), 2.82 -2.76 (m, 2H), 2.72 (dd, *J* = 12.4, 2.7, 1H), 2.68 (d, *J* = 4.5, 1H), 2.63 – 2.57 (m, 1H), 2.51 – 2.46 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 208.17, 154.49, 137.61, 131.20, 122.57, 117.13, 108.32, 104.46, 99.87, 58.53, 55.26, 54.26, 52.14, 45.76, 41.59, 23.77.

Methyl 2-(8-methoxy-1,3,4,6,7,12b-hexahydroindolo[2,3-a]quinolizin-2(12*H*)-ylidene)acetate (32; MA89): In a round-bottom flask, 350 mg of NaH in 60% of mineral oil (8.76 mmol of NaH) was suspended in 20 mL of freshly dried THF and cooled to 0 °C under argon atmosphere. Trimethylphosphonoacetate (1.27 mL; 8.76 mmol) was added dropwise, forming a thick suspension that blocked magnetic stirring. This problem was circumvented by addition of 40 mL of THF and the mixture was agitated for 30 minutes. After this period, 8-methoxy-1,3,4,6,7,12b-hexahydroindolo[2,3-a]quinolizin-2(12*H*)-one (**31**) (790 mg; 2.92 mmol) dissolved in THF was added and the reaction mixture was stirred for 2 hours at room temperature. The excess of NaH was quenched with water and the product was extracted with ethyl acetate, dried with Na_2SO_4, filtered and evaporated. Isolation was carried out with flash chromatography employing a gradient of ethyl acetate/hexanes to obtain 932 mg of a mixture of diastereomers (2.86 mmol; 98% yield). MS [ESI] *m/z* 327.1 [M^+ + H] for both diastereomers.

(Top spot on the TLC) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.03 (t, *J* = 8.0, 1H), 6.92 (d, *J* = 8.1, 1H), 6.47 (d, *J* = 7.8, 1H), 5.76 (s, 1H), 3.88 (s, 3H), 3.84 (d, *J* = 12.2, 1H), 3.73 (s, 3H), 3.35 (d, *J* = 11.2, 1H), 3.21 – 3.10 (m, 3H), 3.04 (dd, *J* = 16.1, 4.0, 1H), 2.65 – 2.59 (m, 2H), 2.50 – 2.44 (m, 3H).

(Top spot on the TLC) ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.79, 154.52, 137.51, 131.25, 122.44, 117.30, 114.97, 108.36, 104.35, 99.87, 60.09, 55.26, 55.02, 52.47, 51.08, 40.58, 29.71, 29.18, 23.41.

Methyl 2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acetate (33; MA91): In a flask designed for hydrogenation was put 100 mL of methanol, 90 mg of palladium on carbon (10%) and then 882 mg (2.7 mmol) of methyl 2-(8-methoxy-1,3,4,6,7,12b-hexahydroindolo[2,3-a]quinolizin-2(12*H*)-ylidene)acetate (**32**) dissolved in methanol. The mixture was agitated for 5 hours under hydrogen at 40 psi, filtered under reduced pressure using Celite to remove the catalyst and the solvent evaporated. Isolation was carried out by flash chromatography using a gradient of ethyl acetate/hexanes. Yield 55% (490 mg; 1.49 mmol). MS (ESI) *m/z* 329.1 [M^+ + H]. HPLC purity 93%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.01 (t, *J* = 7.9, 1H), 6.89 (d, *J* = 8.0, 1H), 6.47 (d, *J* = 7.7, 1H), 3.88 (s, 3H), 3.73 (s, 3H), 3.20 (d, *J* = 11.3, 1H), 3.18 – 3.11 (m, 1H), 3.05 (d, *J* = 6.5, 1H), 3.00 (dd, *J* = 15.9, 4.8, 2H), 2.58 (td, *J* = 11.5, 4.6, 1H), 2.44 – 2.36 (m, 1H), 2.32 (dd, *J* = 13.4, 7.0, 2H), 2.26 (d, *J* = 7.5, 1H), 2.14 (d, *J* = 12.3, 1H), 1.78 (d, *J* = 11.6, 1H), 1.52 (qd, *J* = 12.3, 4.2, 1H), 1.34 – 1.25 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.14 (C19), 154.52 (C9), 137.40 (C13), 132.71 (C2), 122.02 (C11), 117.55 (C7), 108.03 (C8), 104.27 (C12), 99.81 (C10), 59.54 (C3), 55.33 (9-OCH_3), 55.26 (C17), 53.44 (C5), 51.61 (19-OCH_3), 40.98 (C18), 36.13 (C14), 33.04 (C15), 32.04 (C16), 23.76 (C6).

Methyl 2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acetate oxalate (MA91 OXA): In a 20 mL vial were added a solution of 51.0 mg

(0.155 mmol) of methyl 2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acetate dissolved in diethyl ether and a solution of 114 mg (1.27 mmol) of oxalic acid in ether. After addition of the oxalic acid solution, precipitation followed immediately and the mixture was stirred for 30 minutes. The salt was filtered off under reduced pressure and rinsed with ether to remove the excess of oxalic acid. It was obtained 45.6 mg (0.109 mmol; 70% yield) of a white solid.

^{1}H NMR (400 MHz, DMSO) δ 11.06 (s, 1H), 6.97 (t, *J* = 7.8, 1H), 6.91 (d, *J* = 8.0, 1H), 6.46 (d, *J* = 7.5, 1H), 4.34 (br-s, 1H), 3.80 (s, 3H), 3.64 (s, 3H), 3.50 (br-s, 1H), 3.41 (d, *J* = 10.9, 1H), 3.16 – 3.04 (m, 4H), 2.49 (s, 3H), 2.37 (ddd, *J* = 34.7, 15.7, 7.0, 2H), 2.17 (br-s, 1H), 1.86 (d, *J* = 13.7, 1H), 1.54 (dd, *J* = 23.4, 10.6, 1H), 1.41 (dd, *J* = 24.6, 11.8, 1H).

8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizine (34; MA104): To a 10 mL round-bottom flask equipped with a condenser with 4 mL of ethylene glycol, 100 mg (0.34 mmol) of 8-methoxy-1,3,4,6,7,12b-hexahydroindolo[2,3-a]quinolizin-2(12*H*)-one (**31**) was dissolved along with 190 mg (3.4 mmol) of crushed KOH and 0.13 mL (2.7 mmol) of hydrazine hydrate. The mixture was stirred at 190 °C for one hour before the condenser was removed and then stirred for two hours. A solution of ammonium chloride was poured into the flask and the crude extract obtained by extracting with three portions of 30 mL of ethyl acetate, washing with brine. The solution was dried over anhydrous sodium sulfate, filtered and evaporated. After purification with flash chromatography (methanol/dichloromethane), it was obtained 78 mg (0.30 mmol; 84%) of solid. MS (ESI) *m/z* 279.3 [M + Na^+]. HPLC purity 94%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.02 (t, *J* = 7.9, 1H), 6.91 (d, *J* = 8.1, 1H), 6.48 (d, *J* = 7.7, 1H), 3.89 (s, 3H), 3.21 (d, *J* = 11.4, 1H), 3.20 – 3.14 (m, 1H), 3.05 – 2.98 (m, 2H), 2.61 (td, *J* = 11.5, 4.5, 1H), 2.37 (td, *J* = 11.2, 3.5, 1H), 2.02 (d, *J* = 16.8, 2H), 1.89 (d, *J* = 12.2, 1H), 1.77 – 1.73 (m, 2H), 1.59 (ddd, *J* = 15.2, 12.5, 3.2, 1H), 1.52 – 1.44 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 154.50, 137.32, 133.14, 121.93, 117.61, 107.92, 104.22, 99.80, 60.24, 55.33, 53.86, 30.04, 29.96, 25.67, 24.28, 23.56.

8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizine hydrochloride (MA104 HCL): 8-methoxy-1,3,4,6,7,12b-hexahydroindolo[2,3-a]quinolizin-2(12H)-one was dissolved in diethyl ether before a solution of HCl in ether was added and stirred for approximately 30 minutes. A pale yellow solid (23 mg) was obtained after filtering, washing with ether and dried under high vacuum.

^{1}H NMR (400 MHz, DMSO) δ 11.17 (s, 1H), 10.77 (s, 1H), 6.99 (t, *J* = 7.9, 1H), 6.93 (d, *J* = 7.9, 1H), 6.47 (d, *J* = 7.5, 1H), 4.55 (t, *J* = 11.1, 1H), 3.81 (s, 3H), 3.56 (d, *J* = 6.4, 1H), 3.46 (d, *J* = 11.4, 1H), 3.34 (d, *J* = 13.7, 3H), 3.17 (dd, *J* = 23.1, 10.8, 1H), 3.06 (d, *J* = 11.7, 1H), 2.57 (d, *J* = 13.0, 1H), 1.99 (dd, *J* = 26.1, 13.2, 1H), 1.91 – 1.82 (m, 1H), 1.73 (dd, *J* = 26.8, 13.6, 1H), 1.62 (dd, *J* = 24.8, 13.0, 1H).

Methyl 2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)-3-oxopropanoate (35; MA106): To an oven-dried three-neck round-bottom flask equipped with a thermometer was added 40 mL of anhydrous THF was dissolved 0.9 g (2.7 mmol) of methyl 2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acetate (**33**). The solution was brought to -78 °C with a bath of dry ice in acetone while stirring under argon. A solution of lithium diisopropylamide (3.4 mL; 6.8 mmol; 2M in THF)

was added dropwise over 10 minutes while it was observed that the internal temperature did not rise above -65 °C. The mixture was stirred for 30 minutes before the addition of 0.85 mL (13 mmol) of methyl formate and agitation for 3.5 hours at room temperature. A solution of ammonium chloride was poured into the flask and the material was extracted with three portions (30 mL) of ethyl acetate. The solvent was then evaporated and the product purified using silica gel and a gradient of methanol/dichloromethane. After two purifications by chromatography, the solid obtained weighed 93 mg (0.26 mmol; 9.6%). MS (ESI) *m/z* 357.3 [M + 1].

^{1}H NMR (400 MHz, DMSO) δ 10.58 (s, 1H), 7.65 (s, 1H), 6.85 (dt, *J* = 15.2, 7.5, 2H), 6.39 (d, *J* = 7.4, 1H), 3.78 (s, 3H), 3.56 (s, 3H), 3.53 (s, 1H), 3.22 (s, 1H), 3.12 (d, *J* = 11.0, 1H), 2.93 - 2.88 (m, 2H), 2.82 – 2.78 (m, 2H), 2.44 – 2.38 (m, 1H), 2.33 – 2.28 (m, 1H), 2.19 - 2.09 (m, 1H) , 2.03 – 1.98 (m, 1H), 1.95 (t, *J* = 12.2, 1H), 1.39 (d, *J* = 12.5, 1H).

Methyl 3,3-dimethoxy-2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)propanoate (36; MA107): To a round-bottom flask with a condenser was dissolved 93 mg (0.26 mmol) of methyl 2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)-3-oxopropanoate (**35**) in 25 mL of methanol along with 148 mg (0.78 mmol) of *p*-toluenesulfonic acid monohydrate and 0.28 mL (2.6 mmol) of trimethyl orthoformate. The mixture was stirred at reflux temperature under argon for 2.5 hours and the solvent was evaporated under reduced pressure. Extraction with ethyl acetate and aqueous sodium carbonate and purification with silica gel column (3% methanol/dichloromethane) afforded 73 mg (0.18 mmol; 69% yield) of material. MS (ESI) *m/z* 403.2 [M + 1].

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 7.2, 1H), 7.01 (t, *J* = 7.9, 1H), 6.90 (d, *J* = 8.1, 1H), 6.46 (d, *J* = 7.8, 1H), 4.75 (dd, *J* = 16.5, 8.5, 1H), 3.87 (s, 3H), 3.67 (d, *J* = 10.4, 3H), 3.41 (s, 3H), 3.38 (d, *J* = 8.2, 3H), 3.21 (d, *J* = 11.0, 1H), 3.16 – 3.11 (m, 1H), 3.04 – 2.97 (m, 3H), 2.78 (dd, *J* = 8.5, 5.3, 1H), 2.55 (td, *J* = 11.4, 4.5, 1H), 2.43 - 2.34 (m, 1H), 2.17 (d, *J* = 12.8, 1H), 1.83 (d, *J* = 13.8, 1H), 1.72 – 1.63 (m, 1H), 1.57 – 1.52 (m, 1H), 1.43 - 1.37 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.86, 154.47, 137.42, 132.67, 121.97, 117.48, 107.82, 104.35, 99.73, 60.42, 55.28, 55.27, 55.19, 52.88, 51.63, 35.49, 34.61, 32.52, 30.54, 28.24, 23.68, 21.03.

(*E*)-methyl 3-methoxy-2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acrylate (37; MA108): In approximately 1 mL of *N*,*N*-dimethylformamide was dissolved 70.8 mg (0.178 mmol) of methyl 3,3-dimethoxy-2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)propanoate (**36**) and a total of 74 mg (0.6 mmol) of potassium tert-butoxide. The reaction was maintained under agitation at approximately 60 °C for 6 hours before aqueous solution of ammonium chloride was added. The mixture was extracted with three portions of ethyl acetate and washed twice with brine. After drying with sodium sulfate, filtering and purifying twice by chromatography, it was obtained 20.1 mg (0.054 mmol) with a 31% yield. MS (ESI) *m/z* 371.2 [M + 1].

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.32 (s, 1H), 6.99 (t, *J* = 7.8, 1H), 6.87 (d, *J* = 8.1, 1H), 6.44 (d, *J* = 7.6, 1H), 3.84 (s, 3H), 3.75 (s, 3H), 3.70 (s, 3H), 3.25 (d, *J* = 11.6, 1H), 3.11 (d, *J* = 11.5, 2H), 3.00 (d, *J* = 17.4, 1H), 2.87 (t, *J* = 11.8, 1H), 2.61 - 2.55 (m,

1H), 2.42 (t, *J* = 11.8, 1H), 2.34 - 2.25 (m, 1H), 2.14 (dd, *J* = 23.8, 11.8, 1H), 1.92 (d, *J* = 13.4, 1H), 1.61 - 1.56 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.16, 159.99, 154.47, 137.52, 132.64, 121.90, 117.48, 112.94, 107.50, 104.40, 99.65, 61.59, 60.13, 55.67, 55.23, 51.31, 33.68, 33.92, 29.69, 23.35, 22.68.

(*E*)-methyl 3-methoxy-2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acrylate hydrochloride: A few milligrams of (*E*)-methyl 3-methoxy-2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acrylate (**37**) was dissolved in THF before a solution of hydrochloric acid in diethyl ether was added dropwise. The suspension formed was stirred and then the solvents evaporated under reduced pressure to afford an yellowish solid.

^{1}H NMR (500 MHz, DMSO) δ 11.17 (s, 1H), 10.57 (s, 1H), 7.50 (s, 1H), 7.01 (t, *J* = 7.8, 1H), 6.93 (d, *J* = 8.1, 1H), 6.50 (d, *J* = 7.6, 1H), 4.71 (t, *J* = 8.0, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.65 (s, 3H), 3.63 (s, 1H), 3.53 (d, *J* = 11.1, 1H), 3.36 - 3.27 (m, 3H), 3.12 - 3.10 (m, 2H), 2.47 (d, *J* = 11.6, 2H), 2.30 (dd, *J* = 25.2, 12.4, 1H), 1.71 (d, *J* = 12.6, 1H).

methyl 2-((7a)-7a-hydroxy-8-methoxy-1,2,3,4,6,7,7a,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acetate (38; MA116): To a mixture of 2.5 mL of acetonitrile and 0.8mL of water was added 61.0 mg (0.186 mmol) of methyl 2-(8-methoxy-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)acetate (**33**). The solution was brought to 0 °C and stirred under argon before a solution of 79.9 mg (0.186 mmol) of [bis(trifluoroacetoxy)iodo]benzene (PIFA) in 0.75 mL of acetonitrile was added dropwise over a period of five minutes. After stirring for two hours, the mixture was poured into an aqueous solution of K_2CO_3 (pH=9), extracted with three portions of 25 mL of ethyl

acetate, washed with brine, dried with anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The product was purified by preparative HPLC, giving 9.8 mg (0.028 mmol; 15% yield) of an orange solid. HPLC analysis showed 94.3% purity. MS (ESI) *m/z* 345.04 $[M + H]^+$.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (t, *J* = 8.0, 1H), 7.20 (d, *J* = 7.6, 1H), 6.75 (d, *J* = 8.2, 1H), 3.87 (s, 3H), 3.68 (s, 3H), 3.16 (dd, *J* = 11.0, 2.3, 1H), 3.00 (dt, *J* = 11.2, 3.0, 1H), 2.83 (td, *J* = 12.2, 2.6, 1H), 2.72 – 2.70 (m, 1H), 2.68 (s, 1H), 2.64 (t, *J* = 2.2, 1H), 2.45 – 2.37 (m, 2H), 2.27 (dd, *J* = 15.6, 7.6, 1H), 2.15 (dd, *J* = 13.1, 2.9, 1H), 2.05 – 1.95 (m, 1H), 1.78 (d, *J* = 12.6, 1H), 1.68 – 1.59 (m, 2H), 1.37 (td, *J* = 12.6, 3.8, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 183.52, 173.02, 155.91, 154.68, 131.03, 126.31, 114.23, 109.10, 80.88, 59.43, 55.46, 55.38, 51.54, 49.74, 40.96, 35.91, 32.93, 32.39, 29.71.

12. General bioassay procedure

12.1. Materials

All chemicals used were from Sigma-Aldrich (Poole, Dorset, U.K.) with the following exceptions. For the binding experiments, [^{3}H]DAMGO (53.4 Ci/mmol), [^{3}H]U-69,593 (42.7 Ci/mmol), [^{3}H]Enkephlin (45 Ci/mmol), were obtained from Perkin-Elmer Life Sciences Inc. (Boston, MA, U.S.A.). DAMGO, DPDPE, nor-Binaltorphimine were obtained from Tocris Bioscience (Ellisville, Missouri, U.S.A.).

12.2. Cell culture

CHO-K1 cells stably transfected with opioid receptor subtypes μ, δ, and κ were a generous gift from Roth labs. (University of North Carolina at Chapel Hill, Chapel Hill, N.C., U.S.A.). These cells were maintained at 37^{o}C and 5% CO_2 in a DMEM nutrient mixture supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 0.5% penicillin–streptomycin, and either G418 (600 mg/mL) or hygromycin B (300 mg/mL).

Membranes were prepared by scraping the cells in a 50mM Tris buffer, homogenized via sonication and centrifuged for 40 minutes at 13650 rpm at 4^{o}C. These were kept at -80^{o}C. Protein concentration was found via Bio-Rad Protein Assay (Hercules, California, U.S.A), an adaptation of the Bradford method of protein determination[100].

12.3. Radio-ligand binding for opioid receptor subtypes

Opioid binding took place under the following conditions: 10μM of each compound was incubated with [^{3}H]DAMGO (μ), [^{3}H]U-69,593 (κ),or [^{3}H]Enkephlin (δ) for 60 minutes in a 96-well plate . Tritium and membrane concentration for each cell line is determined by saturation experiments performed after each batch of membrane is

scraped. The reaction was terminated via rapid vacuum filtration through GF/B filters presoaked with 0.3% BSA using a Perkin Elmer 96-well Unifilter followed by 10 washes of 50 mM Tris. Plates were read using a Perkin Elmer Topcount. Total binding was defined as binding in the presence of 0.1% DMSO. Non-specific binding was defined as binding observed in the presence of 10μM DAMGO (μ), nor-Binaltorphimine (κ),or DPDPE (δ). Specific binding was the difference between total and non-specific binding. Percent binding was found with the following formula:

100-(Binding of compound- non-specific binding)*100/Specific Binding

To obtain a dose response curve, concentrations of compound ranging from 100μM to 48nM were incubated for 60 minutes in a 96-well plate with a predetermined amount of [^{3}H] specific to each membrane type. Optimal membrane concentration was also predetermine by a saturation experiment. The reaction was terminated via rapid vacuum filtration through GF/B filters presoaked in 0.3% BSA using a Perkin Elmer 96-well Unifilter followed by 10 washes of 50mM Tris. Plates were read using a Perkin Elmer Topcount . Total binding was defined as binding in the presence of 0.1% DMSO. Non-specific binding was defined as binding observed in the presence of 10 μM of the control specific for the receptor of interest. Specific binding was the difference between total and non-specific binding. K_i and IC_{50} values were calculated using the software Graph-Pad Prism 5.

13. References

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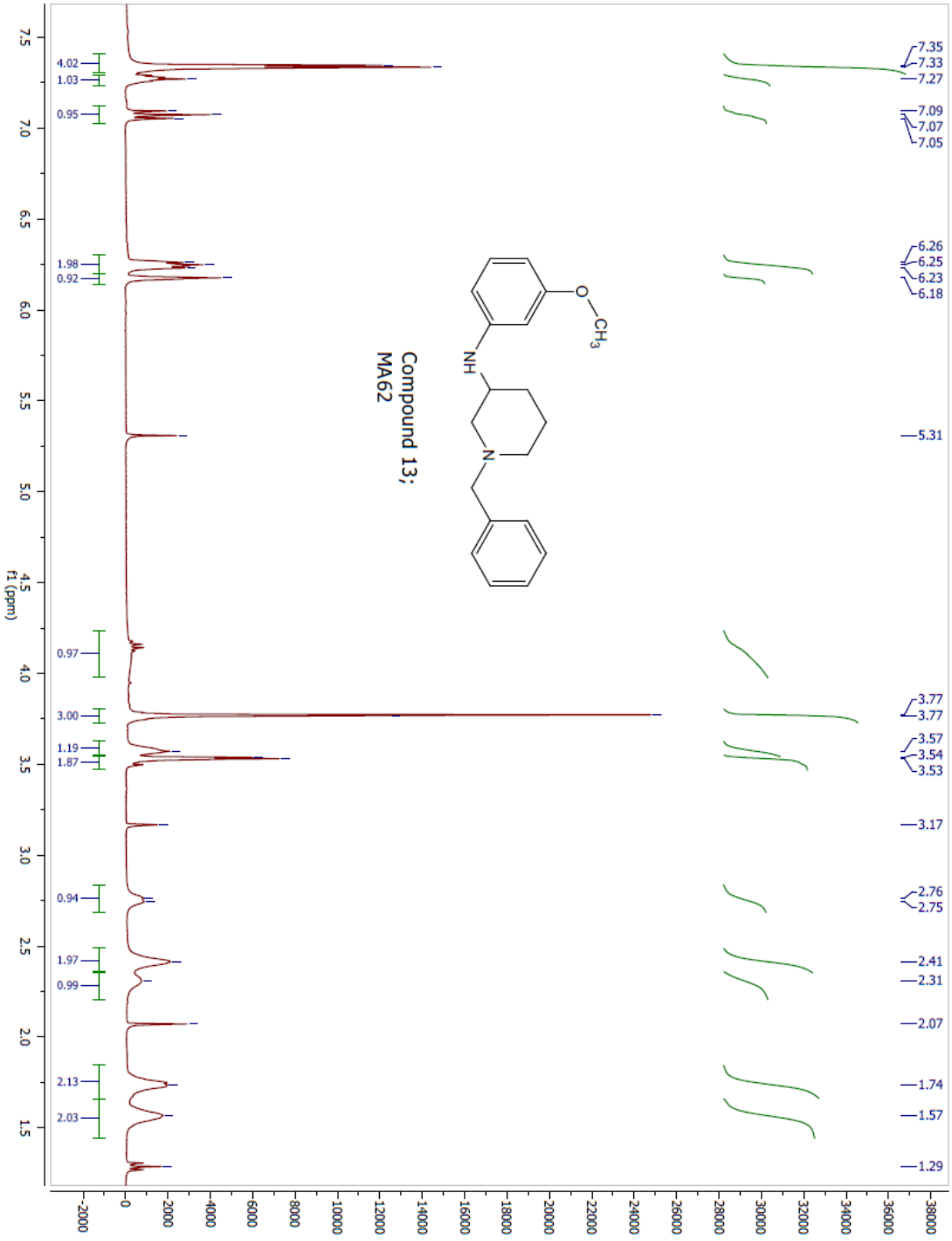
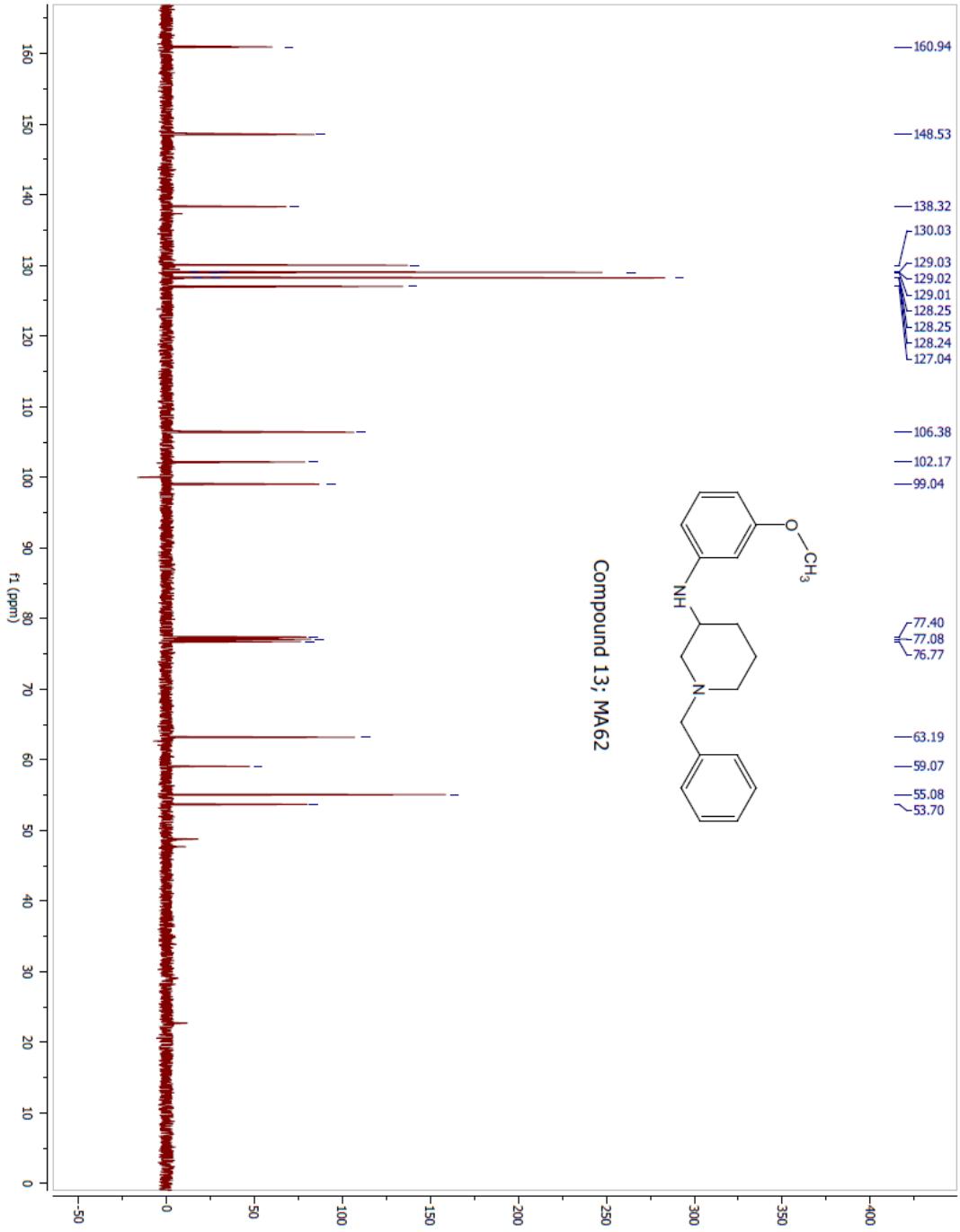
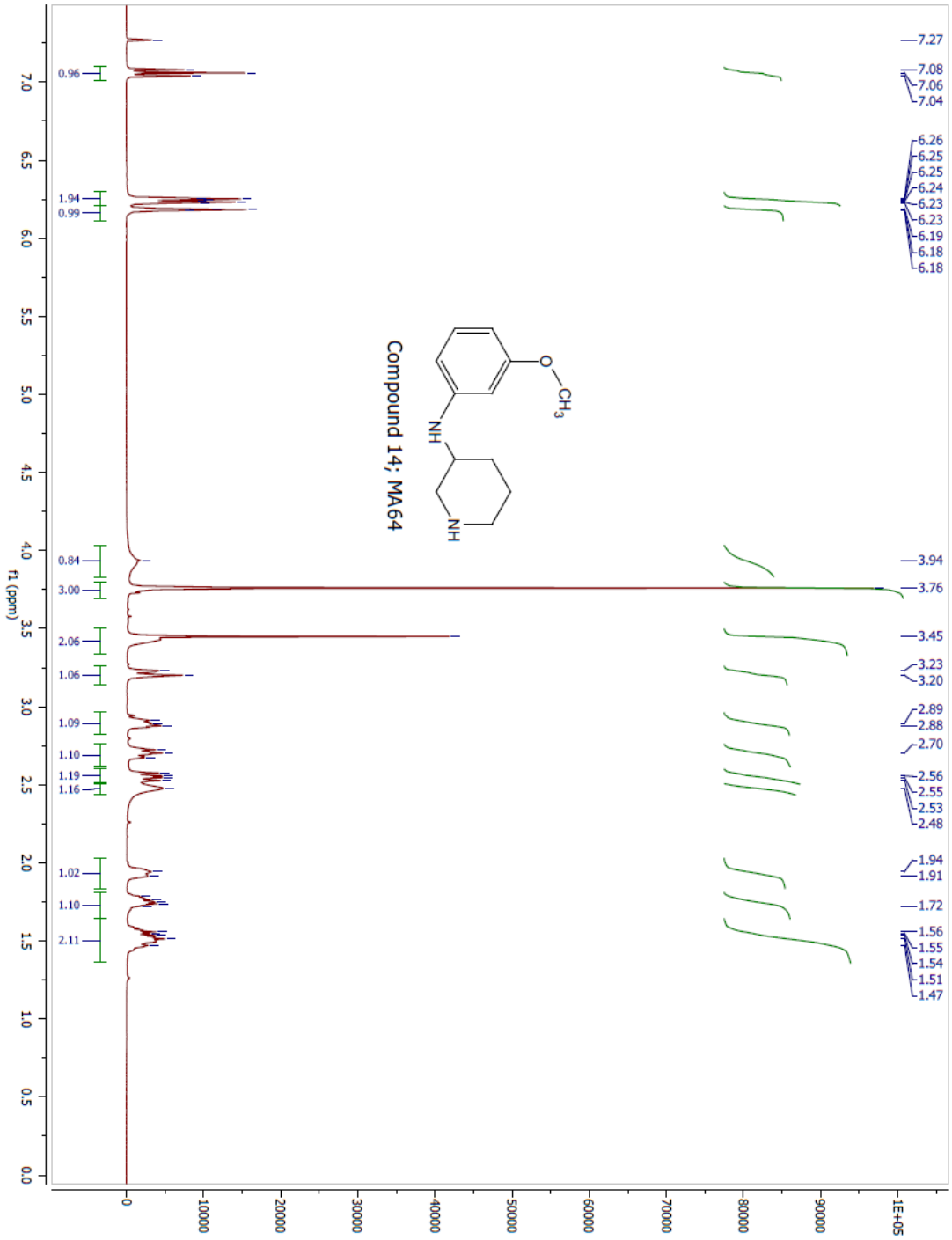
Appendix: NMR spectral data




160.94
148.53
138.32
130.03
129.03
129.02
129.01
128.25
128.25
128.24
127.04
106.38
102.17
99.04
77.40
77.08
76.77
63.19
59.07
55.08
53.70
Compound 13; MA62
NH
N
O
CH3
f1 (ppm)


Compound 14; MA64
NH
NH
O
CH3
f1 (ppm)
7.27
7.08
7.06
7.04
6.26
6.25
6.25
6.24
6.23
6.23
6.19
6.18
6.18
3.94
3.76
3.45
3.23
3.20
2.89
2.88
2.70
2.56
2.55
2.53
2.48
1.94
1.91
1.72
1.56
1.55
1.54
1.51
1.47
0.96
1.94
0.99
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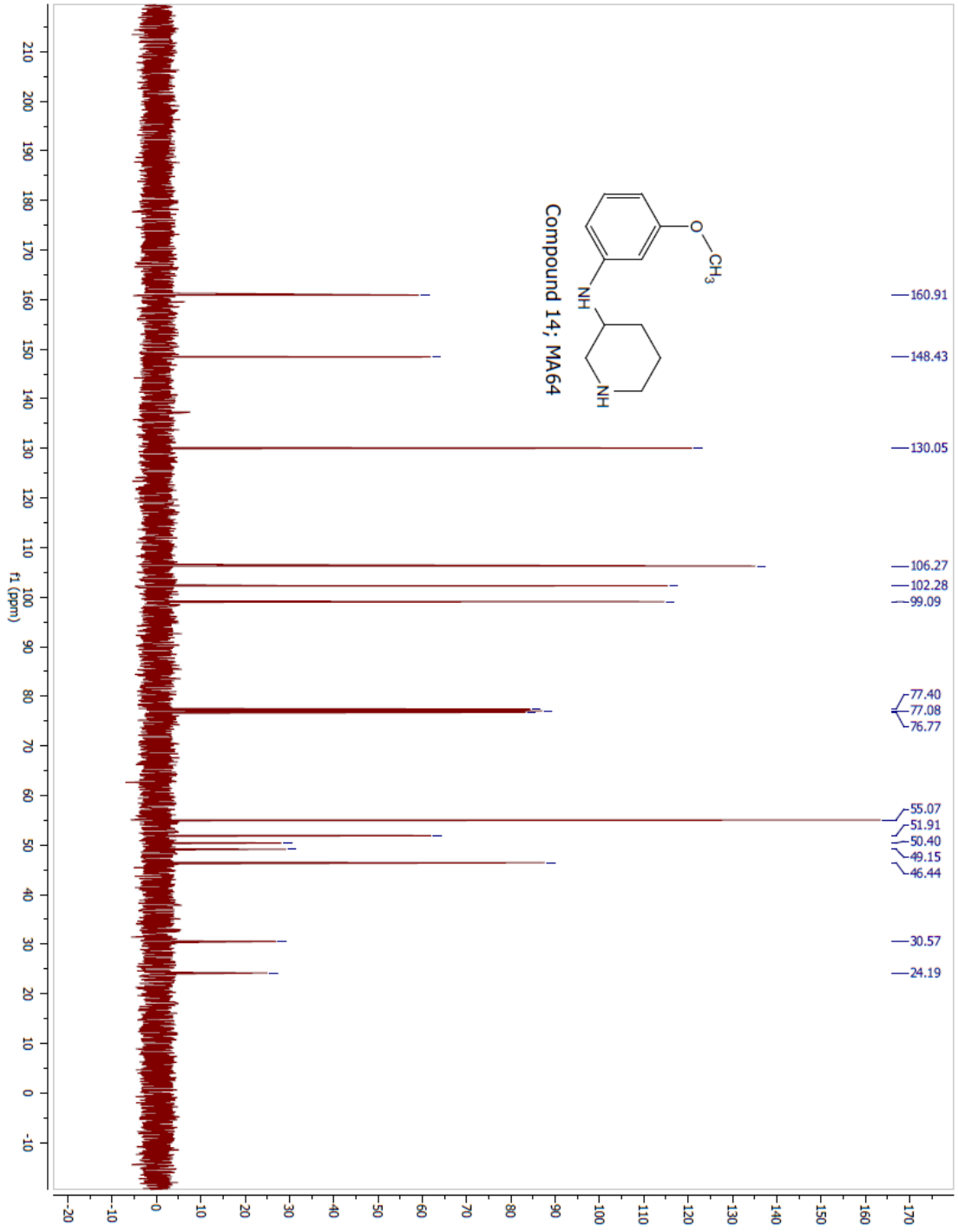
3.00
2.06
1.06
1.09
1.10
1.19
1.16
1.02
1.10
2.11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NH
O
CH3
160.91
148.43
130.05
106.27
102.28
99.09
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50.40
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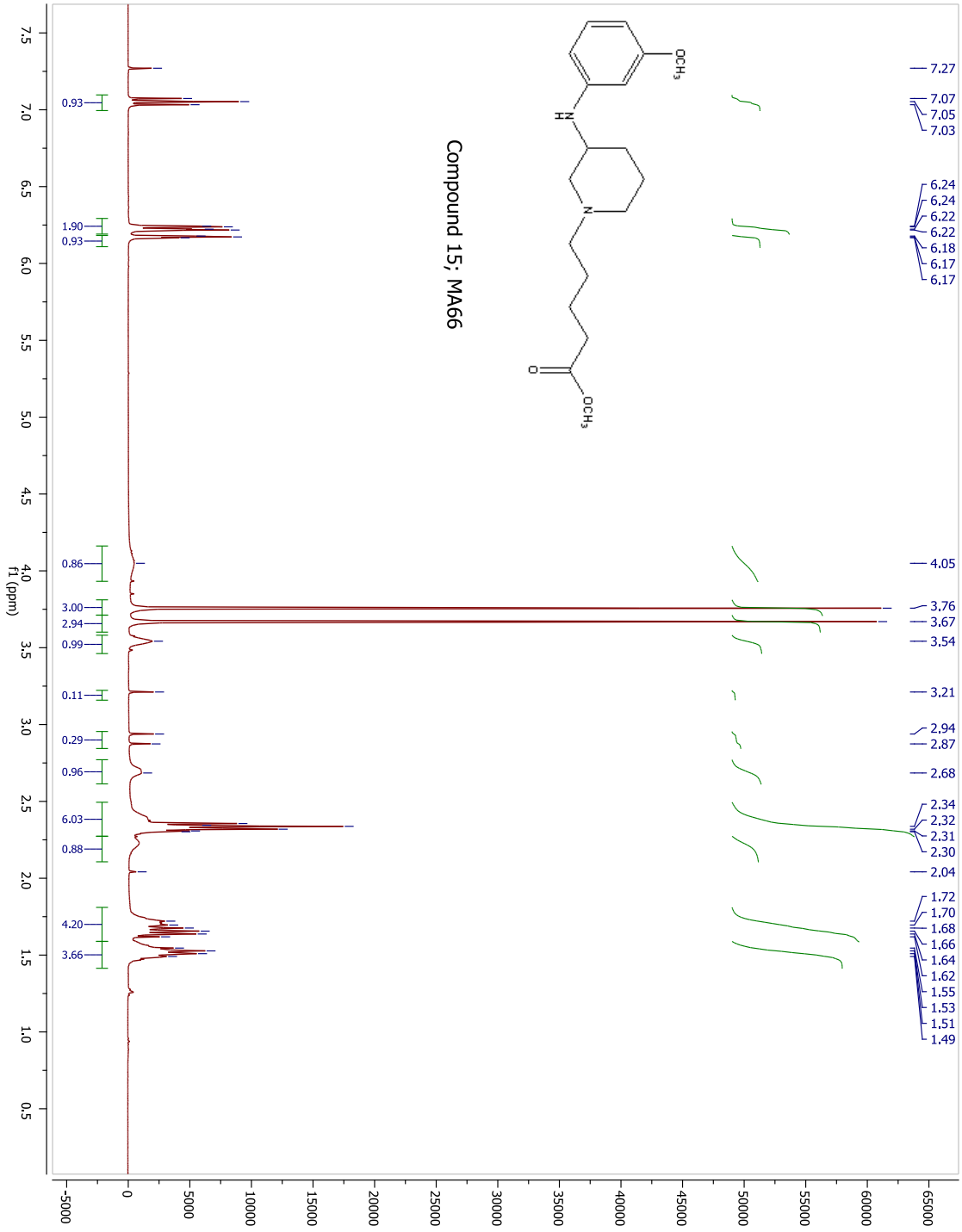
46.44
30.57
24.19
f1 (ppm)


Compound 15; MA66
f1 (ppm)
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7.07
7.05
7.03
6.24
6.24
6.22
6.22
6.18
6.17
6.17
4.05
3.76
3.67
3.54
3.21
2.94
2.87
2.68
2.34
2.32
2.31
2.30
2.04
1.72
1.70
1.68
1.66
1.64
1.62
1.55
1.53
1.51
1.49
0.93
1.90
0.93
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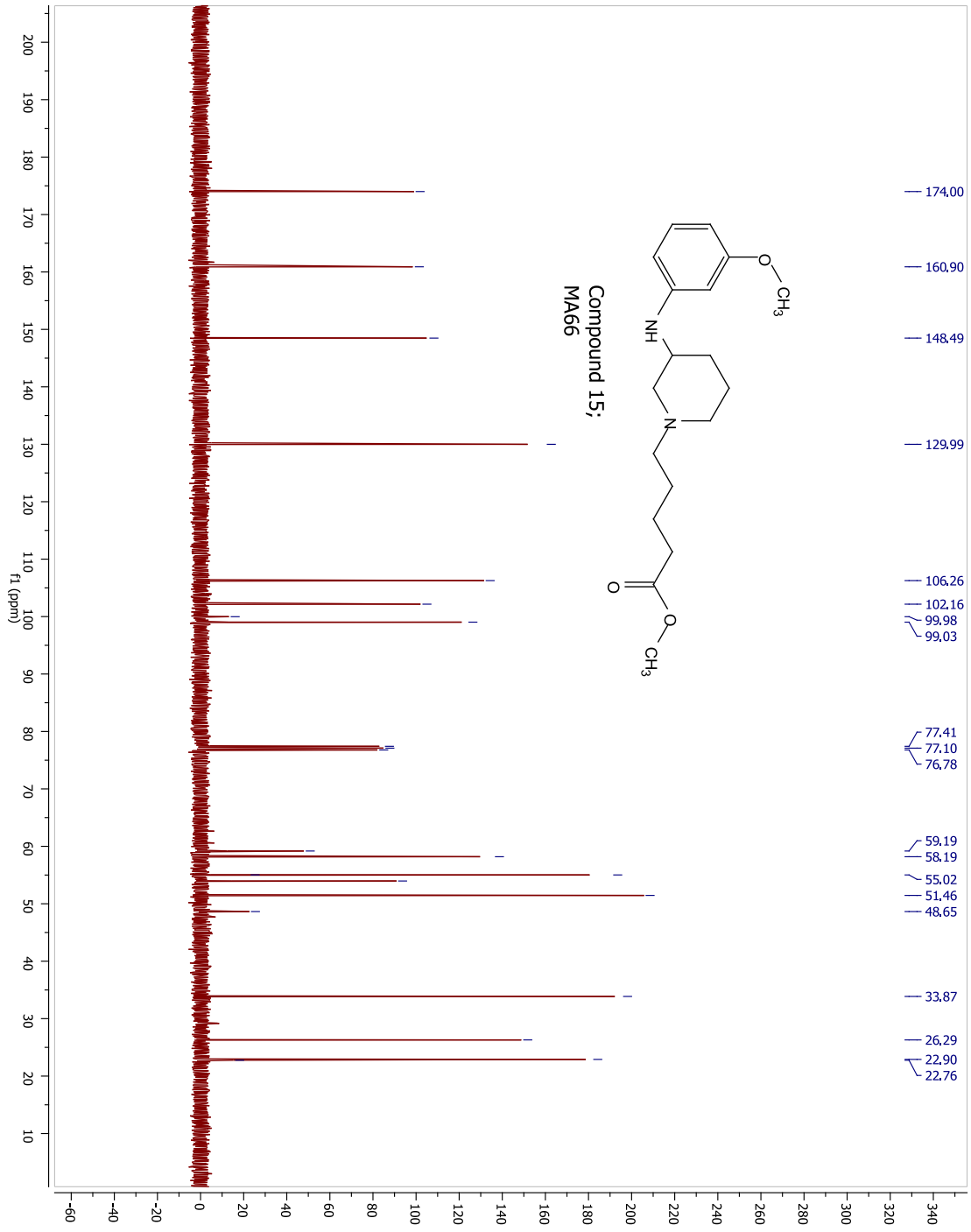
0.99
0.11
0.29
0.96
6.03
0.88
4.20
3.66


Compound 15;
MA66
NH
N
O
O
CH3
O
CH3
174.00
160.90
148.49
129.99
106.26
102.16
99.98
99.03
77.41
77.10
76.78
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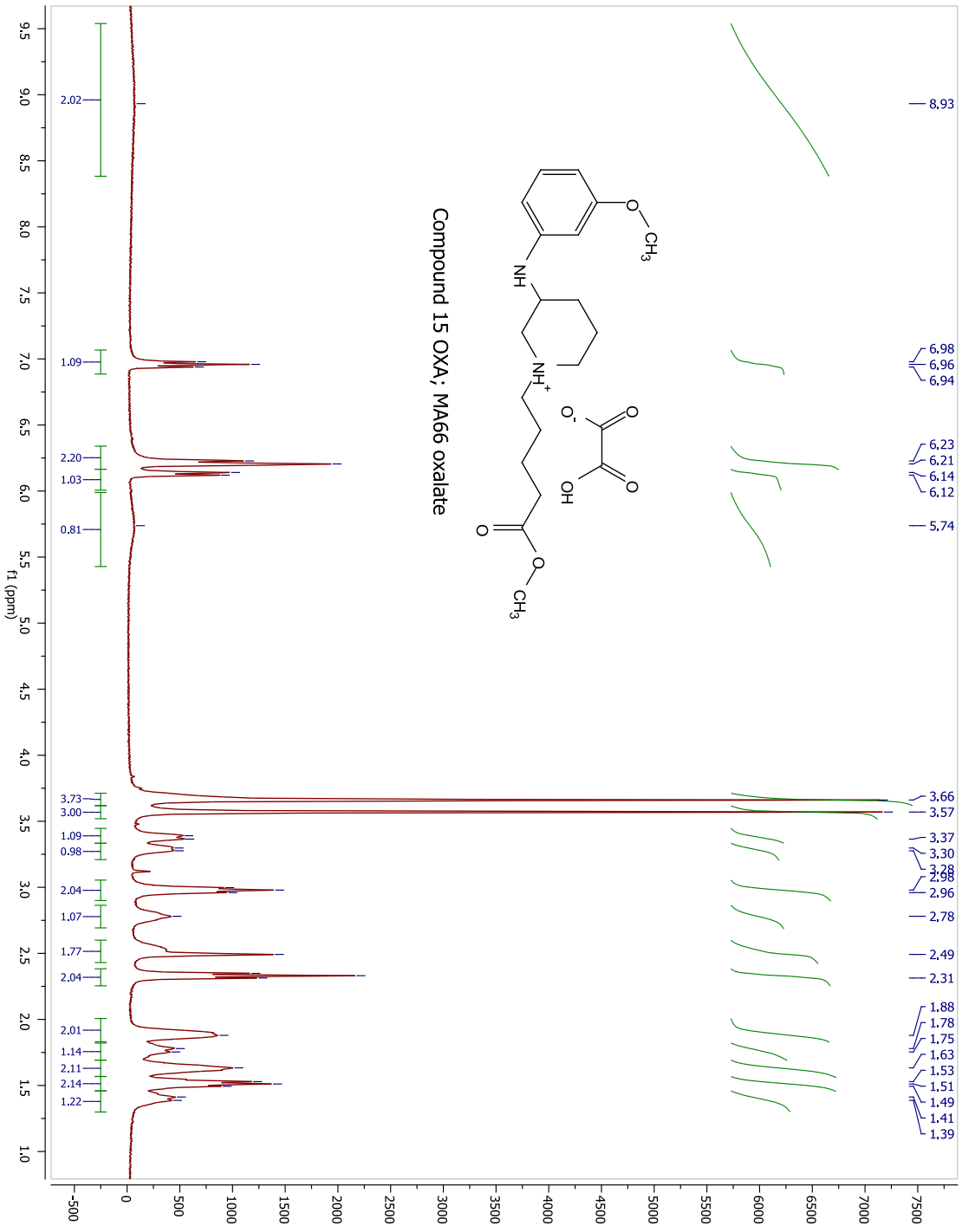
58.19
55.02
51.46
48.65
33.87
26.29
22.90
22.76
f1 (ppm)


Compound 15 OXA; MA66 oxalate
f1 (ppm)
2.02
1.09
2.20
1.03
0.81
3.73
3.00
1.09
0.98
2.04
1.07
1.77
2.04
2.01
1.14
2.11
2.14
1.22
8.93
6.98
6.96
6.94
6.23
6.21
6.14
6.12
5.74
3.66
3.57
3.37
3.30
2.98
2.96
2.78
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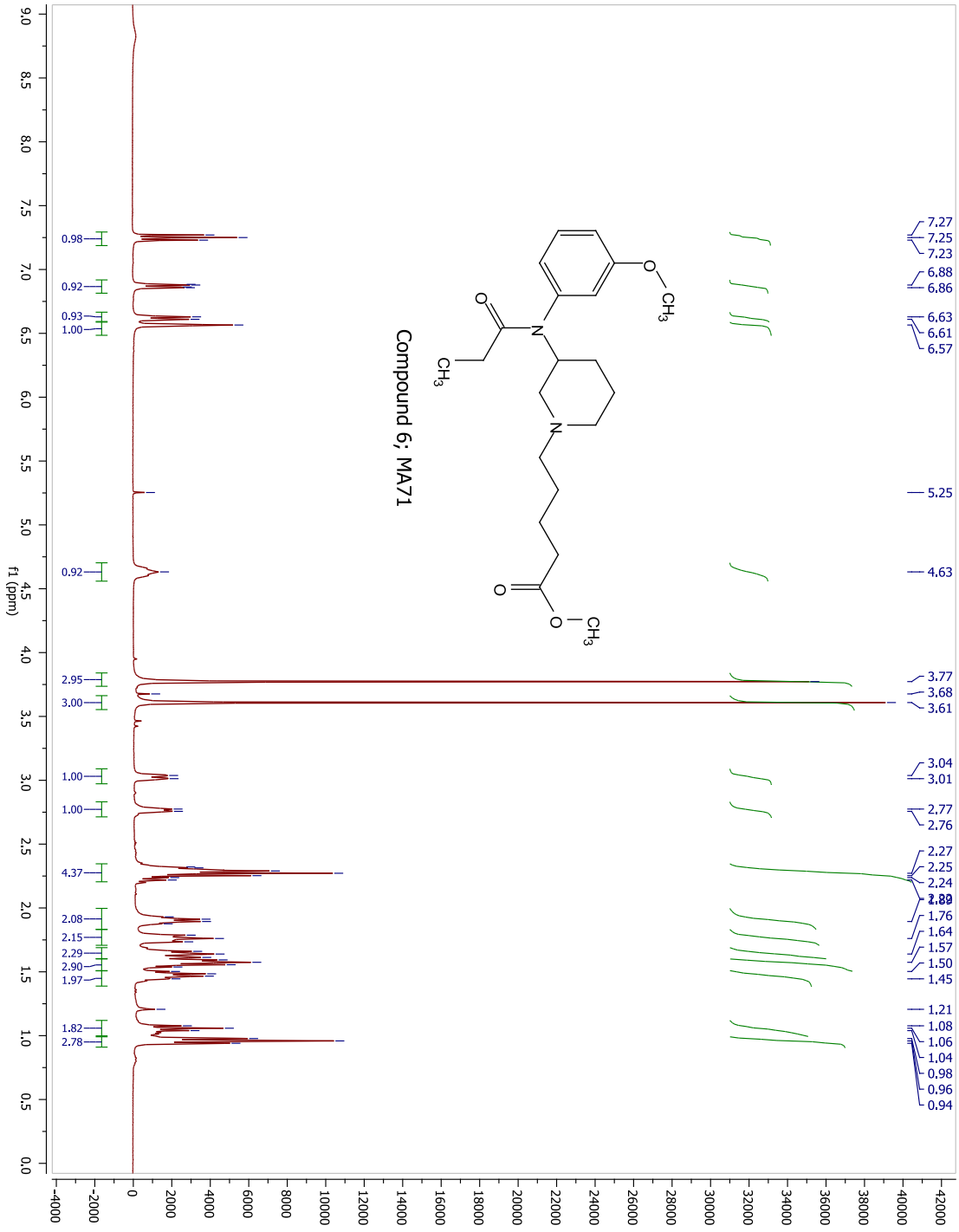
1.53
1.51
1.49
1.41
1.39


Compound 6; MA71
f1 (ppm)
0.98
0.92
0.93
1.00
0.92
2.95
3.00
1.00
1.00
4.37
2.08
2.15
2.29
2.90
1.97
1.82
2.78
7.27
7.25
7.23
6.88
6.86
6.63
6.61
6.57
5.25
4.63
3.77
3.68
3.61
3.04
3.01
2.77
2.76
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2.24
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1.21
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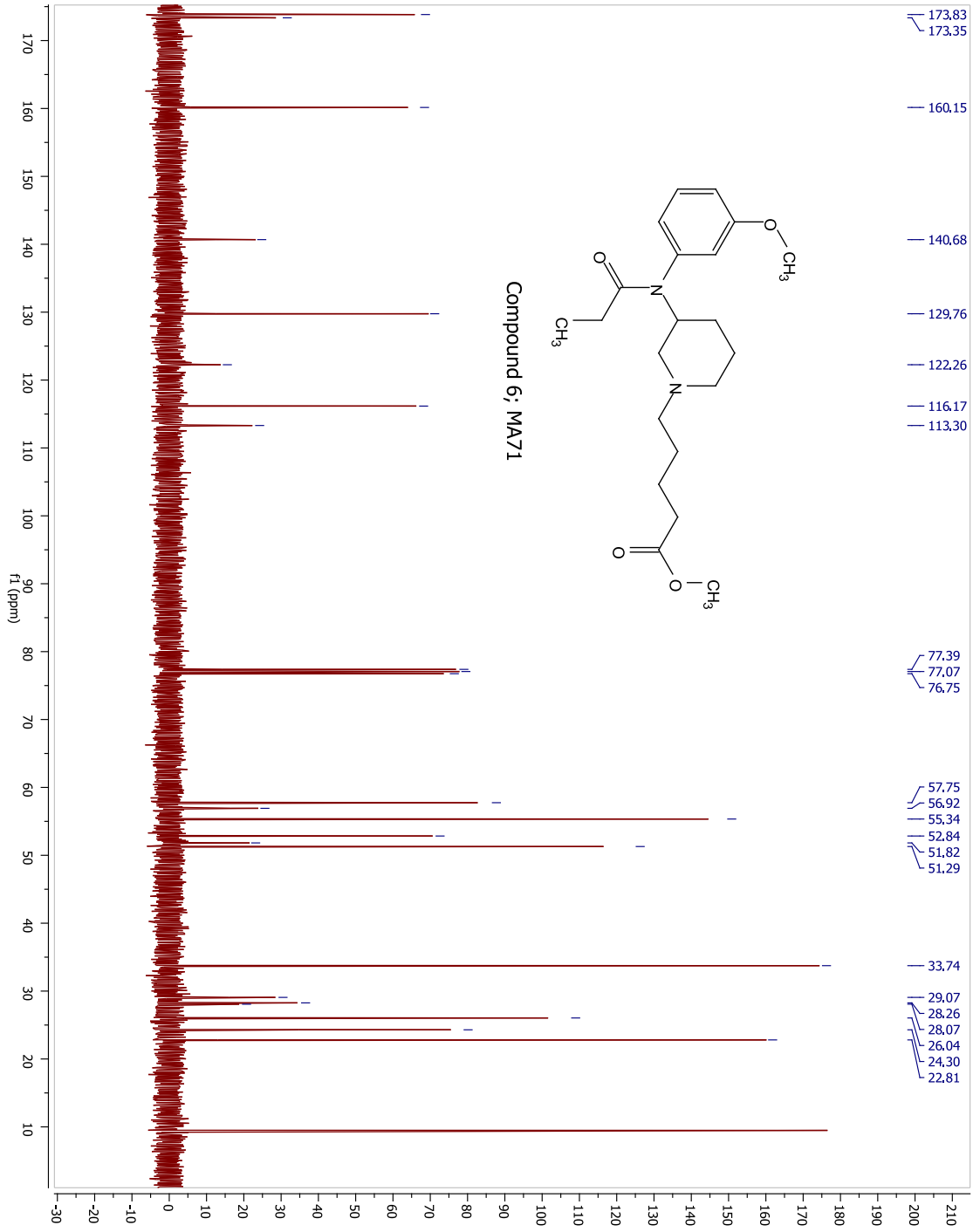
1.06
1.04
0.98
0.96
0.94


Compound 6; MA71
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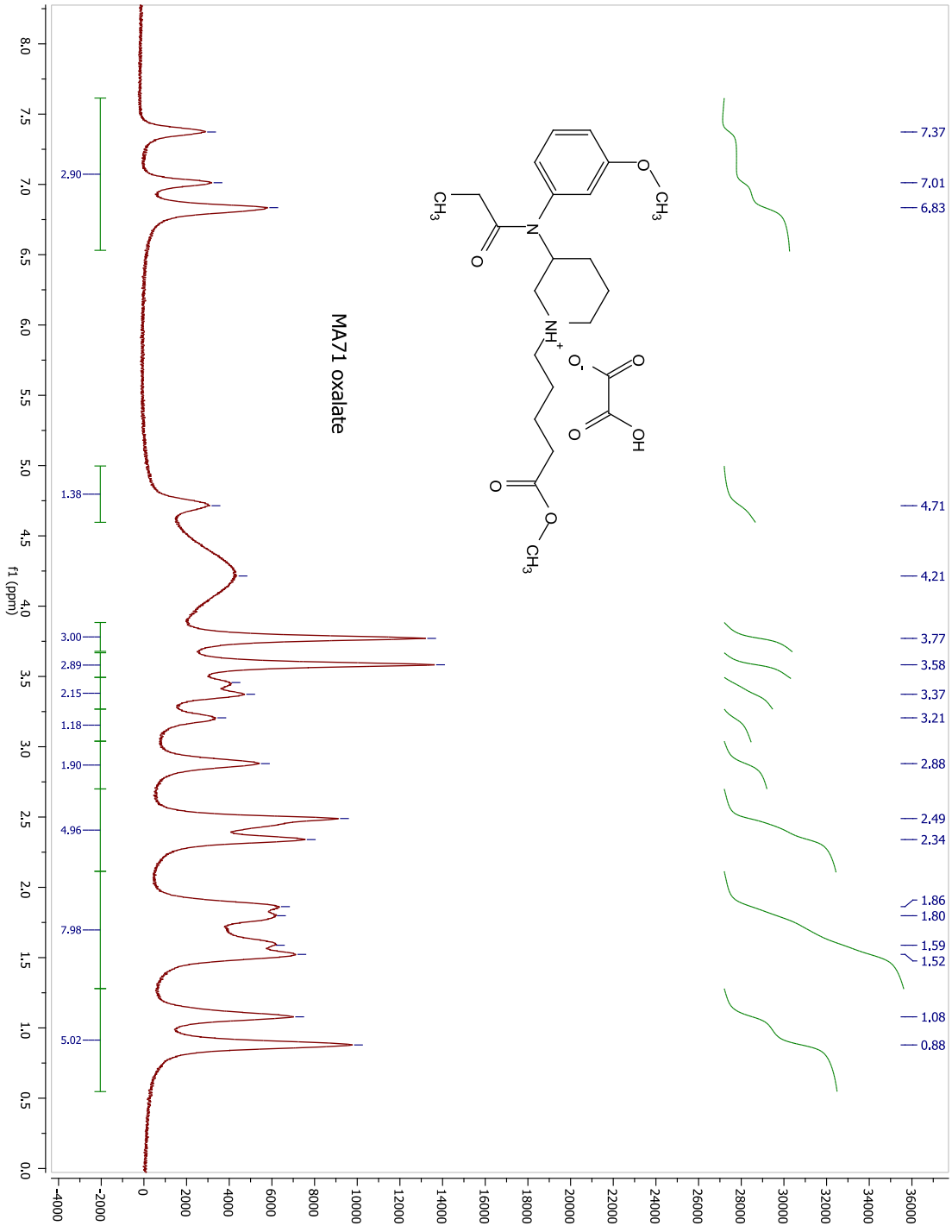
173.35
160.15
140.68
129.76
122.26
116.17
113.30
77.39
77.07
76.75
57.75
56.92
55.34
52.84
51.82
51.29
33.74
29.07
28.26
28.07
26.04
24.30
22.81
f1 (ppm)


MA71 oxalate
f1 (ppm)
2.90
1.38
3.00
2.89
2.15
1.18
1.90
4.96
7.98
5.02
7.37
7.01
6.83
4.71
4.21
3.77
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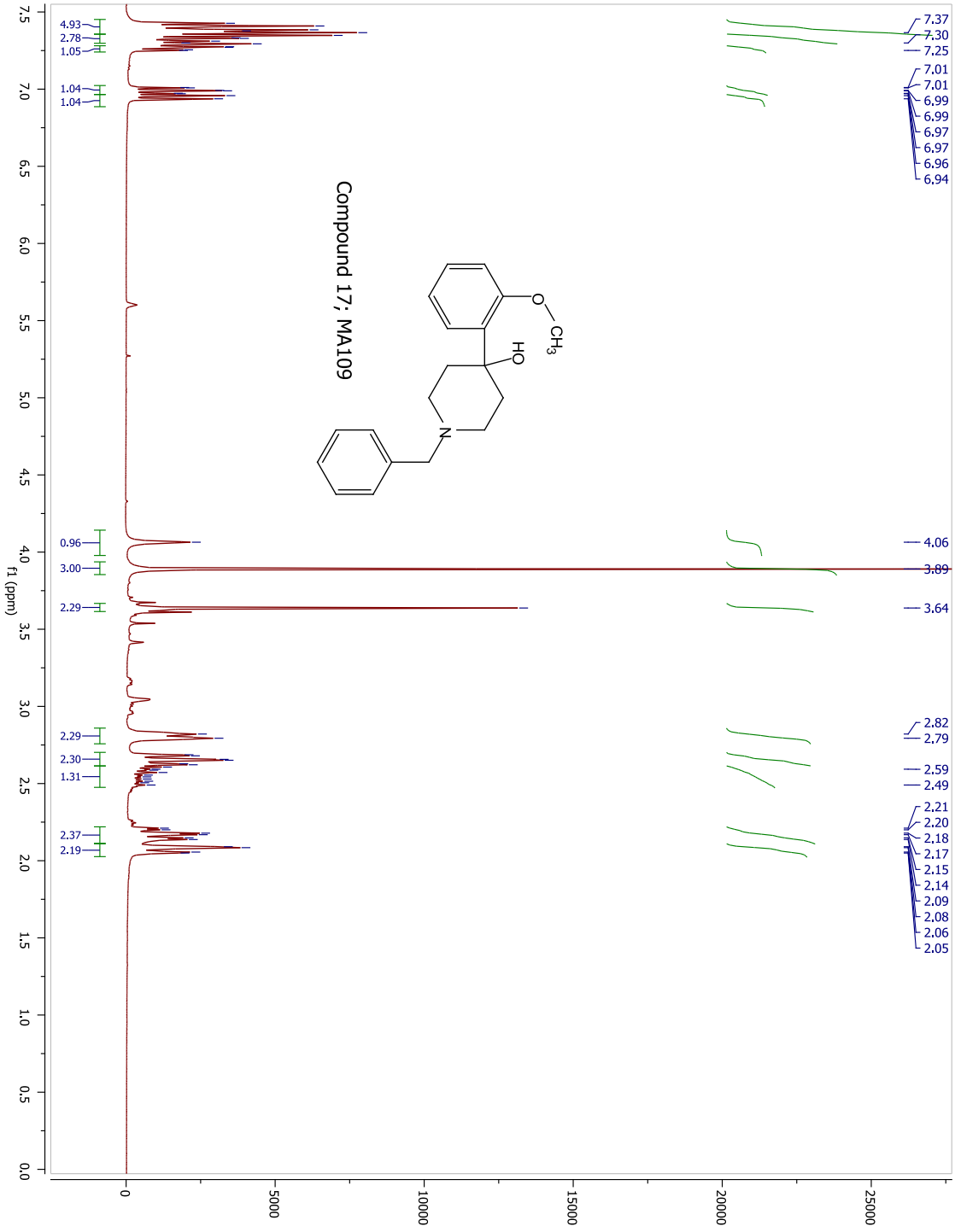
1.08
0.88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1.05
1.04
1.04
0.96
3.00
2.29
2.29
2.30
1.31
2.37
2.19
7.37
7.30
7.25
7.01
7.01
6.99
6.99
6.97
6.97
6.96
6.94
4.06
3.89
3.64
2.82
2.79
2.59
2.49
2.21
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2.17
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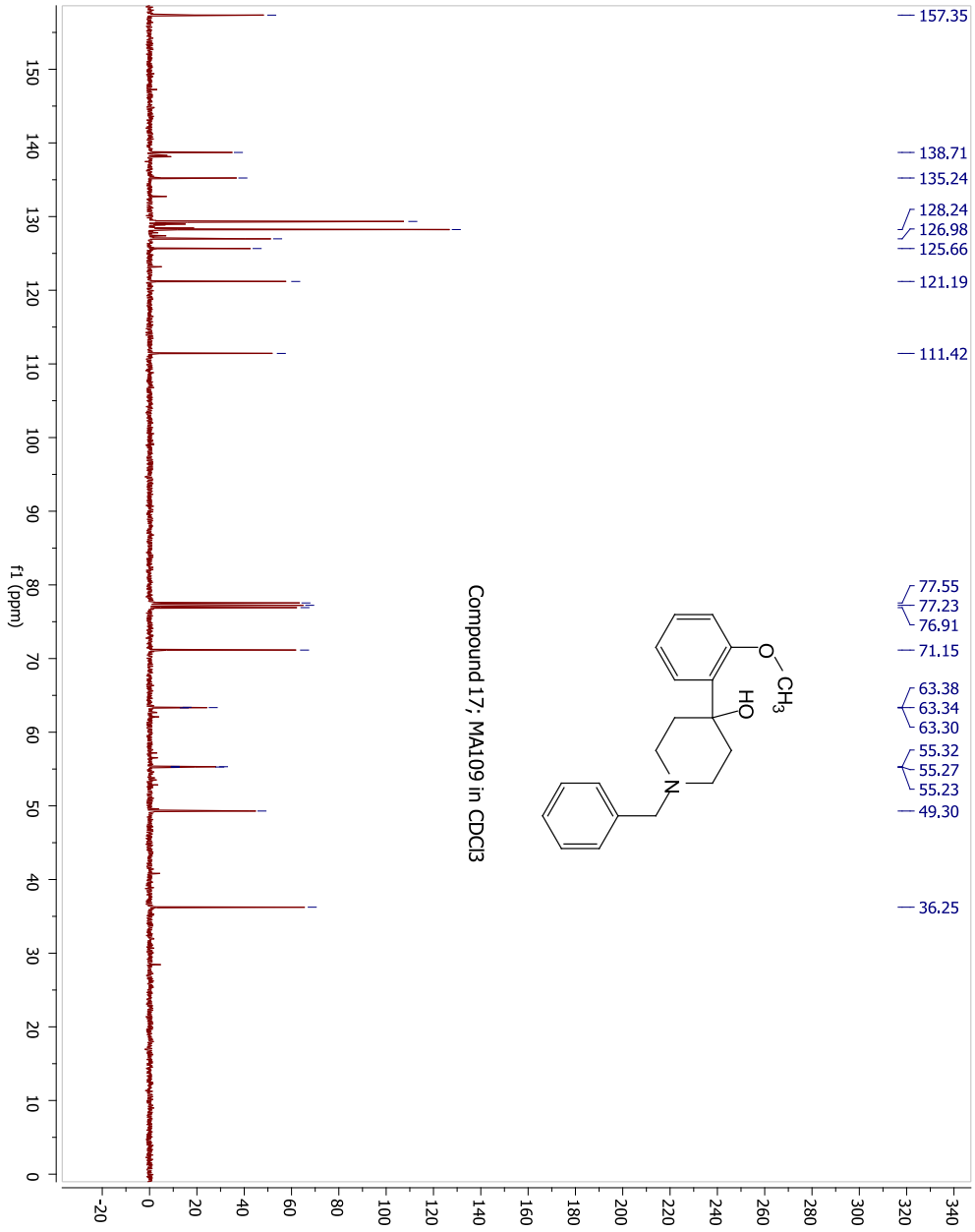
2.06
2.05
O
CH3
HO
N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125.66
121.19
111.42
77.55
77.23
76.91
71.15
63.38
63.34
63.30
55.32
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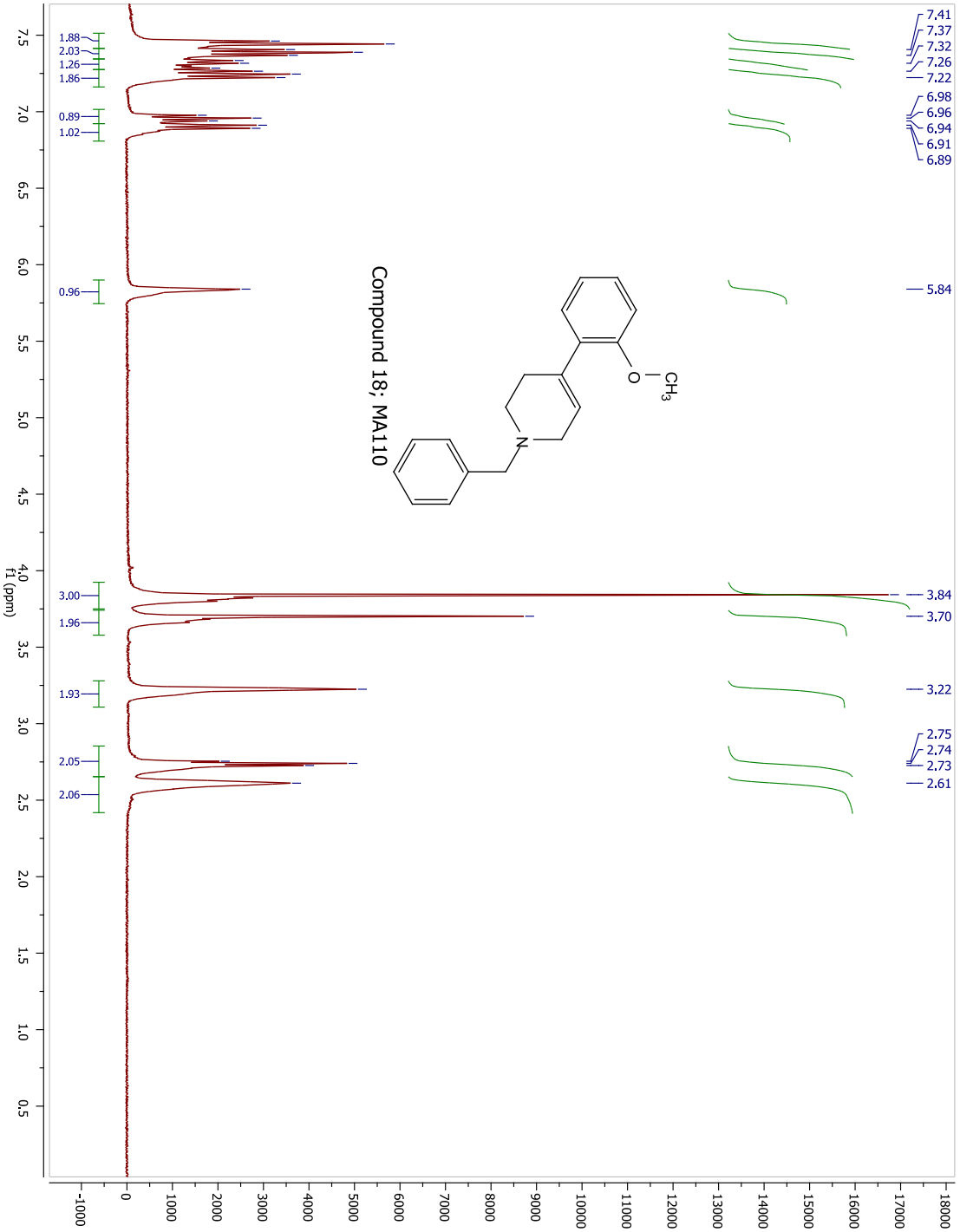
55.23
49.30
36.25
Compound 17; MA109 in CDCl3
f1 (ppm)
O
CH3
HO
N


Compound 18; MA110
f1 (ppm)
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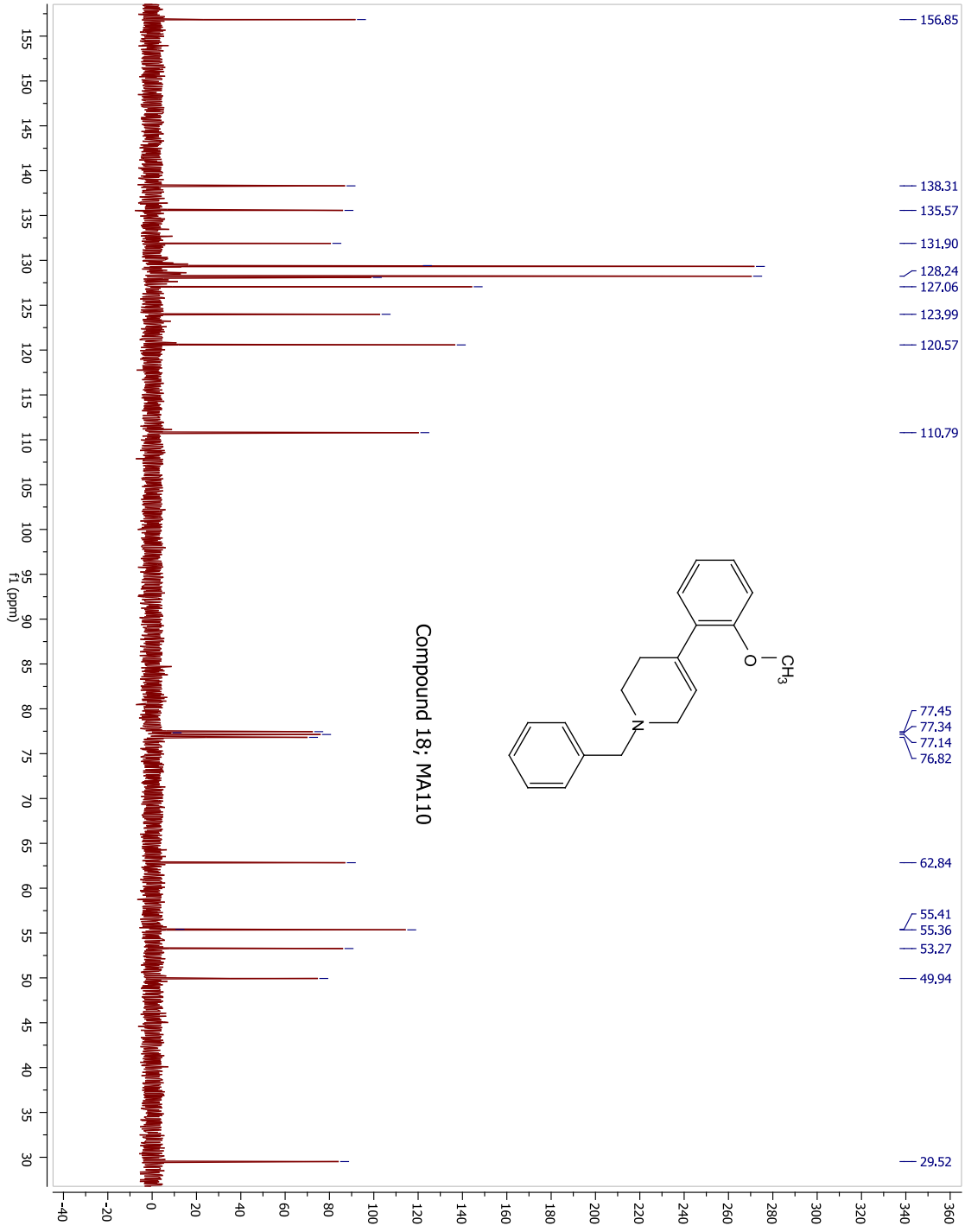
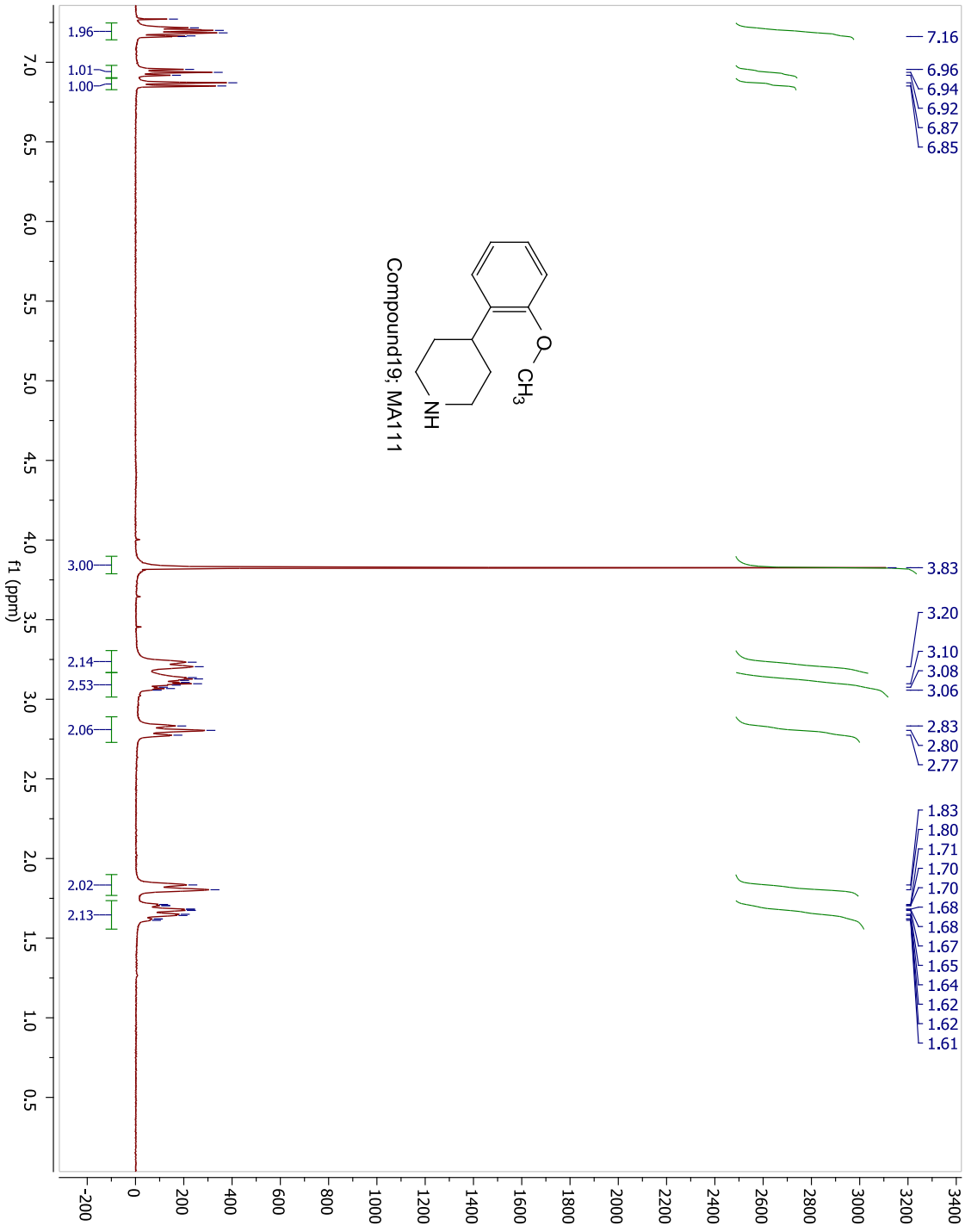
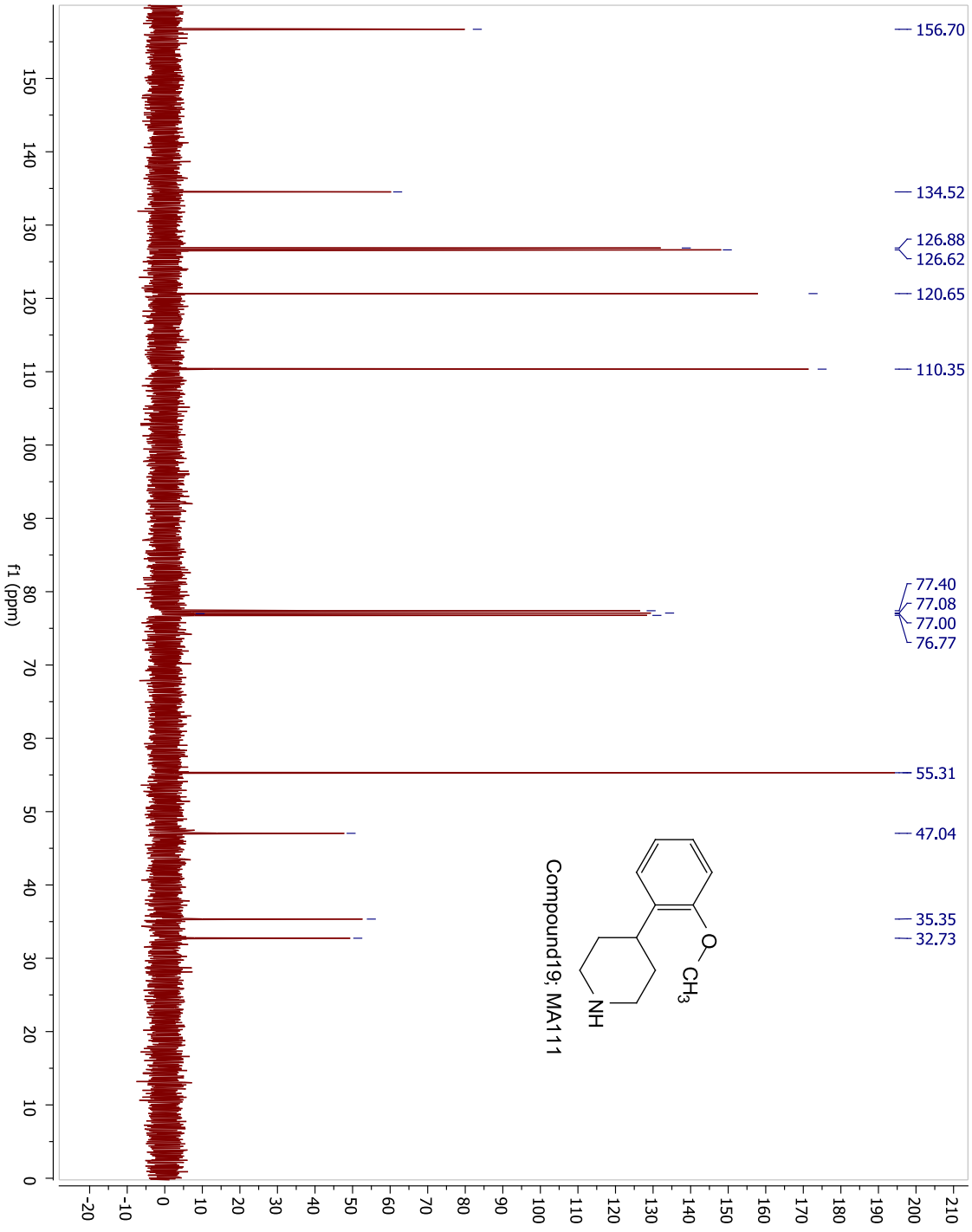
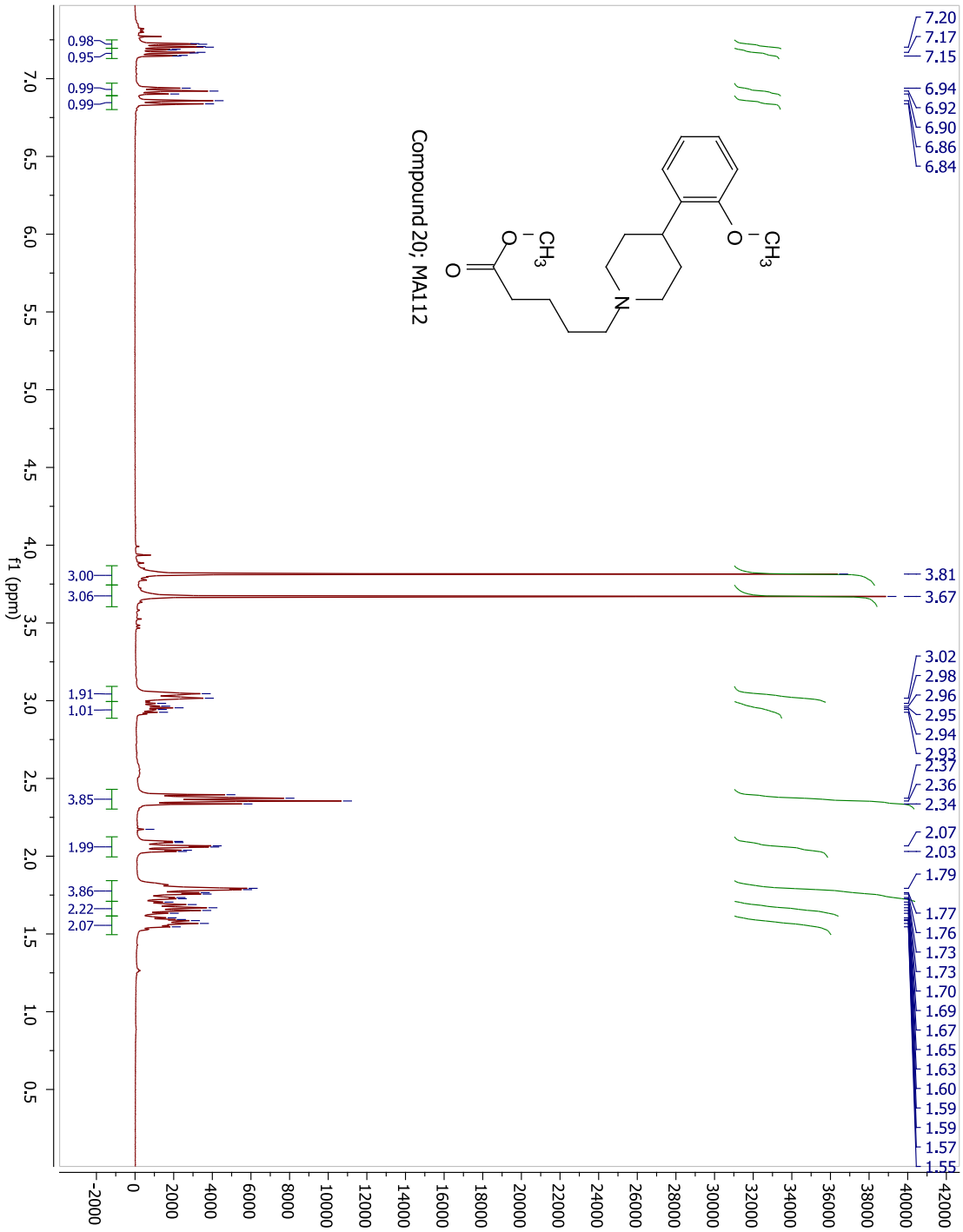
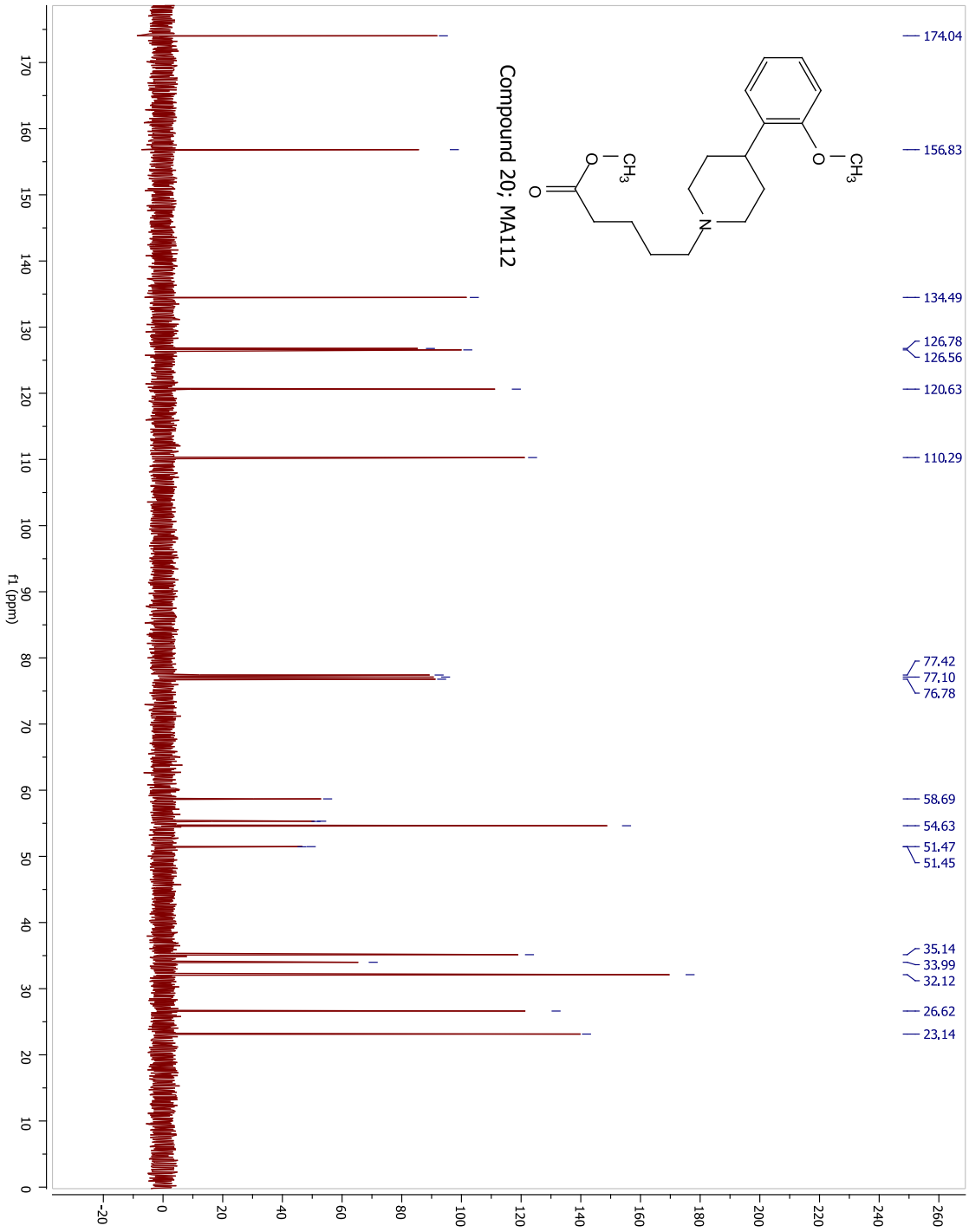
2.03
1.26
1.86
0.89
1.02
0.96
3.00
1.96
1.93
2.05
2.06
7.41
7.37
7.32
7.26
7.22
6.98
6.96
6.94
6.91
6.89
5.84
3.84
3.70
3.22
2.75
2.74
2.73
2.61
O
CH3
N

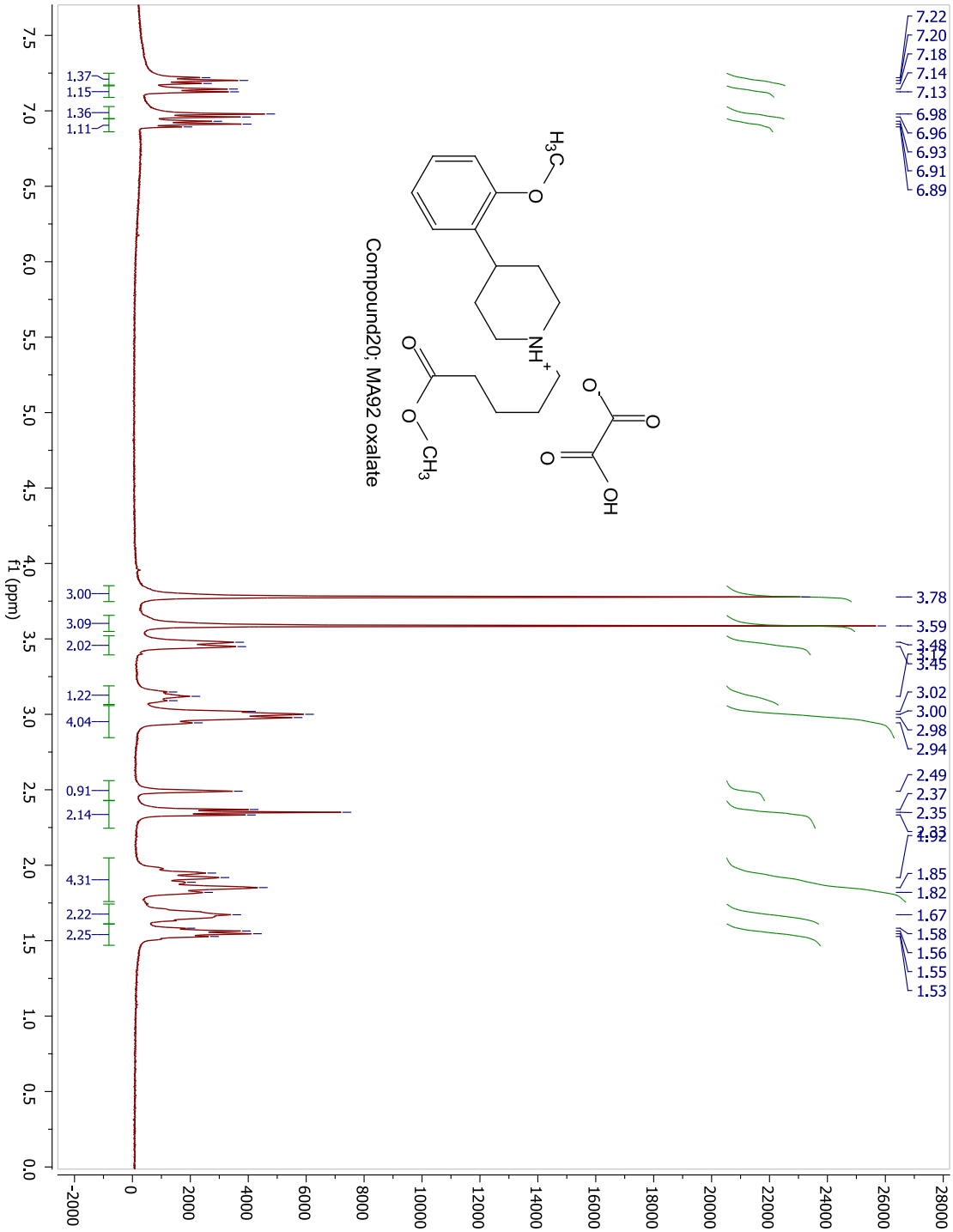

156.85
138.31
135.57
131.90
128.24
127.06
123.99
120.57
110.79
77.45
77.34
77.14
76.82
62.84
55.41
55.36
53.27
49.94
29.52
Compound 18; MA110
O
CH3
N
f1 (ppm)

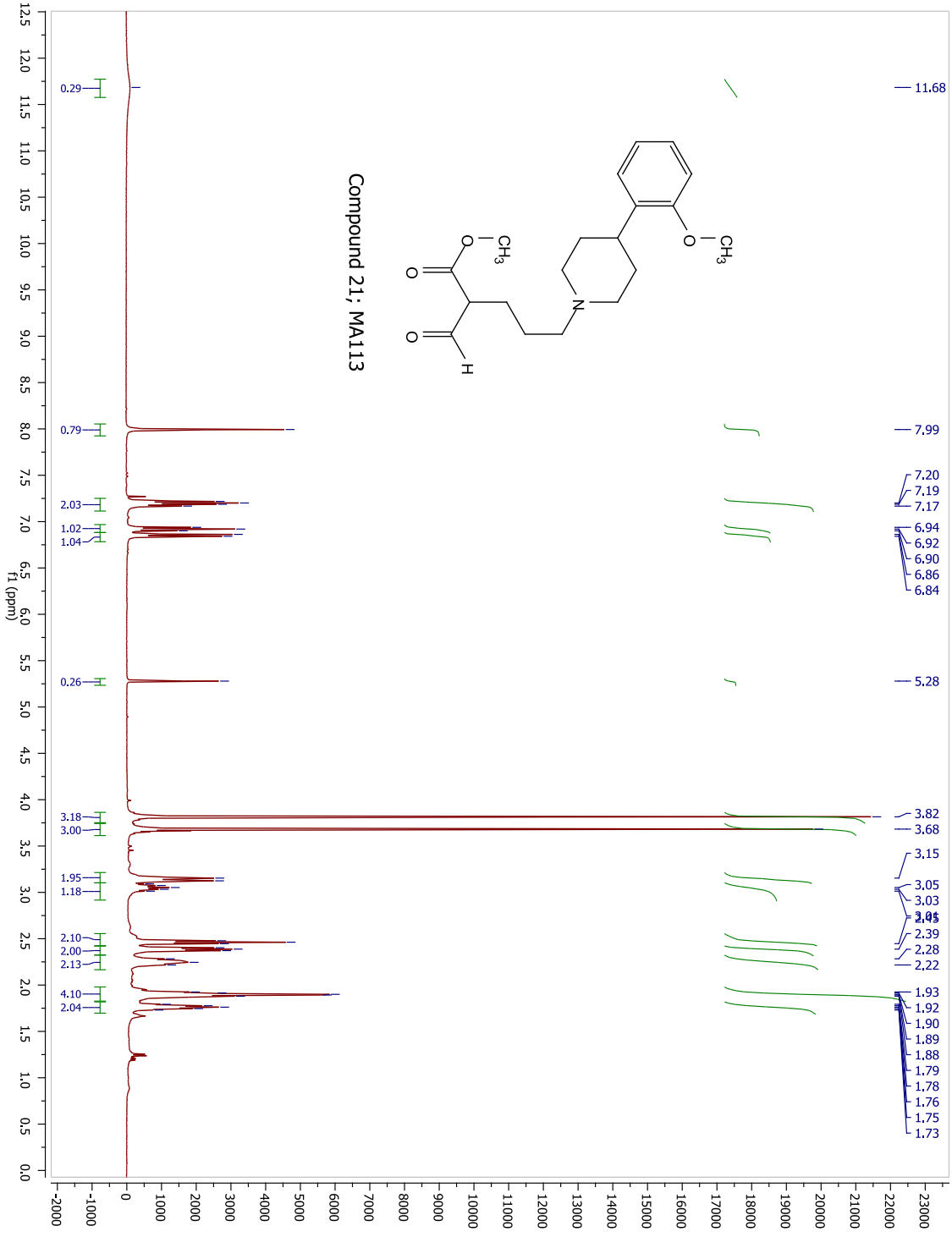

Compound19; MA111
NH
O
CH3
f1 (ppm)
1.96
1.01
1.00
3.00
2.14
2.53
2.06
2.02
2.13
7.16
6.96
6.94
6.92
6.87
6.85
3.83
3.20
3.10
3.08
3.06
2.83
2.80
2.77
1.83
1.80
1.71
1.70
1.70
1.68
1.68
1.67
1.65
1.64
1.62
1.62
1.61

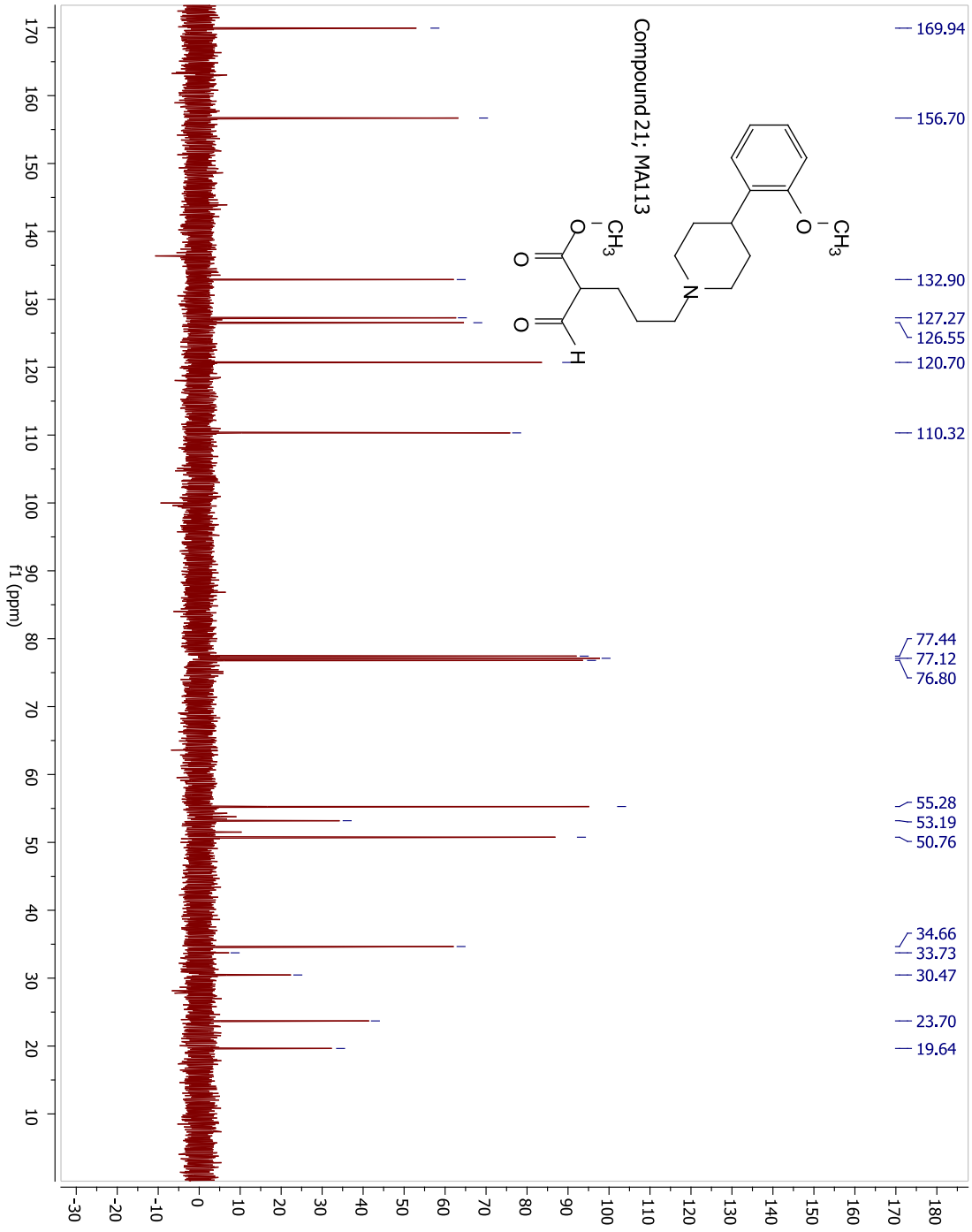

156.70
134.52
126.88
126.62
120.65
110.35
77.40
77.08
77.00
76.77
55.31
47.04
35.35
32.73
f1 (ppm)
Compound19; MA111
NH
O
CH3

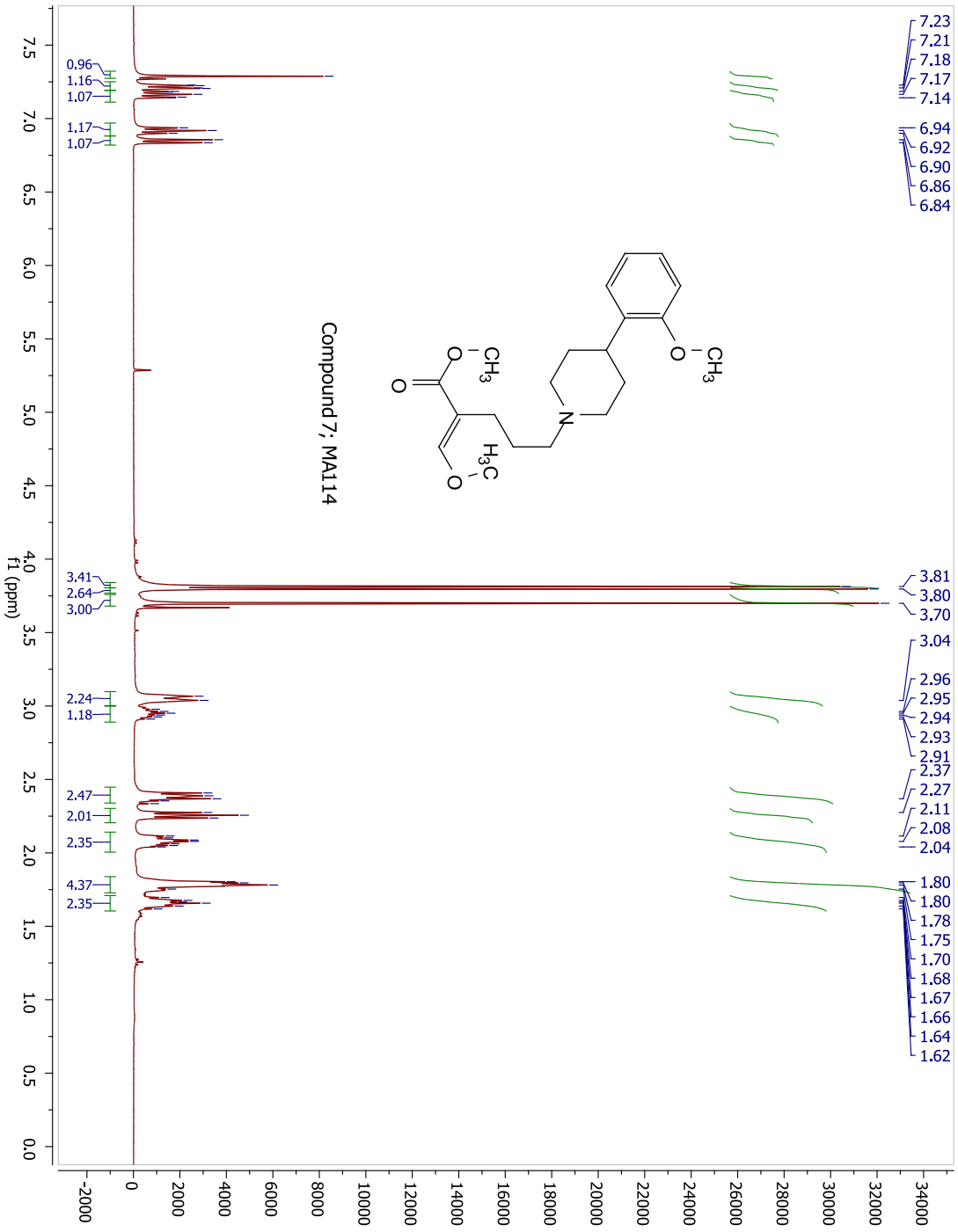

Compound 20; MA112
f1 (ppm)
0.98
0.95
0.99
0.99
3.00
3.06
1.91
1.01
3.85
1.99
3.86
2.22
2.07
7.20
7.17
7.15
6.94
6.92
6.90
6.86
6.84
3.81
3.67
3.02
2.98
2.96
2.95
2.94
2.93
2.37
2.36
2.34
2.07
2.03
1.79
1.77
1.76
1.73
1.73
1.70
1.69
1.67
1.65
1.63
1.60
1.59
1.59
1.57
1.55

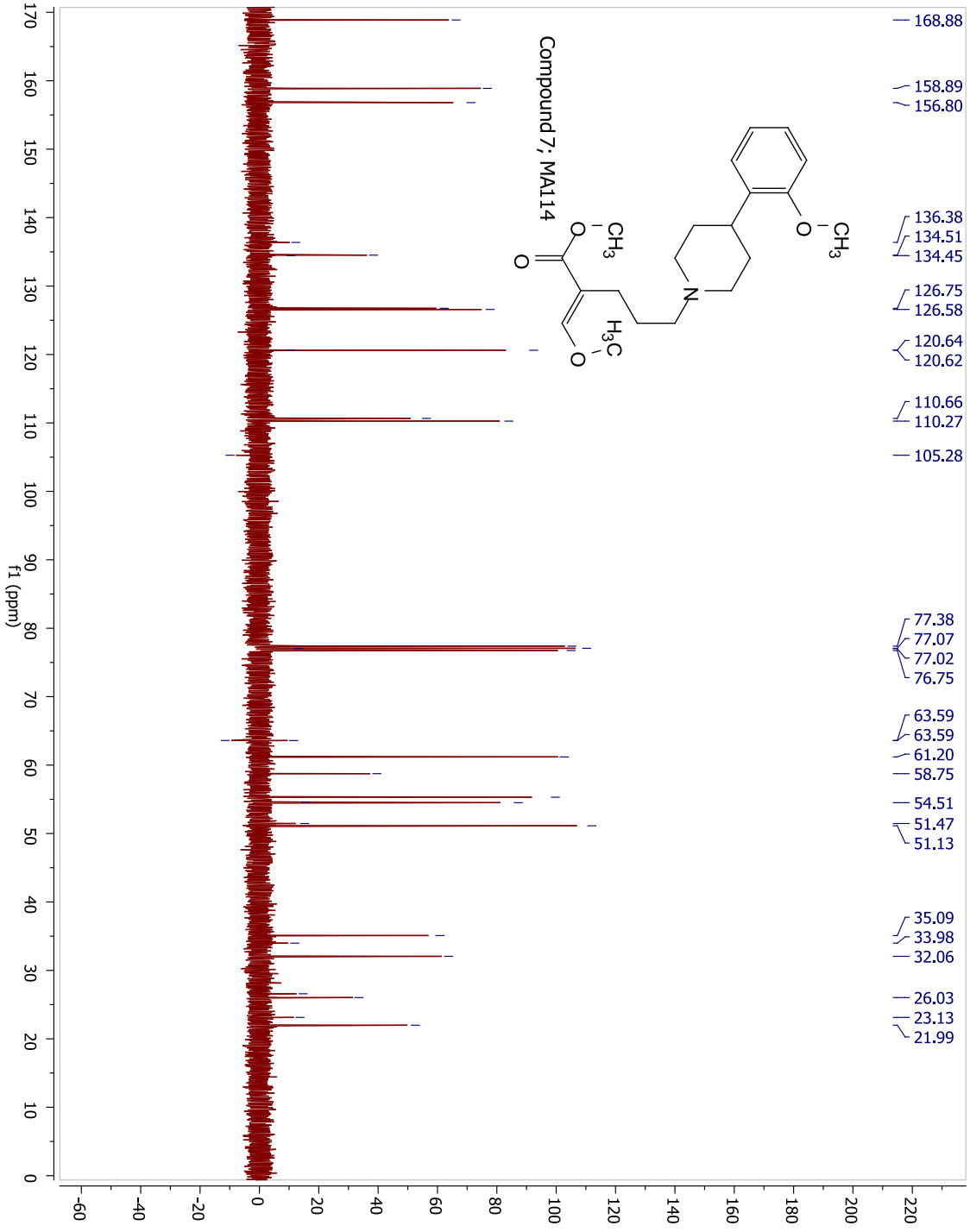

Compound 20; MA112
174.04
156.83
134.49
126.78
126.56
120.63
110.29
77.42
77.10
76.78
58.69
54.63
51.47
51.45
35.14
33.99
32.12
26.62
23.14
f1 (ppm)

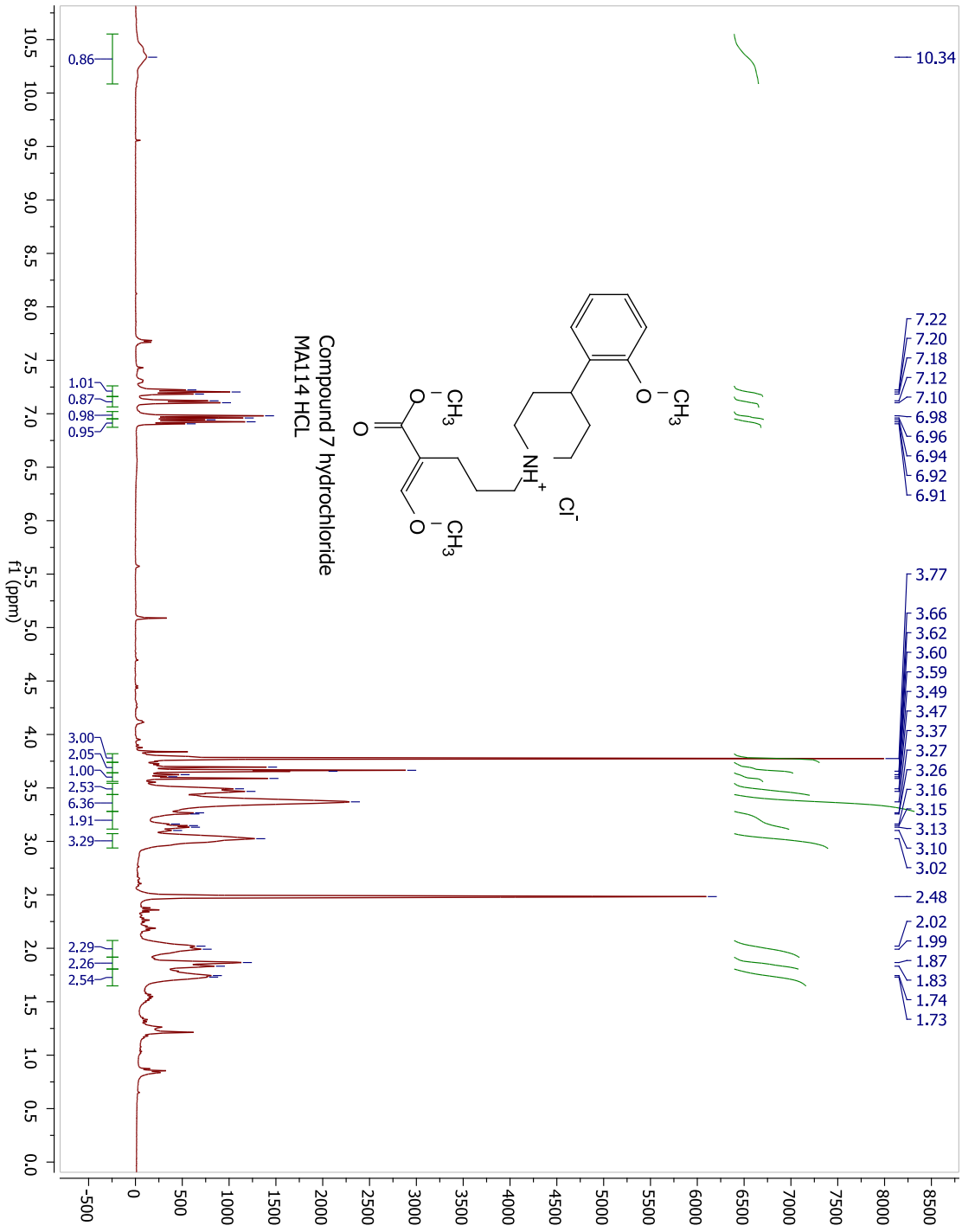

Compound20; MA92 oxalate
f1 (ppm)
7.22
7.20
7.18
7.14
7.13
6.98
6.96
6.93
6.91
6.89
3.78
3.59
3.48
3.45
3.02
3.00
2.98
2.94
2.49
2.37
2.35
1.85
1.82
1.67
1.58
1.56
1.55
1.53
1.37
1.15
1.36
1.11
3.00
3.09
2.02
1.22
4.04
0.91
2.14
4.31
2.22
2.25

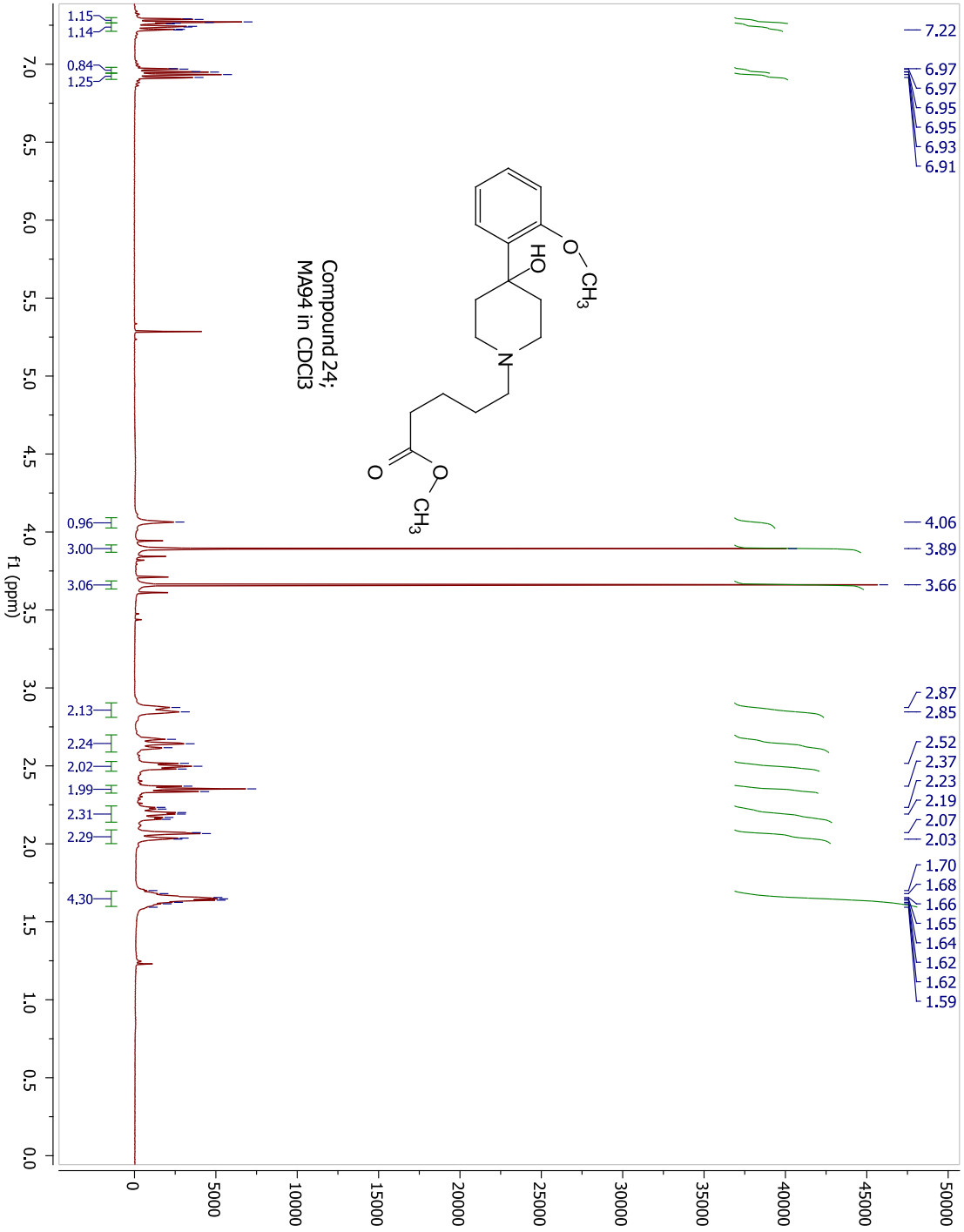

Compound 21; MA113
f1 (ppm)
0.29
0.79
2.03
1.02
1.04
0.26
3.18
3.00
1.95
1.18
2.10
2.00
2.13
4.10
2.04
11.68
7.99
7.20
7.19
7.17
6.94
6.92
6.90
6.86
6.84
5.28
3.82
3.68
3.15
3.05
3.03
2.39
2.28
2.22
1.93
1.92
1.90
1.89
1.88
1.79
1.78
1.76
1.75
1.73

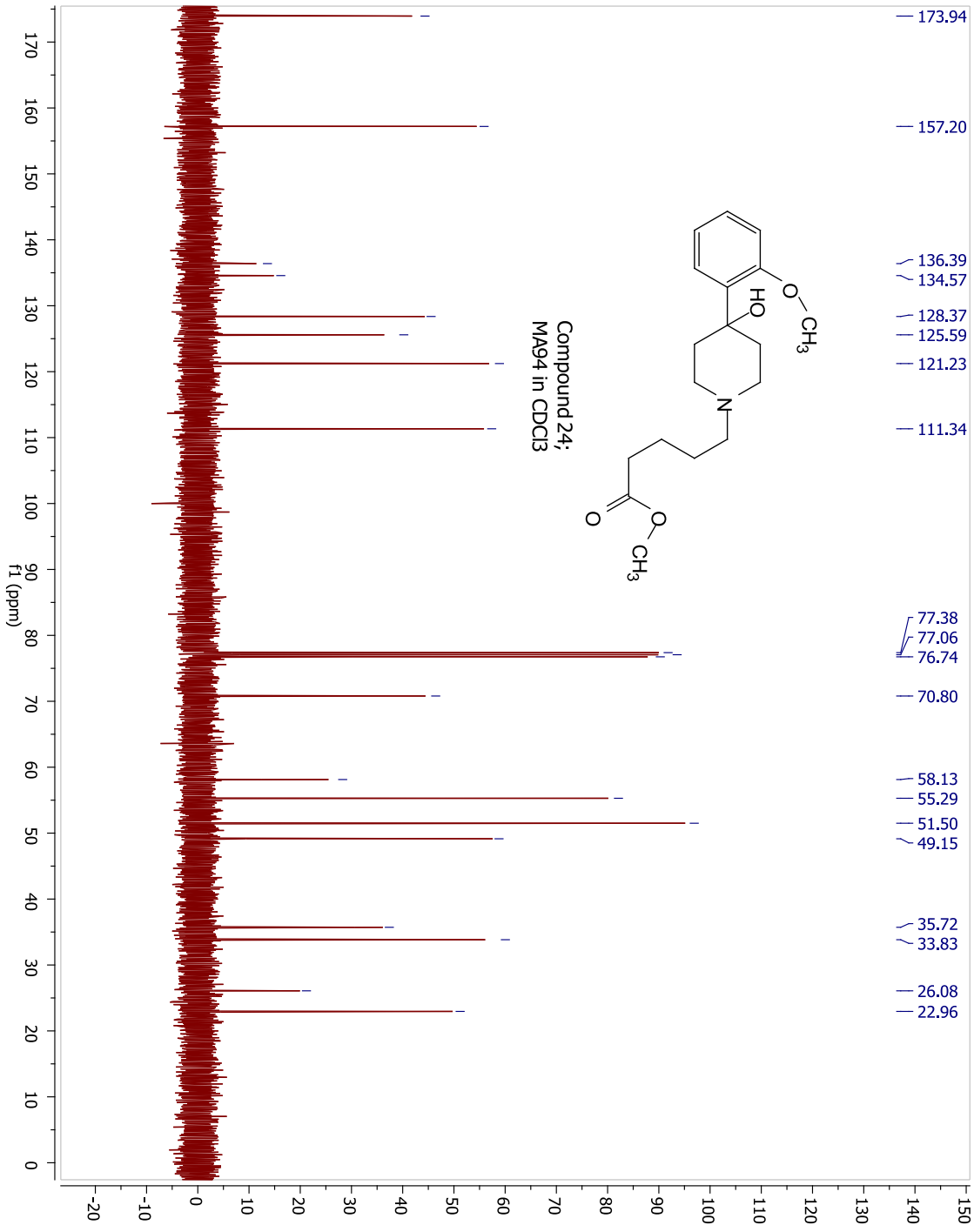

Compound 21; MA113
169.94
156.70
132.90
127.27
126.55
120.70
110.32
77.44
77.12
76.80
55.28
53.19
50.76
34.66
33.73
30.47
23.70
19.64
f1 (ppm)

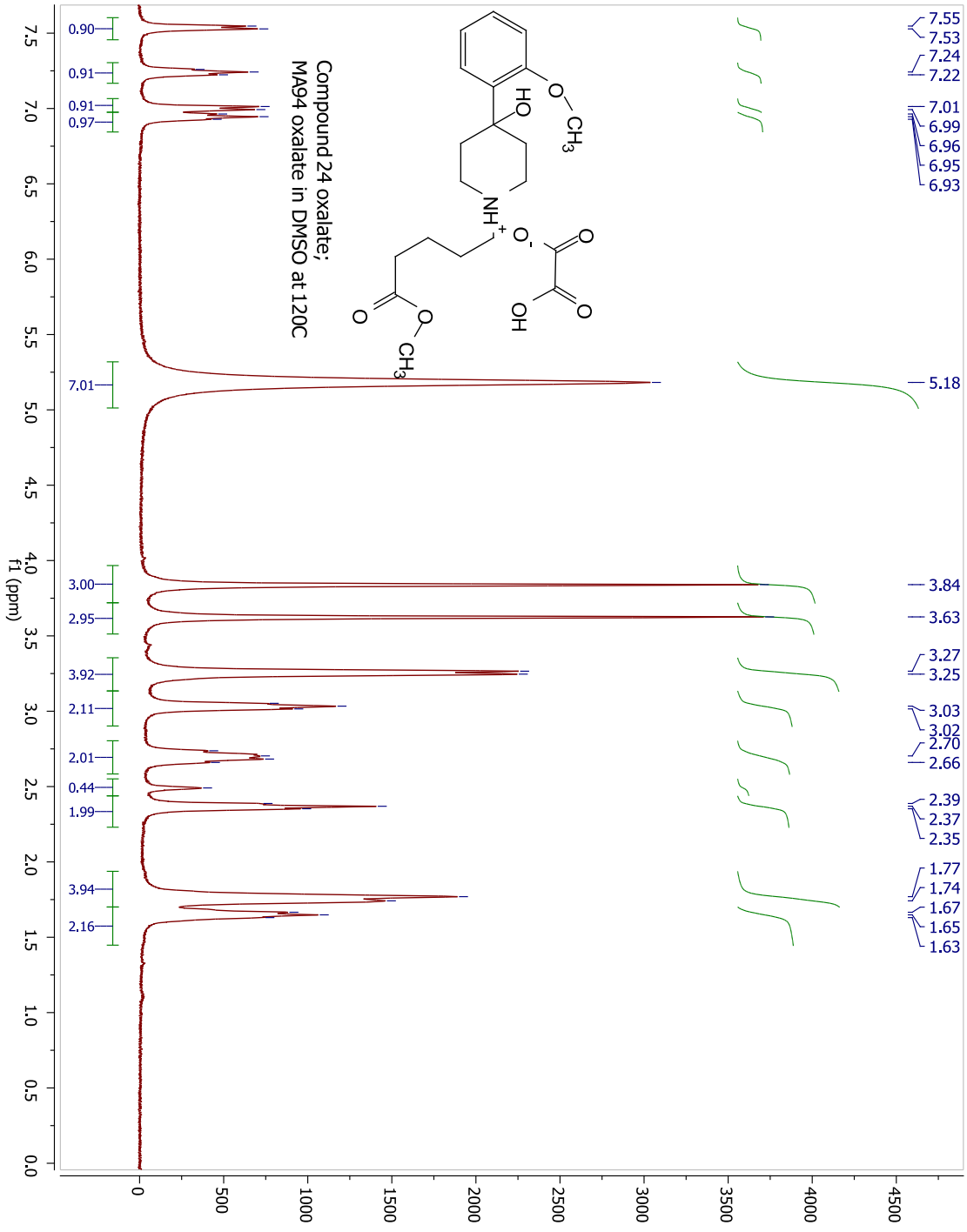

Compound 7; MA114
f1 (ppm)
0.96
1.16
1.07
1.17
1.07
3.41
2.64
3.00
2.24
1.18
2.47
2.01
2.35
4.37
2.35
7.23
7.21
7.18
7.17
7.14
6.94
6.92
6.90
6.86
6.84
3.81
3.80
3.70
3.04
2.96
2.95
2.94
2.93
2.91
2.37
2.27
2.11
2.08
2.04
1.80
1.80
1.78
1.75
1.70
1.68
1.67
1.66
1.64
1.62

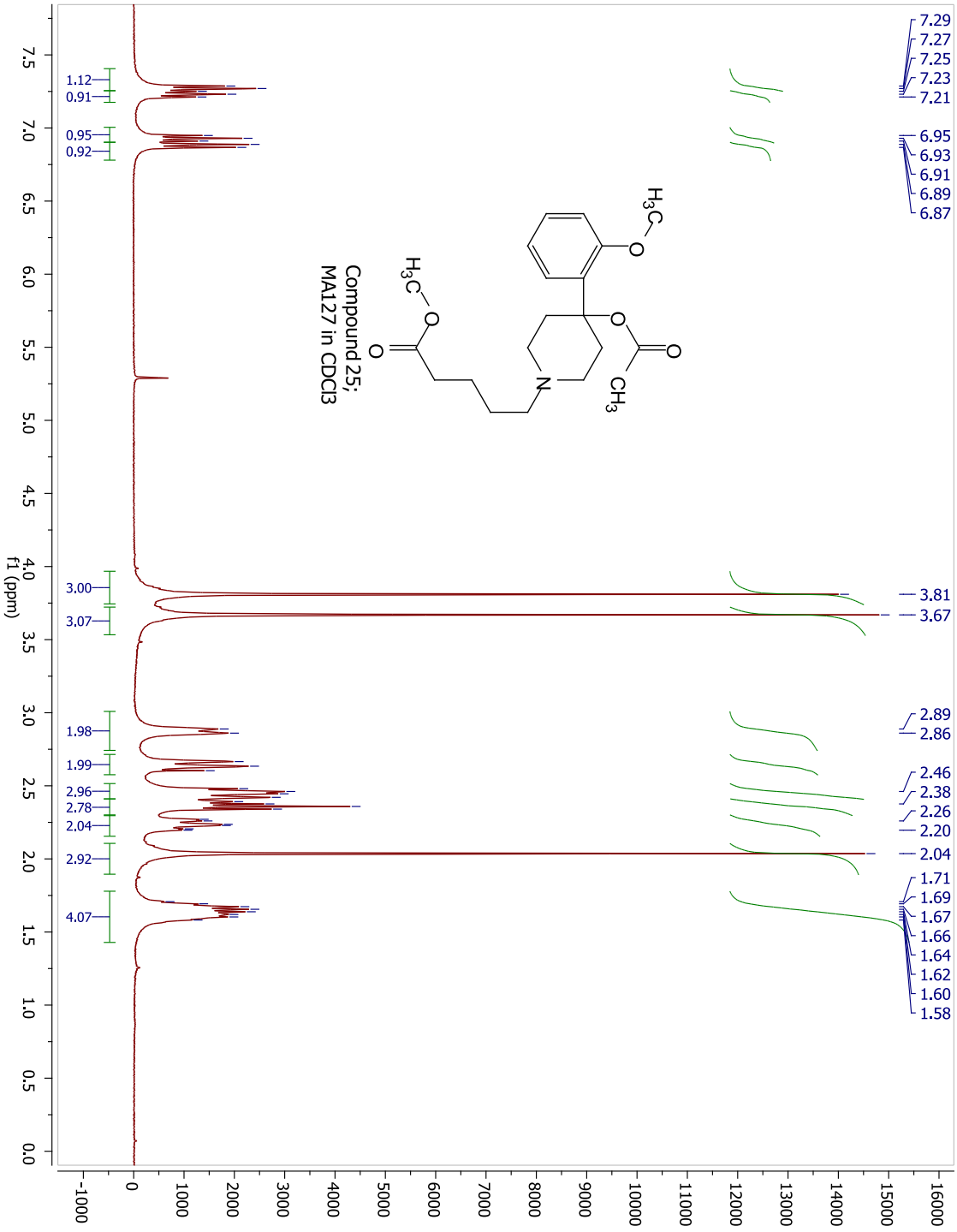

Compound 7; MA114
168.88
158.89
156.80
136.38
134.51
134.45
126.75
126.58
120.64
120.62
110.66
110.27
105.28
77.38
77.07
77.02
76.75
63.59
63.59
61.20
58.75
54.51
51.47
51.13
35.09
33.98
32.06
26.03
23.13
21.99
f1 (ppm)

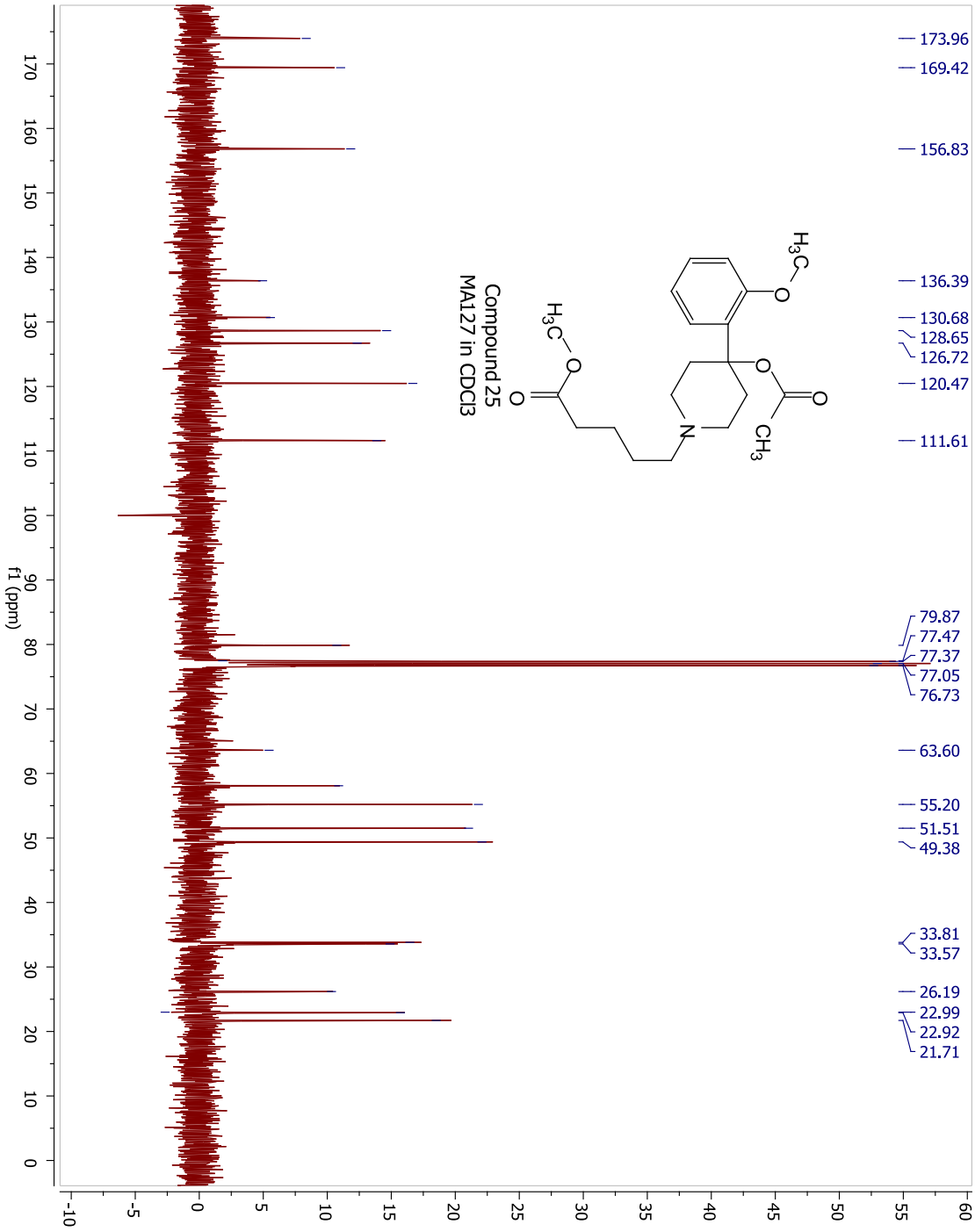

Compound 7 hydrochloride
MA114 HCL
f1 (ppm)
0.86
1.01
0.87
0.98
0.95
3.00
2.05
1.00
2.53
6.36
1.91
3.29
2.29
2.26
2.54
10.34
7.22
7.20
7.18
7.12
7.10
6.98
6.96
6.94
6.92
6.91
3.77
3.66
3.62
3.60
3.59
3.49
3.47
3.37
3.27
3.26
3.16
3.15
3.13
3.10
3.02
2.48
2.02
1.99
1.87
1.83
1.74
1.73

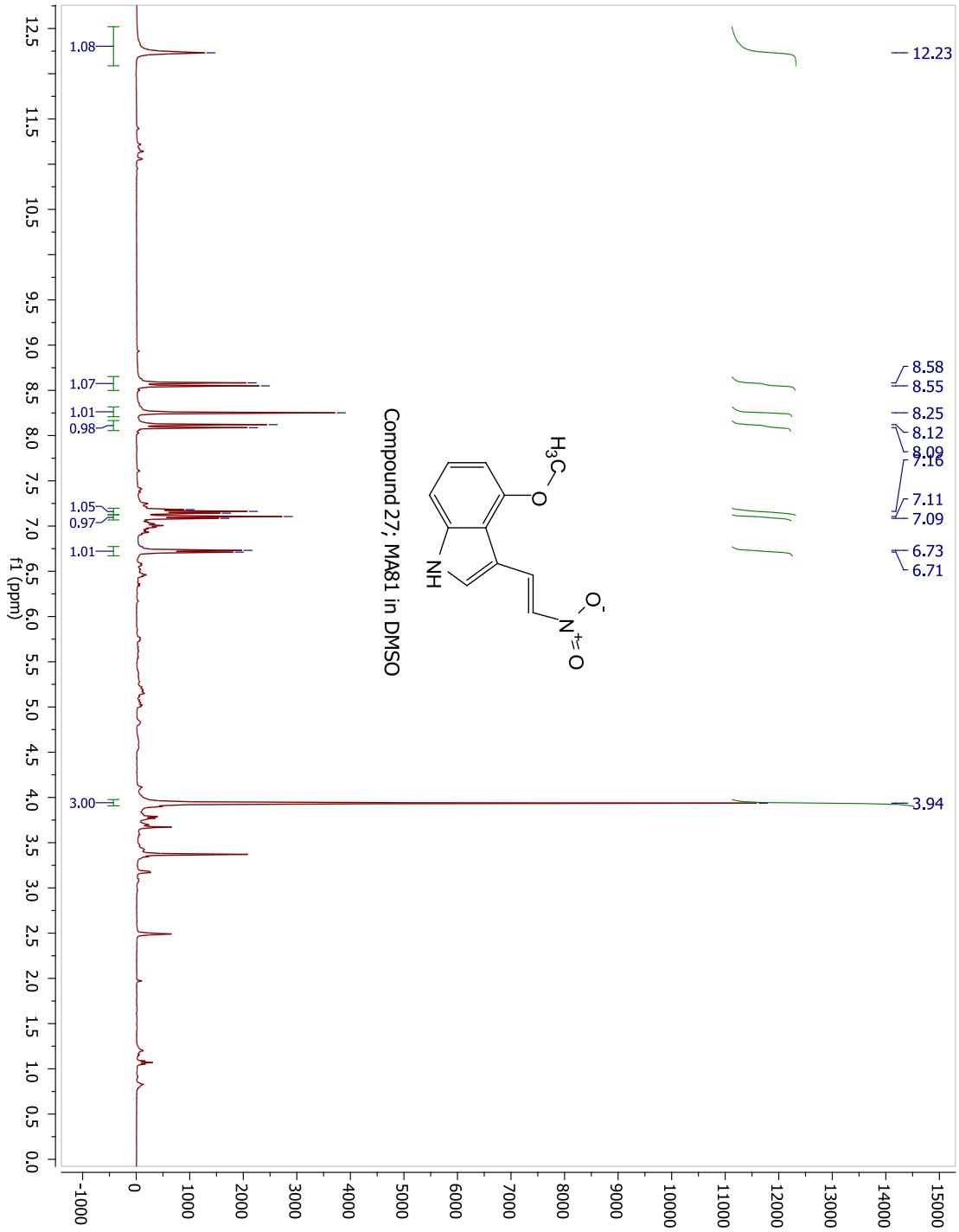

Compound 24;
MA94 in CDCl3
HO
O
CH3
N
O
O
CH3
f1 (ppm)
1.15
1.14
0.84
1.25
0.96
3.00
3.06
2.13
2.24
2.02
1.99
2.31
2.29
4.30
7.22
6.97
6.97
6.95
6.95
6.93
6.91
4.06
3.89
3.66
2.87
2.85
2.52
2.37
2.23
2.19
2.07
2.03
1.70
1.68
1.66
1.65
1.64
1.62
1.62
1.59

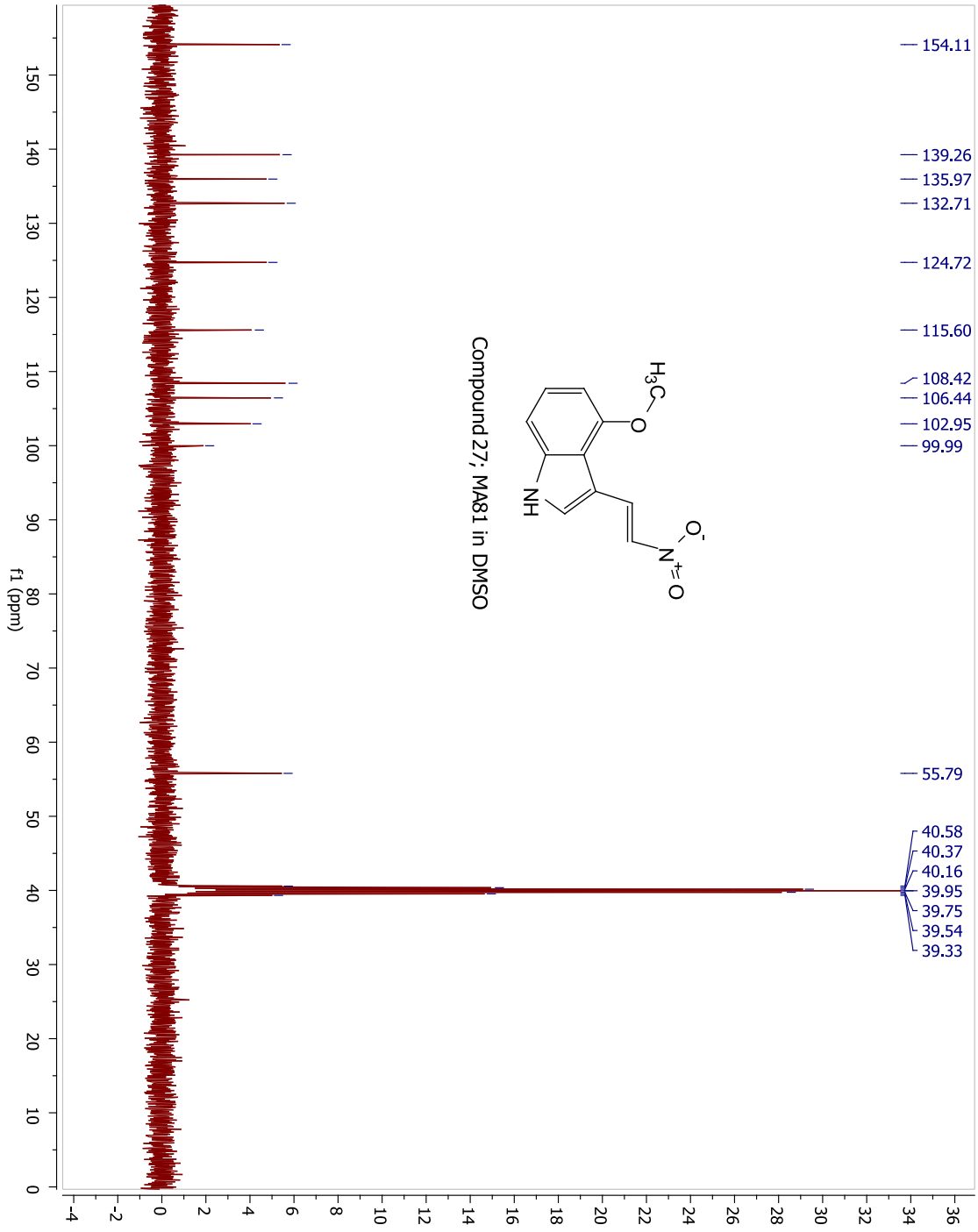

Compound 24;
MA94 in CDCl3
HO
O
CH3
N
O
O
CH3
173.94
157.20
136.39
134.57
128.37
125.59
121.23
111.34
77.38
77.06
76.74
70.80
58.13
55.29
51.50
49.15
35.72
33.83
26.08
22.96
f1 (ppm)

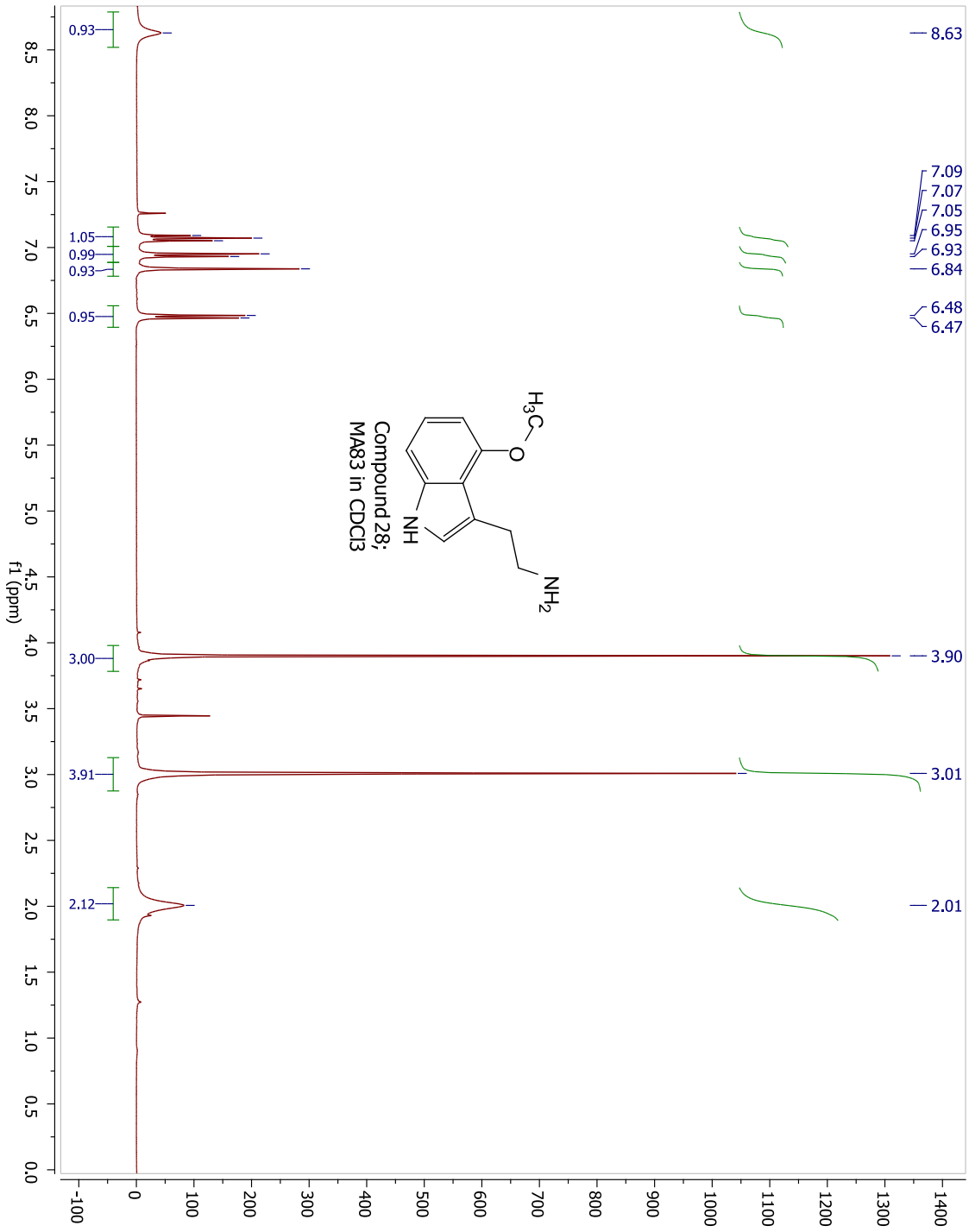

Compound 24 oxalate;
MA94 oxalate in DMSO at 120C
f1 (ppm)
0.90
0.91
0.91
0.97
7.01
3.00
2.95
3.92
2.11
2.01
0.44
1.99
3.94
2.16
7.55
7.53
7.24
7.22
7.01
6.99
6.96
6.95
6.93
5.18
3.84
3.63
3.27
3.25
3.03
3.02
2.70
2.66
2.39
2.37
2.35
1.77
1.74
1.67
1.65
1.63

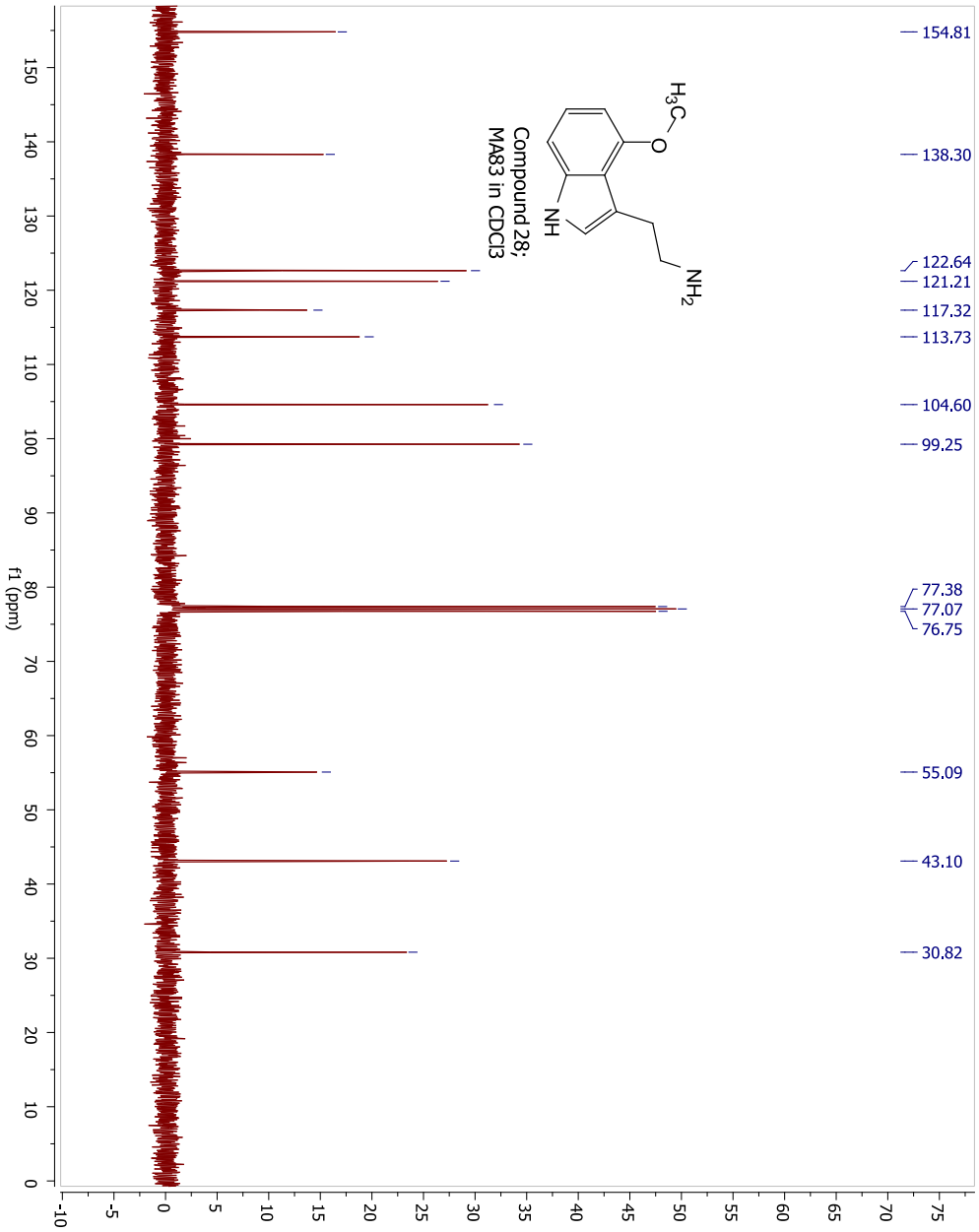

Compound 25;
MA127 in CDCl3
f1 (ppm)
7.29
7.27
7.25
7.23
7.21
6.95
6.93
6.91
6.89
6.87
3.81
3.67
2.89
2.86
2.46
2.38
2.26
2.20
2.04
1.71
1.69
1.67
1.66
1.64
1.62
1.60
1.58
1.12
0.91
0.95
0.92
3.00
3.07
1.98
1.99
2.96
2.78
2.04
2.92
4.07

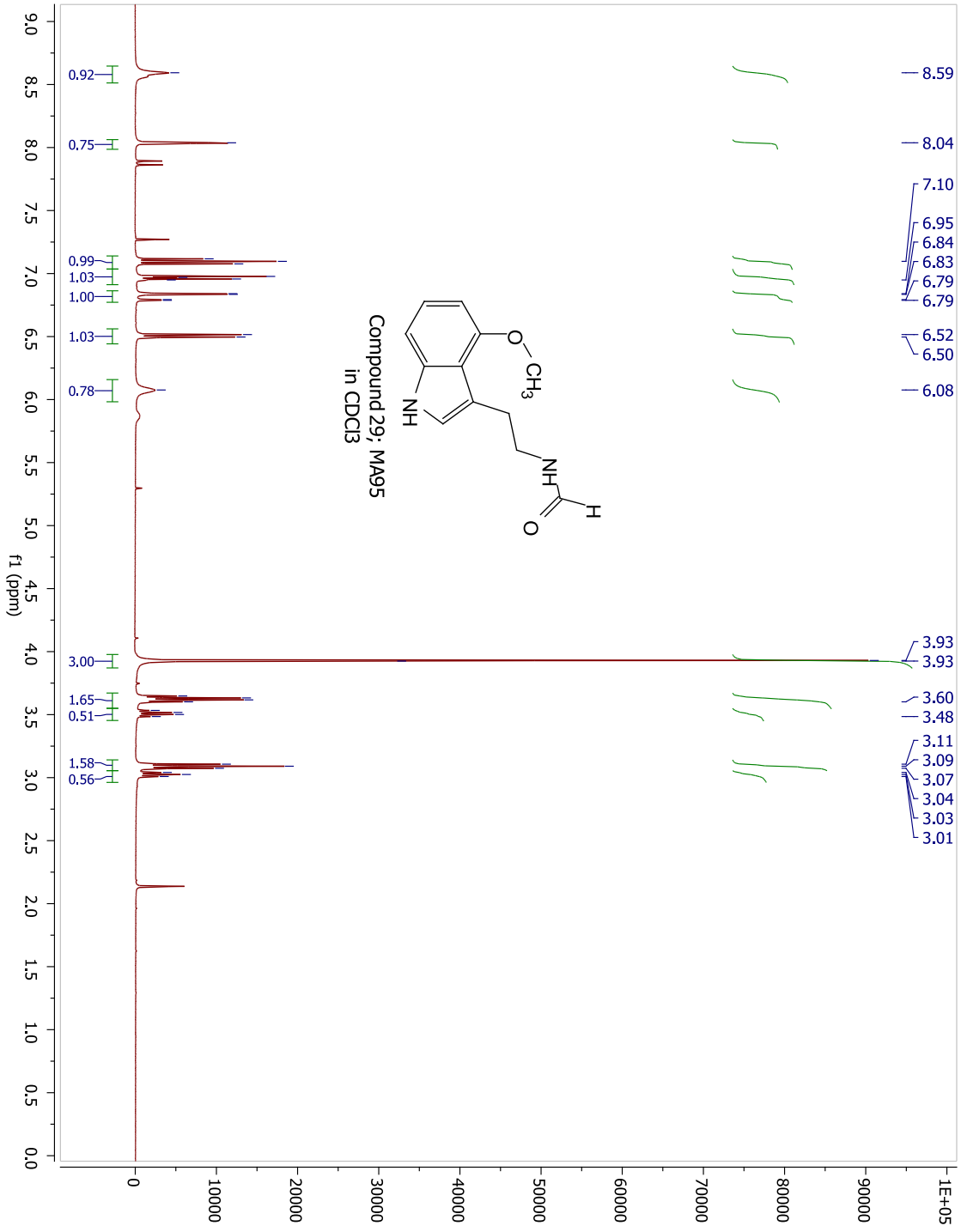

Compound 25
MA127 in CDCl3
f1 (ppm)
173.96
169.42
156.83
136.39
130.68
128.65
126.72
120.47
111.61
79.87
77.47
77.37
77.05
76.73
63.60
55.20
51.51
49.38
33.81
33.57
26.19
22.99
22.92
21.71

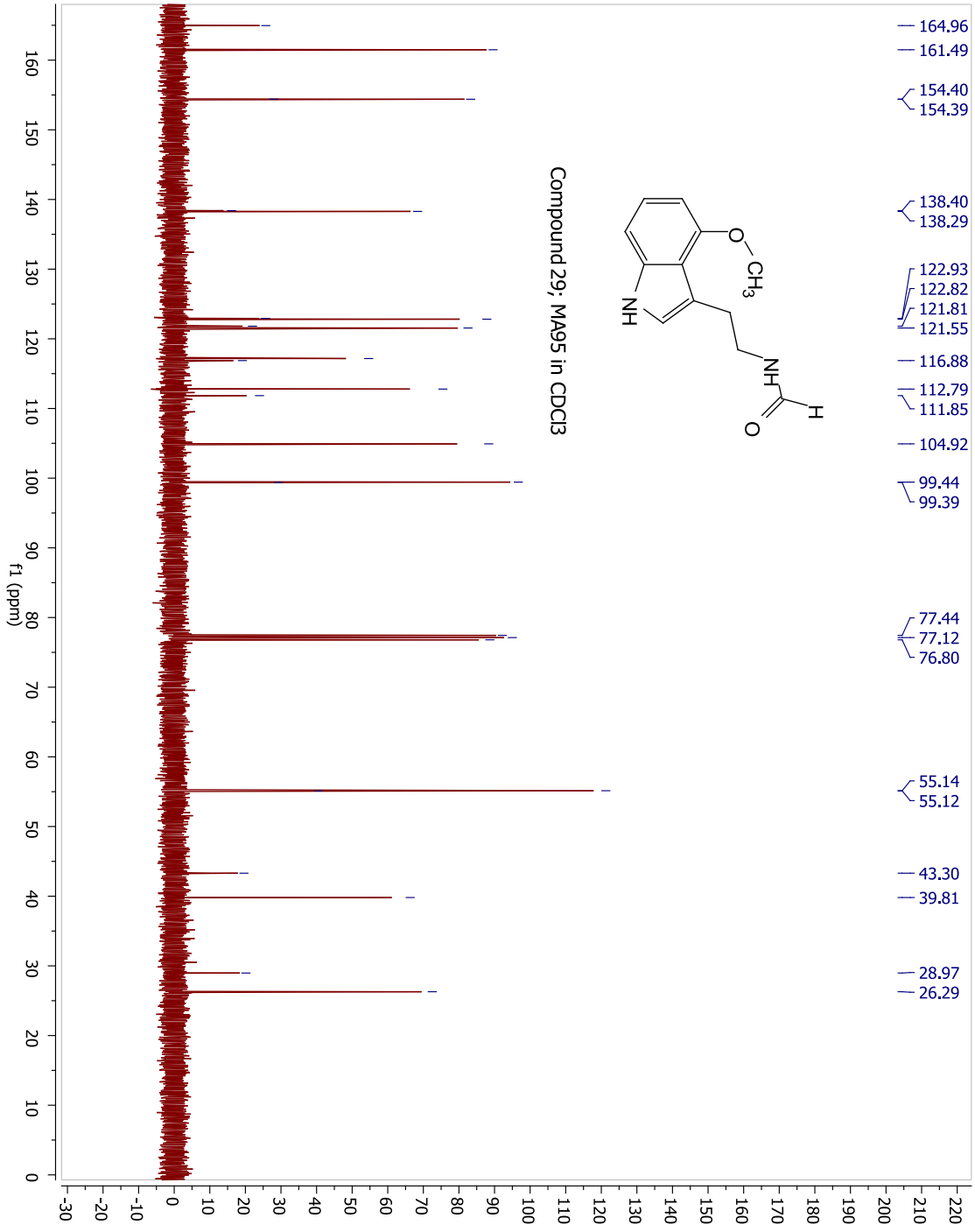

Compound 27; MA81 in DMSO
1.08
1.07
1.01
0.98
1.05
0.97
1.01
3.00
12.23
8.58
8.55
8.25
8.12
8.09
7.16
7.11
7.09
6.73
6.71
3.94
f1 (ppm)

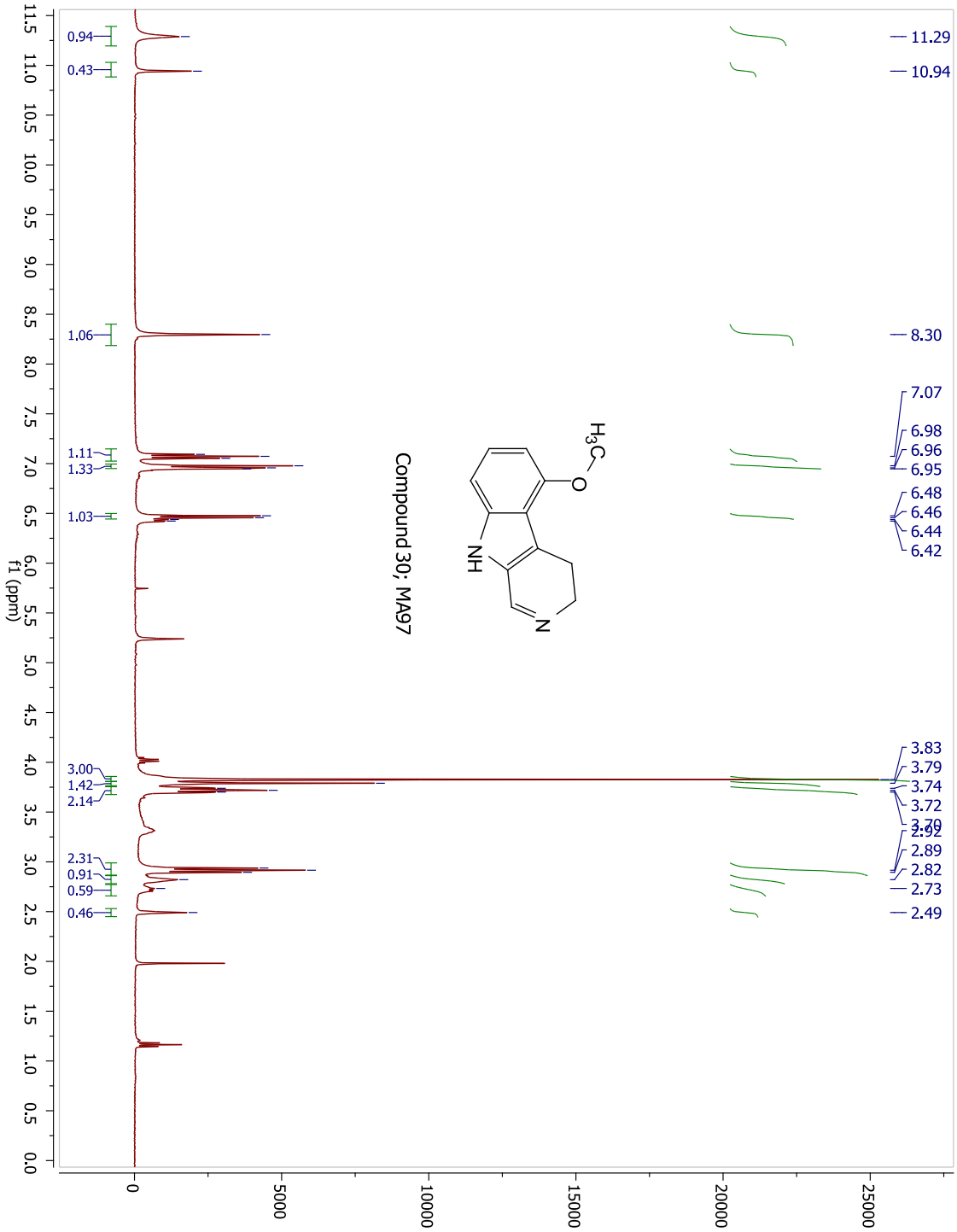

154.11
139.26
135.97
132.71
124.72
115.60
108.42
106.44
102.95
99.99
55.79
40.58
40.37
40.16
39.95
39.75
39.54
39.33
Compound 27; MA81 in DMSO
f1 (ppm)

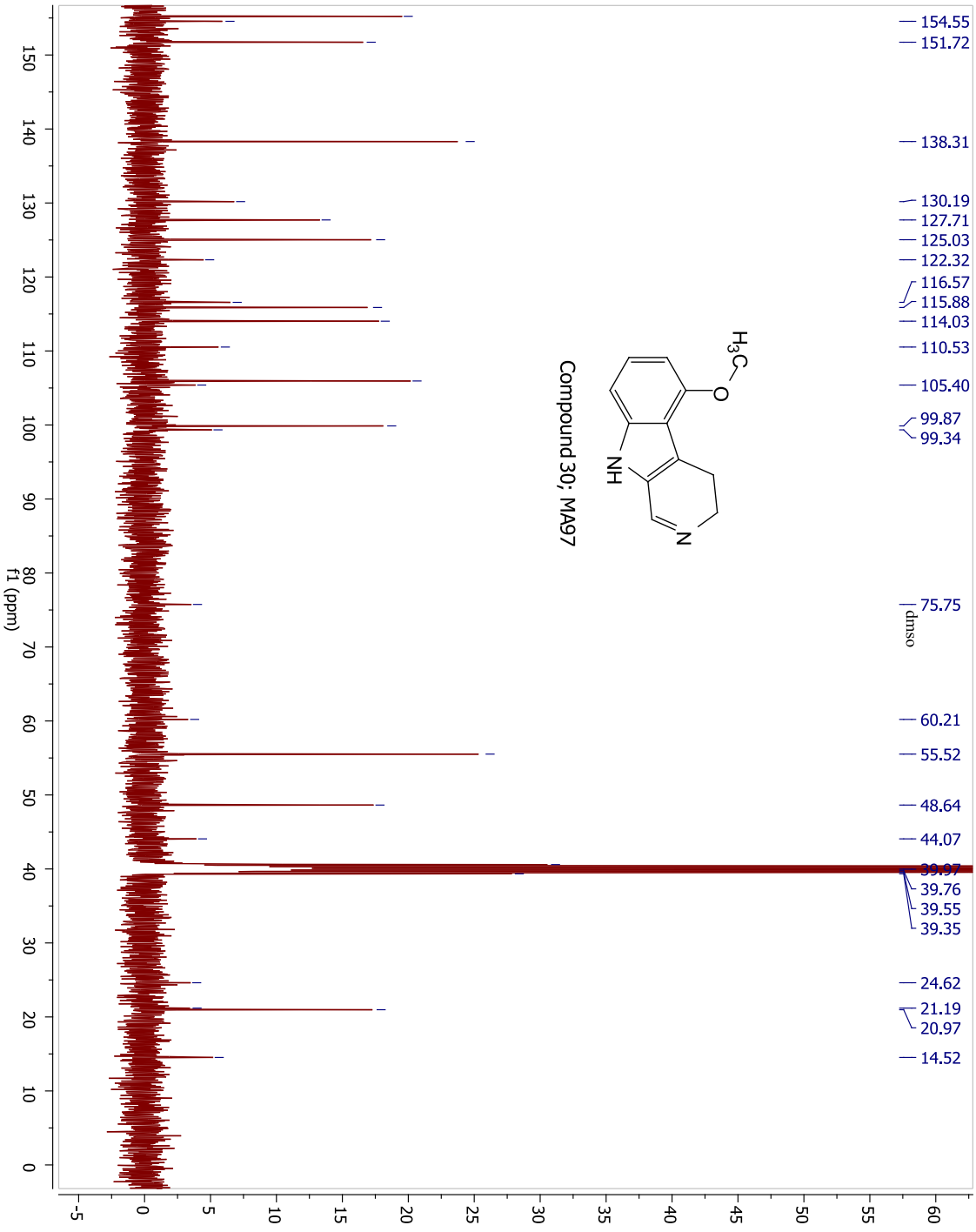

Compound 28;
MA83 in CDCl3
H3C
O
NH
NH2
0.93
1.05
0.99
0.93
0.95
3.00
3.91
2.12
8.63
7.09
7.07
7.05
6.95
6.93
6.84
6.48
6.47
3.90
3.01
2.01
f1 (ppm)

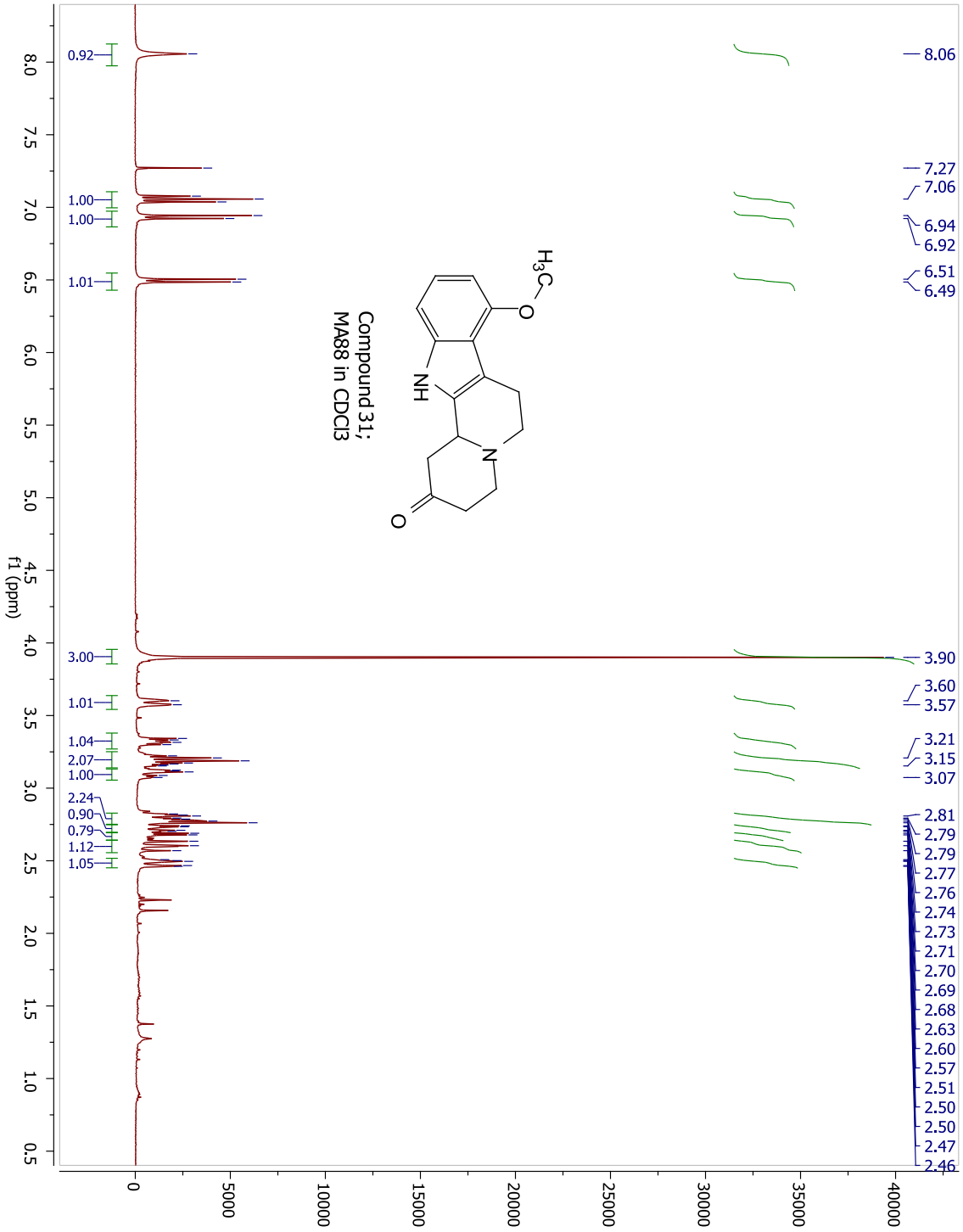

Compound 28;
MA83 in CDCl3
H3C
O
NH
NH2
154.81
138.30
122.64
121.21
117.32
113.73
104.60
99.25
77.38
77.07
76.75
55.09
43.10
30.82
f1 (ppm)

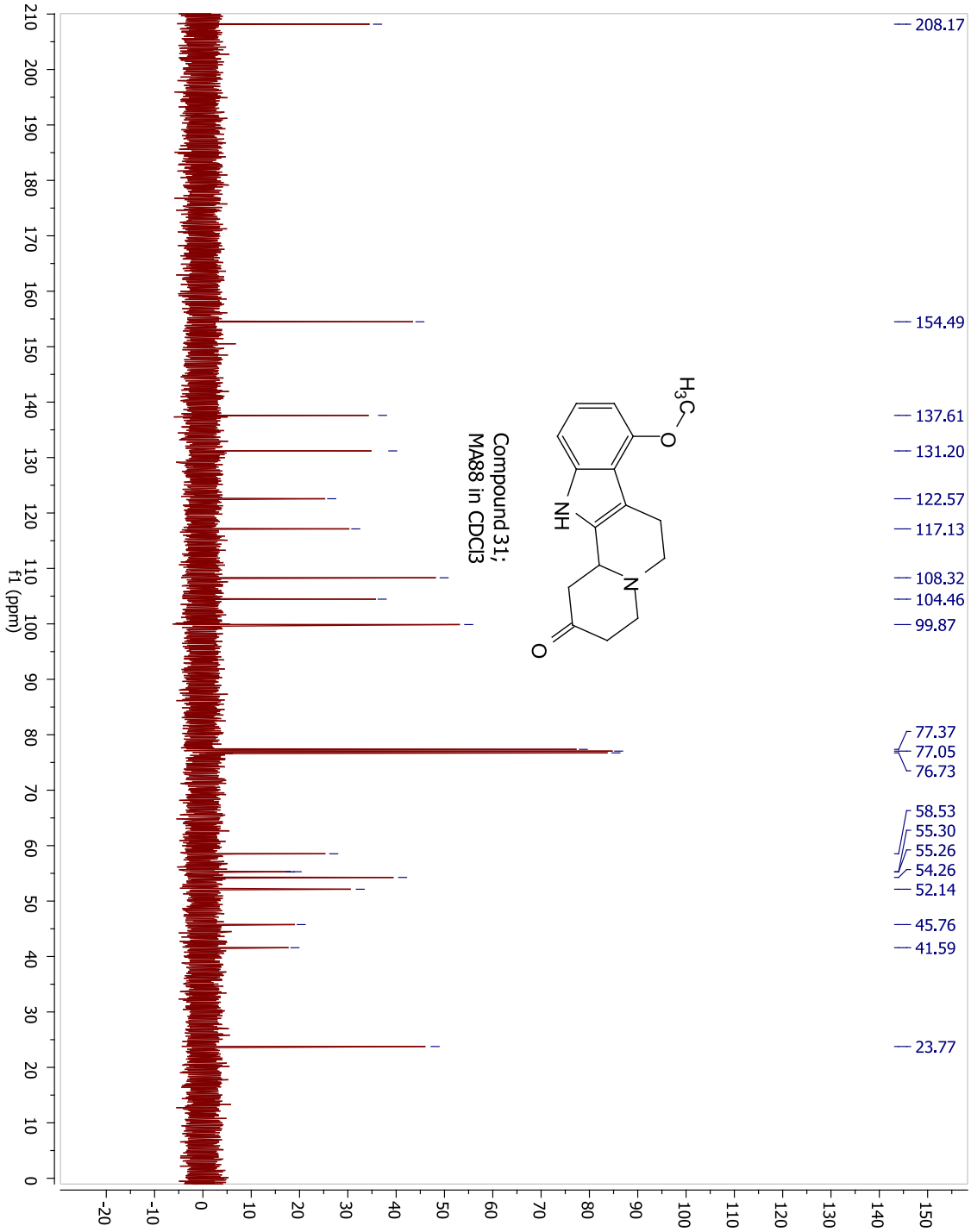

Compound 29; MA95
in CDCl3
NH
O
CH3
NH
O
H
f1 (ppm)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.92
0.75
0.99
1.03
1.00
1.03
0.78
3.00
1.65
0.51
1.58
0.56
8.59
8.04
7.10
6.95
6.84
6.83
6.79
6.79
6.52
6.50
6.08
3.93
3.93
3.60
3.48
3.11
3.09
3.07
3.04
3.03
3.01
0
10000
20000
30000
40000
50000
60000
70000
80000
90000
1E+05

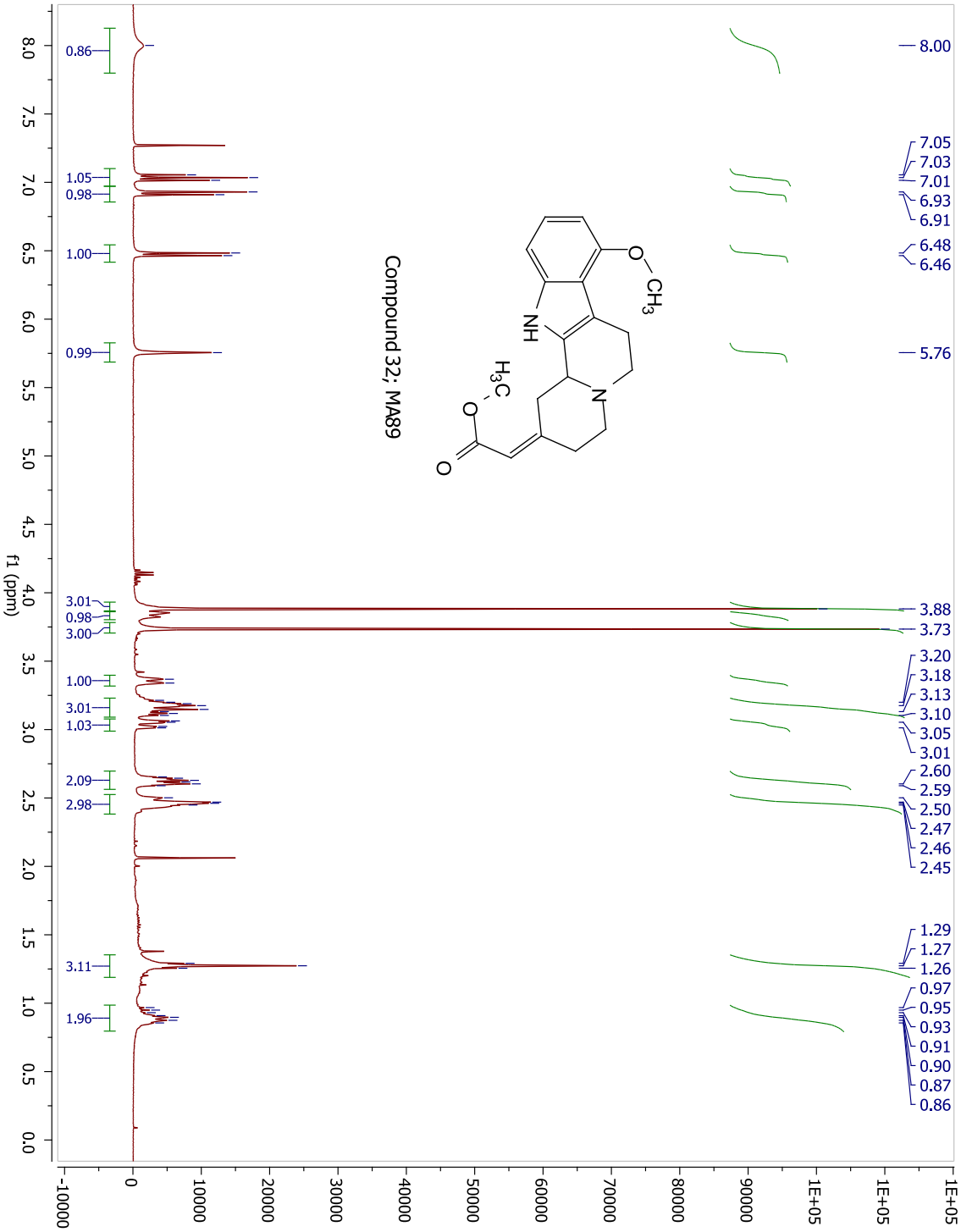

Compound 29; MA95 in CDCl3
164.96
161.49
154.40
154.39
138.40
138.29
122.93
122.82
121.81
121.55
116.88
112.79
111.85
104.92
99.44
99.39
77.44
77.12
76.80
55.14
55.12
43.30
39.81
28.97
26.29
f1 (ppm)

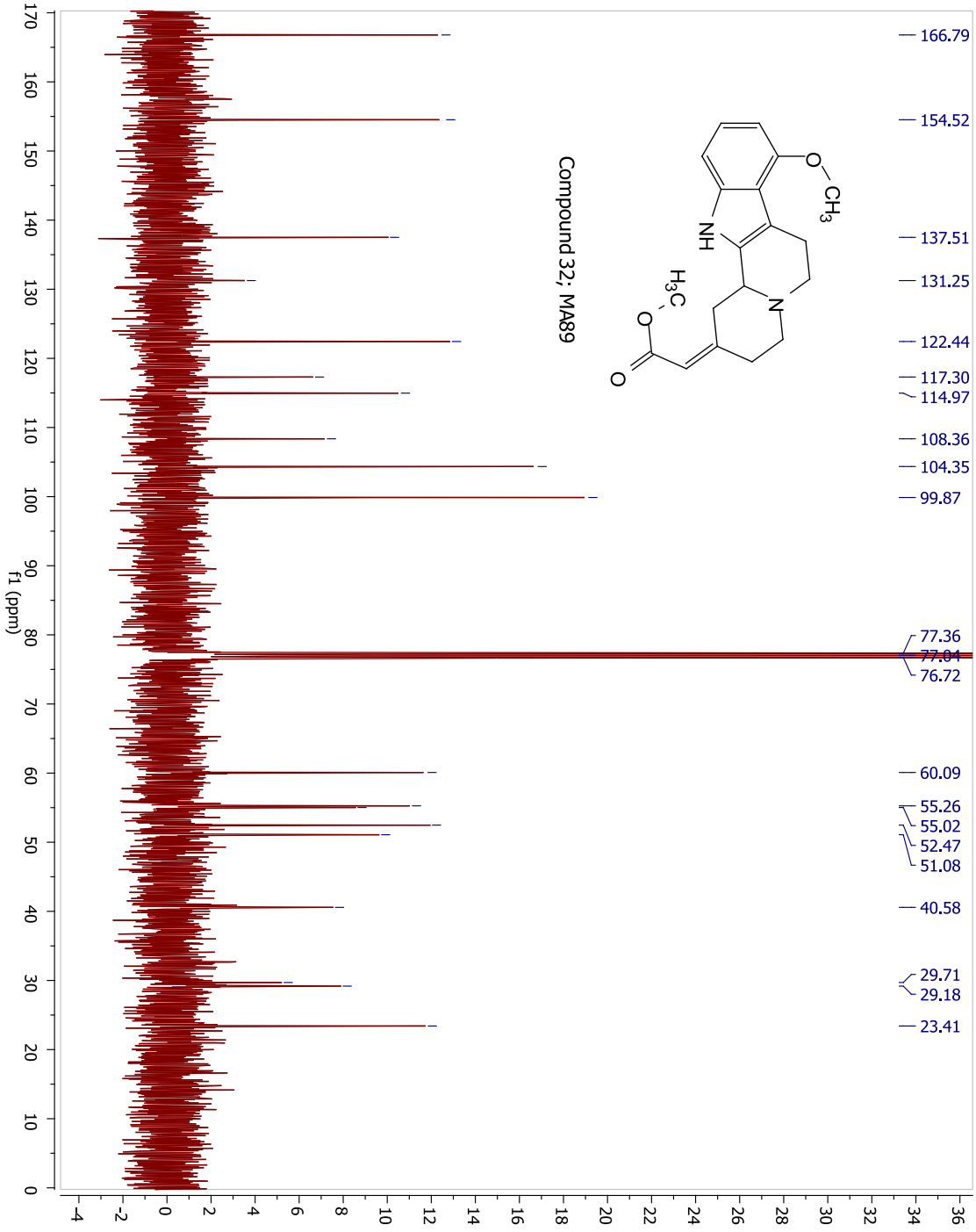

Compound 30; MA97
f1 (ppm)
0.94
0.43
1.06
1.11
1.33
1.03
3.00
1.42
2.14
2.31
0.91
0.59
0.46
11.29
10.94
8.30
7.07
6.98
6.96
6.95
6.48
6.46
6.44
6.42
3.83
3.79
3.74
3.72
2.89
2.82
2.73
2.49
NH
N
O
H3C

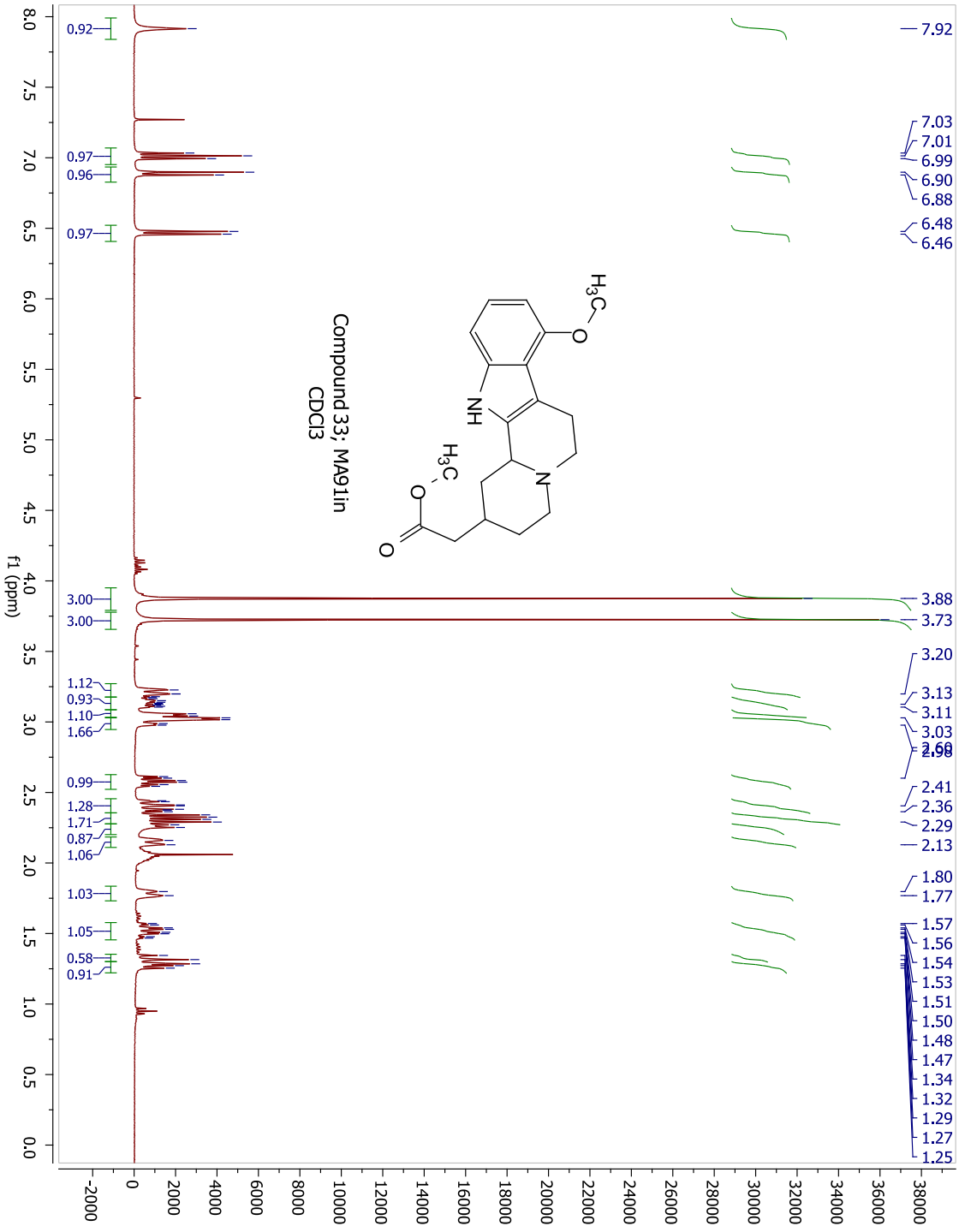

154.55
151.72
138.31
130.19
127.71
125.03
122.32
116.57
115.88
114.03
110.53
105.40
99.87
99.34
75.75
dmso
60.21
55.52
48.64
44.07
39.97
39.76
39.55
39.35
24.62
21.19
20.97
14.52
Compound 30; MA97
NH
N
O
H3C
f1 (ppm)

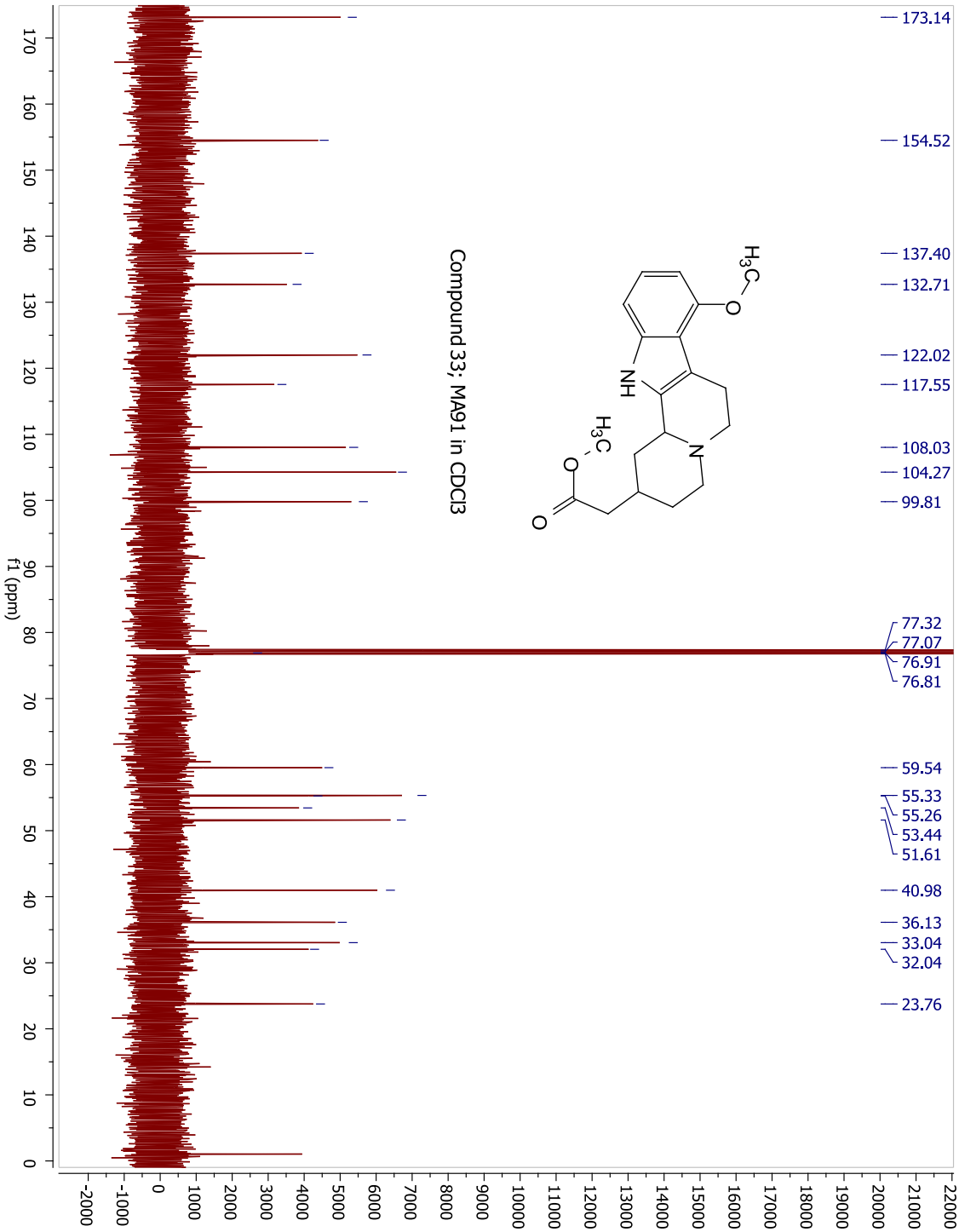

Compound 31;
MA88 in CDCl3
NH
N
O
O
H3C
f1 (ppm)
0.92
1.00
1.00
1.01
3.00
1.01
1.04
2.07
1.00
2.24
0.90
0.79
1.12
1.05
8.06
7.27
7.06
6.94
6.92
6.51
6.49
3.90
3.60
3.57
3.21
3.15
3.07
2.81
2.79
2.79
2.77
2.76
2.74
2.73
2.71
2.70
2.69
2.68
2.63
2.60
2.57
2.51
2.50
2.50
2.47
2.46

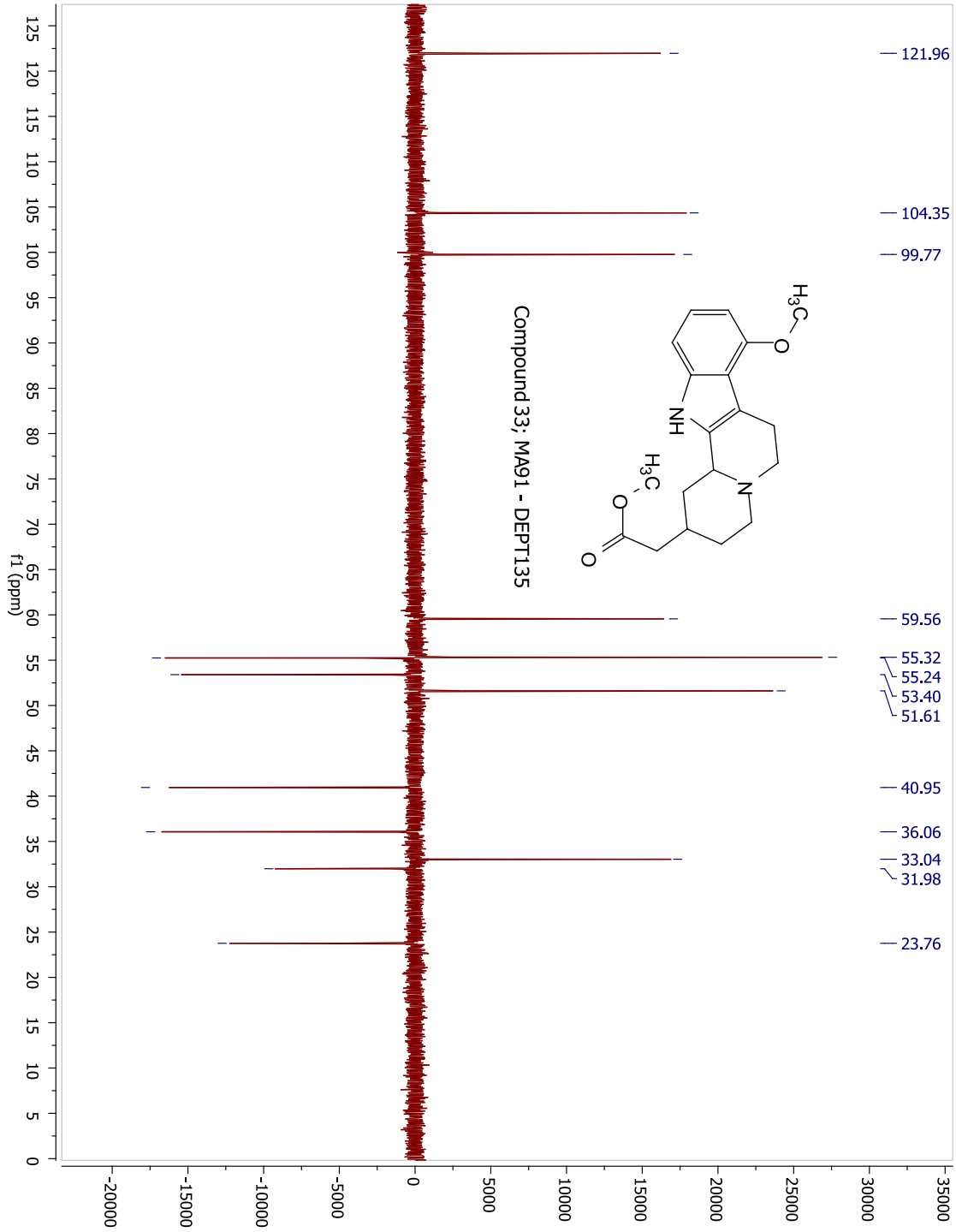

Compound 31;
MA88 in CDCl3
f1 (ppm)
208.17
154.49
137.61
131.20
122.57
117.13
108.32
104.46
99.87
77.37
77.05
76.73
58.53
55.30
55.26
54.26
52.14
45.76
41.59
23.77
NH
N
O
H3C
O

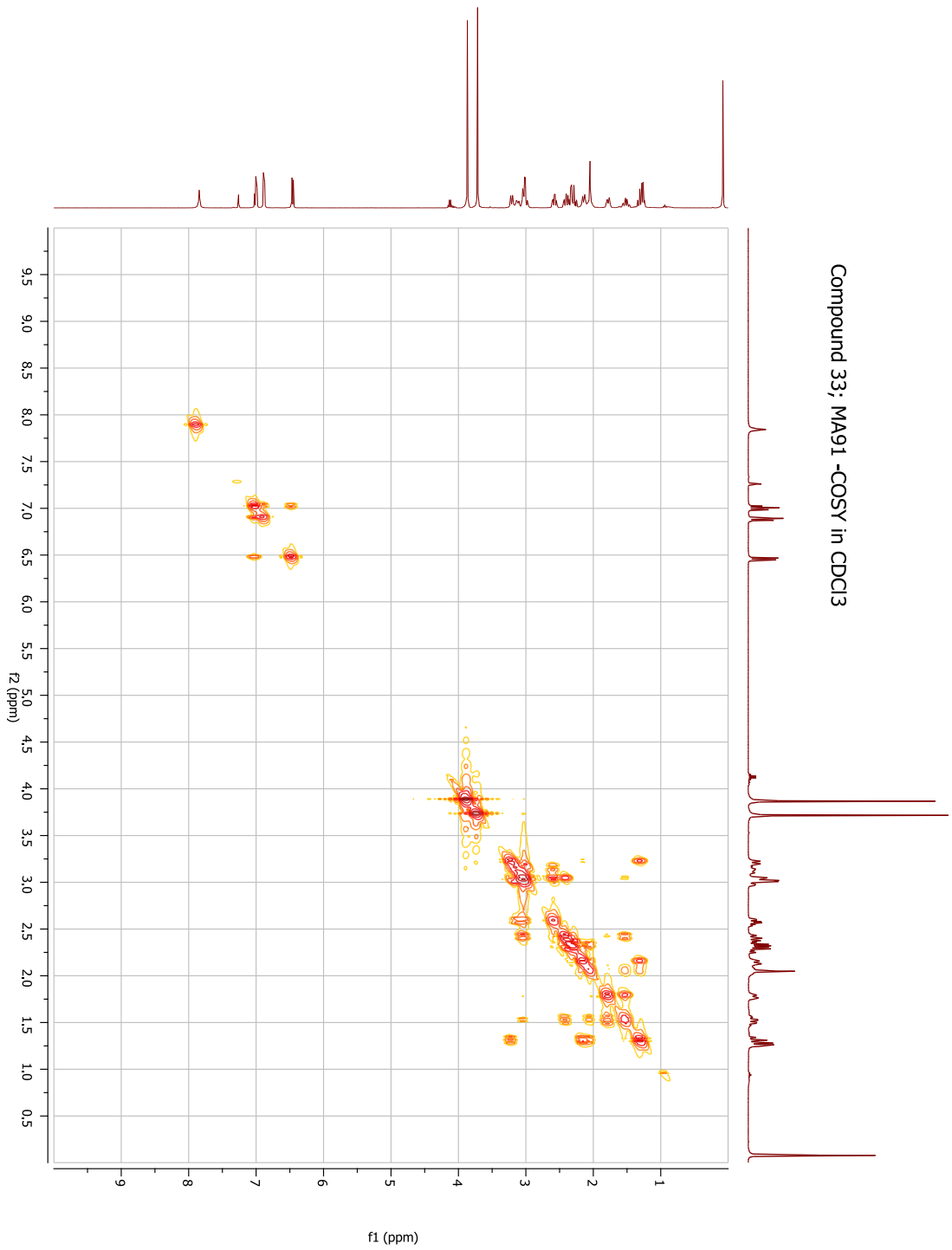

Compound 32; MA89
f1 (ppm)
0.86
1.05
0.98
1.00
0.99
3.01
0.98
3.00
1.00
3.01
1.03
2.09
2.98
3.11
1.96
8.00
7.05
7.03
7.01
6.93
6.91
6.48
6.46
5.76
3.88
3.73
3.20
3.18
3.13
3.10
3.05
3.01
2.60
2.59
2.50
2.47
2.46
2.45
1.29
1.27
1.26
0.97
0.95
0.93
0.91
0.90
0.87
0.86
NH
N
O
CH3
H3C
O
O

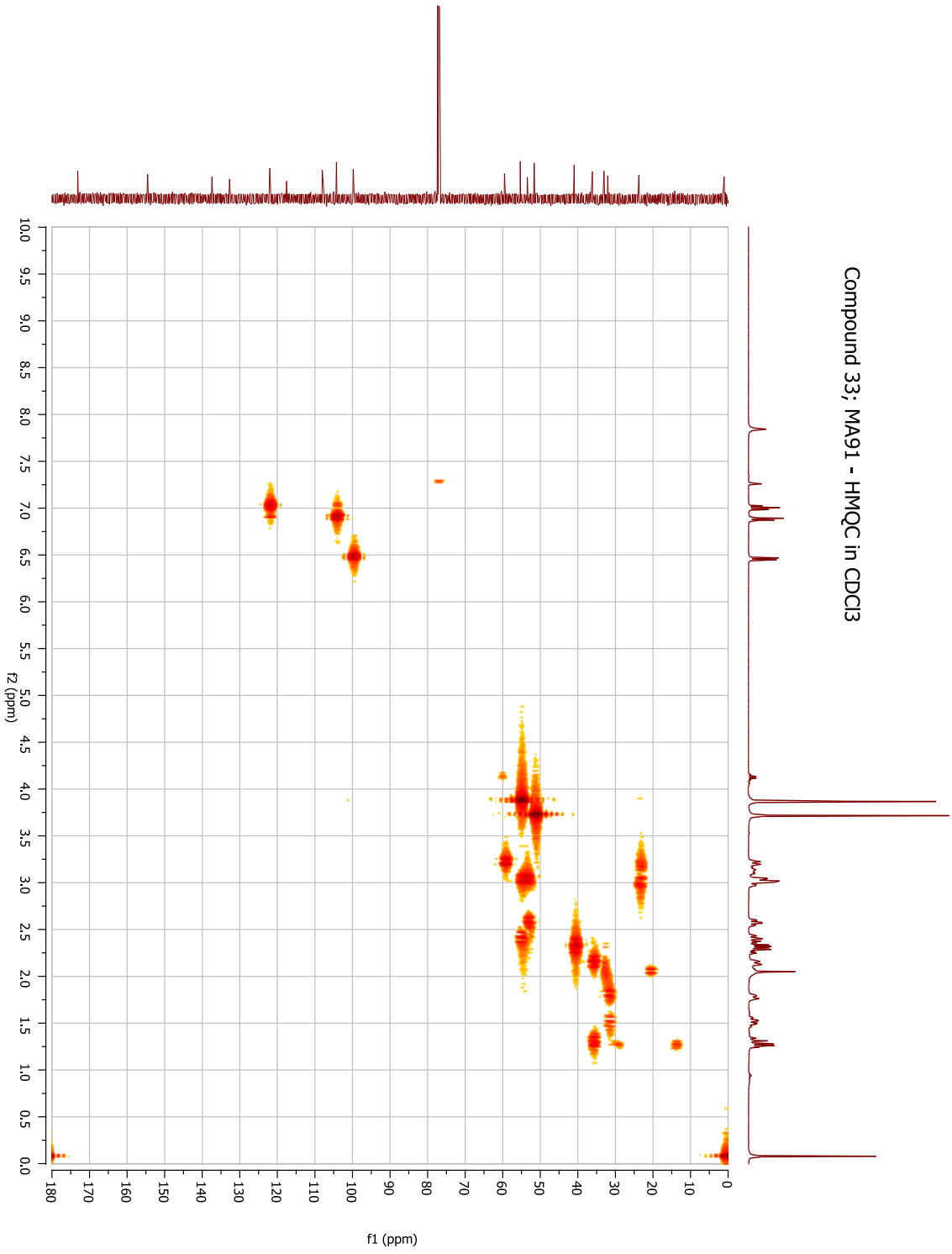

Compound 32; MA89
166.79
154.52
137.51
131.25
122.44
117.30
114.97
108.36
104.35
99.87
77.36
77.04
76.72
60.09
55.26
55.02
52.47
51.08
40.58
29.71
29.18
23.41
f1 (ppm)

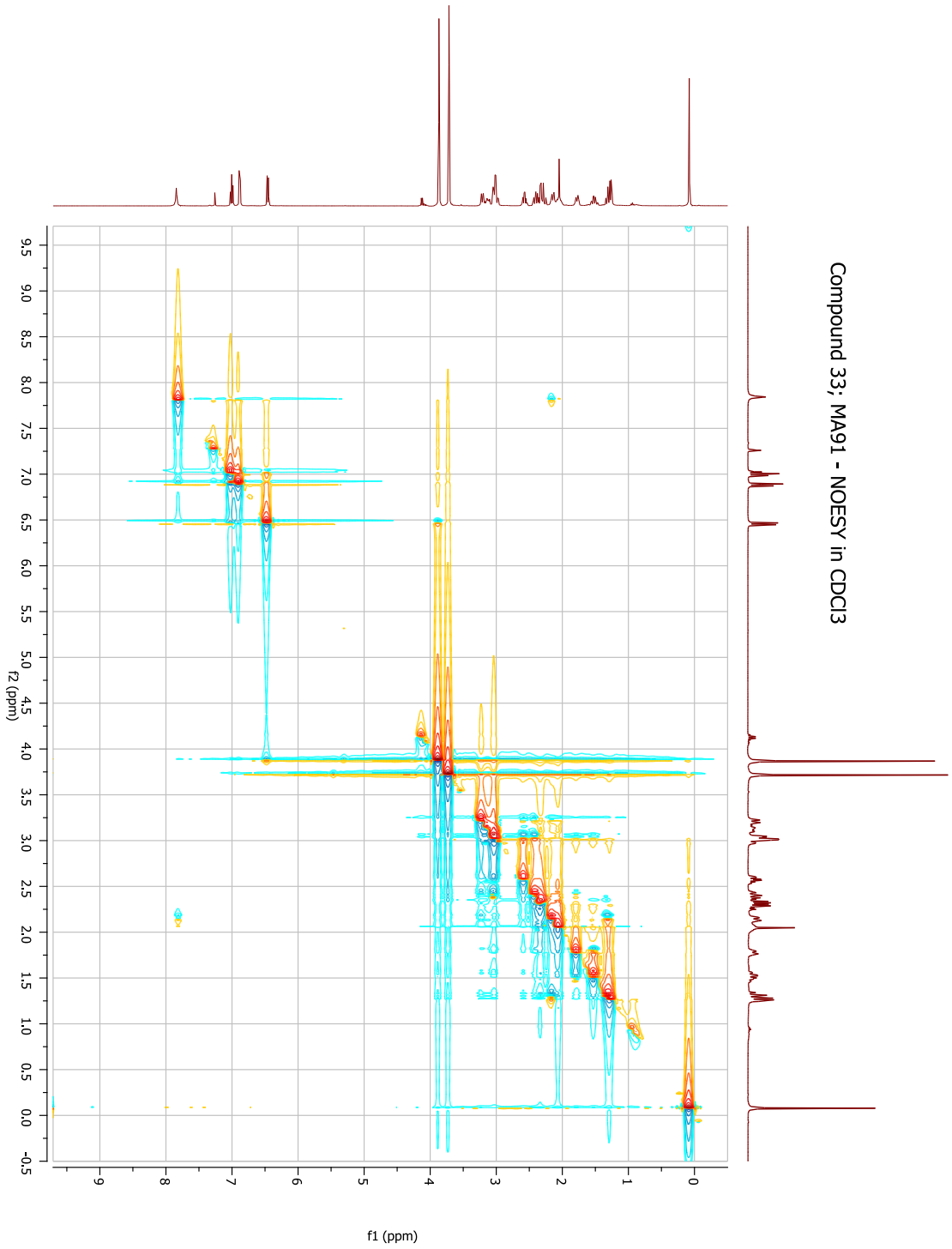

Compound 33; MA91in
CDCl3
NH
N
O
O
O
H3C
H3C
f1 (ppm)
0.92
0.97
0.96
0.97
3.00
3.00
1.12
0.93
1.10
1.66
0.99
1.28
1.71
0.87
1.06
1.03
1.05
0.58
0.91
7.92
7.03
7.01
6.99
6.90
6.88
6.48
6.46
3.88
3.73
3.20
3.13
3.11
3.03
2.41
2.36
2.29
2.13
1.80
1.77
1.57
1.56
1.54
1.53
1.51
1.50
1.48
1.47
1.34
1.32
1.29
1.27
1.25

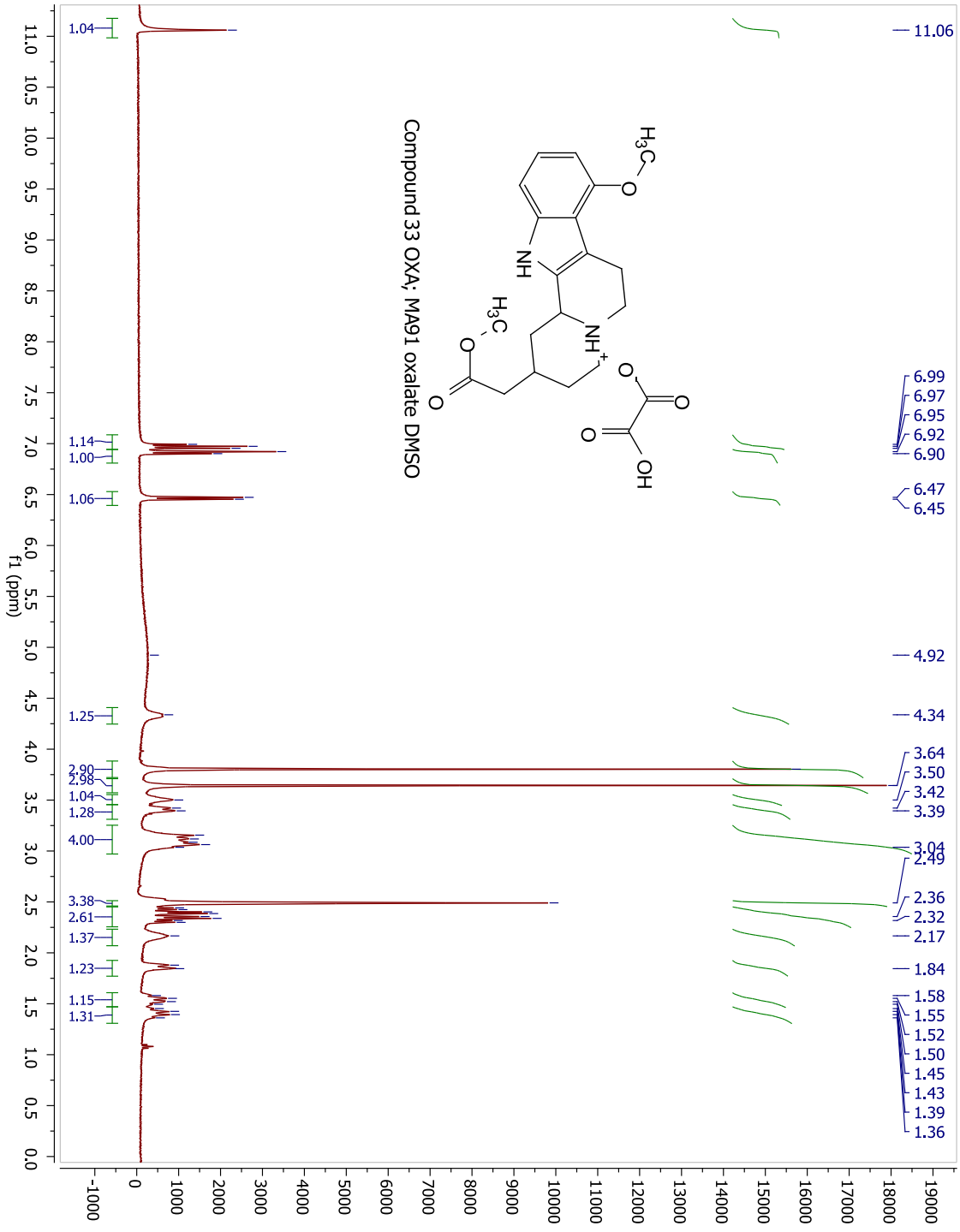

Compound 33; MA91 in CDCl3
173.14
154.52
137.40
132.71
122.02
117.55
108.03
104.27
99.81
77.32
77.07
76.91
76.81
59.54
55.33
55.26
53.44
51.61
40.98
36.13
33.04
32.04
23.76
f1 (ppm)

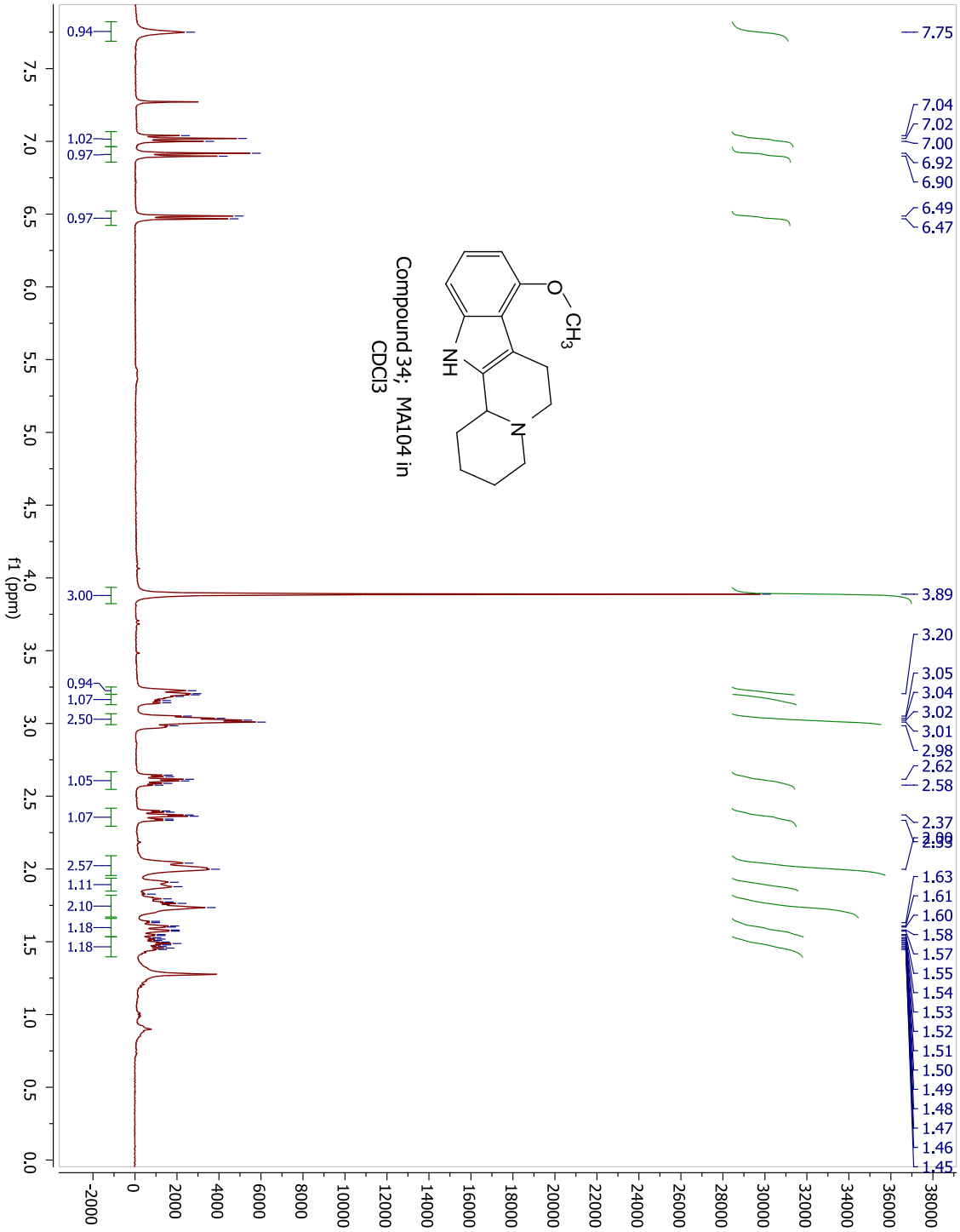

Compound 33; MA91 - DEPT135
f1 (ppm)
121.96
104.35
99.77
59.56
55.32
55.24
53.40
51.61
40.95
36.06
33.04
31.98
23.76
H3C
O
NH
N
O
O
H3C

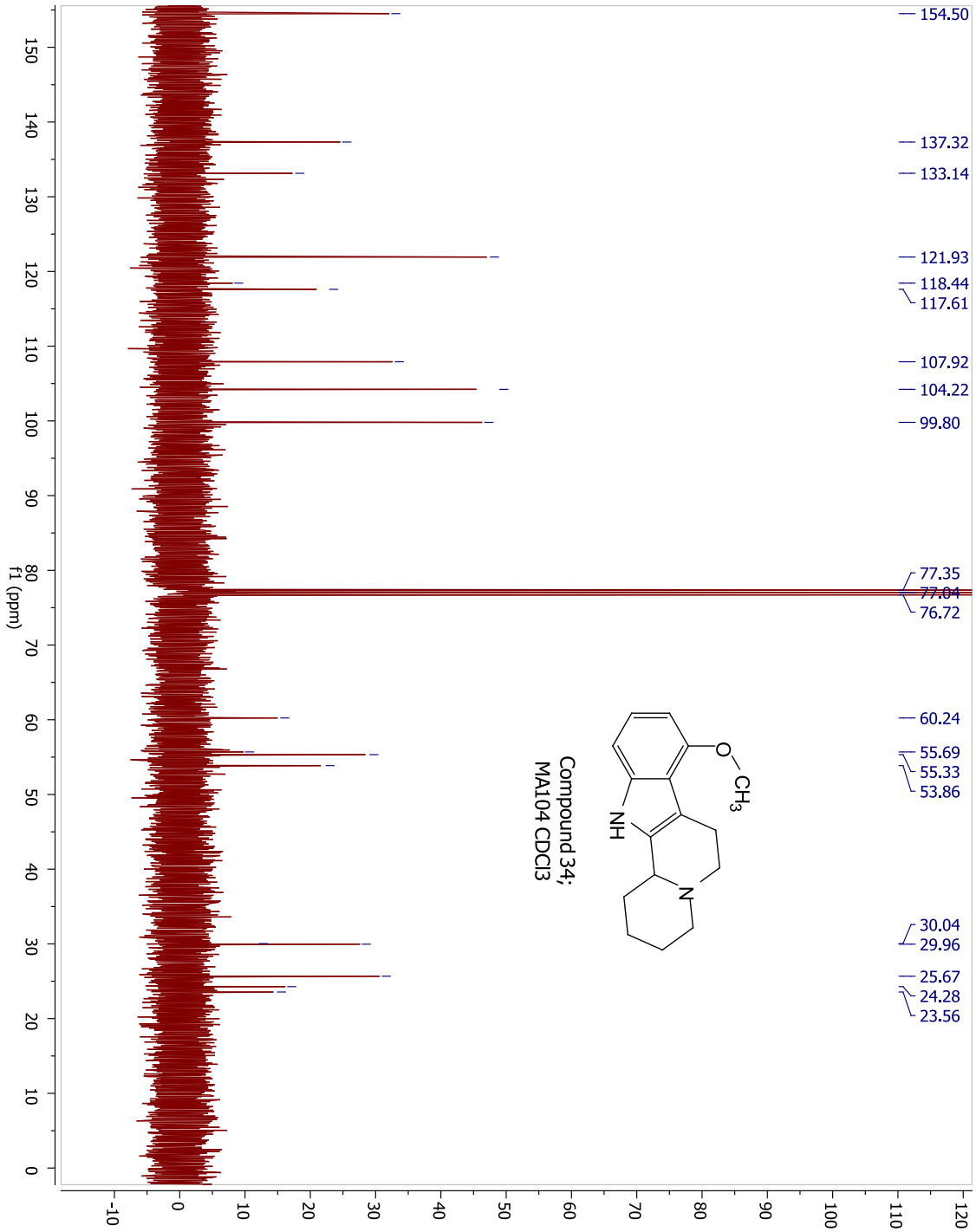

Compound 33; MA91 -COSY in CDCl3
f2 (ppm)
f1 (ppm)

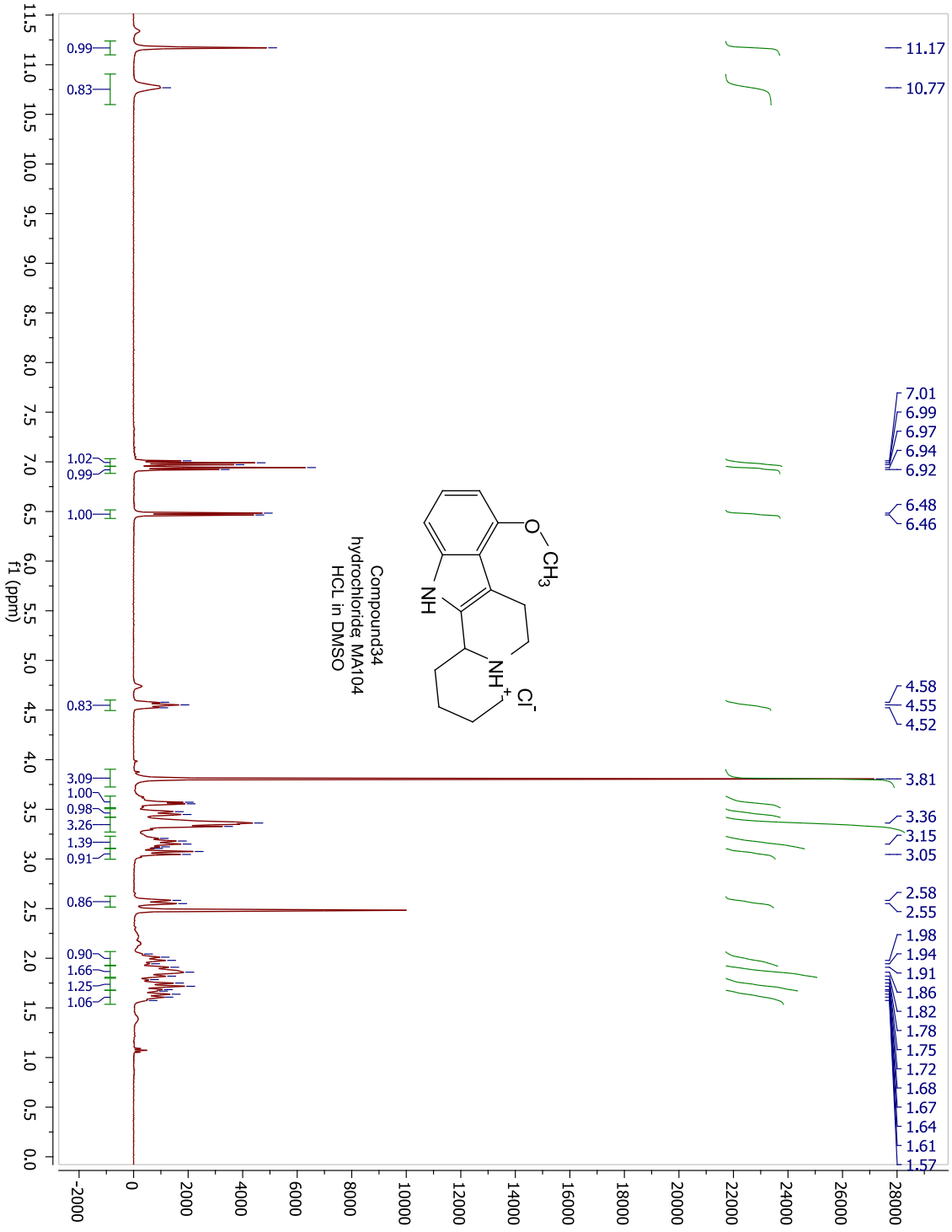

Compound 33; MA91 - HMQC in CDCl3
f2 (ppm)
f1 (ppm)

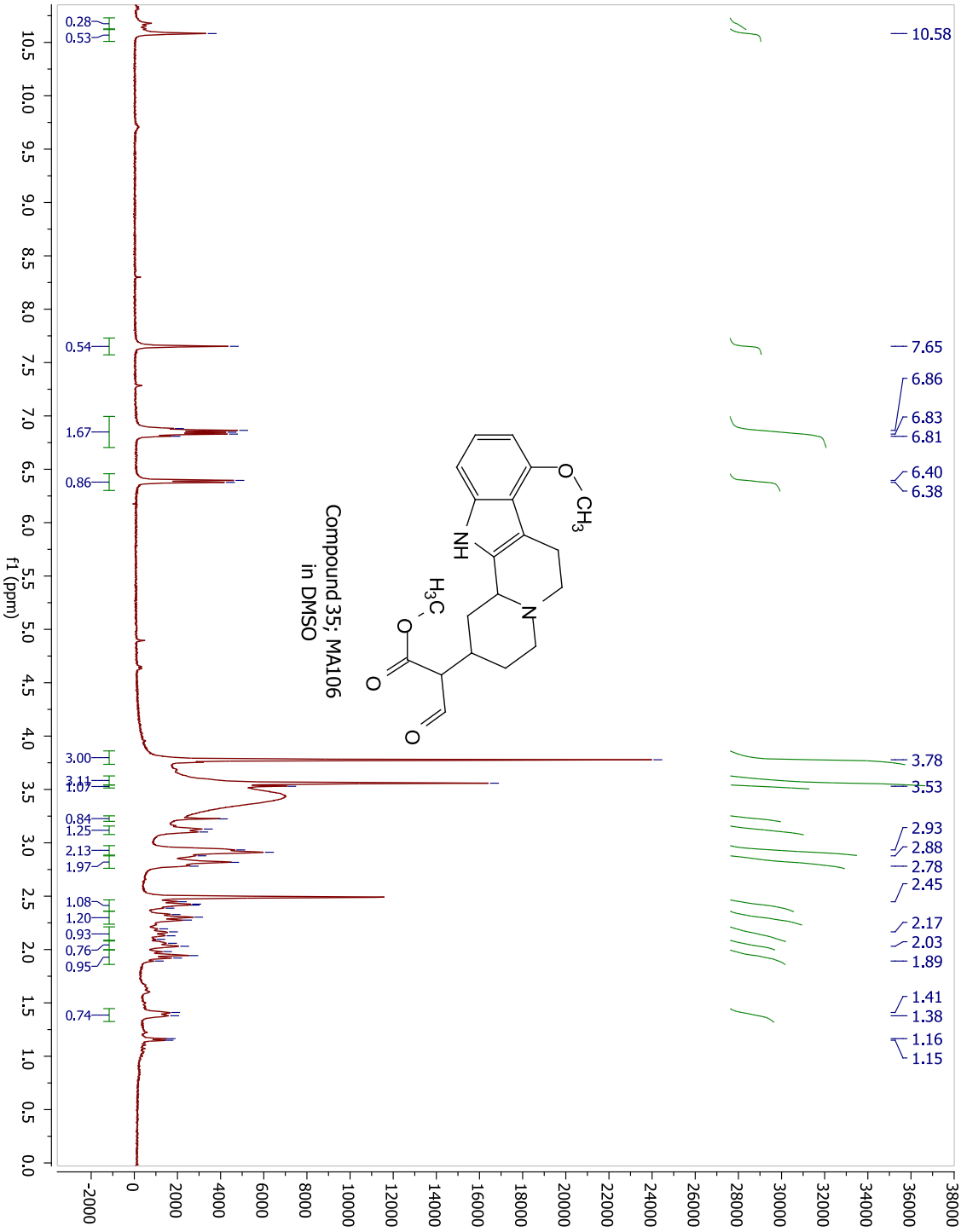

Compound 33; MA91 - NOESY in CDCl3
f2 (ppm)
f1 (ppm)

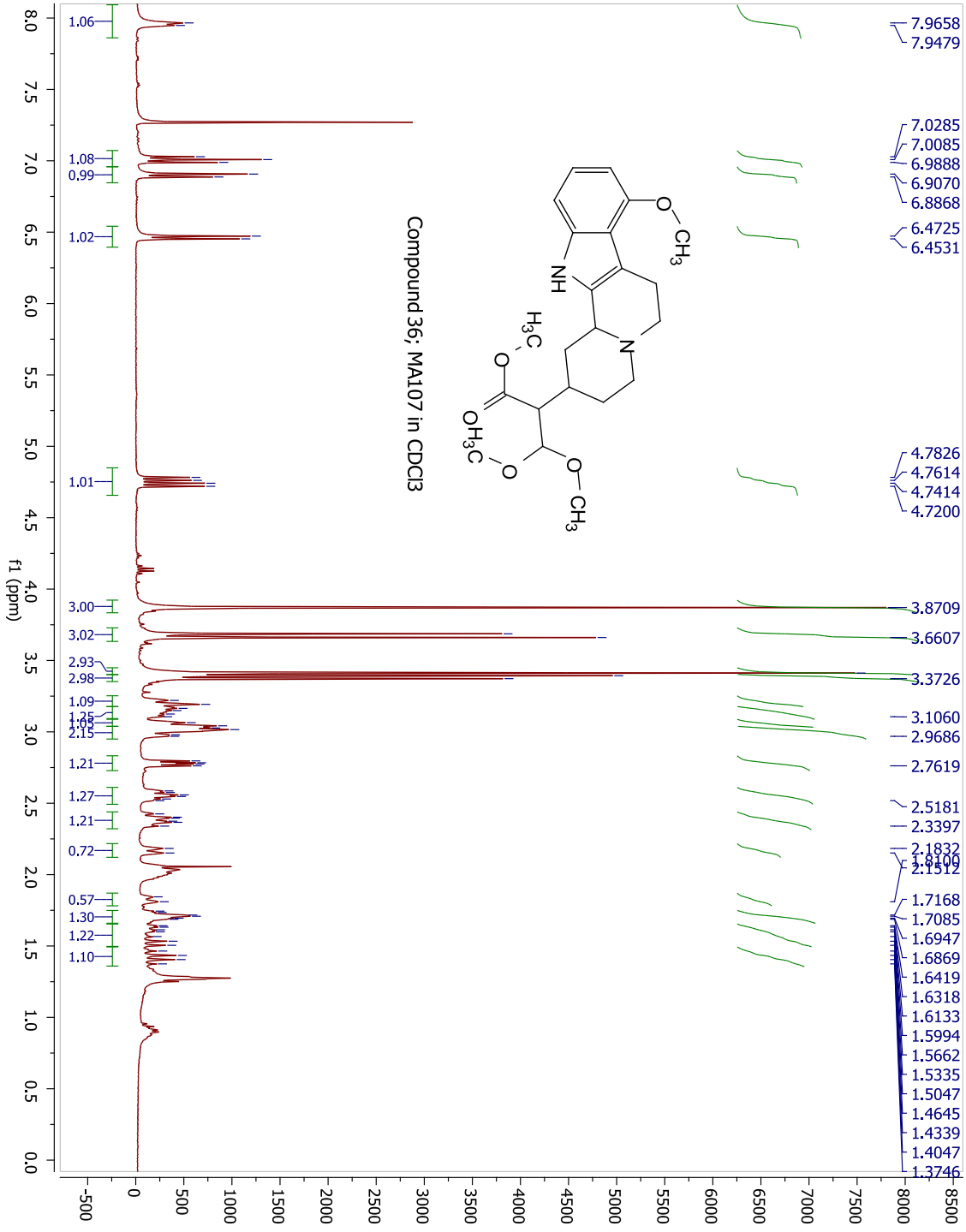

Compound 33 OXA; MA91 oxalate DMSO
f1 (ppm)
1.04
1.14
1.00
1.06
1.25
2.90
2.98
1.04
1.28
4.00
3.38
2.61
1.37
1.23
1.15
1.31
11.06
6.99
6.97
6.95
6.92
6.90
6.47
6.45
4.92
4.34
3.64
3.50
3.42
3.39
3.04
2.49
2.36
2.32
2.17
1.84
1.58
1.55
1.52
1.50
1.45
1.43
1.39
1.36

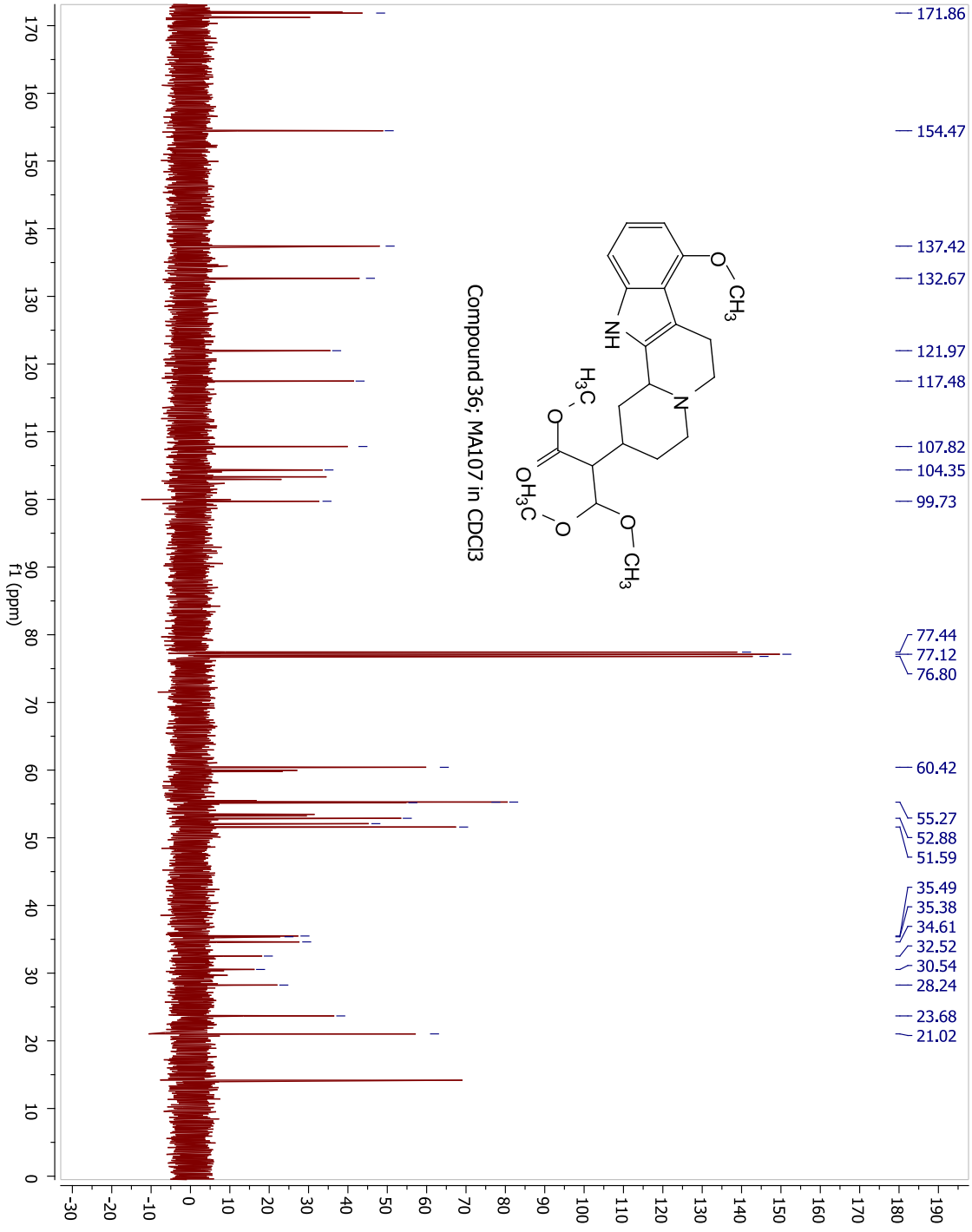

Compound 34; MA104 in CDCl3
NH
O
CH3
N
f1 (ppm)
0.94
1.02
0.97
0.97
3.00
0.94
1.07
2.50
1.05
1.07
2.57
1.11
2.10
1.18
1.18
7.75
7.04
7.02
7.00
6.92
6.90
6.49
6.47
3.89
3.20
3.05
3.04
3.02
3.01
2.98
2.62
2.58
2.37
1.63
1.61
1.60
1.58
1.57
1.55
1.54
1.53
1.52
1.51
1.50
1.49
1.48
1.47
1.46
1.45

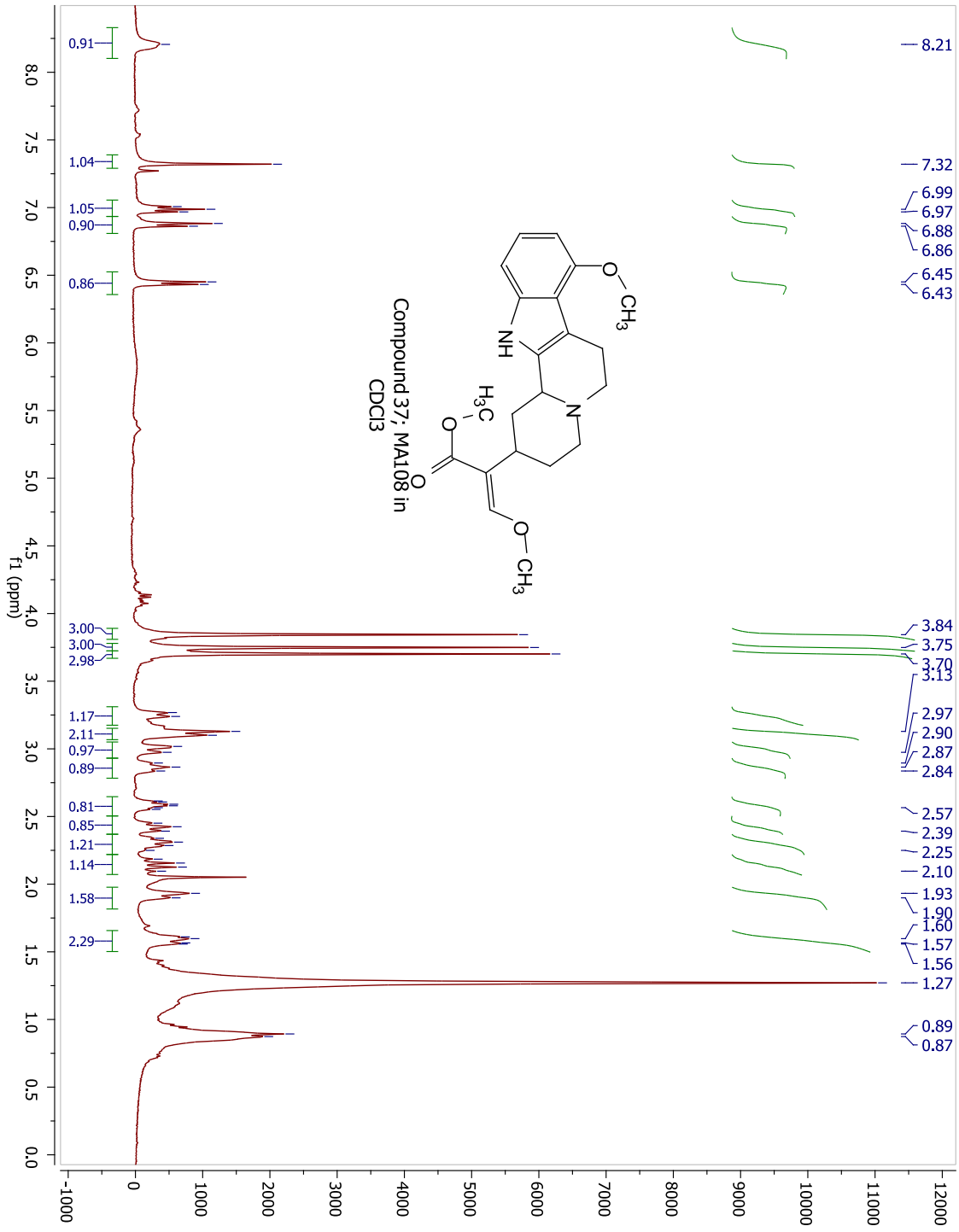

154.50
137.32
133.14
121.93
118.44
117.61
107.92
104.22
99.80
77.35
77.04
76.72
60.24
55.69
55.33
53.86
30.04
29.96
25.67
24.28
23.56
Compound 34;
MA104 CDCl3
NH
O
CH3
N
f1 (ppm)

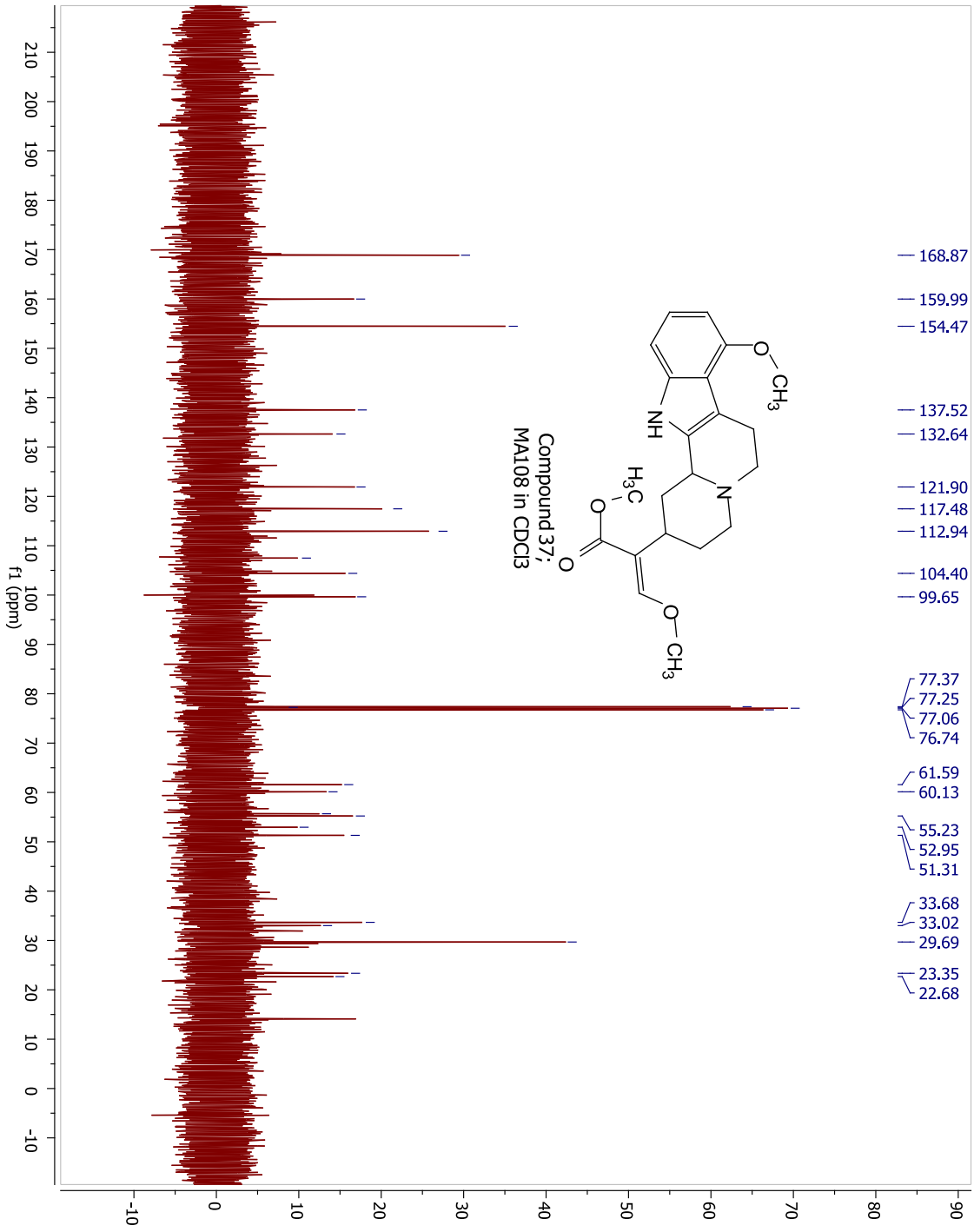

Compound34
hydrochloride MA104
HCL in DMSO
NH
O
CH3
NH+
Cl-
f1 (ppm)
0.99
0.83
1.02
0.99
1.00
0.83
3.09
1.00
0.98
3.26
1.39
0.91
0.86
0.90
1.66
1.25
1.06
11.17
10.77
7.01
6.99
6.97
6.94
6.92
6.48
6.46
4.58
4.55
4.52
3.81
3.36
3.15
3.05
2.58
2.55
1.98
1.94
1.91
1.86
1.82
1.78
1.75
1.72
1.68
1.67
1.64
1.61
1.57

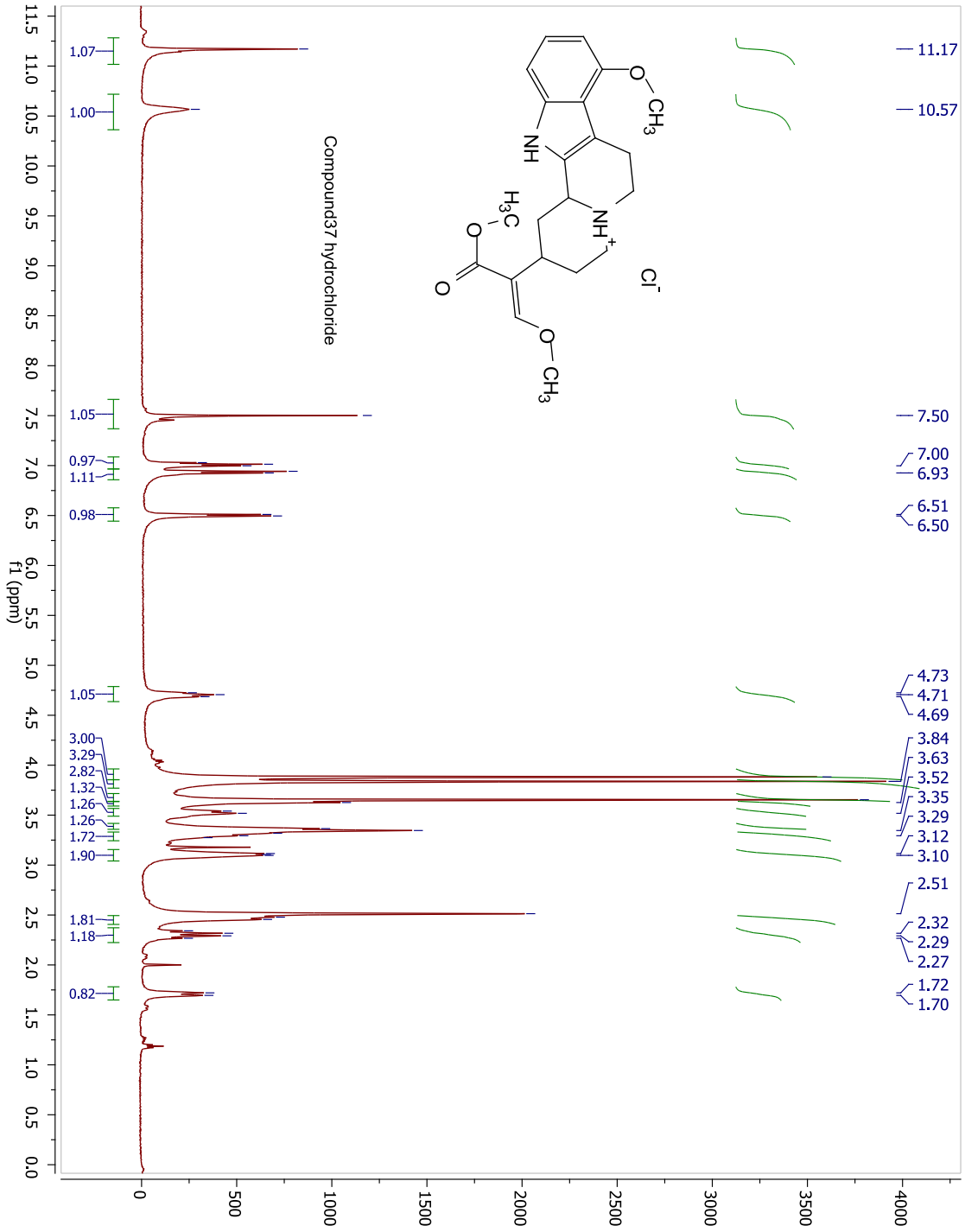

Compound 35; MA106
in DMSO
f1 (ppm)
0.28
0.53
0.54
1.67
0.86
3.00
3.11
1.07
0.84
1.25
2.13
1.97
1.08
1.20
0.93
0.76
0.95
0.74
10.58
7.65
6.86
6.83
6.81
6.40
6.38
3.78
3.53
2.93
2.88
2.78
2.45
2.17
2.03
1.89
1.41
1.38
1.16
1.15

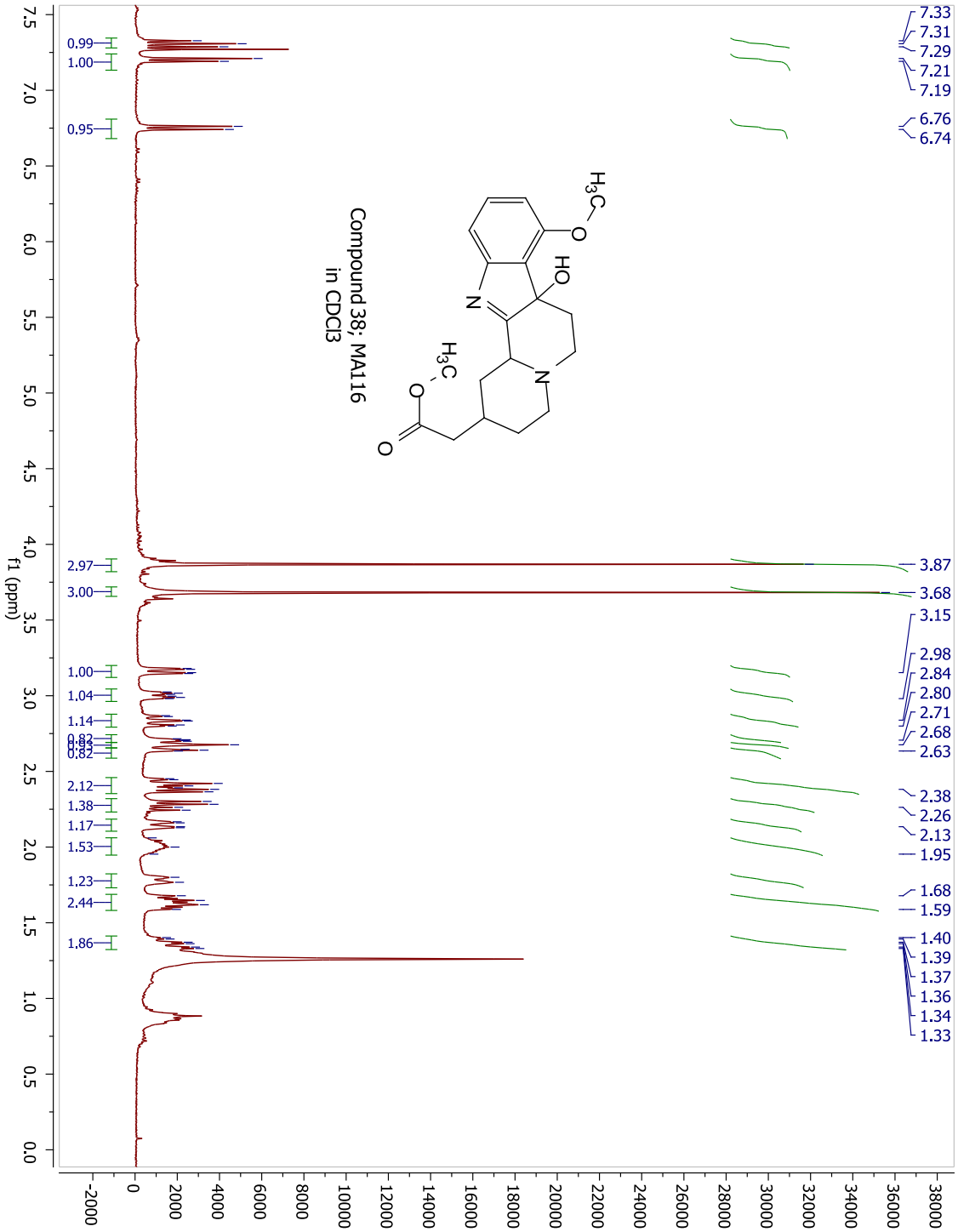

Compound 36; MA107 in CDCl3
f1 (ppm)
1.06
1.08
0.99
1.02
1.01
3.00
3.02
2.93
2.98
1.09
1.25
2.15
1.21
1.27
1.21
0.72
0.57
1.30
1.22
1.10
7.9658
7.9479
7.0285
7.0085
6.9888
6.9070
6.8868
6.4725
6.4531
4.7826
4.7614
4.7414
4.7200
3.8709
3.6607
3.3726
3.1060
2.9686
2.7619
2.5181
2.3397
2.1832
2.1512
1.7168
1.7085
1.6947
1.6869
1.6419
1.6318
1.6133
1.5994
1.5662
1.5335
1.5047
1.4645
1.4339
1.4047
1.3746
NH
N
O
CH3
H3C
OH3C

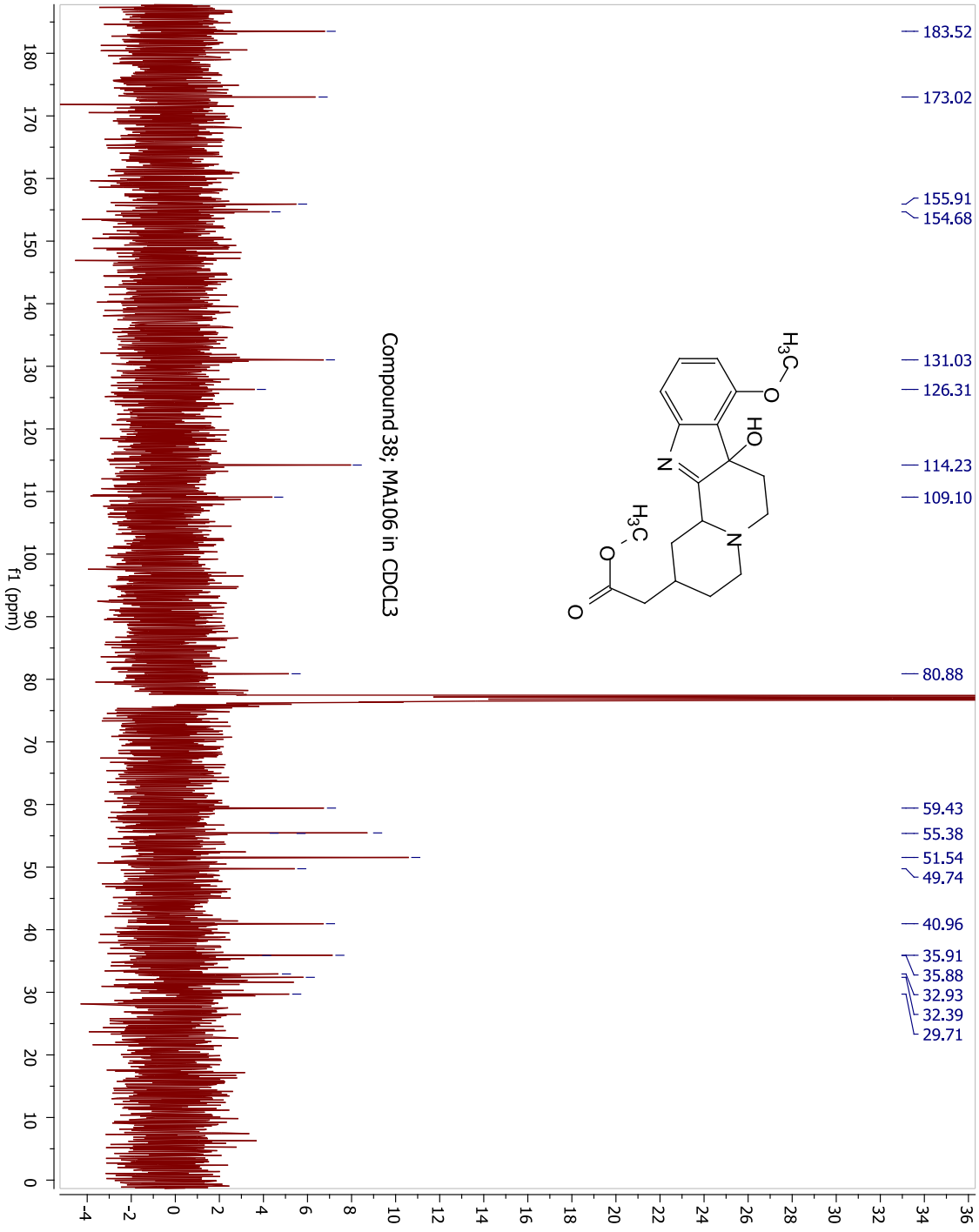

Compound 36; MA107 in CDCl3
f1 (ppm)
171.86
154.47
137.42
132.67
121.97
117.48
107.82
104.35
99.73
77.44
77.12
76.80
60.42
55.27
52.88
51.59
35.49
35.38
34.61
32.52
30.54
28.24
23.68
21.02
NH
N
O
CH3
H3C
OH3C


Compound 37; MA108 in CDCl3
NH
N
O
CH3
H3C
f1 (ppm)
0.91
1.04
1.05
0.90
0.86
3.00
3.00
2.98
1.17
2.11
0.97
0.89
0.81
0.85
1.21
1.14
1.58
2.29
8.21
7.32
6.99
6.97
6.88
6.86
6.45
6.43
3.84
3.75
3.70
3.13
2.97
2.90
2.87
2.84
2.57
2.39
2.25
2.10
1.93
1.90
1.60
1.57
1.56
1.27
0.89
0.87


Compound 37;
MA108 in CDCl3
168.87
159.99
154.47
137.52
132.64
121.90
117.48
112.94
104.40
99.65
77.37
77.25
77.06
76.74
61.59
60.13
55.23
52.95
51.31
33.68
33.02
29.69
23.35
22.68
f1 (ppm)


Compound37 hydrochloride
f1 (ppm)
1.07
1.00
1.05
0.97
1.11
0.98
1.05
3.00
3.29
2.82
1.32
1.26
1.26
1.72
1.90
1.81
1.18
0.82
11.17
10.57
7.50
7.00
6.93
6.51
6.50
4.73
4.71
4.69
3.84
3.63
3.52
3.35
3.29
3.12
3.10
2.51
2.32
2.29
2.27
1.72
1.70
NH
NH+
Cl-
O
CH3
H3C


Compound 38; MA116
in CDCl3
f1 (ppm)
7.33
7.31
7.29
7.21
7.19
6.76
6.74
3.87
3.68
3.15
2.98
2.84
2.80
2.71
2.68
2.63
2.38
2.26
2.13
1.95
1.68
1.59
1.40
1.39
1.37
1.36
1.34
1.33
0.99
1.00
0.95
2.97
3.00
1.00
1.04
1.14
2.12
1.38
1.17
1.53
1.23
2.44
1.86


Compound 38; MA106 in CDCL3
f1 (ppm)
183.52
173.02
155.91
154.68
131.03
126.31
114.23
109.10
80.88
59.43
55.38
51.54
49.74
40.96
35.91
35.88
32.93
32.39
29.71
H3C
O
HO
N
N
O
H3C
O
O

Vita

Marco Aurelio da Graça Arribas was born on January 17, 1977 in São Paulo, State of São Paulo, Brazil. He received a B.S. in Pharmaceutical Sciences from the University of São Paulo, Brazil in 2003 and a Ph.D. in Medicinal Chemistry from the University of Mississippi.