The Dissertation Committee for Matthew James Shashack Certifies that this is the approved version of the following dissertation:

The synthesis of 5-HT_2 receptor designed multiple ligands to investigate the role of the 5-HT_{2A}R and the 5-HT_{2C}R in cocaine addiction

Committee:

Scott R. Gilbertson, Ph.D., Supervisor

Kathryn A. Cunningham, Ph.D.

Cheryl S. Watson, Ph.D.

Amarnath Natarajan, Ph.D.

F. Gerard Moeller, M.D.

Dean, Graduate School

The synthesis of 5-HT_2 receptor designed multiple ligands to investigate the role of the 5-HT_{2A}R and the 5-HT_{2C}R in cocaine addiction

by

Matthew James Shashack, BS

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Dedication

To my wife.

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The synthesis of 5-HT_2 receptor designed multiple ligands to investigate the role of the 5-HT_{2A}R and the 5-HT_{2C}R in cocaine addiction

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Previous studies indicate that the 5-HT_{2A}R and 5-HT_{2C}R play a role in cocaine induced behavior modification through either stimulation or inhibition respectively. Recent research has revealed that dimerization and oligomerization of GPCRs including the 5-HTRs frequently occurs. In order to target these GPCR dimers and oligomers bivalent ligands have been synthesized. Previously synthesized bivalent ligands have demonstrated increased affinity and potency when compared to administration of the monomers. The aim of this project is to synthesis homodimeric 5-HT_{2A} R antagonists, homodimeric 5-HT_{2C}R agonists, and heterodimeric 5-HT_{2A} R antagonist/5-HT_{2C}R agonist that will exhibit improved ability to control cocaine induced behavior modifications compared to the concurrent administration of a 5-HT_{2A} R antagonist and a 5-HT_{2C}R agonist.

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Abbreviations

10% Pd/C	10% palladium on carbon
^{13}C {H} NMR	Proton decoupled ^{13}C nuclear magnetic resonance spectrum
^{1}H NMR	^{1}H nuclear magnetic resonance spectrum
1M BH_3•THF	Borane tetrahydrofuran complex solution
5-HT	Serotonin / 5-hydroxytryptamine
5-HT_{2A}R	Serotonin 2A receptor
5-HT_{2C}R	Serotonin 2C receptor
5-HTR	Serotonin receptor
Å	Angstrom, 10^{-10} meter
aq.	aqueous
Br	Bromide
Ca+2	Calcium ion
Cbz	Carboxybenzyl
CH_2Cl_2	methylene chloride
CH_3CN	acetonitrile
CsOH	Cesium hydroxide
d	Days
DIEA	N,N-Diisopropylethylamine
DMF	Dimethyl formamide
DML	Designed multiple ligand
DMSO	Dimethyl sulfoxide
EC_{50}	Concentration of a substance that induces a 50% response
EDAC	*N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride
EDCI	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
Emax	Maximal response produced by a compound
Et_2O	Diethyl ether
Et_3SiH	triethylsilane
g	Grams
GPCR	G-protein coupled receptor
h	Hour
H_2	Hydrogen gas
HCl	Hydrochloric acid
HOBT	1-Hydroxybenzotriazole hydrate
IC_{50}	Concentration of inhibitor that inhibits 50% response

IP_3	Inositol triphosphate
iPrOH	Isopropanol
K_2CO_3	Potassium carbonate
KH	Potassium hydride
Ki	Binding affinity of a compound at a receptor
KOH	Potassium hydroxide
KOtBu	Potassium tert-butoxide
L	Liter
LAH	Lithium aluminum hydride
MeOH	Methanol
Mg	Magnesium
mg	milligrams
$MgSO_4$	Magnesium sulfate
MHz	Megahertz
mL	Milliliter
mmol	millimole
MTBE	Methyl *tert*-butyl ether
$NaBH_3CN$	Sodium cyanoborohydride
$NaBH_4$	Sodium borohydride
NaH	Sodium hydride
$NaHCO_3$	Sodium bicarbonate
NaHMDS	Sodium bis(trimethylsilyl)amide
$NaNO_2$	Sodium nitrite
NaOH	Sodium hydroxide
nBuLi	n-butyllithium solution
^oC	Degree celcius
PPh_3	Triphenylphosphine
PtO_2	Platinum(IV) oxide
Raney Ni	Raney nickel
Rh(acac)(cod)	(Acetylacetonato)(1,5-cyclooctadiene)rhodium(I) (2)
$RhAl_2O_3$	
rt	Room temperature
SEM	Standard error of the mean
Sn	Tin
$SnCl_2$	Tin (II) chloride
TBAB	Tetrabutylammonium bromide
TBAF	Tetrabutylammonium fluoride
TBAF	Tetrabutylammonium fluoride
TBAHS	Tetrabutylammonium hydrogen sulfate
TBAI	Tetrabutylammonium iodide
TBDPS	*tert*-butyldiphenylsilyl
t-butanol	Tert-butanol
TFA	Trifluoroacetic acid

THF	Tetrahydrfuran
$TiCl_4$	Titanium tetrachloride
TIPS	triisopropylsilyl
Tos	Tosylate
μM	Micromolar

Chapter 1: Introduction

Designed multiple ligands (DMLs) are rationally designed compounds that modulate multiple targets relevant to a single disease.[1] DMLs have been synthesized for a number of different diseases and their receptors, but to date this approach has not been implemented for the study of cocaine addiction and the 5-HT_2 receptors.[1]

The 2009 National survey on drug use and health estimated that in the U.S. alone there were 21.8 million illicit drug users over the age of 12 and 1.6 million current cocaine users.[2] This report illustrated the need for an effective treatment for cocaine addiction. Before a treatment for cocaine addiction could be devised, the etiology of cocaine addiction must be better understood. Recreational drug use becomes an addiction when a user no longer only takes a drug for pleasure but also to fulfill an intense craving.[3] The addict loses the ability to control the amount of drug taken and a dependence forms in which the user enters a negative state if the drug is not available.[3]

Understanding the etiology of cocaine addiction at the cellular level may possibly lead to a treatment for addiction. An effective treatment would allow cocaine addicts to cease their drug seeking behavior and return to normal society. Cocaine acts on the serotonin$_2$ (5-HT_2) receptors in the central nervous system. Targeting 5-HT_2 receptors with designed multiple ligands that modulate receptor activity may lead to a better understanding of cocaine addiction.

Cocaine

Historical Usage

The highly addictive psychoactive stimulant cocaine is a naturally occurring tropane alkaloid found in the *Erythroxylum coca* plant endogenous to South America, Mexico, Indonesia, and the West Indies.[4] The indigenous peoples of South America have chewed coca leaves for hundreds of years for its ability to increase stamina, reduce appetite, and produce feelings of euphoria.[5] In 1855 Albert Niemann isolated cocaine and identified it as the active compound in coca leaves.[4] A few decades later cocaine's anesthetic and vasoconstrictor properties were reported by von Anrep in 1880.[5] At about this time cocaine was introduced to the public in the form of Vin Mariani, a combination of cocaine and wine, and cocaine spiked soft drinks such as Coca-Cola, Rocco Cola, Koca Nola, and Dope.[4, 5] Other products containing cocaine included tonics, toothache medicines, and chocolate cocaine tablets.[4] These products were all available over the counter until the Harrison Narcotics Act was passed in 1914.[4] The passage of this act effectively ended America's first foray into the addictive world of cocaine. The drug culture of the 1960's reintroduced the United States to cocaine, and its illegal usage is still a problem today.

Forms of Cocaine

Today cocaine is a schedule II controlled substance which allows its use in limited medical applications, but its possession and use by the general public is illegal.[5] Cocaine is produced illegally as the hydrochloride salt, the free base, or crack cocaine. The hydrochloride salt is produced by dissolving cocaine in hydrochloric acid.[6] This form

is snorted or dissolved in water and injected.[6] The hydrochloric acid salt of cocaine has a high melting point and decomposes when heated making this form of cocaine unpleasant to smoke.[4] Both free base and crack cocaine are produced by dissolving cocaine hydrochloride in water and adding a base. The free base is produced when ammonia is used as the base.[6] The cocaine is extracted from the basic aqueous layer with an organic solvent such as diethyl ether.[6] This method effectively removes any water soluble impurities, but trace amounts of ether in the cocaine pose a fire risk. Crack cocaine is obtained by using sodium bicarbonate as the base and then heating the alkali solution.[4] The crack cocaine forms a precipitate that can be collected.[4] Although the free base form of cocaine is more pure, crack cocaine is cheaper to produce and can be smoked which makes it a more popular choice.[4]

Pharmacokinetics

The pharmacokinetic properties of cocaine are dependent on the method of administration; smoking, nasal insufflation, or intravenous injection. Cocaine enters the cerebral circulation in 6 to 8 seconds when smoked, 15-20 seconds when injected, and 3 to 5 minutes with maximal levels in 30 to 60 minutes when snorted.[7] Smoking crack produces the most rapid effect because after it enters the circulation in the lungs, it goes directly to the brain. Given equivalent doses intravenous injection has the greatest maximum effect, followed by smoking and then nasal insufflation.[7] The rapid onset of euphoria and the intense high are both reasons that crack cocaine has become the most commonly abused form of cocaine.[4]

Cocaine's mechanism of action affects both the peripheral nervous system and the central nervous system. When administered as a local anesthetic, cocaine acts in the

peripheral nervous system by inhibiting the passage of sodium through sodium channels, which blocks the initiation and transmission of nerve impulses.[6] This blockage is what prevents the perception of pain allowing cocaine to act as an anesthetic. When cocaine is taken for recreational purposes, it acts in the central nervous system at presynaptic nerve terminals.[8] At the presynaptic terminal cocaine blocks the reuptake transporters of dopamine, norepinephrine, and serotonin.[4] Ligand binding studies have shown that cocaine binds the serotonin reuptake transporter with the greatest affinity (0.14 μM), followed by the dopamine reuptake transporter (0.64 μM), and the norepinephrine reuptake transporter (1.60 μM).[9] Blocking the reuptake of neurotransmitters leads to an increased action potential causing a feeling of euphoria.[4] In the presence of cocaine, the neurotransmitters cannot be recycled through their presynaptic reuptake transporters leading to a depletion of neurotransmitters.[5] The intense craving experience by cocaine addicts is thought to be a result of depleted stores of dopamine.[4]

Cocaine's Side Effects

Wide ranging side effects from feelings of euphoria to sudden cardiac arrest can result from cocaine usage. Low doses of cocaine produce pleasurable effects such as euphoria, increased self-confidence, increased alertness, increased energy, and lack of appetite.[4, 5] High acute doses of cocaine or long term cocaine use lead to a variety of undesirable and potentially deadly side effects. These side effects include vasoconstriction which can cause hypertension and cardiac ischemia, acute bronchoconstriction, hyperthermia, tachycardia, seizures, mood disorders, panic attacks, paranoid behavior, and stroke among others.[4, 8] These potentially life threatening side

effects illustrate the dangers that recreational drug users and addicts face when using cocaine.

Serotonin

5-hydroxytryptamine(5-HT) or serotonin is a monoamine neurotransmitter that plays a vital role in regulating a variety of biological functions including sleep, circadian rhythms, mood, cognition, thermoregulation, intestinal peristalsis, and motor, endocrine, cardiovascular, and respiratory function.[10] It also has a role in disorders such as depression, anxiety, schizophrenia, mania, autism, obesity, and drug addiction.[10] Serotonin was first isolated in 1946 as a vasoconstrictor in the serum, and was later identified in the brain as a neurotransmitter.[10] The cell bodies of serotonin synthesizing neurons are located in the raphe nuclei of the brain stem.[11] Seven families of serotonin receptors (5-HT_1-5-HT_7) with at least 14 different subtypes have been identified.[12] All of the 5-HT receptors except the 5-HT_3Rs are metabotrophic receptors which transmit through G-coupled protein receptors.[12] The receptors of the 5-HT_3R family are an ionotropic receptor.[12] Members of the 5-HT_2 family of receptors have been shown to have a role in addiction and will be the focus here.

5-HT_2 Receptors

The 5-HT_2 family is comprised of three receptor subtypes 5-HT_{2A}, 5-HT_{2B}, and 5-$HT_{2C.}$[10] The 5-HT_2Rs are 46-50% homologous, while 5-HT_{2A} and 5-HT_{2C} have greater than 80% sequence homology in the transmembrane regions.[13] The 5-HTRs couple to a Gq/11 protein.[14-16] This protein complex activates phospholipase C, which promotes the production of inositol-1, 4, 5-triphosphate and diacylglycerol resulting in an intracellular

increase of Ca^{+2}.[10, 14-16] Both the 5-HT_{2A}R and 5-HT_{2C}R activate phospholipase A2 leading to a subsequent release of arachidonic acid from the cell membrane.[17]

Serotonin, Dopamine, and the Reward Pathway

The monoamine neurotransmitter dopamine has a critical role in the rewarding effects of addictive drugs.[18] The mesolimbic dopamine pathway which begins in the ventral tegmental area and terminates in the nucleus accumbens, and the mesocortical dopamine pathway which originates in the ventral tegmental region and terminates in the prefrontal cortex both have high concentrations of dopamine and dopamine receptors. [18, 19] These pathways are responsible for the reinforcing effects of natural rewards such as food and sex as well as drugs of abuse.[18, 19] The reward-related associative learning and motivated behaviors associated with the craving, withdrawal, and relapse of addiction may be controlled by dopamine and glutamate in the nucleus accumbens, amygdala, and prefrontal cortex.[20] Although much research supports the role of dopamine in addiction, targeting dopamine receptors has proved to not be an effective pharmacological treatment for addiction. The failure of these treatments could arise from the fact that dopamine is crucial in many essential physiological functions, and manipulating dopamine receptors leads to a wide array of adverse side effects.[21] Targeting dopamine receptors proved unsuccessful in providing effective therapeutics to treat addiction, which led to the examination of serotonin receptors as possible targets for efficacious therapies.

Serotonin has been shown to exhibit some control over the release of both dopamine and glutamate.[18, 22] The effect of serotonin on dopamine release can have an indirect or direct affect on dopamine terminals.[22] The seven 5-HT receptor families are localized to specific dopaminergic brain regions, so targeting specific 5-HT receptor

subtypes could lead to a treatment confined to a specific brain region.[22] The ability to target dopamine release in a particular region of the brain could lead to a treatment with few adverse side effects. The three primary dopamine signaling systems in the brain are the nigrostriatal pathway, the mesolimbic pathway, and the mesocortical pathway.[23] Since the mesolimbic and mesocortical pathways are responsible for natural and drug induced rewards and memory, the effect of serotonin on dopamine release in these pathways will be examined.

The role of 5-HT in the mesolimbic and mesocortical pathways

Mesolimbic and mesocortical dopamine activity is modulated by several 5-HT receptor subtypes including 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3, but the 5-HT_{2A}R and 5-HT_{2C}R are the focus here.[22] Previous research indicates that 5-HT_{2A}Rs are located directly on dopamine-producing cells in the ventral tegmental region.[24] Additionally, the administration of a 5-HT_{2A}R antagonist inhibits 5-HT induced increases in dopamine release in the nucleus accumbens, but 5-HT_{2A}R antagonists do not decrease basal dopamine levels.[25] The effect of the 5-HT_{2A}R antagonist in the nucleus accumbens may be mediated by a direct or indirect effect on the dopamine cells. 5-HT_{2A}Rs are also localized in the prefrontal cortex as indicated by the ability of local administration of a 5-HT_{2A}R antagonist to block 5-HT_2R agonist mediated increases of dopamine.[26] The 5-HT_{2C}R, which has a high level of constitutive activity, is localized in the ventral tegmental area, the substantia nigra, and the nucleus accumbens with some evidence of its existence in the prefrontal cortex.[27, 28] Administration of a 5-HT_{2C}R agonist decreases dopamine efflux in the nucleus accumbens while also decreasing the firing rate in ventral tegmental area.[29] In contrast, a 5-HT_{2C}R antagonist increases firing rate in these brain

areas.[29] Prefrontal cortex dopamine efflux is decreased by 5-HT_{2C}R agonists and increased by antagonists.[30] The localization of 5-HT_{2A}Rs and 5-HT_{2C}Rs in the mesocortical and mesolimbic pathways as well as their ability to regulate dopamine make them prime targets for the development of addiction treatments.

Behavioral effects of cocaine addiction

Directly measuring the physiological and behavioral effects of cocaine in human addicts is not simple or ethical. For this reason, animals have replaced human subjects when studying the effects of cocaine addiction in a laboratory setting. A number of paradigms have been devised to measure distinct behavioral effects in laboratory animals that correlate to a behavior exhibited by human cocaine addicts. These paradigms include the discriminative stimulus effect, hyperactivity, and self-administration.

Discriminative Stimulus Effect of Cocaine

Direct measurements of the emotional effects of cocaine including euphoria, mood elevation, increased confidence, anxiety and nervousness in laboratory animals is not plausible.[31] One paradigm used to measure these effects in rats is the discriminative stimulus effect. The discriminative stimulus effect of cocaine in rats is used to determine if trained rats can differentiate between cocaine administration and placebo administration. 5-HT_2 receptors are involved in the discriminative stimulus effect of cocaine in rats. To measure the discriminative stimulus effect a rat is first trained to associate one lever with cocaine administration and a second lever with placebo administration.[31] When a rat is injected with cocaine, it will press the lever associated with cocaine administration to receive a food or water reinforcement, but when a rat is

injected with saline the lever associated with placebo administration is pressed to receive a food or water reinforcement.[31, 32] The 5-HT_{2A}R and 5-HT_{2C}R have been shown to play opposing roles in the discriminative stimulus effect of cocaine.[31] The 5-HT_{2A}R has been shown to have a stimulatory effect on the discriminative stimulus effect of cocaine. The administration of a 5-HT_{2A}R antagonist has been shown to dose dependently reduce the number of correct lever responses in a drug discrimination assay.[31] These data suggest that a 5-HT_{2A}R antagonist may prevent the rat from experiencing the physiological responses normally associated with cocaine administration. Although a 5-HT_{2A}R antagonist can completely block the ability of a rat to discriminate between saline injections and cocaine injections, a 5-HT_{2A}R agonist cannot fully replicate the response observed from cocaine administration.[31] The inability of a 5-HT_{2A}R agonist to fully mimic the action of cocaine suggests that the 5-HT_{2A}R may only regulate stimulated, but not basal, dopamine neurotransmission. In contrast to the 5-HT_{2A}R, the 5-HT_{2C}R has been shown to have an inhibitory effect on the discriminative stimulatory effect of cocaine. Rats given a 5-HT_{2C}R antagonist prior to cocaine administration exhibited a left shift of the dose response curve for correct lever-response, indicating that a 5-HT_{2C}R antagonist enhances the effect of cocaine leading to an increase in cocaine-lever responding.[31] In contrast, a 5-HT_{2C}R agonist decreases the number of cocaine-lever responses in rats.[31] Therefore, the 5-HT_{2C}R may diminish cocaine's effect by blocking the activation of the dopamine cells that are normally activated during cocaine administration.

Hyperactivity

Another animal addiction model is measuring the activity in rodents after cocaine administration, which corresponds to the motor activity controlled by the mesolimbic and mesocortical pathways.[33] Cocaine administration leads to a state of hyperactivity in rodents, which can easily be quantified by comparing the total horizontal and vertical ambulations of a rat administered cocaine and the total ambulations of a rat administered saline.[33] The cocaine induced increase in activity has been shown to be dose dependent.[34, 35] Both the 5-HT_{2A}R and the 5-HT_{2C}R have exhibited involvement in cocaine induced hyperactivity in rats. The 5-HT_{2A}R enhances cocaine induced hyperactivity after both acute cocaine administration and cocaine administered for five days after ending a sensitization treatment.[33] A 5-HT_{2A}R antagonist such as M-100907 diminishes cocaine induced hyperactivity if given 5-days after a cocaine sensitization treatment, but it has no effect if given during cocaine sensitization treatment.[11] The lack of effect of a 5-HT_{2A}R antagonist during cocaine sensitization treatments may be due to down-regulation and desensitization of 5-HT_{2A}Rs that occurs during repeated use of the antagonist.[36] Studies have suggested that down regulation of 5-HT_{2A}Rs also occurs during the cocaine sensitization regimen since the regimen requires repeated administration of cocaine.[34] These findings suggests that 5-HT_{2A}Rs may play an integral role in cocaine induced hyperactivity during acute administration, but this role changes after repeated cocaine dosing, as is evident in cocaine sensitization regimens. The 5-HT_{2C}R has also been shown to be important in cocaine-induced hyperactivity.[16, 35, 37] In contrast to a 5-HT_{2A}R antagonist, administration of a 5-HT_{2C}R antagonist prior to cocaine administration

enhances cocaine-induced hyperactivity in rats.[16, 37] An animal model similar to hyperactivity is cocaine-induced conditioned hyperactivity in which rats are taught to associate the administration of cocaine with a certain environment (e.g. activity monitor).[35] The 5-HT_{2C}R has also been shown to have a role in cocaine-induced conditioned hyperactivity. When the rats are exposed to the particular environment which is now associated with cocaine administration, they exhibit the conditioned response, hyperactivity. Recent studies indicate that a selective 5-HT_{2C}R agonist inhibits cocaine-induced conditioned hyperactivity, whereas a 5-HT_{2C}R antagonist increases expression of cocaine-induced conditioned hyperactivity.[35] The effect of a 5-HT_{2C}R agonist on cocaine-induced hyperactivity can possibly be explained by the fact that 5-HT_{2C}R inhibition has been shown to increase firing of midbrain dopamine neurons leading to an increase in extracellular dopamine levels.[29] 5-HT_{2C}R antagonists and cocaine may both induce hyperactivity by increasing extracellular dopamine concentration. The role of the 5-HT_{2C}R in cocaine-induced conditioned hyperactivity suggests that the 5-HT_{2C}R may be important in regulating dopamine release in response to cocaine administration.

Self-Administration

Self-administration is an animal model for addiction that measures the motivation to take a drug and its reinforcing effect. Self-administration in rats is used to determine the reinforcement potential of cocaine and to understand drug-seeking behavior. Acute administration of a 5-HT_{2A}R antagonist has been shown to have no effect on the self-administration of cocaine in rats, but it does suppress cocaine-primed reinstatement of cocaine-seeking.[16] Another recent study presents evidence that a 5-HT_{2A}R antagonist can reduce reinstatement of cocaine seeking maintained by cocaine-paired cues, but this study

also supports the inability of a 5-HT_{2A}R antagonist to block the reinforcing effects of cocaine.[38] These studies reveal that although 5-HT_{2A}R antagonists do not attenuate the reinforcing effects of cocaine, their ability to block cue-evoked reinstatement of cocaine self-administration may be able to prevent recovering addicts from cue-evoked elapse.[16, 38]

Although blockade of the 5-HT_{2A}R has no direct effect on self-administration, the 5-HT_{2C}R has exhibited a direct effect on self-administration in rats. Acute administration of a selective 5-HT_{2C}R agonist decreases self-administration, while acute administration of a selective 5-HT_{2C}R antagonist increases cocaine self-administration in rats.[16, 39] 5-HT_{2C}Rs are known to undergo rapid desensitization and internalization following agonist administration.[40] A study in which a 5-HT_{2C}R agonist is given for 8 days was carried out to determine if receptor desensitization or internalization has an effect on cocaine self-administration.[40] This study demonstrates that receptor internalization or desensitization does not attenuate the effect of a 5-HT_{2C}R agonist on cocaine self-administration.[40] These studies support the use of 5-HT_{2C}R agonists to decrease drug seeking behavior in cocaine addicts.

G-protein coupled receptors

G-protein coupled receptors (GPCRs) represent the largest class of cell-surface receptors including more than 1000 unique receptors encoded by more than 1000 genes in the human genome.[41, 42] GPCRs are induced by a variety of ligands including hormones, peptides, amino acids, ions, and even light at the cell membrane.[43,44] Transmembrane events produce a signaling cascade that carries out a wide variety of functions throughout the central nervous system and periphery.[43,44] Due to the wide

ranging functions of GPCRs, mutations and polymorphisms of these receptors have been identified in various diseases.[45] Not surprisingly, almost 50% of modern drugs and 25% of the top 200 best selling drugs of 2000 modulate GPCR activity.[43] The number of successful pharmacotherapies targeting GPCRs illustrates the importance of more fully elucidating their structure and function. GPCRs all share a common motif of seven transmembrane α-helices connected by intracellular and extracellular regions with an extracellular amino-terminal end and an intracellular carboxy-terminal end.[44] The amino acid sequence of GPCRs varies between ~300 and ~1500 residues. The extracellular loops contain two highly conserved cysteine residues that are able to form disulfide bridges which help stabilize the receptor's tertiary structure.[44] Interactions between the seven transmembrane helices may also stabilize the receptors tertiary structure.[44]

GPCRs are separated into six distinct families which although they are structurally similar but contain no sequence homology between families.[43] Class A also known as the *Rhodopsin* family is the largest family of GPCRs and includes the serotonin receptors, which are the focus of this research.[42] The *Rhodopsin* family is further subdivided into α, β, γ, and δ subtypes with the serotonin receptors classified as the α subtype.[42] The *Rhodopsin* family has a high degree of diversity in the transmembrane regions, and it is within the transmembrane regions of α subtype receptors that ligands generally bind.[42]

GPCR activation and signaling cascade

In their inactive state GPCRs are coupled to a GDP complex of G-proteins including Gα and GBγ subunits.[44] Upon GPCR activation by a ligand, the receptor undergoes a conformational change to act as a guanine nucleotide exchange factor to

promote the exchange of guanine diphosphate (GDP) for guanine triphosphate (GTP).[44] The exchange of GDP for GTP activates the Gα subunit, allowing it to dissociate from the GBγ subunit.[44] The free Gα and GBγ subunits are now active and can have downstream effects on various other effector molecules including calcium, potassium channels, adenylyl cyclase, phospholipase C and D, and protein kinases.[44] The active Gα subunit has intrinsic GTPase activity, which converts bound GTP to GDP leading to the inactivation of the Gα subunit.[44]

GPCR Oligomerization

Over the last two decades the classic dogma that GPCRs exists solely as monomeric units has slowly evolved into the widely accepted belief that many GPCRs exist as dimers or oligomers. To date a large number of GPCRs have been shown to form homomeric and heteromeric receptors in variant assays, these receptors include the adenosine A_1, chemokine $CCR_{2,5}$, chemokine $CXCR_4$, dopamine $D_{1,2,3}$, histamine $H_{2,4}$, melatonin $MT_{1,2}$, opioid κ,δ,μ, serotonin 5-HT, somatostatin $SSTR_{1A,1B,1C}$, vasopressin V_2, metabotrophic $mGlu_{1,5}$, $GABA_{B1,B2}$, and other receptors.[46] The formation of dimeric or oligomeric GPCRs has been reported to regulate ligand binding, second messenger activation, and receptor trafficking to the plasma membrane.[47, 48] An example of receptor dimerization controlling membrane trafficking is the heterodimerization of a $GABA_{B1}$ subunit and a $GABA_{B2}$ receptor subunit to mask an endoplasmic reticulum(ER) retention signal which allows the heterodimer to exit the ER and move to the plasma membrane.[48] A heterodimer of α_{1D}- and α_{1B}-adrenoceptors has also been shown to be required for expression at the plasma membrane.[49] In contrast to the constitutive multimeric GPCRs, some research suggests that the formation of some GPCR dimers and oligomers is

promoted by ligand binding.[48, 50-53] The studies which suggest ligand binding prompts oligomer formation are limited in scope and more support is needed for this theory to be universally accepted, but at this time the possibility of ligand induced dimerization cannot be completely discounted.

The identification of GPCR dimers and oligomers challenges the original dogma of a 1:1 stoichiometry between a GPCR and a G-protein complex and instead suggests a more complex 2:1 stoichiometry.[54] This is supported by the three-dimensional structure of both rhodopsin and the G-protein complex composed of the Gα and GB/γ subunits which suggests that a GPCR monomer is not large enough to simultaneously interact with both of the G-protein subunits.[54] If the interpretation of the structural data is correct, then at least some GPCRs must form dimers or oligomers to interact with their G-protein and initiate a signaling cascade. The theory of 2:1 stoichiometry is supported by the existence of 5-HT_{2C}R homodimers that bind two ligands but only one G-protein complex.[47] Additionally, yeast oligomeric α-factor receptors activate G-proteins while a single yeast α -factor receptor cannot fully activate its G-protein.[55] Homodimeric metabotrophic glutamate receptors have also been shown to only optimally activate their G-coupled protein when both receptors have ligand bound.[56] This data supports the theory of the existence of dimeric GPCRs and their regulatory role in G-protein activation.

In addition to GPCR homodimers and heterodimers, GPCR oligomers have also been identified. These GPCR complexes have been identified as tetramers based on immunoblot analysis, but various other oligomeric states may exist.[57, 58] GPCR oligomers may exhibit unique properties not present in GPCR monomers. A heterodimer of a κ-opioid and δ-opioid receptor had significantly decreased affinity for either a κ-opioid

agonist or δ-opioid agonist, but it had high affinity for a partially selective agonist.[59] A CCR2-CCR5 heterodimer is activated by a chemokine agonist at concentrations 10 to 100 times lower than the minimum concentration needed to activate either CCR2 or CCR5 alone.[60] The previous two studies indicate that GPCR heterodimerization is a complex process that may change the pharmacological profile of the individual receptor. GPCR oligomers may be homo-oligomers composed of multiple subunits of the same GPCR, or they may be a hetero-oligomer composed of different GPCRs.[54] The composition of hetero-oligomers becomes even more complex when dimers are taken into account. Both GPCR homodimers and heterodimers could theoretically be building blocks of oligomers which can lead to a very complex arrangement of receptors (**Fig 1.1**).[54]

A hetero-oligomer could be composed of a series of homodimers or heterodimers. A bivalent ligand could bind two different receptors that are both part of one heterodimer in a GPCR oligomer (**Fig 1.1**). The bivalent ligand could also bind two different receptors in a hetero-oligomer that are part of two different homodimers (**Fig 1.1**). GPCR oligomers are associated with multiple G-coupled proteins and when ligand binds to the oligomer multiple G-coupled proteins may be activated.[43] Activation of multiple G-coupled proteins could be the result of ligands binding to multiple GPCRs or ligand binding to one receptor. Receptor activation may lead to a conformational change that leads to the activation of other receptors in the oligomer.[43] Activation of various G-coupled proteins will initiate multiple signaling cascades leading to numerous downstream responses.[43]

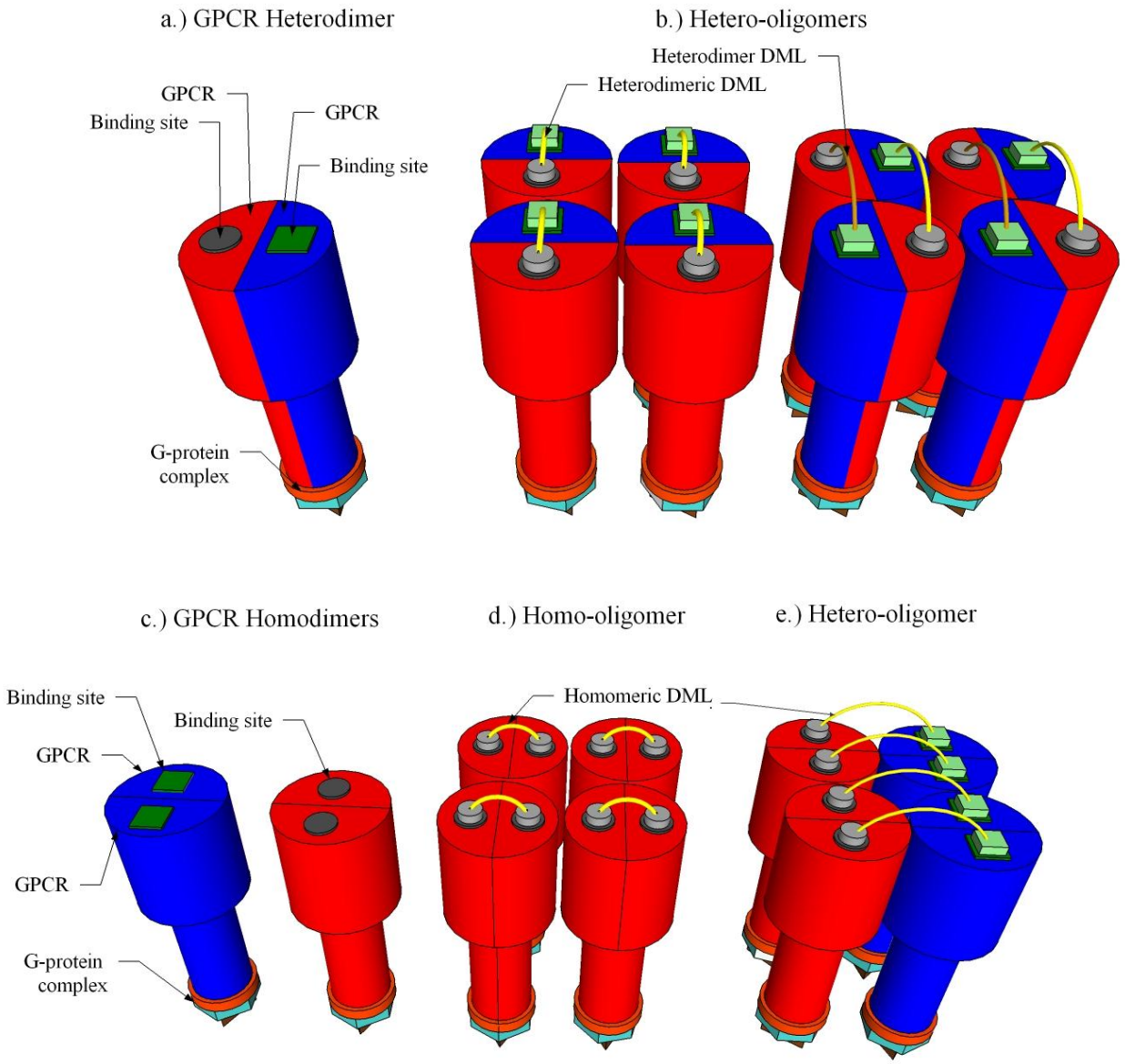


Figure 1. 1 Hypothetical assembly of G protein-coupled receptor oligomers.

Mechanism of GPCR Oligomerization

Disulphide Bonds

The specific type of intramolecular interactions in GPCR oligomerization has yet to be unequivocally determined, but several mechanisms have been suggested. The

suggested mechanisms of oligomerization are the formation of disulphide bonds, transmembrane-domain interactions, and intracellular and extracellular domain interactions.[43] Disulphide bonds between the amino termini of some family 3 GPCRs are crucial for the formation of oligomers.[61] The amino termini of other GPCR families exhibit great diversity, so the existence of amino termini disulphide bonds in GPCRs is not universal. Oligomers from the GPCR family 1 including δ- and κ-opioid receptors, V_2 receptors, M_3 muscarinic-acetylcholine receptors, 5-HT_{1B}R, and 5-HT_{1D}R dissociate when treated with a disulphide reducing agent such as 2-mercaptoethanol.[43] The dissociation is not observed in all family 1 receptors, and the dissociation is not always complete which indicates that another type of interaction is involved in the oligomerization of family 1 GPRCs.[43] The location of disulphide bonds in family 1 GPCRs does not occur at the amino terminus, but it is unclear if the bond is intramolecular or intermolecular.[43]

Transmembrane domain interactions

In addition to disulphide bonds, the GPCR family 1 has been shown to be resistant to dissociation by SDS indicating the possible existence of a hydrophobic transmembrane domain interaction.[43] The nature of the transmembrane domain interaction in GPCRs is currently unknown, but three variations have been proposed. The first variation is domain swapping in which the seven transmembrane domains of each GPCR are interconnected to give GPCRs that are a composite of transmembrane domains from each receptor.[62] In this way binding pockets with unique characteristics are formed. These novel binding pockets may explain the unique properties of some dimers and oligomers. Although transmembrane swapping has not been identified in GPCRs, it has

been reported in a variety of other proteins.[62] The second variation is a contact dimer in which the seven transmembrane domains of each GPCR are prearranged to form separate binding pockets.[43] A hydrophobic interaction between the monomeric GPCRs forms the dimer or oligomer, but each separate binding pocket remains intact.[43] The third type of transmembrane interaction includes both intracellular- and extracellular-domain interactions between the GPCR monomer.[43] The molecular mechanism which forms this type of intermolecular interaction is largely unknown, but these interaction have been recognized in the dimerization of family 3 GPCRs.[43]

5-HT Receptor Oligomers

The isolation and identification of GPCR aggregates of dimers or oligomers is becoming more commonplace, but the isolation and identification of serotonin receptor aggregates is important to the work reported here. Western blot analysis and immunoprecipitation have been used to identify both homo-oligomers and hetero-oligomers of the 5-HT_1 receptor.[63] 5-HT_1 receptors form hetero-oligomers with the closely related 5-HT_{1B}, 5-HT_{1D}, and the distantly related EDG_1, EDG_3, and $GABA_{B2}$ when co-expressed in HEK293 cells.[63] The formation of oligomers between the closely related 5-HT receptors is not surprising, but hetero-oligomer formation between a 5-HT receptor and the EDG and GABA receptors is unexpected. The 5-HT_{1B} and 5-HT_{1D} receptors also exist as monomers, homo-oligomers, and hetero-oligomers.[64] Immunoblot analysis reveals that the concentration of oligomers of both of these receptors increases when incubated with a non-selective 5-HT receptor agonist, but an antagonist had no observed effect.[64] Immunoblot analysis suggests that an agonist does not induce 5-HT_{1B} and 5-HT_{1D} oligomer formation; instead it seems to only stabilize the oligomer.[64] Since

the 5-HT_{2A} and 5-HT_{2C} receptors are the focus of this research, their identification as oligomers is important if they are to be targeted with bivalent ligands. The existence of constitutive 5-HT_{2C} receptor homodimers on the plasma membrane of HEK293 cells has been established.[47] A study with a heterodimer composed of an inactive 5-HT_{2C} receptor and a wild type 5-HT_{2C} receptor reveals that these heterodimers are inactive, suggesting that dimerization may be important to receptor function.[47] Homodimers of the 5-HT_{2C} receptor exist on the plasma membrane of mammalian cells.[65] The identification of 5-HT_{2C} receptor oligomers is important because the research reported here is targeting them with bivalent ligands. Although there is no direct evidence for the existence of 5-HT_{2A} receptor homo-oligomers and 5-HT_{2C}/ 5-HT_{2A} receptor hetero-oligomers, one can speculate that they exist from the multitude of identified GPCR homo-oligomers and hetero-oligomers.

Designed multiple ligands

Over the last few decades, a new paradigm of designed multiple ligands (DMLs) as pharmacological tools has begun to garner support. A DML is a single chemical entity composed of monomers of two distinct compounds that can act simultaneously on multiple receptors.[66] DMLs can be separated into the three classes of fused, merged, and conjugate (**Fig 1.2**).

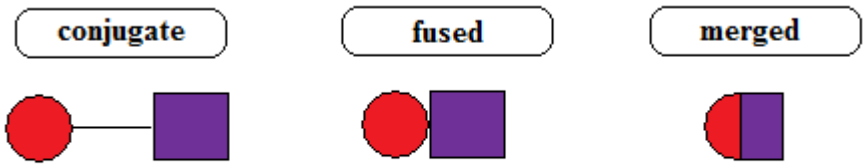


Figure 1.2 Three classes of designed multiple ligands

Fused and merged DMLs are similar in that they are composed of two separate monomers that have some degree of structural similarity and are not separated by a linker.[66] A fused DML is composed of two monomers that are joined at some location but retain much of the structure unique to each monomer.[66] A merged DML is composed of structural aspects of two monomers combined into a single compound.[66] In contrast to a fused or merged DML, a conjugate DML is composed of two highly unmodified monomers connected through a linker.[66] Examples of conjugate DMLs can be found in the primary literature and will be reviewed here. Conjugate DMLs will be the focus of the research presented herein.

Pharmacological benefits of DMLs

Currently drug cocktails composed of multiple single drugs or multiple multi-component drugs containing two or more active compounds are being used to treat some diseases.[66] Although multi-target therapy is currently being implemented, the use of designed multiple ligands in the pharmaceutical industry is severely limited. Current research has demonstrated that designed multiple ligands can provide unique benefits over their parent monomers. If administered as a drug, a single DML may be able to replace a drug cocktail or multi-component drug thus lowering the risk of unexpected

drug-drug interactions.[67] A DML may also exhibit improved binding affinity when compared to the binding affinity of its monomer.[68] If a DML only binds univalently, the unbound ligand will be near a neighboring binding site which equates to a high local concentration of ligand.[68] In theory a DML bound to two receptor binding sites should have an affinity constant equal to the product of the affinity constant for each separate ligand.[68] The increased interest in DMLs is due in part to the emergence of GPCR dimers and oligomers. Targeting these GPCR dimers and oligomers with DMLs may lead to a better understanding of the physiological significance these receptor complexes have on both normal function and disease states.

Literature examples of DMLs

Although the use of bivalent molecules as pharmaceuticals has not been widely examined, examples of successful attempts at creating bivalent molecules can be found in the literature (**Fig 1.3**).

A bivalent 5-HT_{1A}R antagonist-selective serotonin reuptake inhibitor (**1.1)** with improved 5-HT_{1A}R antagonism, but no change in efficacy of the SSRI has been synthesized.[69] A 5-HT_{1A}R agonist / SSRI DML (**1.1**) and a 5-HT_{1D}R agonist DML (**1.2**) with improved activity are two examples of successful DMLs.[70, 71] A bivalent SSRI compound **1.3** exhibits improved selectivity and potency at the serotonin reuptake transporter.[72] Homobivalent ligands of the 5-HT_{1A} antagonist 1-naphthyl-piperazine (**1.4**) and a series of opioid receptor bivalent ligands consisting of a δ-antagonist and a κ-agonist (**1.5**) both have higher affinity binding than their monomers.[73,74,75] A comprehensive review of all DMLs is not the purpose of this report, but a few other examples of DMLs include bivalent SERT/DAT agonists, 5-HT_{1B}R/5-HT_{1D}R agonists, 5-HT_4R agonists, adenosine

A_1R/A_3R agonists, and an adenosine $A_{2A}R$ antagonist/dopamine D_2R agonist among others.[76-80]

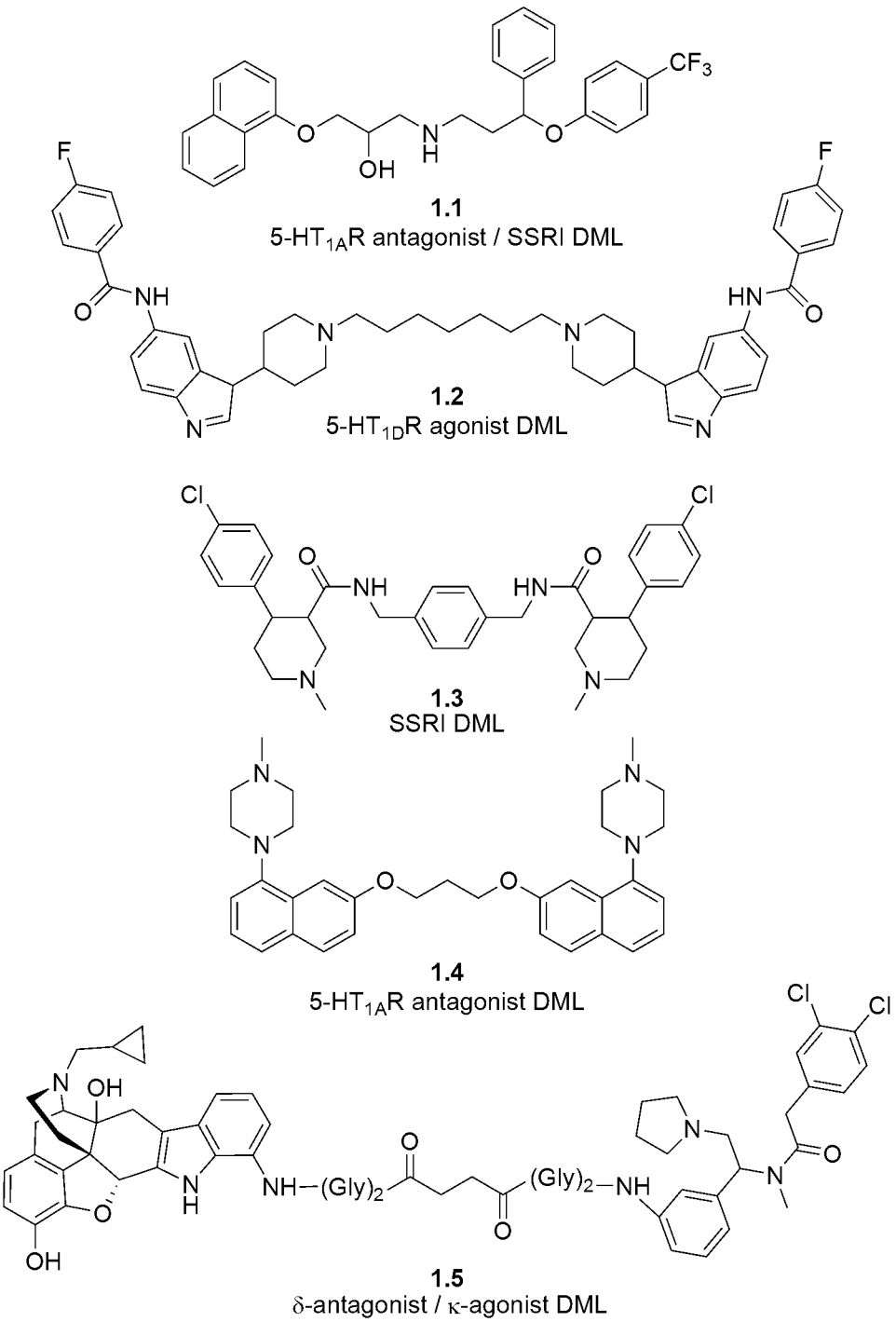


Figure 1.3 Examples of designed multiple ligands

The seminal work in the field of DMLs was done by Portoghese on the opioid receptors.[73, 74, 84, 85] This collection of work is the first to successfully demonstrate that DMLs with improved affinity for their receptors can be synthesized. The work by Portoghese exhibits that functionality along with the length of the linker connecting the two monomers of an opioid receptor DML is critical to optimal binding.[74] Opioid receptor DMLs for a variety of opioid receptor subtypes have been synthesized and characterized. These DMLs include a series of heterodimeric δ-antagonists/κ-agonists and heterodimeric μ/κ antagonists.[73, 74, 84, 85] These examples of successful bivalent molecules in the literature reveal that the use of such molecules is feasible and effective for targeting multiple sites with one molecule.

Steps to synthesize a DML

Identify lead compounds

Synthesizing effective DMLs is a multiple step process that begins with identifying a lead compound and ends with successfully completing the synthesis of a DML. The first step in synthesizing DMLs is to identify a lead compound through either a knowledge based approach or a serendipitous approach.[66] A lead compound chosen using the knowledge based approach will be a compound that has known biological activity and has been previously synthesized.[66] If a compound such as a natural product has known activity but is not accessible in sufficient quantity through a known synthetic route the compound would not be an appropriate choice for a DML. Although a synthetic route could be developed to synthesize a desired natural product, this is undesirable because the goal is to synthesize DMLs and not to develop a novel total synthesis of a natural product. A lead compound that is discovered through a serendipitous approach

involves screening a large library of compounds for activity at a desired target.[66] Screening a large library of compounds may identify novel compounds but the process can be time consuming and expensive. Additionally, once a lead compound is identified through a library screen the synthetic route to the lead compound must be scaled up to produce the devised compound in sufficient quantities to synthesize DMLs. By employing the knowledge based approach a lead compound is quickly and economically selected and the synthesis of a DML can begin. The lead compounds that compose the DMLs synthesized in this work were selected using the knowledge based approach because compounds with known activity at the targeted 5-HT_{2C}R and 5-HT_{2A}R were available. Additionally some data on the absorption, distribution, metabolism, and excretion of the chosen lead compounds was available in the literature.

Once lead compounds that will act as monomers for the DML have been identified, the next step is to determine which type of DML will be synthesized; a conjugate, fused, or merged DML. Since conjugate DMLs can be synthesized through the addition of a linker to lead compound monomers with minimal structural modification, this type of DML will be the focus of this report. In order to synthesize conjugate DMLs an appropriate linker site must be determined for each monomer.

Determine appropriate linker site

To determine an appropriate linker site, each monomer's structure is modified to include functionality which allows for the attachment of a linker. Once the modified monomers have been synthesized a linker will be attached, and the modified monomers with and without a linker will be tested to determine if their activity is commensurate with the original compound. After an appropriate linker location has been identified, a

suitable linker must be selected. An appropriate linker must fulfill several requirements. First, the type of chemical bond between the linker and the monomer must be elucidated. Second, the type of functionality present in the linker must be determined. Last, the optimal length for the linker must be established. Once these criteria have been met the linker may be utilized in the synthesis of DMLs.

Determining type of bond to attach linker

The type of bond used to attach a linker to a monomer varies considerably throughout the literature (**Fig 1.4**). Amine bonds have been utilized to covalently bond a linker to monomers in the synthesis of compound **1.2**.[69, 70, 71] The formation of this bond is simply carried out through an alkylation reaction. The ease of forming this type of bond has lead to its use in multiple 5-HT_{1A} receptor DMLs. Amide bonds are also used to attach a linker to a monomer. An amide bond is used to join a diamine spacer to the selective serotonin reuptake inhibitors (SSRIs) compound **1.3**.[72] Opioid receptor bivalent ligands synthesized by Portoghese contained glycine oligomers connected to the monomers with an amide bond.[73, 74] Amide bonds can be formed through a dehydration reaction between an amine and an acid or a reaction between an amine and an acid halide. Formation of an ester bond through an esterification reaction between an alcohol and an acyl chloride has been used in the synthesis of the DML compound **1.6** which is composed of tropanes that potently inhibit dopamine transporters and serotonin transporters.[76] Ether linkages are used to attach a linker to a monomer in the synthesis of 5-$HT_{1B/1D}$R agonist DMLs and the 5-HT_{1B}R agonist/ antagonist DML compound **1.4**.[75, 77] These types of covalent bonds used to attach a linker to a monomer lend themselves well to the synthesis of DMLs.

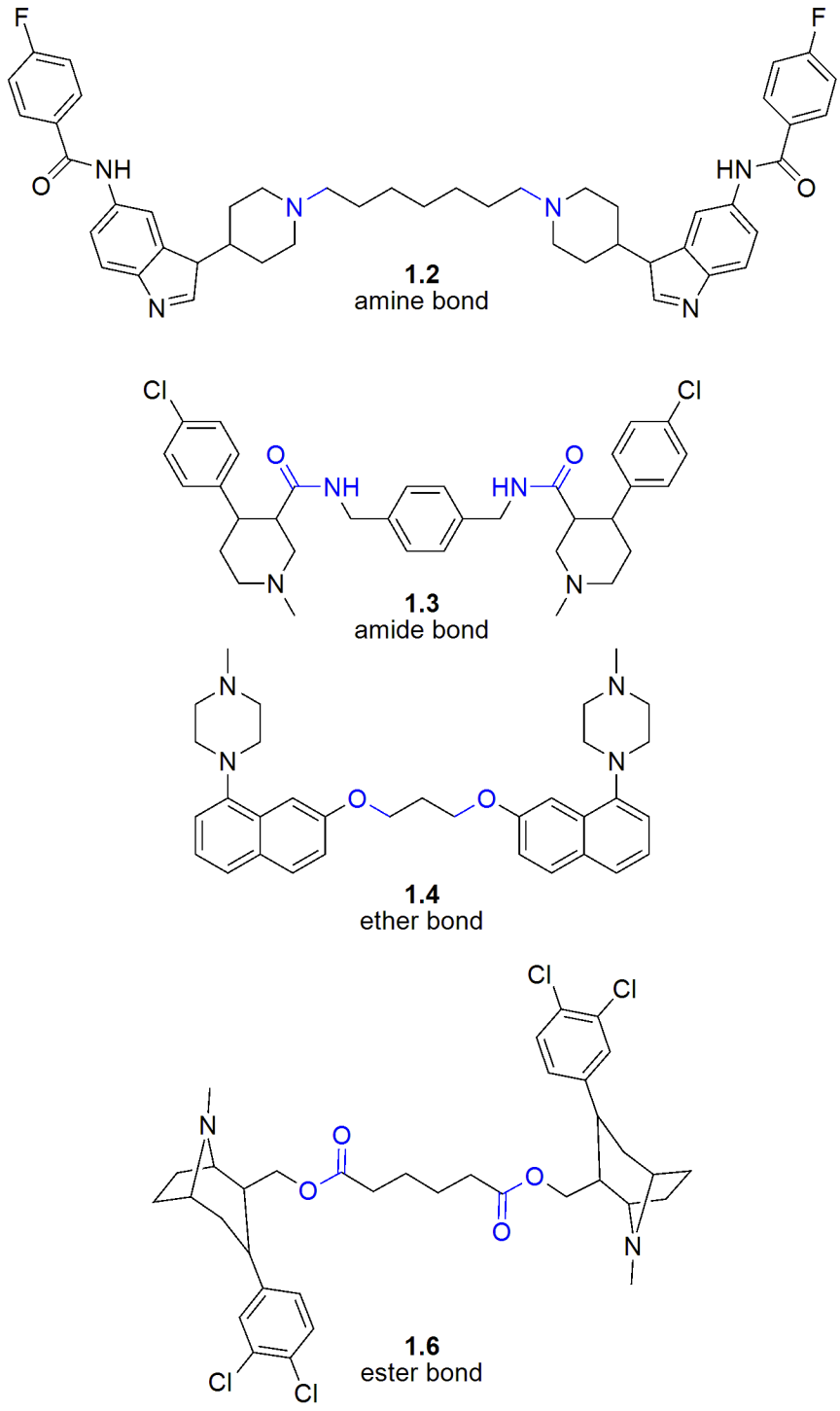


Figure 1.4 Examples of linkages in the literature

Linker Functionality

The functional groups on the linker can vary considerably depending on the desired dimer properties, but typically the linker should be nonreactive and not contain any bulky functional groups (**Fig 1.5**). A linker seen in the literature is a simple alkyl chain of varying lengths such as the linker in compound **1.4**.[69,70,75,76] This type of linker has the advantage of linking two monomers without the addition of any functionality, decreasing the likelihood of affecting the monomers' binding at their targets. Although an alkyl chain linker does not contain any complex functionality, as the alkyl chain length increases the hydrophobicity of the DML also increases. An increase in hydrophobicity may cause a DML to concentrate in the phospholipid bilayer of cells. Another linker that does not contain heteroatoms is an alkyl chain containing an aromatic ring. An aromatic ring linker is similar to an alkyl chain except that it is more planar and is also rigid. The planar nature of the linker decreases the chance of it interfering with the binding of the conjoined monomers in their 3-D binding pockets. The rigidity of aromatic rings limits the amount of rotation in a linker. Aromatic ring linkers have been successfully implemented in the synthesis of a bivalent SSRI DML compound **1.3** as well as 5-$HT_{1B/1D}$R agonist DMLs.[72, 77] Alkynes are also used as linkers because like alkyl chains they do not contain heteroatoms, but unlike alkyl chains they are rigid and they are smaller than aromatic rings. If DMLs with limited flexibility are desired an alkyne linker is often used, which is the case for the 5-HT_4R DML compound **1.**7 and adenosine receptor DMLs.[78, 79] The linker used by Portoghese to synthesize the opioid receptor bivalent ligand compound **1.5** was composed of glycine oligomers.[73, 74]

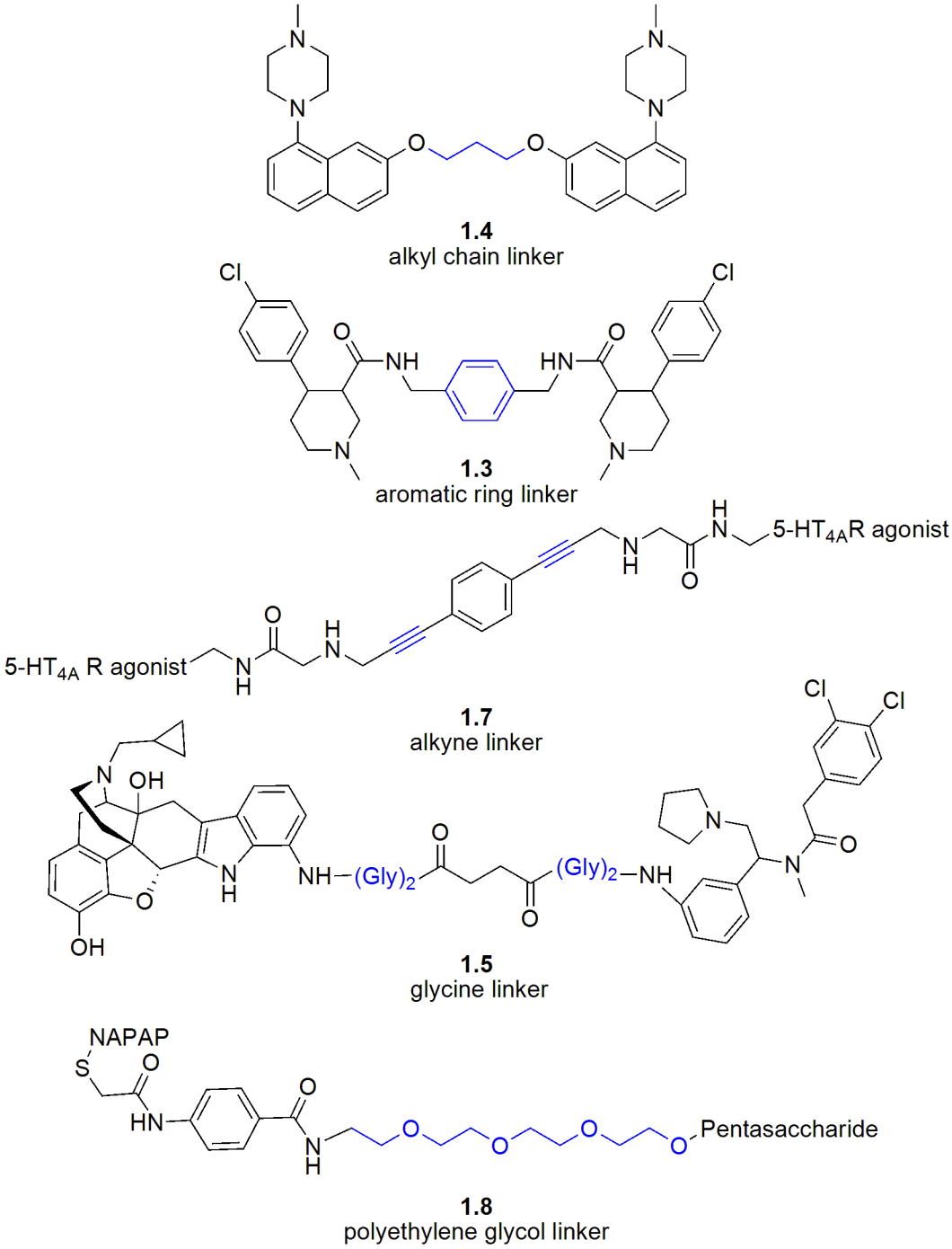


Figure 1.5 Linker functionalities

The amino acid glycine was used because its polar groups do not lead to as significant an increase in hydrophobicity as occurs with the use of alkyl chain linkers. Another benefit of using glycine as a linker is that it can be used to create spacers of various lengths by

simply using multiple glycine subunits to form an amino acid chain. Ethers such as polyethylene glycol (PEG) are also used as linkers because like alkyl chains they are unreactive and compact. Unlike alkyl chains which contain no heteroatoms and are nonpolar, PEG contains ether oxygen atoms which make it more polar than an alkyl chain but less polar than alcohols, esters, or amides. The increased hydrophobicity that a long alkyl chain linker adds to a molecule can be reduced by replacing it with a PEG of equal length. PEG linkers have been successfully employed in the synthesis of an antithrombotic DML and the adenosine A_{2A} receptor antagonist / dopamine D_2 receptor agonist DML compound **1.8**.[80, 81] The varied functionality on the linkers allows the synthesis of dimers with identical monomers but unique properties based on their linkers.

Optimal linker length

Determining optimal linker length is crucial to the development of a functional dimer. Optimal linker length is dependent on the receptors being targeted. The linker of a DML should be just long enough to bridge the gap between the two distinct binding sites on a particular receptor. If the spacer is too short then simultaneous binding at both sites will not occur. A spacer that is too long will prevent the dimer from optimally binding to the two sites, because the confinement space of the dimer would be large.[74] The increased confinement space would cause the dimer to spend less time near the second binding site, leading to a decreased chance of binding both sites. Designing a bivalent molecule that binds two distinct GPCRs may require a linker longer than that needed for a molecule that binds to two sites on a single GPCR oligomer. A long spacer could allow a single bivalent molecule to simultaneously bind recognition sites on two receptors. The effective linker length of DMLs varies throughout the literature and seems to be receptor

specific. Short linkers of only 2-12 atoms have been successfully utilized in the synthesis of SERT/DAT DMLs, antithrombotic DMLs, and 5-HT_1R DMLs.[71, 76, 81, 82] Longer linkers in the range of 20-26 atoms is the optimal length of 5-$HT_{1B/1D}$R agonist DMLs and 5-HT4R DMLs.[77, 78, 83] An adenosine A2AR/dopamine D2R DML with linkers containing 26-66 atoms exhibited increased binding affinities over the monomer.[80] Determining optimal linker length is crucial when designing DMLs.

The optimal linker length for a bivalent molecule targeting both the 5-HT_{2A}R and the 5-HT_{2C}R is currently unknown, but optimal linker length has been determined for bivalent ligands of the 5-HT_4 homodimer and the 5-HT_{1A} homodimer.[78, 82] A chain length of 7-8 carbons confer the best selectivity of 5-HT_{1A} ligands, but 20-24 carbons is the optimal linker length for ligands that bind the 5-HT_4R.[78, 82] Many G-protein coupled receptors have been shown to form homodimers and heterodimers in-vivo but since few crystal structures have been determined, the 5-HT_{2A}R and 5-HT_{2C}R have not been extensively studied in this regard.[83] The DMLs presented in this work will include linkers in the range of 8-24 atoms because this length of linker is in agreement with previously synthesized 5-HT DMLs.[78, 82] Bivalent homodimeric ligands and bivalent heterodimeric ligands may require linkers of different lengths because they are designed to bind distinct receptor dimers.

Aims

The successful synthesis of 5-HTR DMLs is a complex process entailing the successful completion of a number of steps. These steps included choosing parent compounds with activity at either the 5-HT_{2A}R or the 5-HT_{2C}R, determining an appropriate linker location, choosing an appropriate linker, and finally establishing optimal linker length through the synthesis of a series of DMLs. In order to synthesize heterodimeric 5-HT_{2A}R / 5-HT_{2C}R DMLs in addition to homodimeric 5-HT_{2A}R DMLs or homodimeric 5-HT_{2C}R DMLs these steps must be accomplished.

- Aim 1
  - Synthesize heterodimeric DMLs targeting the 5-HT_{2A}R and the 5-HT_{2C}R composed of derivatives of the 5-HT_{2A}R antagonist M-100907 and the 5-HT_{2C}R agonists WAY-163909. These DMLs may have improved affinity and selectivity at their target receptors compared to the monomers.
- Aim 2
  - Synthesize homodimeric DML composed of M-100907 derivatives targeting the 5-HT_{2A}R which may have improved affinity and selectivity at the 5-HT_{2A}R.
- Aim 3
  - Synthesize a homodimeric DML composed of WAY-163909 targeting the 5-HT_{2C}R which may have improved affinity and selectivity at the 5-HT_{2C}R.
- Specific Aims
  - Chose an appropriate 5-HT_{2A}R antagonist and a 5-HT_{2C}R agonist

- Determine appropriate linker attachment points on the 5-HT_{2A}R antagonist M-100907 and the 5-HT_{2C}R agonist WAY-16909.
- Determine an appropriate linker
- Determine appropriate linker length for homodimeric and heterodimeric DMLs.

Chapter 2: Synthesis of 5-HT_{2C} Receptor Agonists

In order to synthesize serotonin receptor DMLs an appropriate 5-HT_{2C} receptor agonist must be selected, synthesized, and derivatized. WAY-470 and WAY-163909 are selective 5-HT_{2C}R agonists originally synthesized by Wyeth.[86, 87, 88] These compounds are an attractive choice for DMLs because their synthesis and structure are amenable to the derivatizations necessary to link them to a spacer.

5-HT_{2C}R affinity of WAY-470 and WAY-163909

High selectivity and affinity to the 5-HT_{2C} receptor as well as high potency as agonists is crucial to molecules used in DMLs. **Table 2.1** shows that both WAY-470 and WAY-163909 have a high selectivity and affinity for the 5-HT_{2C} receptor in addition to being full 5-HT_{2C} agonists. Determination of K_i values of both WAY compounds reveals that they are more selective for the 5-HT_{2C}R over the 5-HT_{2A}R and 5-HT_{2B}R. WAY-470 is three times more selective for the 5-HT_{2C}R than the 5-HT_{2A}R and is 380 time more selective for the 5-HT_{2C}R than the 5-HT_{2B}R.[86] WAY-163909 is 30 times more selective for the 5-HT_{2C}R than the 5-HT_{2A}R and more than 70 times more selective for the 5-HT_{2C}R than the 5-HT_{2B}R.[87, 88] The high affinity of these WAY compounds for the 5-HT_{2C}R means that a lower concentration of agonist can achieve maximum receptor binding. The functional activity of both the WAY compounds is as important as their affinity.

Compound	5-HT_{2C}R Affinity (K_i, nM)	5-HT_{2C} % Emax (5-HT, 100%)	EC_{50} (nM)	5-HT_{2A}R Affinity (K_i, nM)	5-HT_{2B}R Affinity (K_i, nM)
WAY-470	13 nM [89]	120 nM [89]	165 nM [89]	36 nM [86]	>5000 nM [86]
WAY-163909	10.6 nM [87]	110 nM [88]	8 nM [87]	320 nM [87]	736 nM [87]

K_i was determined by measuring [^{125}I] DOI displacement from human 5-HT_2Rs.

E_{max} is the maximal effect of the WAY agonist compared to the effect of 5-HT.

EC_{50} was determined by expressing WAY-stimulated levels of [^{3}H] inositol triphosphate (IP_3).

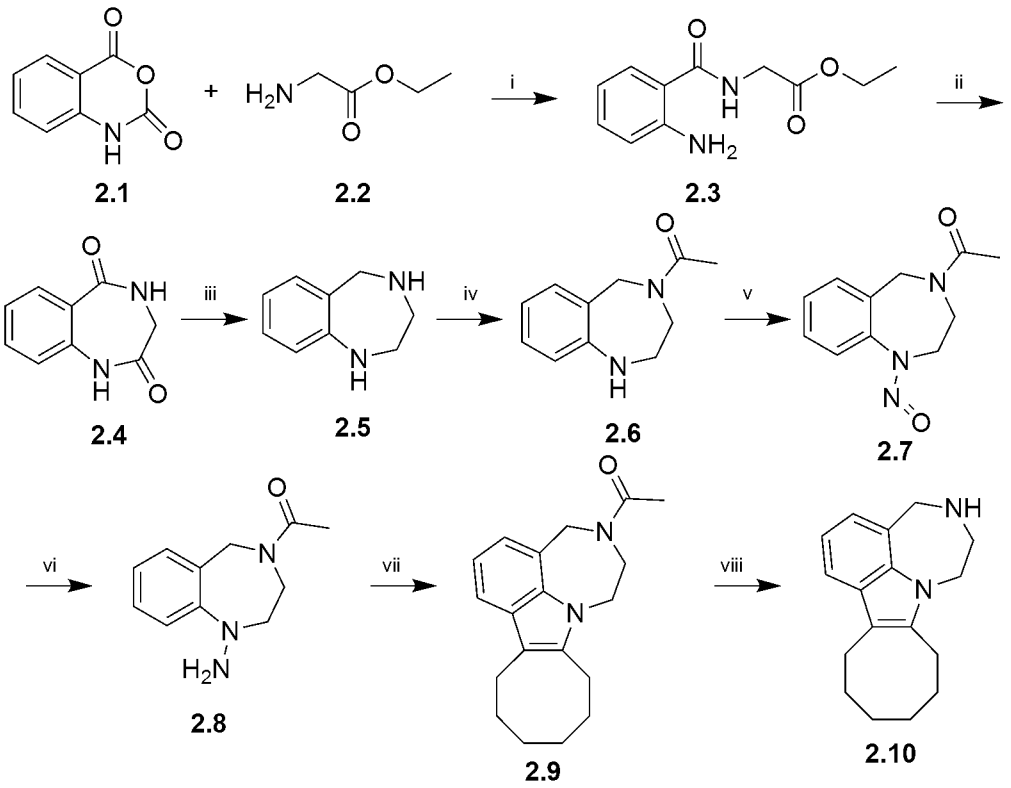
Table 2.1 Affinity of WAY-470 and WAY-163909 for 5-HT_2Rs

Functional activity studies have shown that WAY-470 is a full agonist at the 5-HT_{2C}R and a partial agonist at the 5-HT_{2A}R.[86] Additionally WAY-163909 has been shown to be a full agonist at the 5-HT_{2C}R, while being a partial agonist at the 5-HT_{2B}R (Emax 40%).[87] WAY-163909 has no agonist activity at the 5-HT_{2A}R.[87] The first step in synthesizing DMLs from parent WAY compounds is to reproduce the published synthesis of these 5-HT_{2C}R agonists.

Published synthesis of WAY-470

WAY-470 was chosen as the first compound to synthesize due to its concise synthetic route. Additionally, WAY-470 does not contain any chiral centers which would

lead to the formation of diastereomers upon the coupling of racemic mixtures. Sabb's route to WAY-470 was the initial route chosen to follow. Sabb's route was designed in such a way as to allow for the synthesis of the desired derivatives.[86] **Scheme 2.1** shows the original WAY-470 synthesis. The first step of Sabb's synthesis was the reaction of isatoic anhydride (**2.1**) with glycine ester hydrochloride (**2.2**) under reflux for 24 hours to give the open chain intermediate **2.3**.[86] The second step, a cyclization, was completed by heating the open chain intermediate **2.3** with acetic acid or sulfuric acid for 24 hours to give benzodiazepinedione **2.4** in 16-37.5% yield over the two steps.[86] After completion of the cyclization, the amides were reduced with lithium aluminum hydride to give amine **2.5** in 65-75% yield.[86] The benzylic amine was then selectively protected as an acetyl to give the monoamine **2.6**.[86] The unprotected amine of compound **2.6** was then nitrated with sodium nitrate in aqueous HCl to give the N-nitroso intermediate **2.7** which was reduced with zinc metal in acetic acid to yield the hydrazine **2.8**.[86] The crude hydrazine **2.8** was used directly in a Fischer indole synthesis with cyclooctanone in acetic acid to afford the desired indole **2.9** in a 20% yield.[86] The acetyl protecting group was removed with either NaOH in MeOH or concentrated HCl to give WAY-470 (**2.10**).[86] This reaction sequence could not be replicated in adequate yield in part because the Sabb reference does not contain a detailed experimental section with reaction conditions and purification procedures. In order to obtain WAY-470 **2.10** in adequate quantity to produce DMLs, certain steps in the published synthesis were modified.



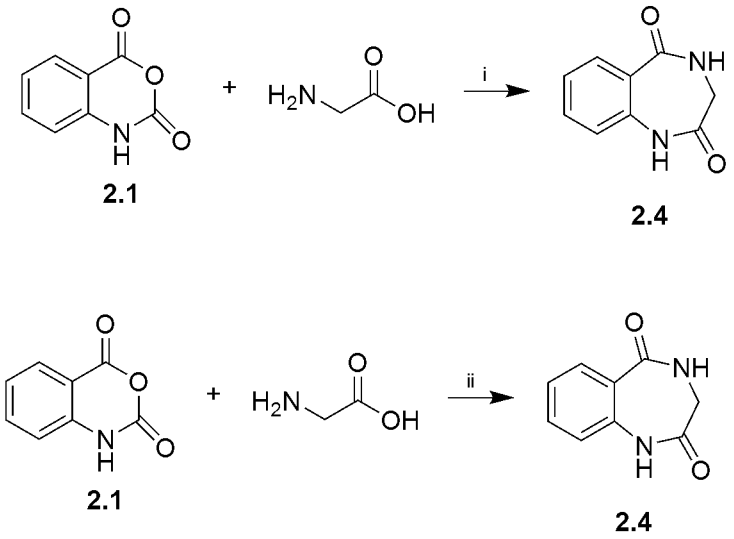
Reagents and conditions: (i) pyridine, reflux, 24 h, 40-50%; (ii) acetic acid, rt, 24 h, 40-70%; (iii) lithium aluminum hydride, THF, rt, 65-75%; (iv) acetic anhydride, triethylamine, ether, rt, 24 h, 90%; (v) sodium nitrite, aq. HCl; (vi) zinc, acetic acid; (vii) cyclooctanone, acetic acid, 75-90 °C, 1.5 h, 20%; (viii) aq. NaOH, MeOH, or conc. HCl

Scheme 2.1 Published synthesis of WAY-470

Modified route to WAY-470

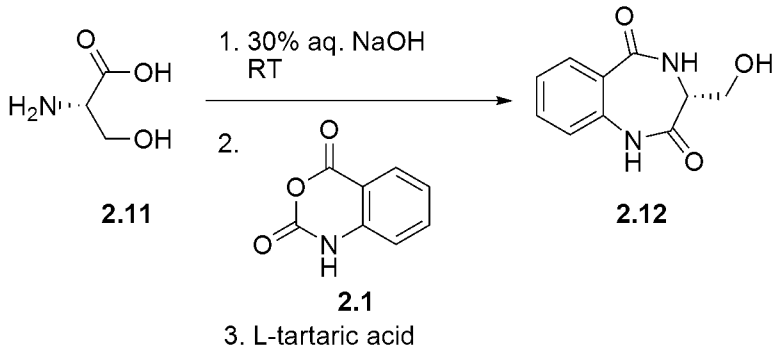
The reaction of isatoic anhydride (**2.1**) with glycine ester hydrochloride (**2.2**) in pyridine in Sabb's published procedure could only be reproduced to give the open chain intermediate in a 20% yield. The low yield of this reaction prompted the examination of other reaction conditions. A procedure in which isatoic anhydride (**2.1**) was reacted with glycine in refluxing acetic acid to give the benzodiazepinedione in one step was attempted, but this reaction failed to give the desired product (**Scheme 2.2**).[90] Reacting isatoic anhydride (**2.1**) with glycine in refluxing DMSO provided the benzodiazepinedione in one step, but the yield was only 36% and the reaction required

column chromatography to obtain pure product (**Scheme 2.2**). [90] Although the desired product could be obtained and the synthesis of WAY-470 (**2.10**) could be carried forward, the low yield and the required column chromatography purification prompted the exploration of a more efficacious reaction. A literature procedure was found in which serine (**2.11**) was reacted with isatoic anhydride (**2.1**) in refluxing aqueous sodium hydroxide.[91] After four hours, L-tartaric acid was added to the reaction mixture to afford the hydroxymethylbenzodiazepinedione **2.12** in a one pot synthesis in a reported 93% yield (**Scheme 2.3**).[91]



Reagents and conditions: (i) acetic acid, reflux, 24 h, 0%; (ii) DMSO, reflux, 3 d, 36%

Scheme 2.2 Attempted ring expansion



Scheme 2.3 Synthesis of 3-(hydroxymethyl)-3,4-dihydro-1H-benzo[e][1,4]diazepine-2,5-dione (2.12)

This reaction was used to give hydroxymethylbenzodiazepinedione **2.12** in good yield. In order to produce the desired benzodiazepinedione (**2.4**), the same reaction conditions were employed but glycine (**2.13**) was used in place of serine (**2.11**). Under these reaction conditions the desired benzodiazepinedione **2.4** was obtained as a beige powder in 86% yield (**Scheme 2.4**). The beauty of this reaction was that it uses inexpensive starting materials and once the reaction was quenched during the aqueous work-up the product crashes out of solution. The reaction mixture was then filtered to collect pure product. Since no column chromatography was required for purification and the starting materials are readily available, this procedure has been successfully scaled up to yield 200 grams of product in a single reaction. Additionally this reaction allowed for the first two steps of Sabb's synthesis to be done in a single reaction vessel in a matter of hours instead of two different reactions over two days.

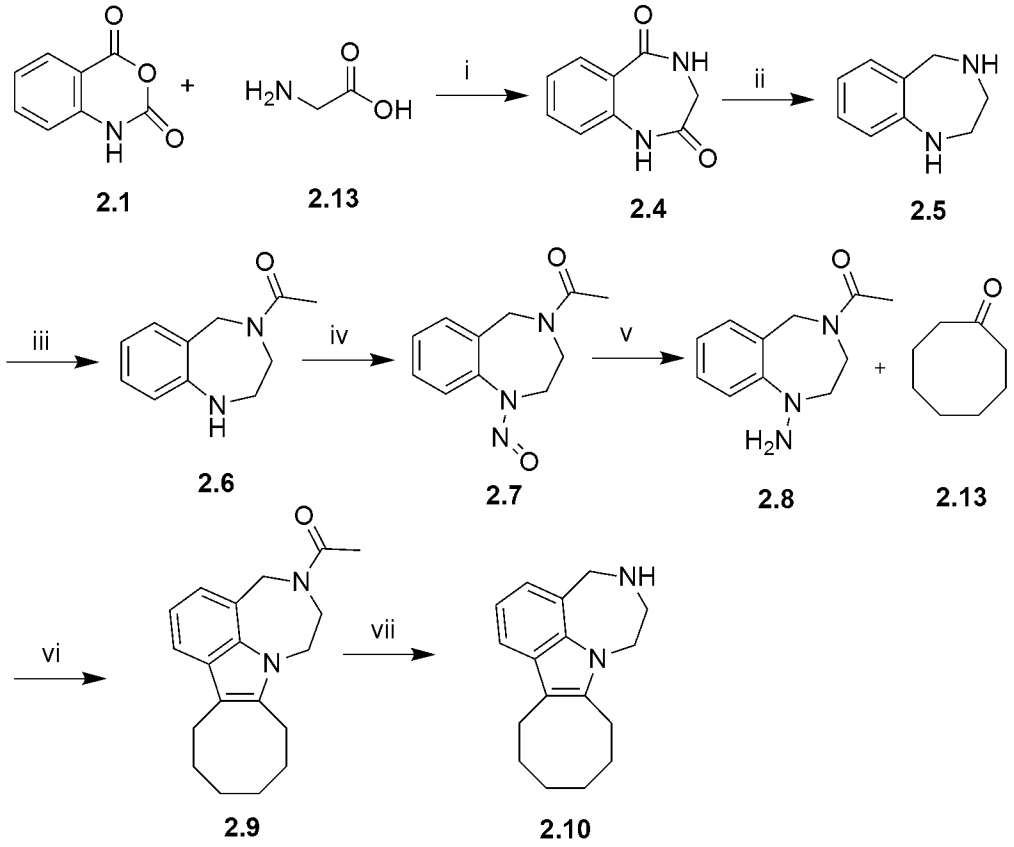
Following Sabb's route toWAY-470, the next step was to reduce the benzodiazepinedione **2.4** to the benzodiazepine **2.5** with either lithium aluminum hydride or 1 molar borane in THF.[86] The lithium aluminum hydride reduction furnishes the benzodiazepine **2.5** in a 93% yield and requires only filtration to collect pure product (**Scheme 2.4**). The Sabb route then selectively protects the benzylic amine in a 90% yield

with an acetyl protecting group using acetic anhydride and triethylamine at room temperature, but reproducing this protection under these conditions proved difficult.[86] Increasing the reaction time and temperature of Sabb's reaction conditions never resulted in a yield in excess of 50%. In order to increase the yield of this reaction various bases and solvent were employed. Replacing triethylamine with the inorganic base potassium carbonate gave the product **2.6** smoothly in an 89% yield (**Scheme 2.4**).

The next step in Sabb's synthesis was the addition of a nitroso group to the unprotected amine using sodium nitrate and aqueous hydrochloric acid to give compound **2.7**.[86] The unpurified N-nitroso intermediate **2.7** was then reduced with powdered zinc in aqueous acetic acid to give the desired hydrazine **2.8**.[86] The hydrazine reaction was filtered to remove the zinc, and then cyclooctanone (**2.13**) was added. The hydrazine (**2.8**) and cyclooctanone (**2.13**) in acetic acid are heated for 1.5 hours to give the desired indole **2.9** in a 20-60% yield over the three steps. In our hands this three step reaction sequence provided the desired product in only a 10% yield. This low yield prompted the exploration of a more efficient route to the desired indole.

The first step in optimizing this three step reaction sequence was to purify the product of each reaction to determine the low yielding reaction or reactions. The addition of the N-nitroso group to the unprotected amine with sodium nitrate in aqueous HCl gave the product **2.7** cleanly in an 86% yield (**Scheme 2.4**). After multiple attempts to reduce the N-nitrosoamine intermediate using the reported conditions of zinc dust and concentrated HCl, the yield of this reaction never exceeded 30% of impure product. Since this reaction was low yielding other reaction conditions were attempted to improve product yield and purity.

The desired hydrazine **2.8** can be synthesized by either reducing the N-nitroso group or by directly aminating the free amine. If direct amination reactions were successful then the N-nitrosoamine step could be eliminated from the synthesis.



Conditions and reagents: (i) 6M NaOH, 100 °C, 4 h, L-tartaric acid, 100 °C, 1 h, 86%; (ii) LAH, THF, reflux, 24 h, 93%; (iii) K_2CO_3, acetic anhydride, CH_3CN, rt, 21 h, 89%; (iv) $NaNO_2$, aq. HCl, rt, 24 h, 86%; (v) $TiCl_4$, Mg, CH_2Cl_2:ethyl ether, 0.75 h, 91%; (vi) acetic acid, reflux, 15 h, 90%; (vii) 6M NaOH, reflux, 5 d, 83%

Scheme 2.4 Modified synthesis of WAY-470

Concurrent to attempting direct amination reactions additional N-nitrosoamine reductions were carried out. Direct amination was attempted with monochloramine in the presence of either NaH or KOtBu in DMF, but none of the desired product formed (**Scheme 2.5**).[92] Reducing the N-nitrosoamine with tin chloride in HCl also failed to give the desired

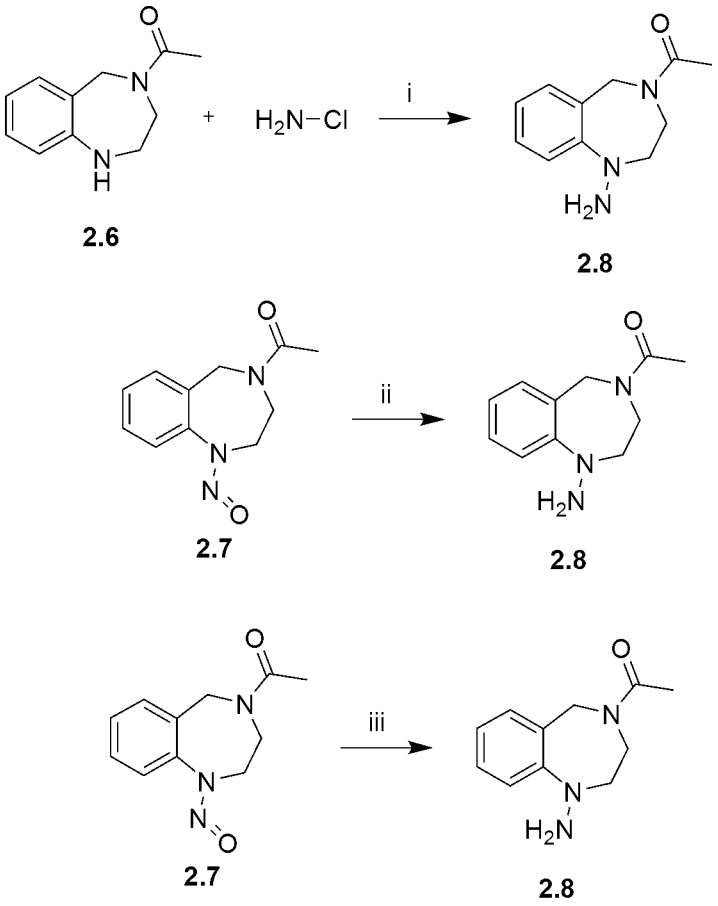
hydrazine, but reducing the N-nitrosamine with magnesium and titanium tetrachloride smoothly gave the product in a 91% yield (**Scheme 2.5**).[93]

The reduction of the N-nitrosoamine **2.7** to the hydrazine **2.8** with $TiCl_4$ and Mg was reported to be a general procedure for selectivity reducing a variety of N-nitrosoamines in the presence of other functional groups.[93] The reaction was carried out by first reacting $TiCl_4$ with Mg in a solution of CH_2Cl_2 /Et_2O.[93] The $TiCl_4$ was reduced by the Mg to form a Ti(II) reagent which reacts with the N-nitrosoamine to form a "nitrenoid" intermediate. Upon hydrolysis with acid the "nitrenoid" intermediate forms the desired hydrazine **2.8** in a 91% yield (**Scheme 2.5**).[93] This reaction produced the desired hydrazine **2.8** and required only an aqueous workup to collect pure product.

An important note for hydrazine **2.8** is that it rapidly decomposes. Upon synthesis, hydrazine **2.8** must be immediately used in a Fischer indole synthesis to afford the desired indole. If hydrazine **2.8** is stored at 0 ^{o}C for only a single night it begins to decompose resulting in low yield of the Fischer indole synthesis.

The Fischer indole synthesis was then carried out by reacting hydrazine **2.8** with cyclooctanone (**2.13**) in hot acetic acid. This reaction provided the desired indole **2.9** in 90% yield (**Scheme 2.4**). The acetyl protecting group was then removed from the benzylic amine by refluxing in aqueous NaOH and MeOH to give WAY-470 (**2.10**) in 83% yield (**Scheme 2.4**).

Gratifyingly, the modifications to Sabb's synthesis of WAY-470 led to reactions with improved yields and decreased reaction times. **Scheme 2.4** shows the complete modified WAY-470 synthesis.



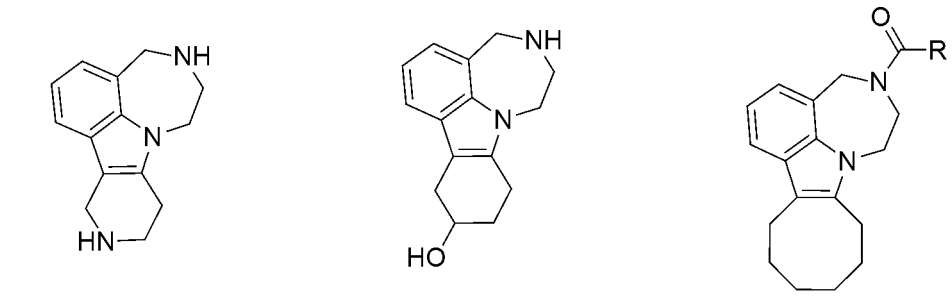
Reagents and conditions: (i) NaH or KOtBu, DMF, rt, 1 h, 0%; (ii) $SnCl_2$, HCl, rt, 3 h, 0%; (iii) $TiCl_4$, Mg, CH_2Cl_2:ethyl ether, 0.75 h, 91%

Scheme 2.5 Synthesis of hydrazine 2.8

Combining the first two steps of Sabb's synthesis to give the ring expanded benzodiazepinedione **2.4** into a one pot reaction decreased the reaction time from 2 days to only 4 hours, and the yield improved from 20% to 87%. Altering the reduction of the N-nitrosoamine **2.7** to the hydrazine **2.8** also improved the yield of the hydrazine and subsequently the yield of the Fischer indole reaction is improved from 20% to 90%. The modifications to the original Sabb route allowed for the facile synthesis of multiple grams of WAY-470 (**2.10**). With WAY-470 (**2.10**) in hand the next step was to synthesize the desired derivatives.

Synthesis of WAY-470 derivatives

The envisioned WAY-470 derivatives include modifying either the cyclooctyl ring or the benzylic amine (**Fig 2.1**). One modification was to replace the cyclooctyl ring with either a piperidine ring (**2.14)** or a cyclohexanol ring (**2.15)**. Work by Sabb indicated that replacing the cyclooctyl ring with a cyclohexyl or a cycloheptyl ring does not significantly affect 5-HT_{2C}R affinity or selectivity.[86] Another modification was the addition of a linker to the benzylic amine through an amide bond. Amide bonds are synthetically accessible and stable to a variety of reaction conditions making them a good candidate for attaching linkers. The downside of attaching a linker to the benzylic amine was that the secondary amine in WAY-470 becomes an amide in the derivative, which could have an adverse effect on its activity at the 5-HT_{2C}R.



WAY-470 derivative1 **2.14**

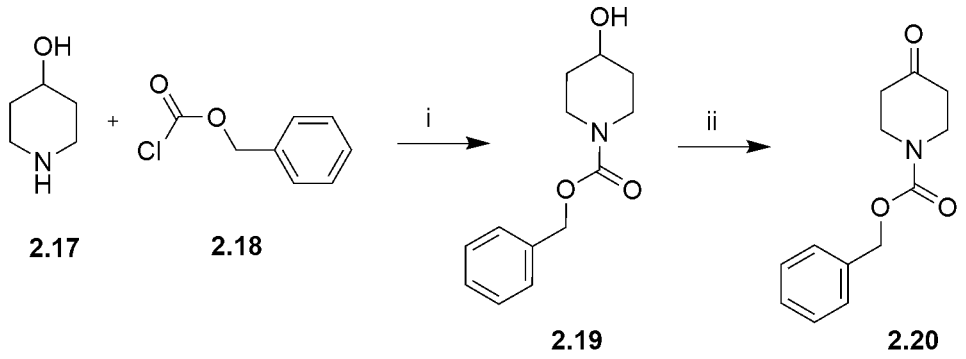
WAY-470 derivative 2 **2.15**

WAY-470 derivative 3 **2.16**

Figure 2.1 WAY-470 derivatives

Synthesizing compound **2.14** required only a slight modification to the WAY-470 synthesis. The change from an eight membered ring to a piperidine ring was accomplished by substituting 1-(benzyloxycarbonyl)-4-piperidinone (**2.20**) for cyclooctanone in the Fischer indole synthesis. The first step in synthesizing this WAY-

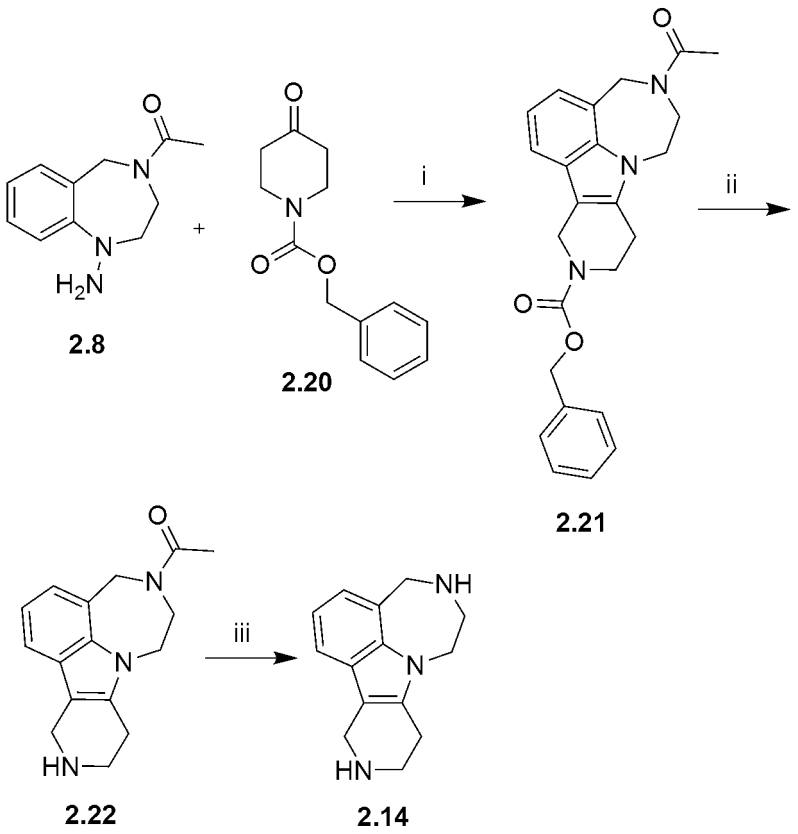
470 derivative was the synthesis of 1-(benzyloxycarbonyl)-4-piperidinone (**2.20**) from 4-hydroxypiperidone (**2.17**) (**Scheme 2.6**). 4-hydroxypiperidone (**2.17**) was reacted with benzyl chloroformate (**2.18**) and N, N-DIEA to give the Cbz-protected amine **2.19** in a 96% yield (**Scheme 2.6**).[95] A Jones' oxidation was then done to convert the hydroxyl group to a ketone to provide 1-(benzyloxycarbonyl)-4-piperidinone (**2.20**) in a 87% yield.[96] Both of the reactions employed to convert 4-hydroxypiperidone to 1-(benzyloxycarbonyl)-4-piperidinone are scalable to produce multi-gram quantities of the desired ketone **2.20**. With the desired ketone in hand, the Fischer indole synthesis and subsequent Cbz and acetyl deprotections were carried out (**Scheme 2.7**).



Conditions and reagents: (i) DIEA, CH_2Cl_2, rt, 22 h, 96%;(ii) Jones' reagent, acetone, 0 °C, 2 h, 87%

Scheme 2.6 Synthesis of 1-(benzyloxycarbonyl)-4-piperidinone 2.20

The Fischer indole synthesis afforded compound **2.21** in a 64% yield (**Scheme 2.7**). The Cbz group was then removed with 10% Pd/C under an atmosphere of hydrogen gas to produce compound **2.22** in a 70% yield (**Scheme 2.7**). At this point in the synthesis, a linker would be attached to the free amine of compound **2.22** if DMLs were going to be synthesized.

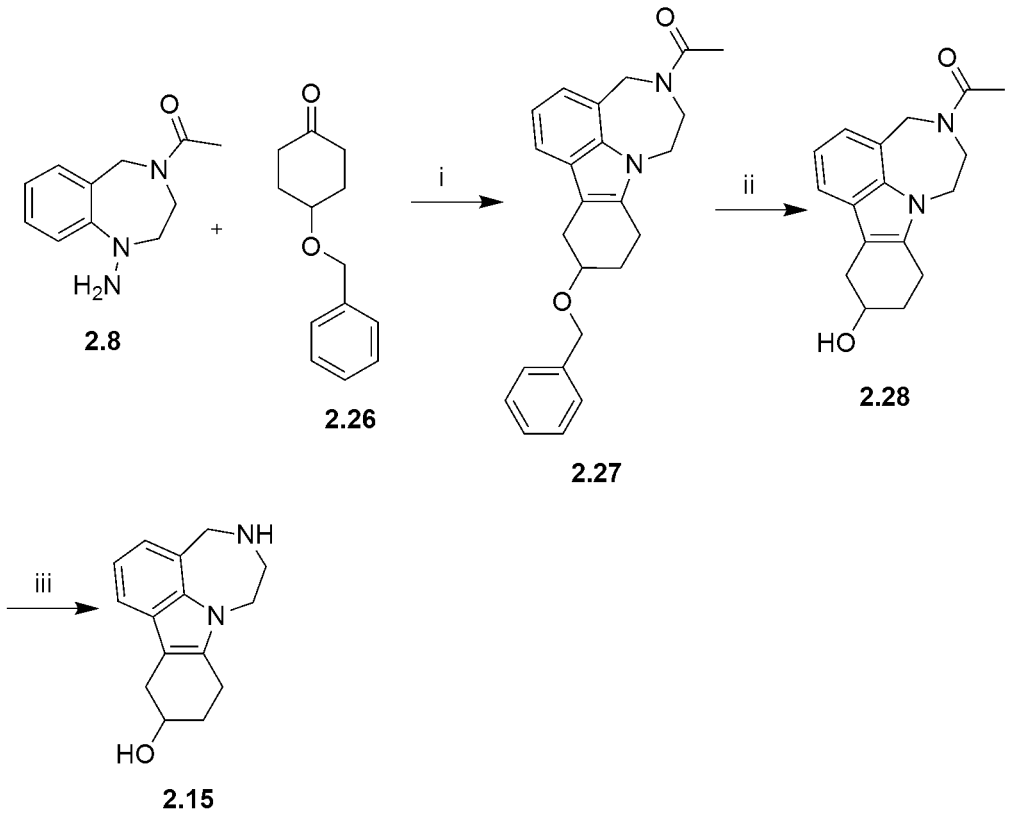


Conditions and reagents: (i) acetic acid, reflux, 18 h, 64%; (ii) 10% Pd/C, EtOH, H_2 gas, 70%; (iii) 6M NaOH, MeOH, reflux, 18 h, 74%

Scheme 2.7 Synthesis of WAY-470 piperidine derivative

The acetyl protecting group was removed from the benzylic amine by refluxing in 6M NaOH and methanol to yield compound **2.14** in a 74% yield (**Scheme 2.7**). The WAY-470 piperidine derivative **2.14** was synthesized in three steps from the hydrazine to yield the first derivative containing the functionality on which a linker can be attached.

Synthesis of compound **2.15** differed from the WAY-470 synthesis in that a different ketone was used in the Fischer indole synthesis and a deprotection step was added. 4-(benzyloxy) cyclohexanone (**2.26**), the ketone required to synthesize the cyclohexanol derivative, can be synthesized from 1, 4-cyclohexanediol in two steps (**Scheme 2.9**).[97]



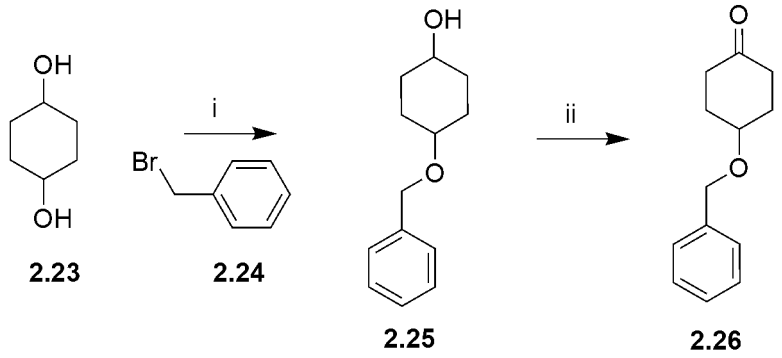
Conditions and reagents: (i) acetic acid, reflux, 16 h, 60%; (ii) Raney Ni 2800, EtOH, H_2 gas, 9 d, 73%; (iii) 6 M NaOH, MeOH, reflux, 18 h, 62%

Scheme 2.8 Synthesis of the cyclohexanol WAY-470 derivative

Benzyl bromide (**2.24**) and NaH were used to benzyl protect one hydroxyl group on 1, 4-cyclohexanediol (**2.23**) in a 36% yield (**Scheme 2.9**).[97] The low yield was due to the fact that only one molar equivalent of benzyl bromide (**2.24**) was used to prevent protection of both alcohols of 1, 4-cyclohexanediol (**2.23**). Even with only one molar equivalent of benzyl bromide (**2.24**) some of the double protected alcohol was formed. 4-(benzyloxy) cyclohexanol (**2.25**) then underwent a Jones' oxidation to furnish 4-(benzyloxy) cyclohexanone (**2.26**) in an 87% yield (**Scheme 2.9**).[98]

Scheme 2.8 shows the synthesis of compound **2.15** from the hydrazine. The previously synthesized hydrazine **2.8** and 4-(benzyloxy) cyclohexanone (**2.26**) in glacial acetic acid generated the desired indole **2.27** in a 60% yield (**Scheme 2.8**). The benzyl

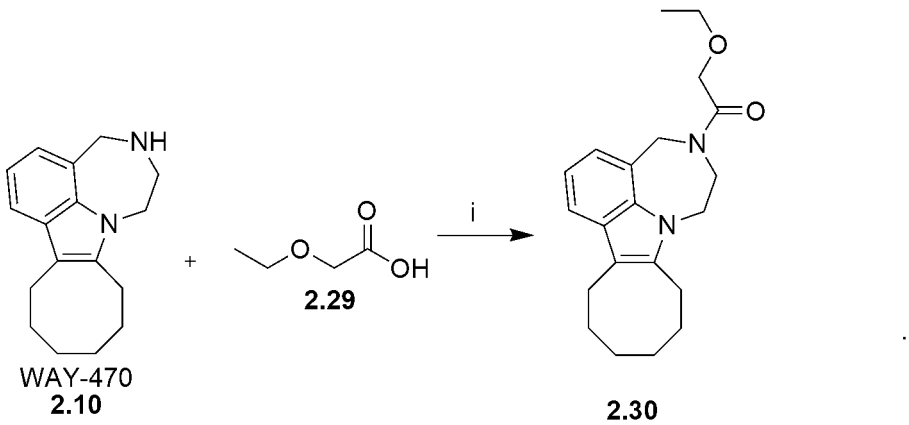
protection group was then removed with Raney nickel under an atmosphere of hydrogen gas to furnish the free alcohol **2.28** in a 73% yield (**Scheme 2.8**). If a linker was to be added it would be done at this step because the acetyl protecting group prevents unwanted alkylation of the benzylic amine. If no linker was to be added then the acetyl protecting group was removed with 6M NaOH in methanol to provide the desired derivative **2.15** in 62% yield (**Scheme 2.8**).



Conditions and reagents: (i) a. NaH, DMF, rt, 1 h; b. **2.24**, 16 h, 36%;(ii) Jones' reagent, acetone, 0 °C, 2 h, 87%

Scheme 2.9 Synthesis of 4-(benzyloxy) cyclohexanone 2.26

The final WAY-470 derivative (**2.16**) was synthesized by attaching a linker to the benzylic amine through an amide bond. The benzylic amine may be crucial to both 5-HT_{2C}R affinity and agonist activity, but since a linker could readily be attached to the nitrogen through an amide bond this derivatives was synthesized. **Scheme 2.10** shows the synthesis of derivative **2.30** from WAY-470(**2.10**). WAY-470 (**2.10**) was reacted with

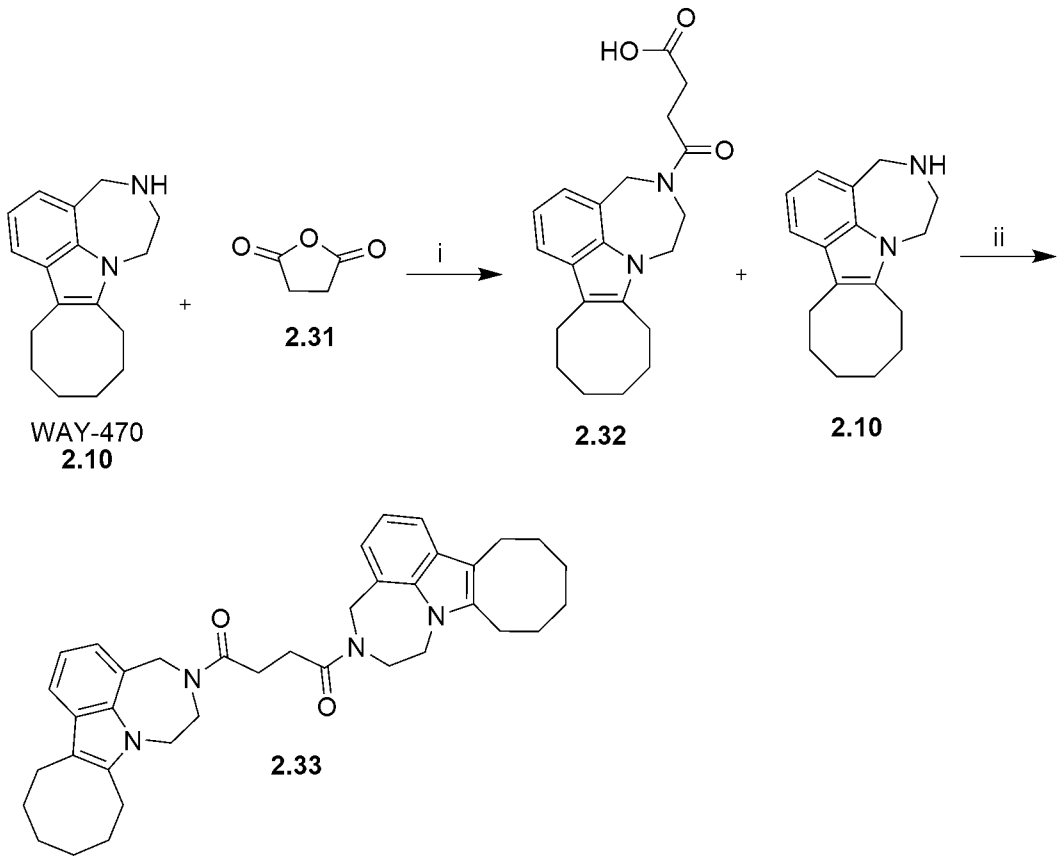


Conditions and reagents: (i) EDAC, DIEA, CH_3CN, rt, 20 h, 54%

Scheme 2.10 Synthesis of WAY-470 derivative 2.30

ethoxyacetic acid (**2.29**) in the presence of the base N,N-DIEA and the coupling reagent EDAC at room temperature to give derivative **2.30** smoothly in a 54% yield (**Scheme 2.10**) . EDAC is a carbodiimide group which reacts with the carboxylic acid of ethoxyacetic acid to form a carboxylic ester. This ester is an activated leaving group that when attacked by the benzylic amine will leave to give the desired amide.

Scheme 2.11 shows the synthesis of the homodimer derivative **2.33**. The synthesis of derivative **2.33** began by reacting WAY-470 (**2.10**) with succinic anhydride (**2.31**) in CH_2Cl_2 at room temperature to give compound **2.32** in an 84% yield (**Scheme 2.11**). The carboxylic acid of **2.32** was then coupled to another molecule of WAY-470 (**2.10**) using the coupling reagent EDAC and the base N,N-DIEA at room temperature to produce derivative **2.33** in a 57% yield (**Scheme 2.11**).

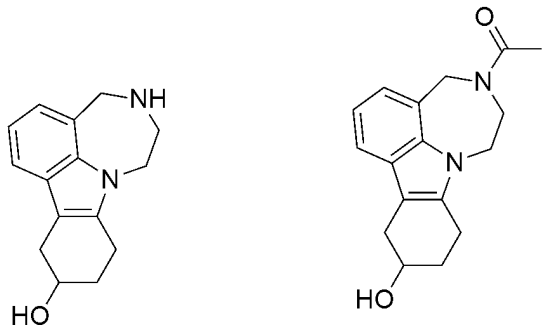


Conditions and reagents: (i) CH_2Cl_2, rt, 24 h, 84%; (ii) EDAC, DIEA, CH_3CN, rt, 20 h, 57%

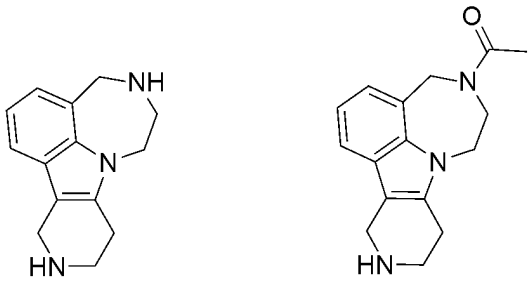
Scheme 2.11 Synthesis of WAY-470 dimer 2.33

Attaching pseudo-tethers to WAY-470 derivatives

With the synthesis of the WAY-470 derivatives **2.14**, **2.15**, **2.30**, and **2.33** complete, pseudo-linkers could be attached to compounds **2.14** and **2.15**. To prevent the unwanted alkylation of the benzylic amine of compounds **2.14** and **2.15** the acetyl protected intermediates **2.28** and **2.22** were utilized in the alkylation reactions (**Fig 2.2**).



2.15 **2.28**

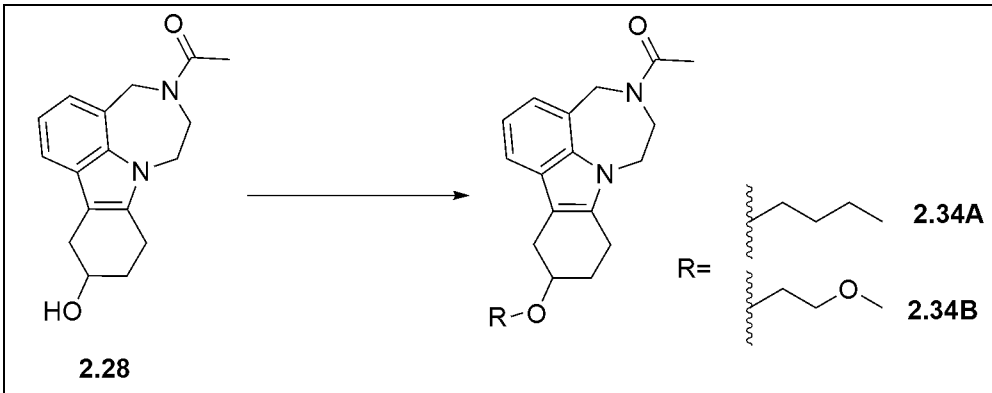


2.14 **2.22**

Figure 2. 2 WAY-470 derivatives on which pseudo-linkers were attached

The secondary alcohol of compound **2.28** was the first location at which the attachment of a pseudo-linker was attempted. The desired alkylated derivative was originally envisioned to be accessible through a simple Williamson ether synthesis using an alkyl halide and a base, but this reaction proved to be problematic.

Using literature precedents, a variety of different organic and inorganic bases including N,N-DIEA, NaH, NaOH, KH, CsOH, nBuLi, NaHMDS, and Na tert-pentoxide were employed under various conditions.[99-103] The various reaction conditions included the solvents DMF, THF, DMSO, H_2O, CH_2Cl_2, and CH_3CN under temperatures ranging from room temperature to reflux including microwave conditions. The reactions were monitored for up to 72 hours. Bromine alkyl halides as well as alkyl tosylates were employed in the ether synthesis, but none produced the desired compound.



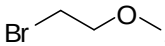
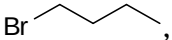
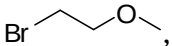
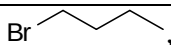
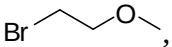
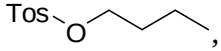
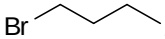
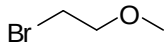
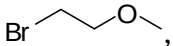
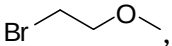
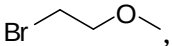
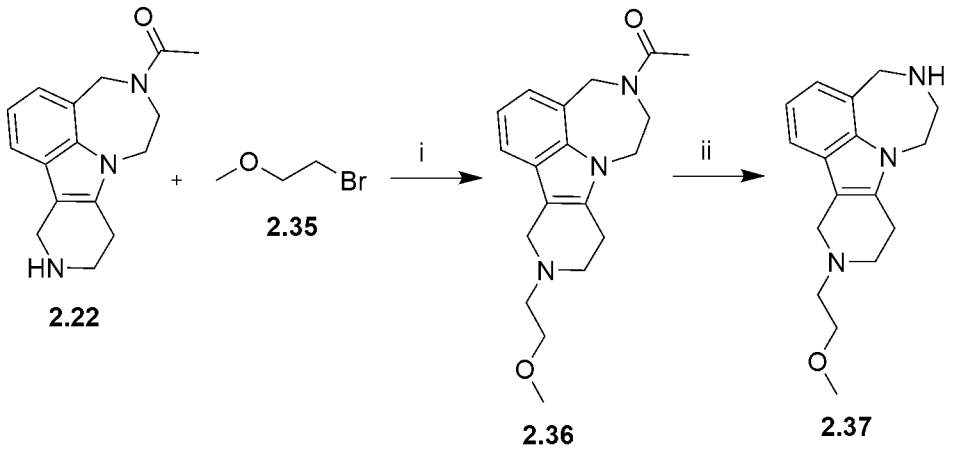
Entry	Conditions	Results
1	 , NaH, DMF, 70 ^{o}C, 24 h	0%
2	 , 6 M NaOH, TBAB, 80 ^{o}C, 48 h	10%
3	 , NaH, DMSO, 80 ^{o}C, 24 h	0%
4	 , DIEA, CH_2Cl_2, reflux, 24 h	0%
5	 , DIEA, toluene, rt, 24 h	0%
6	 , NaH, DMSO, rt, 48 h	0%
7	 , CsOH, DMF, TBAI, 80 ^{o}C, 2 d	0%
8	 , KH, THF, reflux, 24 h	0%
9	 , NaHMDS, TBAI, DMF, 70 ^{o}C, 3 d	0%
10	 , nBuLi, THF, -78 ^{o}C - rt, 18 h	0%
11	 , Na tert-pentoxide, DMF, rt, 5 d	0%
12	 , NaH, DMSO or DMF, MW, 1 h	0%

Table 2. 2 Reaction conditions to alkylate WAY-470 derivative 2.28

None of the combinations of base, solvent, temperature, and reaction time produced the desired ether in any appreciable yield. Since a pseudo-linker could not be attached to the secondary alcohol of **2.15** this route was abandoned, and the attachment of a pseudo-linker to WAY-470 piperidine derivative **2.14** was pursued.

The advantage to using the WAY-470 piperidine derivative **2.14** in place of the WAY-470 cyclohexanol derivative **2.15** to synthesize WAY-470 derivatives was that the chiral carbon of **2.15** was replaced with a nonchiral nitrogen atom. The absence of a chiral carbon in compound **2.14** simplified the synthesis because separation of diastereomers was not required. The synthetic challenge of selectively attaching a pseudo-linker to the piperidine nitrogen and not the benzylic nitrogen was avoided by using compound **2.22** in which the benzylic amine was acetyl protected. Alkylating the piperidine nitrogen gives compound **2.36** in a 31% yield (**Scheme 2.12**).



Conditions and reagents: (i) DIEA, CH_3CN, reflux, 16 h, 31%; (ii) 6 M NaOH, MeOH, 68%

Scheme 2.12 Synthesis of WAY-470 piperidine derivative 2.36

The acetate was cleaved under basic conditions to give the desired N-alkylated derivative **2.37** in a 68% yield (**Scheme 2.12**). Compound **2.37** was not submitted for initial testing

due to the fact that a shift from the parent compound WAY-470 to WAY-163909 was made at this time.

Concurrently to synthesizing **2.37**, a set of WAY-470 derivatives were submitted to Dr. Cunningham's lab for testing. Compound **2.21** which contains both a Cbz and acetyl protecting group was submitted to determine if either protecting group has a detrimental effect on 5-HT_{2C}R affinity and agonist activity. In addition to compound **2.21**, compounds **2.9**, **2.10**, **2.30**, and **2.33** were also submitted for testing to determine if 5-HT_{2C}R affinity and agonist activity were retained when the benzylic nitrogen is alkylated. The Ca^{+2} stimulation assay results revealed that none of the compounds tested stimulated Ca^{+2} release and were therefore not 5-HT_{2C}R agonists (**Fig 2.3**). EC_{50} values were not determined for these compounds because they all would have been over the cutoff of >10 μM. These results suggest that the benzylic amine is critical to 5-HT_{2A}R agonist activity. Protecting the benzylic amine as an amide greatly decreased the basicity of the nitrogen due to the electron withdrawing effect of the carbonyl group. Due to the importance of the benzylic amine to agonist activity, this location was not an appropriate linker attachment site. An alternative linker attachment point was the piperidine ring nitrogen of compound **2.22**, which was alkylated with the pseudo-linker ethyl methyl ether to give compound **2.37**. Prior to submission of compound **2.37** for testing a switch to the alternative 5-HT_{2C}R agonist WAY-163909 was made.

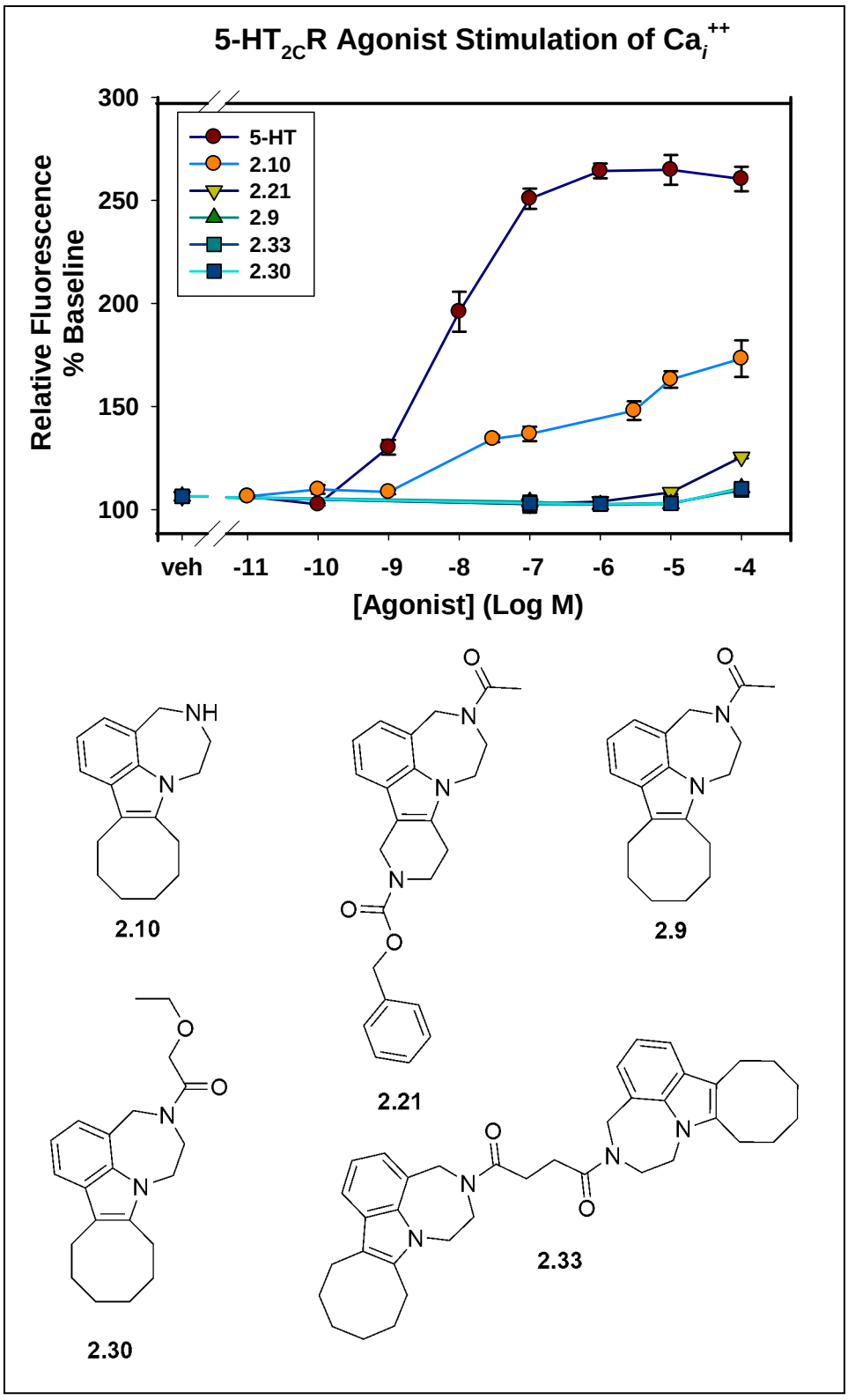


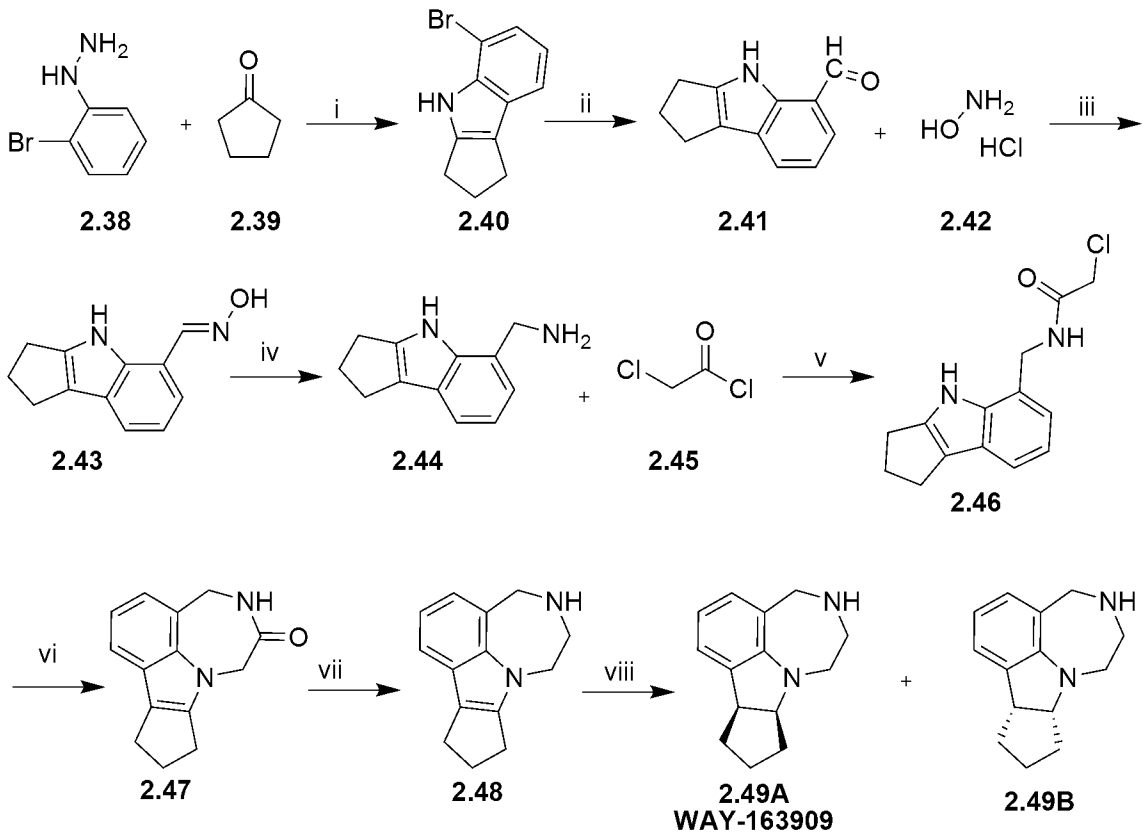
Figure 2.3 Stimulation of Ca++ by WAY-470 derivatives measured as increased fluorescence

Published Synthesis of WAY-163909

The 5-HT_{2C}R agonist WAY-163909 has a higher affinity and better selectivity for the 5-HT_{2C}R than WAY-470.[87] WAY-163909 was structurally similar to WAY-470 with the only differences being the size of the cycloalkyl ring and the presence of an indole double bond in WAY-470 that has been reduced in WAY-163909. Although the two compounds were structurally similar, the absence of the indole double bond had a significant effect on the pharmacological properties of WAY-163909. WAY-470 had an unspecified short half-life while WAY-163909 had a half-life in humans of 300 minutes.[87] Additionally WAY-163909 was not metabolized by small intestinal microsomes, which suggests that small intestine first pass metabolism will not limit oral bioavailability of WAY-163909.[87] The slower clearance of WAY-163909 along with its potentially high oral bioavailability made WAY-163909 an attractive alternative to WAY-470.

WAY-163909 was structurally similar to WAY-470, but the published synthesis of WAY-163909 varied considerably from that of WAY-470.[104] The published synthesis of WAY-470 began with the synthesis of the benzodiazepine core and the indole ring was formed in the latter part of the synthesis. In contrast to WAY-470, the published synthesis of WAY-163909 began with the formation of the indole and the benzodiazepine was formed towards the end of the synthesis (**Scheme 2.13**). The published synthesis of WAY-163909 began with 2-bromophenylhydrazine hydrochloride (**2.38**) and cyclopentanone (**2.39**) in dilute sulfuric acid underwent a Fischer indole synthesis to give the bromoindole **2.40** in a 59% yield (**Scheme 2.13**).[104] The bromoindole **2.40** was metalated with nBuLi and then formylated with DMF to produce the crude indole

aldehyde **2.41** in a quantitative yield (**Scheme 2.13**) .[104] The indole oxime **2.43** was generated in a 58% yield from crude **2.41** and hydroxylamine (**2.42**) in pyridine (**Scheme 2.13**) .[104]



Conditions and reagents: (i) 4% sulfuric acid, 100 °C, 6 h, 59%; (ii) a. nBuLi, MTBE, 0 °C , 20 min, b. DMF, 1 h; (iii) 2:1 pyridine:H_2O, rt, 16 h, 58% over two steps; (iv) LAH, THF, 0 °C - rt, 16 h, 69%; (v) pyridine, rt, 12 h, 57%; (vi) NaH, DMF, rt, 16 h, 58%; (vii) LAH, Et_2O, rt, 16 h, 70%; (viii) 1 M BH_3.THF, TFA, 0 °C, 4h, 58%; diastereomers separated with flash chromatography

Scheme 2.13 Published synthesis of WAY-163909

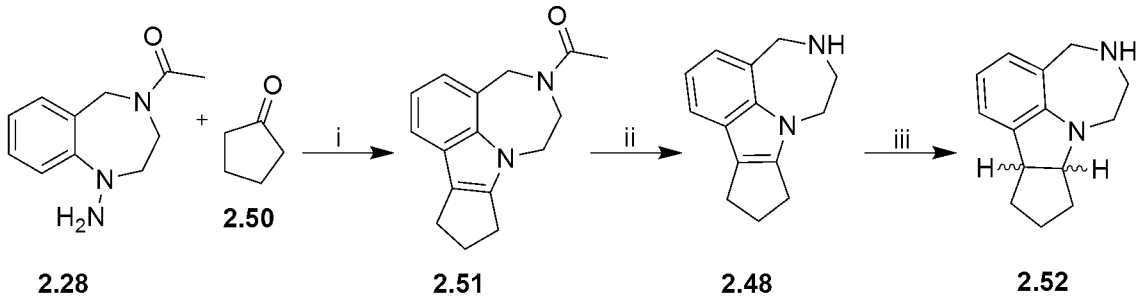
The indole oxime **2.43** was then reduced with LAH to the indole amine **2.44** in a 69% yield (**Scheme 2.13**) .[104] Chloroacetyl chloride (**2.45**) in the presence of pyridine was used to acylate the indole amine **2.44** to generate acyl indole **2.46** in a 57% yield (**Scheme 2.13**).[104] With **2.46** in hand, the benzodiazepine ring was accessed through a cyclization by treating **2.46** with NaH to give benzodiazepinone **2.47** in a 58% yield

(**Scheme 2.13**).[104] The amide bond of **2.47** was then reduced with LAH to produce the benzodiazepine **2.48** in a 70% yield (**Scheme 2.13**).[104] The indole bond of benzodiazepine **2.48** was reduced with BH_3•THF in TFA to give the diastereomers **2.49A** and **2.49B** which were separated by flash chromatography (**Scheme 2.13**).[104] The synthesis of WAY-163909 (**2.49A**) was completed in eight steps following the published procedure.

Although the published synthesis of WAY-163909 (**2.49A**) in **Scheme 2.13** was effective, it did not utilize any of the reactions employed in the synthesis of WAY-470. Additionally the published reaction sequence was not amenable to the synthesis of derivatives in which the cyclopentyl ring of WAY-163909 was modified. The cyclopentyl ring was introduced through a Fischer indole synthesis in the first step of the published procedure. Since the cyclopentyl ring was introduced in the first step of the reaction sequence, any derivatization of this ring would also have to occur in the first step of the synthesis. Since any variation in this ring would have to be introduced in the first step of this reaction sequence, there is no common intermediate from which multiple derivatives can be synthesized. Each derivative with a variation in this ring would require a complete synthesis beginning at the first reaction in **Scheme 2.13**. Derivatives synthesized in this manner would be very time and labor intensive because each reaction for each derivative would have to be optimized and in some cases the published reaction conditions may not be favorable for the desired derivatives. In order to produce derivatives in an efficient manner the published reaction sequence in **Scheme 2.13** was not pursued.

Modified Synthesis of WAY-163909

Since WAY-470 and WAY-163909 were structurally similar, the published synthesis of WAY-163909 was compared to the synthesis of WAY-470 to determine if WAY-163909 could be synthesized by modifying the WAY-470 synthesis. The WAY-163909 intermediate compound **2.48** only differed from WAY-470 in the size of the cycloalkyl ring. The previous synthesis of the WAY-470 derivative intermediates **2.21** and **2.27** suggested that hydrazine **2.28** could be reacted with a variety of cyclic ketones in a Fischer indole synthesis to give compounds containing different cycloalkyl rings or even rings such as piperidine containing heteroatoms. The successful synthesis of the Fischer indole derivatives **2.21** and **2.27** suggested that cyclopentanone may be successfully employed in this reaction. The reaction sequence to furnish WAY-163909 can be seen in **Scheme 2.14**. A Fischer indole synthesis was carried out with hydrazine **2.28** and cyclopentanone (**2.50**) in acetic acid under standard conditions to give the desired indole **2.51** in a 29% yield (**Scheme 2.14)** . The acetyl protecting group was then removed with 6M NaOH in MeOH under reflux for 24 hours to give compound **2.48** in an 80% yield (**Scheme 2.14**). Intermediate **2.48** was then reduced with 1M BH_3•THF in TFA to give racemic WAY-163909 (**2.52)** in a 99% yield (**Scheme 2.14**).



Conditions and reagents: (i) acetic acid, 100 °C, 6 h, 29%; (ii) 6 M NaOH, MeOH, reflux, 24 h, 80%; (iii) 1 M BH_3.THF, TFA, rt, 48 h, 99%

Scheme 2.14 Route to racemic WAY-163909 from hydrazine 2.28

Once the synthesis of racemic WAY-163909 (**2.52**) was complete, the synthesis of WAY-163909 derivatives with linker attachment points was started. The desired derivatives were similar to the WAY-470 derivatives synthesized previously. The first derivative, compound **2.53,** had a piperidine ring in place of the cyclopentyl ring (**Fig 2.4**). The second derivative, compound **2.54**, had a cyclohexanol ring in place of the cyclopentyl ring (**Fig 2.4**). The third derivative, compound **2.55** had bromine on the aromatic ring (**Fig 2.4**). Derivatives **2.53** and **2.54** both contained six membered rings with a heteroatom that could be used as a linker attachment point. The piperidine nitrogen of compound **2.53** allowed for a linker attachment site that did not introduce a chiral carbon. In contrast, compound **2.54** contained a chiral carbon in the six membered ring on which a hydroxy group was attached. The introduction of the chiral carbon in compound **2.54** led to the formation of diastereomers. These diastereomers led to a mixture of products that were not separable. The synthesis of **2.53** and **2.54** was similar to the modified synthesis of WAY-163909 as seen in **Scheme 2.14** except that different cyclic ketones were required for the Fischer indole synthesis.

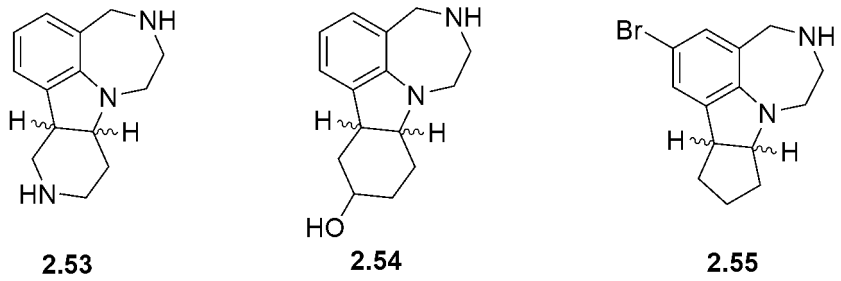
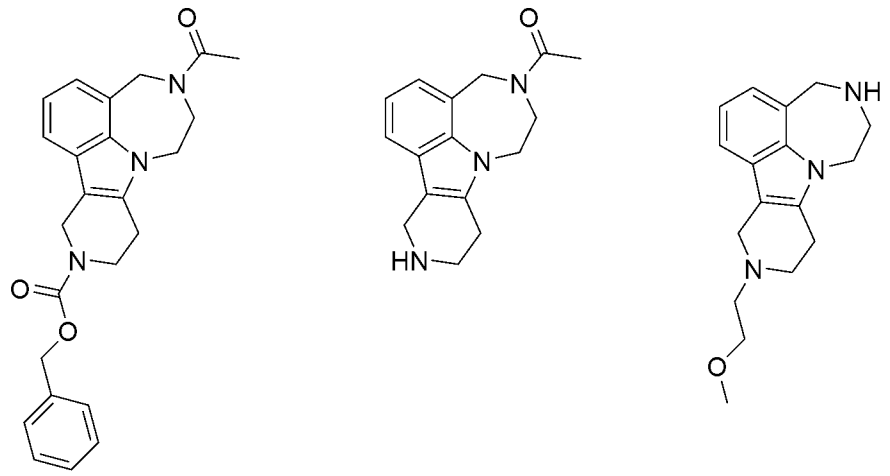


Figure 2.4 WAY-163909 derivatives

The synthesis of derivative **2.53** utilizes the previously synthesized Cbz-piperidine indole **2.21**. The next step was the reduction of the indole bond to give compound **2.58**. The reduction of the indole bond in the published procedure was carried out with 1M BH_3•THF in TFA to give the reduced indole in good yield. These conditions could not successfully reduce the indole double bond of compound **2.21**. The failure of these reaction conditions to reduce the indole bond could be attributed to the presence of the nitrogen atom in the piperidine ring. If the piperidine was causing the reduction to fail, then derivative **2.53** will be unable to be synthesized. In order to determine if the indole can be reduced, three unique substrates were exposed to various reaction conditions. The three unique substrates were compounds **2.21**, **2.22**, and **2.37** because these compounds all contained a piperidine ring but also containing different protecting groups.

Compound **2.21** contained both a Cbz-protecting group on the piperidine nitrogen and an acetyl protecting group on the benzylic nitrogen. Compound **2.22** contained an acetyl protecting on the benzylic nitrogen, but the piperidine was unprotected. Compound **2.37** contained an ethyl methyl ether pseudo-linker on the piperidine nitrogen and the benzylic nitrogen was unprotected. Numerous attempts to reduce the indole bond of compounds **2.21**, **2.22**, and **2.37** utilizing a variety of reaction conditions failed to

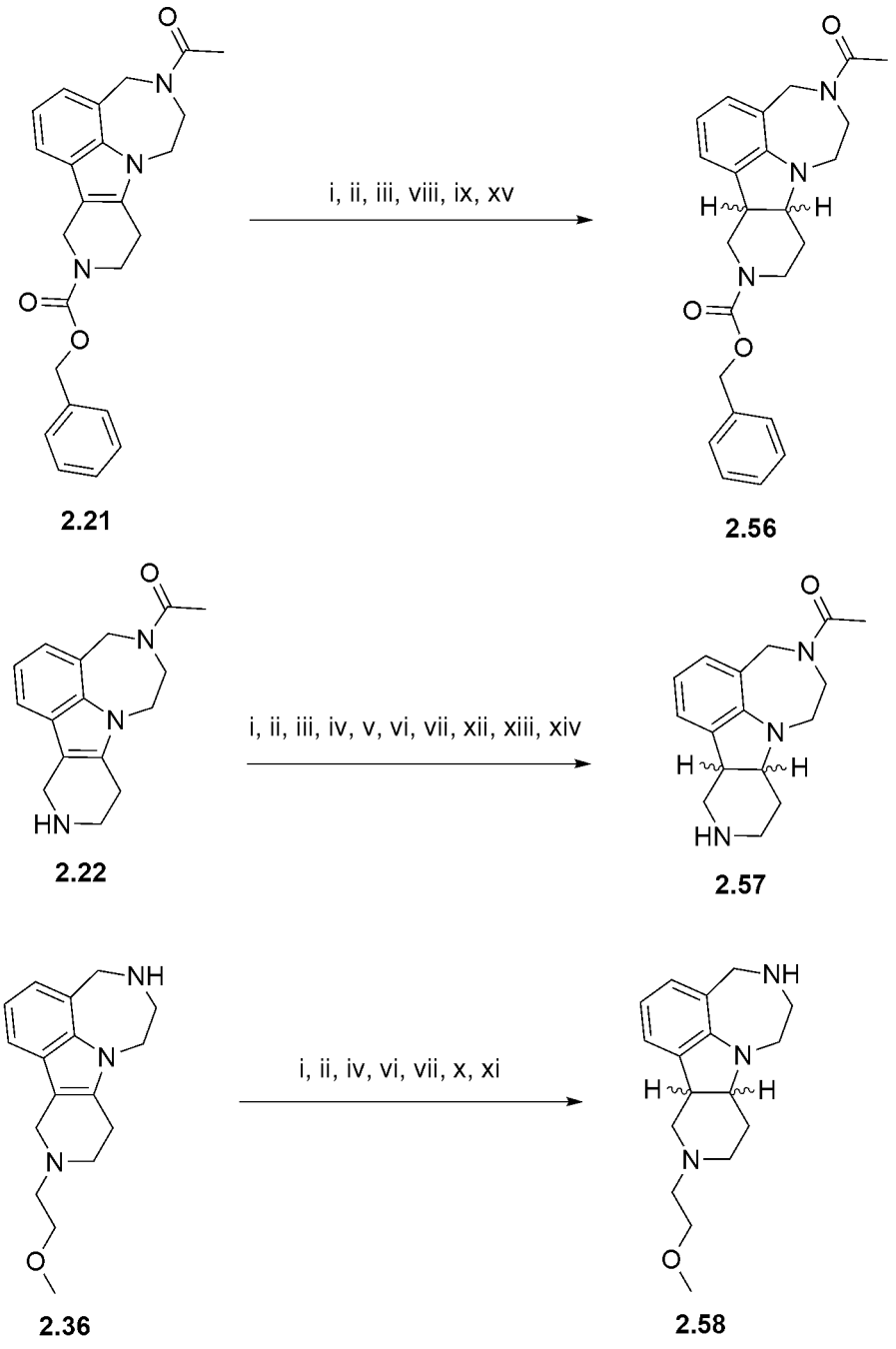
produce the desired product (**Scheme 2.15**). The successful reduction of the indole bond was carried out on compound **2.21** with $NaBH_4$ in TFA over 18 hours.



2.21 **2.22** **2.37**

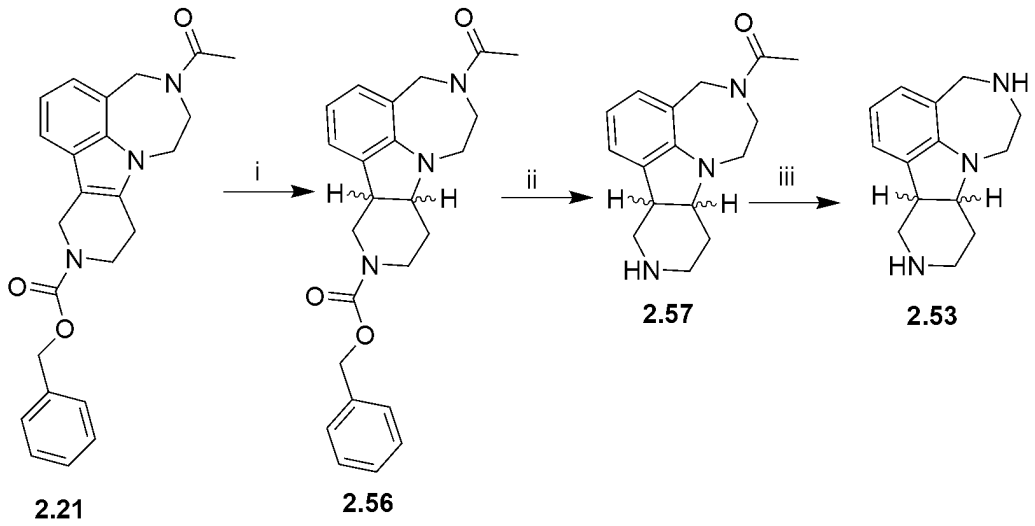
Figure 2.5 WAY-163909 piperidine intermediates employed in the study of an indole bond reduction

Once appropriate reactions conditions to reduce the indole bond of compound **2.21** had been determined, the synthesis of derivative **2.53** was carried forward (**Scheme 2.16**). Reducing compound **2.21** with $NaBH_4$ in TFA at room temperature over 18 hours produced compound **2.56** in a 92% yield (**Scheme 2.16**). The Cbz-protecting group on the piperidine was then removed with 10% Pd/C in EtOH under an atmosphere of hydrogen gas to furnish **2.57** in a 72% yield (**Scheme 2.16**). Acetyl protected **2.57** was then deprotected in 6 M NaOH and MeOH under reflux for 24 hours to provide the WAY-163909 piperidine derivative **2.53** in a 75% yield (**Scheme 2.16**) .



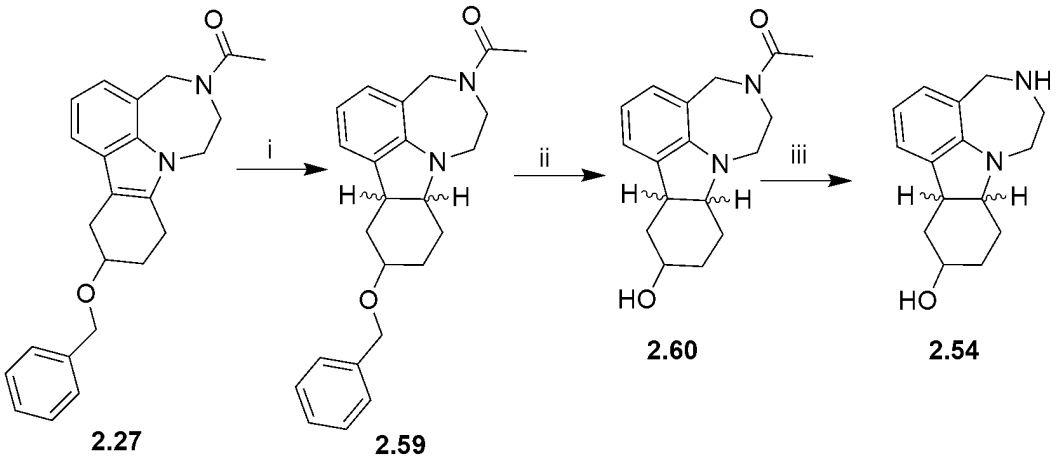
Reagents and conditions: (i) $NaBH_3CN$, TFA, rt, 48 h; (ii) TFA, Et_3SiH, 60 °C, 24 h; (iii) Rh(acac)(cod), PPh_3, MeOH, 80 °C, 24 h; (iv) Sn, HCl, EtOH, 80 °C, 48 h; (v) Mg, MeOH, rt, 24 h; (vi) 10% Pd/C, TFA, H_2 gas, rt, 24 h; (vii) Raney Ni, 400psi, H_2 gas, rt, 6 d; (viii) $RhAl_2O_3$, TFA, EtOH, H_2 gas, rt, 24 h; (ix) $NaBH_4$, acetic acid, rt, 24 h; (x) 1 M BH_3.THF, TFA, rt, 24 h; (xi) PtO_2, H_2 gas, acetic acid, rt, 48 h; (xii) 10% Pd/C, 500psi, H_2 gas, MeOH, HCl, rt, 24 h; (xiii) 10% Pt/C, 500psi, H_2 gas, EtOH, rt, 48 h; (xiv) PtO_2, H_2 gas, EtOH, rt, 48 h; (xv) $NaBH_4$, TFA, rt, 18 h

Scheme 2.15 Reduction of the indole bond



Conditions and reagents: (i) $NaBH_4$, TFA, 18 h, rt, 92%; (ii) 10% Pd/C, EtOH, rt, 48 h, H_2, 72%; (iii) 6 M NaOH, MeOH, reflux, 24 h, 74%

Scheme 2.16 Reduction and deprotection of the WAY-163909 derivative 2.53



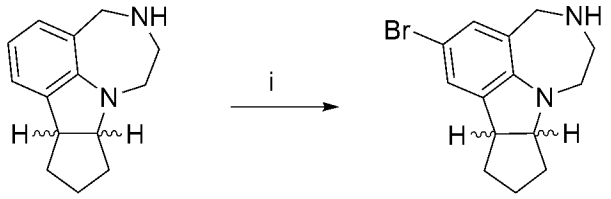
Conditions and reagents: (i) $NaBH_4$, TFA, 18h, rt, 70%; (ii) Raney Ni 2800, EtOH, RT, 48 h, H_2, 85%; (iii) 6M NaOH, MeOH, reflux, 24h, 62%

Scheme 2.17 Reduction and deprotection of the WAY-163909 derivative 2.54

The synthesis of the cyclohexanol derivative **2.54** began with the previously synthesized intermediate **2.27**. The indole of intermediate **2.27** was reduced with $NaBH_4$

in TFA in a reaction analogous to the reduction of compound **2.21** to smoothly produce the reduced indole **2.59** in a 70% yield (**Scheme 2.17**). The benzyl protecting group of **2.59** was removed with Raney Ni in EtOH under an atmosphere of hydrogen gas to give compound **2.60** with a free hydroxyl group in a 55% yield (**Scheme 2.17**). The last step was the removal of the acetyl protecting group in 6 M NaOH in MeOH under reflux for 24 hours to afford the WAY-163909 cyclohexanol derivative **2.54** in a 62% yield (**Scheme 2.17**).

The final WAY-163909 derivative synthesized was the bromine containing derivative compound **2.55**. This derivative was synthesized in one step by reacting racemic WAY-163909 (**2.52**) with bromine in CH_2Cl_2 at 0 ^oC for 4 hours and then warming to room temperature and stirring an additional 18 hours to afford the bromine derivative **2.55** in a 60% yield (**Scheme 2.18**).



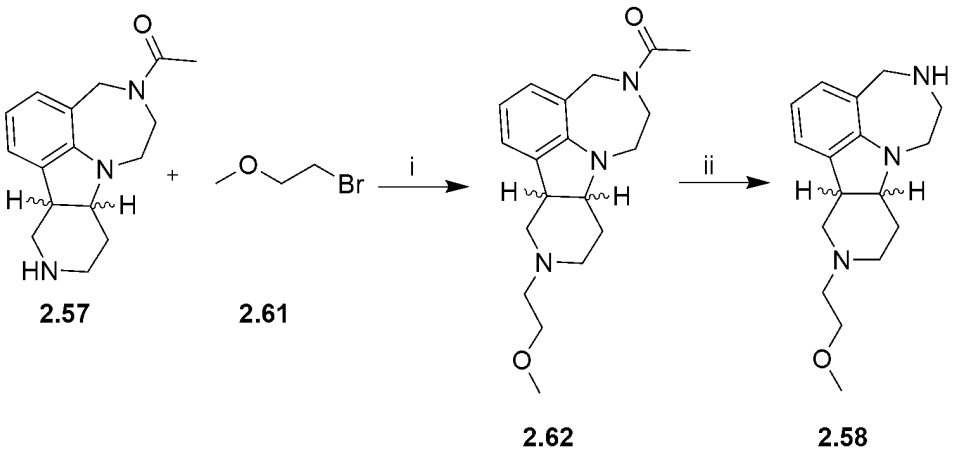
2.52 **2.55**

Conditions and reagents: (i)Br_2, CH_2Cl_2, 0 ^oC 4 h, rt 18 h, 60%

Scheme 2.18 Synthesis of the WAY-163909 derivative 2.55

In addition to compounds **2.53**, **2.54**, and **2.55**, a derivative of compound **2.53** with a pseudo-linker was synthesized. Addition of a pseudo-linker to the piperidine derivative **2.53** began with the acetyl protected compound **2.57** (**Scheme 2.19**). Compound **2.57** was N-alkylated with 2-bromoethyl methyl ether **2.61** in the presence of

N,N-DIEA in acetonitrile at 66 ^{o}C for 24 hours to generate **2.62** in a 40% yield (**Scheme 2.19**). The acetyl protected compound **2.57** was employed in this reaction to prevent unwanted alkylation at the benzylic amine. The acetyl protecting group of compound **2.62** was removed with 6 M NaOH in MeOH under reflux to furnish the alkylated piperidine derivative **2.58** in an 81% yield (**Scheme 2.19**).



Conditions and reagents: (i) DIEA, CH_3CN, 66 °C, 24 h, 40%; (ii) 6 M NaOH, MeOH, reflux, 20 h, 81%

Scheme 2.19 Synthesis of WAY-163909 derivative 2.62 containing a pseudo-tether

The WAY-163909 derivatives **2.53**, **2.54**, **2.55**, and **2.58** were submitted to the Cunningham lab for EC_{50} determination. **Table 2.3** shows the experimentally determined EC_{50} values for these compounds. The EC_{50} value for each derivative tested exceeded the cutoff of 10μM, which was more than 100 times that of the authentic WAY-163909 sample tested. This data suggest that modifying the cycloalkyl ring to either a piperidine or a cyclohexanol ring had a detrimental effect on 5-HT_{2C}R agonist activity. The decrease in activity could be due to the presence of a heteroatom in the cycloalkyl ring or the

bromine on the aromatic ring, both which may change the orientation of the WAY-163909 derivatives in the 5-HT_{2C}R binding pocket.

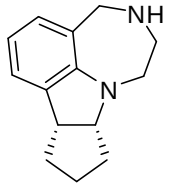
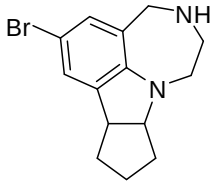
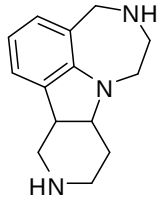
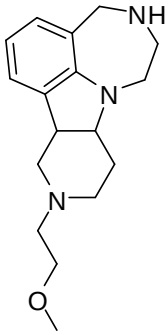
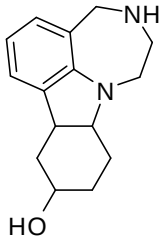
Compound	EC_{50} ± SEM	Compound	EC_{50} ± SEM
 **WAY-163909**	71 ± 15 nM	 **2.55**	>10 μM
 **2.53**	30 ± 14 μM	 **2.58**	>10 μM
 **2.54**	> 10 μM		

Table 2.3 EC_{50}s of WAY-163909 derivatives

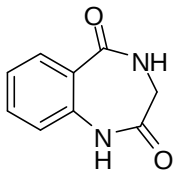
Conclusion

The lack of biological activity of the WAY-163909 derivatives **2.53**, **2.54**, **2.55**, and **2.58** suggest that this benzodiazepine scaffold was not tolerant to the modifications

necessary to synthesize DMLs. In order to synthesize DMLs with the desired activity at the 5-HT_{2A}R and the 5-HT_{2C}R a different 5-HT2cR agonist must be chosen.

Experimental

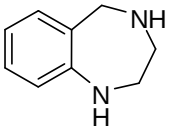
3,4-dihydro-1H-benzo[e][1,4]diazepine-2,5-dione (2.4)



2.4

To a suspension of glycine (5 g, 67 mmol) in 30 mL water was added 6 M sodium hydroxide to a pH of 8. Then isatoic anhydride (10.8 g, 66.7 mmol) was added in 10 portions over 3 hours, adjusting the pH back to 8 after each addition. After the addition of the isatoic anhydride, reflux for 4 hours. After 4 hours a solution of L-tartaric acid (25 g, 167 mmol) in 35 mL water was added and refluxed for an additional hour. After one hour, the water was distilled off, and the remaining residue was cooled to 50 ^{o}C. Aqueous ammonia hydroxide was slowly added until an off-white precipitate crashed out of solution. The precipitate was collected and dried under vacuum to give 9.5 g of 3,4-dihydro-1H-benzo[e][1,4]diazepine-2,5-dione (**2.4)** as a off-white solid **(**86%). ^{1}H NMR (500 MHz, DMSO-D_6) δ 10.34 (bs, 1H), 8.53 (t, J = 5.7 Hz, 1H), 7.72 (dd, J = 8.1, 1.8 Hz, 1H), 7.47 (ddd, J = 8.0, 8.0, 1.8 Hz, 1H), 7.18 (ddd, J = 8.0, 8.0, 1.1 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 3.55 (d, J = 5.8 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-D_6) δ 171.7, 168.6, 137.7, 132.8, 131.3, 126.0, 124.4, 121.4, 45.0

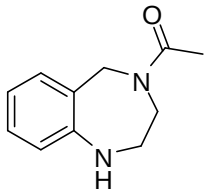
2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine (2.5)



2.5

A slurry of 3,4-dihydro-1H-benzo[e][1,4]diazepine-2,5-dione (**2.4)** (0.42 g, 2.35 mmol) in 20 mL THF was cooled to 0 ^{o}C. Lithium aluminum hydride (0.44 g, 12 mmol) was added portionwise with stirring. The slurry was refluxed for 20 hours. After 20 hours the reaction mixture was cooled to 0 ^{o}C and 0.5 mL water was added. The reaction is stirred for 30 minutes, and then 0.5 mL 1M sodium hydroxide was added. The reaction mixture was warmed to room temperature and stirred 30 minutes. After which 0.5 mL water was added and stirring was continued for 30 minutes, after which celite was added and the reaction was filter. The filter cake was washed with ethyl acetate. The filtrate was concentrated under reduced pressure to afford 0.31 g of 2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine (**2.5**) as a red-orange solid (93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11-7.06 (m, 2H), 6.82 (dd, J = 7.3, 7.3 Hz, 1H), 6.76 (d, J = 7.3 Hz, 1H), 3.89 (s, 2H), 3.09-3.04 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 150.1, 132.8, 129.8, 127.7, 120.7, 119.0, 54.6, 52.0, 51.2

1-(2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (2.6)

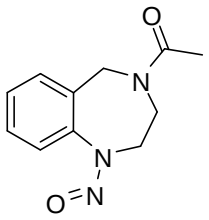


2.6

A solution of 2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine (**2.5)** (2.16 g, 14.6 mmol) and potassium carbonate (2.12 g, 15.3 mmol) in 38 mL acetonitrile was cooled to 0 ^{o}C. Then acetic anhydride (1.5 g, 14.6 mmol) was added over 30 minutes to the cooled reaction mixture. The ice bath was removed and the reaction was stirred at room temperature for 21 hours. After 21 hours, the acetonitrile was removed under reduced pressure. The

residue was taken up in water and extracted 4 times with CH_2Cl_2. The combined organic layers are dried over $MgSO_4$, filtered, and concentrated. The crude reaction mixture was purified by column chromatography (100% CH_2Cl_2 to 9% MeOH in CH_2Cl_2) to afford 2.2 g of 1-(2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (**2.6**) as a red-orange solid (89% as amide isomers). ^{1}H NMR (500 MHz, $CDCl_3$) δ .34 (d, J = 7.5 Hz, 0.44H), 7.14 (d, J = 7.5Hz, 1.56H), 7.08 (dd, J = 8.0, 7.5 Hz, 0.24H), 6.87 (dd, J = 7.4, 7.2 Hz, 0.76H), 6.79 (d, J = 8.0Hz, 0.76H), 6.76 (d, J = 8.0 Hz, 0.24H), 4.55 (s, 0.6H), 4.43 (s, 1.4H), 3.81 (dd, J = 4.6, 4.6 Hz, 1.4H), 3.68 (dd, J = 5.1, 4.6 Hz, 0.6H), 3.21 (dd, J = 5.2, 4.6 Hz, 0.6H), 3.17 (dd, J = 5.2, 4.6 Hz, 1.4H), 2.17 (s, 2H), 2.05 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 149.5, 149.0, 131.0, 129.5, 129.0, 128.7, 128.2, 127.8, 121.4, 120.8, 119.7, 119.2, 53.4, 52.2, 49.4, 48.9, 48.3, 47.8, 22.2, 22.0

1-(1-nitroso-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (2.7)

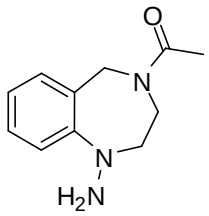


2.7

A solution of 1-(2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (**2.6**) (2.5 g, 13.14 mmol) in 25 mL of 0.8M HCl was cooled to 0 ^{o}C, and then a solution of sodium nitrite (1.1 g, 15.8 mmol) in 3 mL water was added dropwise. The reaction was warmed to room temperature and stirred for 16 hours. After 16 hours the reaction mixture was extracted 4 times with CH_2Cl_2. The combined organic layers are dried over $MgSO_4$, filtered, and concentrated to give 2.1 g of 1-(1-nitroso-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (**2.7**) as a red-brown oil (86% as amide

isomers). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42-7.22 (m, 4H), 4.47 (s, 0.9H), 4.38 (s, 1.1H), 4.02 (bs, 1H), 3.93 (bs, 1H), 3.62 (bs, 1H), 3.55 (dd, J = 5.7, 5.1 Hz, 1H), 2.02 (s, 1.6H), 1.89 (s, 1.4H);^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 169.3, 141.2, 140.7, 131.1, 130.2, 129.1, 129.1, 128.8, 128.6, 128.5, 125.1, 123.9, 51.6, 47.5, 46.9, 44.3, 43.9, 43.6, 21.5, 21.4

1-(1-amino-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (2.8)

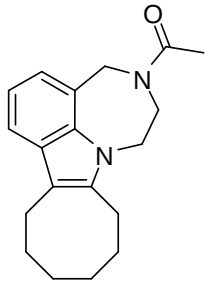


2.8

Titanium tetrachloride (7.05 g, 37 mmol) was added to a solution of CH_2Cl_2 and diethyl ether (60 mL, 4:1 v:v) at 0 ^{o}C. Then the magnesium powder (0.90 g, 37 mmol) was added in five portions over 30 minutes with stirring. The reaction mixture was stirred at 0 ^{o}C for 30 minutes. The reaction was then warmed to room temperature and stirred an additional for 4 hours. 1-(1-nitroso-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (**2.7**) (2.05 g, 9.3 mmol) in 5 mL CH_2Cl_2 was added, and the reaction was stirred at room temperature an additional 45 minutes. After 45 minutes the reaction was cooled to 0 ^{o}C, quenched with 30 mL of 0.3M HCl, and stirred an additional hour. 6M sodium hydroxide was then added to make the reaction mixture basic, the reaction turned blue. The layers were separated, and the aqueous layer was extracted 3 times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give 1.7 g of 1-(1-amino-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (**2.8**) as a red-orange

oil (91% as two ring conformations). ^{1}H NMR (500MHz, $CDCl_3$) δ 7.35-7.25 (m, 2H), 7.13 (dd, J = 7.5, 1.2 Hz, 1H), 6.94 (ddd, J = 7.5, 7.5, 1.2 Hz, 1H), 4.52 (s, 0.8H), 4.41 (s, 1.2H), 3.88 (bs, 1H), 3.84 (dd, J = 5.2, 5.1 Hz, 1.3H), 3.67 (dd, J = 5.2, 4.5 Hz, 0.7H), 3.27 (dd, J = 5.2, 5.2 Hz, 0.7H), 3.24 (dd, J = 5.2, 5.2 Hz, 1.3H), 2.16 (s, 2H), 2.02 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 168.9, 152.8, 152.4, 130.7, 129.3, 128.8, 128.4, 127.6, 127.0, 122.2, 121.7, 116.7, 115.8, 62.1, 61.7, 52.8, 49.0, 48.9, 45.0, 21.9, 21.8

1-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)ethanone (2.9)

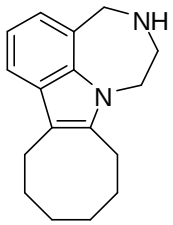


2.9

A solution of of 1-(1-amino-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (**2.8**) (1.47 g, 7.2 mmol) and cyclooctanone (**2.13)** (2.7 g, 21.6 mmol) in 40 mL acetic acid was refluxed for 15 hours, cooled to room temperature, and diluted with 100 mL water. The brown aqueous solution was extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The brown residue was purified by column chromatography (1% MeOH in CH_2Cl_2) to afford 1.9 g of 1-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)ethanone (**2.9**) as a red-brown solid (90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, J = 7.3 Hz, 1H), 7.09 (dd, J = 7.8, 7.3 Hz, 1H), 6.96 (d, J = 6.9 Hz, 1H), 4.89 (s, 2H), 4.25-

4.22 (m, 2H), 4.15-4.13 (m, 2H), 2.93 (dd, J = 6.4, 6.4 Hz, 2H), 2.88 (dd, J = 6.4, 6.4 Hz, 2H), 2.24 (s, 3H), 1.81-1.69 (m, 4H), 1.54-1.48 (m, 2H), 1.44-1.41 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 188.0, 170.3, 138.5, 135.5, 129.4, 121.7, 118.9, 118.8, 117.2, 113.9, 54.1, 47.7, 45.5, 30.8, 28.7, 26.3, 26.1, 23.3, 22.2

1,2,3,4,8,9,10,11,12,13-decahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indole (2.10)

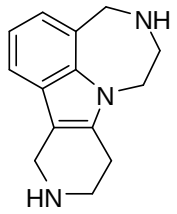


2.10

1-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)ethanone (**2.9**) (1.14 g, 3.8 mmol) was dissolved in 25 mL MeOH and 40 mL of a 6M sodium hydroxide solution. The reaction mixture is refluxed for 24 hours, cooled to room temperature, and concentrated to remove the MeOH. The remaining aqueous residue is extracted 3 times with CH_2Cl_2. The combined organic layers are dried over $MgSO_4$, filtered, and concentrated to give 802 mg of 1,2,3,4,8,9,10,11,12,13-decahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indole (**2.10)** as a red-orange solid (83% as two different conformations). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, J = 7.4 Hz, 1H), 6.94 (dd, J = 7.5, 7.5 Hz, 1H), 6.80 (d, J = 7.5 Hz, 1H), 4.22 (s, 1.4H), 4.14 (s, 0.6H), 3.98 (dd, J = 5.2, 5.1 Hz, 1.4H), 3.93 (dd, J = 5.2, 5.1 Hz, 0.6H), 3.31 (dd, J = 5.2, 5.1 Hz, 1.4H), 3.27 (dd, J = 5.2, 5.1 Hz, 0.6H), 2.81 (dd, J = 6.9, 6.3 Hz, 2H), 2.77 (dd, J = 6.3, 6.3 Hz, 2H), 1.66-1.58 (m, 6H), 1.40-1.37 (m, 2H), 1.32-1.27 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.1, 138.0, 136.5, 136.0, 129.2, 128.8, 126.7, 124.0, 120.2, 119.3,

118.8, 118.7, 116.0, 115.9, 113.9, 113.6, 75.6, 59.1, 54.7, 53.2, 50.8, 50.3, 49.9, 46.7, 30.8, 28.9, 28.8, 26.3, 26.0, 25.9, 23.4, 23.3, 23.2

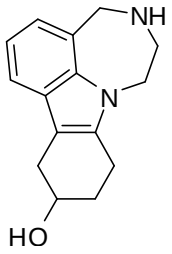
1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.14)



2.14

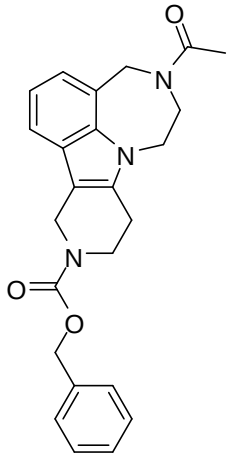
1-(1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (**2.22**) (65 mg, 0.24mmol) and NaOH (38 mg, 0.96 mmol) in 6 mL MeOH and 6 mL 6M NaOH was refluxed for 19 hours and then cooled to room temperature. The MeOH was removed under reduced pressure and the remaining residue was diluted with H_2O and extracted 3 times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated to afford 23.6 mg of 1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (**2.14**) as a yellow solid (43%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (d, J = 7.4 Hz, 1H), 7.00 (dd, J = 7.4, 7.4 Hz, 1H), 6.90 (d, J = 8.0 Hz, 1H), 4.25 (s, 2H), 4.04 (s, 2H), 3.92 (dd, J = 5.2, 5.2 Hz, 2H), 3.34 (dd, J = 5.2, 4.5 Hz, 2H), 3.24 (dd, J = 5.7, 5.7 Hz, 2H), 2.69 (dd, J = 6.0, 5.4 Hz, 2H), 1.86 (bs, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.3, 135.2, 127.6, 127.3, 119.7, 119.2, 116.0, 110.8, 55.2, 50.2, 50.0, 43.8, 42.5, 24.1; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{14}H_{17}N_3$: 228.1501, measured 228.1505.

1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-9-ol (2.15)



1-(9-hydroxy-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (**2.28**) (97 mg, 0.34 mmol) and NaOH (55 mg, 1.36 mmol) in 7 mL MeOH and 7 mL 6M NaOH was refluxed for 19 hours and then cooled to room temperature. The MeOH was removed under reduced pressure and the remaining residue was diluted with H_2O and extracted 3 times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated to afford 64.6 mg of 1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-9-ol (**2.15**) as a yellow solid (78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (d, J = 8.0 Hz, 1H), 6.99 (dd, J = 7.4, 7.4 Hz, 1H), 6.86 (d, J = 6.8 Hz, 1H), 4.20-4.15 (m, 3H), 3.85 (dd, J = 5.1, 5.1 Hz, 2H), 3.31 (dd, J = 9.7, 4.0 Hz, 2H), 3.02 (dd, J = 14.4, 6.3 Hz, 1H), 2.80 (ddd, J = 16.0, 5.2, 5.2 Hz, 1H), 2.70-2.64 (m, 2H), 2.30 (bs, 1H), 2.10-2.04 (m, 1H), 2.03-1.94 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.9, 135.5, 129.0, 126.9, 119.8, 119.1, 116.0, 108.0, 67.1, 55.0, 50.0, 31.2, 30.6, 20.2; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{15}H_{18}N_2O$: 243.1497, measured 243.1488.

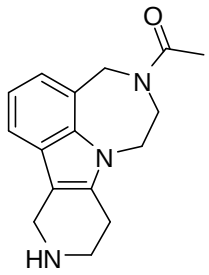
Benzyl 3-acetyl-1,2,3,4,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(8H)-carboxylate (2.21)



2.21

1-(1-amino-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (**2.8**) (5.58 g, 27.2 mmol) and benzyl 4-oxopiperidine-1-carboxylate (**2.20**) (19 g, 81.6 mmol) was dissolved in 85 mL acetic acid. The reaction is refluxed for 18 hours, cooled to room temperature, and concentrated to remove the acetic acid. The residue was neutralized with an aqueous solution of $NaHCO_3$ and extracted 3 times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (100% CH_2Cl to 4% MeOH in CH_2Cl) to afford 7 g of benzyl 3-acetyl-1,2,3,4,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(8H)-carboxylate (**2.21**) as a yellow solid (64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (bs, 6H), 7.10 (dd, J = 7.3, 7.3 Hz, 1H), 6.91 (d, J = 6.9 Hz, 1H), 5.19 (s, 2H), 4.82 (s, 2H), 4.71 (bs, 2H), 4.08 (bs, 4H), 3.91 (bs, 2H), 2.81 (bs, 2H), 2.16 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 170.4, 155.9, 136.8, 135.7, 135.5, 135.3, 128.6, 128.2, 128.1, 122.2, 119.8, 119.7, 119.6, 117.2, 117.1, 108.0, 67.4, 54.1, 53.7, 47.3, 45.4, 41.5, 23.2, 22.0

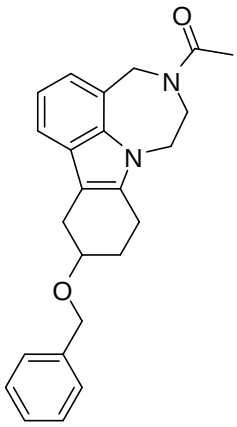
1-(1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.22)



2.22

10% Pd/C (216 mg) was added to a solution of benzyl 3-acetyl-1,2,3,4,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(8H)-carboxylate (**2.21**) (720 mg, 1.79 mmol) in 25 mL EtOH. The reaction mixture was stirred under an atmosphere of hydrogen gas for 24 hours. The reaction mixture was filter through a plug of celite and then concentrated. Column chromatography (100% CH_2Cl to 10% MeOH in CH_2Cl_2) gave 337 mg of 1-(1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (**2.22**) as an orange oil (70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (d, J = 8.0 Hz, 1H), 7.03 (dd, J = 7.4, 7.4 Hz, 1H), 6.94 (d, J = 7.4 Hz, 1H), 4.80 (s, 2H), 4.08 (d, J = 3.7 Hz, 2H), 4.07 (s, 4H), 3.25 (dd, J = 5.7, 5.7 Hz, 2H), 2.72 (dd, J = 5.8, 5.8 Hz, 2H), 2.15 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 135.4, 135.3, 127.7, 122.0, 119.4, 119.3, 117.1, 110.1, 54.3, 47.3, 45.4, 43.5, 42.1, 23.6, 22.1

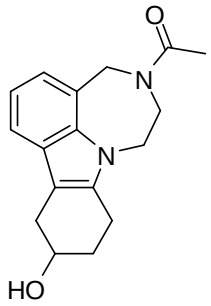
1-(9-(benzyloxy)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.27)



2.27

1-(1-amino-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (**2.8**) (5.48 g, 26.7 mmol) and 4-(benzyloxy)cyclohexanone (**2.26**) (16.4 g, 80.1 mmol) were dissolved in 175 mL acetic acid. The reaction mixture was refluxed for 16 hours and then cooled to room temperature. The cooled reaction mixture was concentrated to remove the acetic acid, and the residue was neutralized with an aqueous solution of $NaHCO_3$ and extracted 3 times with EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated to give a brown oil. The brown oil was purified by column chromatography (100% CH_2Cl to 1% MeOH in CH_2Cl_2) to yield 5.7 g of 1-(9-(benzyloxy)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (**2.27**) as a brown oil (60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.21 (m, 6H), 7.01 (dd, J = 8.0, 8.0 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H), 4.77 (s, 2H), 4.65 (s, 2H), 4.08 (dd, J = 12.8, 12.0 Hz, 2H), 4.00-3.89 (m, 2H), 3.10 (dd, J = 15.0, 4.5 Hz, 1H), 2.88-2.63 (m, 3H), 2.23-1.93 (m, 3H), 2.11 (s, 3H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 170.0, 138.8, 136.1, 129.2, 128.6, 128.4, 127.7, 127.6, 121.8, 120.6, 119.5, 119.2, 119.1, 117.2, 108.5, 74.2, 70.6, 54.5, 47.6, 45.7, 28.6, 27.6, 22.1, 20.9; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{24}H_{26}N_2O_2$: 397.1892, measured 397.1874.

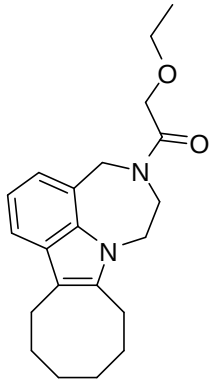
1-(9-hydroxy-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.28)



2.28

1-(9-(benzyloxy)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (**2.27**) (538 mg, 1.43 mmol) was dissolved in 20 mL EtOH. To the reaction mixture was added 12ml of a slurry of Raney Ni 2800 in water. The suspension was stirred under an atmosphere of hydrogen for 8 days. After 8 days the reaction mixture was filtered through a plug of celite. The filtrate was concentrated and purified by column chromatography (100% CH_2Cl to 5% MeOH in CH_2Cl_2) to afford 295 mg of 1-(9-hydroxy-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (**2.28**) as pale yellow solid (73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, J = 8.1 Hz, 1H), 7.02 (dd, J = 7.5, 7.5 Hz, 1H), 6.92 (d, J = 7.0 Hz, 1H), 4.80 (s, 2H), 4.21-4.29 (m, 1H), 4.18-4.00 (m, 3H), 3.68 (dd, J = 15.5, 4.5 Hz, 1H), 2.90-2.67 (m, 3H), 2.15 (s, 3H), 2.14-2.02 (m, 1H), 1.68 (s, 2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 170.0, 135.8, 129.2, 121.8, 119.4, 119.2, 117.2, 116.8, 108.1, 67.4, 54.6, 47.6, 46.3, 31.3, 30.7, 22.2, 20.4; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{17}H_{20}N_2O_2$: 307.1422, measured 307.1398.

2-ethoxy-1-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)ethanone (2.30)

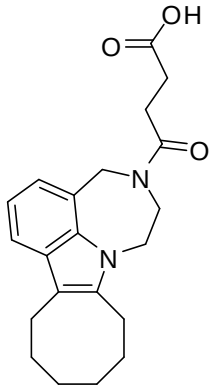


2.30

A solution of 1,2,3,4,8,9,10,11,12,13-decahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indole (**2.10**) (95.7 mg, 0.38 mmol), EDAC (87.4 mg, 0.46 mmol), ethoxyacetic acid (**2.29**) (47.9 mg, 0.46 mmol), and N,N-DIEA (98.2 mg, 0.76 mmol) was dissolved in 7 mL CH_3CN and stirred at room temperature for 24 hours. After 24 hours, the solvent was removed under reduced pressure and the residue was taken up in water and extracted 3 times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, concentrated, and purified by column chromatography (100% CH_2Cl to 5% MeOH in CH_2Cl_2) to produce 70 mg of 2-ethoxy-1-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)ethanone (**2.30**) as an orange oil (77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (d, J = 6.9 Hz, 1H), 7.02 (dd, J = 7.7, 7.2 Hz, 1H), 6.90 (d, J = 6.6 Hz, 1H), 4.89 (s, 2H), 4.22 (s, 2H), 4.19-4.17 (m, 2H), 4.09-4.07 (m, 2H), 3.52 (q, J = 6.9 Hz, 2H), 2.86 (dd, J = 6.3, 6.3 Hz, 2H), 2.82 (dd, J = 6.3, 5.7 Hz, 2H), 1.72-1.65 (m, 4H), 1.48-1.43 (m, 2H), 1.36-1.32 (m, 2H), 1.18 (t, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.0, 138.3, 135.3, 129.2, 121.5, 119.1,

118.9, 117.1, 113.9, 70.3, 67.2, 52.2, 48.2, 45.3, 30.8, 28.8, 26.3, 25.9, 23.3, 23.2, 15.1; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{21}H_{28}N_2O_2$: 363.2048, measured 363.2039.

4-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)-4-oxobutanoic acid (2.32)

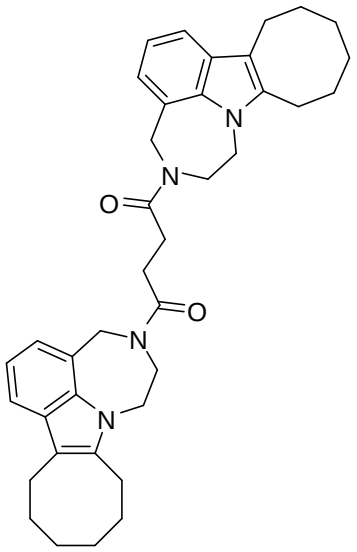


2.32

A solution of 1,2,3,4,8,9,10,11,12,13-decahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indole (**2.10**) (106.4 mg, 0.42 mmol) and succinic anhydride (**2.31**) (84 mg, 0.84 mmol) in 10 mL CH_2Cl_2 was stirred at room temperature for 24 hours. After 24 hours the reaction was diluted with H_2O and extracted 3 times with CH_2Cl_2. The organic layers were dried over $MgSO_4$, concentrated, and purified by column chromatography (100% CH_2Cl to 9% MeOH in CH_2Cl_2) to yield 125 mg of 4-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)-4-oxobutanoic acid (**2.32**) as a yellow solid (84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 7.3 Hz, 1H), 7.01 (dd, J = 7.3, 7.3 Hz, 1H), 6.90 (d, J = 6.9 Hz, 1H), 4.82 (s, 2H), 4.13 (dd, J = 4.6, 4.6 Hz, 2H), 4.04 (dd, J = 4.6, 4.6 Hz, 2H), 2.85 (dd, J = 6.4, 6.0 Hz, 2H), 2.82 (dd, J = 6.2, 5.7 Hz, 2H), 2.75 (dd, J = 6.9, 6.4 Hz, 2H), 1.62-1.72 (m, 4H), 1.48-1.41 (m, 2H), 1.39-1.31 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 177.3, 171.3, 138.3, 135.3, 129.3, 121.1, 119.0,

118.9, 117.2, 113.8, 52.8, 48.1, 45.1, 30.7, 29.3, 28.7, 28.6, 26.2, 25.8, 23.2, 23.1; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{21}H_{26}N_2O_3$: 377.1841, measured 377.1823.

1,4-bis(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)butane-1,4-dione (2.33)

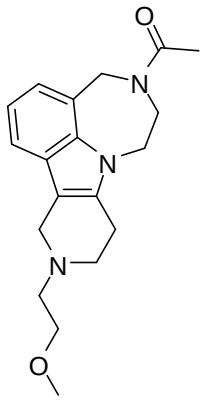


2.33

A solution of 4-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)-4-oxobutanoic acid (**2.32**) (111.2 mg, 0.31 mmol), EDAC (72 mg, 0.38 mmol), 1,2,3,4,8,9,10,11,12,13-decahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indole (**2.10**) (96 mg, 0.38 mmol), and N,N-DIEA (81 mg, 0.63 mmol) in 12 mL CH_3CN was stirred at room temperature for 43 hours. After 43 hours, the solvent was removed under reduced pressure, and the residue was taken up in H_2O and extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (100% CH_2Cl_2 to 9% MeOH in CH_2Cl_2) to yield 104 mg 1,4-bis(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)butane-1,4-dione (**2.33**) as a yellow-orange solid (57%). ^{1}H NMR (400

MHz, $CDCl_3$) δ 7.40 (d, J = 7.8 Hz, 2H), 6.97 (dd, J = 7.3, 7.3 Hz, 2H), 6.87 (d, J = 6.8 Hz, 2H), 4.84 (s, 3H), 4.13 (dd, J = 4.6, 1.8 Hz, 4H), 4.04 (dd, J = 6.4, 1.4 Hz, 4H), 2.85-2.77 (m, 10H), 1.72-1.61 (m, 10H), 1.47-1.40 (m, 4H), 1.38-1.30 (m, 4H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.5, 138.2, 135.4, 129.2, 121.7, 119.1, 118.8, 117.0, 113.7, 52.7, 47.9, 45.3, 30.7, 28.7, 28.6, 26.2, 25.8, 23.2, 23.1; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{38}H_{46}N_4O_2$: 613.3518, measured 613.3502.

1-(9-(2-methoxyethyl)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.36)

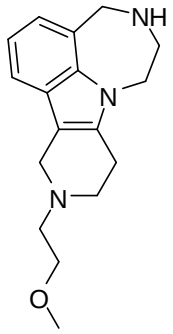


2.36

A solution of 1-(1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (**2.22**) (540 mg, 2.0 mmol), 2-bromoethyl methyl ether (**2.34**) (335 mg, 2.4 mmol), and N,N'-DIEA (624 mg, 4.8 mmol) in 80 mL CH_3CN was stirred at 60 ^oC for 40 hours. After 40 hours the reaction was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was taken up in aqueous NH_4Cl and extracted three times with EtOAc. The combined organic extracts were dried over $MgSO_4$, concentrated, and purified by column chromatography (100% CH_2Cl_2 to 9% MeOH in CH_2Cl_2) to afford 191 mg of 1-(9-(2-methoxyethyl)-

1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (**2.36**) as a red-orange oil (31%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (d, J = 7.5 Hz, 1H), 7.03 (dd, J = 7.5, 7.5 Hz, 1H), 6.93 (d, J = 6.3 Hz, 1H), 4.81 (s, 2H), 4.07 (d, J = 1.7 Hz, 2H), 3.77 (s, 2H), 3.64 (dd, J = 5.7, 5.2 Hz, 2H), 3.39 (s, 3H), 2.98 (dd, J = 5.7, 5.7 Hz, 2H), 2.87 (dd, J = 5.7, 5.7 Hz, 2H), 2.81 (dd, J = 5.1, 5.1 Hz, 2H), 2.16 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 135.8, 135.3, 127.9, 122.0, 119.3, 119.2, 117.1, 109.4, 71.0, 59.1, 57.0, 54.5, 51.2, 49.9, 47.5, 45.7, 23.2, 22.1; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{19}H_{25}N_3O_2$: 328.2025, measured 328.2013.

9-(2-methoxyethyl)-1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.37)

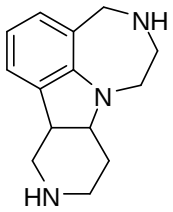


2.37

A solution of 12 mL MeOH, 10 mL 6M NaOH, and 1-(9-(2-methoxyethyl)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (**2.36**) (191 mg, 0.58 mmol) was refluxed for 24 hours. After which the reaction was cooled to room temperature and the MeOH was removed under reduced pressure. The remaining residue was diluted with H_2O and extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated to afford 113 mg of 9-(2-methoxyethyl)-1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (**2.37**) as a red-

brown oil (68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, J = 8.0 Hz, 1H), 6.99 (dd, J = 7.5, 7.5 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 4,25 (s, 2H), 3.92 (dd, J = 5.2, 5.1 Hz, 2H), 3.77 (s, 2H), 3.64 (dd, J = 5.7, 5.7 Hz, 2H), 3.39 (s, 3H), 3.33 (dd, J = 4.6, 5.1 Hz, 2H), 2.97 (dd, J = 5.7, 5.7 Hz, 2H), 2.86 (t, J = 5.7 Hz, 2H), 2.81 (t, J = 5.7 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.8, 134.9, 127.7 127.1, 119.6, 119.1, 115.9, 109.5, 71.0, 59.1, 57.1, 55.1, 51.3, 50.2, 50.1, 50.0, 23.2

1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.53)

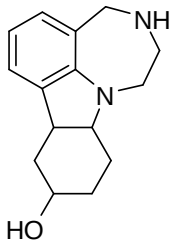


2.53

A solution of 1-(1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (**2.57**) (83 mg, 0.31 mmol) in 6 mL MeOH and 6 mL 6M NaOH is refluxed for 48 hours and then cooled to room temperature. The MeOH was removed under reduced pressure and the remaining residue was diluted with H_2O and extracted three times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated to give 53 mg of 1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (**2.53**) as a yellow solid (74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.95 (d, J = 6.9 Hz, 1H), 6.86 (d, J = 6.9 Hz, 1H), 6.69 (dd, J = 7.7, 7.7 Hz, 1H), 4.02 (d, J = 16.1 Hz, 1H), 3.83 (dd, J = 4.6, 4.0 Hz, 1H), 3.35-3.32 (m, 1H), 3.30 (s, 1H), 3.28 (s, 1H), 3.15 (ddd, J = 10.3, 6.3, 4.0 Hz, 1H), 3.03-2.97 (m, 2H), 2.87 (dd, J = 3.5, 2.8 Hz, 1H), 2.85 (d, J = 3.5 Hz, 1H), 2.60 (dd, J = 10.9, 10.3 Hz, 1H), 2.45 (dd, J

= 12.6, 10.3 Hz, 1H), 1.91 (dq, J = 14.4, 2.9 Hz, 1H), 1.82-1.77 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.3, 133.4, 127.4, 127.2, 121.9, 119.4, 64.3, 54.3, 54.0, 50.5, 49.6, 41.8, 41.2, 26.0; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{14}H_{19}N_3$: 230.1657, measured 230.1650.

1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-jk]carbazol-9-ol (2.54)

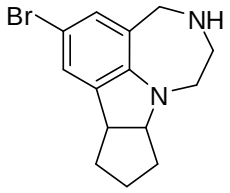


2.54

A solution 1-(9-hydroxy-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (**2.60**) (68 mg, 0.24 mmol) in 5 mL MeOH and 5 mL 6M NaOH was refluxed for 24 hours and then cooled to room temperature. The MeOH was removed under reduced pressure and the remaining residue was diluted with H_2O and extracted three times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated to give 36 mg of 1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-jk]carbazol-9-ol (**2.54**) as an orange solid (62%). ^{1}H NMR (500 MHz, $CDCl_3$) mixture of diastereomers δ 6.97 (t, J = 7.4 Hz, 1H), 6.84 (dd, J = 9.7, 8.0 Hz, 1H), 6.69 (dd, J = 7.4, 7.4 Hz, 1H), 4.24-4.20 (m, 1H), 3.98-3.92 (m, 2H), 3.85 (d, J = 15.5 Hz, 0.5H), 3.78 (d, J = 15.5 Hz, 0.5H), 3.62 (tt, J = 10.8, 3.4 Hz, 1H), 3.39-3.21 (m, 4H), 3.14-2.95 (m, 2H), 2.76-2.67 (m, 1H), 2.16-1.93 (m, 5H), 1.85-1.74 (m, 1H), 1.70-1.60 (m, 1H), 1.58-1.42 (m, 1H), 1.11 (q, J = 12.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.8, 151.7, 137.0, 135.8, 135.6, 135.5, 129.0, 127.2, 127.0, 126.7, 121.6,

121.5, 119.8, 119.7, 119.3, 119.2, 116.1, 108.3, 68.8, 67.2, 65.6, 65.6, 64.9, 55.0, 54.6, 54.0, 53.9, 53.8, 50.5, 50.2, 50.1, 41.1, 40.2, 37.9, 36.0, 31.3, 30.7, 30.2, 28.6, 24.1, 20.4, 20.2; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{15}H_{20}N_2O$: 245.1654, measured 245.1649.

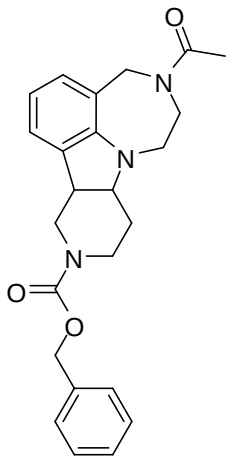
6-bromo-2,3,4,7b,8,9,10,10a-octahydro-1H-cyclopenta[b][1,4]diazepino[6,7,1-hi]indole (2.55)



2.55

2,3,4,7b,8,9,10,10a-octahydro-1H-cyclopenta[b][1,4]diazepino[6,7,1-hi]indole (**2.52**) (138 mg, 0.64 mmol) was dissolved in 3 mL CH_2Cl_2 and cooled to 0 ^oC. Then add Br_2 in 1 mL CH_2Cl_2 dropwise. Stir at 0 ^oC for three hours and then stirre at room temperature for 18 hours. After 18 hours the reaction mixture was concentrated under reduced pressure to yield 123 mg of 6-bromo-2,3,4,7b,8,9,10,10a-octahydro-1H-cyclopenta[b][1,4] diazepino[6,7,1-hi]indole (**2.55**) as a brown oil (60%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.14 (s, 1H), 7.04 (s, 1H), 4.34 (d, J = 14.3 Hz, 1H), 4.01 (dd, J = 8.0, 5.1 Hz, 1H), 3.88 (d, J = 15.5 Hz, 1H), 3.81 (dd, J = 8.6, 7.5 Hz, 1H), 3.70 (d, J = 13.2 Hz, 1H), 3.39-3.29 (m, 2H), 3.12 (dd, J = 10.9, 10.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.6, 138.9, 130.7, 128.1, 115.9, 111.6, 73.6, 50.3, 49.4, 48.8, 46.2, 35.1, 34.5, 24.4; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{14}H_{17}BrN_2$: 293.0653, measured 293.0642.

Benzyl 3-acetyl-1,2,3,4,7b,8,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(10H)-carboxylate (2.56)

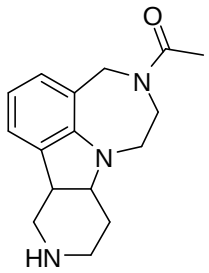


2.56

Benzyl 3-acetyl-1,2,3,4,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(8H)-carboxylate (**2.21**) (4.1 g, 10.2 mmol) was dissolved in 60 mL TFA and cooled to 0 ^{o}C. To the cooled solution $NaBH_4$ (887 mg, 23.5 mmol) was added portionwise over 15 minutes with stirring. The reaction was allowed to warm to room temperature and then stirred an additional 90 minutes. After 90 minutes the reaction was cooled back to 0 ^{o}C and 60 mL H_2O was added. The stirred reaction was then neutralized with dropwise addition of 6M NaOH. The neutralized reaction was extracted three times with CH_2Cl_2, and the combined organic layers are dried over $MgSO_4$ and concentrated to yield 3.8 g of benzyl 3-acetyl-1,2,3,4,7b,8,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(10H)-carboxylate (**2.56**) as a yellow solid (92% as a mixture of diastereomers). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.28 (m, 10H), 7.06 (d, J = 7.4 Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 6.73 (bs, 1H), 5.11 (s, 2H), 5.13-5.08 (m, 2H), 4.58 (d, J = 16.6 Hz, 1H), 4.43 (bs, 1H), 4.27 (d, J = 15.9 Hz, 1H), 4.08 (bs, 1H), 3.96-3.82 (m, 2H), 3.68-3.63 (m, 1H), 3.51 (bs, 1H), 3.40-3.33 (m, 1H), 3.29 (d, J = 11.4 Hz, 0.5H), 3.21 (d,

J = 12.6 Hz, 0.5H), 3.13 (td, J = 3.5, 12.6 Hz, 1H), 3.02-2.95 (m, 1H), 2.86 (dd, J = 10.9 Hz, 1H), 2.21 (s, 2H), 2.09 (2, 1H), 2.0-1.89 (m, 5H), 1.48 (bs, 1H); ^{13}C NMR (500MHz, $CDCl_3$) δ 170.0, 155.3, 137.0, 136.9, 128.7, 128.6, 128.5, 128.1, 128.0, 128.0, 127.9, 127.4, 122.0, 67.1, 67.0, 66.9, 64.0, 63.9, 53.7, 53.4, 50.9, 50.6, 47.5, 44.6, 41.5, 39.9, 39.8, 22.2, 21.9; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{24}H_{27}N_3O_3$: 428.1950, measured 428.1953.

1-(1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.57)

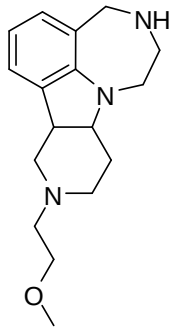


2.57

10% Pd/C (2.2 g) was added to a stirred solution of benzyl 3-acetyl-1,2,3,4,7b,8,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(10H)-carboxylate (**2.56**) (3.72 g, 9.2 mmol) in 110 mL EtOH. The reaction mixture was stirred under an atmosphere of hydrogen for 48 hours and then filtered through a plug of celite. The filtrate was concentrated and then purified by column chromatography using KP-NH silica gel (100% CH_2Cl_2 to 4% MeOH in CH_2Cl_2) to yield 1.8 g 1-(1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (**2.57**) as a yellow solid (72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (d, J = 6.9 Hz, 1H), 6.95 (d, J = 7.5 Hz, 1H), 6.78 (dd, J = 7.5, 7.5 Hz, 1H), 5.5 (s, 1H), 4.64 (d, J = 16.1 Hz, 1H), 4.39-4.24 (m, 2H),

3.45-3.37 (m, 1H), 3.34-3.21 (m, 3H), 3.05 (ddd, J = 12.6, 2.8, 2.8 Hz, 1H), 2.93-2.71 (m, 2H), 2.49 (dd, J = 12.6, 11.5 Hz, 1H), 2.29-2.06 (m, 2H), 2.20 (s, 3H), 1.95-1.82 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 151.3, 132.0, 127.7, 123.3, 122.7, 120.4, 63.0, 53.1, 50.0, 47.1, 46.5, 40.0, 38.4, 23.1, 21.9, 22.0; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{16}H_{21}N_3O$: 272.1763, measured 272.1741.

9-(2-methoxyethyl)-1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.58)

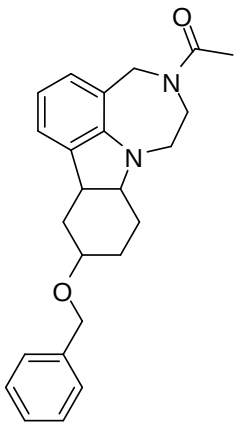


2.58

A solution of 1-(9-(2-methoxyethyl)-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (**2.62**) (59 mg, 0.18 mmol) in 3 mL MeOH and 3 mL 6M NaOH was refluxed for 24 hours and then cooled to room temperature. The MeOH was removed under reduced pressure and the residue was diluted with H_2O and extracted three times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated to give 42 mg of 9-(2-methoxyethyl)-1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (**2.58**) as an orange oil (81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 (d, J = 7.4H, 1H), 6.86 (d, J = 7.4 Hz, 1H), 6.69 (dd, J = 7.4, 6.9 Hz, 1H), 4.02 (d, J = 15.5 Hz, 1H), 3.78 (d, J = 16.0 Hz, 1H), 3.51-3.46 (m,2H), 3.34 (s, 3H), 3.33-3.23 (m, 4H), 2.99-2.95 (m, 1H), 2.85-2.82

(m, 1H), 2.73 (bs, 1H), 2.58-2.48 (m, 3H), 2.27-2.22 (m, 1H), 2.01 (bs, 2H), 1.98-1.92 (m, 3H), 1.77 (dd, J = 11.4, 10.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.5, 133.7, 127.6, 127.3, 122.1, 119.6, 70.3, 64.0, 59.1, 58.3, 57.9, 54.7, 54.2, 50.6, 49.6, 41.1, 25.5

1-(9-(benzyloxy)-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.59)

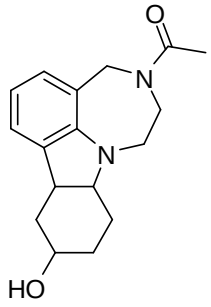


2.59

1-(9-(benzyloxy)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (**2.27**) (616 mg, 1.64 mmol) was dissolved in 20 mL TFA and cooled to 0 ^{o}C. To the cooled reaction mixture was added $NaBH_4$ portionwise over 10 minutes. The reaction was then warmed to room temperature and stirred an additional 18 hours. After 18 hours the reaction was cooled back to 0 ^{o}C and then neutralized with dropwise addition of 6M NaOH. The neutralized reaction was extracted three times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, concentrated, and purified by column chromatography (100% CH_2Cl to 8% MeOH in CH_2Cl_2) to yield 430 mg of 1-(9-(benzyloxy)-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (**2.59**) as a red-orange oil (70% as a mixture of diastereomers). ^{1}H NMR (500

MHz, $CDCl_3$) δ 7.38-7.22 (m, 5H), 7.01 (dd, J = 14.9, 6.9 Hz, 1H), 6.87 (dd, J = 9.7, 7.4 Hz, 1H), 6.73 (dd, J = 9.7, 7.4 Hz, 1H), 4.60-4.49 (m, 3H), 4.32-4.22 (m, 1H), 3.65 (bs, 1H), 3.48-3.38 (m, 1H), 3.34-3.22 (m, 2H), 3.11-3.05 (m, 1H), 2.83-2.65 (m, 1H), 2.13 (d, J = 5.7 Hz, 3H), 2.08 (d, J = 1.2 Hz, 1H), 2.03-1.88 (m, 2H), 1.76-1.68 (m, 1H), 1.59 (ddd, J = 9.1, 2.3, 2.3 Hz, 1H), 1.17 (dd, J = 12.6, 11.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 170.1, 151.3, 151.2, 139.1, 136.5, 136.4, 128.5, 128.4, 127.5, 127.5, 126.7, 126.5, 122.6, 122.2, 121.7, 119.8, 119.5, 75.4, 72.4, 70.1, 69.7, 65.6, 65.2, 53.6, 53.1, 50.5, 49.7, 47.4, 40.8, 37.6, 36.7, 33.5, 26.9, 24.8, 24.0, 22.2, 22.0, 21.9, 20.3; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{24}H_{28}N_2O_2$: 399.2048, measured 399.2034.

1-(9-hydroxy-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.60)

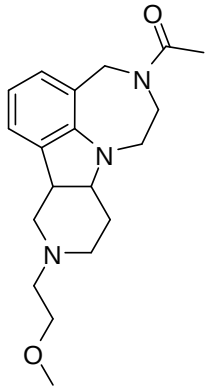


2.60

To a stirred solution of 1-(9-(benzyloxy)-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (**2.59**) (430 mg, 1.14 mmol) in 20 mL EtOH was added 4.0 mL of Raney Ni 2800. This suspension was stirred under an atmosphere of hydrogen for 6 days. After 6 days the reaction mixture was filtered through a plug of celite. The filtrate was concentrated and purified by column chromatography (100% CH_2Cl to 8% MeOH in CH_2Cl_2) to afford 180 mg of 1-(9-

hydroxy-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (**2.60**) as an orange solid (55% as a mixture of diastereomers). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02-6.90 (m, 1H), 6.86 (dd, J = 13.1, 6.9 Hz, 1H), 6.70 (ddd, J = 13.7, 7.5, 7.5 Hz, 1H), 4.59 (d, J = 15.5 Hz, 1H), 4.50 (d, J = 16.6 Hz, 1H), 4.36 (d, J = 16.6 Hz, 1H), 4.25 (d, J = 16.1 Hz, 1H), 4.22-4.17 (m, 1H), 4.12-3.97 (m, 1H), 3.92-3.88 (m, 1H), 3.69-3.57 (m, 1H), 3.38-3.20 (m, 2H), 3.10-3.01 (m, 1H), 2.91-2.86 (m, 1H), 2.72-2.64 (m, 1H), 2.54 (bs, 1H), 2.12 (dd, J = 11.5, 9.1 Hz, 4H), 2.02-1.94 (m, 1H), 1.81-1.73 (m, 1H), 1.67-1.57 (m, 1H), 1.53-1.40 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.4, 170.3, 151.2, 151.1, 136.4, 136.1, 136.0, 135.9, 129.3, 127.8, 127.6, 126.7, 126.4, 122.6, 122.5, 122.1, 122.0, 121.9, 121.7, 121.4, 120.3, 119.9, 119.8, 119.4, 119.3, 119.1, 117.2, 108.4, 68.7, 68.6, 67.1, 65.6, 65.5, 64.9, 54.4, 53.6, 53.3, 52.9, 50.9, 50.8, 50.8, 50.2, 50.1, 49.3, 48.0, 47.5, 47.4, 47.3, 45.5,41.0, 40.9, 39.9, 37.8, 35.7, 35.6, 31.2, 30.4, 30.0, 28.6, 28.5, 24.1, 24.0, 22.1, 22.0, 21.9, 20.5, 20.4; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{17}H_{22}N_2O_2$: 309.1579, measured 309.1554.

1-(9-(2-methoxyethyl)-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.62)



2.62

1-(1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (**2.57**) (104 mg, 0.38 mmol) 2-bromoethyl methyl ether (**2.61**) (107 mg, 0.77 mmol), and N,N-DIEA (200 mg, 1.54 mmol) in 12 mL CH_3CN was stirred at 66 ^oC for 24 hours and then cooled to room temperature. The solvent was removed under reduced pressure and the residue was taken up in aqueous $NaHCO_3$ and extracted three times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, concentrated, and purified by column chromatography (100% CH_2Cl to 3% MeOH in CH_2Cl_2) to afford 59 mg of 1-(9-(2-methoxyethyl)-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (**2.62**) as a yellow oil (40%). ^1H NMR (500 MHz, $CDCl_3$) δ 6.96 (d, J = 7.4H, 1H), 6.84 (d, J = 7.5 Hz, 1H), 6.67 (dd, J = 7.2, 7.2 Hz, 1H), 4.55 (d, J = 16.1 Hz, 1H), 4.32 (d, J = 13.8 Hz, 1H), 4.23 (d, J = 16.0 Hz, 1H), 3.44 (t, J = 5.7 Hz, 2H), 3.28 (s, 3H), 3.26-3.17 (m, 3H), 2.77 (ddd, J = 11.5, 6.3, 1.8 Hz, 1H), 2.71-2.65 (m, 2H), 2.50-2.41 (m, 2H), 2.22-2.15 (m, 1H), 2.04 (s, 3H), 1.97-1.85 (m, 2H), 1.72 (t, J = 11.5 Hz, 1), 1.18 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 151.8, 134.2, 127.0, 123.0, 122.1, 119.7, 70.2, 63.9, 59.0, 58.1, 57.5, 53.3, 50.1, 49.4, 47.3, 40.7, 25.3, 22.0; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{19}H_{27}N_3O_2$: 330.2182, measured 330.2173.

Chapter 3: M-100907 Derivatives

In order to synthesize serotonin receptor DMLs an appropriate 5-HT_{2A} receptor antagonist must be selected, synthesized, and derivatized. M-100907 (**3.1**) also known as volinanserin is a highly selective 5-HT_{2A} receptor antagonist originally developed by Sanofi-Aventis for the treatment of schizophrenia and has undergone clinical trails for sleep disorders.[105, 106] M-100907 (**3.1**) was the 5-HT_{2A}R antagonist of choice because it is selective for its target receptor, it is accessible through a known synthetic route, and its structure lends itself well to the desired derivatizations.

5-HT_{2A}R affinity of M-100907

Since the known 5-HT_{2A} antagonist M-100907 (**3.1**) was to be used to synthesize novel DMLs, high affinity to the 5-HT_{2A} receptor as well as potency as an antagonist was important. M-100907 (**3.1**) is greater than 100 times more selective for the 5-HT_{2A}R (K_i = 0.85nM) than the 5-HT_{2C}R (K_i = 88nM).[107] In addition to being selective for the 5-HT_{2A}R, both *in vitro* and *in vivo* experiments demonstrated that M-100907 (**3.1**) is a highly potent 5-HT_{2A}R antagonist.[105, 107] Clinical trials revealed that a 6 mg dose of M-100907 (**3.1**) resulted in 90% 5-HT_{2A}R occupancy in the prefrontal cortex.[105] Additionally, the only reported side effects of M-100907 (**3.1**) during a phase III clinical trial in Europe were headaches and constipation.[105] The ability of M-100907 (**3.1**) to bind selectivity to the 5-HT_{2A}R both *in vitro* and *in vivo* in addition to its low level of side effects in human patients supported its use in 5-HT receptor DMLs.

M-100907 SAR Data

In addition to a favorable pharmacological profile, the structure of M-100907 (**3.1**) was well suited to the manipulations necessary to synthesize DMLs including the

addition of a tether. The two potential linker locations were at the 3'-methoxy on one aromatic ring and the 4'-fluorine on the other aromatic ring (**Fig 3.1**). Limited data existed on the SAR of M-100907 (**3.1**) at either linker location. The 3'-methoxy on the aromatic ring was not critical for activity.[105] The removal of that group occurs during first pass metabolism to yield M-105725 (**3.2**) a known 5-HT_{2A}R antagonist with a Ki of 1.3 nM but limited blood brain barrier permeability (**Fig 3.1**).[108] The increase in polarity from the additional hydroxy group decreases the lipophilicity of M-105725 (**3.2**).[108] The difference in lipophilicity partially explained why the blood brain barrier permeability of M-100907 (**3.1**) is 4 times that of M-105725 (**3.2**).[108] Limited data existed for M-100907 derivatives in which the 4'-fluorine was modified. [105, 109] Since only a small number of

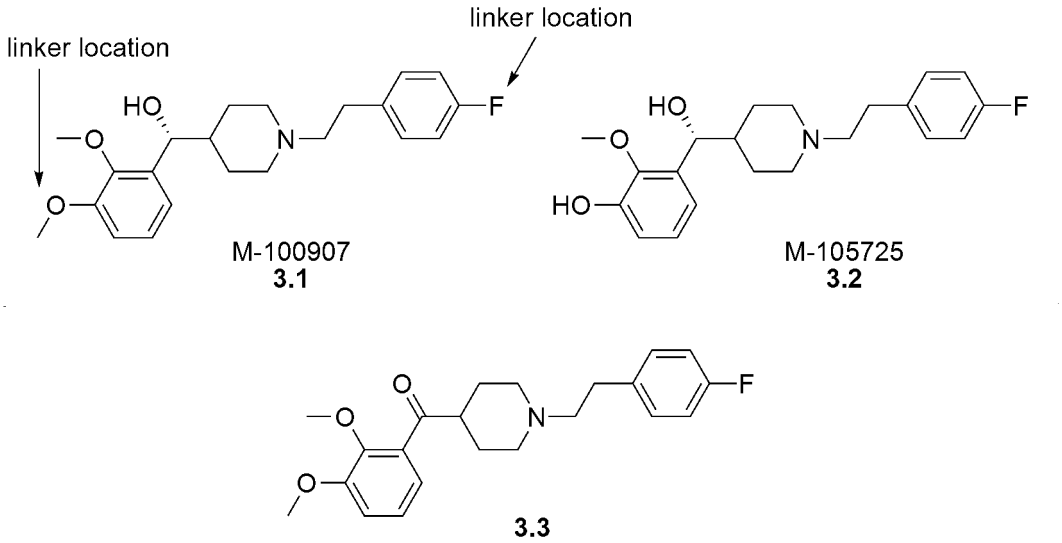


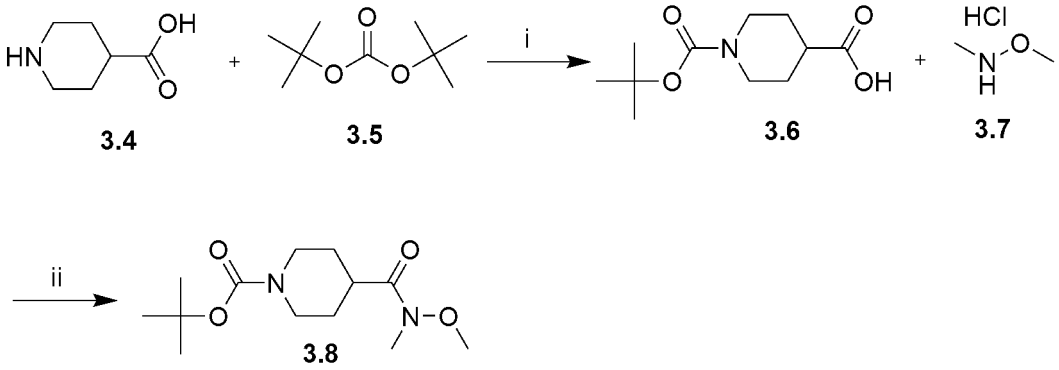
Figure 3. 1 Structure of M-100907, M-105725, and 3.3

of fluorine derivatives with limited variation had been examined, further modifications at this position were investigated to determine if this was an appropriate linker attachment site. SAR data on a series of compound **3.3** derivatives with an unreduced piperidnyl carbonyl revealed that the piperidnyl alcohol was not critical for 5-HT_{2A}R activity.[109] The 5-HT_{2A}R activity of compound **3.3** was only 2 fold less than that of M-100907 (**3.1**), but

it retained the more than 100 fold selectivity for the 5-HT_{2A}R over the 5-HT_{2C}R.[109] Since compound **3.3** retained selectivity for the 5-HT_{2A}R and was only slightly less active than M-100907, it was chosen as an additional compound on which linkers would be attached. The selectivity for a specific 5-HT receptor and amenability to functional group modification made M-100907 a good candidate for use in DMLs.

Published M-100907 synthesis

In order to synthesize M-100907 derivatives containing the appropriate linker attachment points, the parent compound M-100907 (**3.1**) was first synthesized. The route to M-100907 published by Rice was utilized because it is a linear straightforward route that was designed in such a way as to allow the modifications required to synthesize the M-100907 derivatives we desired. The synthesis of M-100907 (**3.1**) began by synthesizing the Weinreb amide of isonipecotic acid (**3.4**) (**Scheme 3.1**)



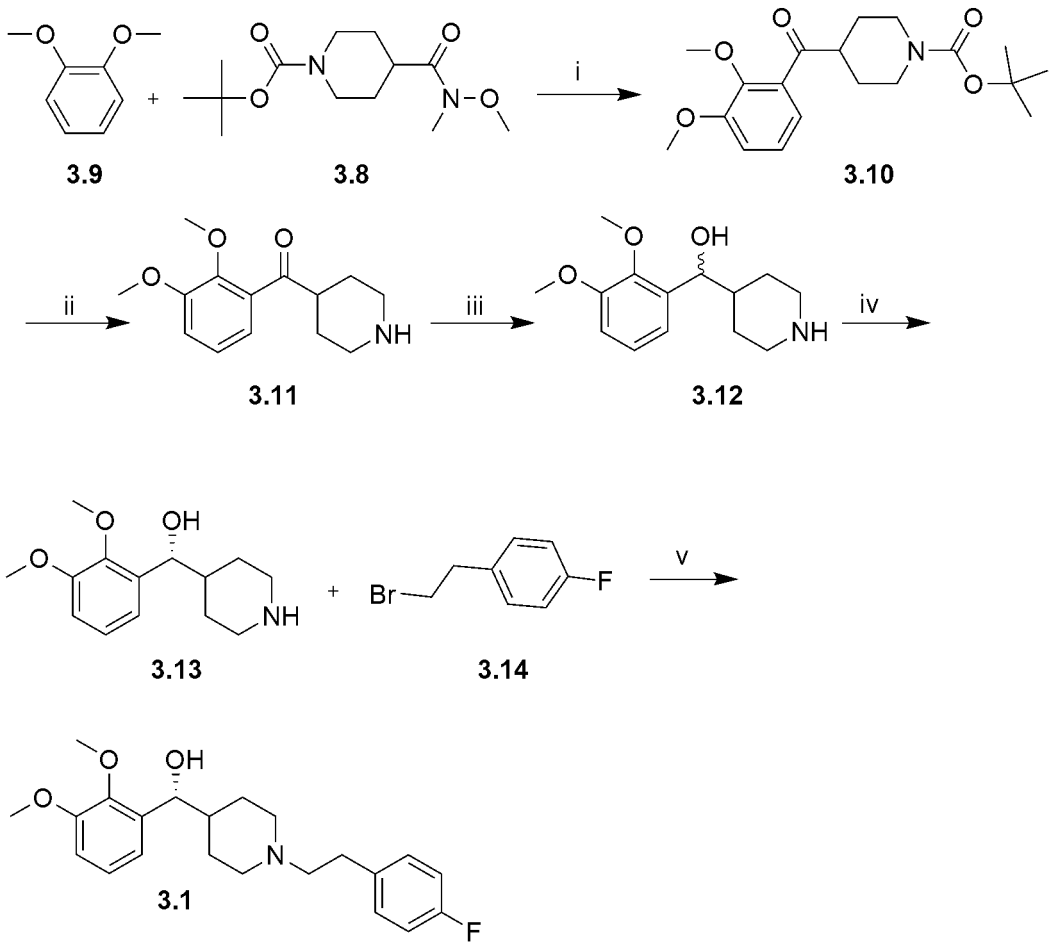
Reagents and conditions: (i) 1,4-dioxane, CH_3CN, 1 M NaOH, rt, 18 h, 86%; (ii) a. EDCI, HOBT, DIEA, **3.7**, rt, 18 h, 80%

Scheme 3.1 Synthesis of Weinreb's amide 3.8

Boc-protected isonipecotic acid (**3.6)** was synthesized from isonipecotic acid and di-tertbutyldicarbonate in 1, 4-dioxaone, acetonitrile, and 1 M aqueous NaOH to give Boc-protected isonipecotic acid (**3.6**) smoothly in 86% yield without column

chromatography.[111] Boc-protected isonipecotic acid (**3.6**) was reacted with N,O-dimethylhydroxylamine hydrochloride, and the amino acid coupling reagents EDCI and HOBT with N,N-DIEA to give the desired amide **3.8** in an 80% yield.[111] The advantages of using the amino acid coupling reagents EDCI and HOBT was that they are water soluble and can be removed during an aqueous reaction work-up. Removal of the coupling reagents during an aqueous work-up circumvented the need for purification by column chromatography, which allowed this reaction to be done on a large scale.

With amide **3.8** in hand, the next step in the Rice synthesis of M-100907 (**3.1**) was to react **3.8** with veratrole (**3.9)**. Veratrole (**3.9**) was metalated with n-butyl lithium over two hours at room temperature then **3.8** was added and the reaction was stirred at room temperature to give the desired benzylpiperidine **3.10** in an 84% yield (**Scheme 3.2**).[111] The Boc protecting group of the benzylpiperidine **3.10** was removed by treatment with TFA at room temperature over 30 minutes to give the free piperidine **3.11** (**Scheme 3.2**).[111] The yield of compound **3.11** was not determined because the crude reaction mixture was reduction to a secondary alcohol to yield **3.12** in a 71% yield over two steps from compound **3.10** (**Scheme 3.2**).[111] Compound **3.12** was optically resolved through the formation of diastereomeric salts from (+)- and (-)-di-O,O'-p-toluyltartaric acid (**Scheme 3.2**).[111] Compound **3.13** the (R)-(+) enantiomer was then N-alkylated with 4-fluorophenethyl bromide **3.14** to yield M-100907 (**3.1)** in a 72% yield (**Scheme 3.2 v**).[111] The Rice synthesis of M-100907 was reproduced with exception of the optical resolution of compound **3.12**. The optical resolution was omitted because it was not required to synthesize first generation M-100907 derivatives.



Scheme 3.2. Reagents and conditions:(i) n-BuLi, THF, -50 °C, 84%; (ii) TFA, rt, 30mins; (iii) $NaBH_4$, MeOH, rt, 71%; (iv) (+)-di-O,O'-p-toluyl-D-tartaric acid, iPrOH, MeOH, 76% (v) $NaHCO_3$, DMF, 80 °C, 72%;

Scheme 3.2 Published synthesis of M-100907

Synthesis of M-100907 derivatives

With the synthesis of racemic M-100907 complete, the synthesis of the M-100907 derivatives required for the development of DMLs was begun. The SAR data for M-100907 and its linear structure suggested that modifications at the 3'-methoxy of one aromatic ring and the 4'-fluorine of the other aromatic ring may be tolerated (**Fig 3.2**).

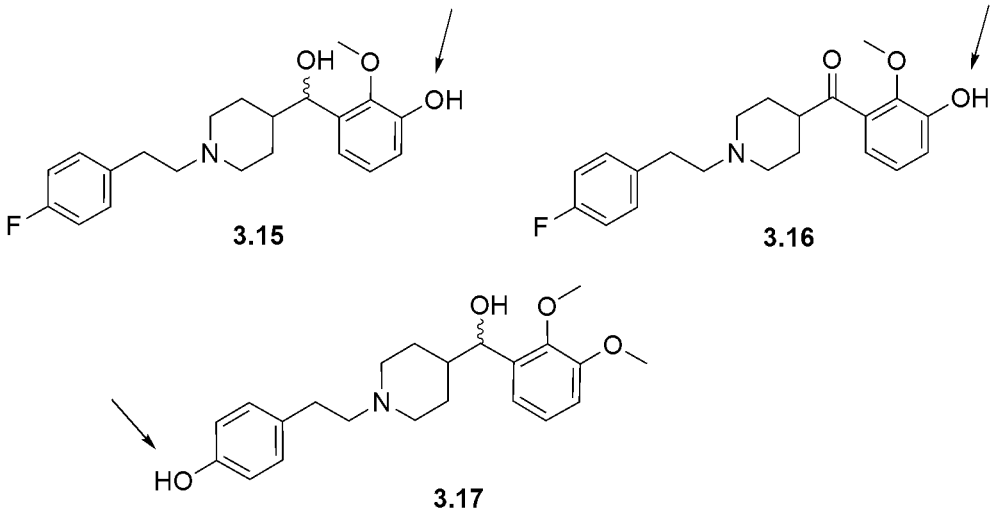


Figure 3.2 M-100907 derivatives

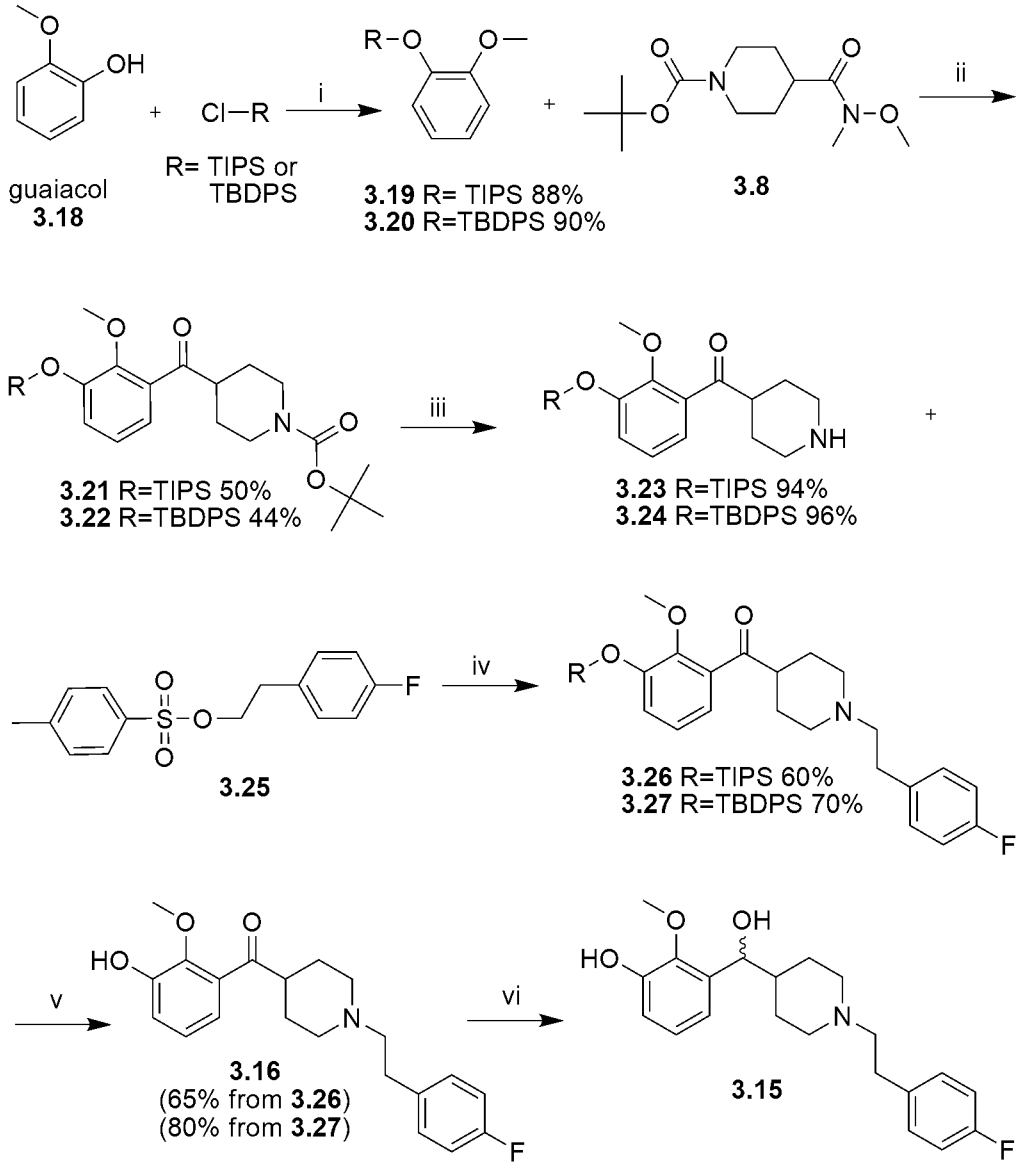
An additional M-100907 derivative included modifying the 3-position methoxy of unreduced M-100907 (**3.16**) (**Fig 3.2**). Derivatives **3.15**, **3.16**, and **3.17** were accessible through slight modifications of previous syntheses.[110, 112]

The synthesis of compounds **3.15** and **3.16** began with the addition of a silyl protecting group to guaiacol (**3.18**) (**Scheme 3.3**). In addition to the t-butyldiphenylsilyl protecting group used by Rice, a triisopropyl silyl protecting group was also used. Guaiacol was reacted with either triisopropylsilyl chloride or t-butyldiphenylsilyl chloride and the organic base imidazole in DMF at room temperature over 24 hours to produce **3.19** in a 88% yield and **3.20** in a 90% yield (**Scheme 3.3**).[112] The silyl protected compounds **3.19** and **3.20** were reacted with the Weinreb amide **3.8** under the reaction conditions reported by Rice. Under these conditions compounds **3.21** and **3.22** were not isolated in greater than a 20% yield. Allowing the silyl protected **3.19** or **3.20** and n-BuLi to slowly warm from -78 ^{o}C to 0 ^{o}C over one hour, then warming from 0 ^{o}C to room temperature over 3 hours, and finally refluxing for 3 hours increased the yields of products **3.21** and **3.22** to 50% and 44% yields (**Scheme 3.3**).[112] The Boc group is then

removed with TFA over 2 hours to give the unprotected piperidines **3.23** and **3.24** in a 94% and 96% yield (**Scheme 3.3**). Rice used 4-fluorophenethyl bromide and $NaHCO_3$ in DMF at 80 ^oC to smoothly give the alkylated amine **3.1** in a 72% yield, but this reaction could not be reproduced in an appreciable yield. Replacing the bromine leaving group with a tosylate, changing the base from $NaHCO_3$ to N,N-DIEA, and changing the solvent from DMF to CH_3CN produced the desired N-alkylated products **3.26** and **3.27** in 60% and 70% yield respectively (**Scheme 3.3**). The silyl protecting groups were removed with TBAF to afford the unprotected product **3.16** in a 60% yield from **3.26** and an 80% yield from **3.27** (**Scheme 3.3**). A sodium borohydride reduction of the carbonyl smoothly yielded derivative **3.15** in a 70% yield (**Scheme 3.3**).

Guaiacol (**3.18**) was protected with two different silyl ether protecting groups because the yield of the metalation reaction varied based on the protecting group. The yield of the benzylpiperidine product was 50% when TIPS protected guaiacol was used, but the yield was only 44% when TBDPS protected guaiacol was used. The difference in yield could be explained by differences in steric bulk between the t-butyldiphenylsilyl and the triisopropylsilyl groups.

An important difference between the two groups was the published separation of the diastereomers. The TIPS protected piperidine **3.23** was reduced with sodium borohydride to a mixture of racemic alcohols. According to a published procedure the racemic alcohol was resolved by reacting it with (S)-(+)-α-methoxyacetic acid to yield two diastereomeric esters which were then separated by column chromatography.[112]



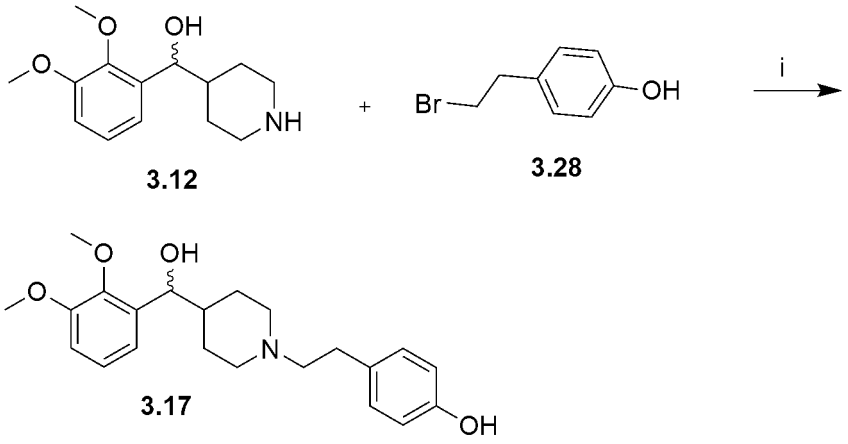
Reagents and conditions: (i) imidazole, DMF, rt, 24 h; (ii) a. nBuLi, -78 °C - reflux, 6 h: b. **3.8**, rt, 18 h;(iii) TFA, rt, 2 h; (iv) DIEA, CH_3CN, reflux, 24 h; (v) TBAF, THF, rt, 4.5 h; (vi) $NaBH_4$, EtOH, rt, 24 h, 70%

Scheme 3.3 Synthesis of M-100907 derivatives 3.16 and 3.15

The TBDPS protected piperidine **3.24** was reduced with sodium borohydride to a mixture of racemic alcohols. The mixture of alcohols were separated through the formation of diastereomeric salts from (+)- and (-)-di-O,O'-p-toluyltartaric acid.[110] These

diastereomeric salts were separated through recrystallization on a multigram scale, which removed the limitation on the scale of the reaction when separation on column chromatography was required. The different methods to separate enantiomers prompted the synthesis of both the Tips and TBDPS protected intermediates.

With the synthesis of derivatives **3.15** and **3.16** complete, the synthesis of derivative **3.17** was pursued. Derivative **3.17** was synthesized through a slight modification of the published M-100907 synthesis. The racemic alcohol **3.12** was alkylated with 4-hydroxyphenethyl bromide (**3.28**) and the base K_2CO_3 in DMF to give the desired phenol derivative **3.17** in a 53% yield (**Scheme 3.4**). With the synthesis of the three M-100907 derivatives **3.15**, **3.16**, and **3.17** complete, the next step was to attach pseudo-linkers to the designed linker attachment points.



Reagents and conditions: (i) $NaHCO_3$, DMF, 80 °C, 53%

Scheme 3.4 Synthesis of the M-100907 derivative 3.17

Attaching pseudo-tethers to M-100907 derivatives

The linker attachment points of the three M-100907 derivatives were either the 3-position hydroxy group on one aromatic ring or the 4-position hydroxy group on the

second aromatic ring. The pseudo-linkers attached to these locations were the short alkyl chain butane and a methyl ethyl ether which both mimic possible linkers used in the synthesis of DMLs (**Figure 3.3**). Attaching these pseudo-linkers to M-100907 derivatives produced a series of compounds that provided data on which site on M-100907 tolerates modification as well as which type of linker should be used to synthesize DMLs. The pseudo-linkers were attached to the M-100907 derivatives by alkylating agent containing a leaving group.

The general procedure for the alkylation of M-100907 derivatives used the inorganic base K_2CO_3 and a tosylated alkylating agent in refluxing acetone to give the desired products in good to excellent yields. Acetone is miscible with water so it was removed after the reaction was complete on a rotary evaporator. The tosylate leaving group was employed in this reaction because it was a good leaving group and affords the desired products in high yields. Additionally the linkers envisioned for use in the synthesis of DMLs were commercially available as terminal alcohols that can be tosylated, but the envisioned linkers were not commercially available with the terminal halide leaving groups bromine or iodine.

Derivative **3.29** containing a butyl chain pseudo-linker was synthesized from compound **3.11** in three steps (**Scheme 3.5**). Compound **3.11** was alkylated with bromide **3.28** in a 53% yield to produce intermediate **3.35** containing the desired phenol. Compound **3.35** was then alkylated with **3.36** in the presence of K_2CO_3 in acetone under reflux to give the desired alkylated intermediate in a 70% yield. The intermediate **3.37** was then reduced with $NaBH_4$ to afford **3.29** in a 62% yield.

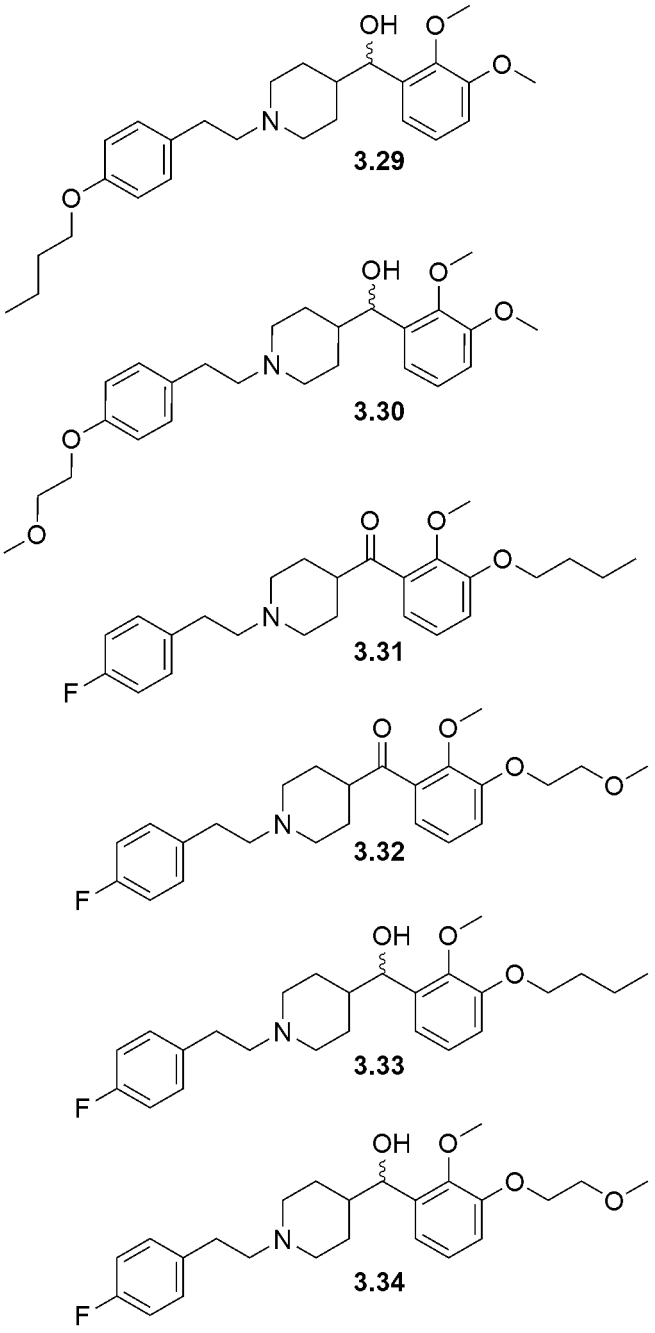
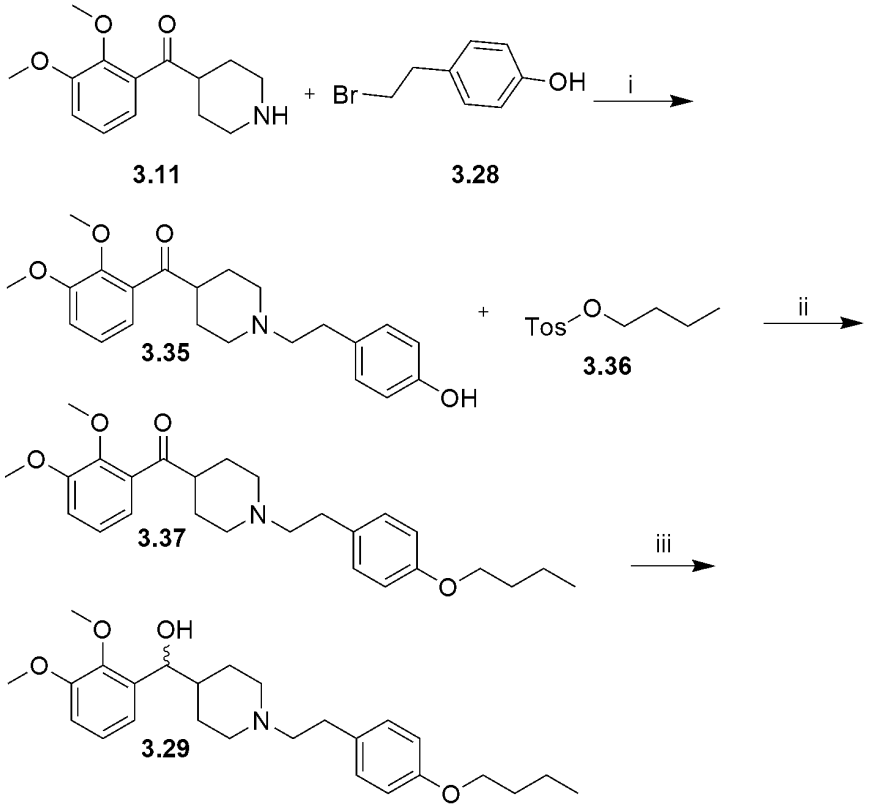


Figure 3.3 M-100907 derivatives containing pseudo-linkers

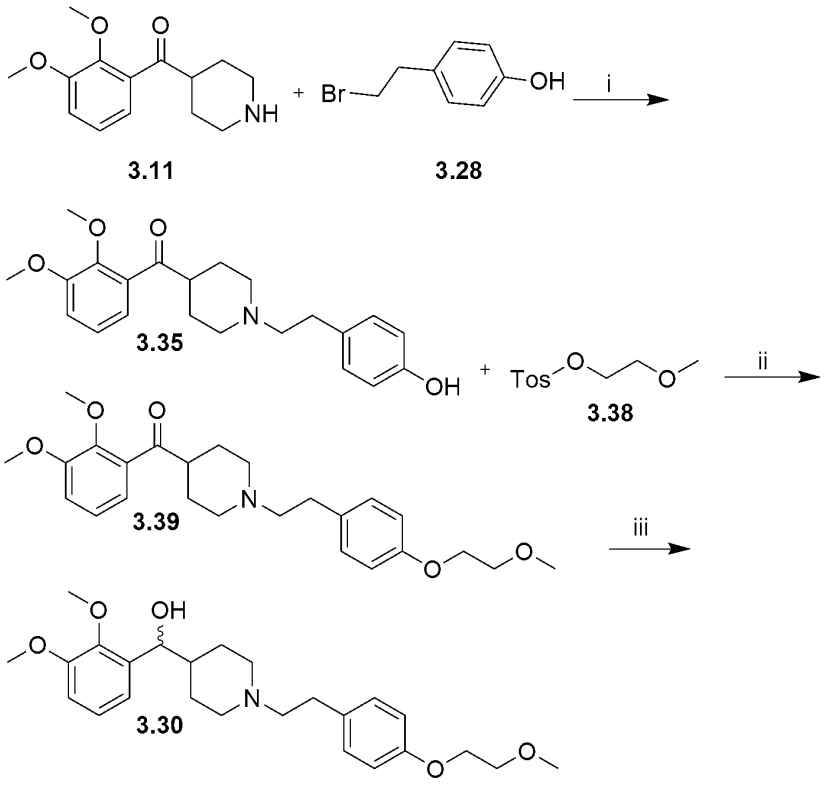


Reagents and conditions:(i) $NaHCO_3$, DMF, 110 °C, 48 h, 53%; (ii) K_2CO_3, acetone, reflux, 2 d, 70%; (iii) $NaBH_4$, EtOH, rt, 1 h, 62%

Scheme 3.5 Synthesis of derivative 3.29

Derivative **3.30** was synthesized from phenol **3.35** by alkylating with compound **3.38** to afford compound **3.39**. Reduction with $NaBH_4$ produced the desired derivative **3.30**.

Compound **3.16** was alkylated with butyl-4-methylbenzenesulfonate in the presence of K_2CO_3 in acetone under reflux to give derivative **3.31** containing a butane pseudo-linker in a 97% yield (**Scheme 3.7**). Compound **3.31** was reduced with $NaBH_4$ in MeOH to produce derivative **3.33** containing a butane pseudo-linker in a 77% yield (**Scheme 3.7**).

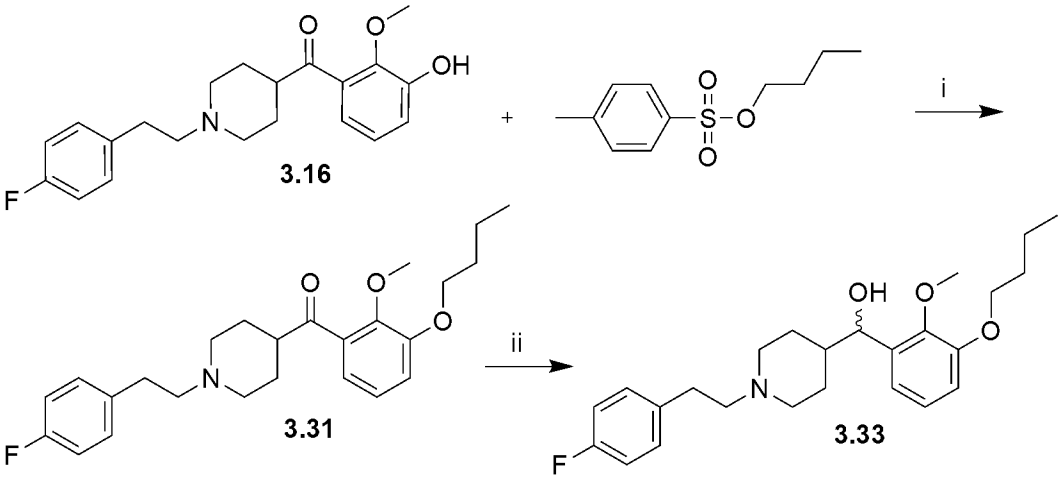


Reagents and conditions:(i) $NaHCO_3$, DMF, 110 °C, 48 h, 53%; (ii) K_2CO_3, acetone, reflux, 2 d, 62%; (iii) $NaBH_4$, EtOH, rt, 1 h, 61%

Scheme 3.6 Synthesis of derivative 3.30

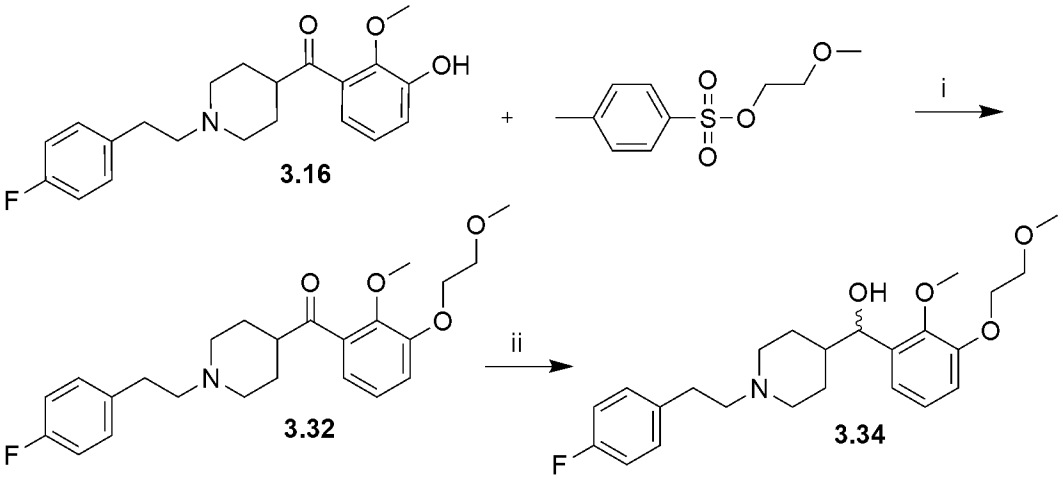
Under similar reaction conditions compound **3.16** was alkylated with 2-methoxyethyl-4-methylbenzenesulfonate to produce derivative **3.32** containing an ethyl methyl ether pseudo-linker in a 77% yield (**Scheme 3.8**). Compound **3.32** was reduced with $NaBH_4$ to give derivative **3.34** in a 74% yield (**Scheme 3.8**).

The M-100907 derivatives **3.15**, **3.16**, and **3.17** as well as derivatives **3.29**, **3.30**, **3.31**, **3.32**, **3.33**, and **3.34** were tested to determine if they retain 5-HT_{2A}R antagonist activity (**Table 3.1**). The IC_{50} value of each derivative was determined, and this data revealed that one of the linker attachment sites was preferred over the other. Additionally, one of the pseudo-linkers also exhibits an improved antagonist profile over the other pseudo-linker.



Reagents and conditions: (i) K_2CO_3, acetone, reflux, 18 h, 97%; (ii) $NaBH_4$, MeOH, rt, 2 h, 77%

Scheme 3.7 Synthesis of M-100907 derivative 3.33



Reagents and conditions: (i) K_2CO_3, acetone, reflux, 18 h, 77%; (ii) $NaBH_4$, MeOH, rt, 2 h, 74%

Scheme 3.8 Synthesis of M-100907 derivatives 3.32 and 3.34

IC50s of M-100907 derivatives containing pseudo-linkers

Compound **3.17** which contains a hydroxy group in place of the 4'-fluorine had the highest IC_{50} value of the three derivatives at 90 nM. The two 3'-methoxy derivatives **3.15** and **3.16** had IC_{50} values of 5.6 nM+/-0.9 nM and 2.7+/-1.6 nM. The IC_{50}s of these

three derivatives suggested that replacing the 3'-methoxy with a hydroxy group was favored over replacing the 4'-fluorine with a hydroxy group. This was not surprising due to the fact that **3.15** was the racemic mixture of the known active first pass metabolite M-105725.[108] Although replacing the 4'-fluorine atom with a hydroxy group did lead to an increased IC_{50} value, the increase was small enough that attaching a pseudo-linker at this location was still warranted. With this data in mind, the IC_{50} values of the derivatives containing pseudo-linkers were examined. The derivatives with pseudo-linkers at the 4'-fluorine were compounds **3.29** with a butane pseudo-linker and **3.30** with an ethyl methyl ether pseudo-linker. These two derivatives had IC_{50} values of >100 μM and 9 μM respectively. Since the IC_{50} values for these two derivatives were in the micromolar range, their antagonist activity was not sufficient to justify the synthesis of any additional derivatives with pseudo-linkers attached to the 4'-hydroxy group of compound **3.17**.

The IC_{50} values for compounds **3.31**, **3.32**, **3.33**, and **3.34** clearly showed that attaching a linker to the 3'-hydroxy group was favored over the 4'-hydroxy on the other terminal aromatic ring. Although this site did tolerate modifications while retaining 5-HT_{2A}R antagonist activity, the type of pseudo-linker attached at this position affected the activity. The difference in antagonist activity between derivatives **3.31** containing a butane pseudo-linker and derivative **3.32** containing an ethyl methyl ether pseudo-linker was over 25 fold, while the antagonist activity of the racemic alcohol derivatives **3.33** and **3.34** was comparable. The ethyl methyl ether pseudo-linker had less of a deleterious effect on antagonist activity than did the butyl chain linker. The difference in activity may be due in part to the difference in polarity between the two pseudo-linkers.

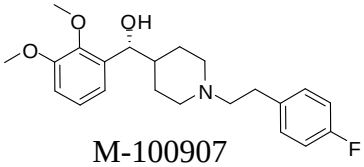
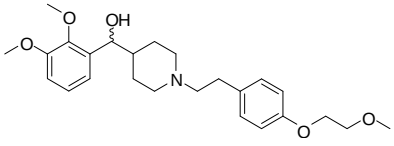
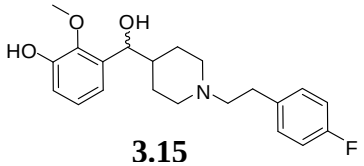
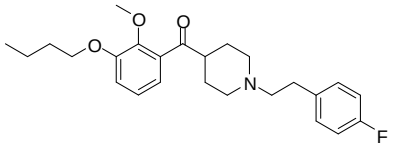
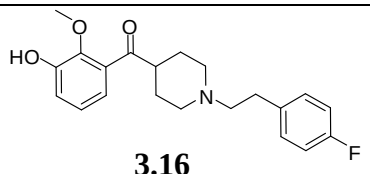
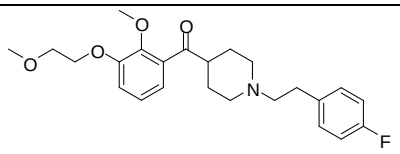
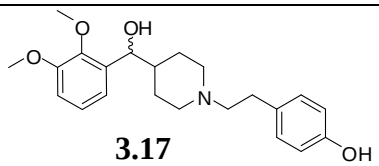
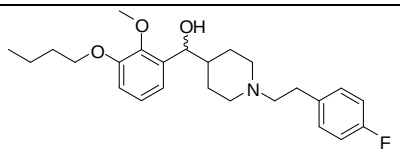
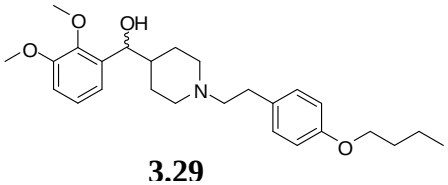
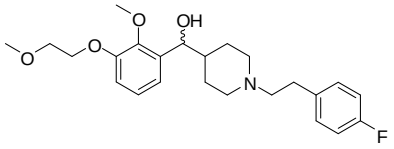
Compound	IC50 ± SEM	Compound	IC50 ± SEM
 M-100907	3.3 nM	 **3.30**	9.0 μM
 **3.15**	5.6 ± 0.9 nM	 **3.31**	873 ± 339 nM
 **3.16**	2.7 ± 1.6 nM	 **3.32**	34 ± 16 nM
 **3.17**	90 nM	 **3.33**	134 ± 30 nM
 **3.29**	>100 μM (partial agonist)	 **3.34**	120 nM

Table 3.1 IC50s of M-100907 derivatives containing pseudo-linkers

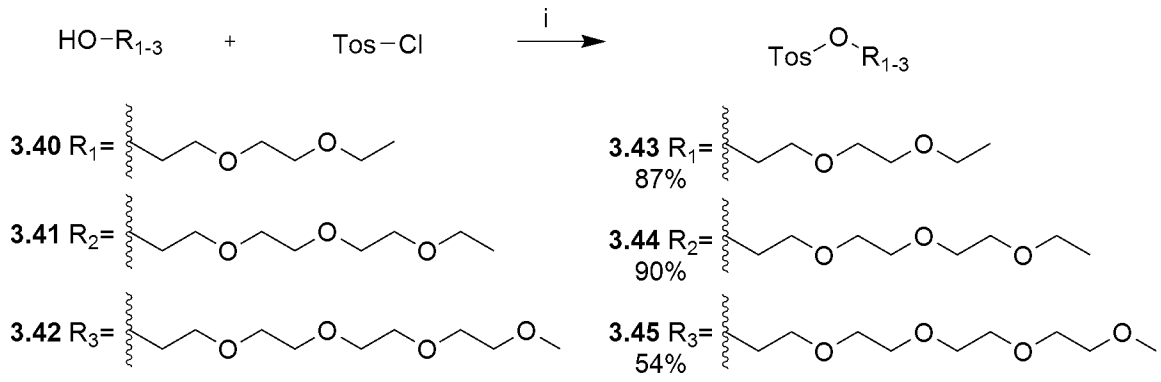
The butyl chain was simply an aliphatic hydrocarbon while ethyl methyl ether contained a polar oxygen atom. The ether oxygen atom may form hydrogen bonds with amino acid residues in or around the binding pocket which may account for the improved antagonist activity over the alkyl chain pseudo-linker. Since the ethyl methyl ether

pseudo-linker had a better antagonist profile than the alkyl chain pseudo-linkers the use of ethers as linkers in DMLs was further examined.

The IC_{50} values also showed a marked difference in antagonist activity between the carbonyl containing compounds **3.31** and **3.32** and the racemic alcohol derivatives **3.33**, and **3.34**. Compound **3.31** contained a butyl pseudo-linker and an unreduced carbonyl group, and compound **3.33** was a racemic mixture of secondary alcohols containing a butyl pseudo-linker. The racemic derivative **3.33** had better antagonist activity than the carbonyl derivative **3.31**. In contrast to these results, the carbonyl derivative **3.32** containing an ethyl methyl ether pseudo-linker was a four fold better antagonist than the racemic alcohol derivative **3.34** containing an ethyl methyl ether pseudo-linker. When an alkyl pseudo-linker was used the racemic alcohol derivative **3.33** was a better antagonist, but when ethyl methyl ether was used the unreduced derivative **3.32** was the better antagonist. The difference in activity could be explained by the different pseudo-linkers. Both of the ethyl methyl ether derivatives had better antagonist activity than either of the derivatives containing a butyl pseudo-linker. Since a clear trend between antagonist activity and a carbonyl group or an alcohol was not present, additional derivatives of compounds **3.15** and **3.16** will be synthesized.

Synthesis of M-100907 derivatives containing polyethylene glycol pseudo-linkers

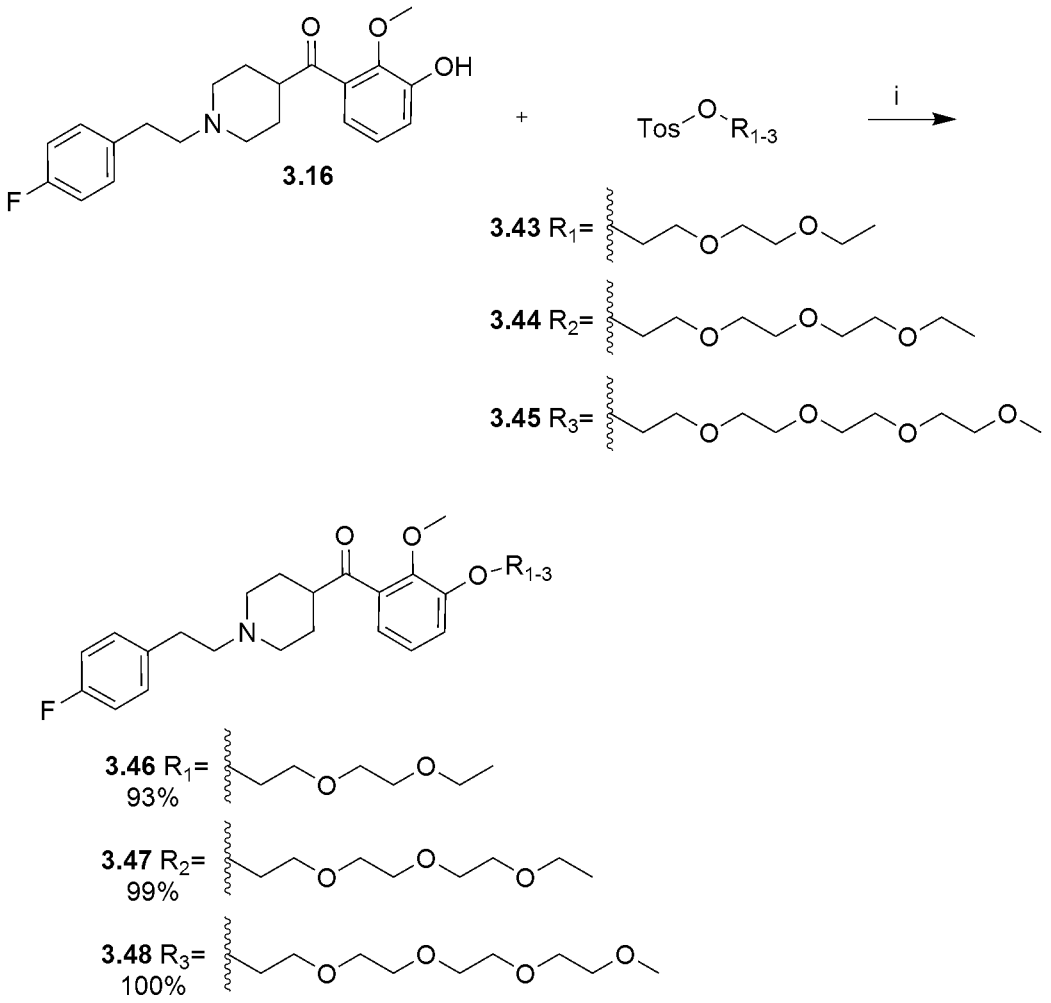
To determine the effect an increase in pseudo-linker length had on antagonist activity a series of derivatives of compound **3.16** containing polyethylene glycol groups were synthesized. In order to synthesize derivatives of compound **3.16** with longer polyethers, the ethers were first synthesized.



Reagents and conditions: (i) KOH, THF, 0 °C - rt, 24 h

Scheme 3.9 Synthesis of polyethylene glycol pseudo-linkers

The polyethers were synthesized by tosylating the glycol ethers **3.40**, **3.41**, and **3.42** with p-toluenesulfonyl chloride and KOH in THF and H_2O at room temperature over 24 hours to give **3.43**, **3.44**, and **3.45** in 87%, 90%, and 54% respectively (**Scheme 3.9**). With the tosylated polyethylene glycol pseudo-liners in hand, M-100907 derivatives were synthesized. These molecules were synthesized by alkylating the 3'-hydroxy group of compound **3.16** with one of the tosylated polyethylene glycol pseudo-linkers. The reaction conditions were identical to those previously used to alkylate **3.16**. The alkylation was done in the presence of K_2CO_3 in refluxing acetone over 24 hours to produce the desired derivatives **3.46**, **3.47**, and **3.48** in 93%, 99%, and 100% yield respectively (**Scheme 3.10**). The next compounds synthesized were DMLs of compound **3.16** containing linkers between 6 and 24 atoms in length.



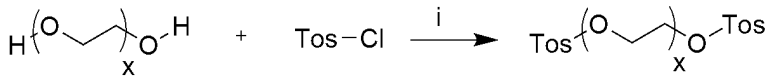
Reagents and conditions: (i) K_2CO_3, acetone, reflux, 20 h

Scheme 3.10 Synthesis of M-100907 derivatives with polyethylene glycol pseudo-linkers

Synthesis of M-100907 derivative homodimers

The synthesis of homobivalent DMLs from compound **3.16** began with the synthesis of the required linkers. Once the linkers were synthesized, they were used to join two molecules of **3.16** over two steps. The synthesis of the desired linkers began with commercially available polyethylene glycols **3.49**, **3.50**, **3.51**, **3.52**, and **3.53**. These

polyethylene glycols were tosylated in a single step using 3 molar equivalents of p-toluenesulfonyl chloride and excess KOH in THF and H_2O (**Scheme 3.11**).[113]



3.49 x=2
3.50 x=3
3.51 x=4
3.52 x=5
3.53 x=6

3.54 x=2, 74%
3.55 x=3, 80%
3.56 x=4, 75%
3.57 x=5, 95%
3.58 x=6, 73%

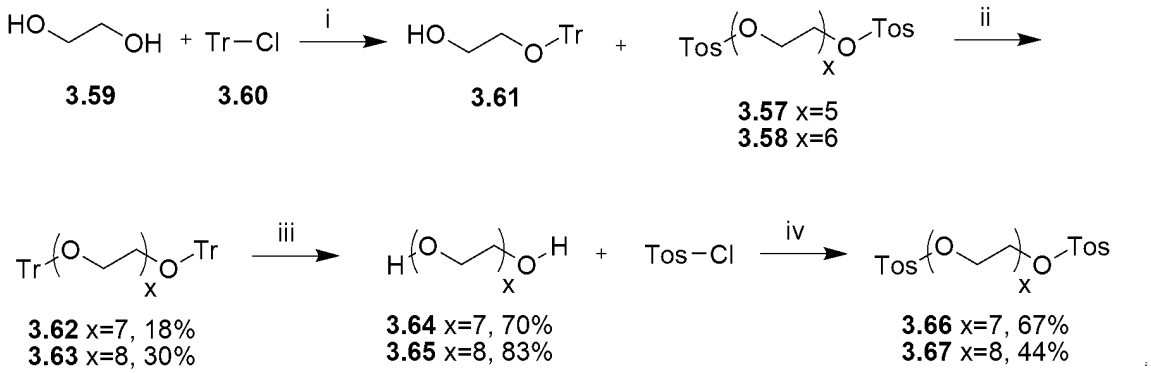
Reagents and conditions: (i) KOH, THF, H_2O, rt, 21 h

Scheme 3.11 Synthesis of ditosylated polyethylene glycol linkers

The desired tosylated compounds **3.54**, **3.55**, **3.56**, **3.57**, and **3.58** were produced in 74%, 80%, 75%, 95%, and 73% yields respectively (**Scheme 3.11**). Although most of the polyethylene glycols needed for linkers were available from commercial suppliers at a reasonable cost, heptaethylene glycol and octaethylene glycol were not available in sufficient quantities at a reasonable price. Therefore, both heptaethylene glycol and octaethylene glycol were synthesized from readily available short chain polyethylene glycols.

The synthesis of heptaethylene glycol and octaethylene glycol began by protecting one hydroxyl group of ethylene glycol with a trityl group. The reaction of ethylene glycol **3.59** and trityl chloride **3.60** in the presence of pyridine gave the desired mono-protected ethylene glycol **3.61** in a 95% yield (**Scheme 3.12**).[114] Compound **3.61** was reacted with the ditosylated compounds **3.57** or **3.58** and the phase transfer catalyst TBAHS (tetrabutylammonium hydrogen sulfate) in 6 M NaOH and toluene to give the trityl protected elongated polyethylene glycols **3.57** and **3.58** in 18% and 30% yield (**Scheme 3.12**). The two terminal trityl protecting groups were removed in a single step

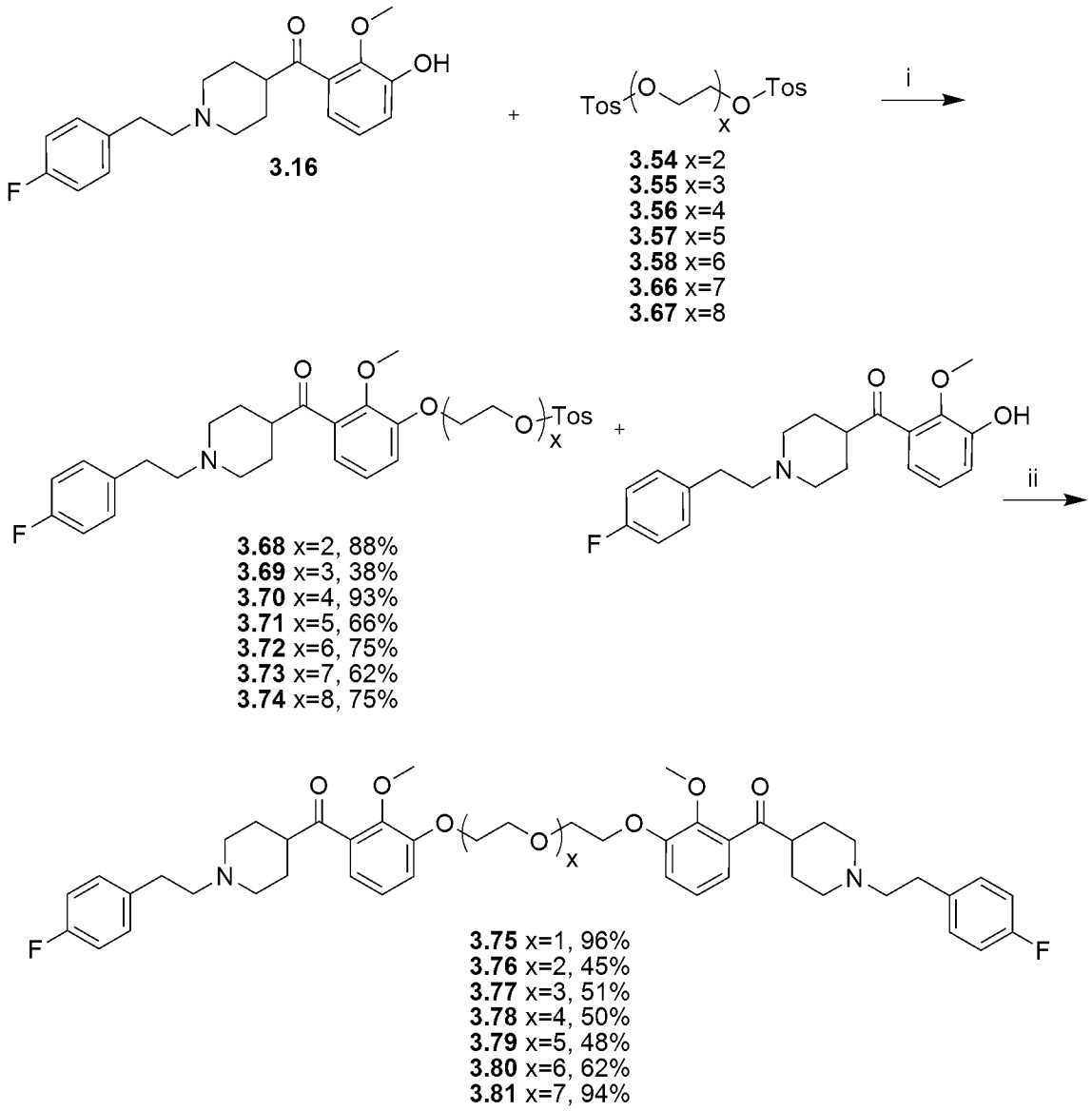
with 10% Pd/C in CH_2Cl_2 under hydrogen gas to produce heptaethylene glycol **3.64** in 70% yield and octaethylene glycol **3.65** in 83% yield (**Scheme 3.12**). Compounds **3.64** and **3.65** were then ditosylated under reaction conditions identical to those used in **Scheme 3.11** to give the ditosylated heptaethylene glycol **3.66** in 67% yield and the ditosylated octaethylene glycol **3.67** in 44% yield (**Scheme 3.12**).



Reagents and conditions: (i) pyridine, 45 °C, 19 h, 95%; (ii) TBAHS, 6 M NaOH, toluene, 65 °C, 16 h; (iii) 10% Pd/C, CH_2Cl_2, H_2 gas, 40 bar, rt, 5 d; (iv) KOH, THF, H_2O, rt, 21 h

Scheme 3.12 Synthesis of ditosylated polyethylene glycol linkers from short chain polyethylene glycols

Once the synthesis of the desired ditosylated polyethylene glycols was complete, these linkers were used to synthesize M-100907 DMLs from derivative **3.16**. The reaction conditions employed in the synthesis of the DMLs were the same conditions used to attach pseudo-linkers to compound **3.16**. The DMLs were synthesized over two steps by first alkylating **3.16** with one of the ditosylated polyethylene glycol linkers in the presence of K_2CO_3 in refluxing acetone over 20 hours to give compounds **3.68-3.74** (**Scheme 3.13**). Compounds **3.68-3.74** which now contain a tosylated linker were reacted with compound **3.16** in the presence of K_2CO_3 in refluxing acetone over 20 hours to yield the desired DMLs **3.75-3.81** (**Scheme 3.13**).



Reagents and conditions: (i) K_2CO_3, acetone, reflux, 20 h; (ii) K_2CO_3, acetone, reflux, 20 h

Scheme 3. 13 Synthesis of M-100907 homodimers

Carefully controlling the stoichiometric ratio of both derivative **3.16** and the ditosylated linkers allowed for the DMLs to be synthesized in either a single reaction or stepwise over two reactions. Reacting one molar equivalent of **3.16** with half a molar equivalent of a ditosylated linker resulted in the formation of the DML in a single step. In contrast if one molar equivalent of derivative **3.16** was reacted with four molar

equivalents of a ditosylated linker, then compounds **3.68-3.74** containing a tosylated linker were predominantly formed. Even with a four molar excess of the ditosylated linker, a small amount of dimer was still formed. Although formation of DMLs from **3.16** in one step was synthetically simpler than the two step reaction sequence, the two step sequence was preferred. The two step sequence was preferred because it will eventually allow for the synthesis of heterodimeric DMLs in addition to homodimeric DMLs. The alkylated derivative containing a tosylated linker was isolated and then used to alkylate different molecules to yield a variety of heterodimeric DMLs. One goal of this project was to synthesize heterodimeric DMLs, so developing the chemistry necessary to synthesize DMLs over two steps will be useful in the future.

IC50s of M-100907 derivatives with polyethylene glycol pseudo-linkers and homodimeric DMLs

The M-100907 derivatives with extended pseudo-linkers **3.46**, **3.47** and **3.48** were tested to determine if they retained 5-HT_{2A}R antagonist activity. The IC_{50} value of each derivative was determined, and from these results the effect of an extended pseudo-linker was examined. Derivatives with extended polyethylene glycol linkers of 8, 11, and 13 atoms in length retained nanomolar activity, but they were 20-30 times less active than the parent compound **3.16** and 2-3 times less active than the compound **3.32** with a short pseudo-linker.

Compound **3.47** with an 11 atom pseudo-linker retained the most antagonist activity with an IC_{50} value of 63 nM (**Table 3.2)**. Compound **3.46** with an 8 atom pseudo-linker had an IC_{50} value of 74 nM, and compound **3.48** with a 13 atom pseudo-linker had an IC_{50} value of 103 nM (**Table 3.2)**. The IC_{50} values of these three compounds

suggested that a linker between 8 and 11 atoms was the optimal length for homodimer DMLs.

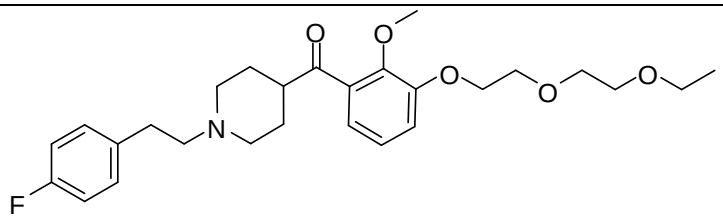
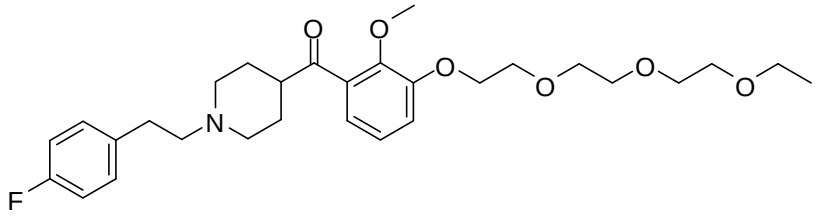
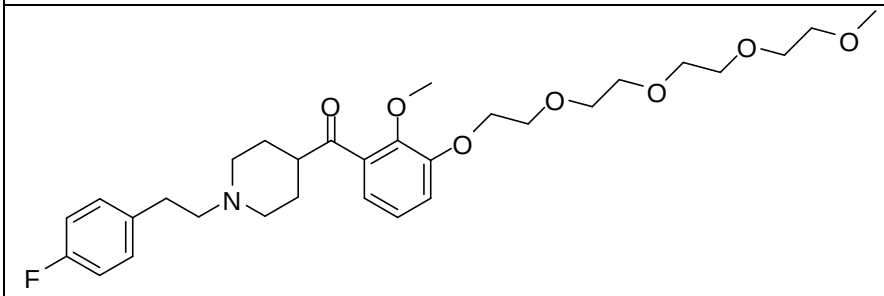
Compound	IC_{50} ± SEM
 **3.46**	74 ± 20 nM
 **3.47**	63 ± 7 nM
 **3.48**	103 ± 30 nM

Table 3.2 IC_{50} of M-100907 derivatives containing polyethylene glycol pseudo-linkers

The oxygen atoms of the polyethylene glycol chains could form hydrogen bonds to amino acid residues in or around the binding pocket of the 5-HT_{2A}R. This interaction could disrupt the antagonist activity by changing the orientation in which these derivatives sit in the binding pocket. The slight decrease in antagonist activity of compounds **3.46**, **3.47** and **3.48** suggested that polyethylene glycol linkers could be used

in the synthesis DMLs, and the linker will not contribute to any observed increase in antagonist activity.

The M-100907 homodimeric DML derivatives **3.75**, **3.76**, **3.77**, **3.78**, **3.79**, **3.80** and **3.81** were tested to determine if they retain 5-HT_{2A}R antagonist activity. The IC_{50} value of each derivative was determined and was tabulated in **Table 3.3**. Upon review of the IC_{50} values, a correlation between linker length and antagonist activity began to emerge. As linker length increased from 5 atoms to 11 atoms antagonist activity gradually improved. As linker length increased from 11 atoms to 23 atoms antagonist activity began to decrease. Compound **3.77** with a linker of 11 atoms and an IC_{50} value of 28 nM was the most potent antagonist synthesized to date. Compound **3.76** with an 8 atom linker and IC_{50} value of 56 nM, compound **3.78** with a 14 atom linker and an IC_{50} value of 32 nM, and compound **3.79** with a 17 atom linker and an IC_{50} value of 34 nM were comparable in antagonist activity to compound **3.77**. The linker lengths of compounds **3.76**, **3.77**, **3.78**, and **3.79** were 7.4Å, 9.7Å, 15.4Å, and 18.2Å respectively. This data suggested that two distinct binding sites on a 5-HT_{2A}R homodimer could be bridged by linkers between 8 and 17 atoms in length. DMLs with linkers shorter than 8 atoms may not be able to bridge the distance between two receptors. The inability of shorter linkers to bridge two receptors was supported by the increased IC_{50} value of compound **3.75** with a 5 atom linker that is 4.7Å in length.

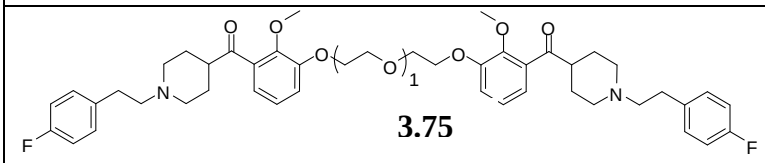
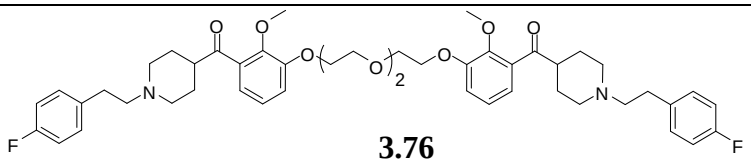
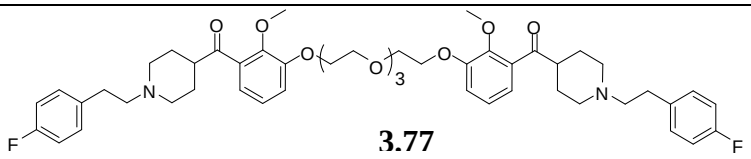
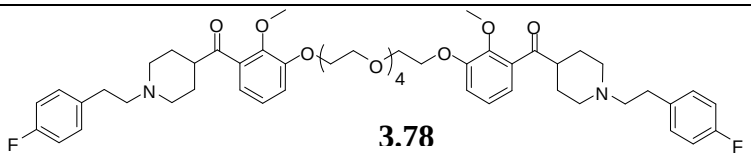
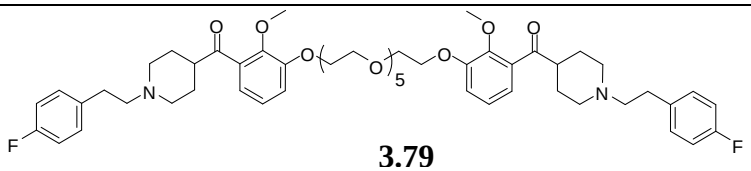
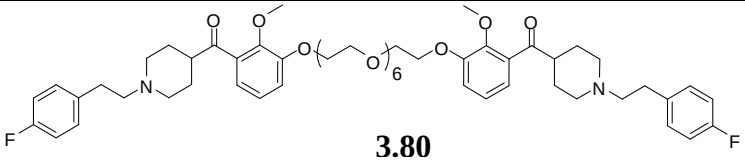
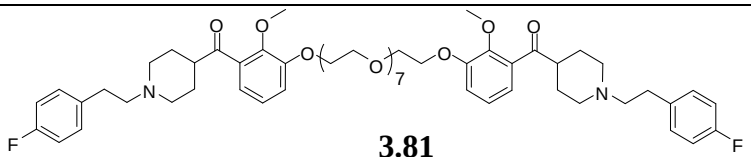
Compound	IC_{50} ± SEM	Length of linker (atoms)
 **3.75**	181 ± 71 nM	5
 **3.76**	56 ± 14 nM	8
 **3.77**	28 ± 16 nM	11
 **3.78**	32 ± 6 nM	14
 **3.79**	34 ± 10 nM	17
 **3.80**	154 ± 25 nM	20
 **3.81**	373 ± 153 nM	23

Table 3.3 EC50 of M-100907 homodimers

Taken together the linker length measurements in angstroms and IC_{50} values suggested that the antagonist binding sites on 5-HT_{2A}R dimers were separated by at least

7.4Å since at this linker length a sharp increase in IC_{50} was observed. Compound **3.80** with a 20 atom (20 Å) linker and compound **3.81** with a 23 atom (23.9Å) linker both had higher IC_{50} values than the derivatives with the 8-17atom linkers. These compounds were 5-13 times less active than compound **3.77** with an 11 atom linker. The decrease in antagonist activity was explained by the increased confinement volume of long linkers. Confinement volume is the total three dimensional space that a linker can occupy when bond rotation is taken into account.[75] At optimal linker length the confinement volume will be low and will correspond to a high local concentration at the receptor. An increase in linker length of flexible linkers such as polyethylene glycol led to an increase in confinement volume. As linker length increases, confinement volume increases to a point where it is so large that there is no longer an increase in local concentration from the binding of a DML.

As stated previously, the data presented here suggested that the optimal linker length was between 8 and 17 atoms, corresponding to a length of 7.4Å to 18.2Å in space. Retention of antagonist activity over a range of linker lengths may be in part due to the flexibility of the polyethylene glycol linker. The bonds of the polyethylene glycol linkers were able to freely rotate allowing the linkers to adopt different conformations. These conformations allowed linkers of different lengths to contort in such a way as to bridge two distinct GPCR binding sites. A linker length of between 8 and 17 atoms may also be able to bridge two binding sites while having a small enough confinement volume that a high local concentration was present at the dimeric receptors. The derivatives tested with linker length between 8 and 17 atoms produced the most active 5-HT_{2A}R antagonist.

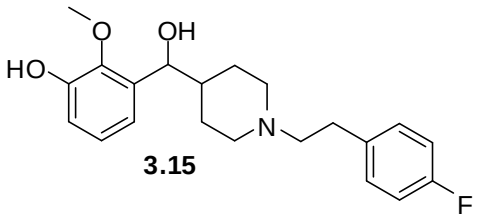
Conclusion

A range of optimal linker lengths for a bivalent antagonist targeting the 5-HT_{2A}R was determined. Optimal linker lengths of previously synthesized DMLs for other 5-HT receptors were determined. A chain length of 7-8 carbons conferred the best selectivity of 5-HT_{1A} ligands, while 20-24 carbons was the optimal linker length for ligands that bind the 5-HT_4 receptor.[78, 82] The optimal linker length for the M-100907 homodimers synthesized here was between 8 and 17 atoms. This linker length was in the range of previously determined optimal linker lengths for DMLs for other 5-HT receptors.

In summary, the 5-HT_{2A}R antagonist M-100907 was derivatized in order to synthesize a series of novel designed multiple ligands. The M-100907 derivatives **3.15** and **3.16** which contain a hydroxy group in place of the 3'-position methoxy retained antagonist activity while containing functionality amenable to linker attachment. These derivatives were used to determine that polyethylene glycol linkers may be successfully employed in the synthesis of homodimeric M-100907 DMLs with nanomolar antagonist activity at the 5-HT_{2A}R. First generation homodimers suggested that a linker length of between 8 and 17 atoms is optimal for 5-HT_{2A}R antagonist DMLs.

Experimental

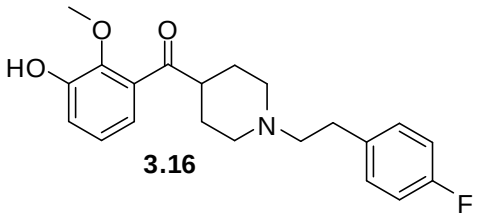
3-((1-(4-fluorophenethyl)piperidin-4-yl)(hydroxy)methyl)-2-methoxyphenol (3.15)



3.15

A stirred solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (565 mg, 1.58 mmol) in 15 mL anhydrous EtOH was cooled to 0 ^{o}C. To this solution was added $NaBH_4$ (239 mg, 6.32 mmol) portionwise over 5 minutes. The reaction was allowed to warm to room temperature and stirred an additional for 2 hours. After 2 hours, the EtOH was removed under reduced pressure and the residue was quenched with aqueous NH_4Cl. The aqueous solution was extracted 3 times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated to give 403 mg of 3-((1-(4-fluorophenethyl)piperidin-4yl)(hydroxy)methyl)-2-methoxyphenol (**3.15**) as an orange solid (71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (dd, J = 8.0, 5.1 Hz, 2H), 7.03 (dd, J = 8.0, 8.0 Hz, 1H), 6.95 (dd, J = 8.6, 8.6 Hz, 1H), 6.91-6.87 (m, 3H), 4.66 (d, J = 8.0 Hz, 1H), 3.83 (s, 3H), 3.10 (d, J = 10.9 Hz, 1H), 2.94 (d, J = 10.9 Hz, 1H), 2.77 (dd, J = 9.8, 6.9 Hz, 2H), 2.54 (dd, J = 8.6, 8.0 Hz, 2H), 2.09 (d, J = 13.2 Hz, 1H), 2.01 (t, J = 10.9 Hz, 1H), 1.92 (t, J = 9.1 Hz, 1H), 1.73-1.69 (m, 1H), 1.50 (qd, J = 12.0, 3.4 Hz, 1H), 1.40-1.23 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.5, 149.1, 145.3, 136.2, 135.9, 130.1, 130.0, 125.1, 118.9, 115.6, 115.3, 115.1, 73.4, 61.7, 60.9, 53.7, 42.7, 32.8, 28.8, 28.4; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{21}H_{26}FNO_3$: 360.1975, measured 360.1952.

(1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (3.16)



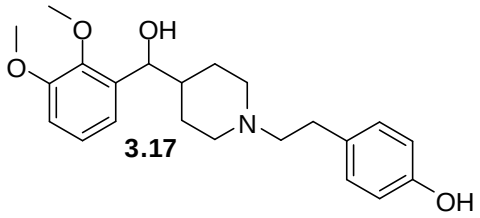
3.16

From **3.26**: To a solution of (1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(triisopropylsilyloxy)phenyl)methanone (**3.26**) (500 mg, 1.0 mmol) in 3 mL anhydrous THF at room temperature was added 1M TBAF (1.3 mL, 1.3 mmol) dropwise over 5 minutes. The reaction was stirred at room temperature for 2 hours. After 2 hours the reaction was diluted with brine and extracted 3 times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 232 mg of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) as a red-orange oil (65%).

From **3.27**: To a solution (3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (**3.27**) (595 mg, 1.0 mmol) in 3 mL anhydrous THF at room temperature was added 1M TBAF (1.3 mL, 1.3 mmol) dropwise over 5 minutes. The reaction was stirred at room temperature for 2 hours. After 2 hours the reaction was diluted with brine and extracted 3 times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 286 mg of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) as a red-orange oil (80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14-7.11 (m, 2H), 7.06-7.02 (m, 2H), 6.96-6.93 (m, 3H), 3.80 (s, 3H), 3.09 (tt, J = 10.1, 4.0 Hz, 1H), 3.00 (d, J = 11.4 Hz,

2H), 2.77 (dd, J = 8.0, 5.7 Hz, 2H), 2.58 (dd, J = 8.0, 5.2, Hz, 2H), 2.16 (dd, J = 10.0, 10 Hz, 2H), 1.90 (d, J = 12 Hz, 2H), 1.88 (qd, J = 3.5, 10.9 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.5, 162.5, 160.5, 149.4, 145.3, 132.8, 130.2, 130.1, 125.0, 120.3, 118.9, 115.3, 115.2, 62.9, 60.7, 53.2, 32.8, 28.1; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{21}H_{24}FNO_3$: 358.1818, measured 358.1824.

4-(2-(4-((2,3-dimethoxyphenyl)(hydroxy)methyl)piperidin-1-yl)ethyl)phenol (3.17)

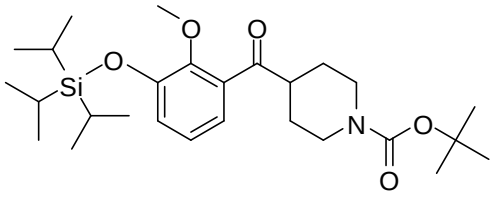


3.17

To a stirred solution of (2,3-dimethoxyphenyl)(piperidin-4-yl)methanol (**3.12**) (190 mg, 0.76 mmol) and K_2CO_3 (105 mg, 0.76 mmol) in 7 mL DMF was added 4-(2-bromoethyl)phenol (**3.28**) (169 mg, 0.84 mmol). The reaction was stirred at 75 ^oC for 24 hours. After the reaction was complete the DMF was removed under reduced pressure. The residue was taken up in brine and extracted three times with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated to give a residue that was purified by flash chromatography (100% CH_2Cl_2 to 40% MeOH in CH_2Cl_2) to afford 149 mg of 4-(2-(4-((2,3-dimethoxyphenyl)(hydroxy)methyl)piperidin-1-yl)ethyl)phenol (**3.17**) as a white solid (53%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.01 (dd, J = 8.0, 8.0 Hz, 1H), 6.91 (d, J = 8.0 Hz, 2H), 6.86 (dd, J = 8.1, 1.2 Hz, 1H), 6.81 (dd, J = 8.0, 1.2 Hz, 1H), 6.67 (d, J = 8.6 Hz, 2H), 4.59 (d, J = 8.0 Hz, 1H), 3.84 (s, 3H), 3.82 (s, 3H), 3.10 (d, J = 10.9 Hz, 1H), 2.96 (d, J = 10.9 Hz, 1H), 2.68 (dd, J = 9.7, 5.8 Hz, 2H), 2.52 (dd, J = 8.6, 7.4, 2H), 2.04 (dd, J = 11.5, 9.1 Hz, 2H), 1.94 (dd, J = 12.0, 9.7 Hz, 1H), 1.71-1.64

(m, 1H), 1.51 (q, J = 10.5 Hz, 1H), 1.41 (ddd, J = 13.2, 13.2, 3.5 Hz, 1H), 1.25 (dd, J = 6.9, 3.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.1, 152.5, 146.5, 136.4, 131.0, 130.0, 129.7, 124.1, 119.8, 116.1, 115.9, 111.5, 74.3, 61.0, 55.8, 53.5, 42.7, 32.2, 28.3, 28.1; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{22}H_{29}NO_4$: 372.2175, measured 372.2174.

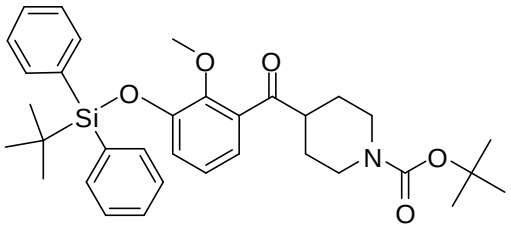
tert-butyl 4-(2-methoxy-3-(triisopropylsilyloxy)benzoyl)piperidine-1-carboxylate (3.21)



A solution of triisopropyl(2-methoxyphenoxy)silane (**3.19**) (8.0 g, 28.5 mmol) in 65 mL freshly distilled THF was cooled to -78 ^{o}C. 2.5M nBuLi (63 mL, 156.8 mmol) was added dropwise and stirred an additional an additional 10 minutes at -78 ^{o}C. The solution was then stirred at 0 ^{o}C for 2 hours and then warmed to room temperature and stirred an additional 2 hours. After stirring at room temperature the solution was refluxed for 2 hours and then cooled to -78 ^{o}C. Tert-butyl 4-(methoxy(methyl)carbamoyl)piperidine-1-carboxylate (**3.8**) (9.3 g, 34.2 mmol) in 10 mL freshly distilled THF was added and the reaction mixture was warmed to room temperature. The reaction was stirred for 18 hours at room temperature, cooled to 0 ^{o}C, and quenched with aqueous NH_4Cl. The aqueous solution was extracted 3 times with CH_2Cl_2, and the organic extracts were dried over $MgSO_4$ and concentrated. The crude reaction mixture was purified by flash chromatography (100% hexane to 20% EtOAc in hexane) to afford 7 g of tert-butyl 4-(2-methoxy-3-(triisopropylsilyloxy)benzoyl)piperidine-1-carboxylate (**3.21**) as a yellow oil

(50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99-6.93 (m, 3H), 4.06 (bs, 2H), 3.84 (s, 3H), 3.23 (tt, J = 11.0, 3.7 Hz, 1H), 2.84 (dd, J = 12.4, 9.3 Hz, 2H), 1.83 (d, J = 10.5 Hz, 2H), 1.63-1.53 (m, 2H), 1.45 (s, 9H), 1.30 (septet, J = 7.8 Hz, 3H), 1.11 (d, J = 7.3 Hz, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.3, 154.8, 149.5, 134.8, 124.3, 123.4, 121.0, 79.6, 61.7, 48.0, 28.5, 27.9, 18.0, 12.9, 12.4

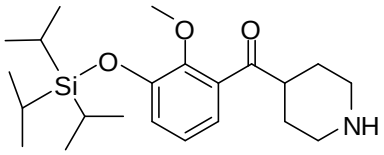
tert-butyl 4-(3-(tert-butyldiphenylsilyloxy)-2-methoxybenzoyl)piperidine-1-carboxylate (3.22)



A solution of tert-butyl(2-methoxyphenoxy)diphenylsilane (**3.20**) (8.0 g, 21.9 mmol) in 55 mL freshly distilled THF was cooled to -78 ^{o}C. 2.5M nBuLi in THF (11.4 mL, 26.3 mmol) was added dropwise and stirred an additional an additional 10 minutes at -78 ^{o}C. The solution was then stirred at 0 ^{o}C for 2 hours and then warmed to room temperature and stirred an additional 2 hours. After stirring at room temperature the solution was refluxed for 2 hours and then cooled to -78 ^{o}C. Tert-butyl 4-(methoxy(methyl)carbamoyl)piperidine-1-carboxylate (**3.8**) (5.68 g, 20.8 mmol) in 10 mL freshly distilled THF was added and the reaction mixture was warmed to room temperature. The reaction was stirred for 18 hours at room temperature, cooled to 0 ^{o}C, and quenched with aqueous NH_4Cl. The aqueous solution was extracted 3 times with CH_2Cl_2, and the organic extracts were dried over $MgSO_4$ and concentrated. The crude reaction mixture was purified by flash chromatography (100% hexane to 20% EtOAc in

hexane) to afford 5.2 g of tert-butyl 4-(3-(tert-butyldiphenylsilyloxy)-2-methoxybenzoyl)piperidine-1-carboxylate (**3.22**) as a light orange solid (44%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, J = 6.8 Hz, 4H), 7.43 (dd, J = 7.5, 7.5 Hz, 2H), 7.37 (dd, J = 7.5, 6.9 Hz, 4H), 6.85 (d, J = 6.3 Hz, 1H), 6.69 (dd, J = 8.0, 7.5 Hz, 1H), 6.64 (d, J = 6.9 Hz, 1H), 4.06 (bs, 2H), 3.92 (s, 3H), 3.18 (tt, J = 11.4, 3.4 Hz, 1H), 2.84 (bs, 2H), 1.80 (d, J = 11.5 Hz, 2H), 1.56 (d, J = 10.3 Hz, 2H), 1.46 (s, 9H), 1.13 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.2, 154.8, 149.0, 148.6, 135.6, 134.8, 132.3, 130.2, 127.9, 124.1, 123.7, 121.0, 79.6, 62.1, 47.9, 28.5, 27.9, 26.6, 19.6

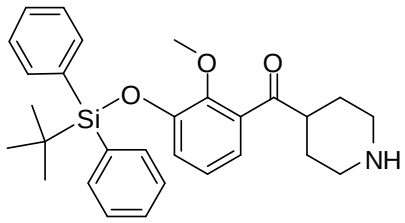
(2-methoxy-3-(triisopropylsilyloxy)phenyl)(piperidin-4-yl)methanone (3.23)



tert-butyl 4-(2-methoxy-3-(triisopropylsilyloxy)benzoyl)piperidine-1-carboxylate (**3.21**) (7.5 g, 15.3 mmol) was cooled to 0 ^{o}C and then 40 mL TFA (58 g, 505 mmol) was added dropwise with stirring. The reaction was warmed to room temperature and stirred an additional 2 hours. After 2 hours the reaction was cooled to 0 ^{o}C and then quench with 6 M NaOH. The neutralized reaction was extracted 3 times with CH_2Cl_2, the combined extracts were dried over $MgSO_4$ and concentrated to afford 5.6 g of (2-methoxy-3-(triisopropylsilyloxy)phenyl)(piperidin-4-yl)methanone (**3.23**) as a red oil (94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.98-6.93 (m, 3H), 3.84 (s, 3H), 3.24 (tt, J = 11.0, 3.7 Hz, 1H), 3.16 (ddd, J = 12.8, 3.7, 3.7 Hz, 2H), 2.74 (ddd, J = 11.5, 2.7, 2.7 Hz, 2H), 1.90 (dd, J = 13.7, 2.7 Hz, 2H), 1.63 (qd, J = 11.4, 4.1 Hz, 2H), 1.35-1.25 (m, 3H), 1.11 (d, J = 7.3

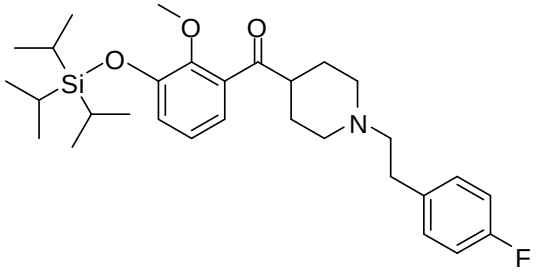
Hz, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.3, 149.5, 148.9, 134.7, 124.3, 123.3, 121.0, 61.7, 47.6, 45.5, 28.4, 18.0, 12.9

(3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl)(piperidin-4-yl)methanone (3.24)



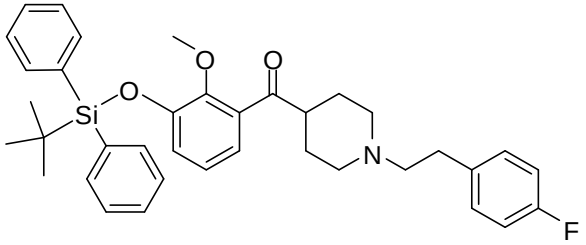
tert-butyl 4-(3-(tert-butyldiphenylsilyloxy)-2-methoxybenzoyl)piperidine-1-carboxylate (**3.22**) (6.1 g, 10.7 mmol) was cooled to 0 ^{o}C and then 30 mL TFA (40.2 g, 353 mmol) was added dropwise with stirring. The reaction was warmed to room temperature and stirred an additional 2 hours. After 2 hours the reaction was cooled to 0 ^{o}C and then quench with 6 M NaOH. The neutralized reaction was extracted 3 times with CH_2Cl_2, the combined extracts were dried over $MgSO_4$ and concentrated to afford 4.9 g of (3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl)(piperidin-4-yl)methanone (**3.24**) as a red oil (96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, J = 6.9 Hz, 4H), 7.40 (dd, J = 7.5, 6.9 Hz, 2H), 7.35 (dd, J = 7.5, 6.8 Hz, 4H), 6.92 (dd, J = 7.5, 1.2 Hz, 1H), 6.71 (dd, J = 8.0, 1.7 Hz, 1H), 6.67 (dd, J = 8.0, 7.5 Hz, 1H), 3.96 (s, 3H), 3.49-3.42 (m, 2H), 3.13 (dd, J = 10.3, 9.8 Hz, 2H), 2.12 (d, J = 10.8 Hz, 2H), 1.99 (dd, J = 10.3, 9.8 Hz, 2H), 1.15 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.1, 149.0, 148.9, 135.6, 133.5, 132.2, 130.3, 128.0,124.4, 124.3, 121.4, 118.0, 115.7, 62.2, 50.1, 44.7, 43.2, 26.6, 24.7, 19.5

(1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3- (triisopropylsilyloxy)phenyl) methanone (3.26)



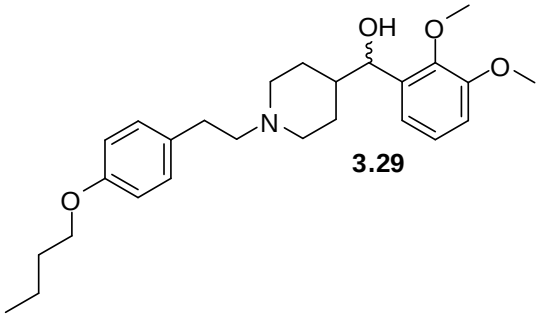
A solution of 2-methoxy-3-(triisopropylsilyloxy)phenyl)(piperidin-4-yl)methanone (**3.23**) (822 mg, 2.1 mmol), 4-fluorophenethyl 4-methylbenzenesulfonate (**3.25**) (550 mg, 2.5 mmol), and N,N-DIEA (650 mg, 5.0 mmol) in 12 mL CH_3CN was refluxed for 24 hours and then cooled to room temperature. The solvent was removed under reduced pressure and the residue was taken up in a saturated solution of $NaHCO_3$. The aqueous solution was extracted 3 times with CH_2Cl_2. The organic extracts were dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 631 mg of (1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(triisopropylsilyloxy)phenyl)methanone (**3.26**) as an orange oil (60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (dd, J = 5.7, 5.2 Hz, 2H), 6.99-6.93 (m, 5H), 3.83 (s, 3H), 3.08 (tt, J = 10.9, 4.0 Hz, 1H), 2.97 (d, J = 11.4 Hz, 2H), 2.76 (dd, J = 8.6, 7.5 Hz, 2H), 2.55 (dd, J = 8.6, 8.0 Hz, 2H), 2.13 (dd, J = 11.5, 9.7 Hz, 2H), 1.90 (d, J = 11.5 Hz, 2H), 1.75 (qd, J = 11.4, 3.5 Hz, 2H), 1.30 (septet, J = 7.4 Hz, 3H), 1.12 (d, J = 7.5 Hz, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.7, 162.3, 160.4, 149.4, 148.8, 136.1, 136.1, 135.0, 130.5, 130.1, 130.0, 124.2, 123.1, 120.9, 115.3, 115.2, 115.0, 63.5, 61.2, 60.8, 53.5, 53.3, 47.9, 38.5, 32.9, 28.1, 17.9, 13.1, 12.9, 12.6

(3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (3.27)



A solution of (3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl)(piperidin-4-yl)methanone (**3.24**) (2.6 g, 5.5 mmol), 4-fluorophenethyl 4-methylbenzenesulfonate (**3.25**) (1.9 g, 6.6 mmol), and N,N-DIEA (1.7 g, 13.2 mmol) in 28 mL CH_3CN was refluxed for 24 hours and then cooled to room temperature. The solvent was removed under reduced pressure and the residue was taken up in a saturated solution of $NaHCO_3$. The aqueous solution was extracted 3 times with CH_2Cl_2. The organic extracts were dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 2.3 g of (3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (3.27) as a red oil (70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, J = 8.6 Hz, 4H), 7.43 (dd, J = 7.5, 7.4 Hz, 2H), 7.37 (dd, J = 7.5, 6.8 Hz, 4H), 7.15 (dd, J = 8.6, 6.9 Hz, 2H), 6.96 (dd, J = 9.2, 8.6 Hz, 2H), 6.86 (dd, J = 7.4, 1.7 Hz, 1H), 6.69 (dd, J = 8.0 Hz, 1H), 6.62 (dd, J = 8.0, 1.7 Hz, 1H), 3.93 (s, 3H), 3.06 (tt, J = 10.9, 3.5 Hz, 1H), 2.98 (d, J = 11.5 Hz, 2H), 2.78 (dd, J = 8.0, 5.2 Hz, 2H), 2.56 (dd, J = 8.5, 5.7 Hz, 2H), 2.13 (ddd, J = 11.4, 2.2, 2.2 Hz, 2H), 1.87 (d, J = 10.8 Hz, 2H), 1.74 (qd, J = 13.2, 3.5 Hz, 2H), 1.13 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.8, 149.0, 148.5, 135.6, 135.1, 132.4, 130.2, 130.1, 128.0, 124.0, 123.4, 121.0, 115.4, 115.3, 115.1, 62.1, 61.0, 53.4, 33.0, 28.2, 26.6, 19.6

(1-(4-butoxyphenethyl)piperidin-4-yl)(2,3-dimethoxyphenyl)methanol (3.29)

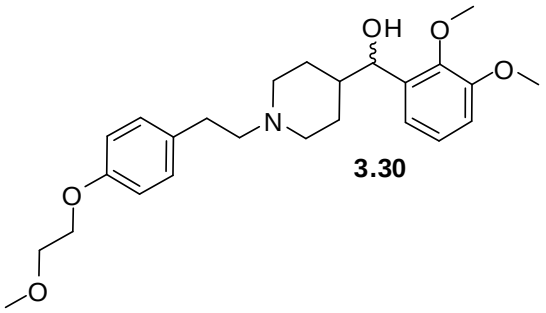


3.29

A solution of (2,3-dimethoxyphenyl)(1-(4-hydroxyphenethyl)piperidin-4-yl)methanone (**3.37)** (172 mg, 0.40 mmol) in 8 mL EtOH is cooled to 0 ^{o}C. Then $NaBH_4$ (75 mg, 2.0 mmol) was added portionwise with stirring. The reaction was warmed to room temperature and allowed to stir for 1 hour. After 1 hour the reaction was quenched with aqueous NH_4Cl. The EtOH was removed under reduced pressure. The residue was diluted with H_2O and extracted 3 times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to provide 105 mg of (1-(4-butoxyphenethyl)piperidin-4-yl)(2,3-dimethoxyphenyl)methanol (**3.29**) as a white solid (62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (d, J = 8.2 Hz, 2H), 7.04 (dd, J = 8.3, 8.3 Hz, 1H), 6.90 (dd, J = 7.8, 0.9 Hz, 1H), 6.83 (dd, J = 7.8, 0.9 Hz, 1H), 6.79 (d, J = 8.7 Hz, 2H), 4.63 (d, J = 8.2 Hz, 1H), 3.92 (t, J = 6.4 Hz, 2H), 3.85 (s, 6H), 3.07 (d, J = 11.5 Hz, 1H), 2.92 (d, J = 11.4 Hz, 1H), 2.71 (dd, J = 8.7, 7.8 Hz, 2H), 2.49 (dd, 8.7, 7.8 Hz, 2H), 2.07 (d, J = 12.8 Hz, 1H), 1.95 (ddd, J = 11.4, 2.3, 2.3 Hz, 1H), 1.87 (ddd, J = 11.4, 2.3, 2.3 Hz, 1H), 1.74 (quintet, J = 6.4 Hz, 2H), 1.65 (tt, J = 3.6, 11.4 Hz, 1H), 1.47 (sextet, J = 7.8 Hz, 3H), 1.35 (ddd, J = 9.7, 3.2, 3.2 Hz, 1H), 1.27 (d, J = 14.6 Hz, 1H), 0.95 (t, J = 7.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 152.5, 146.6, 136.6, 132.4, 129.6, 124.1, 119.8, 114.5, 111.4, 74.4, 67.8, 61.3, 61.0, 55.8, 53.8, 43.0, 32.9, 31.4, 28.9, 19.4,

14.0; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{26}H_{37}NO_4$: 428.2801, measured 428.2788.

(2,3-dimethoxyphenyl)(1-(4-(2-methoxyethoxy)phenethyl)piperidin-4-yl)methanol (3.30)

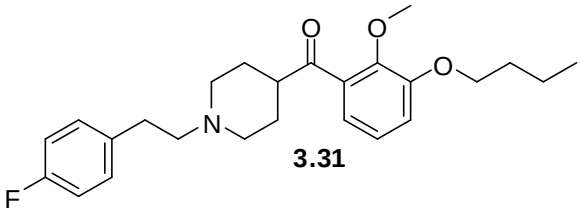


3.30

(2,3-dimethoxyphenyl)(1-(4-(2-methoxyethoxy)phenethyl)piperidin-4-yl)methanone , (**3.39**) (20 mg, 0.05 mmol) in 0.7 mL EtOH was cooled to 0 ^oC. $NaBH_4$ (8.8 mg, 0.25 mmol) was added, and the reaction mixture was warmed to room temperature. The reaction was stirred 1 hour and then quenched with aqueous NH_4Cl. The EtOH was removed under reduced pressure. The residue was diluted with H_2O and extracted 3 times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to provide 13.0 mg of (2,3-dimethoxyphenyl)(1-(4-(2-methoxyethoxy)phenethyl)piperidin-4-yl)methanol (**3.30**) as a white solid (61%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.08 (d, J = 9.2 Hz, 2H), 7.04 (dd, J = 7.5, 7.5 Hz, 2H), 6.90 (dd, J = 7.5, 1.2 Hz, 1H), 6.83 (d, J = 6.8 Hz, 3H), 4.64 (d, J = 8.1 Hz, 1H), 4.08 (dd, J = 4.5, 4.5 Hz, 2H), 3.86 (s, 6H), 3.73 (dd, J = 4.5, 4.5 Hz, 2H), 3.44 (s, 3H), 3.12 (d, J = 11.4 Hz, 1H), 2.97 (d, J = 11.4 Hz, 1H), 2.76 (dd, J = 10.3, 6.3 Hz, 2H), 2.55 (dd, J = 8.6, 8.0 Hz, 2H), 2.08 (dd, J = 12.6, 5.8 Hz, 1H), 2.01 (dd, J = 9.4, 9.4 Hz, 1H), 1.95 (t, J = 10.3 Hz, 1H), 1.69 (qdd, J = 12.0, 4.0 Hz, 1H), 1.53 (qd, J = 12.6, 4.1 Hz, 1H), 1.43 (qd, J

= 12.6, 4.0 Hz, 1H), 1.31 (d, J = 13.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.2, 152.6, 146.6, 136.5, 132.5, 129.7, 124.1, 119.8, 114.7, 111.5, 74.4, 71.2, 67.4, 61.0, 60.9, 59.3, 55.8, 53.7, 42.8, 32.6, 28.6; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{25}H_{25}NO_5$: 430.2593, measured 430.2578.

(3-butoxy-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (3.31)

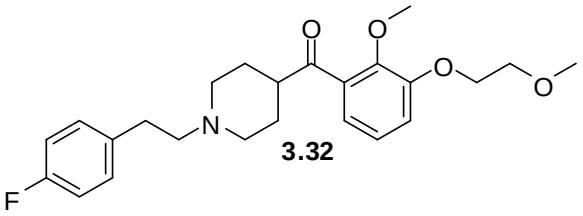


3.31

To a solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2 methoxyphenyl) methanone (**3.16**) (181.5 mg, 0.51 mmol) in 8 mL acetone was added butyl 4-methylbenzenesulfonate (232.9 mg, 1.02 mmol) and K_2CO_3 (141 mg, 1.02 mmol). The reaction mixture was refluxed for 22 hours, and then cooled to room temperature. The solvent was then removed under reduced pressure. Flash chromatography (100% CH_2Cl_2 to 8% MeOH in CH_2Cl_2) isolated 205 mg of (3-butoxy-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (**3.31**) as an orange oil (97%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16-7.13 (m, 2H), 7.06-6.99 (m, 2H), 6.96-6.92 (m, 3H), 4.01 (t, J = 6.3 Hz, 2H), 3.88 (s, 3H), 3.11-3.08 (m, 1H), 2.97 (d, J = 11.4 Hz, 2H), 2.78-2.74 (m, 2H), 2.56-2.53 (m, 2H), 2.11 (t, J = 9.8 Hz, 2H), 1.91 (d, J = 11.5 Hz, 2H), 1.85-1.82 (m, 2H), 1.80-1.74 (m, 2), 1.53 (sextet, J = 7.4 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.6, 162.3, 160.4, 152.3 147.1, 136.2, 136.2, 134.4, 130.1, 124.2,

120.1, 115.8, 115.2, 115.0, 68.5, 61.6, 60.8, 53.5, 53.4, 48.2, 32.9, 31.4, 28.1, 19.4, 13.9; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{25}H_{32}FNO_3$: 414.2444, measured 414.2438.

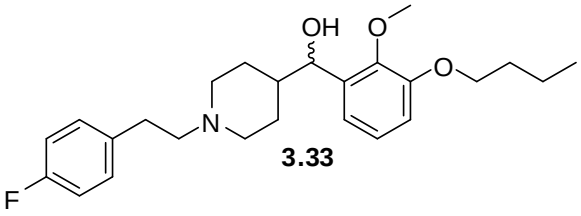
(1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(2-methoxyethoxy)phenyl) methanone (3.32)



3.32

(1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2 methoxyphenyl)methanone (**3.16**) (85.4 mg, 0.24 mmol), 2-methoxyethyl 4-methylbenzenesulfonate (110 mg, 0.48 mmol), and K_2CO_3 (66 mg, 0.48 mmol) in 5 mL acetone was refluxed for 22 hours then cooled to room temperature. The solvent was removed under reduced pressure and the crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 8% MeOH in CH_2Cl_2) to afford 75.3 mg of (1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(2-methoxyethoxy)phenyl)methanone (**3.32**) as an orange oil (75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17-7.13 (m, 2H), 7.08-7.02 (m, 2H), 7.00-6.93 (m, 3H), 4.17 (dd, J = 6.0, 4.6 Hz, 2H), 3.90 (s, 3H), 3.80 (dd, J = 6.0, 4.6 Hz, 2H), 3.45 (s, 3H), 3.14-3.09 (m, 1H), 3.00-2.98 (m, 2H), 2.81-2.77 (m, 2H), 2.60-2.56 (m, 2H), 2.18 (dd, J = 10.1, 10.1 Hz, 2H), 1.93 (dd, J = 13.7, 3.2 Hz, 2H), 1.81-1.72 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 162.7, 160.2, 152.0, 147.5, 136.0, 134.5, 130.2, 130.1, 124.3, 120.9, 116.7, 115.3, 115.1, 76.8, 71.1, 70.7, 68.5, 61.7, 60.7, 59.3, 53.2, 47.9, 32.8, 27.9; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{24}H_{30}FNO_4$: 416.2237, measured 416.2239.

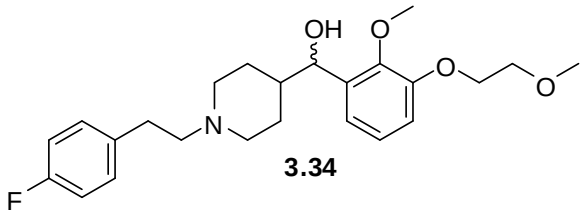
(3-butoxy-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanol (3.33)



3.33

(3-butoxy-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (**3.31**) (116.2 mg, 0.28 mmol) in 5 mL anhydrous EtOH was cooled to 0 ^{o}C. $NaBH_4$ was added portionwise with stirring over 5 minutes. The reaction was stirred at 0 ^{o}C an additional 10 minutes and then stirred at room temperature for 1 hour. The solvent was removed under reduced pressure, and the residue was taken up in aqueous NH_4Cl and extracted three times with CH_2Cl_2. The combined organic fractions were dried over $MgSO_4$ and concentrated to give 89.1 mg of (3-butoxy-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanol (**3.33**) as an orange solid (77%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17-7.12 (m, 2H), 7.03-6.92 (m, 3H), 6.88 (dd, J = 7.8, 1.3 Hz, 1H), 6.82 (dd, J = 8.2, 1.3 Hz, 1H), 4.66 (d, J = 7.3 Hz, 1H), 3.99 (ddd, J = 6.4, 1.8, 1.8 Hz, 2H), 3.88 (s, 3H), 3.20 (d, J = 11.4 Hz, 1H), 3.85 (d, J = 11.0 Hz, 1H), 2.89-2.85 (m, 2H), 2.68-2.64 (m, 2H), 2.19-2.07 (m, 3H), 1.89-1.79 (m, 2H), 1.77-1.61 (m, 2H), 1.60-1.49 (m, 3H), 1.38 (d, J = 12.8 Hz, 1H), 0.99 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.1, 162.8, 160.3, 152.0, 146.5, 136.2, 135.1, 130.2, 130.1, 124.0, 119.5, 115.4, 115.2, 112.4, 74.1, 68.3, 60.9, 60.2, 53.5, 42.3, 32.0, 31.5, 28.1, 27.7, 19.5, 13.9; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{25}H_{34}FNO_3$: 416.2601, measured 416.2587.

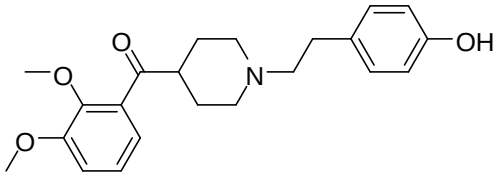
(1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(2-methoxyethoxy)phenyl) methanol (3.34)



3.34

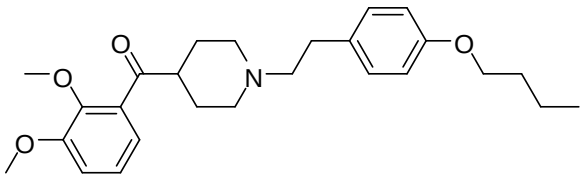
(1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(2-methoxyethoxy)phenyl)methanone (**3.32**) (11.2 mg, 0.028 mmol) in 0.6 mL anhydrous EtOH was cooled to 0 ^{o}C. $NaBH_4$ was added with stirring. The reaction was stirred an additional 10 minutes and then stirred at room temperature for 1 hour. The solvent was removed under reduced pressure, and the residue was taken up in aqueous NH_4Cl and extracted three times with CH_2Cl_2. The combined organic fractions were dried over $MgSO_4$ and concentrated to give 6.1 mg of (1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(2-methoxyethoxy)phenyl)methanol (**3.34**) as an orange solid (55%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.12 (dd, J = 8.6, 5.7 Hz, 2H), 7.01 (q, J = 8.0 Hz, 1H), 6.96-6.89 (m, 3H), 6.84 (dd, J = 8.0, 1.7 Hz, 1H), 4.60 (d, J = 8.0 Hz, 1H), 4.14 (dd, J = 5.2, 4.0 Hz, 2H), 3.90 (s, 3H), 3.78 (dd, J = 6.3, 4.0 Hz, 2H), 3.44 (s, 3H), 3.05 (d, J = 11.4 Hz, 1H), 2.91 (d, J = 11.4 Hz, 1H), 2.75 (dd, J = 8.6, 5.8 Hz, 2H), 2.50 (dd, J = 8.6, 8.3 Hz, 2H), 2.05 (d, J = 13.2 Hz, 1H), 1.94 (ddd, J = 12.0, 2.3, 2.3 Hz, 1H), 1.87 (dd, J = 11.4, 2.8 Hz, 1H), 1.66 (qt, J = 11.5, 4.0 Hz, 1H), 1.45 (qd, J = 12.6, 3.5 Hz, 1H), 1.35 (qd, J = 11.8, 3.5 Hz, 1H), 1.27 (d, J = 11.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.3, 160.4, 151.7, 146.9, 136.6, 136.1, 130.1, 130.0, 123.8, 120.3, 115.2, 115.0, 113.0, 74.6, 71.1, 68.1, 60.9, 59.2, 53.8, 53.5, 42.9, 32.9, 28.8; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{24}H_{32}FNO_4$: 418.2394, measured 418.2379.

(2,3-dimethoxyphenyl)(1-(4-hydroxyphenethyl)piperidin-4-yl)methanone (3.35)



A solution of (2,3-dimethoxyphenyl)(piperidin-4-yl)methanone (**3.11**) (3.94 g, 15.8 mmol), 4-hydroxyphenethyl bromide (3.49 g, 17.4 mmol), and $NaHCO_3$ (1.99 g, 23.7 mmol) in 70 mL DMF was stirred at 110 ^oC for 48 hours. The reaction was then cooled to room temperature and the DMF was removed under reduced pressure. The residue was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 3.0 g of (2,3-dimethoxyphenyl)(1-(4-hydroxyphenethyl)piperidin-4-yl)methanone (**3.35**) as a red solid (53%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.10-7.00 (m, 3H), 6.95 (d, J = 8.0 Hz, 2H), 6.71 (d, J = 8.0 Hz, 2H), 3.90 (s, 3H), 3.85 (s, 3H), 3.17-3.13 (m, 1H), 3.03 (d, J = 10.9 Hz, 2H), 2.73 (dd, J = 9.2, 4.6 Hz, 2H), 2.61 (dd, J = 9.5, 4.5 Hz, 2H), 2.27 (dd, J = 10.3, 10.3 Hz, 2H), 1.96 (d, J = 11.5 Hz, 2H), 1.82 (qd, J = 13.7, 3.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 154.9, 152.8, 147.0, 134.1, 131.0, 130.2, 129.8, 124.6, 120.5, 115.9, 115.7, 115.1, 63.9, 61.9, 60.8, 56.1, 53.0, 47.5, 38.4, 32.1, 27.3; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{22}H_{27}NO_4$: 370.2018, measured 320.2001.

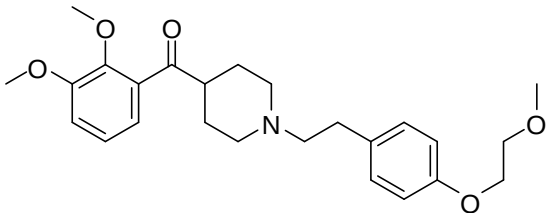
(1-(4-butoxyphenethyl)piperidin-4-yl)(2,3-dimethoxyphenyl)methanone (3.37)



A solution of (2,3-dimethoxyphenyl)(1-(4-hydroxyphenethyl)piperidin-4-yl)methanone (**3.35**) (215 mg, 0.58 mmol), butyl 4-methylbenzenesulfonate (**3.36**) (399 mg, 1.75 mmol), and K_2CO_3 (160 mg, 1.16 mmol) in 7 mL acetone was refluxed for 2 days. The

reaction was cooled to room temperature and the acetone was removed under reduced pressure. Flash chromatography (100% CH_2Cl_2 to 10% MeOH) isolated 173 mg of -(4-butoxyphenethyl)piperidin-4-yl)(2,3-dimethoxyphenyl)methanone (**3.37**) as a red solid (70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (dd, J = 8.7, 6.9 Hz, 3H), 6.99 (ddd, J = 14.6, 8.2, 1.8 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 3.93 (t, J = 6.9 Hz, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.08 (tt, J = 11.0, 3.7 Hz, 1H), 2.96 (d, J = 11.9 Hz, 2H), 2.73 (dd, J = 7.2, 5.0 Hz, 2H), 2.57-2.52 (m, 2H), 2.11 (ddd, J = 11.5, 2.3, 2.3 Hz, 2H), 1.91 (dd, J = 13.8, 2.8 Hz, 2H), 1.78-1.71 (m, 4H), 1,47 (sextet, J = 7.8 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.6, 157.5, 152.8, 147.0, 134.5, 132.3, 129.6, 124.4, 120.3, 114.8, 114.5, 67.7, 61.8, 61.1, 56.0, 53.4, 48.2, 32.8, 31.5, 28.1, 19.3, 14.0; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{26}H_{35}NO_4$: 426.2644, measured 426.2629.

(2,3-dimethoxyphenyl)(1-(4-(2-methoxyethoxy)phenethyl)piperidin-4-yl)methanone (3.39)



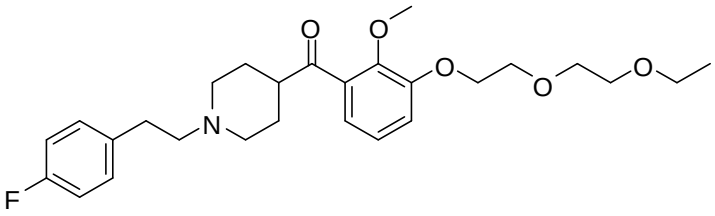
A solution of (2,3-dimethoxyphenyl)(1-(4-hydroxyphenethyl)piperidin-4-yl)methanone (**3.35**) (214 mg, 0.58 mmol), 2-methoxyethyl 4-methylbenzenesulfonate (**3.38**) (401 mg, 1.74 mmol), and K_2CO_3 (160 mg, 1.16 mmol) in 12 mL acetone was refluxed for 2 days. The reaction was cooled to room temperature and the acetone was removed under reduced pressure. Flash chromatography (100% CH_2Cl_2 to 10% MeOH) isolated 155 mg of (2,3-dimethoxyphenyl)(1-(4-(2-methoxyethoxy)phenethyl)piperidin-4-yl)methanone

(**3.39**) as a red oil (62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (dd, J = 8.2, 7.3 Hz, 3H), 6.98 (ddd, J = 16.1, 8.3, 1.3 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 4.09 (dd, J = 4.5, 3.2 Hz, 2H), 3.88 (s, 3H), 3.84 (s, 3H), 3.73 (dd, J = 4.5, 3.2 Hz, 2H), 3.44 (s, 3H), 3.08 (tt, J = 11.0, 4.1 Hz, 1H), 2.99 (d, J = 11.5 Hz, 2H), 2.74 (dd, J = 7.7, 5.1 Hz, 2H), 2.54 (dd, J = 7.7, 5.0 Hz, 2H), 2.11 (ddd, J = 11.0, 1.8, 1.8 Hz, 2H), 1.91 (d, J = 11.5 Hz, 2H), 1.75 (ddd, J = 11.0, 11.0, 3.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.6, 157.2, 152.8, 146.9, 134.4, 132.8, 129.7, 124.4, 120.3, 114.8, 114.6, 71.2, 67.3, 61.8, 61.1, 59.3, 56.0, 53.4, 48.2, 32.8, 28.1; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{25}H_{33}NO_5$: 428.2437, measured 428.2453.

General procedure for O-alkylation of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) with a tosylated alkylating agent.

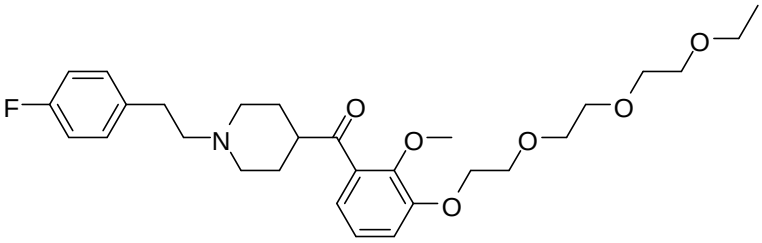
A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (105 mg, 0.30 mmol), a tosylated alkylating agent **3.43**, **3.44**, or **3.45** (0.60 mmol, 190-218 mg), and K_2CO_3 (62 mg, 0.45 mmol) in 8 mL acetone was refluxed for 24 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2).

(3-(2-(2-ethoxyethoxy)ethoxy)-2-methoxyphenyl)(1-(4-fluorophenethyl) piperidin-4-yl)methanone (3.46)



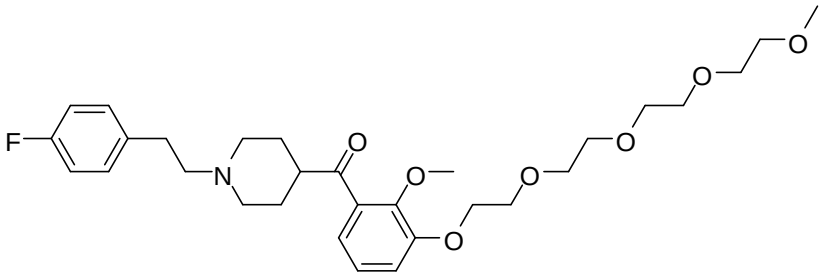
The above procedure with 2-(2-ethoxyethoxy)ethyl 4-methylbenzenesulfonate (**3.43**) as the alkylating agent produced 145 mg of (3-(2-(2-ethoxyethoxy)ethoxy)-2-methoxyphenyl)(1-(4-fluorophenethyl) piperidin-4-yl)methanone (**3.46**) (93%) as a red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17-7.13 (m, 2H), 7.07-7.01 (m, 2H), 7.0-6.93 (m, 3), 4.20 (dd, J = 5.1, 4.6 Hz, 2H), 3.91 (dd, J = 6.9, 2.9 Hz, 2H), 3.91 (s, 3H), 3.72 (dd, J = 6.3, 4.6 Hz, 2H), 3.61 (dd, J = 6.3, 4.5 Hz, 2H), 3.52 (q, J = 7.5 Hz, 2H), 3.12 (tt, J = 10.9, 4.0 Hz, 1H), 3.00 (d, J = 11.5 Hz, 2H), 2.78 (dd. J = 8.5, 5.8 Hz, 2H), 2.57 (dd, J = 8.6, 5.8 Hz, 2H), 2.17 (dd, J = 11.5, 9.7 Hz, 2H), 1.93 (d, J = 10.9 Hz, 2H), 1.79 (qd, J = 10.9, 2.9 Hz, 2H). 1.21 (t, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.2, 162.1, 160.2, 151.7, 147.1, 134.1, 129.9, 129.8, 124.0, 120.5, 116.3, 115.0, 114.9, 70.7, 69.7, 69.5, 68.2, 66.5, 61.5, 60.4, 53.0, 32.5, 27.7, 15.0; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{27}H_{36}FNO_5$: 496.2475, measured 496.2455.

(3-(2-(2-(2-ethoxyethoxy)ethoxy)ethoxy)-2-methoxyphenyl)(1-(4-fluorophenethyl) piperidin-4-yl)methanone (3.47)



The above procedure with 2-(2-(2-ethoxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**3.44**) as the alkylating agent produced 153 mg of (3-(2-(2-(2-ethoxyethoxy)ethoxy)ethoxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone **(3.47**) (99%) as a red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16-7.13 (m, 2H), 7.08-7.02 (m, 2H), 7.00-6.93 (m, 3H), 4.19 (dd, J = 5.1, 4.6 Hz, 2H), 3.90 (dd, J = 5.7, 3.4 Hz, 2H), 3.90 (s, 3H), 3.74 (dd, J = 5.8, 2.9 Hz 2H), 3.68 (dd, J = 5.8, 2.9 Hz, 2H), 3.65 (dd, J = 5.4, 2.9 Hz, 2H), 3.58(dd, J = 5.4, 2.9 Hz, 2H) 3.52 (q, J = 6.9 Hz, 2H), 3.13 (tt, J = 10.9, 4.0 Hz, 1H), 3.01 (d, 11.5 Hz, 2H), 2.78 (dd, J = 8.0, 5.1 Hz, 2H), 2.61 (dd, J = 8.3, 5.2 Hz, 2H), 2.19 (t, J = 10.2 Hz, 2H), 1.93 (d, J = 11.4 Hz, 2H), 1.79 (qd, J = 10.3, 3.4 Hz, 2H), 1.21 (t, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.1, 162.1, 160.2, 151.7, 147.1, 135.6, 134.0, 129.9, 129.8, 124.0, 120.5, 116.4, 115.0, 114.8, 70.6, 70.5, 70.4, 69.6, 69.4, 68.2, 66.4, 61.5, 60.2, 52.8, 47.6, 32.3, 27.5, 15.0; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{29}H_{40}FNO_6$: 518.2918, measured 518.2904.

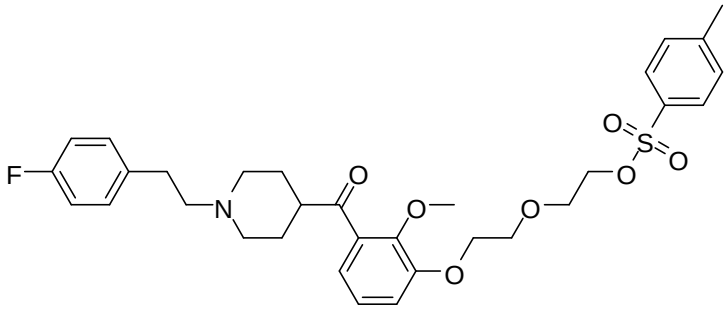
(3-(2,5,8,11-tetraoxatridecan-13-yloxy)-2-methoxyphenyl)(1-(4-fluorophenethyl) piperidin-4-yl)methanone (3.48)



The above procedure with 2,5,8,11-tetraoxatridecan-13-yl 4-methylbenzenesulfonate (**3.45**) as the alkylating agent produced 164 mg of (3-(2,5,8,11-tetraoxatridecan-13-yloxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (**3.48**) (100%)

as a red oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (dd, J = 8.6, 5.1 Hz, 2H), 7.07-7.02 (m, 2H), 7.00-6.93 (m, 3H), 4.18 (dd, J = 5.2, 4.6 Hz, 2H), 3.90 (dd, J = 5.2, 4.6 Hz, 2H), 3.90 (s, 3H), 3.73 (dd, J = 6.3, 4.0 Hz, 2H), 3.69-3.62 (m, 8H), 3.54 (dd, J = 6.3, 4.0 Hz, 2H), 3.37 (s, 3H), 3.11 (tt, J = 10.8, 4.0 Hz, 1.0H), 2.99 (d, J = 11.4 Hz, 2H), 2.77 (dd, J = 8.1, 5.2 Hz, 2H), 2.57 (dd, J = 8.1, 5.7 Hz, 2H), 2.15 (t, J = 10.9 Hz, 2H), 1.92 (d, J = 10.9 Hz, 2H), 1.77 (qd, J = 10.9, 3.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.1, 162.0, 160.0, 151.6, 147.1, 135.6, 134.0, 129.9, 129.8, 123.9, 120.4, 116.2, 114.9, 114.7, 71.6, 70.5, 70.4, 70.3, 70.2, 69.4, 68.1, 62.1, 61.4, 58.8, 47.9, 32.4, 27.6; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{30}H_{42}FNO_7$: 548.3024, measured 548.3030.

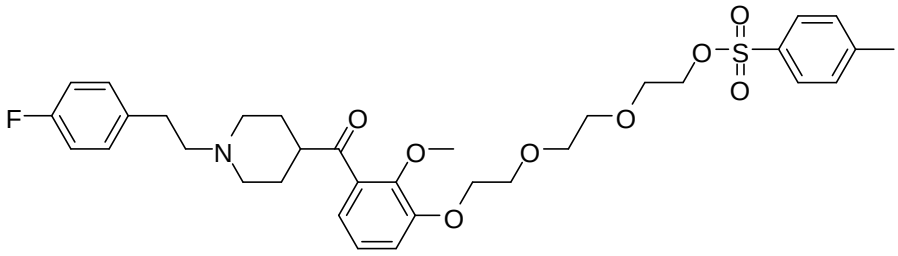
2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy) ethoxy) ethyl 4-methylbenzenesulfonate (3.68)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (295 mg, 0.82 mmol), 2,2'-oxybis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (**3.54**) (1.4 g, 3.3 mmol), and K_2CO_3 (228 mg, 1.65 mmol) in 20 mL acetone was refluxed for 24 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 437 mg of 2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-

2-methoxyphenoxy) ethoxy) ethyl 4-methylbenzenesulfonate (**3.68**) as a red oil (89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.1, 2H), 7.15 (dd, J = 8.6, 5.7 Hz, 2H), 7.07-7.01 (m, 1H), 6.95 (tt, J = 8.6, 1.7 Hz, 2H), 4.17 (dd, J = 4.6, 4.5 Hz, 2H), 4.10 (dd, J = 4.6, 4.5 Hz, 2H), 3.87 (s, 3H), 3.82 (dd, J = 4.6, 3.8 Hz, 2H), 3.75 (dd, J = 4.6, 2.9 Hz, 2H), 3.11 (tt, J = 10.9, 4.1 Hz, 1H), 3.00 (d, J = 1.4 Hz, 2H), 2.78 (dd, J = 10.9, 5.1 Hz, 2H), 2.57 (dd, J = 10.8, 5.2 Hz, 2H), 2.41 (s, 3H), 2.15(t, J = 10.3 Hz, 2H), 1.92 (d, J = 11.4 Hz, 2H), 1.76 (qd, J = 11.5, 3.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.2,162.2, 160.3, 151.8, 147.3, 144.9, 135.9, 134.2, 132.8, 130.1, 130.0, 129.8, 127.9, 124.1, 120.7, 116.5, 115.1, 114.9, 69.6, 69.3, 68.7, 68.3, 61.6, 60.6, 53.5, 53.2, 47.9, 32.7, 27.9, 21.6; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{32}H_{38}FNO_7S$: 600.2431, measured 600.2437.

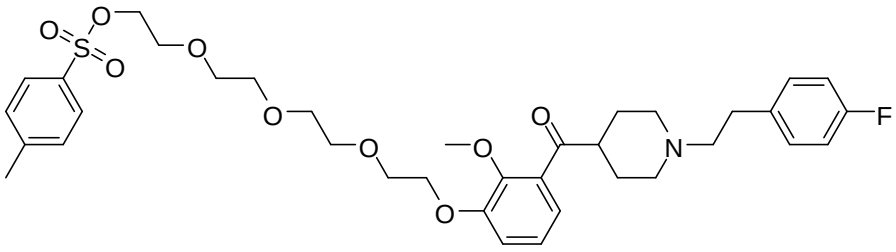
2-(2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)ethoxy) ethoxy)ethyl 4-methylbenzenesulfonate (3.69)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (78.3 mg, 0.22 mmol), 2,2'-(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (**3.55**) (215 mg, 0.44 mmol), and K_2CO_3 (61 mg, 0.44 mmol) in 7 mL acetone was refluxed for 24 hours. The reaction mixture was cooled to room temperature and the solvent was removed under

reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 53 mg of 2-(2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**3.69**) as an orange oil (38%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 8.0 Hz, 2H), 7.33 (dd, J = 8.0, 5.5 Hz, 2H), 7.14 (dd, J = 8.6, 5.2 Hz, 2H), 7.07-7.01 (m, 2H), 6.99-6.94 (m, 3H), 4.19-4.13 (m, 4H), 3.88 (dd, J = 9.6, 5.6 Hz, 2H), 3.88 (s, 3H), 3.70-3.68 (m, 4H), 3.61 (dd, J = 6.6, 4.0 Hz, 2H), 3.09 (tt, J = 10.8, 3.4 Hz, 1H), 2.98 (d, J = 12.9 Hz, 2H), 2.77 (dd, J = 8.6, 5.1 Hz, 2H), 2.56 (dd, J = 8.6, 5.1 Hz 2H), 2.43 (s, 3H), 2.13 (t, J = 10.9 Hz, 2H), 1.91 (d, J = 11.5 Hz, 2H), 1.75 (qd, J = 11.5, 3.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 162.4, 160.4, 152.0, 147.4, 145.0, 136.1, 134.5, 133.0, 130.2, 130.1, 130.0, 128.0, 124.3, 120.8, 116.6, 115.2, 115.1, 70.9, 70.8, 69.8, 69.3, 68.8, 68.5, 61.7, 60.8, 53.3, 48.1, 32.9, 28.1, 21.7; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{34}H_{42}FNO_8S$: 644.2693, measured 644.2697.

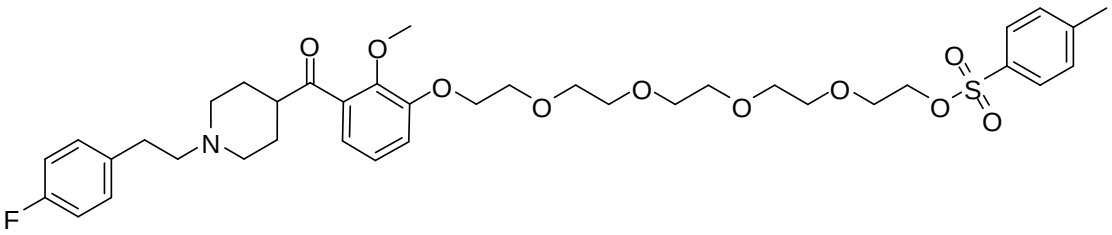
2-(2-(2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy) ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (3.70)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (80 mg, 0.22 mmol), 2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (**3.56**) (452 mg, 0.90

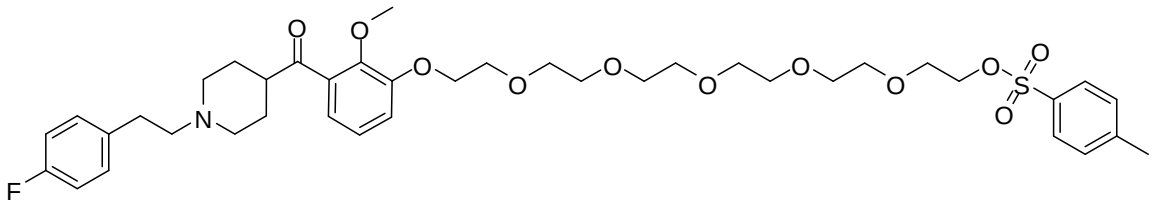
mmol), and K_2CO_3 (62 mg, 0.45 mmol) in 7 mL acetone was refluxed for 24 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 143 mg of 2-(2-(2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**3.70**) as an orange oil (93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J = 8.6 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.15 (dd, J = 8.6, 5.1 Hz, 2H), 7.07-7.02 (m, 2H), 6.99-6.92 (m, 3H), 4.18 (dd, J = 5.2, 4.5 Hz, 2H), 4.15 (dd, J = 4.6, 4.2 Hz, 2H), 3.90 (dd, J = 5.7, 4.0 Hz, 2H), 3.90 (s, 3H), 3.72 (dd, J = 6.3, 4.0 Hz, 2H), 3.67 (dd, J = 5.2, 4.6 Hz, 2H), 3.64 (dd, J = 6.3, 4.0 Hz, 2H), 3.58 (septet, J = 2.9 Hz, 4H), 3.10 (tt, J = 10.9, 4.0 Hz, 1H), 2.98 (d, J = 11.5 Hz, 2H), 2.77 (dd, J = 10.3, 5.7 Hz), 2.56 (dd, J = 10.3, 5.7 Hz, 2H), 2.43 (s, 3H), 2.12 (dd, J = 11.4, 9.8 Hz, 2H), 1.91 (d, J = 11.5 Hz, 2H), 1.75 (qd, J = 10.9, 3.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.4, 162.3, 160.4, 151.9, 147.3, 144.9, 136.1, 134.4, 132.9, 130.1, 130.0, 129.9, 128.0, 124.2, 120.7, 116.5, 115.2, 115.0, 70.8, 70.7, 70.6, 70.5, 69.7, 69.3, 68.7, 68.4; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{36}H_{46}FNO_9S$: 710.2775, measured 710.2780.

14-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate (3.71)



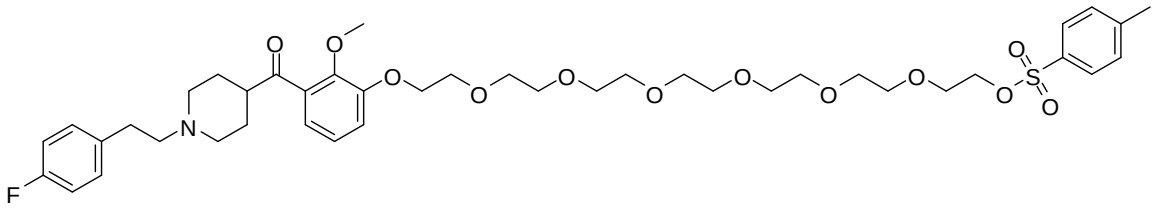
A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (119 mg, 0.34 mmol), 3,6,9,12-tetraoxatetradecane-1,14-diyl bis(4-methylbenzenesulfonate) (**3.57**) (738 mg, 1.35 mmol), and K_2CO_3 (94 mg, 0.68 mmol) in 11 mL acetone was refluxed for 24 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 163 mg of 14-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate (**3.71**) as an orange oil (66%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.14 (dd, J = 8.6, 5.8 Hz, 2H), 7.07-7.01 (m, 2H), 6.95 (q, J = 8.6 Hz, 3H), 4.18 (dd, J = 5.2, 4.6 Hz, 2H), 4.15 (dd, J = 5.2, 4.5 Hz, 2H), 3.90 (dd, J = 5.1, 4.5 Hz, 2H), 3.89 (s, 3H), 3.73 (dd, J = 5.1, 4.0 Hz, 2H), 3.69-3.65 (m, 2H), 3.65-3.60 (m, 2H), 3.58 (s, 4H), 3.09 (tt, J = 10.9, 4.0 Hz, 1H), 2.98 (d, J = 11.5 Hz, 2H), 2.77 (dd, J = 8.6, 7.5 Hz, 2H), 2.54 (dd, J = 8.6, 7.5 Hz, 2H), 2.44 (s, 3H), 2.12 (t, J = 10.8 Hz, 2H), 1.90 (d, J = 11.4 Hz, 2H), 1.76 (qd, J = 11.4, 3.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.6, 162.4, 160.5, 152.0, 147.4, 145.0, 136.2, 134.5, 133.1, 130.2, 130.1, 129.9, 128.1, 124.3, 120.8, 116.6, 115.3, 115.1, 70.9, 70.8, 70.7, 70.6, 69.8, 69.4, 68.8, 68.5, 61.8, 60.8, 53.4, 48.2, 33.0, 28.1, 21.8; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{38}H_{50}FNO_{10}S$: 754.3037, measured 754.3030.

17-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (3.72)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (130 mg, 0.36 mmol), 3,6,9,12,15-pentaoxaheptadecane-1,17-diyl bis(4-methylbenzenesulfonate) (**3.58**) (850 mg, 1.44 mmol), and K_2CO_3 (100 mg, 0.72 mmol) in 10 mL acetone was refluxed for 24 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 209 mg of 17-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (**3.72**) as an orange-yellow oil (75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J = 6.9 Hz, 2H), 7.33 (d, J = 6.9 Hz, 2H), 7.14 (dd, J = 6.3, 5.8 Hz, 2H), 7.07-7.03 (m, 2H), 6.98-6.93 (m, 3H), 4.16 (ddd, J = 16.6, 6.3, 3.4 Hz, 4H), 3.90 (s, 3H), 3.90 (dd, J = 4.6, 1.1 Hz, 2H), 3.72 (d, J = 3.4 Hz, 2H), 3.66 (dd, J = 12.6, 12.6 Hz, 14H), 3.56 (dd, J = 4.6, 1.1 Hz, 2H), 3.09 (m, 1H), 2.98 (d, J = 9.7 Hz, 2H), 2.77 (dd, J = 8.6, 6.3 Hz, 2H), 2.55 (dd, J = 9.2, 5.7 Hz, 2H), 2.43 (s, 3H), 2.11 (t, J = 10.9 Hz, 2H), 1.90 (d, J = 12.0 Hz, 2H), 1.75 (dd, J = 10.9, 10.3 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5,162.3, 160.4, 152.0, 147.4, 144.9, 136.2, 134.4, 133.0, 130.1, 130.0, 129.9, 128.0, 124.2, 120.7, 116.6, 115.2, 115.0, 70.8, 70.7, 70.6, 70.6, 70.5, 69.4, 68.7, 61.7, 60.7, 53.6, 53.3, 48.1, 32.9, 28.1, 21.7; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{40}H_{54}FNO_{11}S$: 798.3299, measured 798.3286.

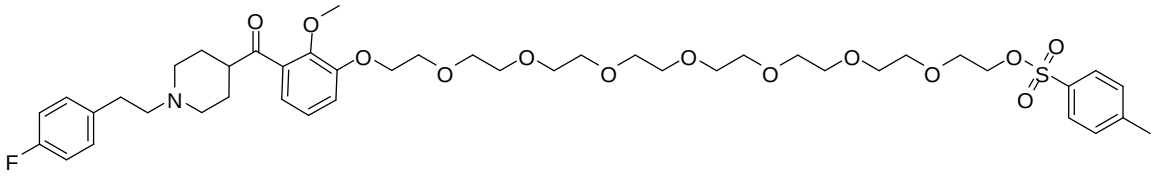
20-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15,18-hexaoxaicosyl 4-methylbenzenesulfonate (3.73)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (55 mg, 0.15 mmol), 3,6,9,12,15,18-hexaoxaicosane-1,20-diyl bis(4-methylbenzenesulfonate) (**3.66**) (198 mg, 0.31 mmol), and K_2CO_3 (43 mg, 0.31 mmol) in 5 mL acetone was refluxed for 24 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 78 mg of 20-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15,18-hexaoxaicosyl 4-methylbenzenesulfonate (**3.73**) as an orange-yellow oil (62%). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.80 (dd, J = 1.8, 8.2 Hz, 2.0H), 7.34 (dd, J = 7.7, 2.0 Hz, 2H), 7.15 (dd, J = 8.7, 5.5 Hz, 2H), 7.08-7.01 (m, 2H), 6.99-6.93 (m, 3H), 4.19-4.14 (m, 4H), 3.89 (dd, J = 6.8, 2.8 Hz, 2H), 3.89 (s, 3H), 3.75-3.57 (m, 22H), 3.09 (tt, J = 11.0, 3.7 Hz, 1H), 2.98 (d, J = 11.9 Hz, 2H), 2.77 (dd, J = 10.7, 7.3 Hz, 2H), 2.56 (dd, J = 11.0, 7.8 Hz, 2H), 2.44 (s, 3H), 2.13 (t, J = 10.9 Hz, 2H), 1.91 (d, J = 11.5 Hz, 2H), 1.75 (qd, J = 11.0, 3.6 Hz, 2H); ^{13}C NMR (400MHz, $CDCl_3$) δ 151.9, 146.4, 144.9, 136.1, 134.5, 133.0, 130.2, 130.1, 129.9, 128.0, 124.3, 120.8, 116.5, 115.3, 115.1, 72.6, 70.9, 70.8, 70.7, 70.6, 70.3, 69.7, 69.3, 68.7, 68.5, 61.7,

60.8, 53.6, 53.3, 48.1, 32.9, 28.1, 21.7; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{42}H_{58}FNO_{12}S$: 842.3561, measured 842.3548.

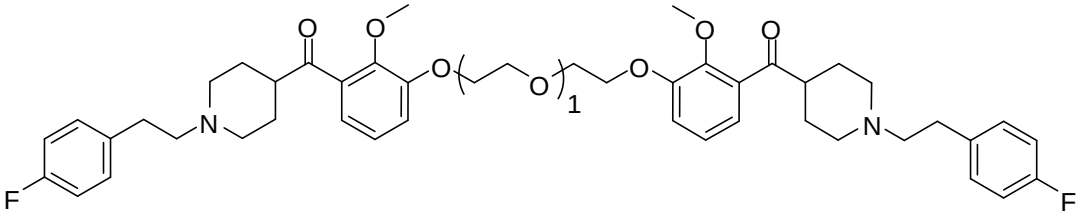
23-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15,18,21-heptaoxatricosyl 4-methylbenzenesulfonate (3.74)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (62 mg, 0.17 mmol), 3,6,9,12,15,18,21-heptaoxatricosane-1,23-diyl bis(4-methylbenzenesulfonate) (**3.67**) (350 mg, 0.52 mmol), and K_2CO_3 (47 mg, 0.34 mmol) in 10 mL acetone is refluxed for 24 hours. The reaction mixture is cooled to room temperature and the solvent is removed under reduced pressure. The crude reaction mixture is purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 110 mg of 23-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15,18,21-heptaoxatricosyl 4-methylbenzenesulfonate (**3.74**) as an orange-yellow oil (75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 8.6 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.16 (dd, J = 8.6, 5.2 Hz, 2H), 7.07-7.02 (m, 2H), 6.99-6.93 (m, 3H), 4.18 (dd, J = 5.1, 4.6 Hz, 2H), 4.15 (dd, J = 5.2, 4.0 Hz, 2H), 3.90 (dd, J = 5.7, 4.0 Hz, 2H), 3.90 (s, 3H), 3.72 (dd, J = 6.3, 4.0 Hz, 2H), 3.69-3.60 (m, 20H), 3.58 (s, 4H), 3.10 (tt, J = 10.9, 4.0 Hz, 1H), 2.98 (d, J = 11.5 Hz, 2H), 2.77 (dd, J = 8.6, 5.1 Hz, 2H), 2.56 (dd, J = 8.3, 5.7 Hz, 2H), 2.44 (s, 3H), 2.13 (t, J = 10.9 Hz, 2H), 1.91 (d, J = 12 Hz, 2H), 1.75 (qd, J = 11.4, 3.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 162.4, 160.4, 152.0, 147.4, 144.9, 136.1, 134.4, 133.0, 130.2,

130.1, 129.9, 128.0, 124.2, 120.7, 116.5, 115.2, 115.1, 70.9, 70.8, 70.7, 70.7, 70.6, 70.5, 69.7, 69.3, 68.7, 68.5, 61.7, 60.8, 53.6, 53.3, 48.1, 32.9, 28.1, 21.7; HRMS – ESI: m/z [M + Na]$^+$ calculated for $C_{44}H_{62}FNO_{13}S$: 886.3824, measured 886.3818.

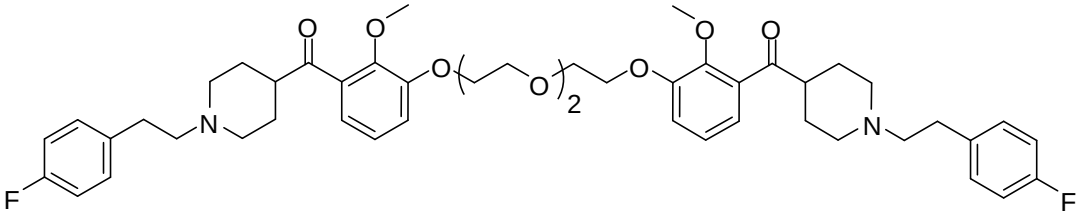
(3,3'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.75)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (68 mg, 0.19 mmol), 2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy) ethoxy)ethyl 4-methylbenzenesulfonate (**3.68**) (57 mg, 0.096 mmol), and K_2CO_3 (26 mg, 0.19 mmol) in 5 mL acetone was refluxed for 48 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 72 mg of (3,3'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (**3.75**) as an orange oil (96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (dd, J = 8.7, 5.5 Hz, 4H), 7.08-7.01 (m, 4H), 7.00-6.93 (m, 6H), 4.20 (dd, J = 5.1, 4.6 Hz, 4H), 3.98 (dd, J = 5.1, 4.6 Hz, 4H), 3.88(s, 6H), 3.07 (tt, J = 11.0, 4.2, Hz, 2H), 2.97(d, J = 11.5 Hz, 4H), 2.77 (dd, J = 8.7, 5.5 Hz, 4H), 2.56 (dd, J = 8.3, 5.2 Hz, 4H), 2.12 (t, J = 10.3 Hz, 4H), 1.90 (d, J = 11 Hz, 4H), 1.75 (qd, J = 11.0, 3.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.5, 162.7, 160.3,

152.0, 147.5, 136.1, 134.7, 130.3, 130.2, 124.4, 121.0, 116.7, 115.4, 115.2, 70.1, 69.0, 61.8, 60.9, 53.4, 48.2, 33.0, 28.2; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{46}H_{54}F_2N_2O_7$: 785.3977, measured 785.3966.

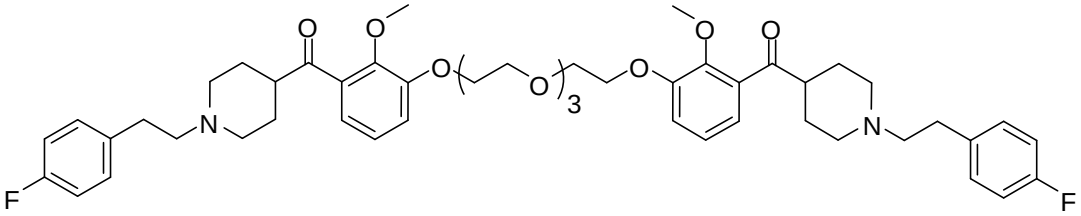
(3,3'-(2,2'-(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.76)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (59 mg, 0.164 mmol), 2-(2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**3.69**) (53 mg, 0.082 mmol), and K_2CO_3 (23 mg, 0.164 mmol) in 5 mL acetone was refluxed for 48 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 31 mg of (3,3'-(2,2'-(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (**3.76**) as an orange oil (44%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (dd, J = 8.3, 5.5 Hz, 4H), 7.07-7.01 (m, 4H), 7.00-6.94 (m, 6H), 4.18 (dd, J = 5.0, 4.6 Hz, 4H), 3.90 (dd, J = 5.0, 4.6 Hz, 4H), 3.88 (s, 6H), 3.75 (s, 6H), 3.09 (tt, J = 10.9, 4.0 Hz, 2H), 2.99 (d, J = 11.5 Hz, 4H), 2.79 (dd, J = 11.0, 5.0 Hz, 4H), 2.58 (dd, J = 11.0, 5.5 Hz, 4H), 2.16 (t, J = 10.3 Hz, 4H), 1.92 (d, J = 11 Hz, 4H), 1.76 (qd, J = 11.4, 3.6, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ

206.4, 162.7, 151.9, 147.4, 135.9, 134.5, 130.2, 130.1, 124.3, 120.8, 116.5, 115.3, 115.1, 71.0, 69.8, 68.5, 61.7, 60.6, 53.2, 47.9, 32.7, 27.9; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{48}H_{58}F_2N_2O_8$: 851.4059, measured 851.4045.

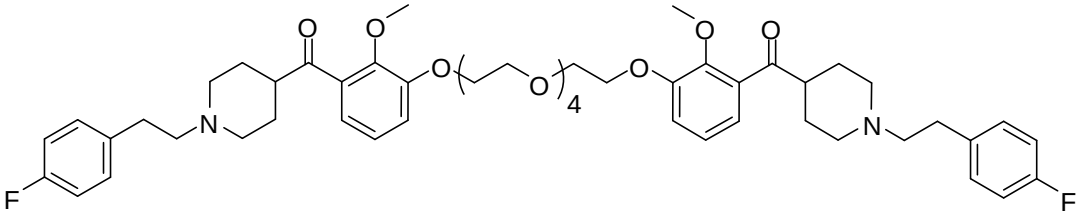
(3,3'-(2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.77)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (44 mg, 0.12 mmol), 2-(2-(2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy) ethoxy) ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**3.70**) (42 mg, 0.062 mmol), and K_2CO_3 (17 mg, 0.124 mmol) in 5 mL acetone was refluxed for 48 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 28 mg of (3,3'-(2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (**3.77**) as an red oil (51%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16-7.13 (m,4H), 7.07-7.00 (m, 4H), 6.99-6.94 (m, 6H), 4.17 (dd, J = 5.2, 4.6 Hz, 4H), 3.89 (dd, J = 5.2, 4.6 Hz, 4H), 3.89 (s, 6H), 3.73 (dd, J = 3.4, 3.4 Hz, 2H), 3.72 (dd, J = 3.5, 3.5 Hz, 2H), 3.68 (dd, J = 2.3, 2.3 Hz, 2H), 3.67 (dd, J = 3.5, 3.5 Hz, 2H), 3.10 (tt, J = 10.9, 4.0 Hz, 2H), 2.98 (d, J = 11.5 Hz, 4H), 2.79 (dd, J = 8.0, 8.0 Hz, 4H), 2.57 (dd, J = 8.0, 8.0

Hz, 4H), 2.15 (bs, 4H), 1.92 (d, J = 12.0 Hz, 4H), 1.76 (q, J = 10.9 Hz, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 162.5, 160.6, 152.1, 147.5, 136.0, 134.6, 130.3, 130.2, 124.4, 120.9, 116.7, 115.4, 115.2, 71.0, 70.9, 69.8, 68.6, 61.8, 60.8, 53.3, 48.0, 32.8, 28.0; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{50}H_{62}F_2N_2O_9$: 895.4321, measured 895.4341.

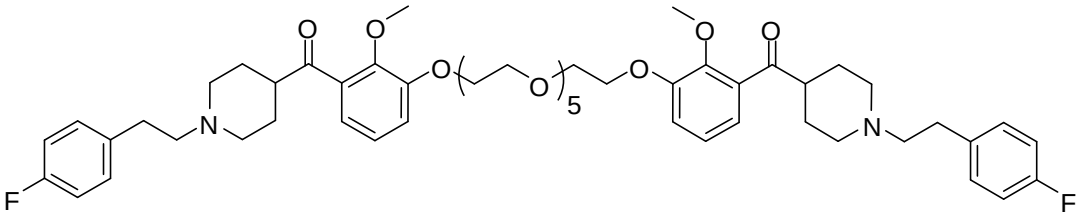
(3,3'-(3,6,9,12-tetraoxatetradecane-1,14-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.78)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (157 mg, 0.44 mmol), 14-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate (**3.71**) (163 mg, 0.22 mmol), and K_2CO_3 (61 mg, 0.44 mmol) in 12 mL acetone was refluxed for 48 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 102 mg of (3,3'-(3,6,9,12-tetraoxatetradecane-1,14-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (**3.78**) as an red-brown oil (51%). ^1H NMR (500 MHz, $CDCl_3$) δ 7.15 (dd, J = 8.0, 5.1 Hz, 4H), 7.069-7.01 (m, 4H), 6.99-6.97 (m, 6H), 4.18 (dd, J = 4.6, 4.6 Hz, 4H), 3.89 (dd, J = 4.6, 4.6 Hz,

4H), 3.89 (s, 6H), 3.73 (dd, J = 6.8, 4.0 Hz, 4H), 3.67 (dd, J = 5.1, 2.9 Hz, 4H), 3.64 (s, 4H) 3.08 (tt, J = 10.9, 4.19 Hz, 2H), 2.98 (d, J = 11.4 Hz, 4H), 2.78 (dd, J = 8.6, 7.4 Hz, 4H), 2.56 (dd, J = 7.4, 5.4 Hz, 4H), 2.13 (t, J = 10.9 Hz, 4H), 1.91 (d, J = 11.4 Hz, 4H), 1.77 (qd, J = 10.8, 2.9 Hz, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 162.5, 160.5, 152.0, 147.4, 136.1, 134.6, 130.2, 124.3, 120.8, 116.6, 115.3, 115.1, 70.9, 70.8, 70.7, 69.8, 68.5, 61.8, 60.8, 53.4, 48.1, 32.9, 28.1; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{52}H_{66}F_2N_2O_{10}$: 939.4583, measured 939.4551.

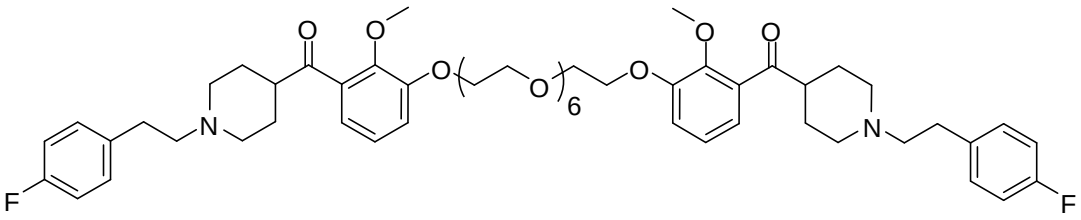
(3,3'-(3,6,9,12,15-pentaoxaheptadecane-1,17-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.79)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (193 mg, 0.54 mmol), 17-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (**3.72**) (209 mg, 0.27 mmol), and K_2CO_3 (75 mg, 0.54 mmol) in 12 mL acetone was refluxed for 48 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 125 mg of (3,3'-(3,6,9,12,15-pentaoxaheptadecane-1,17-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (**3.79**) as an red-brown oil (48%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (dd,

J = 8.6, 5.2 Hz, 4H), 7.07-7.01 (m, 4H), 6.99-6.94 (m, 6H), 4.18 (dd, J = 5.2, 4.6 Hz, 4H), 3.89 (dd, J = 5.2, 4.6 Hz, 4H), 3.89 (s, 6H), 3.73 (dd, J = 6.3, 4.0 Hz, 4H), 3.66 (dd, J = 6.9, 4.0 Hz, 4H), 3.64 (s, 8H), 3.09 (tt, J = 10.9, 4.0 Hz, 2H), 2.98 (d, J = 11.4 Hz, 4H), 2.78 (dd, J = 8.6, 5.5 Hz, 4H), 2.56 (dd, J = 8.6, 5.2 Hz, 4H), 2.1 (dd, J = 10.9, 10.9 Hz, 4H), 1.91 (d, J = 11.4 Hz, 4H), 1.76 (qd, J = 10.9, 3.4 Hz, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 162.5, 160.5, 152.0, 147.4, 136.1, 134.6, 130.2, 130.1, 124.3, 120.8, 116.6, 115.3, 115.5, 70.9, 70.8, 70.7, 70.7, 69.8, 68.5, 61.8, 60.8, 53.3, 48.1, 32.9, 28.1; HRMS – ESI: m/z $[M + Na]^+$ calculated for $C_{54}H_{70}F_2N_2O_{11}$: 983.4845, measured 938.4855.

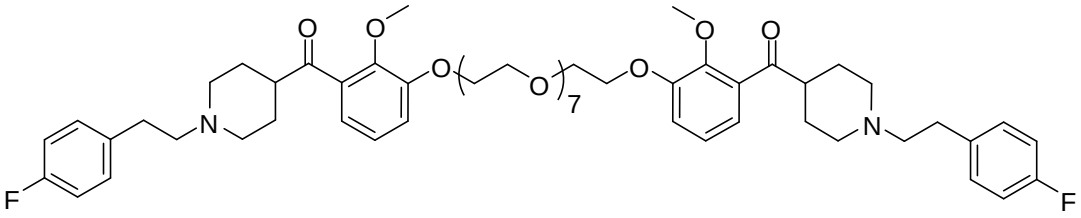
(3,3'-(3,6,9,12,15,18-hexaoxaicosane-1,20-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.80)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (71 mg, 0.20 mmol), 20-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15,18-hexaoxaicosyl 4-methylbenzenesulfonate (**3.73**) (84 mg, 0.10 mmol), and K_2CO_3 (28 mg, 0.20 mmol) in 8 mL acetone was refluxed for 48 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2) to afford 62 mg of (3,3'-(3,6,9,12,15,18-hexaoxaicosane-1,20-diylbis(oxy))bis(2-

methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (**3.80**) as an red-brown oil (62%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (dd, J = 8.6, 5.1 Hz, 4H), 7.07-7.01 (m, 4H), 6.99-6.94 (m, 6H), 4.18 (dd, J = 4.7, 4.7 Hz, 4H), 3.89 (dd, J = 4.7, 4.7 Hz, 4H), 3.89 (s, 6H), 3.73 (dd, J = 6.3, 2.9 Hz, 4H), 3.67 (dd, J = 5.2, 2.9 Hz, 4H), 3.64-3.62 (m, 12H), 3.11-3.06 (tt, J = 10.9, 3.4 Hz, 2H), 2.98 (d, H = 11.5 Hz), 2.77 (dd, J = 8.5, 5.1 Hz, 4H), 2.55 (dd, J = 8.6, 5.7 Hz, 4H), 2.11 (t, J = 9.7 Hz, 4H), 1.90 (d, J = 11.5 Hz, 4H), 1.75 (qd, J = 11.4, 3.4 Hz, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 162.4, 160.5, 152.0, 147.4, 136.1, 134.6, 130.2, 124.3, 120.8, 116.5, 115.3, 115.1, 70.9, 70.7, 70.7, 70.6, 69.7, 68.5, 61.7, 60.8, 53.4, 48.2, 32.9, 28.1; HRMS – ESI: m/z [M + Na]$^{+}$ calculated for $C_{56}H_{74}F_2N_2O_{12}$: 1027.5108, measured 1027.5078.

(3,3'-(3,6,9,12,15,18,21-heptaoxatricosane-1,23-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.81)



A solution of (1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (**3.16**) (91 mg, 0.25 mmol), 23-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15,18,21-heptaoxatricosyl 4-methylbenzenesulfonate (**3.74**) (110 mg, 0.125 mmol), and K_2CO_3 (35 mg, 0.25 mmol) in 8 mL acetone was refluxed for 48 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (100% CH_2Cl_2 to 10%

MeOH in CH_2Cl_2) to afford 69 mg of (3,3'-(3,6,9,12,15,18,21-heptaoxatricosane-1,23-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (**3.81**) as an red-brown oil (68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (dd, J = 8.5, 5.7 Hz, 4H), 7.07-7.01 (m, 4H), 6.99-6.93 (m, 6H), 4.18 (dd, J = 5.2, 5.2 Hz, 4H), 3.89 (dd, J = 5.2, .2 Hz, 4H), 3.89 (s, 6H), 3.72 (dd, J = 5.8, 4.0 Hz, 4H), 3.67 (dd, J = 5.1, 4.6 Hz, 4H), 3.65-3.63 (m, 16H), 3.10 (tt, J = 10.9, 4.0 Hz, 2H), 2.98 (d, J = 11.5 Hz, 4H), 2.78 (dd, J = 8.6, 5.1 Hz, 4H), 2.56 (dd, J = 8.5, 5.1 Hz, 4H), 2.13 (t, J = 11.4 Hz, 4H), 1.92 (d, J = 11.5 Hz, 4H), 1.75 (qd, J = 11.4, 3.4 Hz, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.5, 162.4, 160.4, 152.0, 147.4, 136.1, 134.5, 130.2, 130.1, 124.3, 120.8, 116.5, 115.3, 115.1, 70.9, 70.7, 70.7, 70.6, 69.7, 68.5, 61.7, 60.8, 53.6, 53.3, 48.1, 32.9, 28.1; HRMS – ESI: m/z $[M + H]^+$ calculated for $C_{58}H_{78}F_2N_2O_{13}$: 1071.3570, measured 1071.5347.

Chapter 4: Summary / Future Work

The successful synthesis of 5-HTR DMLs was a complex process involving the completion of a number of steps. These steps included choosing parent compounds with activity at either the 5-HT_{2A}R or the 5-HT_{2C}R, determining an appropriate linker location, choosing an appropriate linker, and finally establishing optimal linker length through the synthesis of a series of DMLs. In order to synthesize heterodimeric 5-HT_{2A}R / 5-HT_{2C}R DMLs in addition to homodimeric 5-HT_{2A}R DMLs or homodimeric 5-HT_{2C}R DMLs some of these steps were accomplished while others are still under investigation.

Specific Aim 1: Chose appropriate parent compounds

The first step in the synthesis of DML was to choose an appropriate parent compound. This step was accomplished through the use of compounds with known activity at the 5-HT_{2A}R or the 5-HT_{2C}R. The 5-HT_{2A}R antagonist M-100907 and the 5-HT_{2C}R agonists WAY-470 and WAY-163909 were chosen as the initial compounds on which structural modifications were attempted. These molecules were chosen as parent molecules for DMLs because they have known 5-HTR activity and their structures were amenable to the modifications necessary for linker attachment.

Specific Aim 2: Determine appropriate linker locations

The next step was to determine an appropriate linker location for the 5-HT_{2A}R antagonist M-100907 and the 5-HT_{2C}R agonists WAY-470 and WAY-163909. This aim was completed for the 5-HT_{2A}R antagonist M-100907 but not the 5-HT_{2C}R agonists WAY-470 and WAY-163909. M-100907 was shown to tolerate modification at the 3'-methoxy position. The 3'-methoxy can be replaced with a hydroxy group which can be used as a linker attachment point for polyethylene glycol linkers. The addition of linkers

at this location produced only a minimal decrease in IC_{50}. The M-100907 derivatives were also tested in an ERK activation assay to determine the ability of these derivatives to antagonize DOI-induced ERK activation. The data from the ERK activation assay was in agreement with the calcium release assay. This validated the data suggesting that the 3'-methoxy position was an appropriate location to attach a linker. A variety of structural modifications of WAY-470 and WAY-163909 were made in order to determine an appropriate linker attachment point. These modifications included the addition of a linker through an amide bond to the benzylic nitrogen, the addition of a bromine atom to the aryl ring, and changing the cycloalkyl ring to either a piperidine ring or a cyclohexanol ring, but all modifications have led to the loss of biological activity. Successfully determining an appropriate linker location for M-100907 allowed for the synthesis of a series of derivatives with different linkers. Since a linker location could not be determined for the 5-HT_{2C}R agonists, these compounds were not used to synthesize additional derivatives or DMLs.

Specific Aim 3: Determine appropriate linker

The series of M-100907 derivatives containing pseudo-tethers that were synthesized were used to determine which type of linker could be employed in the synthesis of DMLs. The derivatives synthesized contain either an alky chain pseudo-tether or an ethyl methyl ether pseudo-tether. The IC_{50} for these derivatives containing pseudo-tethers was determined, and from this data the ethyl methyl ether pseudo-tether was shown to be the preferred choice for a linker over a simple alkyl chain. Since ethyl methyl ether was shown to be an acceptable pseudo-tether, tethers similar to ethyl methyl ether were used to synthesize homodimeric M-100907 derivative DMLs.

Specific Aim 4: Determine appropriate linker lengths

The appropriate linker length was determined for homodimeric M-100907 derivative DMLs but not for homodimeric 5-HT_{2C}R agonist DMLs or heterodimeric 5-HT_{2A}R / 5-HT_{2C}R DMLs. To determine appropriate linker length for homodimeric M-100907 derivative DMLS a series of DMLs containing linkers of various lengths was synthesized. The linkers were composed of polyethylene glycols which are structurally similar to the pseudo-tether ethyl methyl ether. Polyethylene glycol linkers varying in length from 5 atoms to 23 atoms were used to synthesize a series of seven homodimeric M-100907 derivative DMLs. The IC_{50} value for these seven DMLs was determined, and from this data the optimal linker length was determined to be between 8 and 17 atoms in length.

Discussion

The IC_{50} data of the synthesized M-100907 homodimers supports the existence of 5-HT_{2A}R homodimers in-vivo. Previous research indicates that the 5-HT_{2A}R forms homodimers through non-covalent interactions that are not SDS-PAGE resistant.[115] In order to determine if the newly synthesized M-100907 homodimers bind to the homodimeric or oligomeric receptors the ligand-receptor complexes could be isolated.

In order to isolate the ligand-receptor complex a variety of techniques may be used. One method to isolate the desired complexes is to extract a variety of proteins from the cells of interest through sonication, homogenization, or permeabilizing the cell membrane with numerous freeze-thaw cycles. Once the proteins have been extracted they could be isolated using HPLC. The complex of interest could then be identified by molecular weight. Another method to isolate the desired ligand-receptor complex is the

use of ultracentrifuge. This method employs centrifugal force to separate components of a sample based on differences in molecular weight.[116] The sample is added to the top of a concentration gradient of a sucrose solution in a centrifuge tube. [116] When the sample is spun at increasing centrifugal force components with higher molecular weights move to the bottom of the gradient while the component with lower molecular weight are distributed throughout the sucrose gradient. [116] The layer containing compounds corresponding to the molecular weight of the protein complex of interest can then be removed and the ligand-receptor complex isolated. Gel electrophoresis could also be used to isolate the desired ligand-receptor complex. The use of a non-denaturing gel would allow for the isolation of the desired ligand-receptor without disrupting the interaction between the ligand and its receptor.

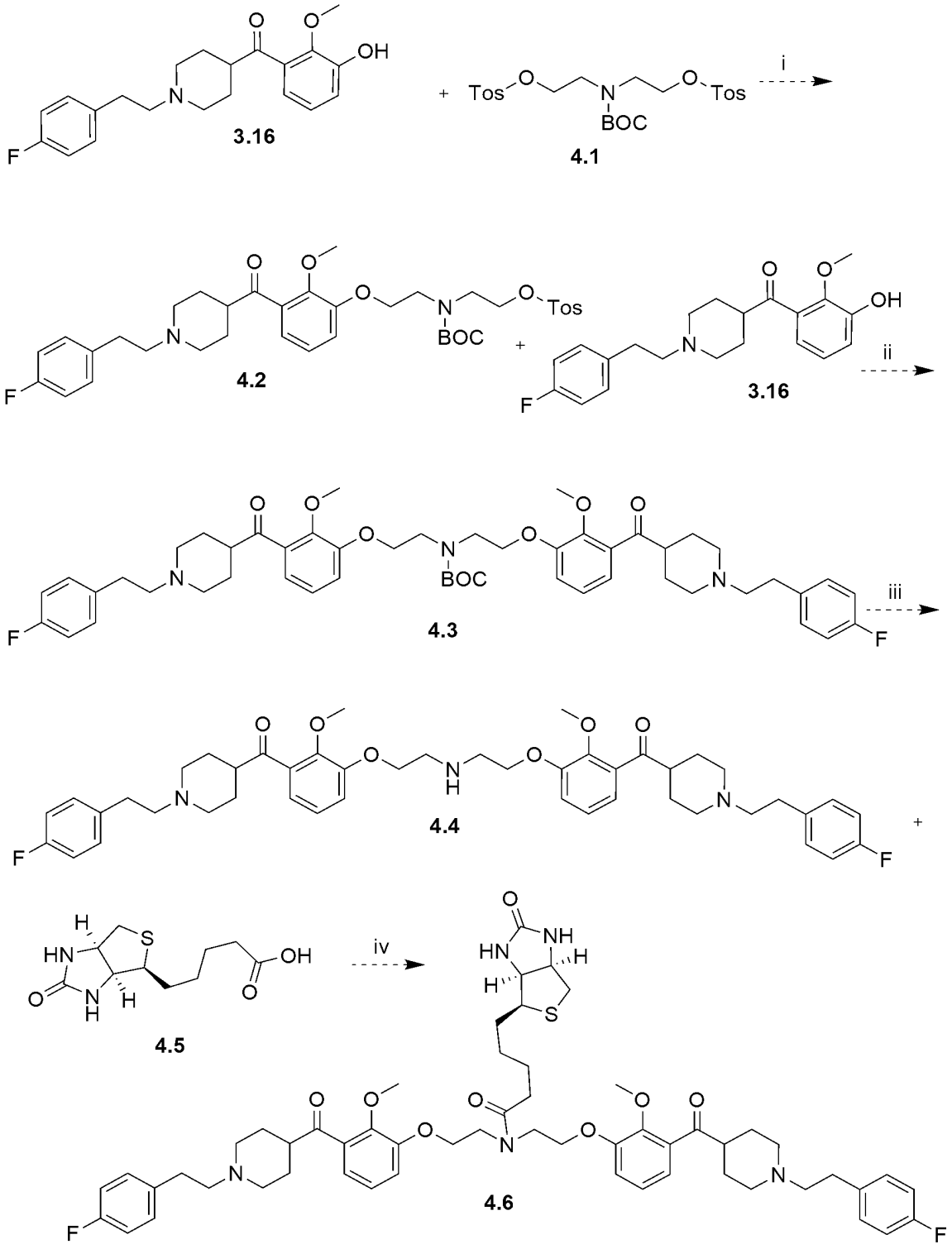
Introducing biotin to the M-100907 homodimers would also allow for the isolation of the ligand-receptor complex by exploiting the highly stable interaction between biotin and avidin.[117] The linker connecting two M-100907 derivatives is the ideal place to introduce biotin because this circumvents further structural modifications to the M-100907 derivatives. Additionally N-BOC-diethanolamine could be used in place of the polyethylene glycol linkers when synthesizing M-100907 homodimers.

The commercially available N-Boc-diethanolamine could be elongated to produce long chain linkers using chemistry previously described (**Scheme 3.12**). The di-tosylated N-BOC-diethanolamine **4.1** could then be employed as a linker in the synthesis of M-100907 derivative homodimers (**Scheme 4.1**). Derivative **3.16** could be alkylated with **4.1** in the presence of K_2CO_3 in acetone under reflux to afford intermediate **4.2**. Intermediate **4.2** would then be alkylated with another molecule of **3.16** to give

homodimer **4.3** containing a BOC protected amine. The BOC protecting group would then be removed with TFA at room temperature to give the free amine **4.4**. The free amine **4.4** could be coupled to biotin using EDAC and DIEA in acetonitrile to give the desired biotinylated M-100907 homodimer **4.6** (**Scheme 4.1**).

The biotinylated M-100907 homodimer **4.6** could be used to identify 5-HT_{2A}R dimers and oligomers. After treating cells with compound **4.6**, proteins including the complex of compound **4.6** and 5-HT_{2A}Rs would be extracted. The protein extract would then be added to beads containing avidin. [117] The proteins complexes containing biotin would bind to the avidin beads and the remaining proteins could be washed away. [117] The remaining proteins are then eluted from the avidin beads and collected and identified. In this way compound **4.6** could be used to determine if these M-100907 homodimers bind to 5-HT_{2A}R monomers, dimers, or oligomers.

These novel 5-HT_{2A}R antagonist homodimers could also be used to probe the role of 5-HT_{2A}R antagonists when bound to 5-HT_{2A}R oligomers. A recent study has revealed that the 5-HT_{2A}R antagonist M-100907 exhibits a biphasic binding curve when it binds to 5-HT_{2A}R dimers or oligomers.[115] This biphasic binding curve suggests that M-100907 binds to these dimer and oligomers with negative cooperativity.[115] When a single molecule of M-100907 binds to a 5-HT_{2A}R dimer or oligomer a second molecule will have diminished affinity to bind the receptor. The biphasic binding curve and negative cooperativity suggests that 5-HT_{2A}R monomers and 5-HT_{2A}R dimers or oligomers may have variations in their signaling pathways. The M-100907 homodimers synthesized in this work could be used to probe the theory of negative cooperativity by determining if these homodimers exhibit a biphasic binding curve similar to M-100907.



Reagents and conditions: (i) K_2CO_3, acetone, reflux, 20 h; (ii) K_2CO_3, acetone, reflux, 20 h; (iii) TFA, rt; (iv) EDAC, DIEA, CH_3CN, rt

Scheme 4. 1 Synthesis of a biotinylated M-100907 homodimer

Conclusion

The work reported here represents the first steps to the successful synthesis of 5-HT receptor DMLs. First generation homodimeric 5-HT_{2A}R antagonist DMLs were synthesized, but the IC_{50}s of these compounds may be improved by reducing the ketone to an alcohol and resolving the diastereomers. Additional modifications could be made to the linker to alter the polarity of the DMLs which may also improve the IC_{50}. In order to synthesize homodimeric 5-HT_{2C}R agonist DMLs and heterodimeric 5-HT_{2A}R / 5-HT_{2C}R DMLs more work must be done. An appropriate 5-HT_{2C}R agonist must be chosen and a linker site determined, and then heterodimeric DMLs may be synthesized. The synthesis of DMLs is not a straightforward process, but this work lays the groundwork for continuing exploration into production of 5-HT receptor DMLs.

Appendix A: Chapter 2 Spectral Data ^{1}H NMR and ^{13}C NMR

Figure A. 1: ^{1}H-NMR Spectra (500 MHz, DMSO-D_6)
3,4-dihydro-1H-benzo[e][1,4]diazepine-2,5-dione (2.4)

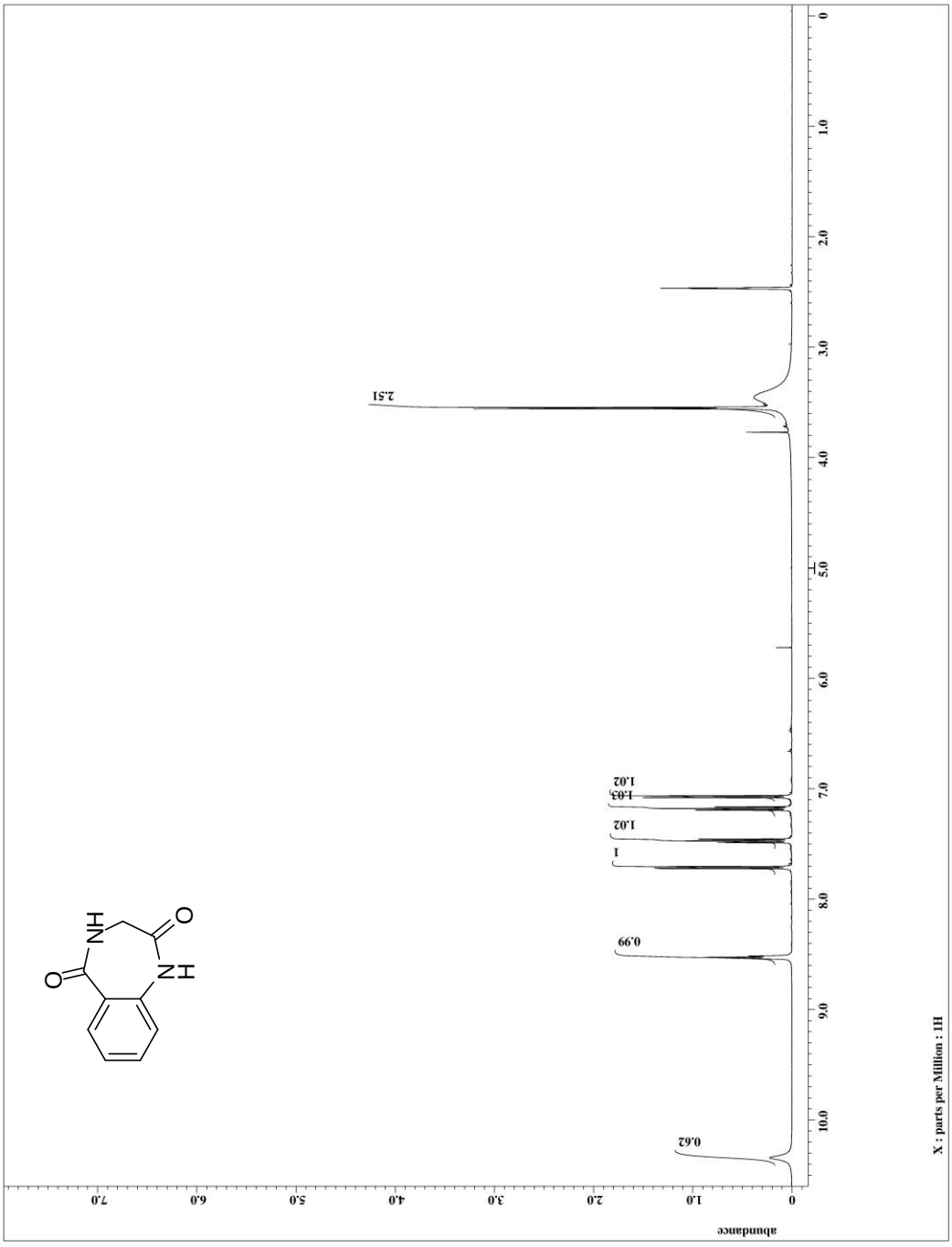


Figure A. 2: ^{13}C-NMR Spectra (125 MHz, DMSO-D_6)
3,4-dihydro-1H-benzo[e][1,4]diazepine-2,5-dione (2.4)

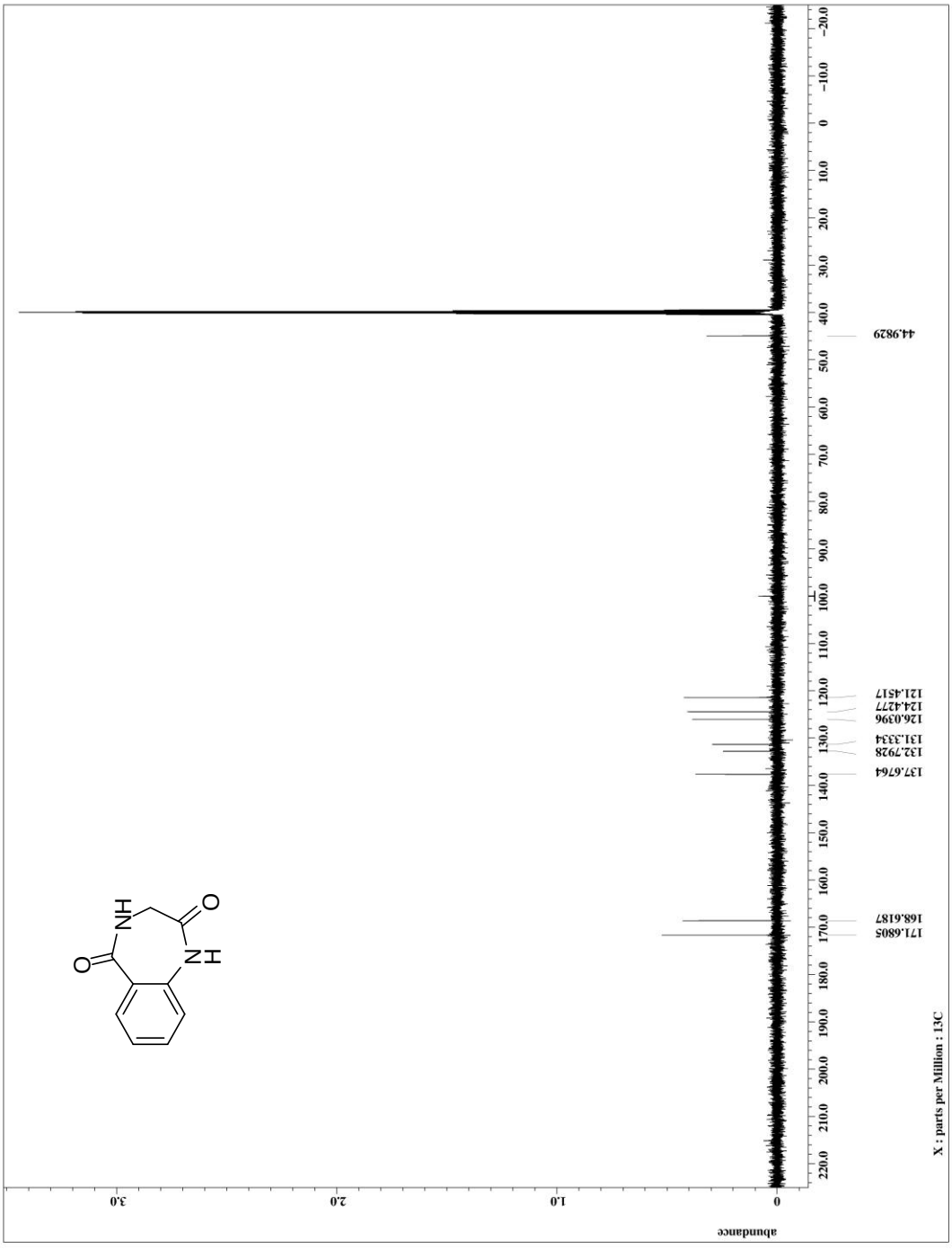


Figure A. 3: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine (2.5)

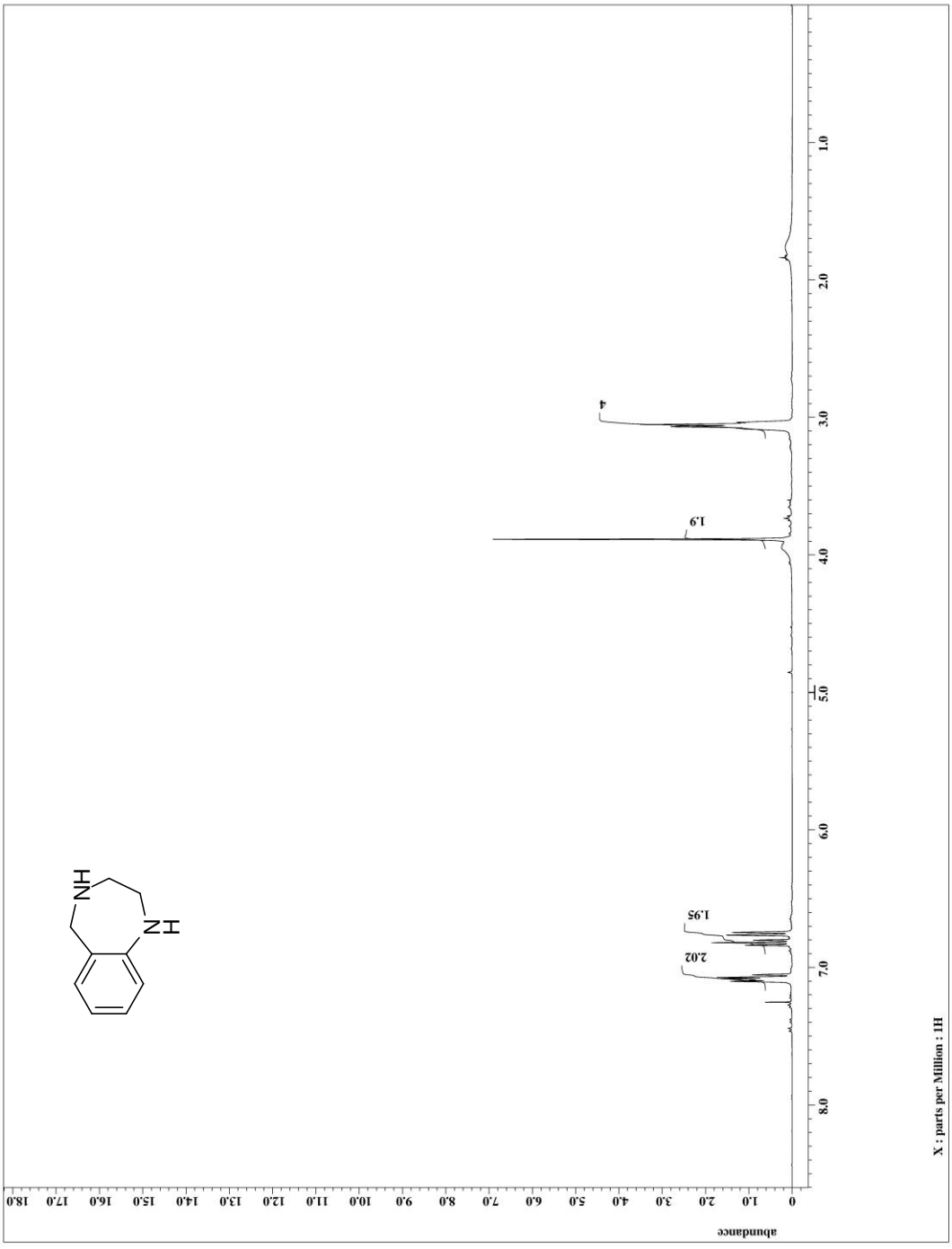


Figure A. 4: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine (2.5)

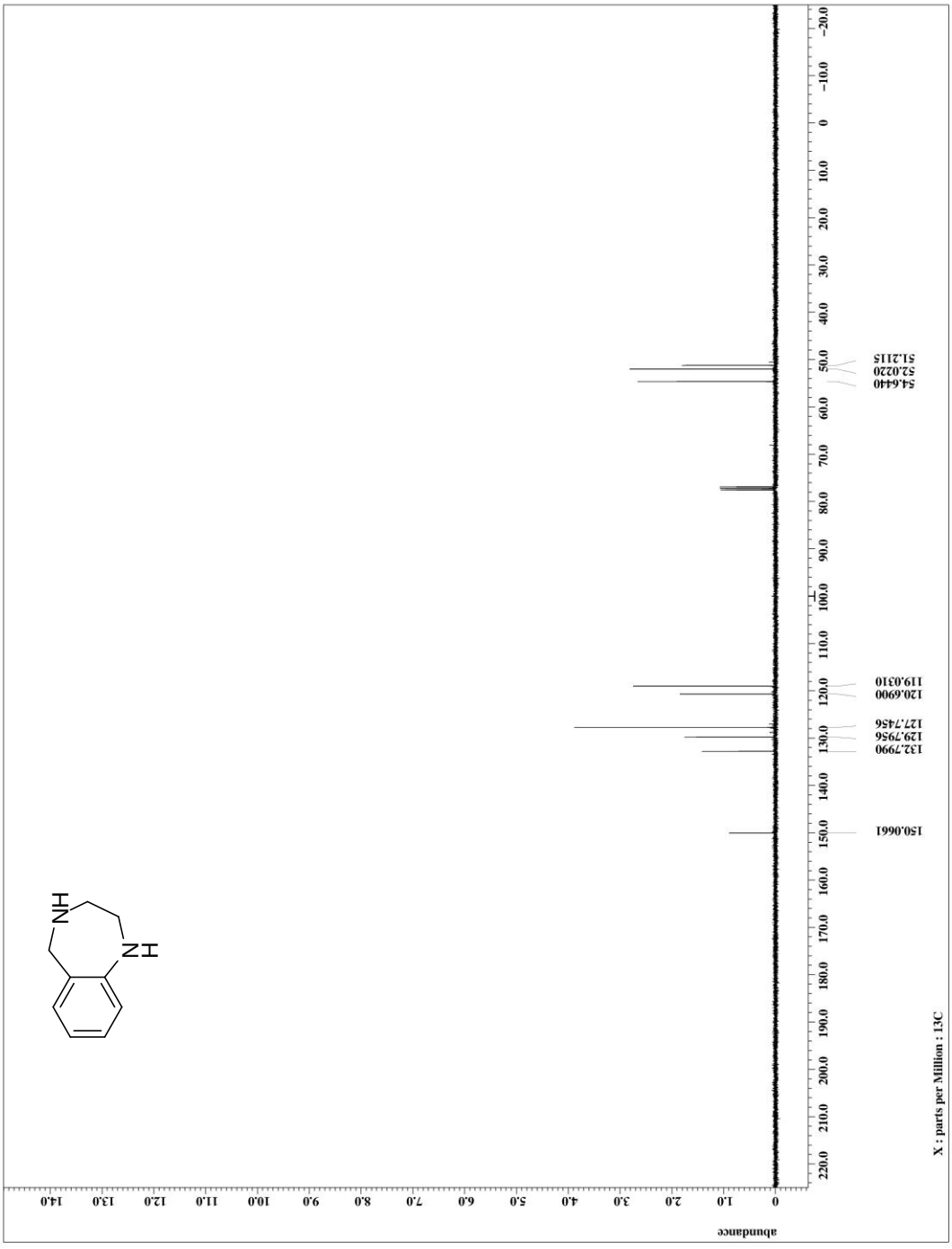


Figure A. 5: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1-(2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (2.6)

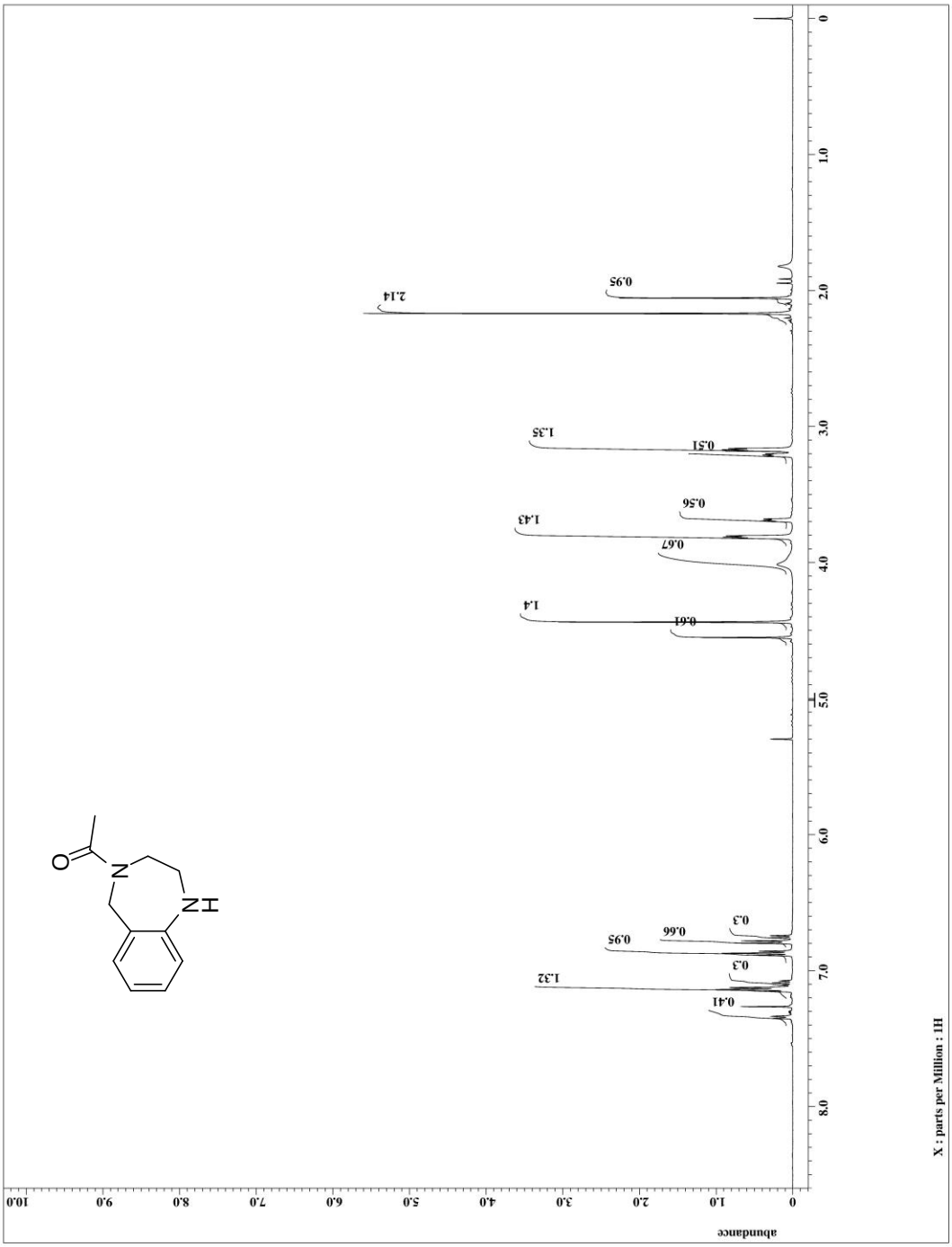


Figure A. 6: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1-(2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (2.6)

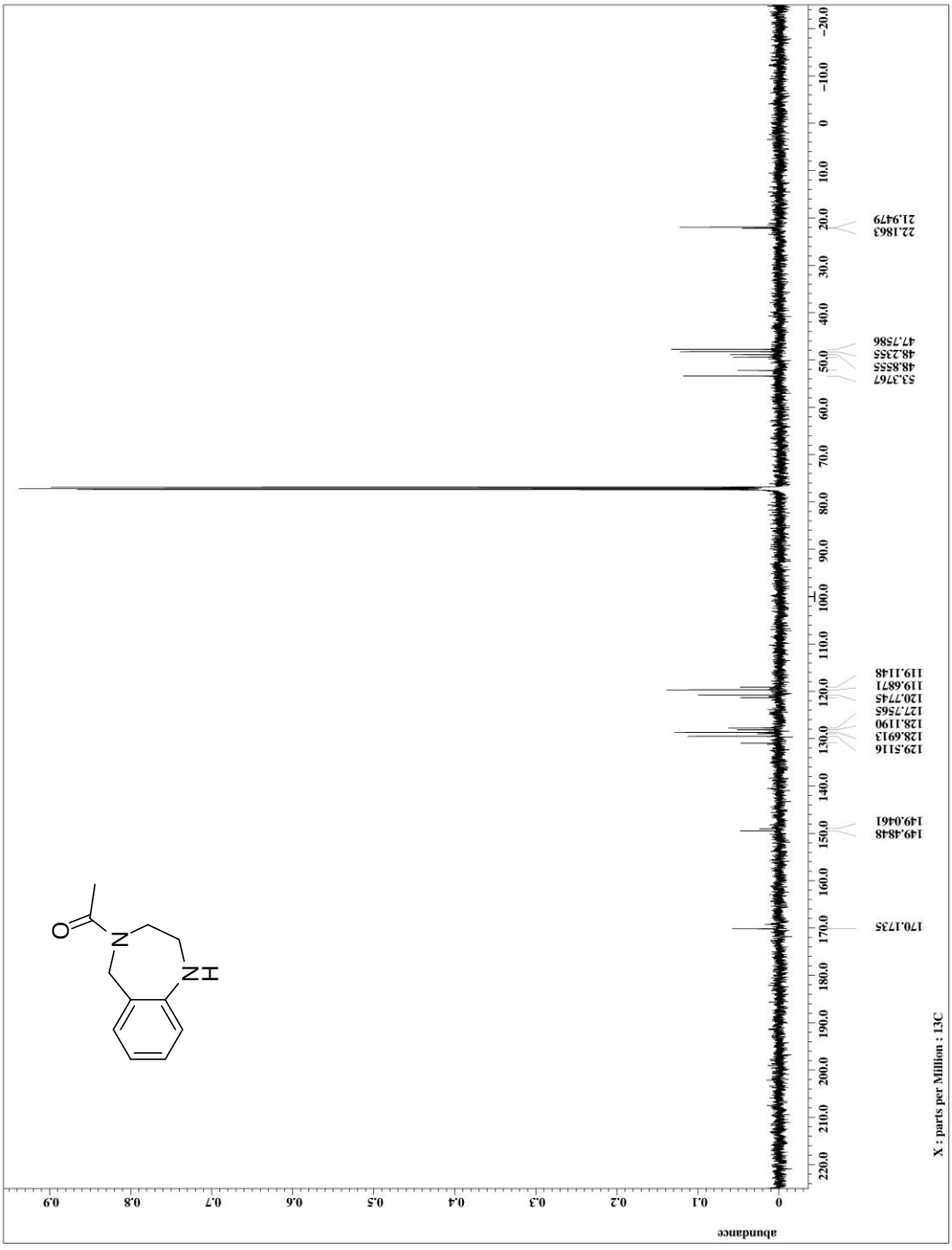


Figure A. 7: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1-(1-nitroso-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (2.7)

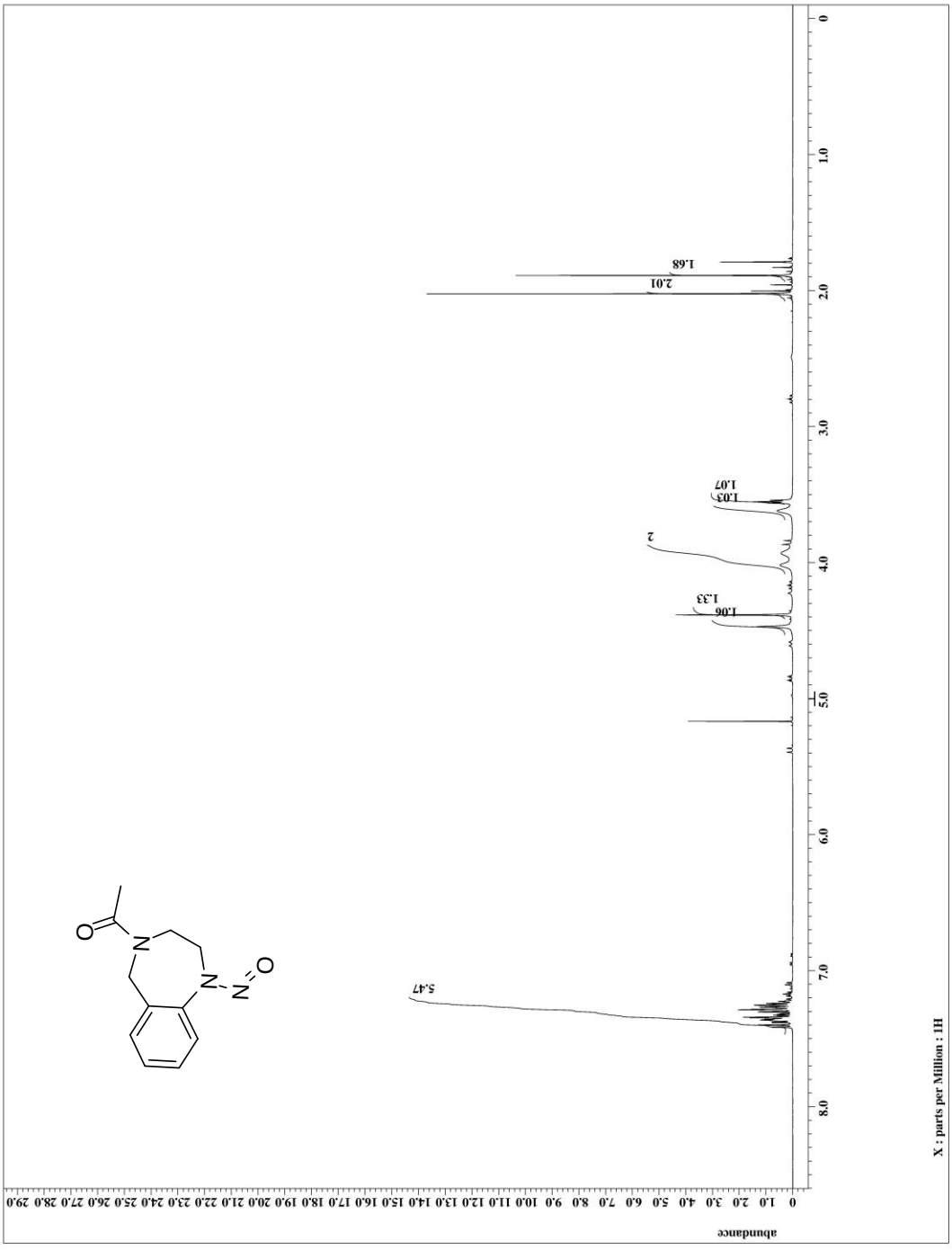


Figure A. 8: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1-(1-nitroso-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (2.7)

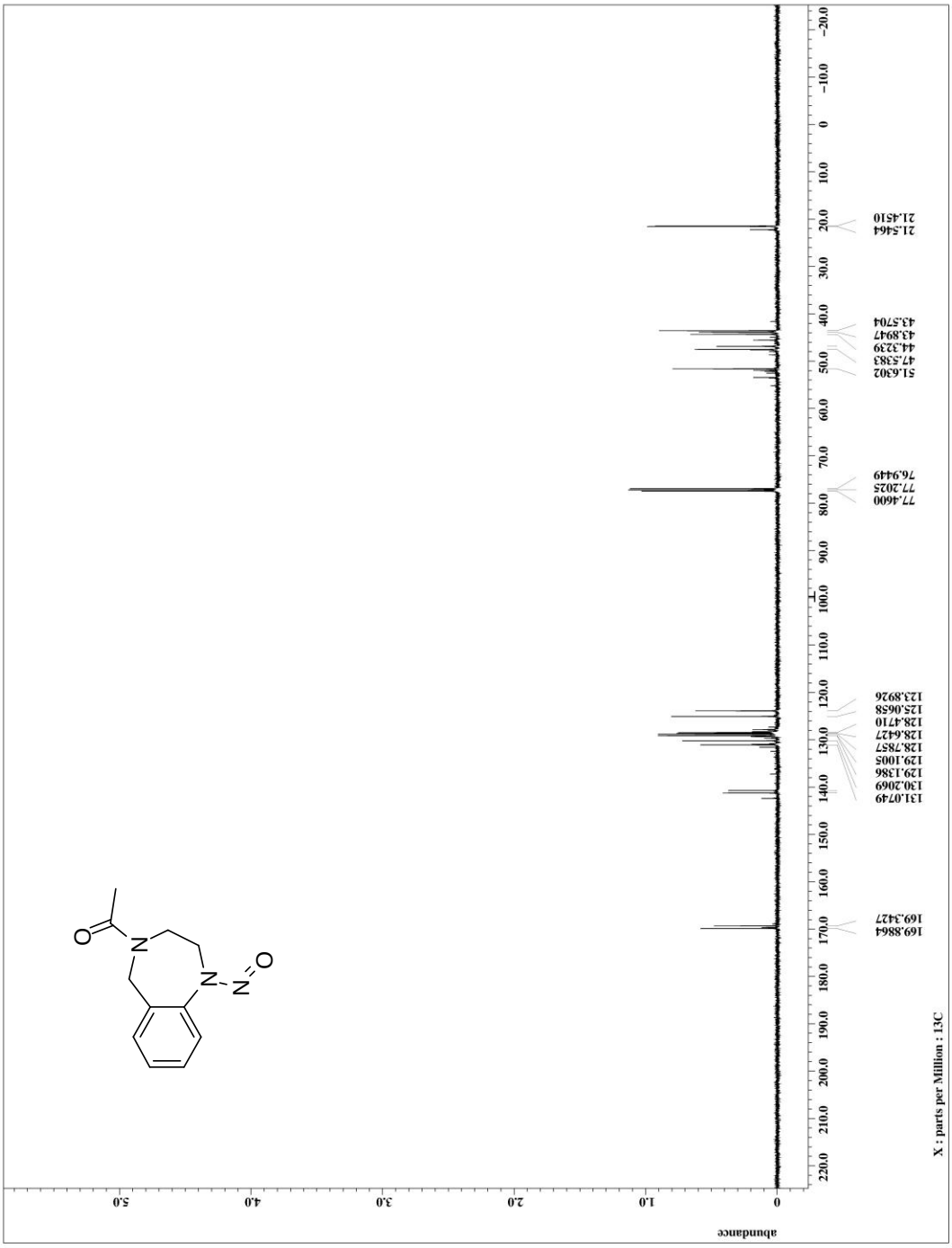


Figure A. 9: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1-(1-amino-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (2.8)

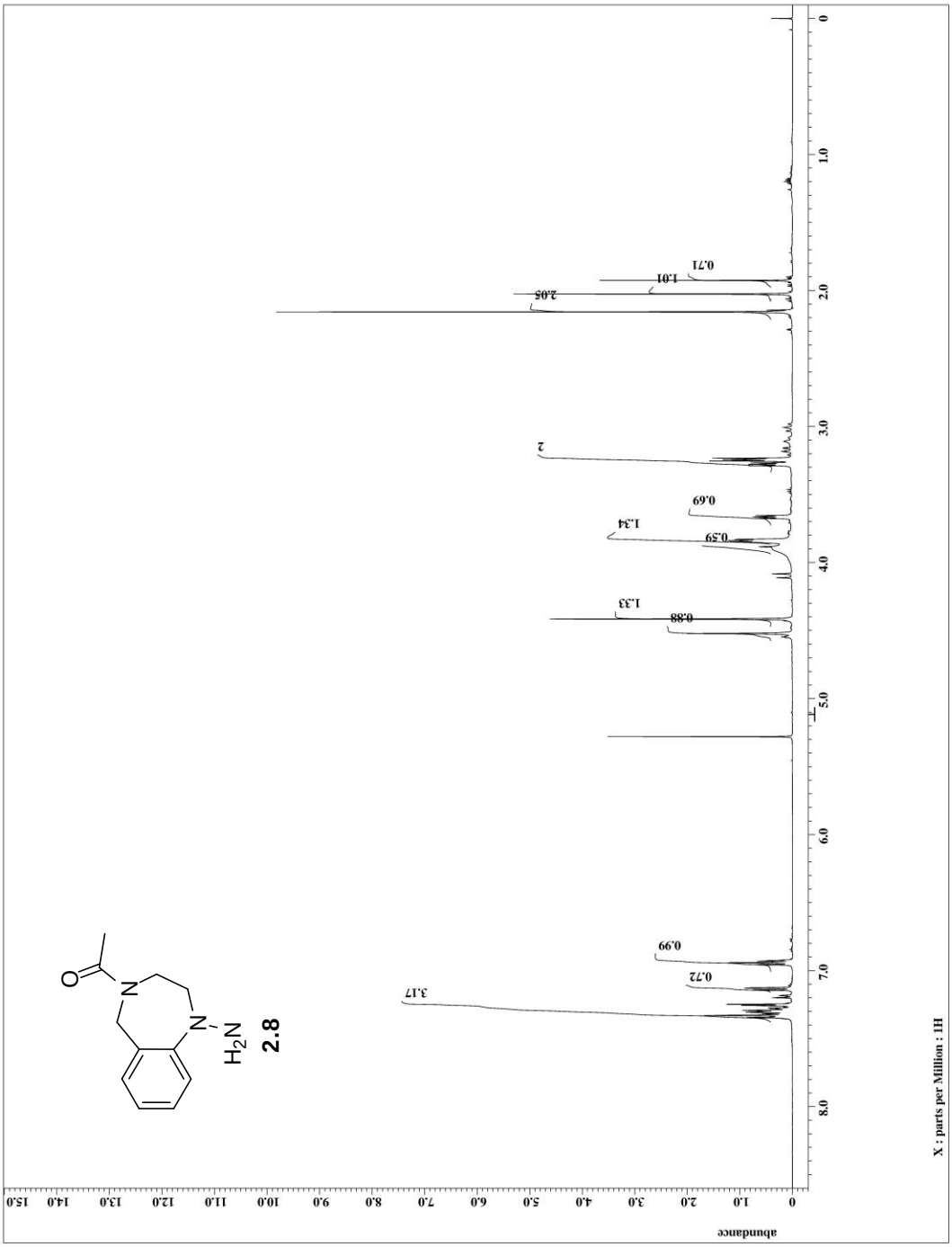


Figure A. 10: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1-(1-amino-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (2.8)

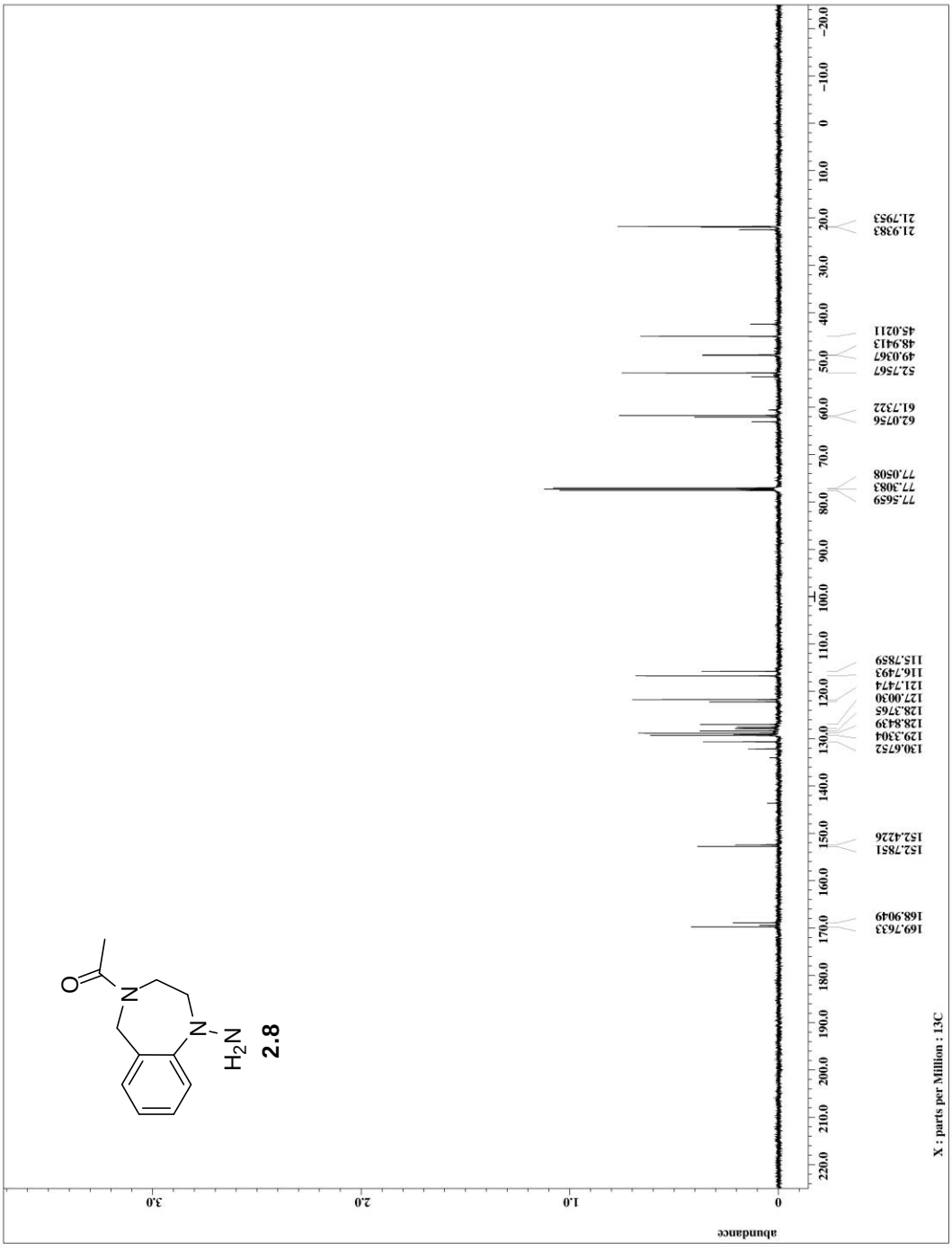


Figure A. 11: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)ethanone (2.9)

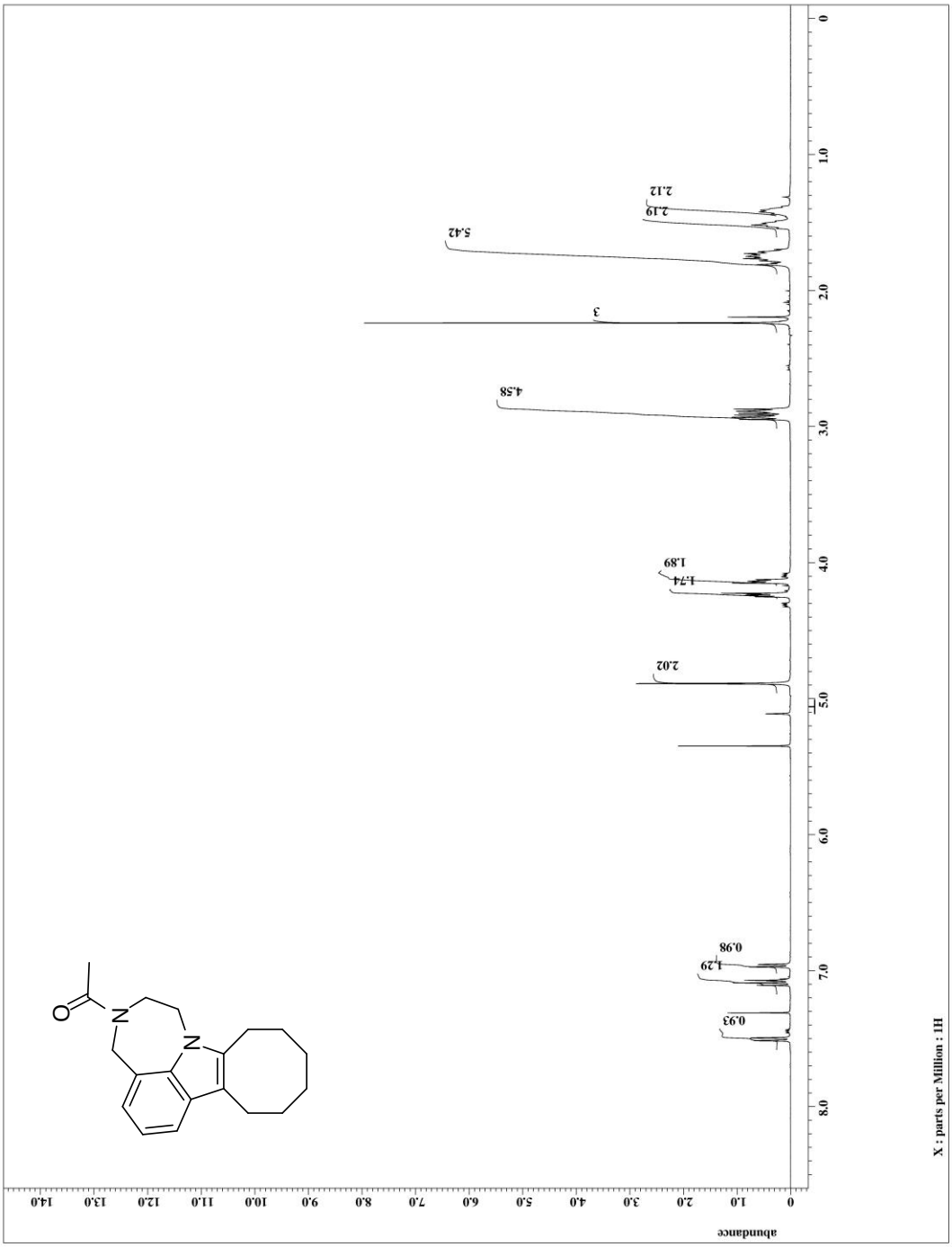


Figure A. 12: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl) ethanone (2.9)

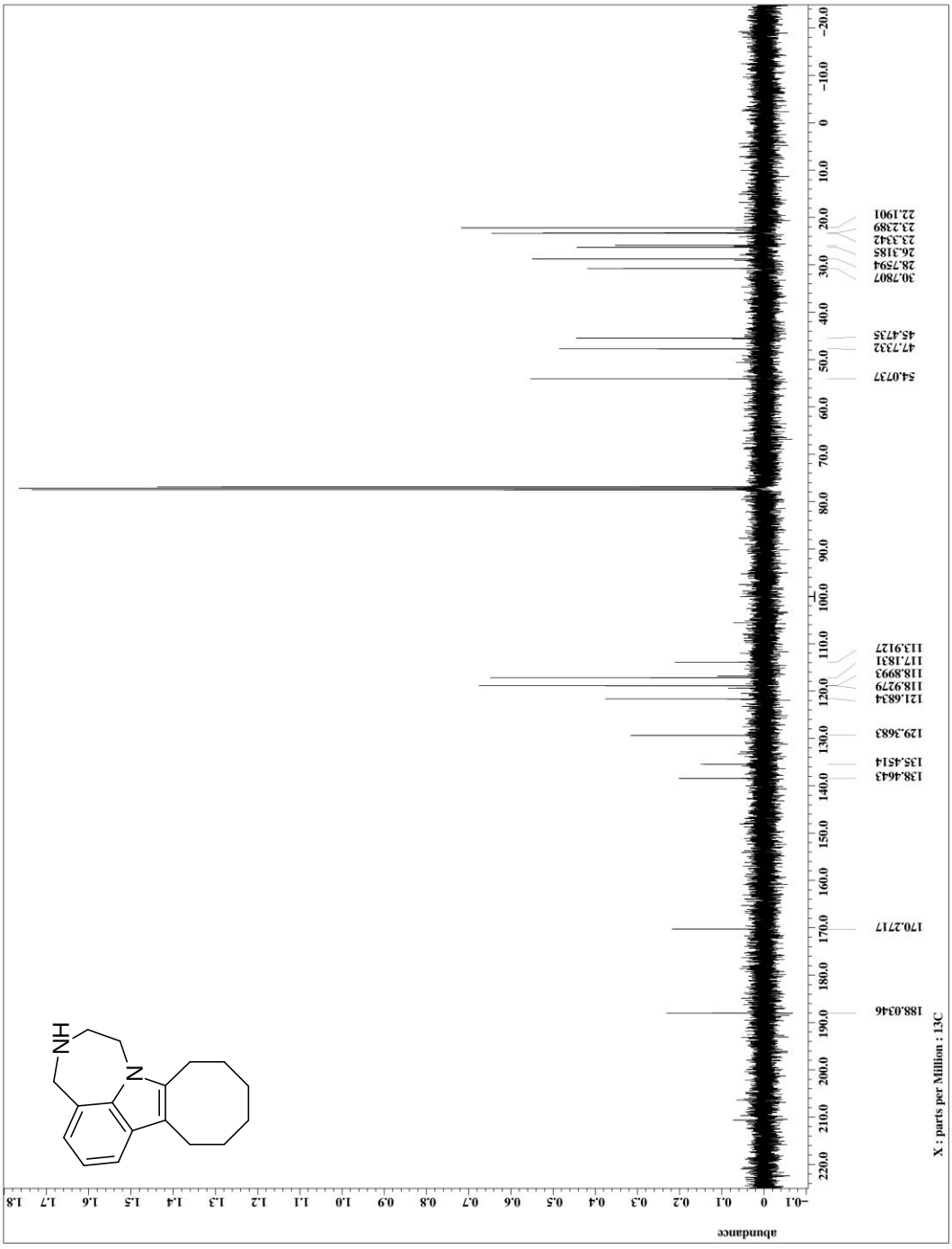


Figure A. 13: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1,2,3,4,8,9,10,11,12,13-decahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indole (2.10)

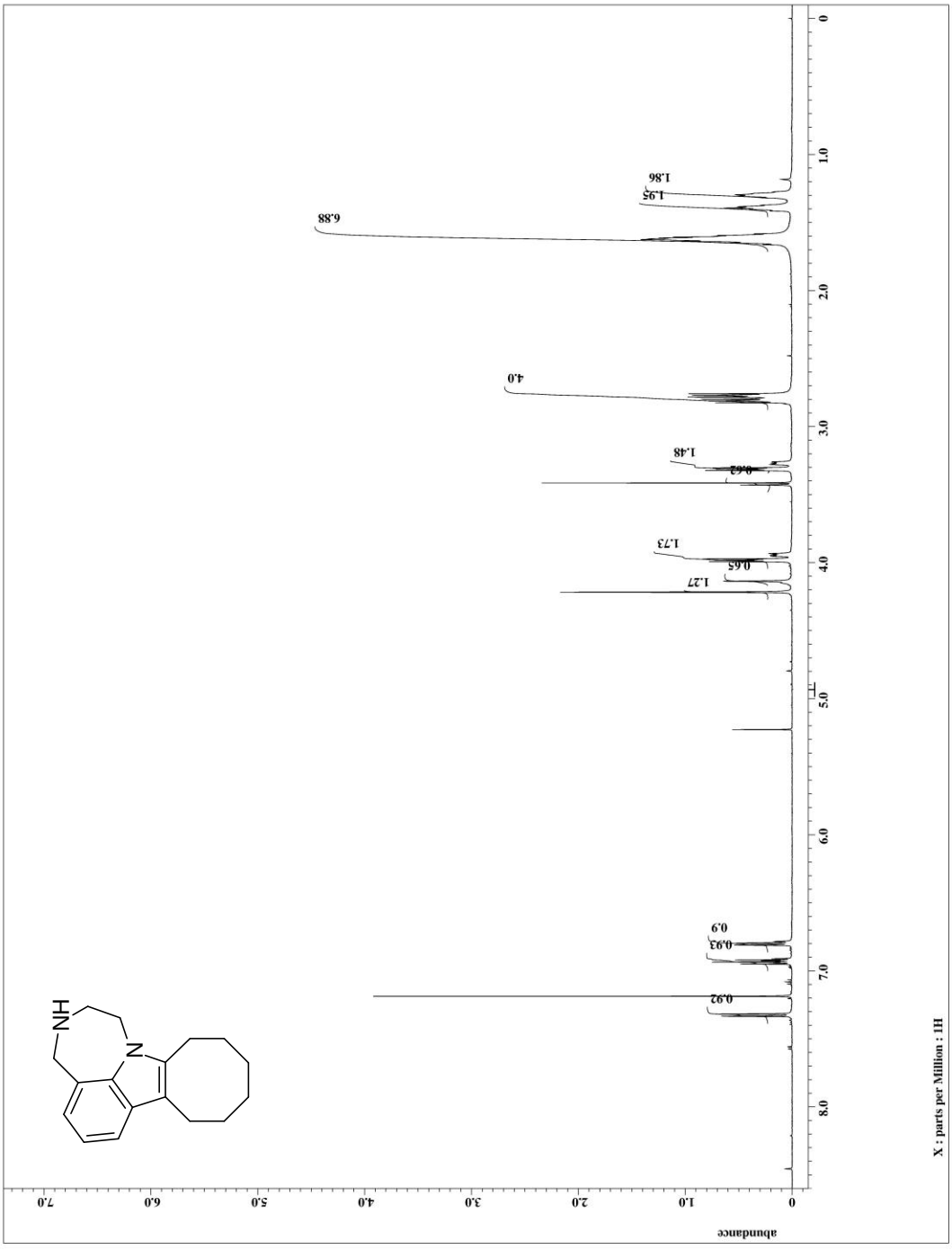


Figure A. 14: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1,2,3,4,8,9,10,11,12,13-decahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indole (2.10)

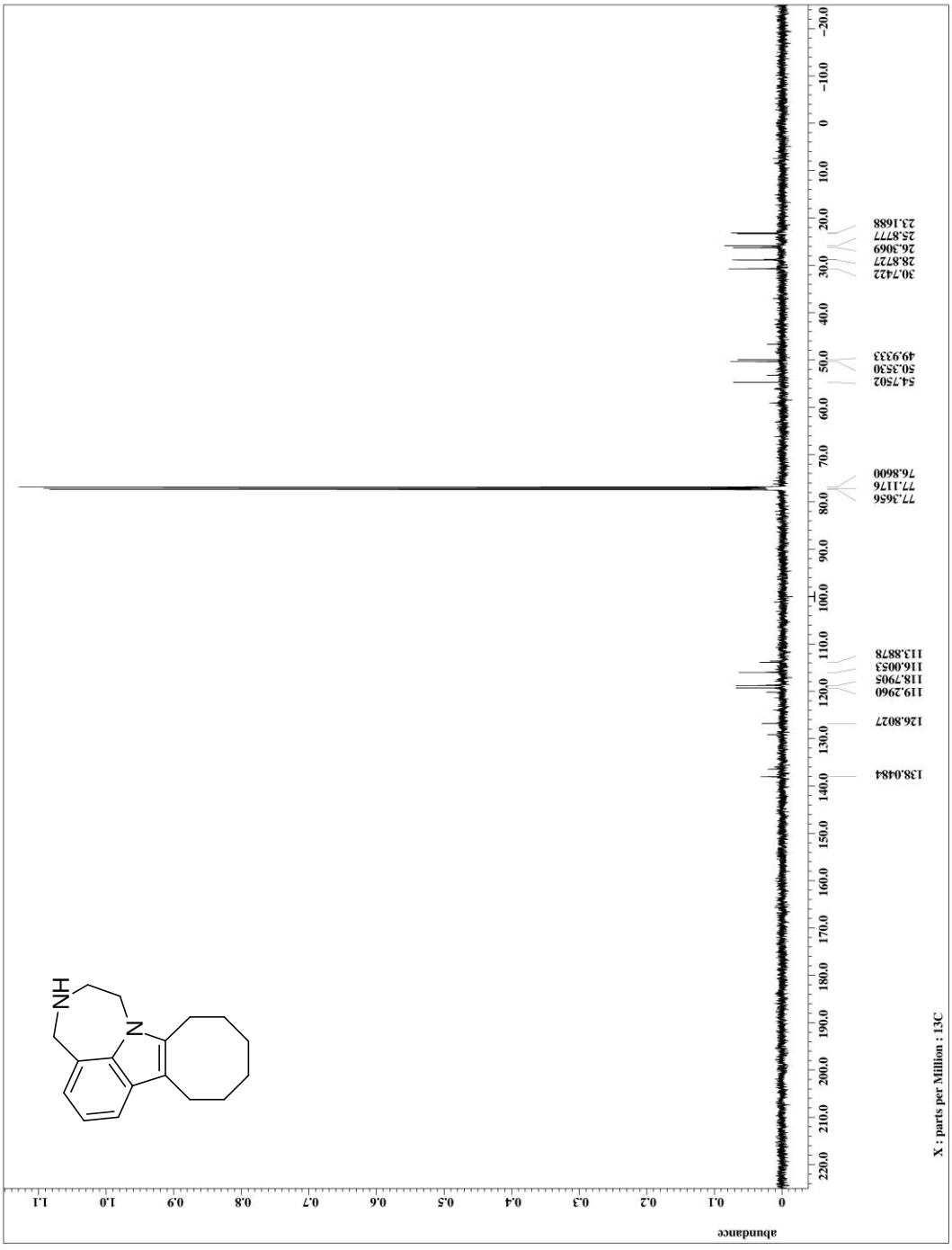


Figure A. 15: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.14)

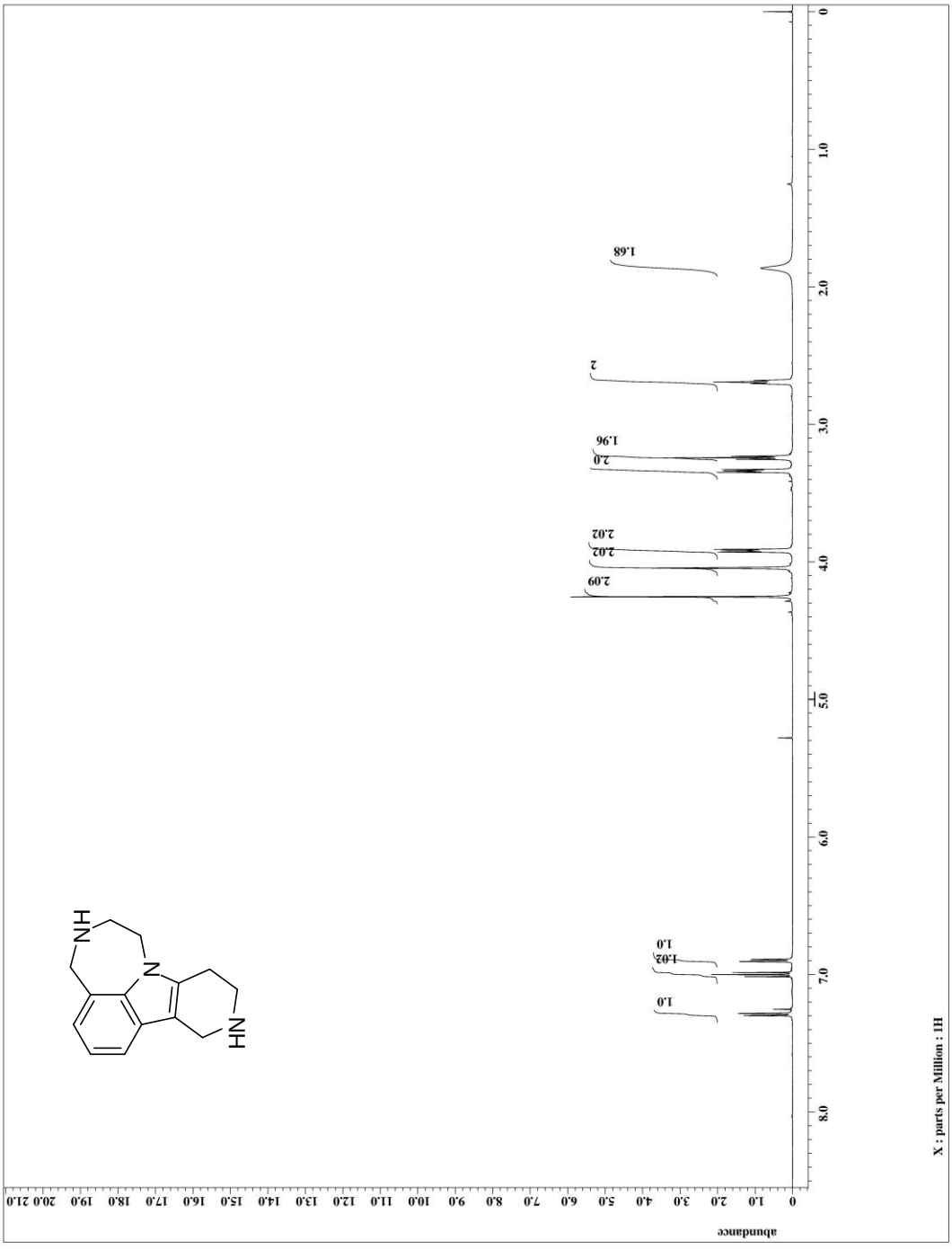


Figure A. 16: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.14)

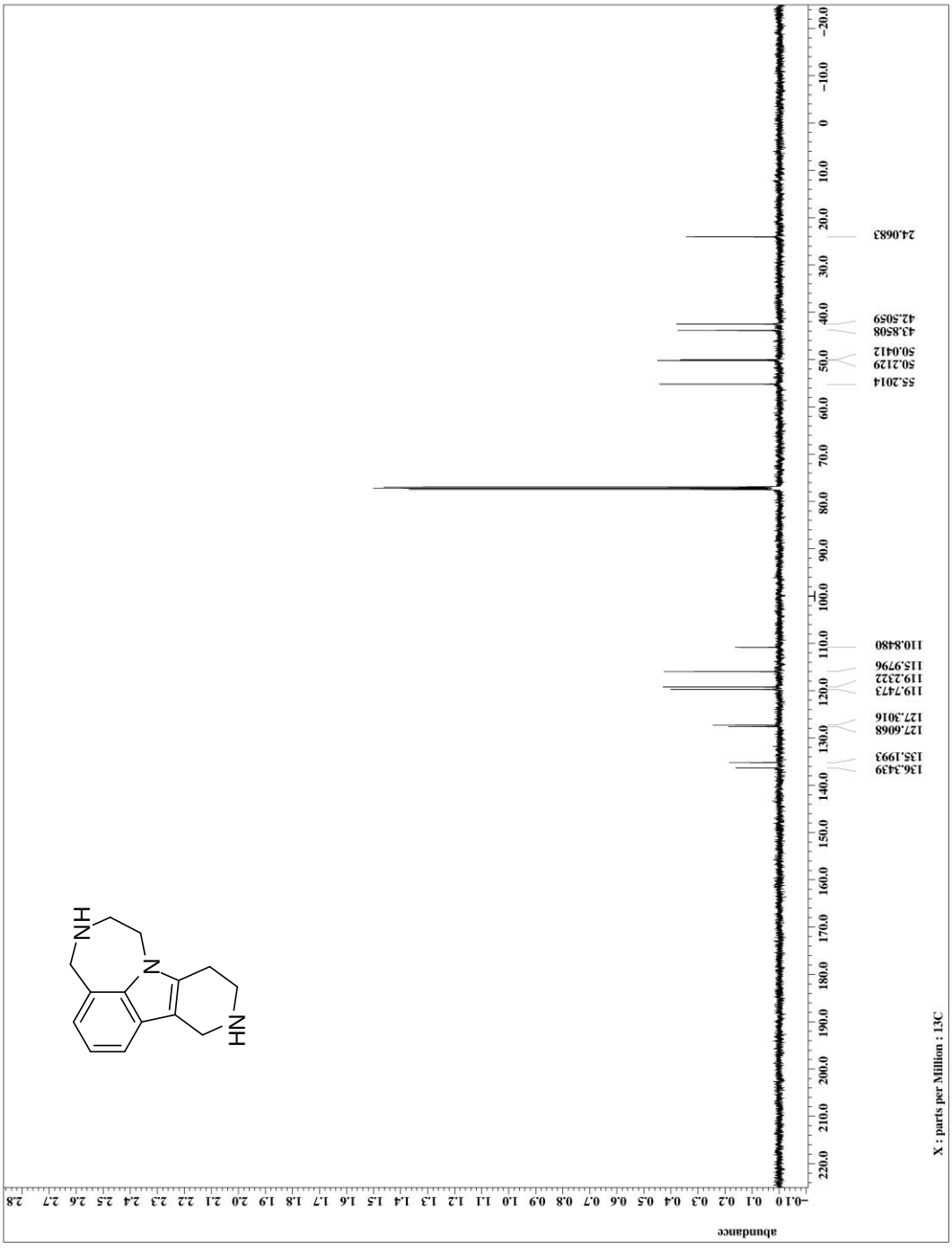


Figure A. 17: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-9-ol (2.15)

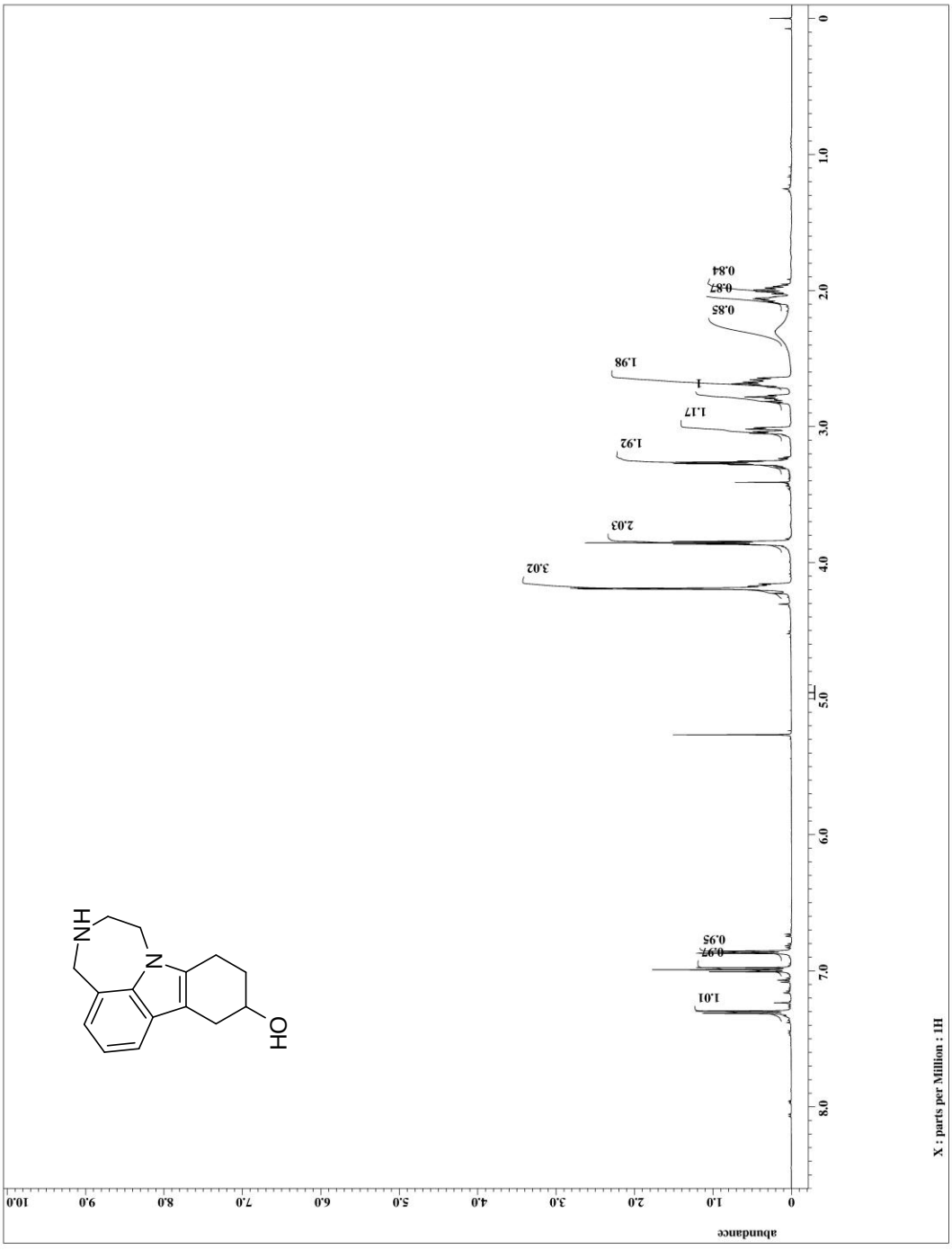


Figure A. 18: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-9-ol (2.15)

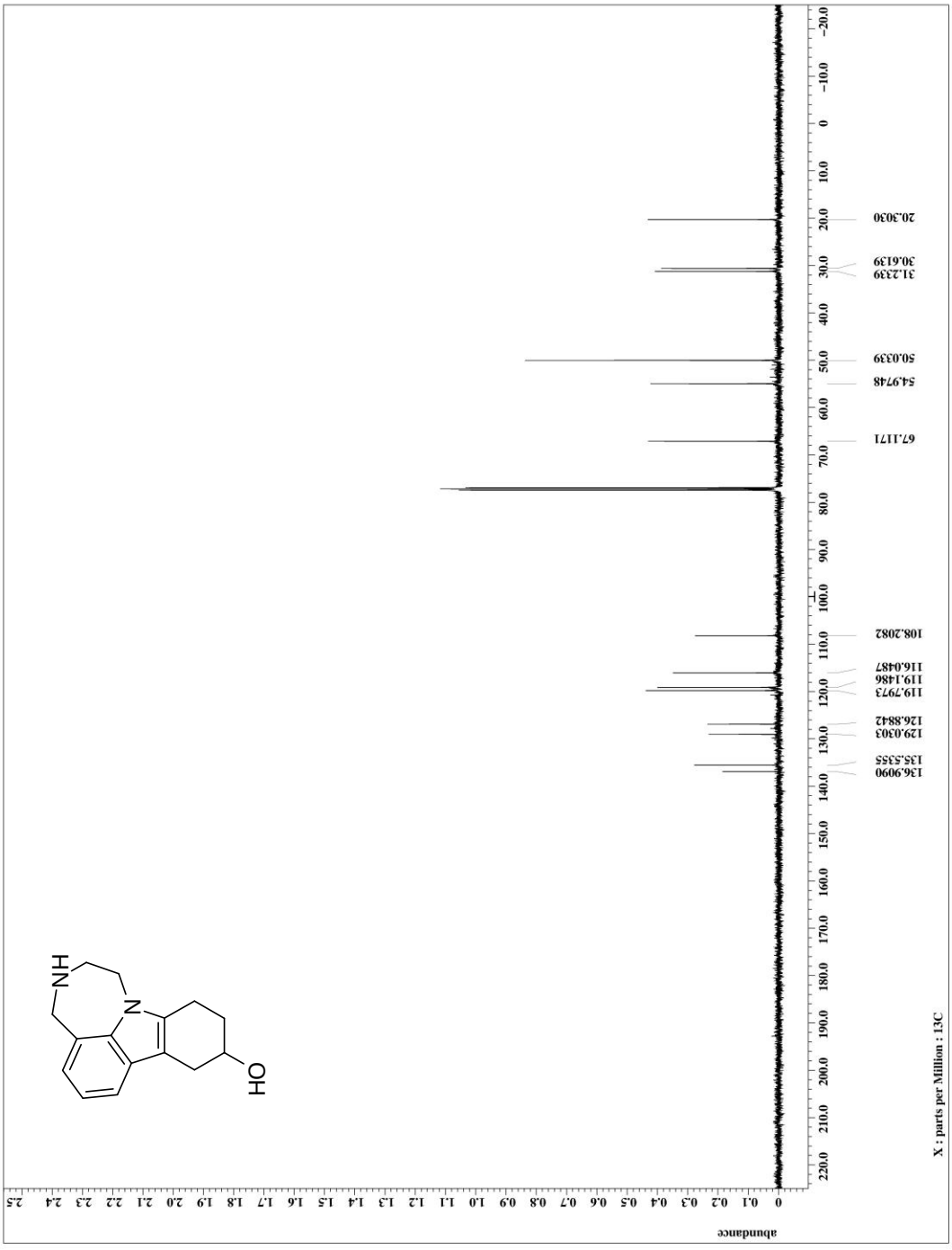


Figure A. 19: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
benzyl 3-acetyl-1,2,3,4,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(8H)-carboxylate (2.21)

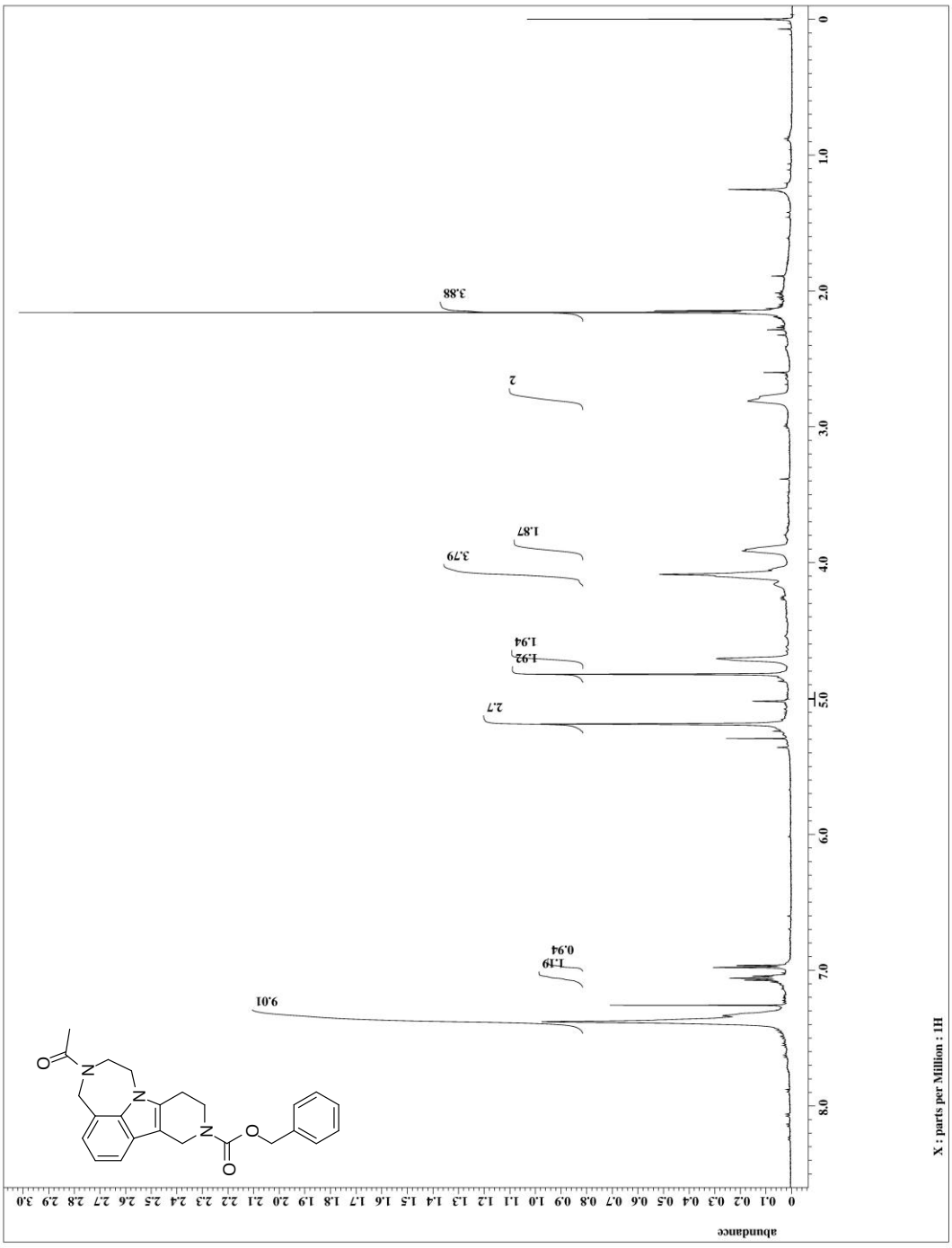


Figure A. 20: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
benzyl 3-acetyl-1,2,3,4,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(8H)-carboxylate (2.21)

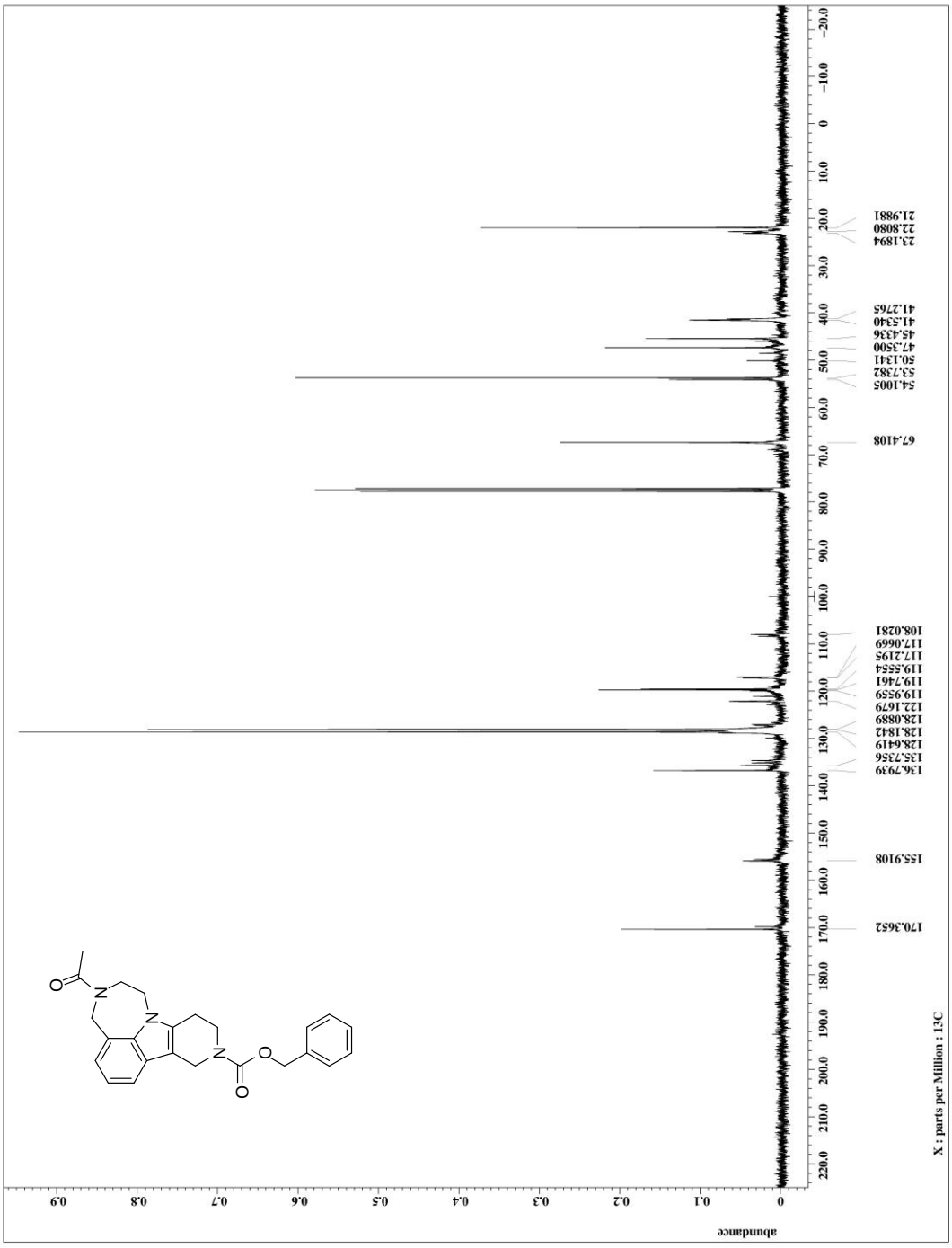


Figure A. 21: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1-(1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.22)

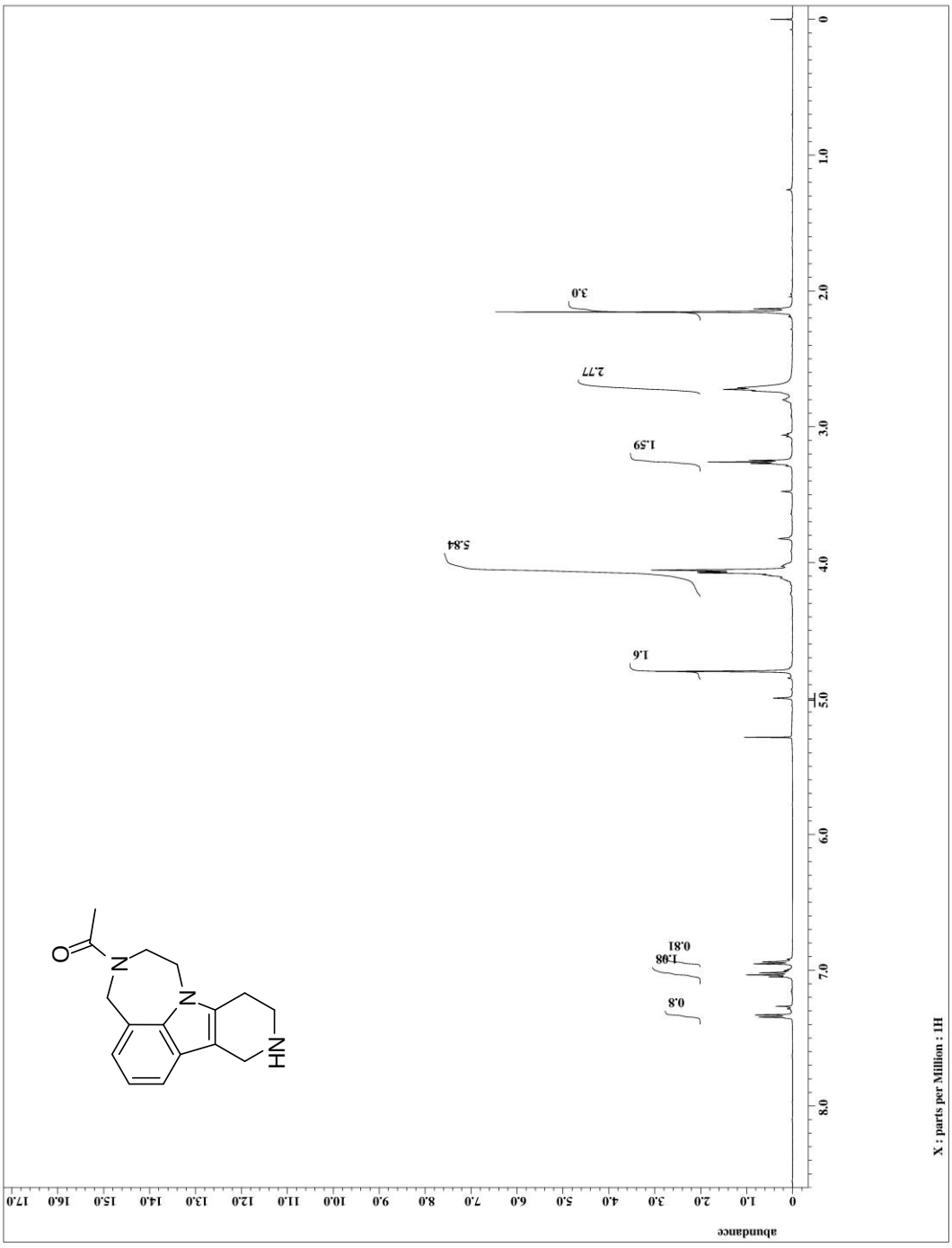


Figure A. 22: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1-(1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.22)

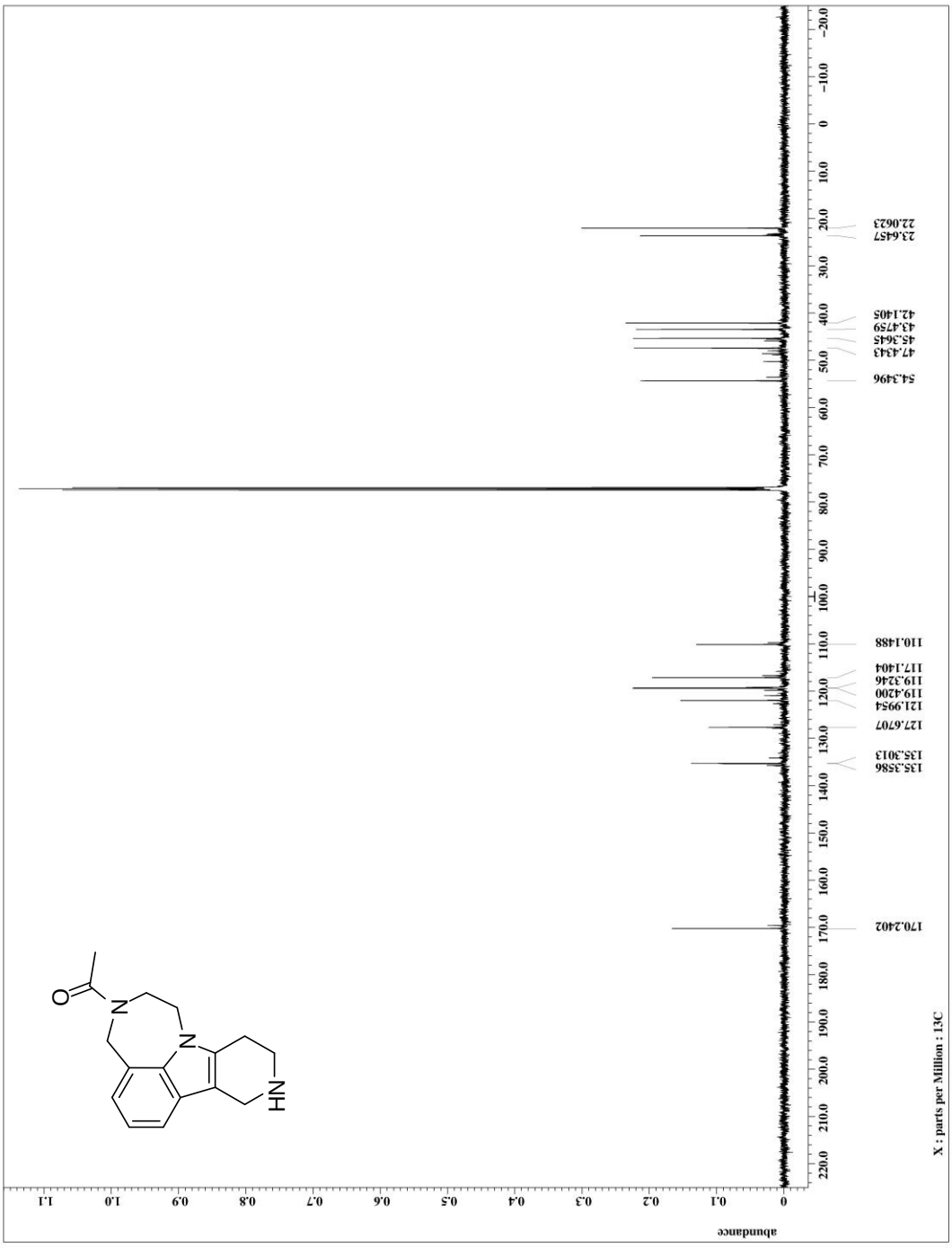


Figure A. 23: ^{1}H-NMR Spectra (300 MHz, $CDCl_3$)
1-(9-(benzyloxy)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.27)

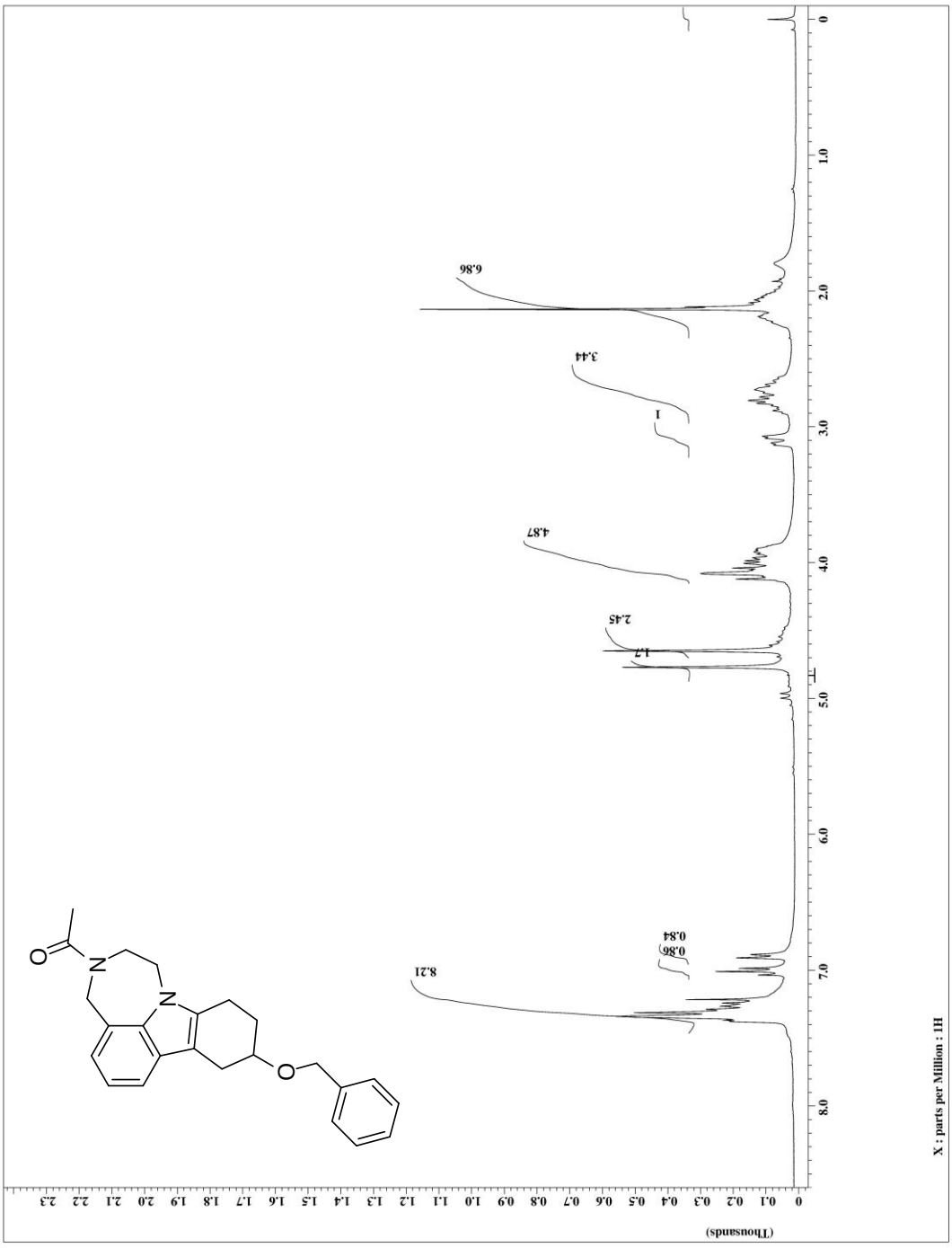


Figure A. 24: ^{13}C-NMR Spectra (300 MHz, $CDCl_3$)
1-(9-(benzyloxy)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.27)

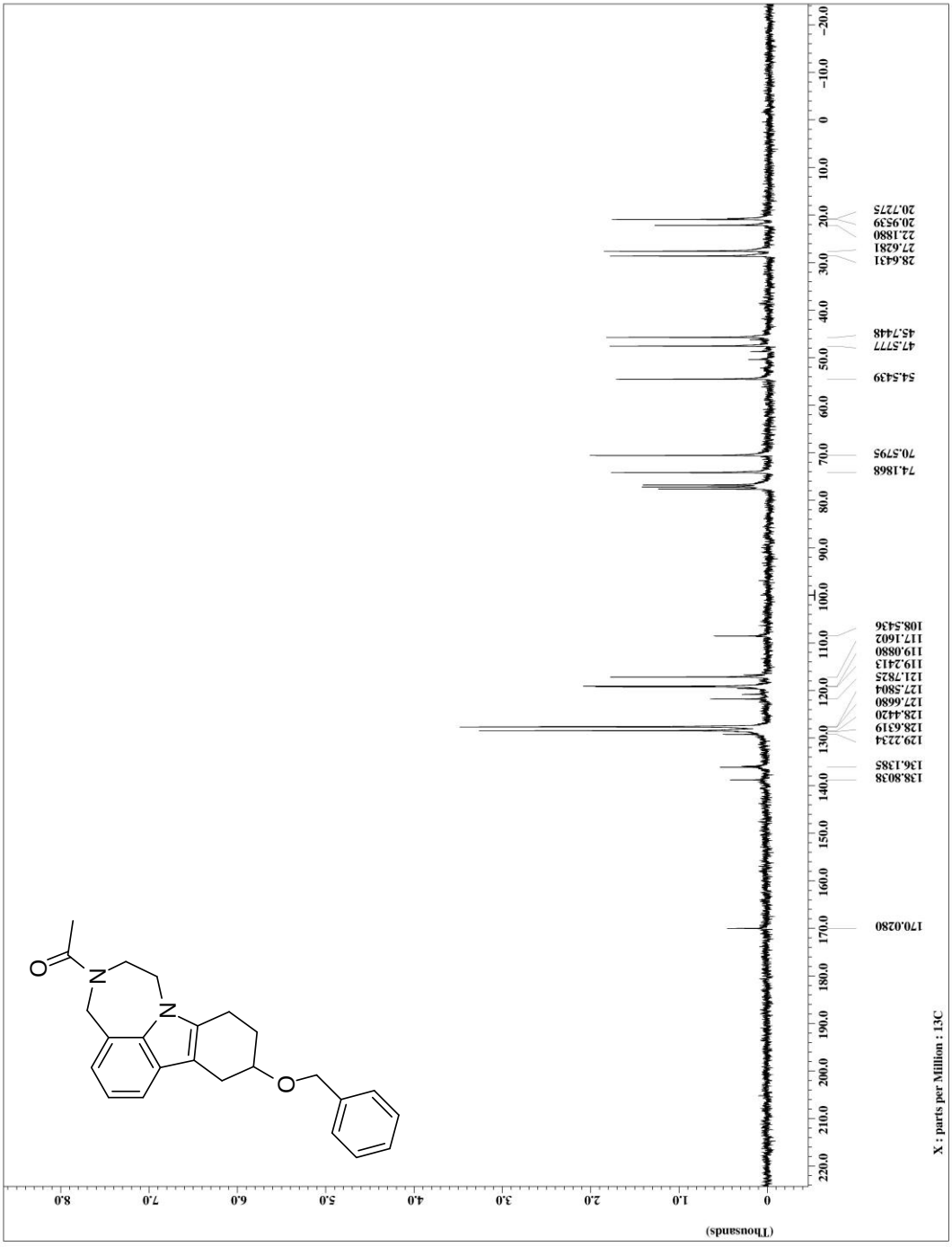


Figure A. 25: ^{1}H-NMR Spectra (300 MHz, $CDCl_3$)
1-(9-hydroxy-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.28)

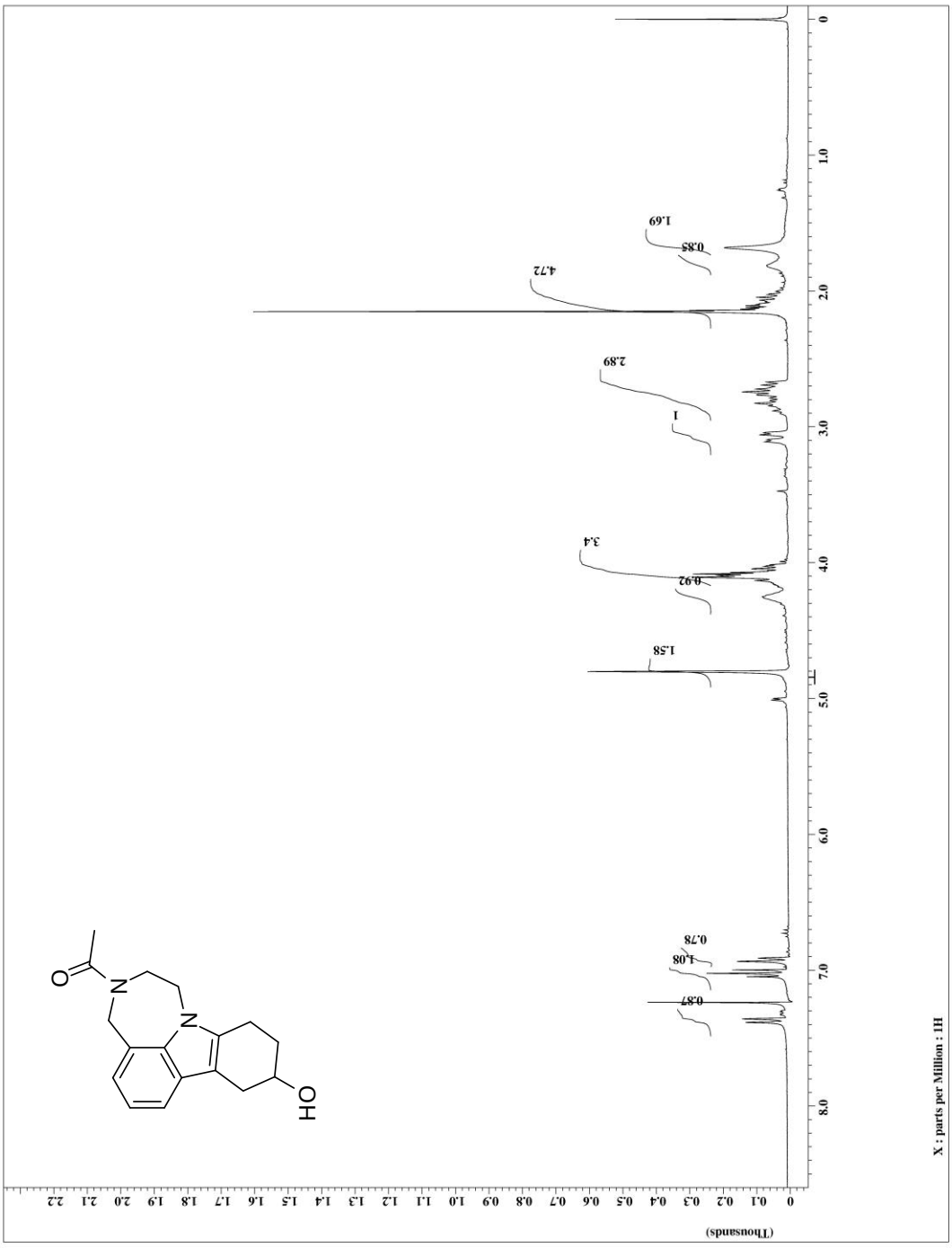


Figure A. 26: ^{13}C-NMR Spectra (300 MHz, $CDCl_3$)
1-(9-hydroxy-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.28)

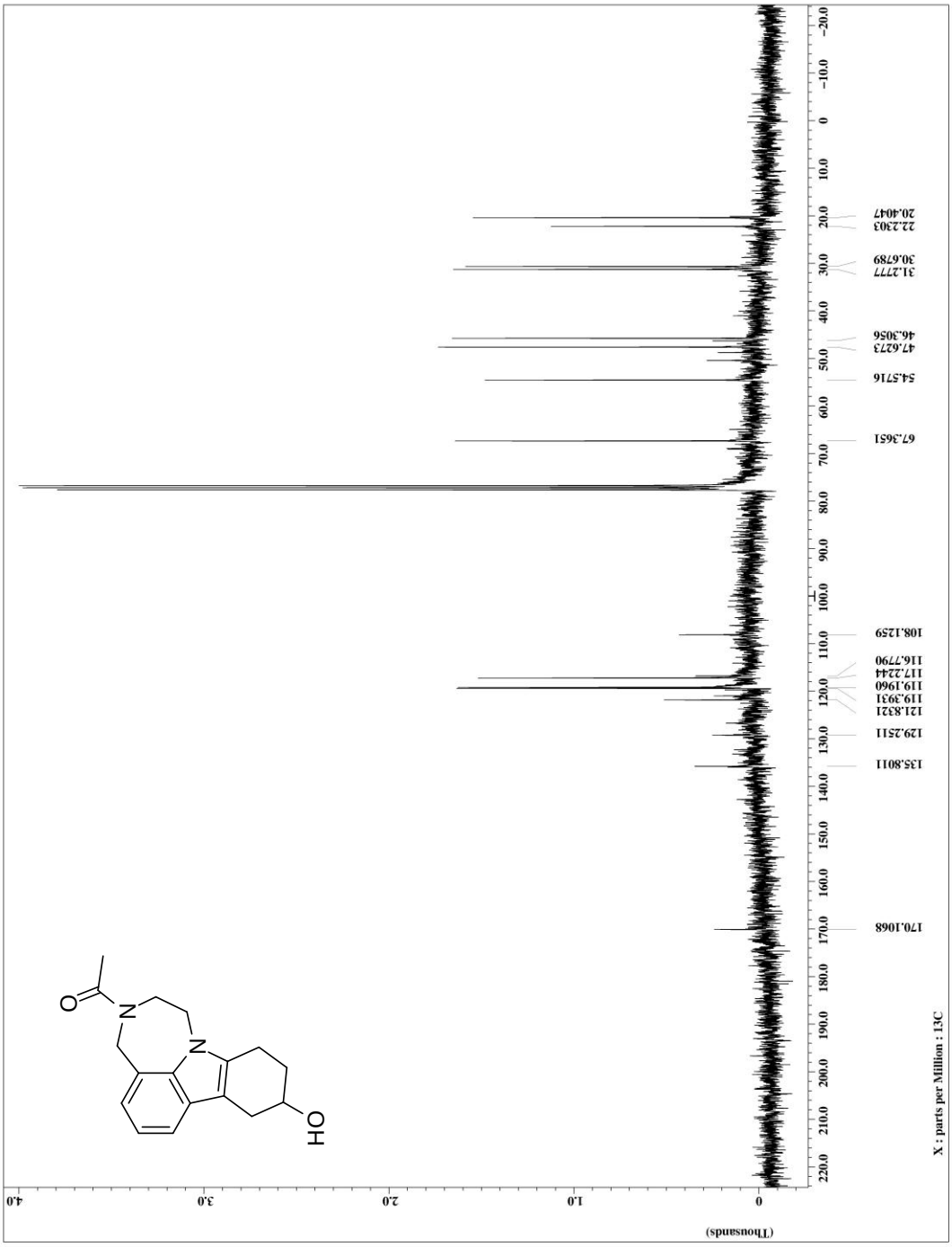


Figure A. 27: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
2-ethoxy-1-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)ethanone (2.30)

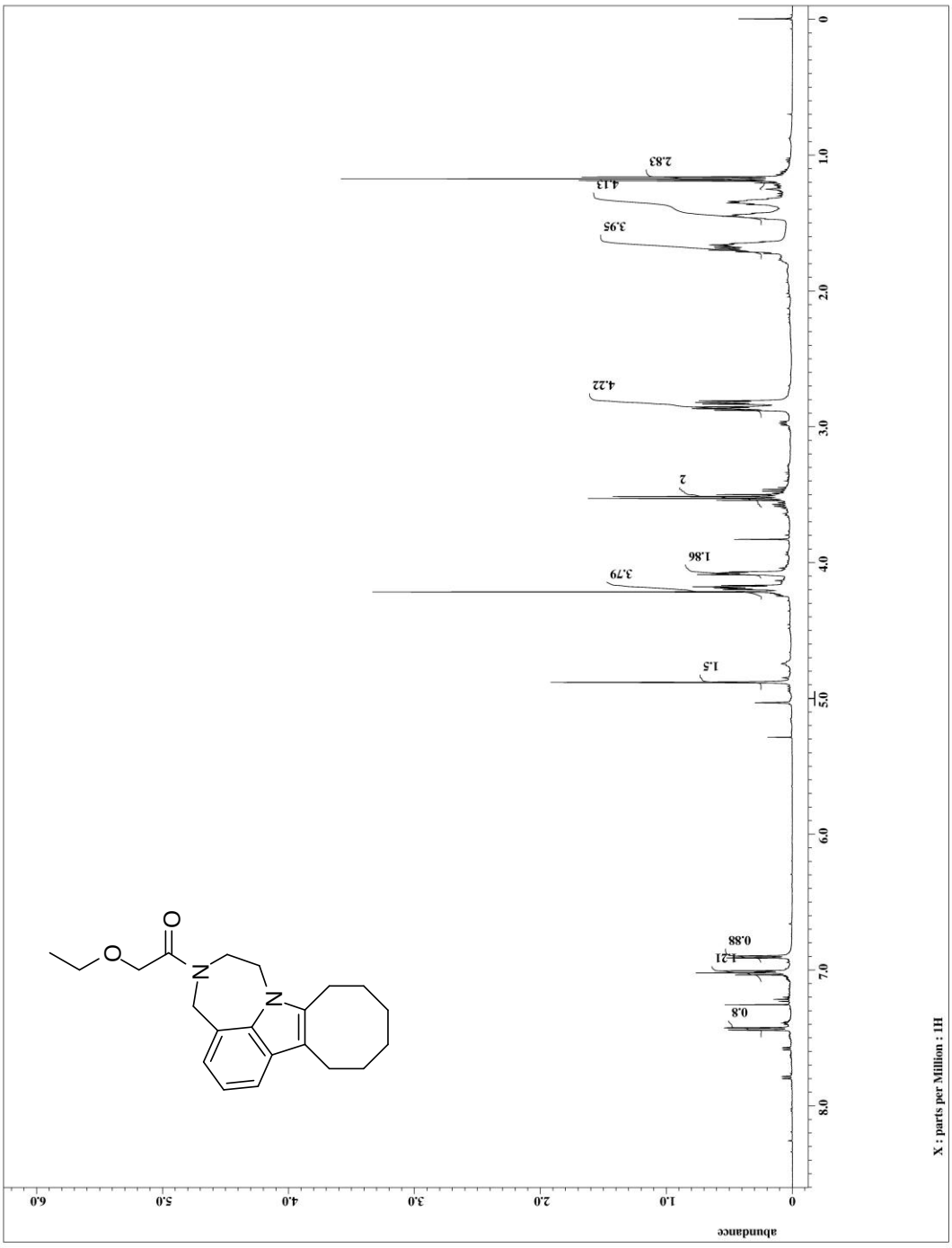


Figure A. 28: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
2-ethoxy-1-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)ethanone (2.30)

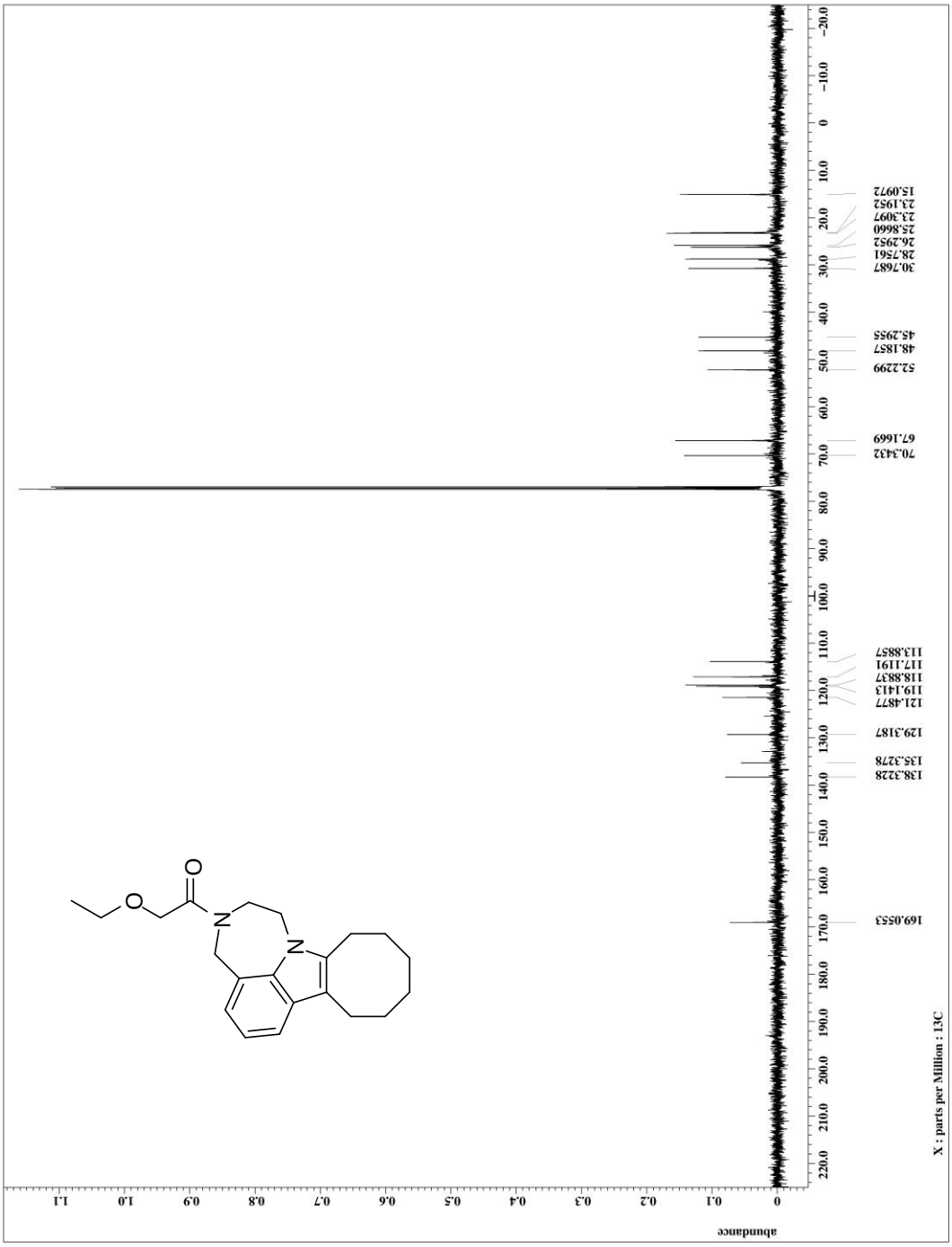


Figure A. 29: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
4-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)-4-oxobutanoic acid (2.32)

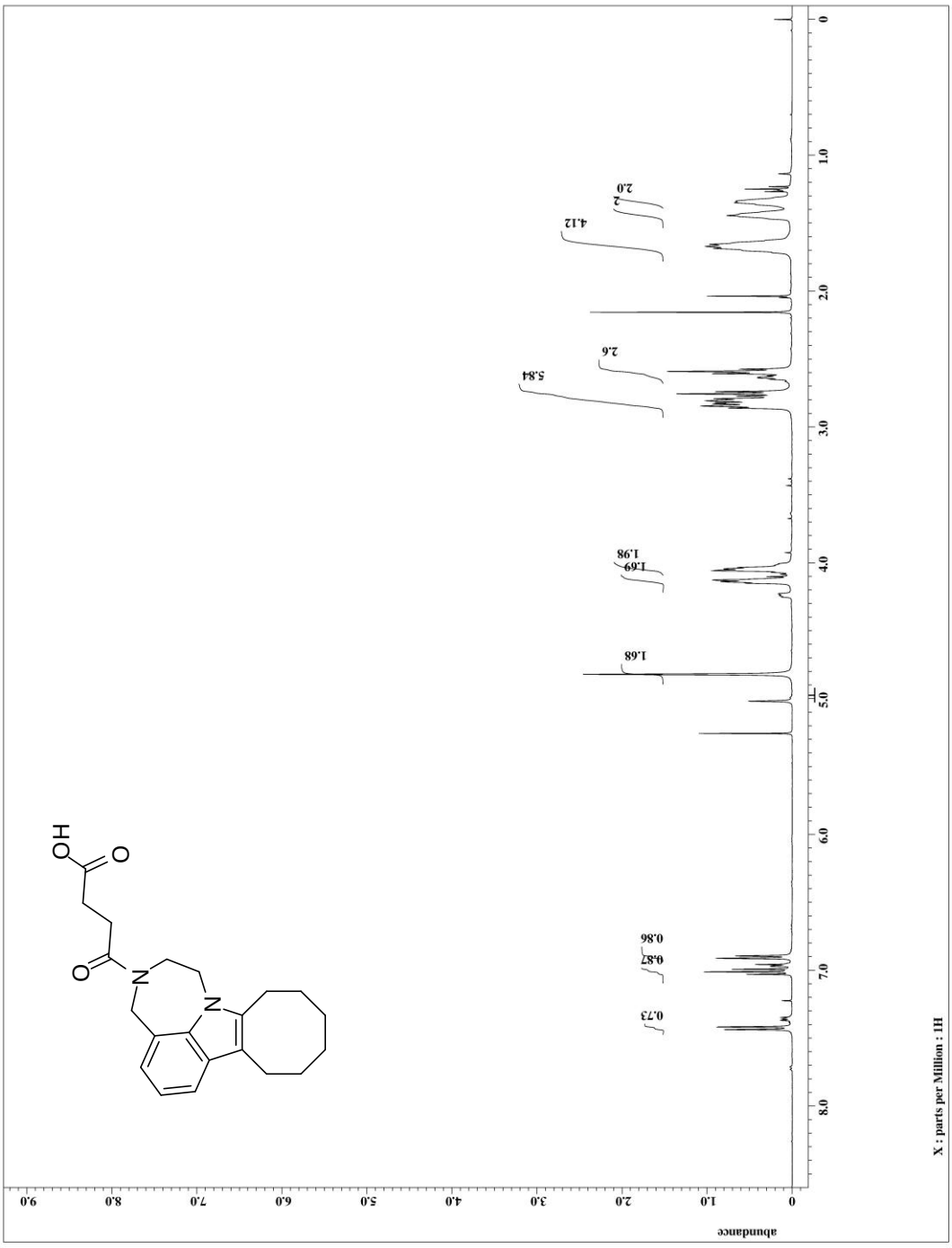


Figure A. 30: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
4-(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)-4-oxobutanoic acid (2.32)

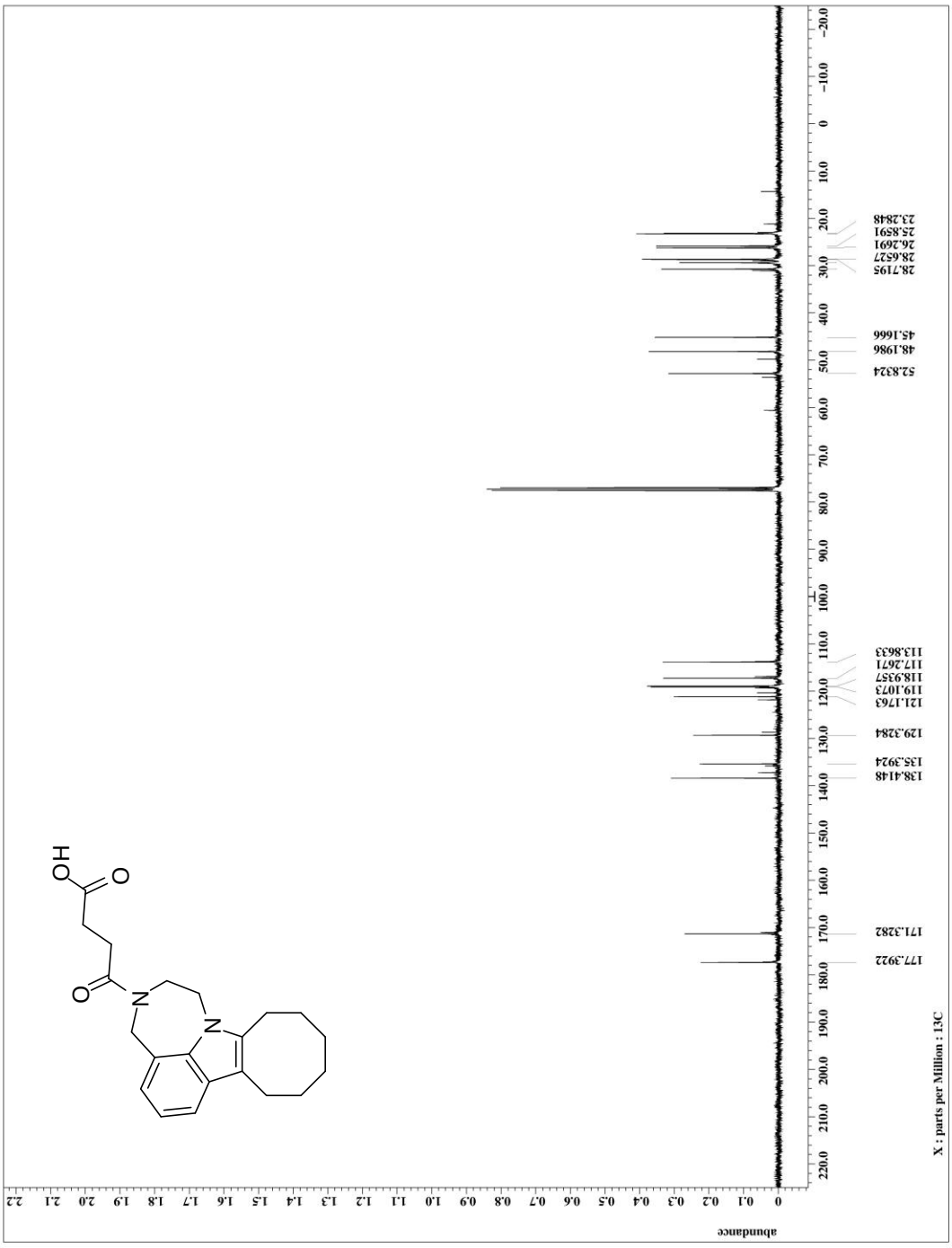


Figure A. 31: ^{1}H-NMR Spectra (400vMHz, $CDCl_3$)
1,4-bis(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)butane-1,4-dione (2.33)

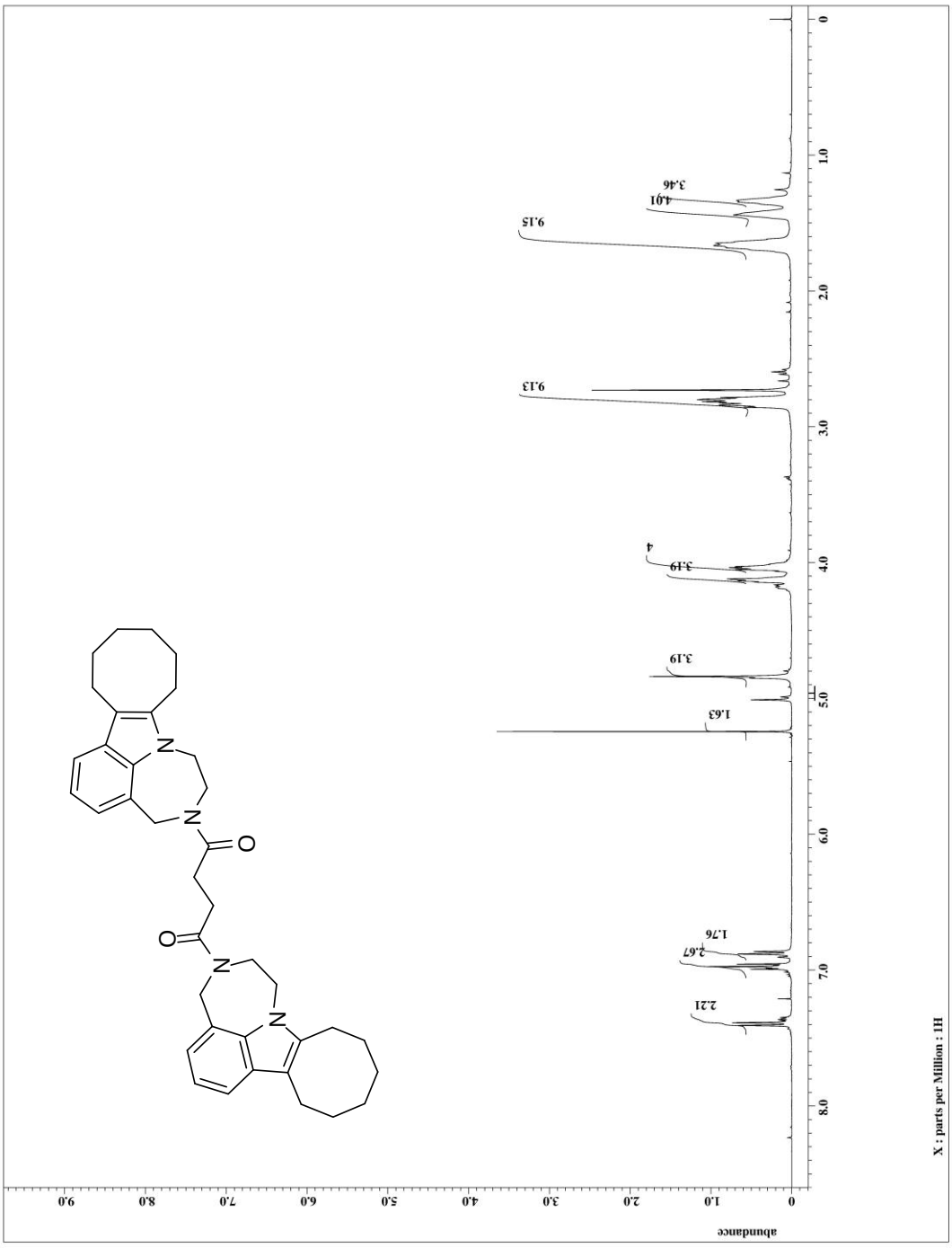


Figure A. 32: ^{13}C-NMR Spectra (400vMHz, $CDCl_3$)
1,4-bis(1,2,8,9,10,11,12,13-octahydrocycloocta[b][1,4]diazepino[6,7,1-hi]indol-3(4H)-yl)butane-1,4-dione (2.33)

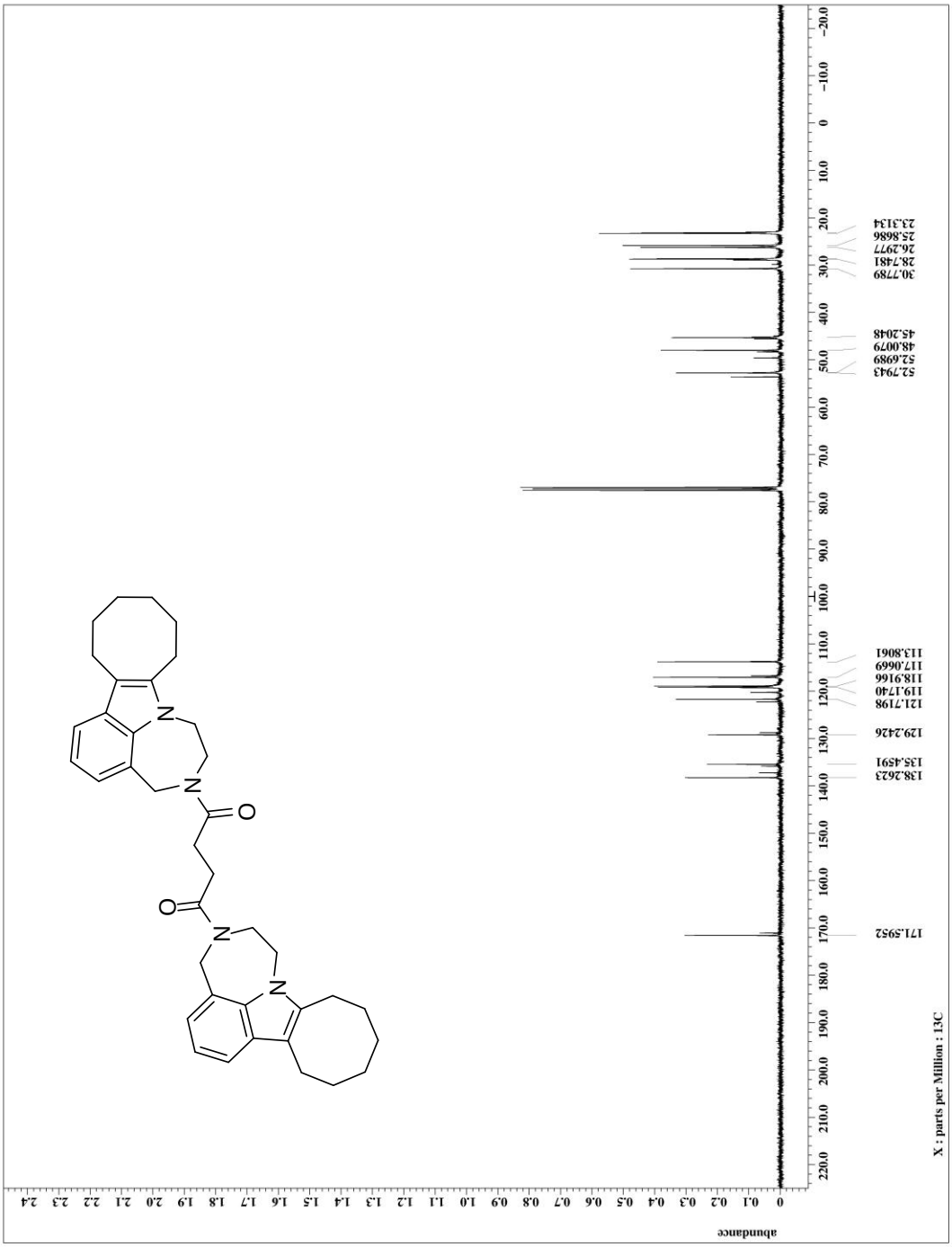


Figure A. 33: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1-(9-(2-methoxyethyl)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.35)

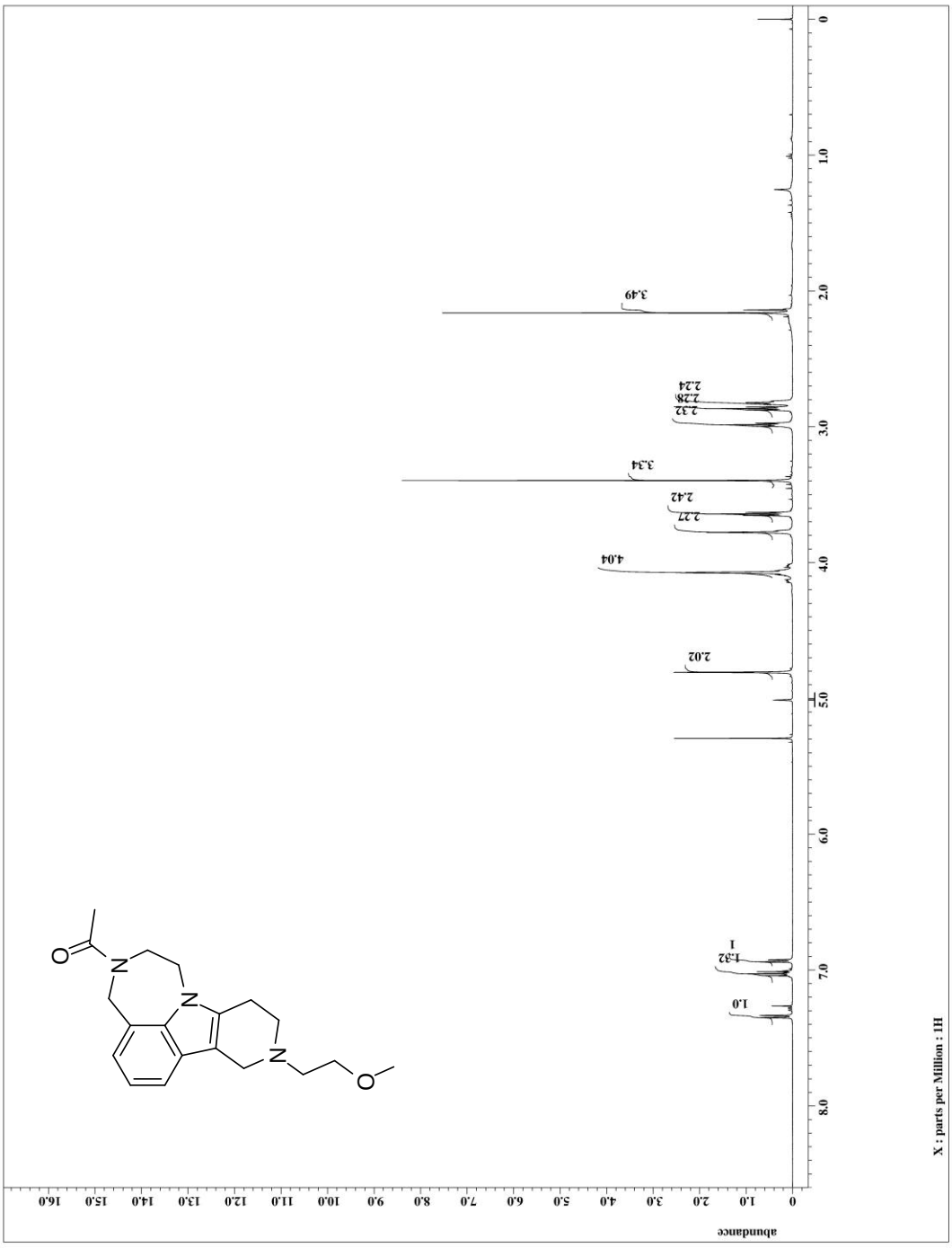


Figure A. 34: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1-(9-(2-methoxyethyl)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.35)

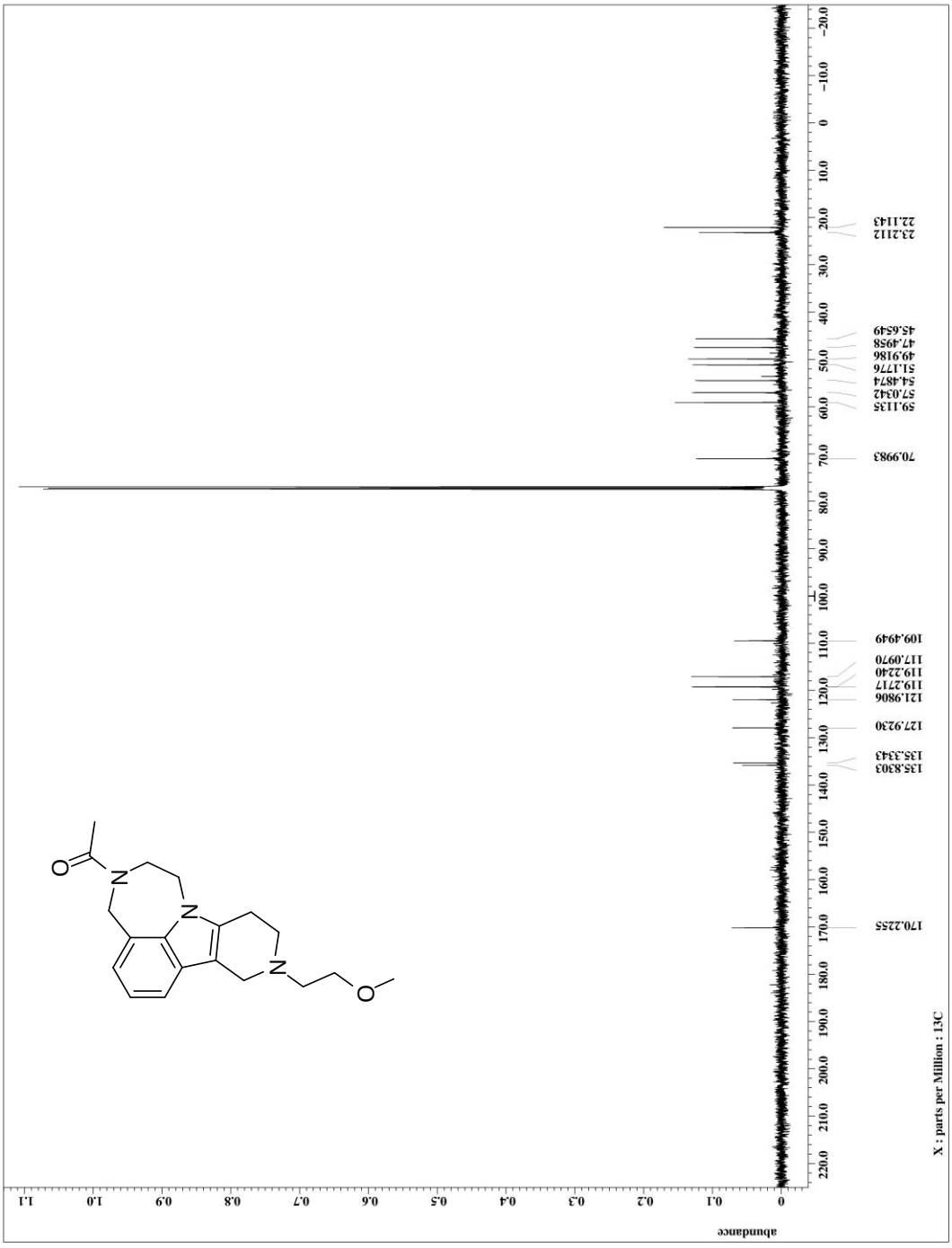


Figure A. 35: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
9-(2-methoxyethyl)-1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.36)

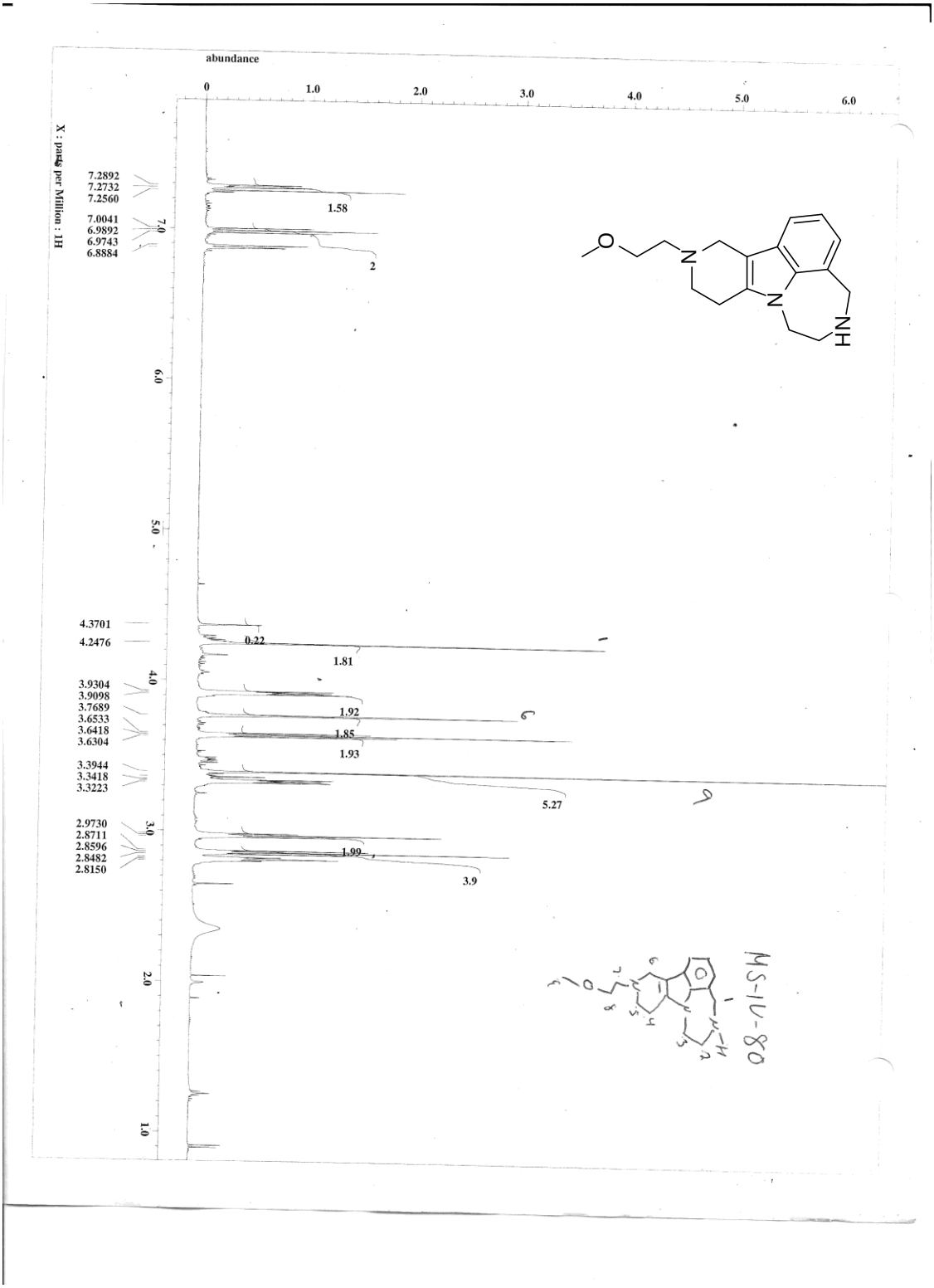


Figure A. 36: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
9-(2-methoxyethyl)-1,2,3,4,8,9,10,11-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.36)

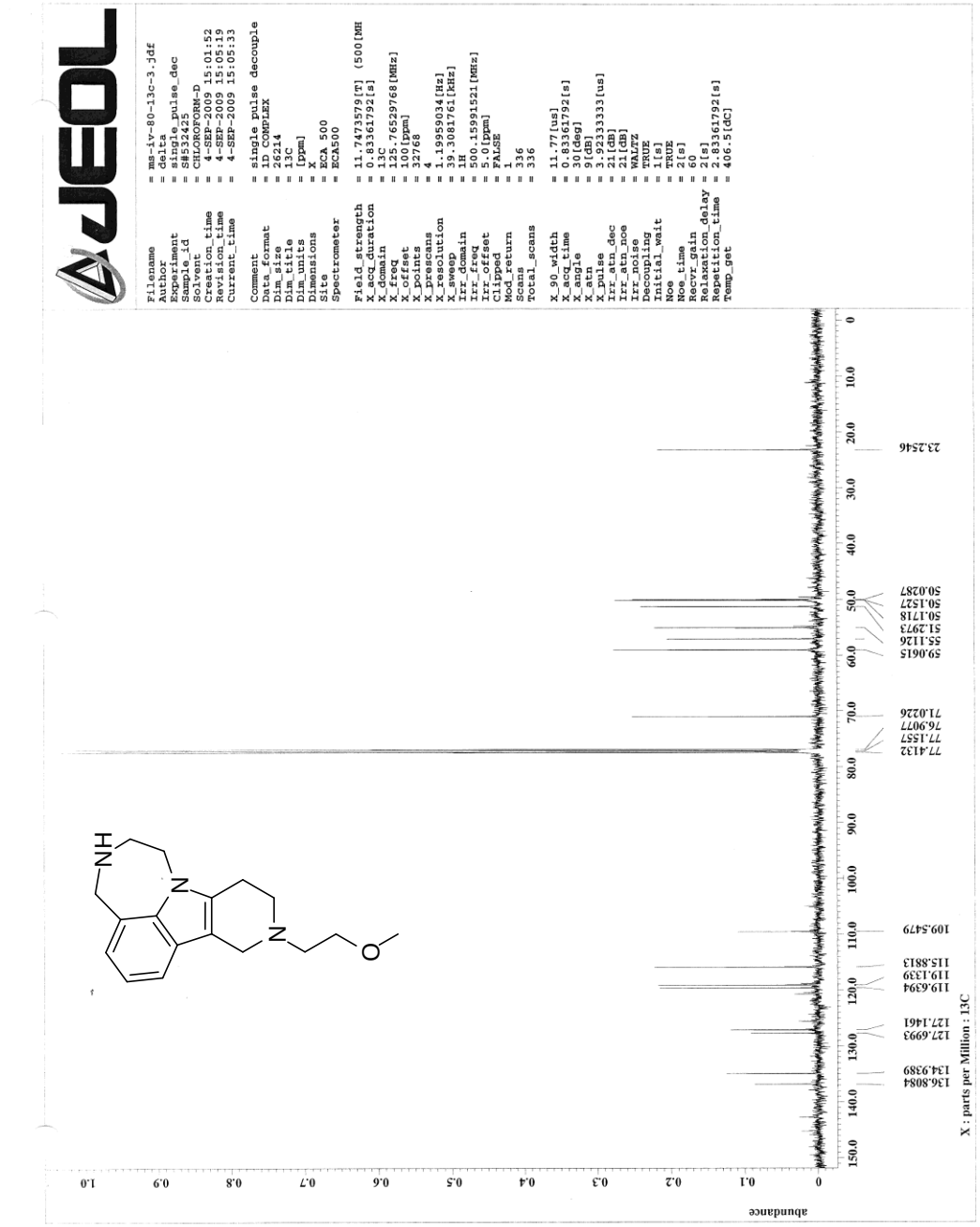


Figure A. 37: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.53)

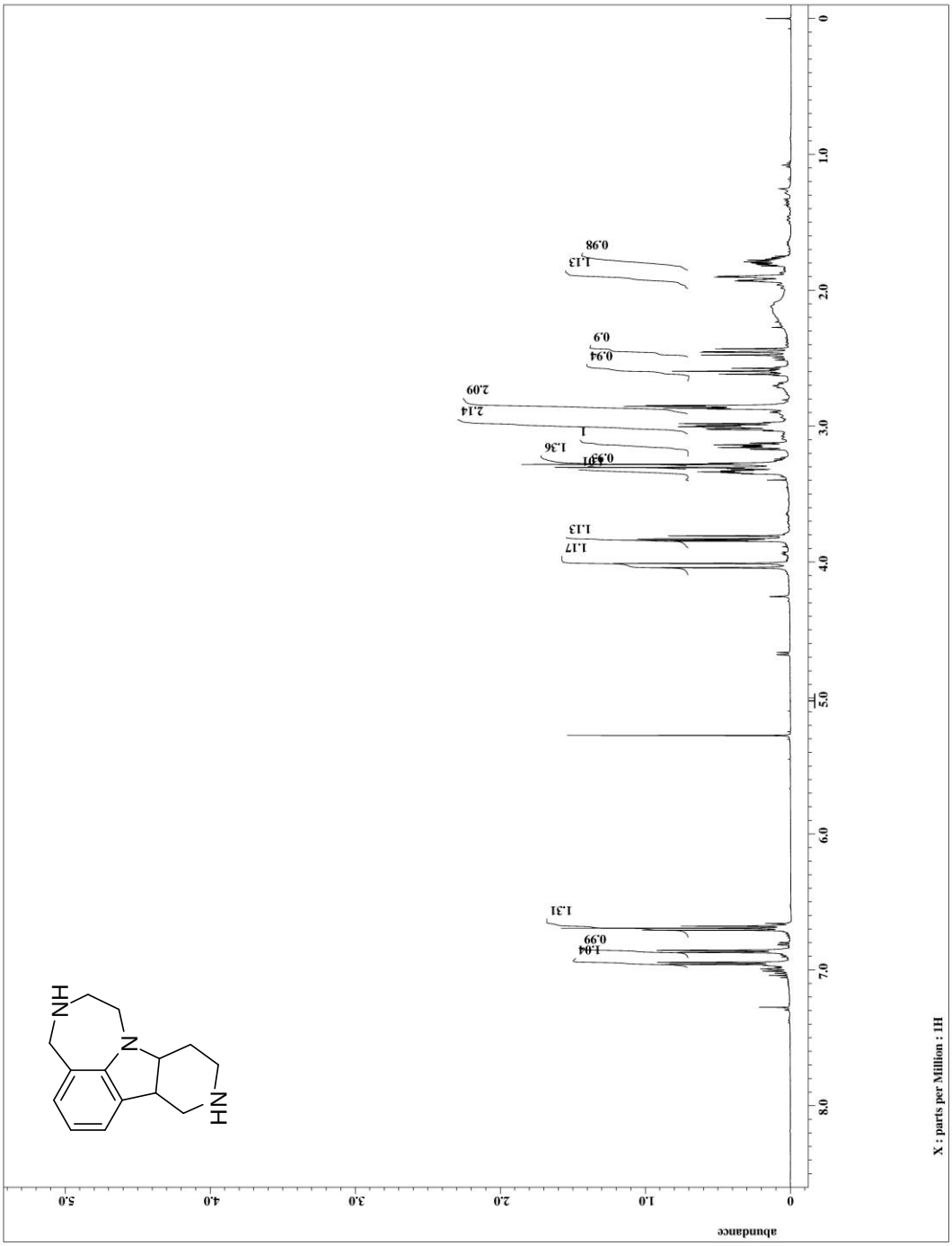


Figure A. 38: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.53)

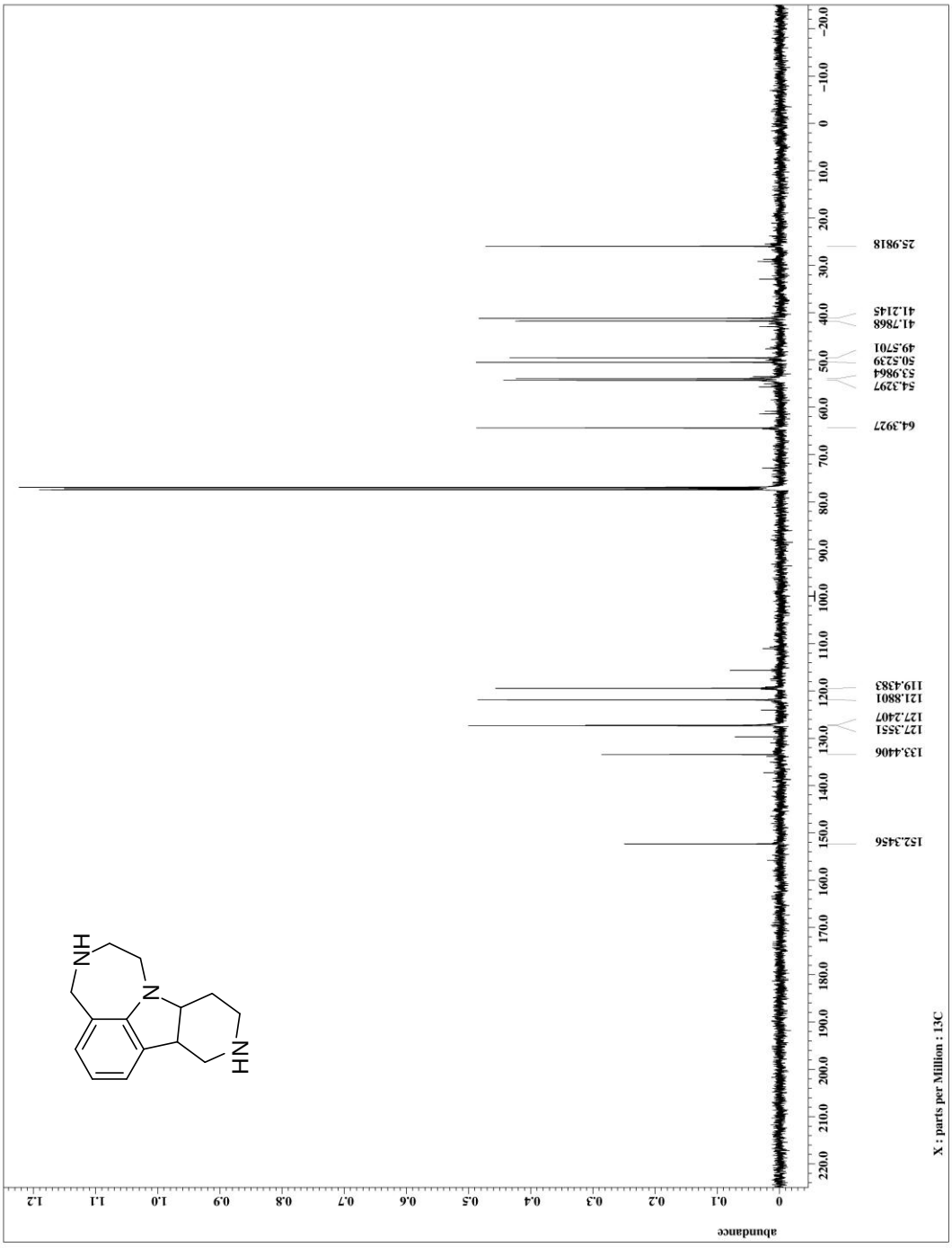


Figure A. 39: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-jk]carbazol-9-ol (2.54)

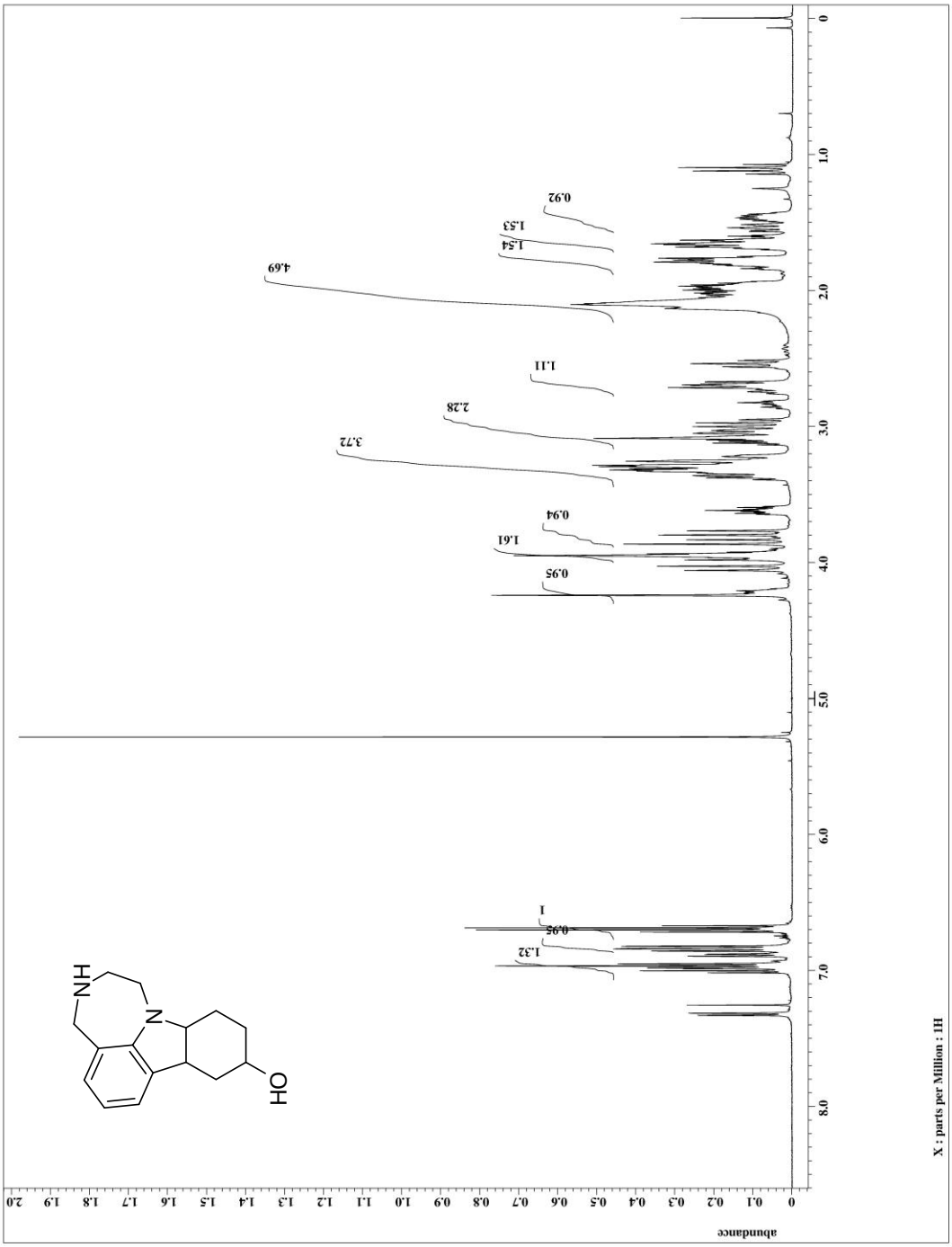


Figure A. 40: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-jk]carbazol-9-ol (2.54)

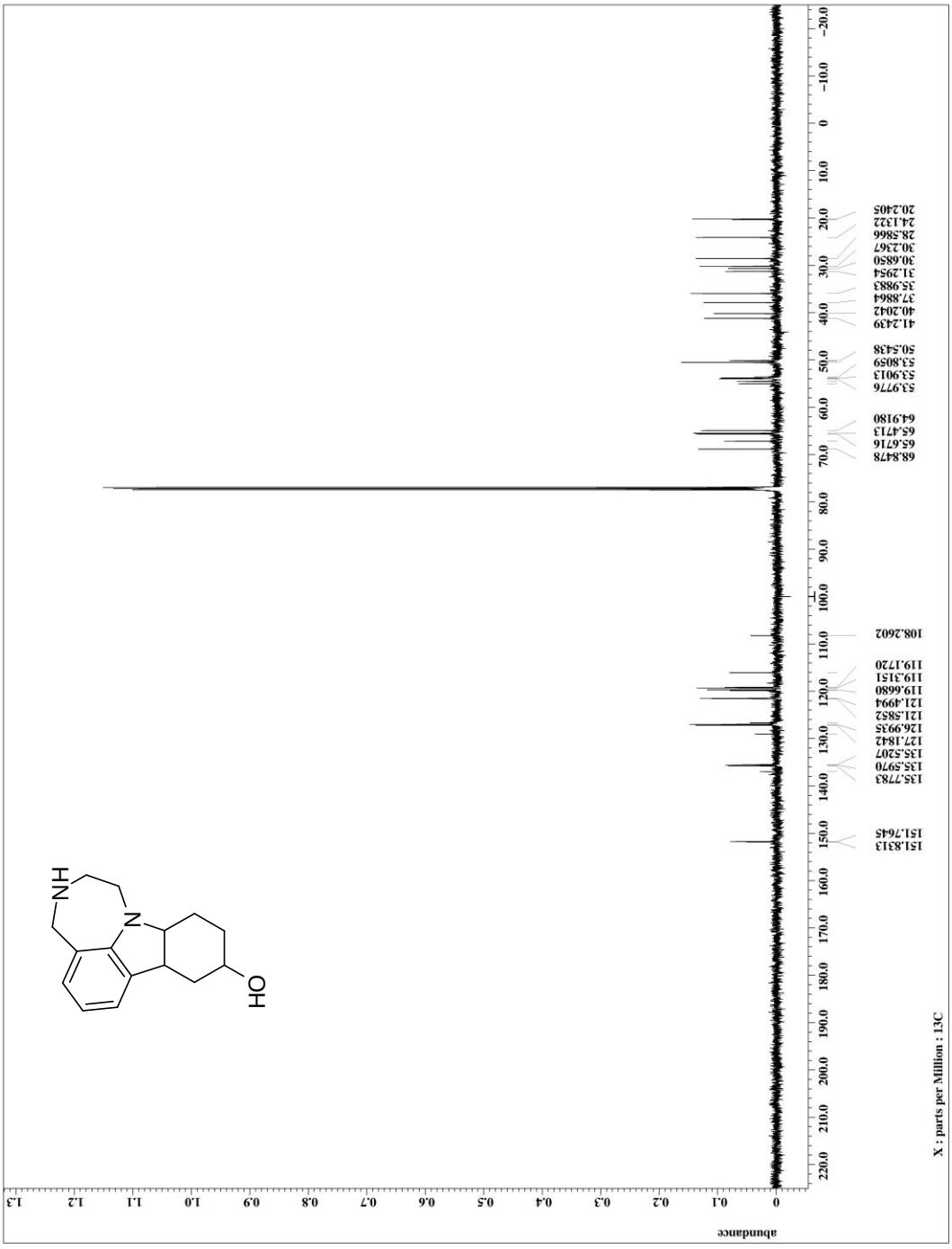


Figure A. 41: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
6-bromo-2,3,4,7b,8,9,10,10a-octahydro-1H-cyclopenta[b][1,4]diazepino[6,7,1-hi]indole (2.55)

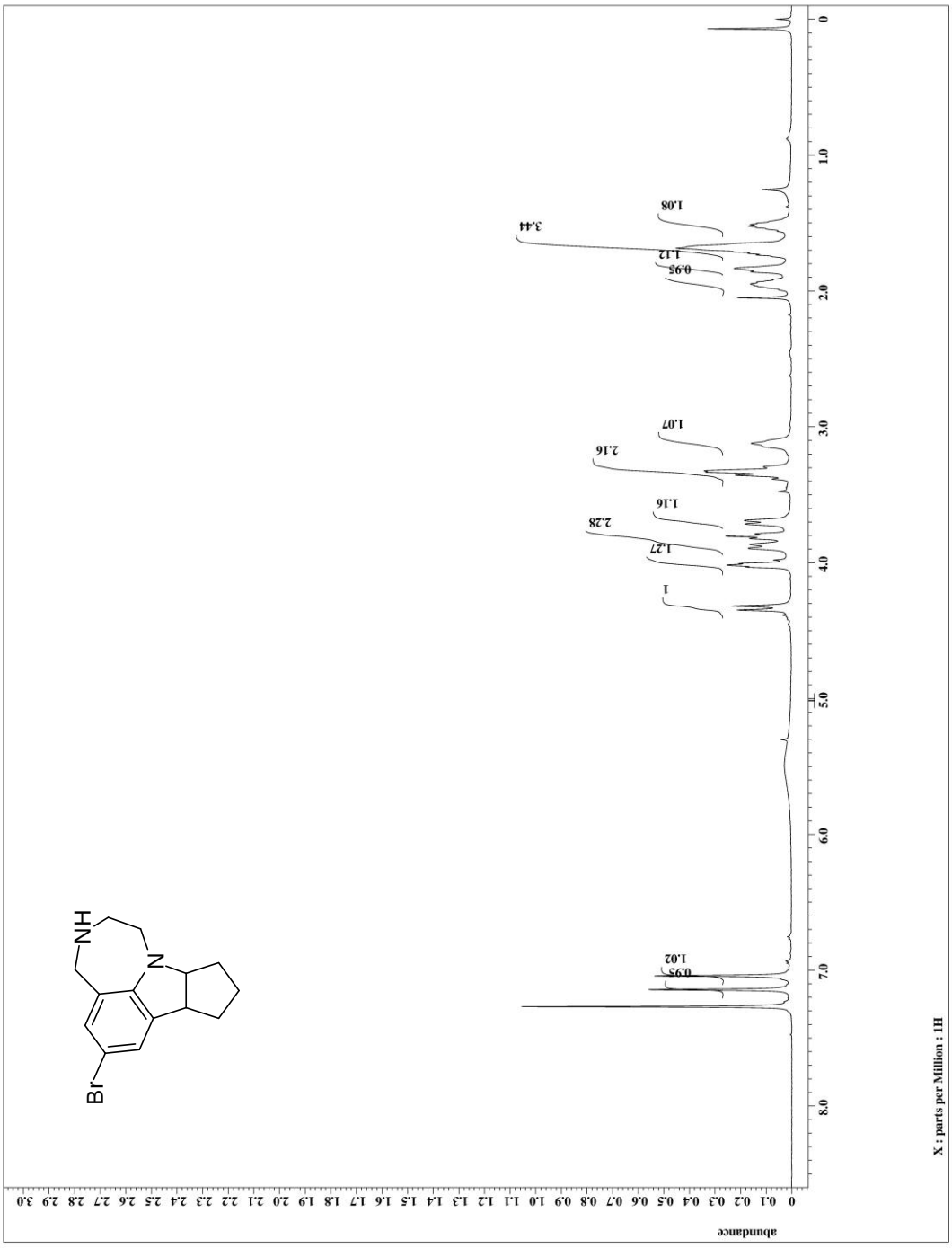


Figure A. 42: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
6-bromo-2,3,4,7b,8,9,10,10a-octahydro-1H-cyclopenta[b][1,4]diazepino[6,7,1-hi]indole (2.55)

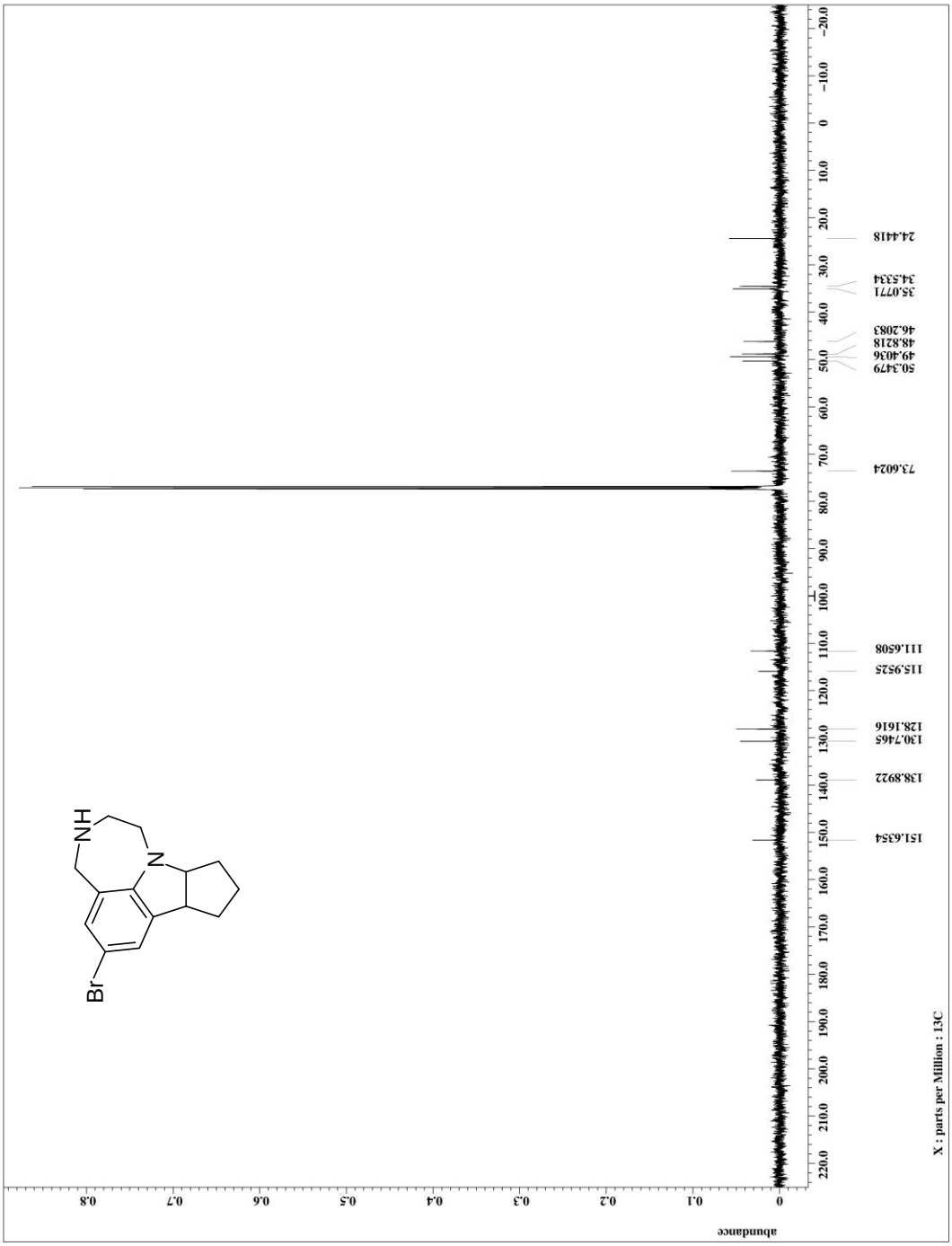


Figure A. 43: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
benzyl 3-acetyl-1,2,3,4,7b,8,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(10H)-carboxylate (2.56)

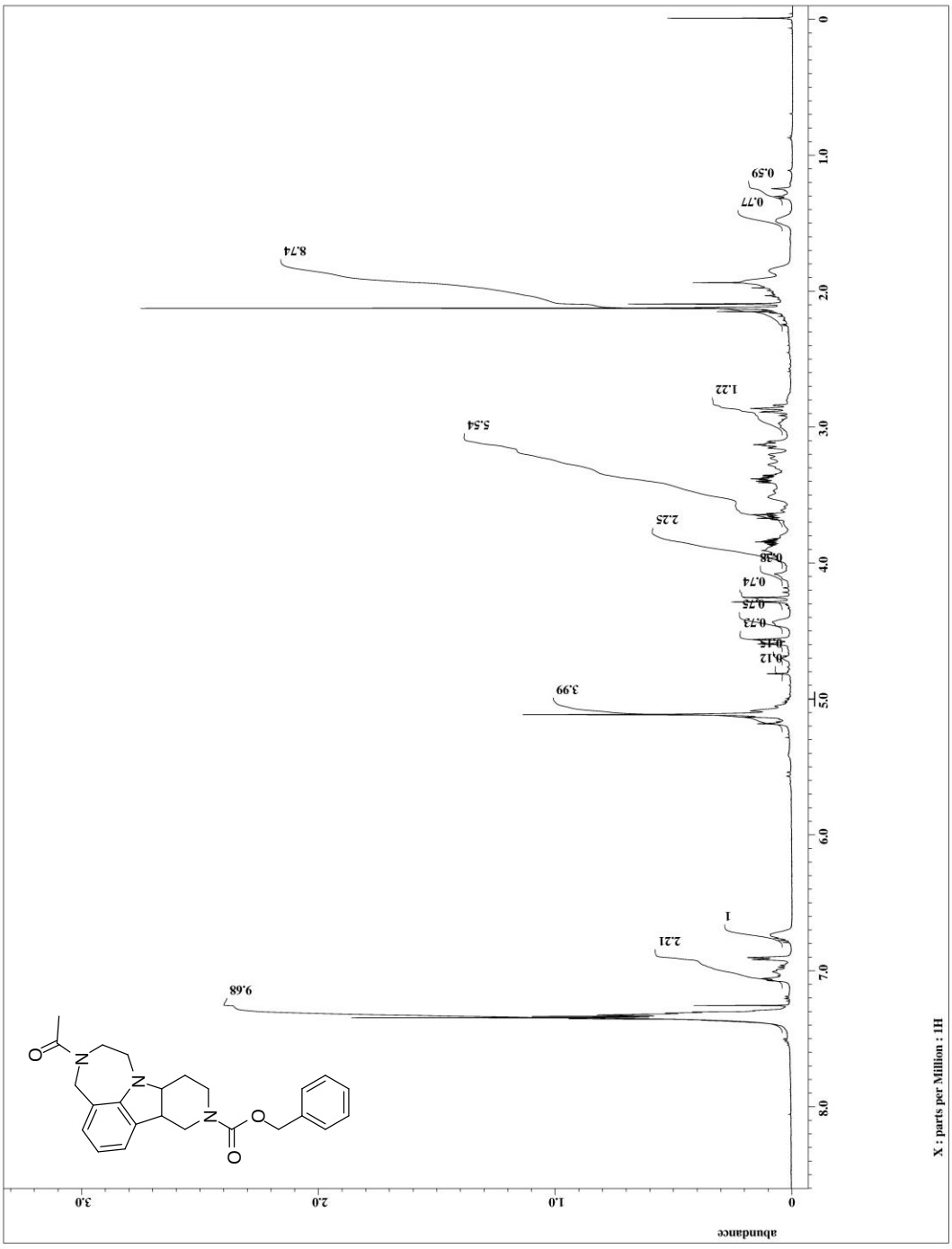


Figure A. 44: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
benzyl 3-acetyl-1,2,3,4,7b,8,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole-9(10H)-carboxylate (2.56)

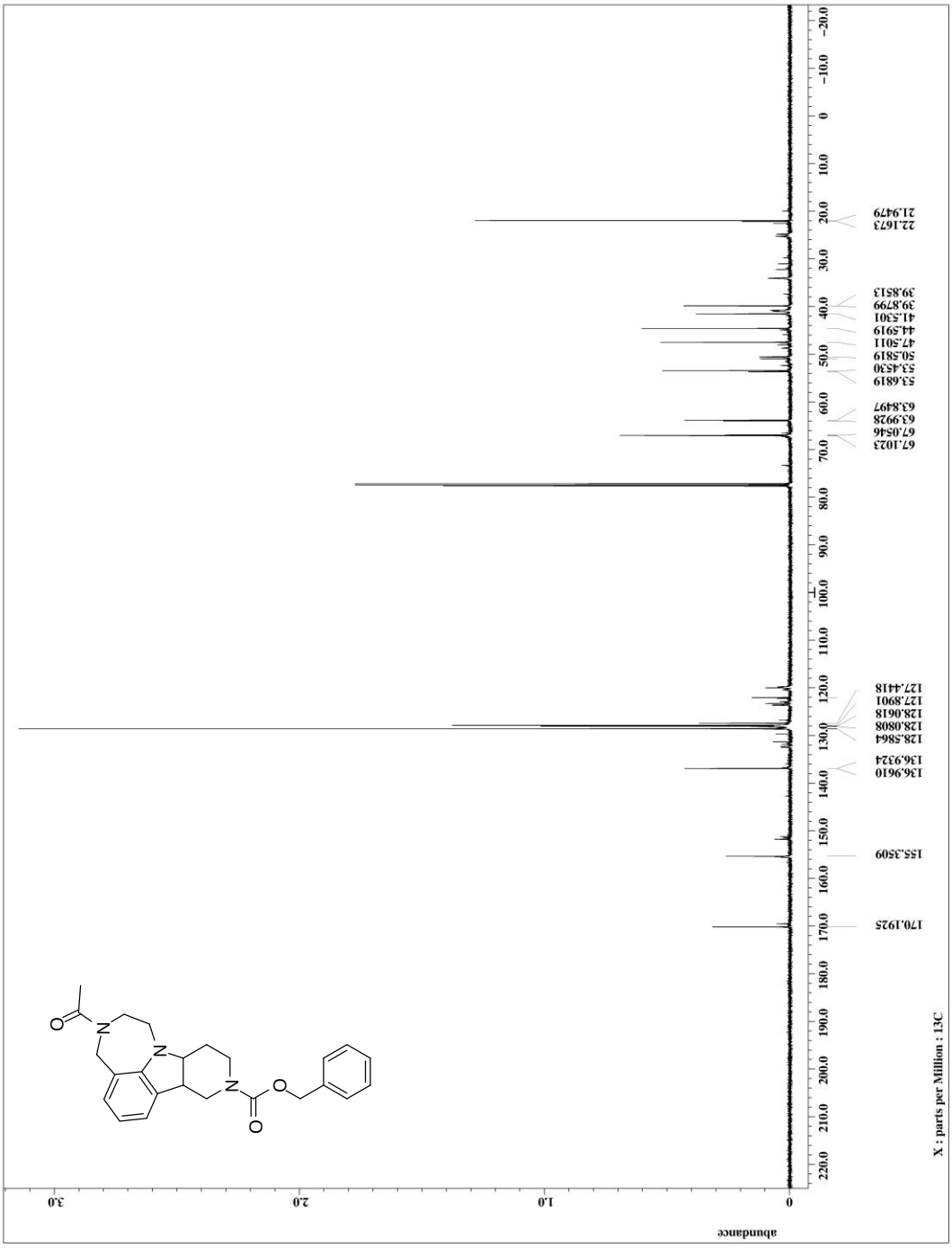


Figure A. 45: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1-(1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.57)

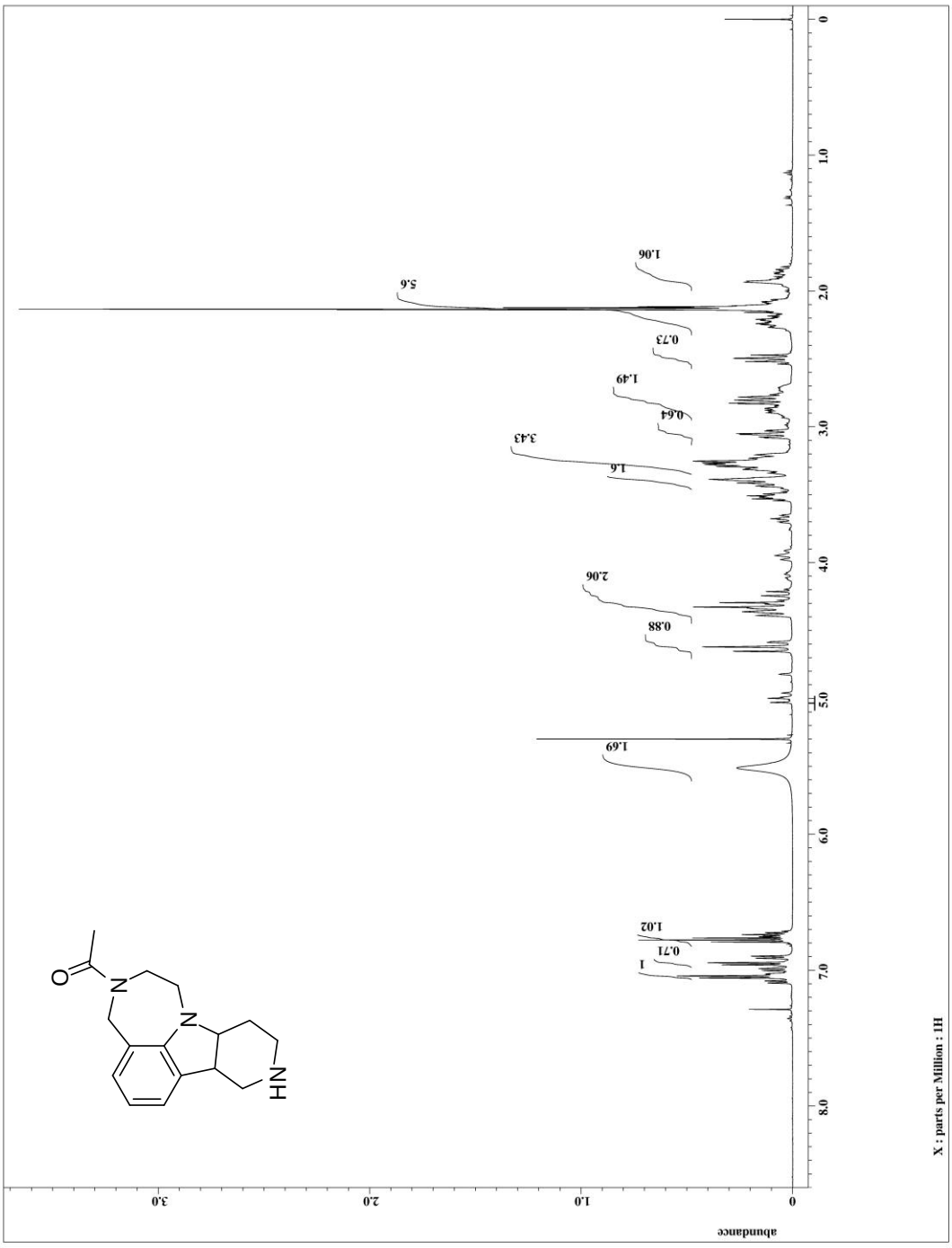


Figure A. 46: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1-(1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.57)

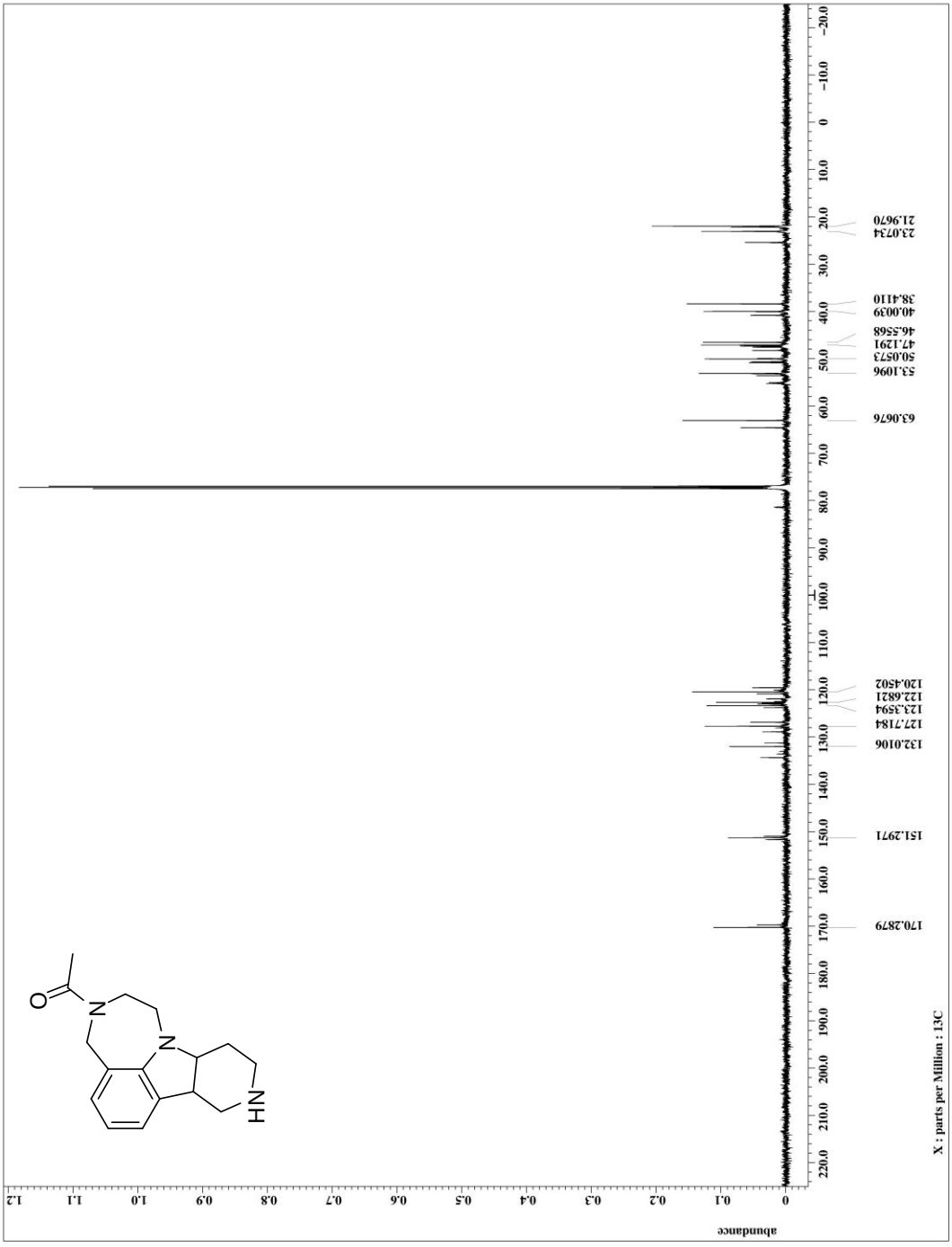


Figure A. 47: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
9-(2-methoxyethyl)-1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.58)

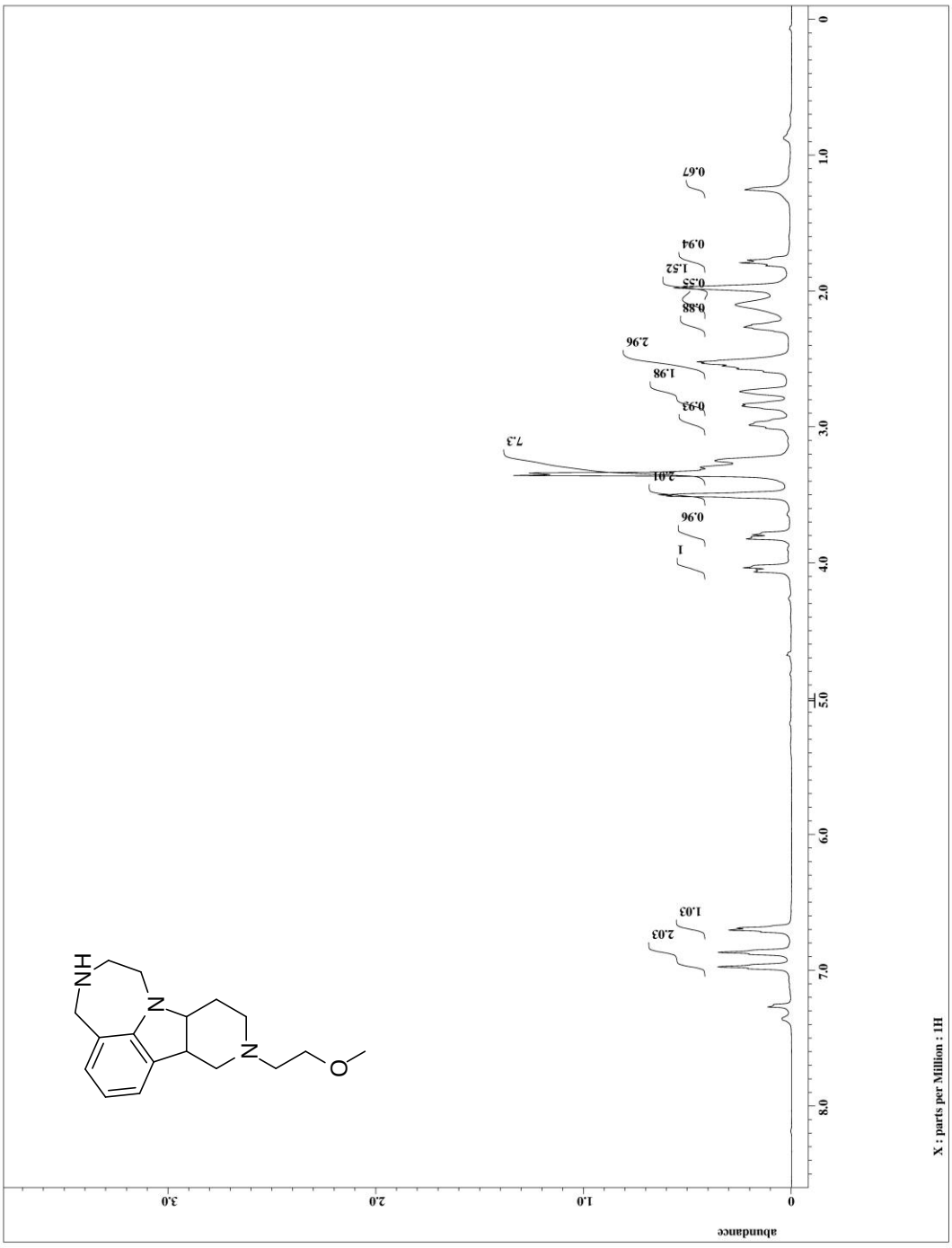


Figure A. 48: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
9-(2-methoxyethyl)-1,2,3,4,7b,8,9,10,11,11a-decahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indole (2.58)

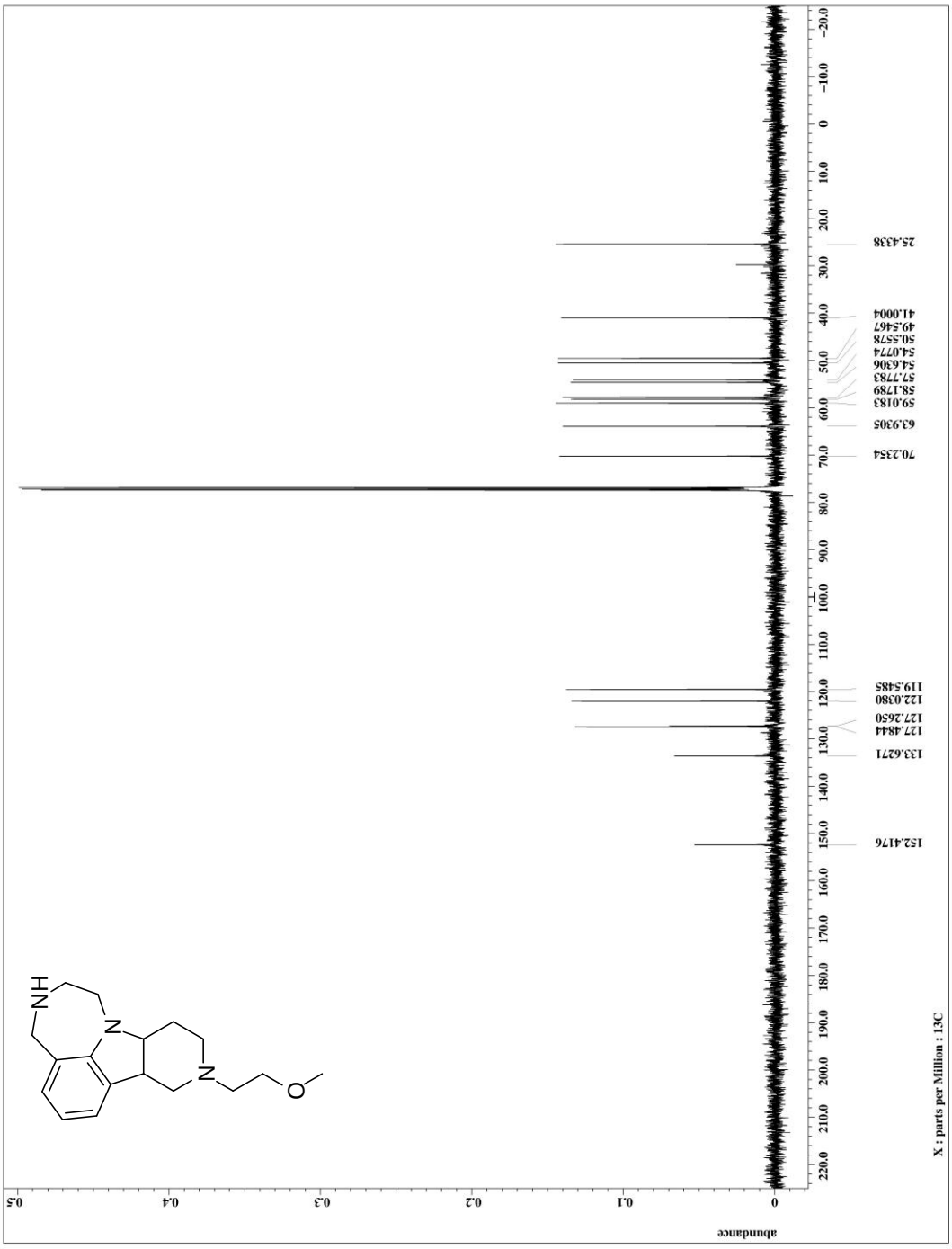


Figure A. 49: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1-(9-(benzyloxy)-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.59)

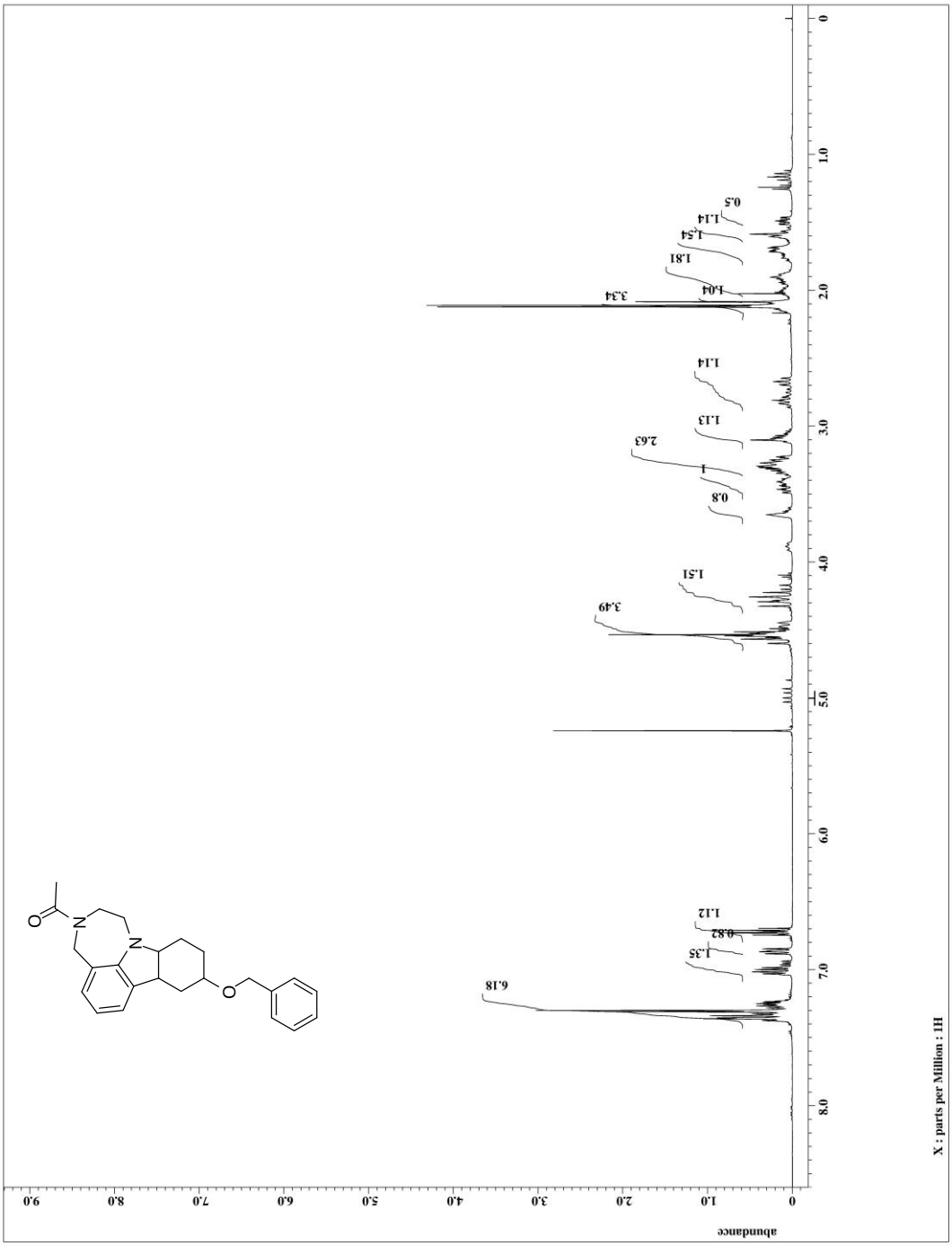


Figure A. 50: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1-(9-(benzyloxy)-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.59)

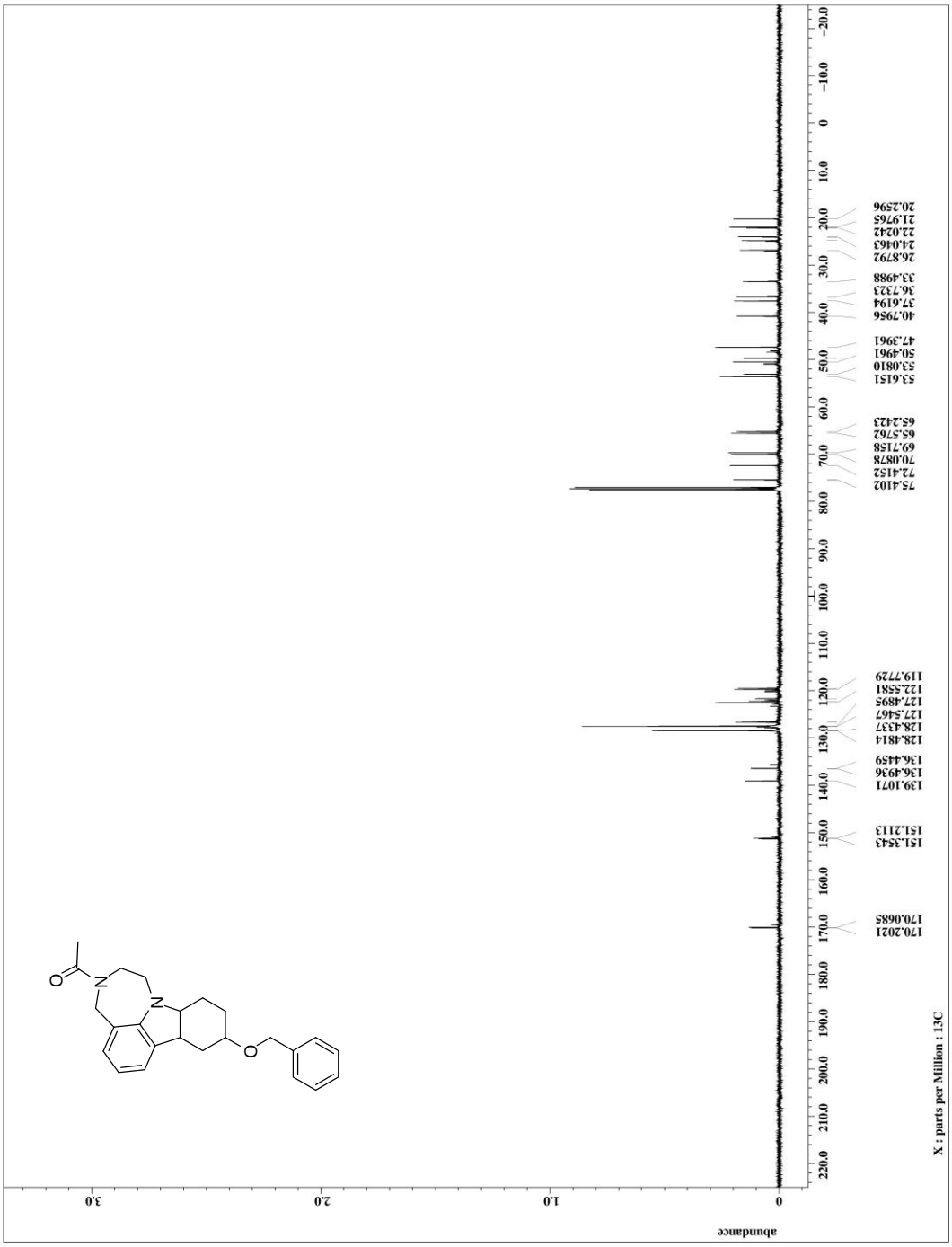


Figure A. 51: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1-(9-hydroxy-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.60)

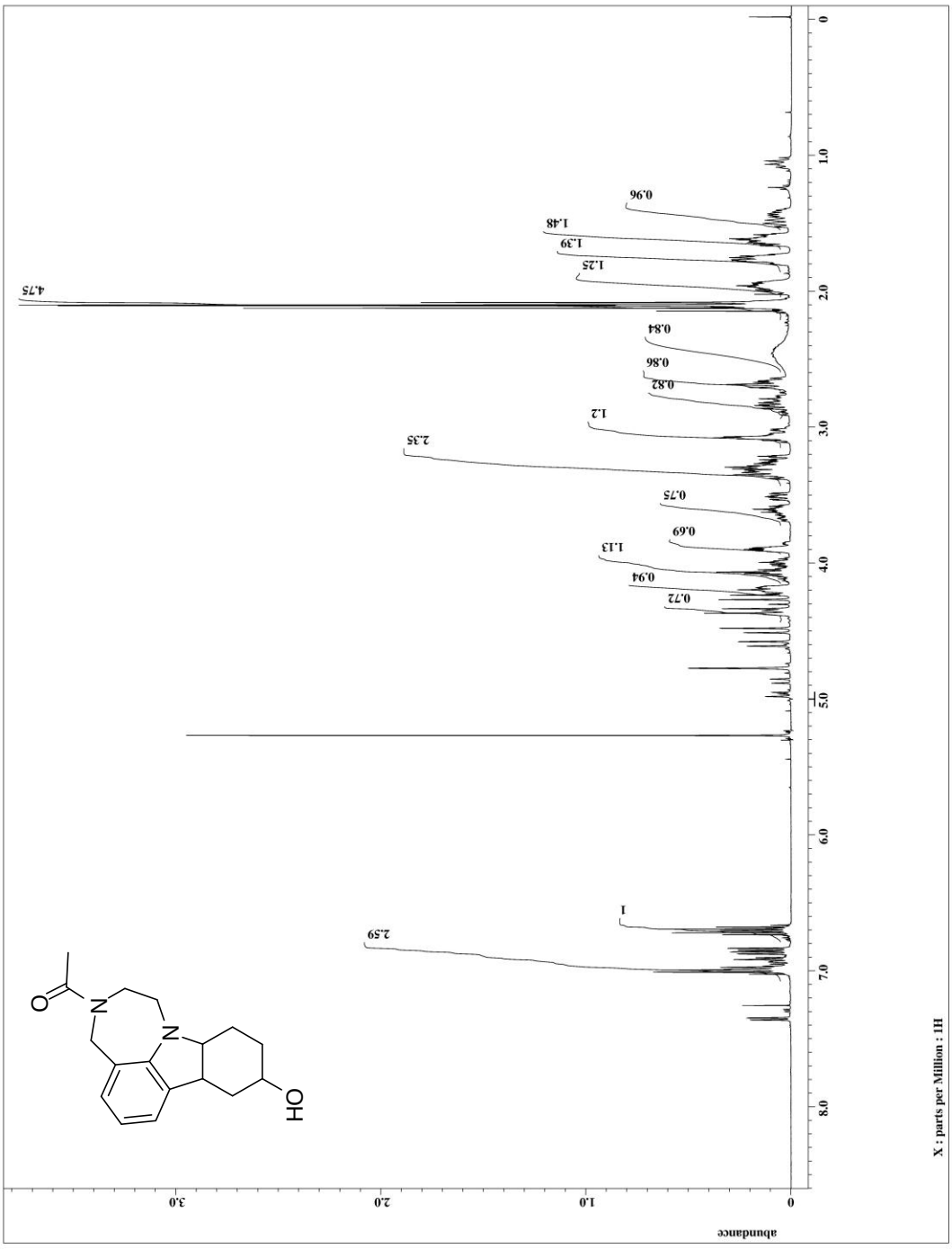


Figure A. 52: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1-(9-hydroxy-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (2.60)

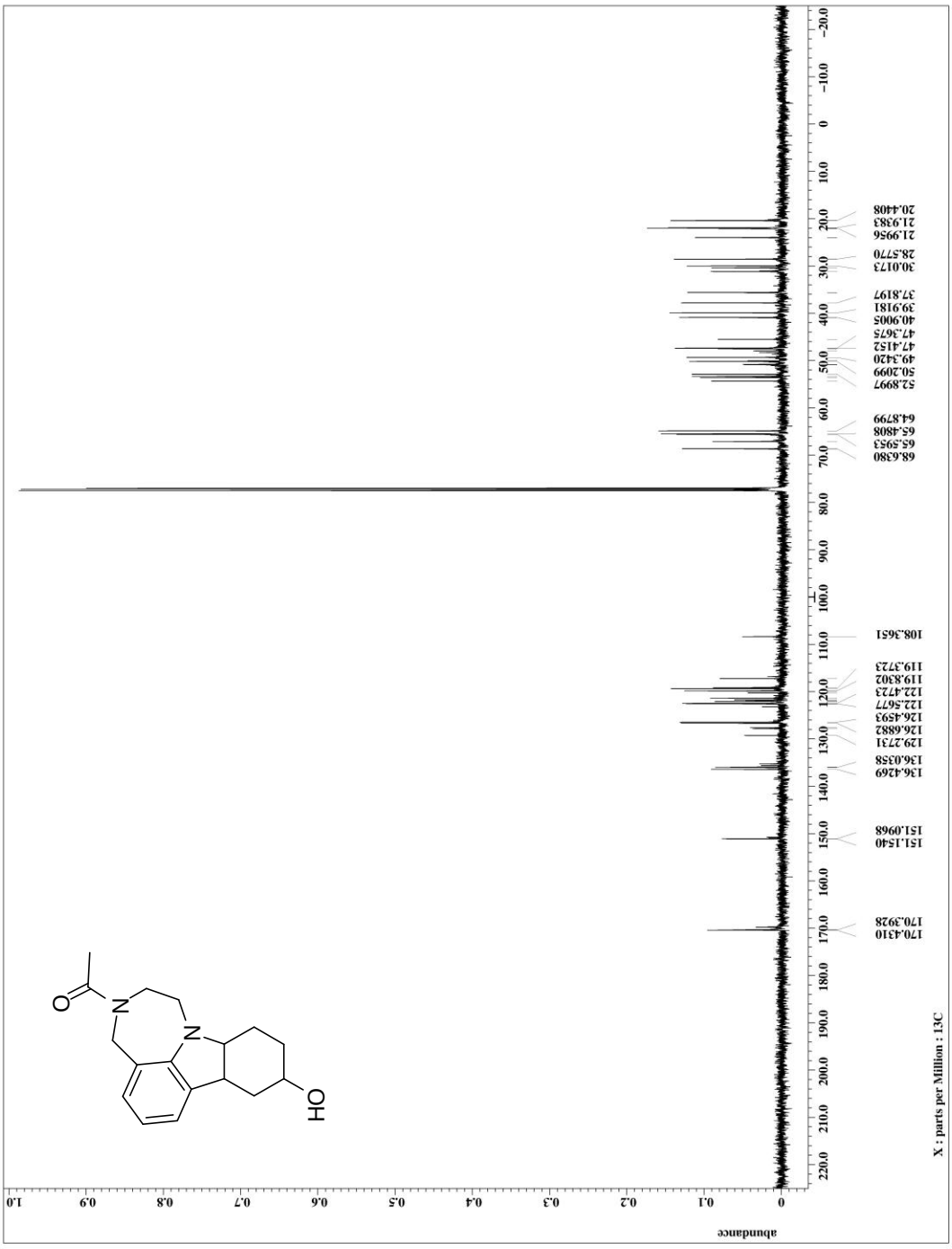


Figure A. 53: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
1-(9-(2-methoxyethyl)-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.62)

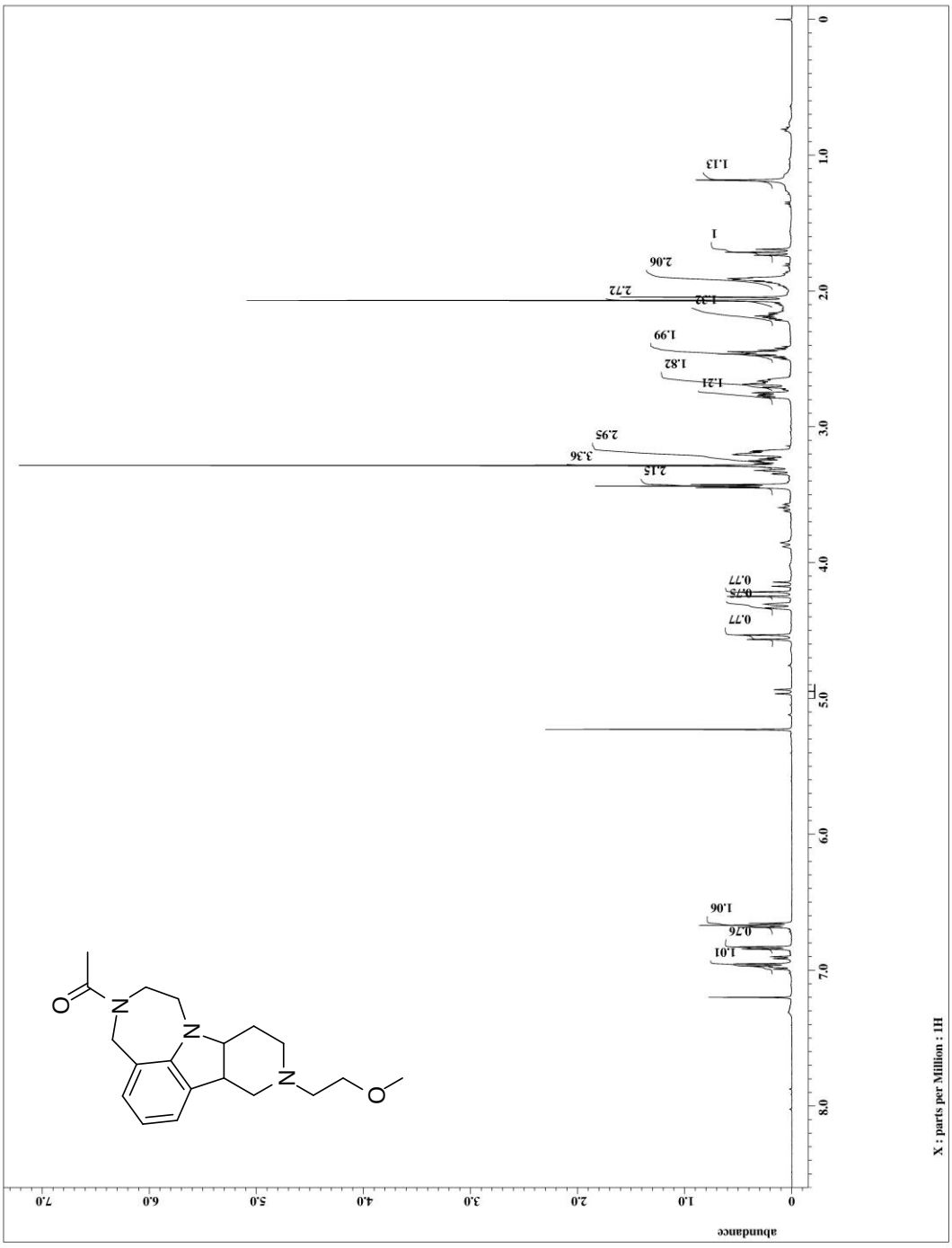
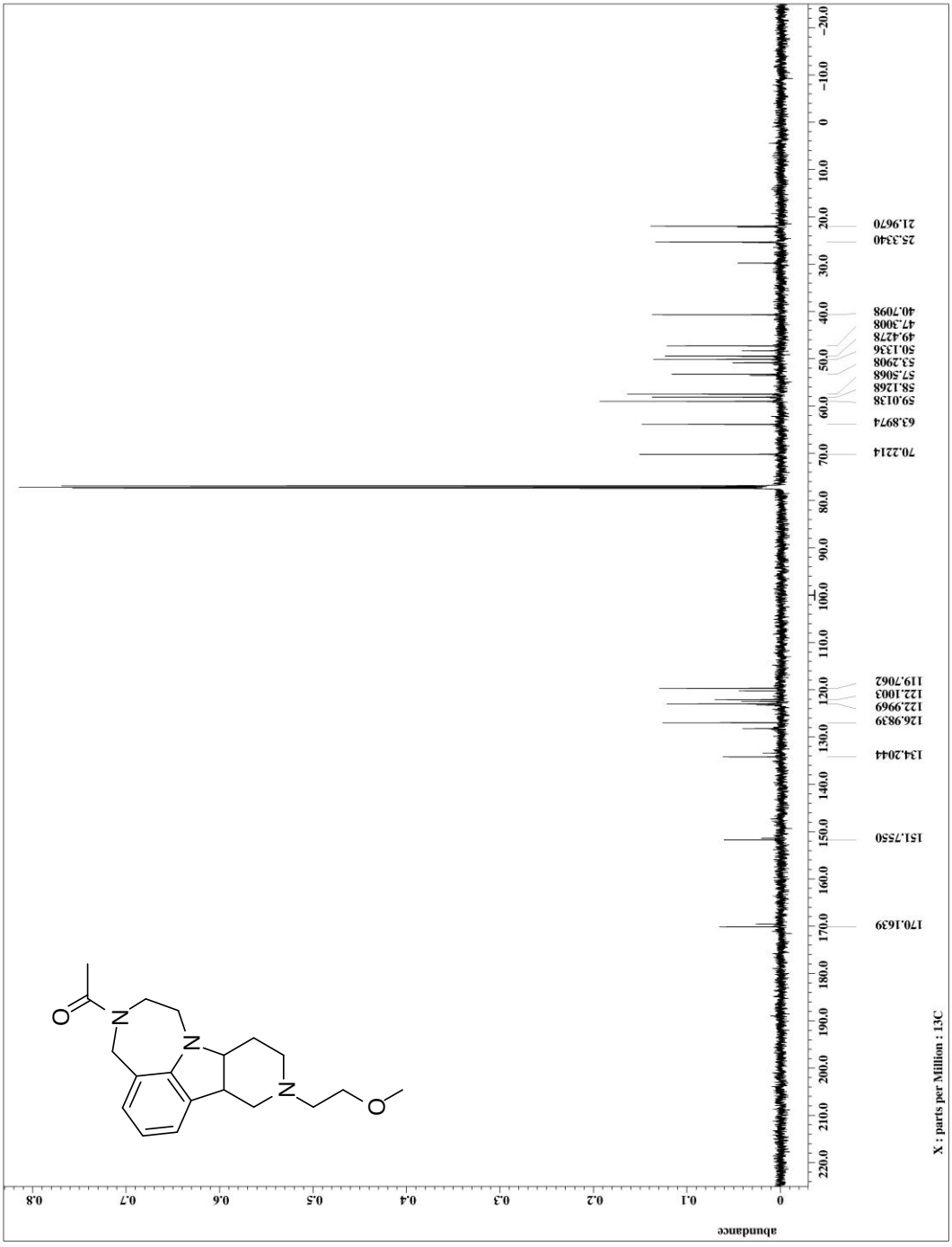


Figure A. 54: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
1-(9-(2-methoxyethyl)-1,2,7b,8,9,10,11,11a-octahydro-[1,4]diazepino[6,7,1-hi]pyrido[4,3-b]indol-3(4H)-yl)ethanone (2.62)



Appendix B: Chapter 3 Spectral Data ^{1}H NMR and ^{13}C NMR

Figure B. 1: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)

3-((1-(4-fluorophenethyl)piperidin-4-yl)(hydroxy)methyl)-2-methoxyphenol (3.15)

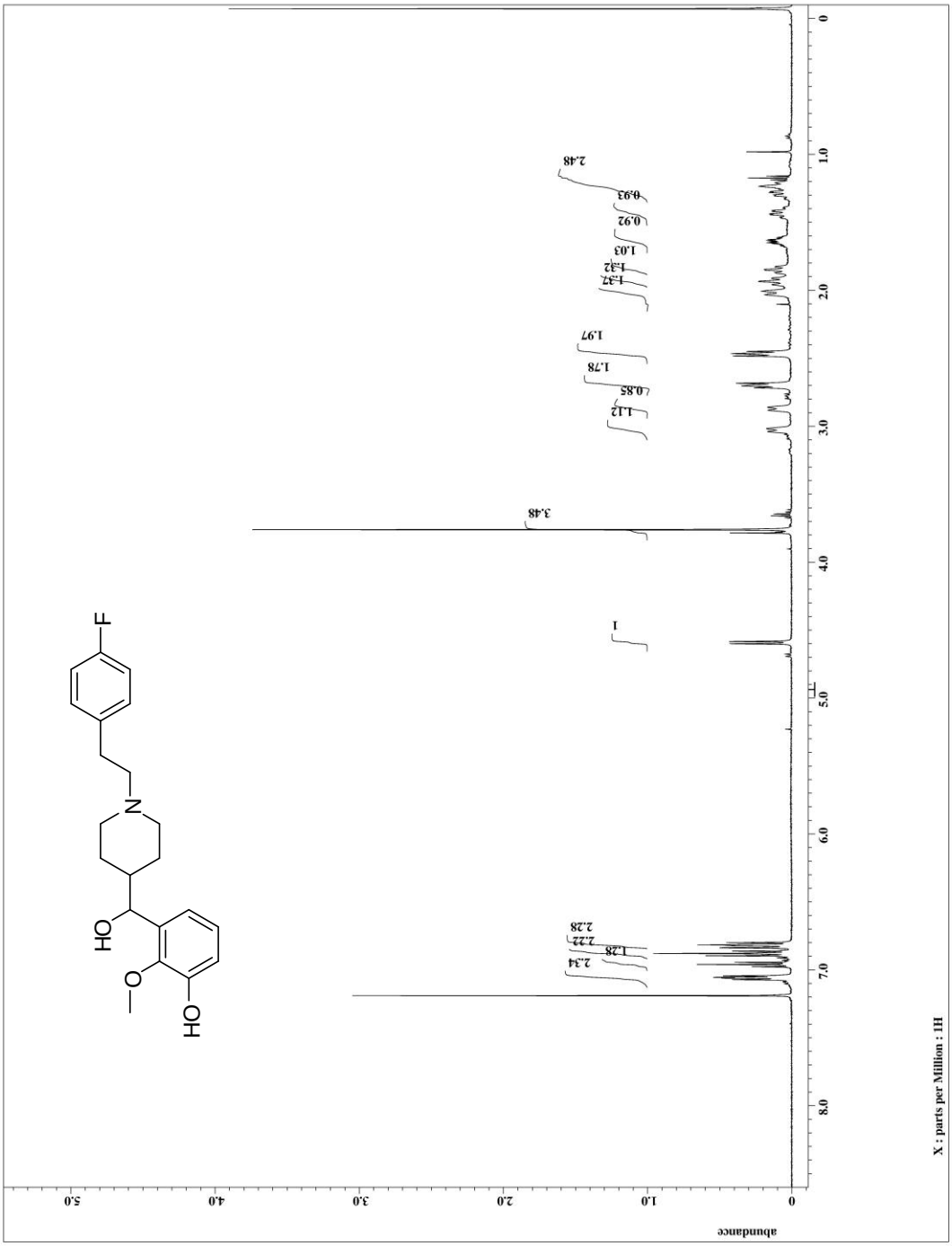


Figure B. 2: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
3-((1-(4-fluorophenethyl)piperidin-4-yl)(hydroxy)methyl)-2-methoxyphenol (3.15)

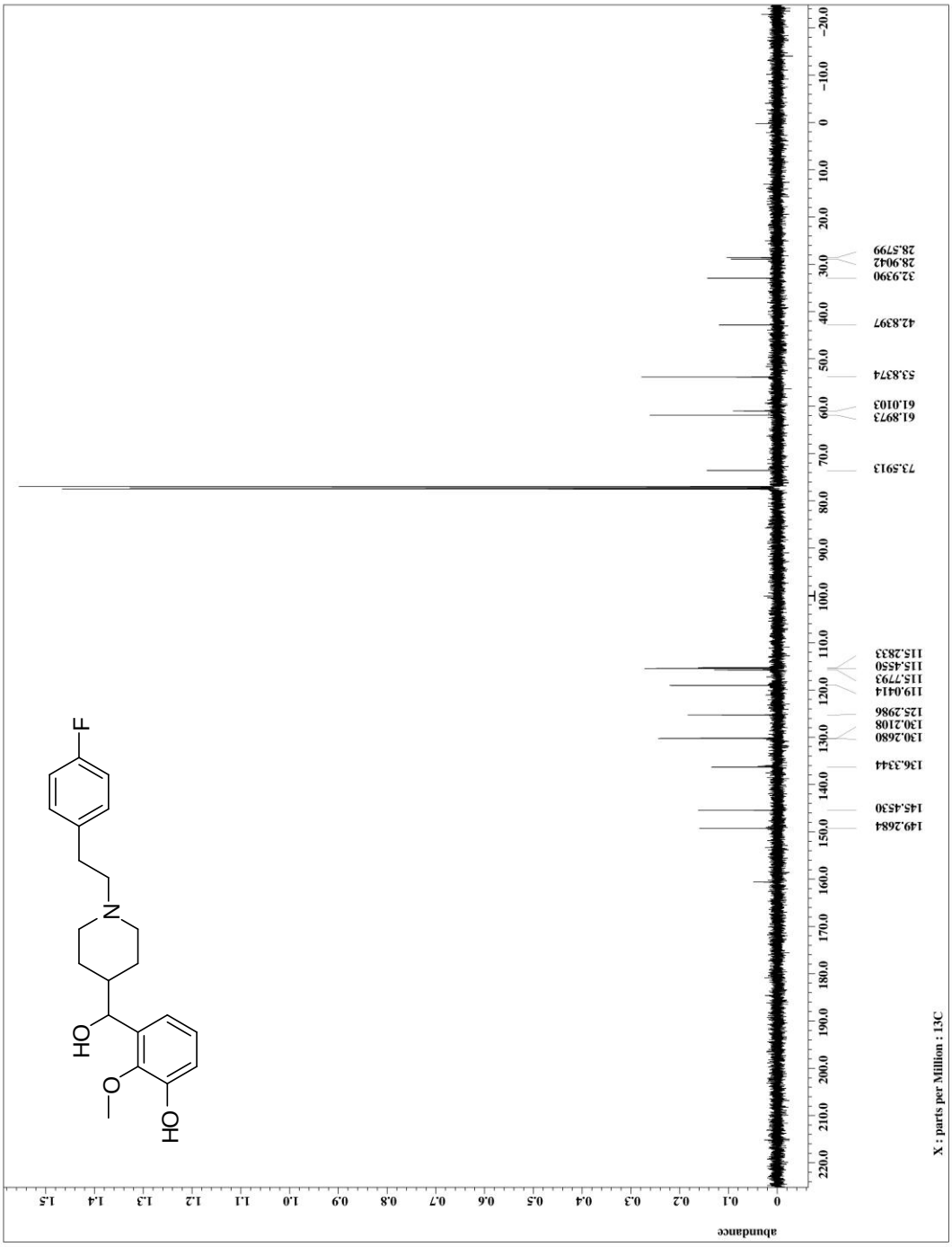


Figure B. 3: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (3.16)

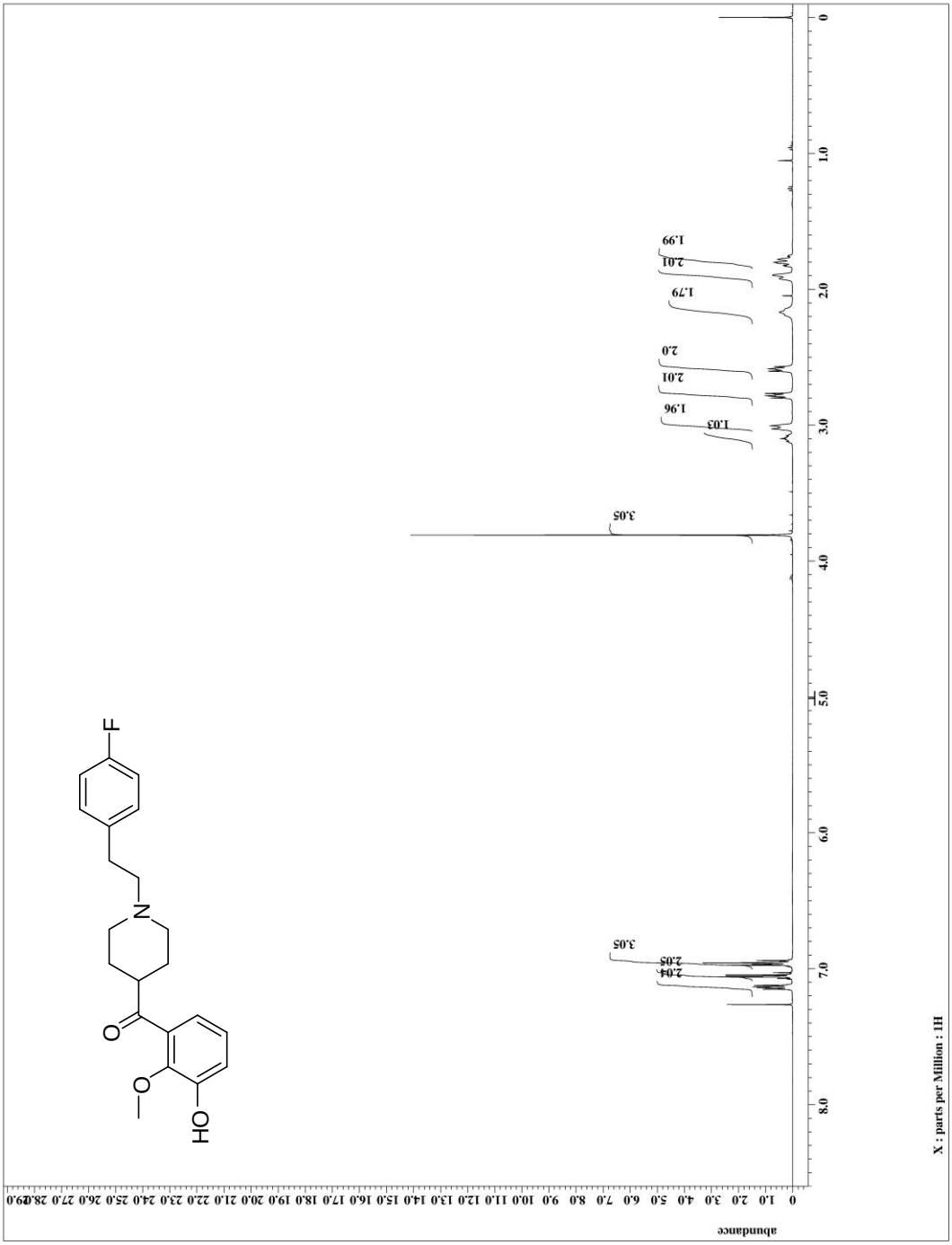


Figure B. 4: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(1-(4-fluorophenethyl)piperidin-4-yl)(3-hydroxy-2-methoxyphenyl)methanone (3.16)

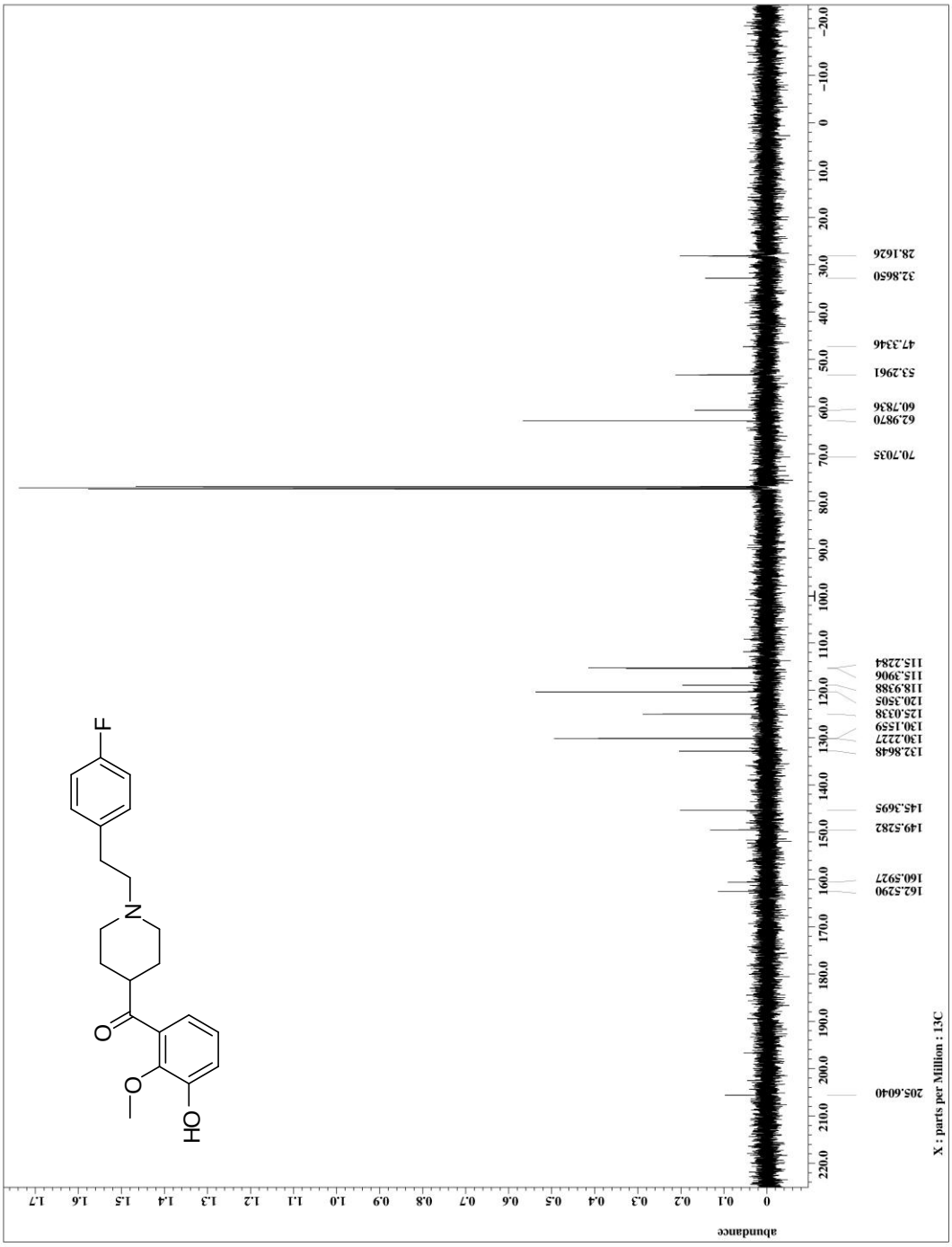


Figure B. 5: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
4-(2-(4-((2,3-dimethoxyphenyl)(hydroxy)methyl)piperidin-1-yl)ethyl)phenol (3.17)

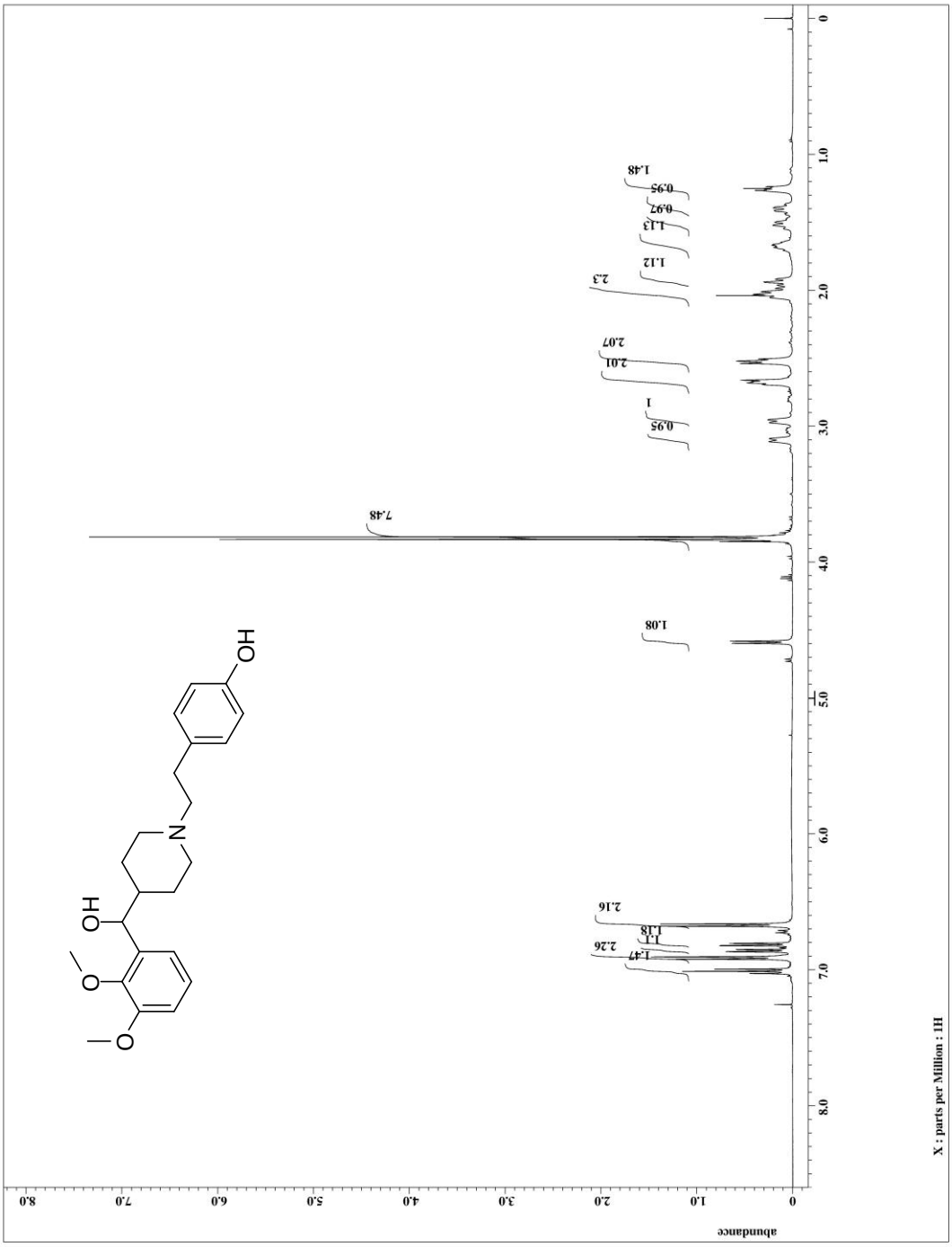


Figure B. 6: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
4-(2-(4-((2,3-dimethoxyphenyl)(hydroxy)methyl)piperidin-1-yl)ethyl)phenol (3.17)

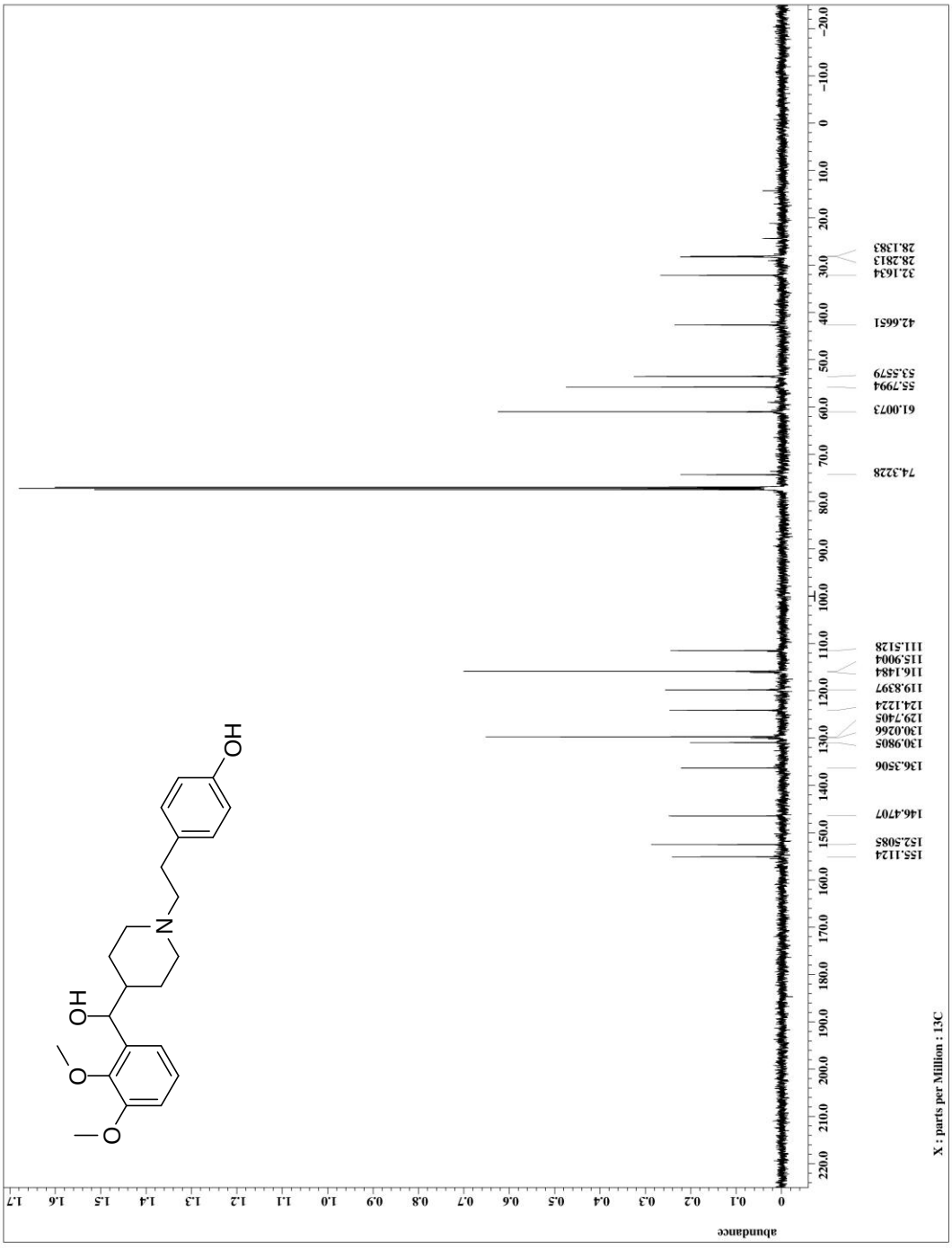


Figure B. 7: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
tert-butyl 4-(2-methoxy-3-(triisopropylsilyloxy)benzoyl)piperidine-1-carboxylate (3.21)

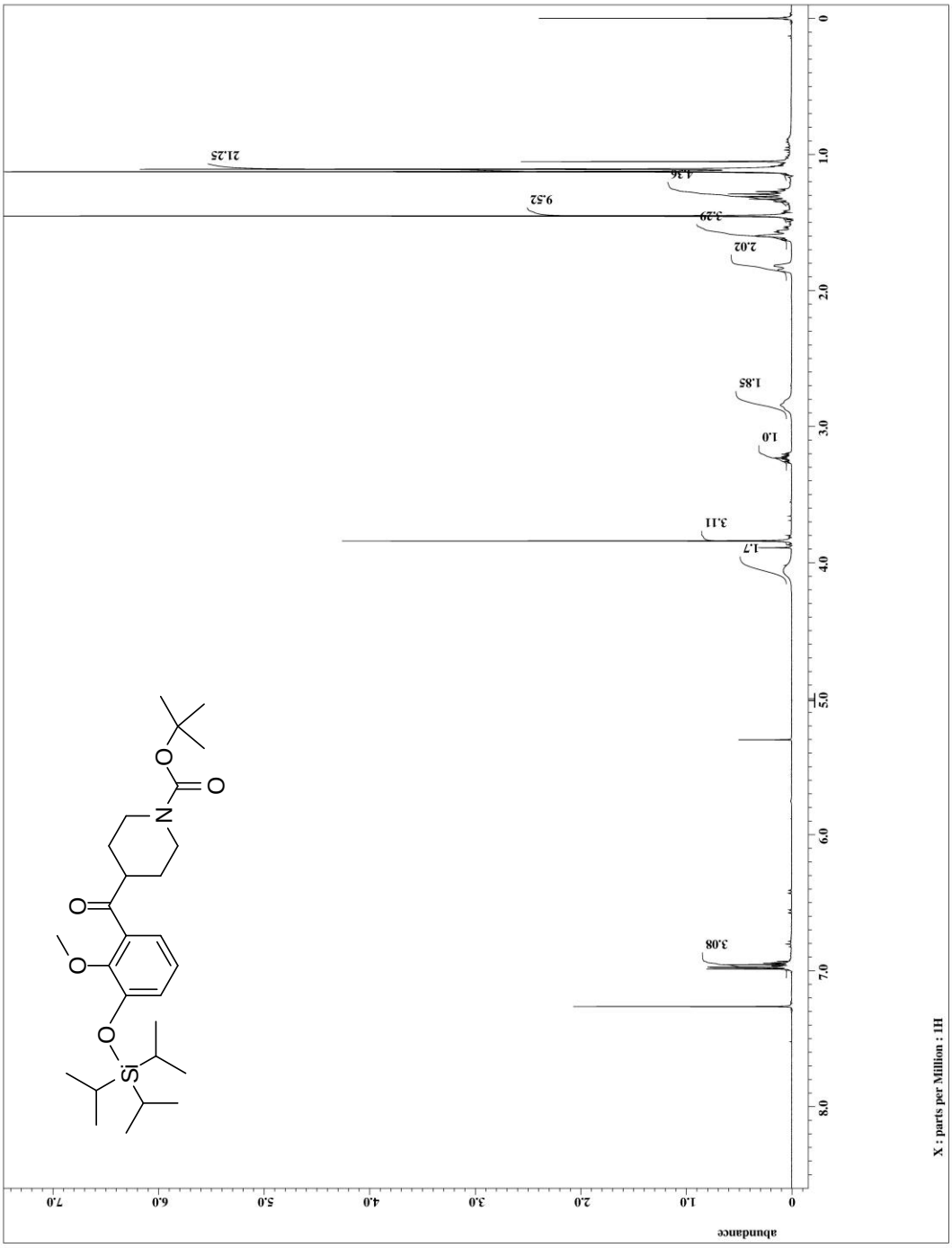


Figure B. 8: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
tert-butyl 4-(2-methoxy-3-(triisopropylsilyloxy)benzoyl)piperidine-1-carboxylate (3.21)

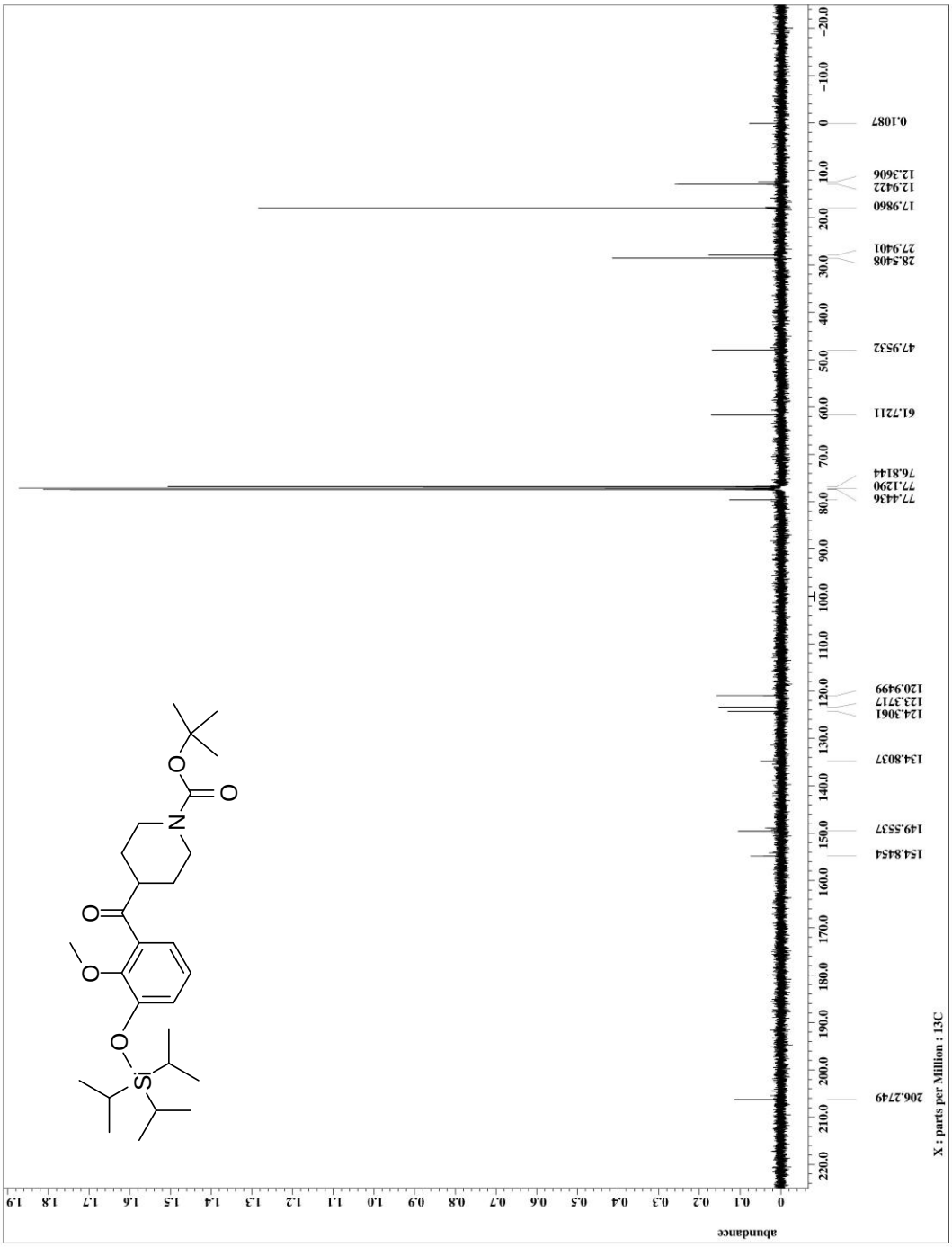


Figure B. 9: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
tert-butyl 4-(3-(tert-butyldiphenylsilyloxy)-2-methoxybenzoyl)piperidine-1-carboxylate (3.22)

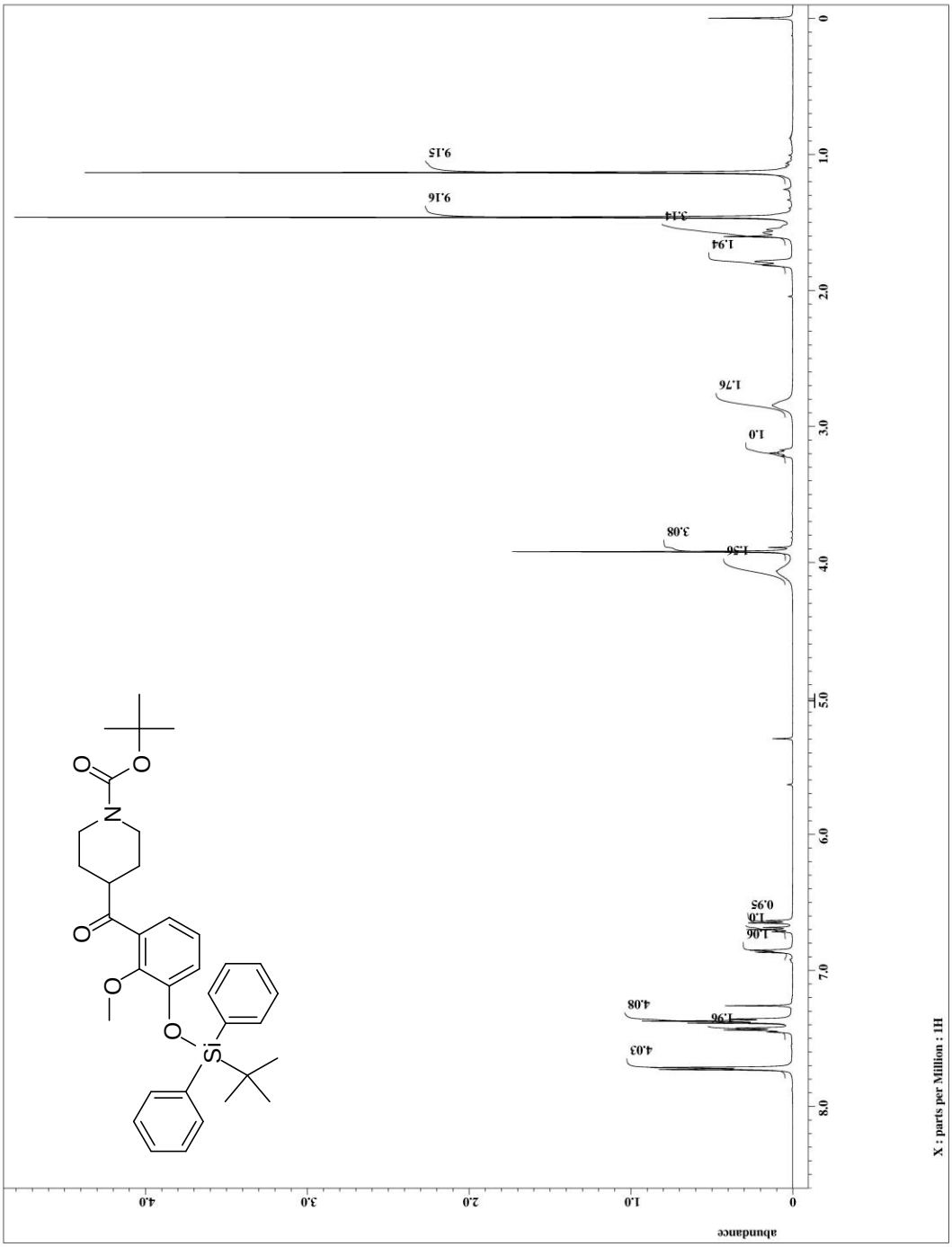


Figure B. 10: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
tert-butyl 4-(3-(tert-butyldiphenylsilyloxy)-2-methoxybenzoyl)piperidine-1-carboxylate (3.22)

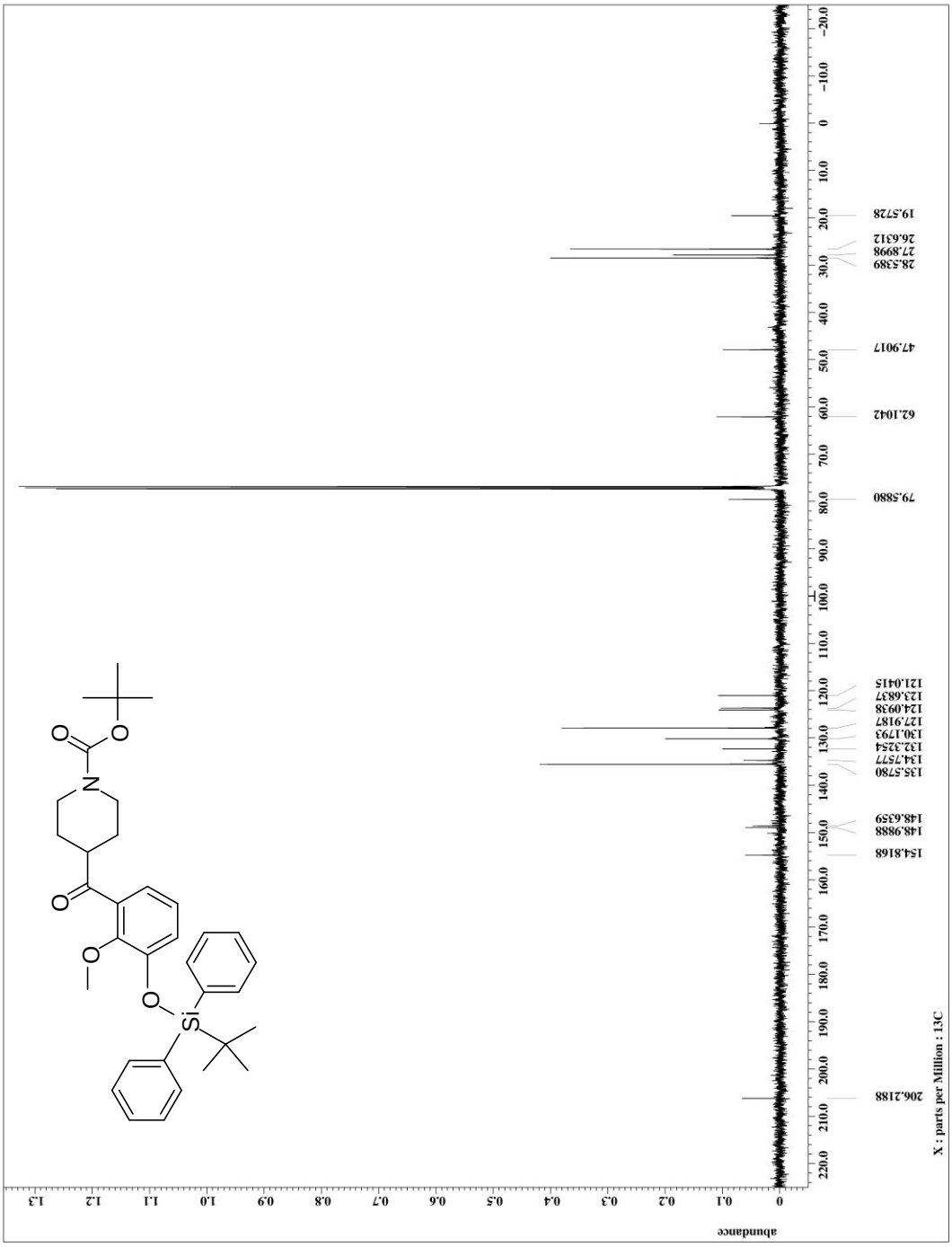


Figure B. 11: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(2-methoxy-3-(triisopropylsilyloxy)phenyl)(piperidin-4-yl)methanone (3.23)

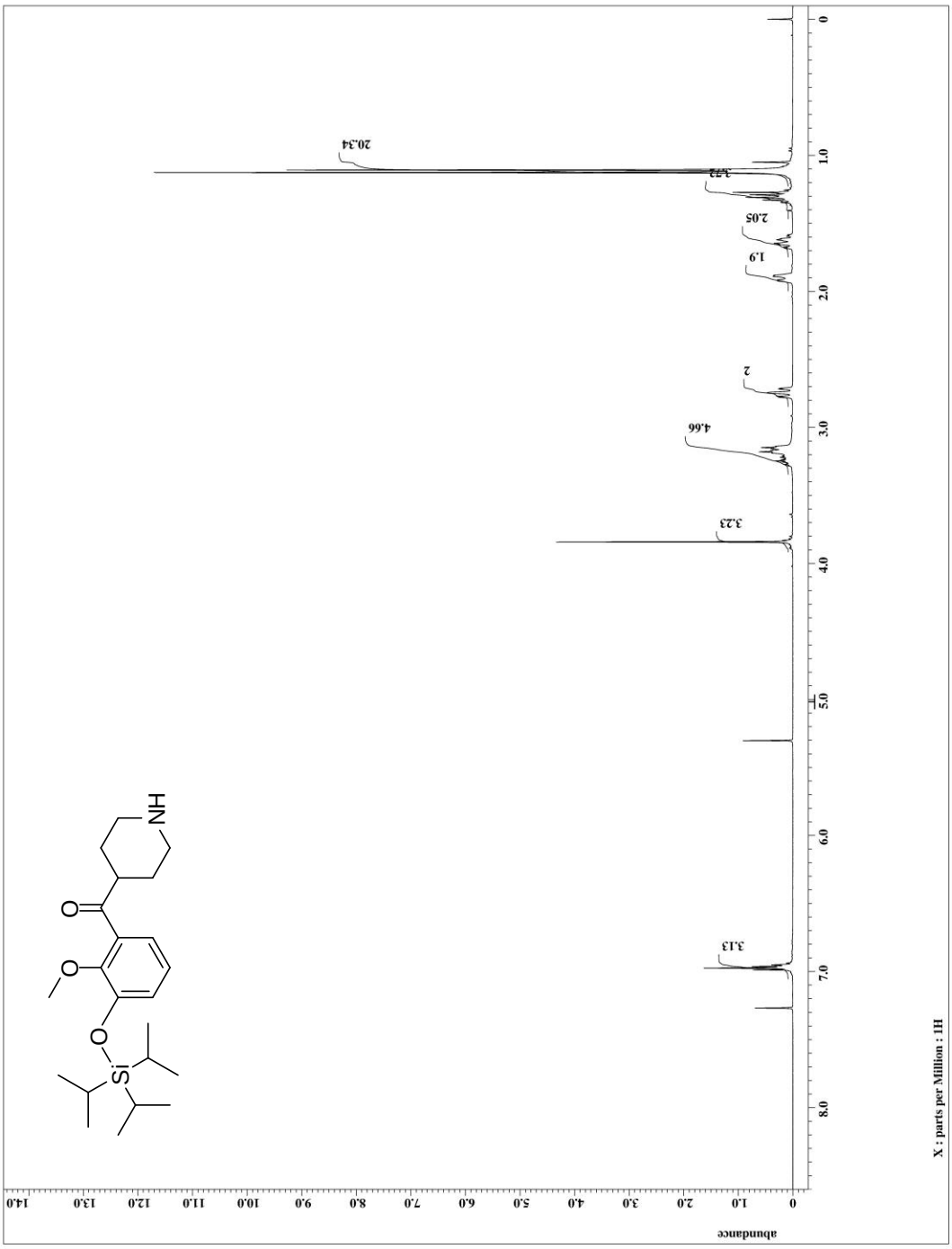


Figure B. 12: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(2-methoxy-3-(triisopropylsilyloxy)phenyl)(piperidin-4-yl)methanone (3.23)

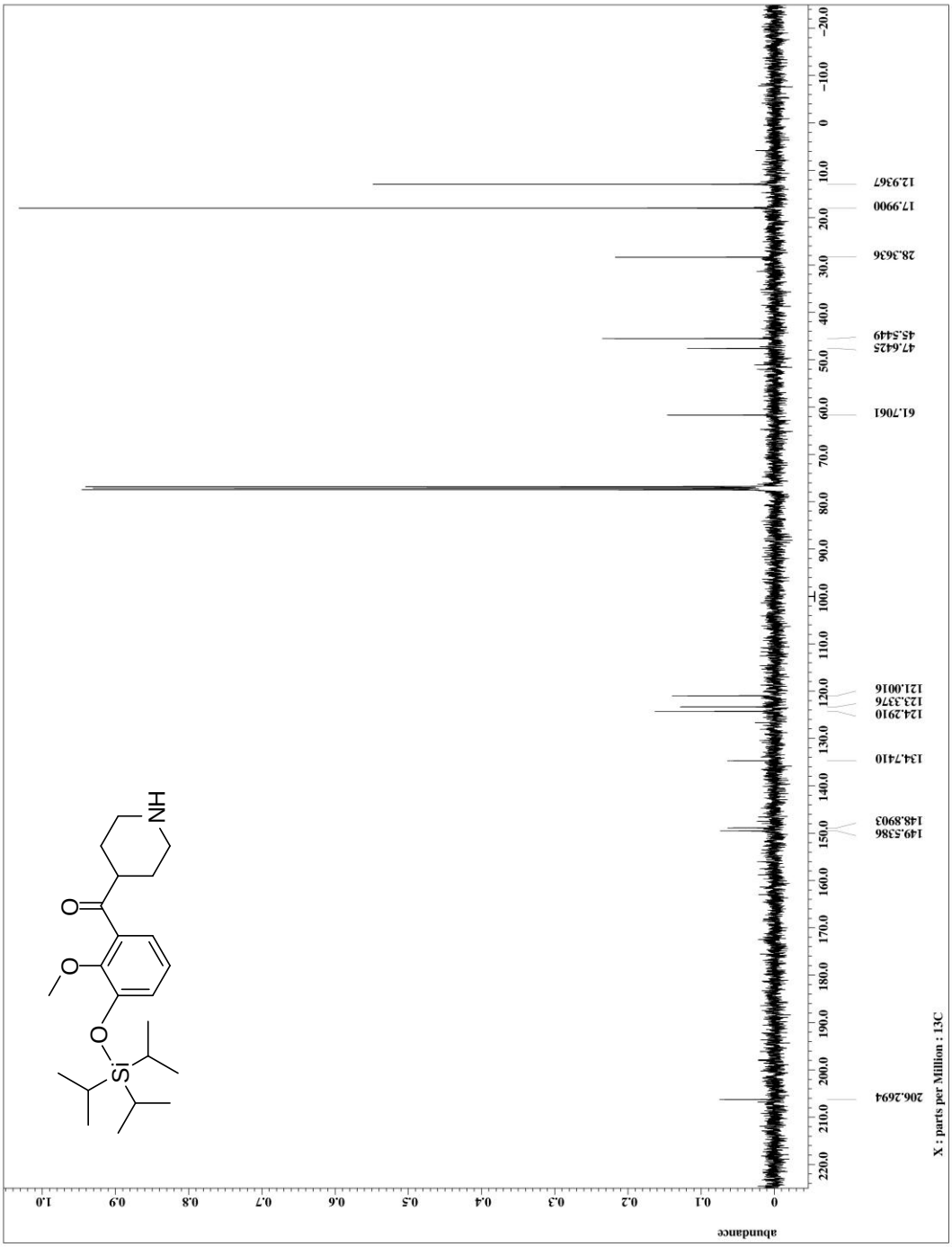


Figure B. 13: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl)(piperidin-4-yl)methanone (3.24)

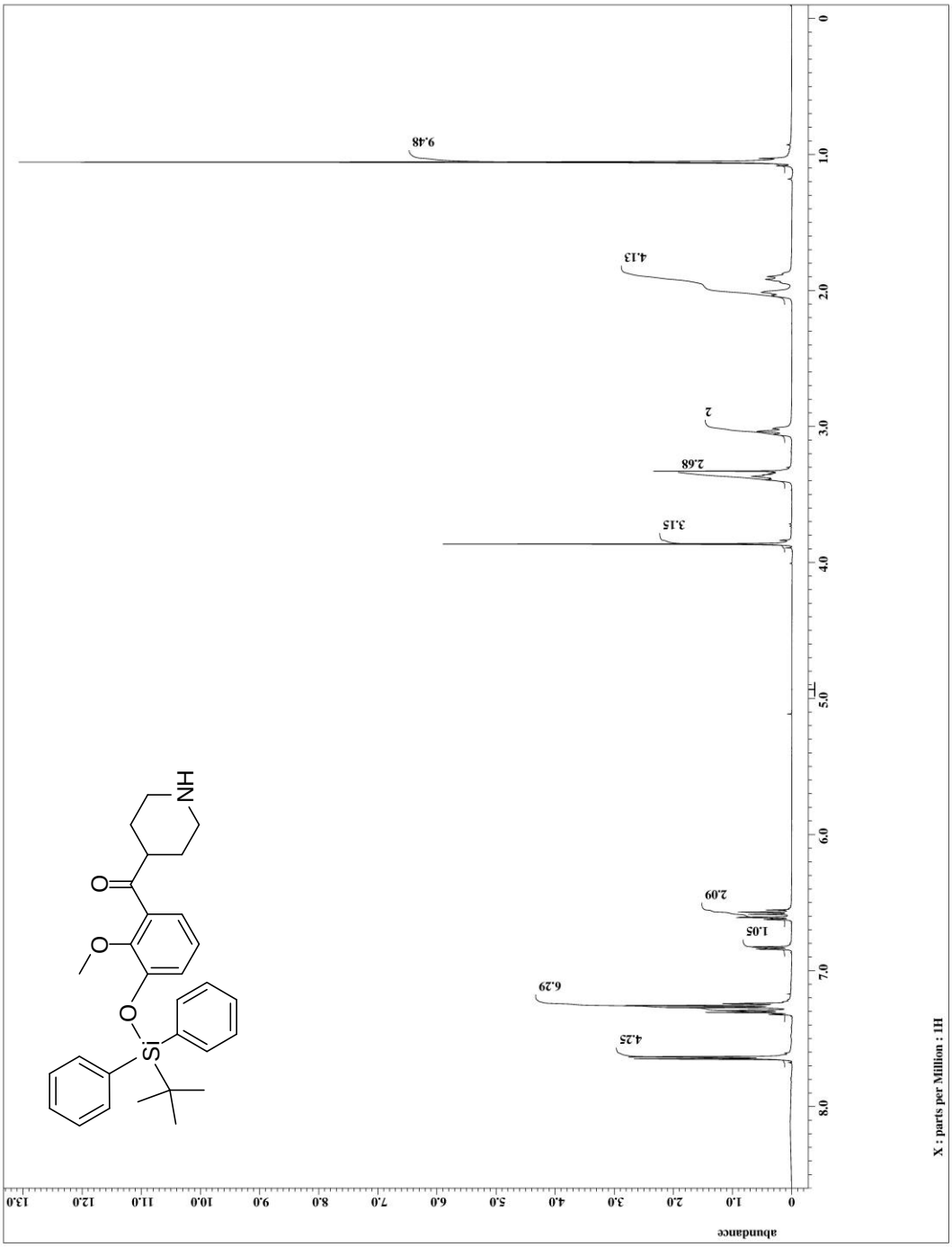


Figure B. 14: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl)(piperidin-4-yl)methanone (3.24)

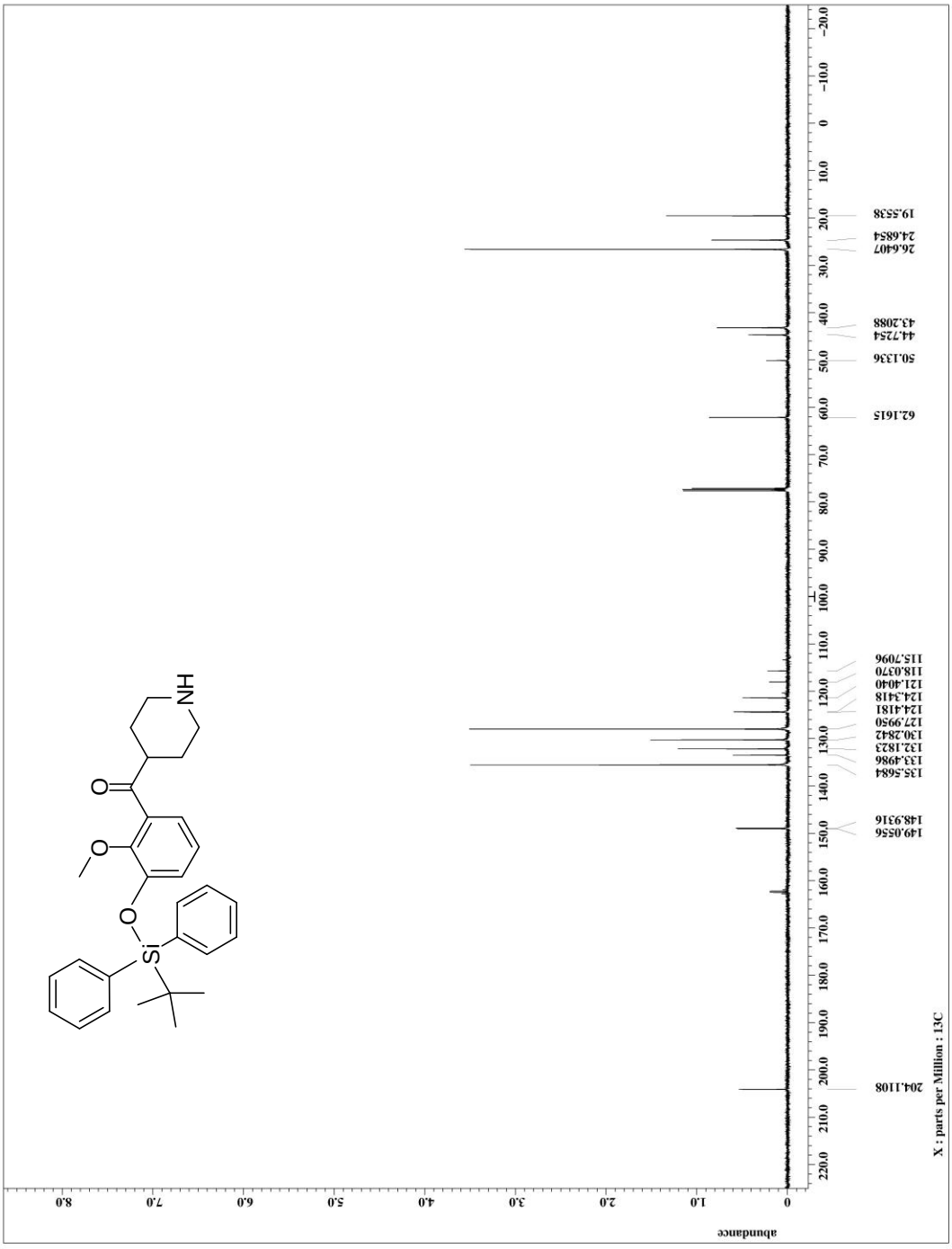


Figure B. 15: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(triisopropylsilyloxy)phenyl) methanone (3.26)

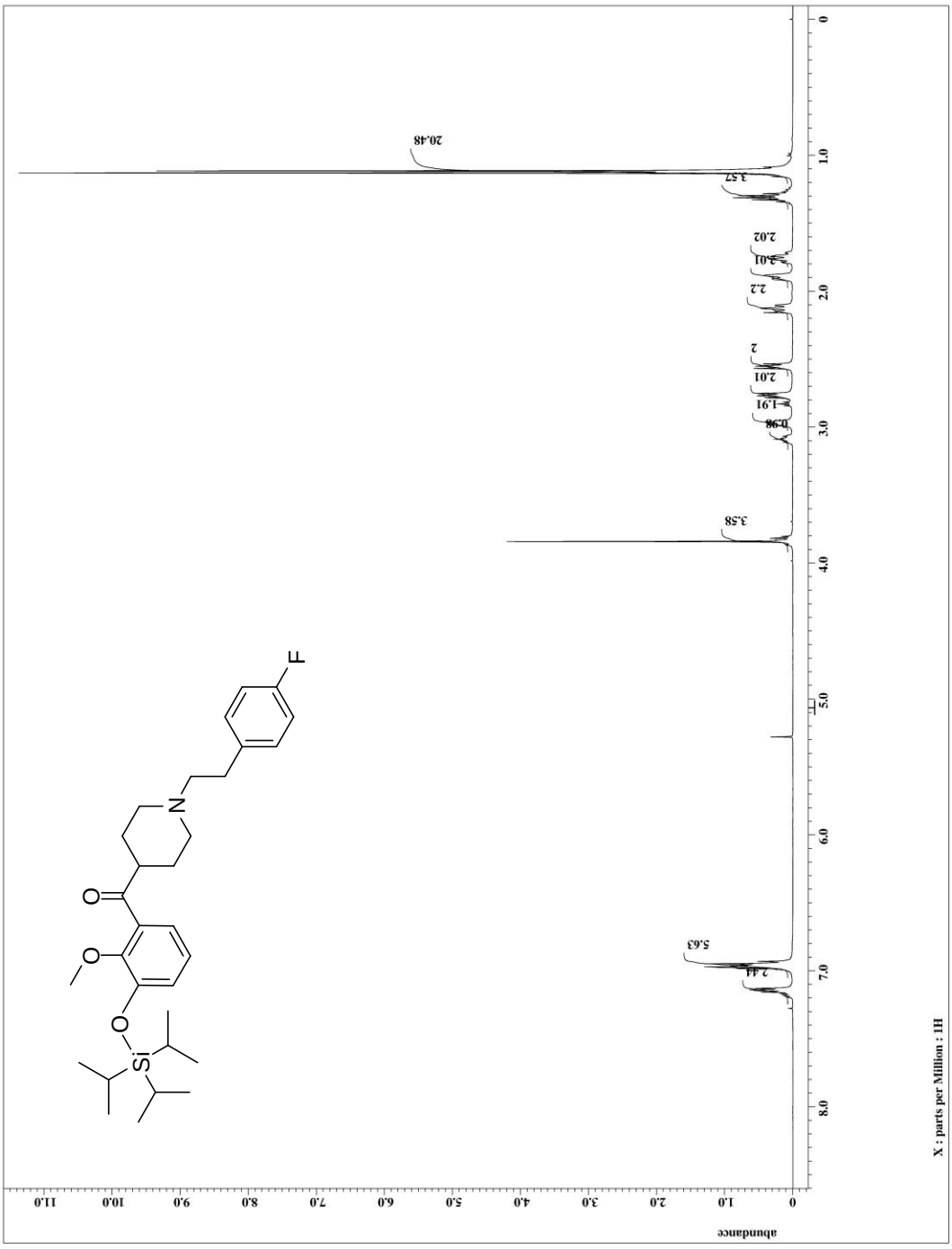


Figure B. 16: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(triisopropylsilyloxy)phenyl) methanone (3.26)

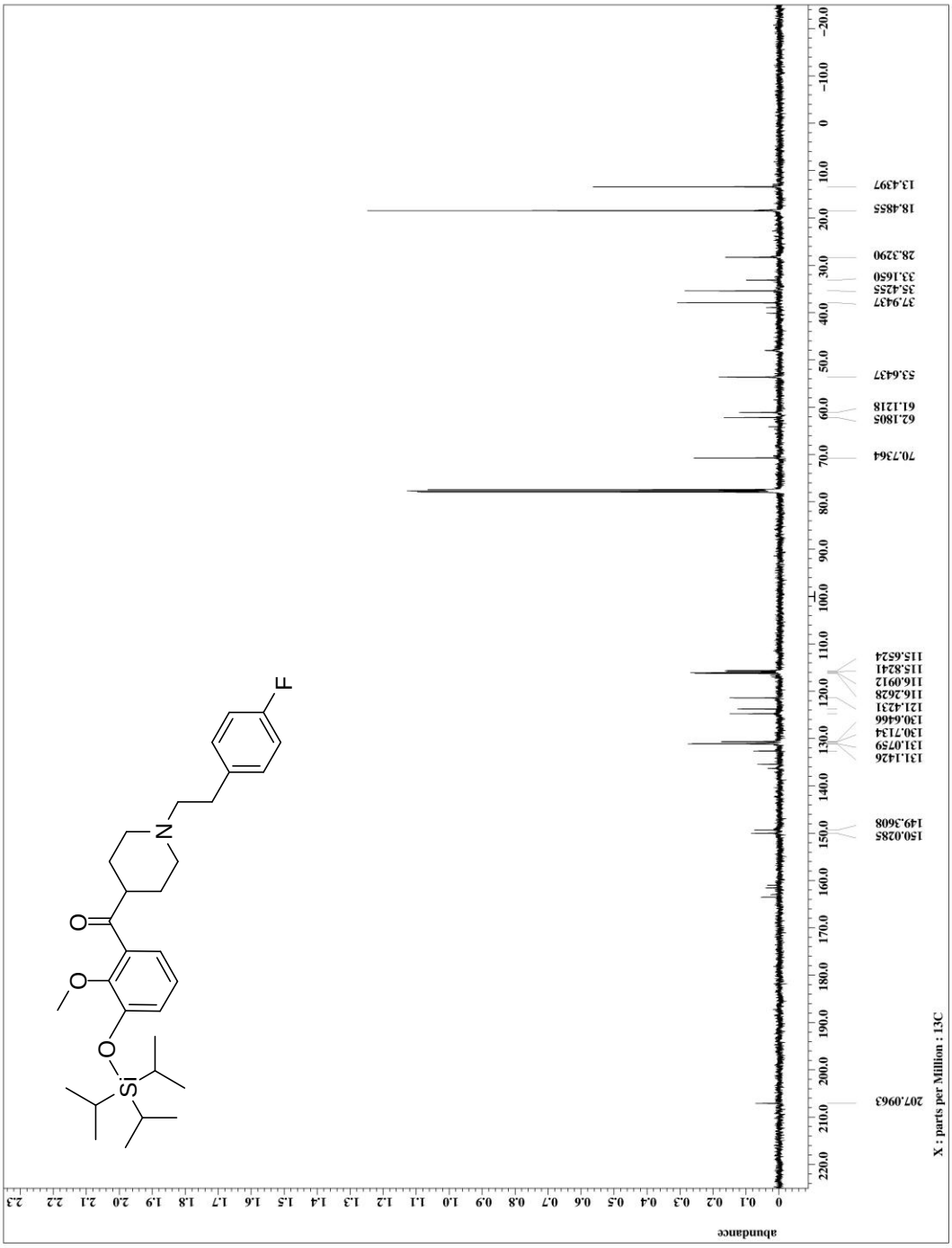


Figure B. 17: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (3.27)

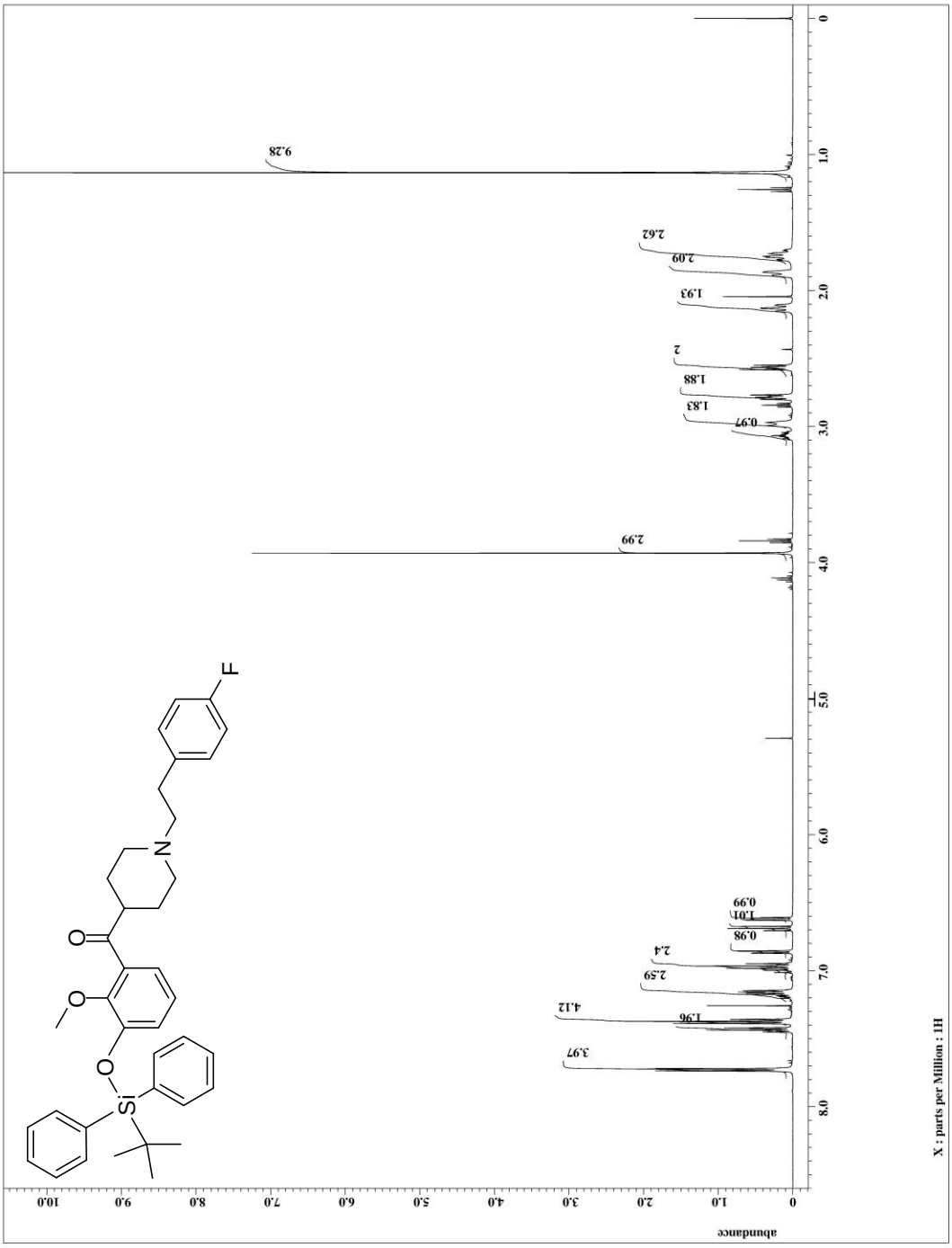


Figure B. 18: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (3.27)

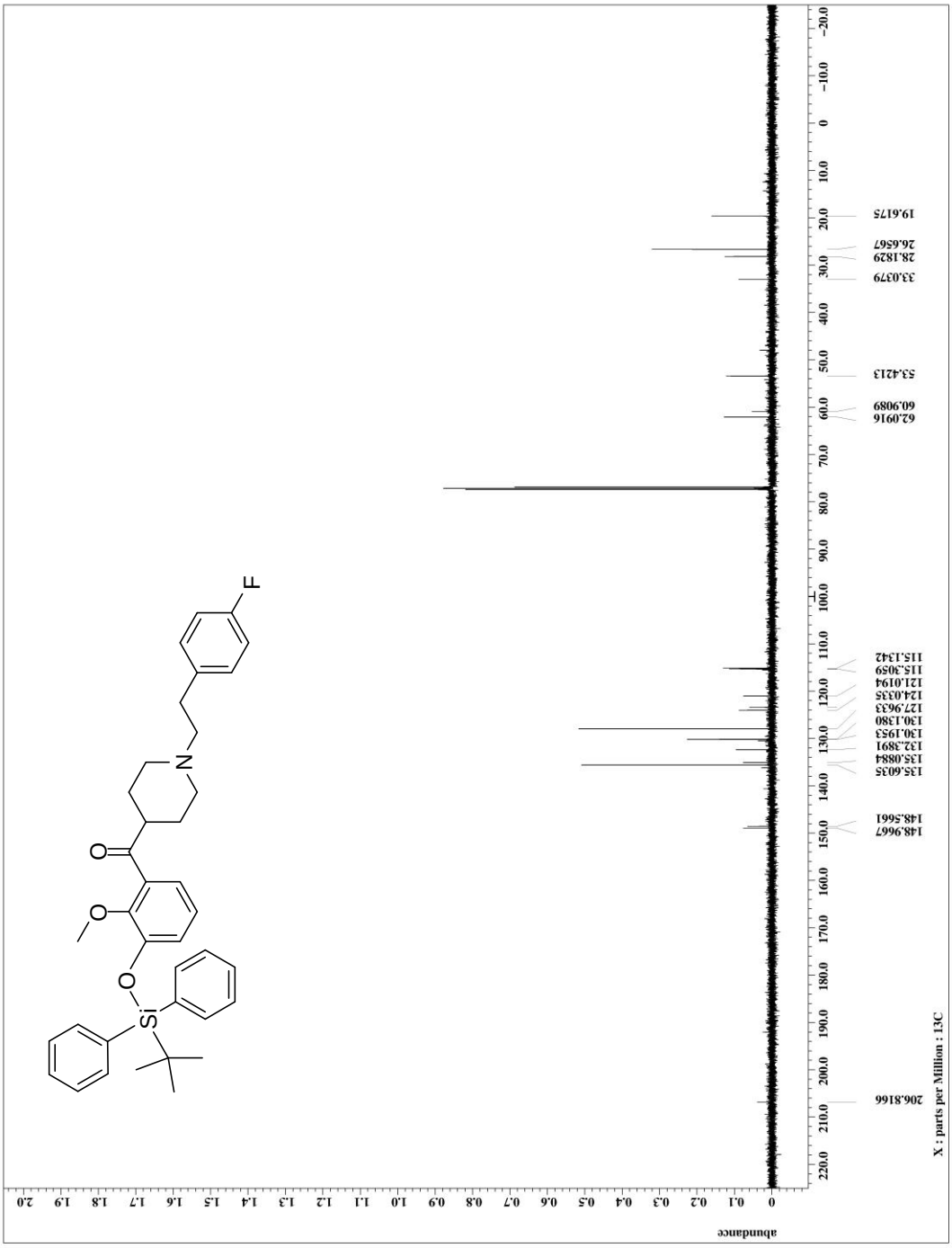


Figure B. 19: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
(1-(4-butoxyphenethyl)piperidin-4-yl)(2,3-dimethoxyphenyl)methanol (3.29)

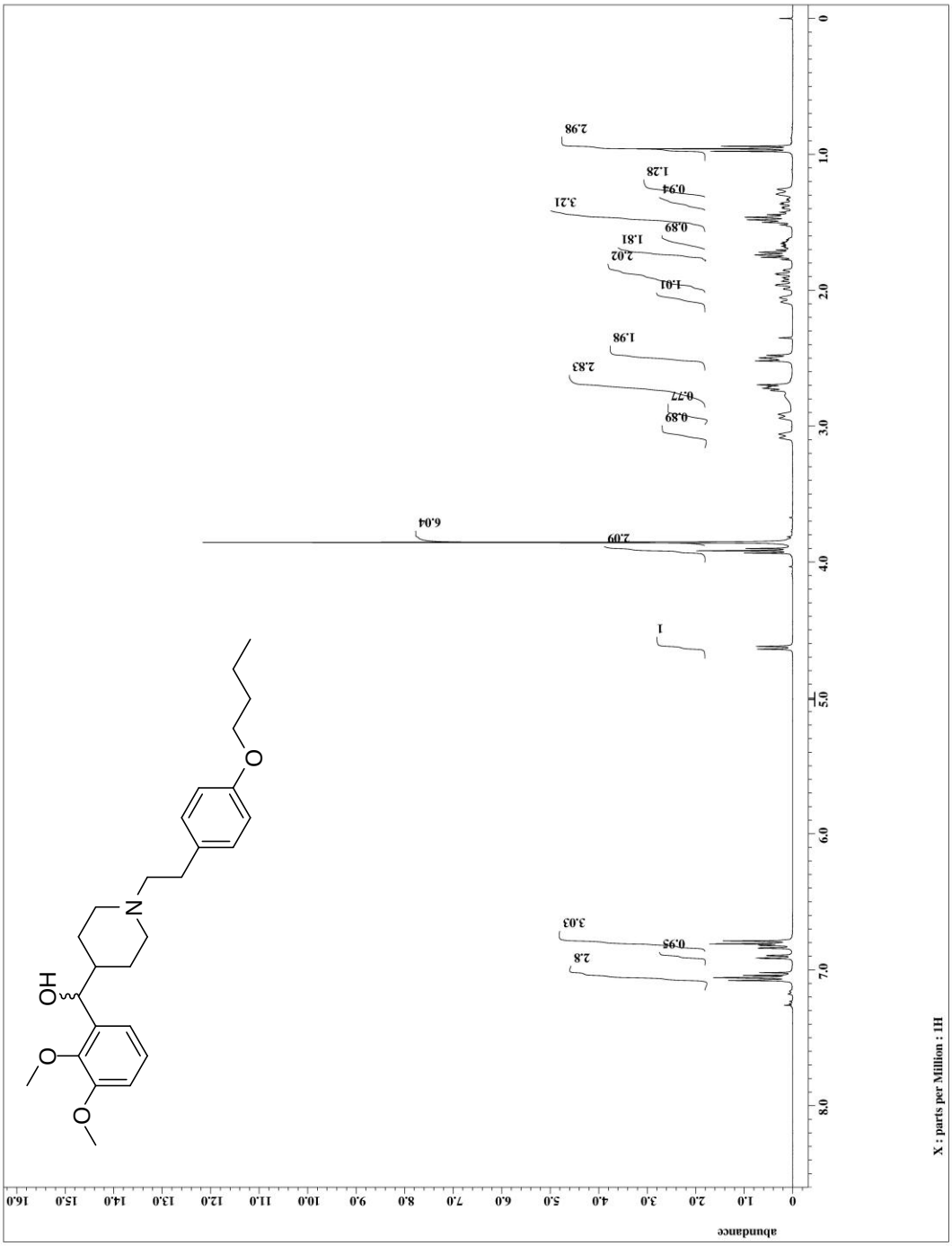


Figure B. 20: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
(1-(4-butoxyphenethyl)piperidin-4-yl)(2,3-dimethoxyphenyl)methanol (3.29)

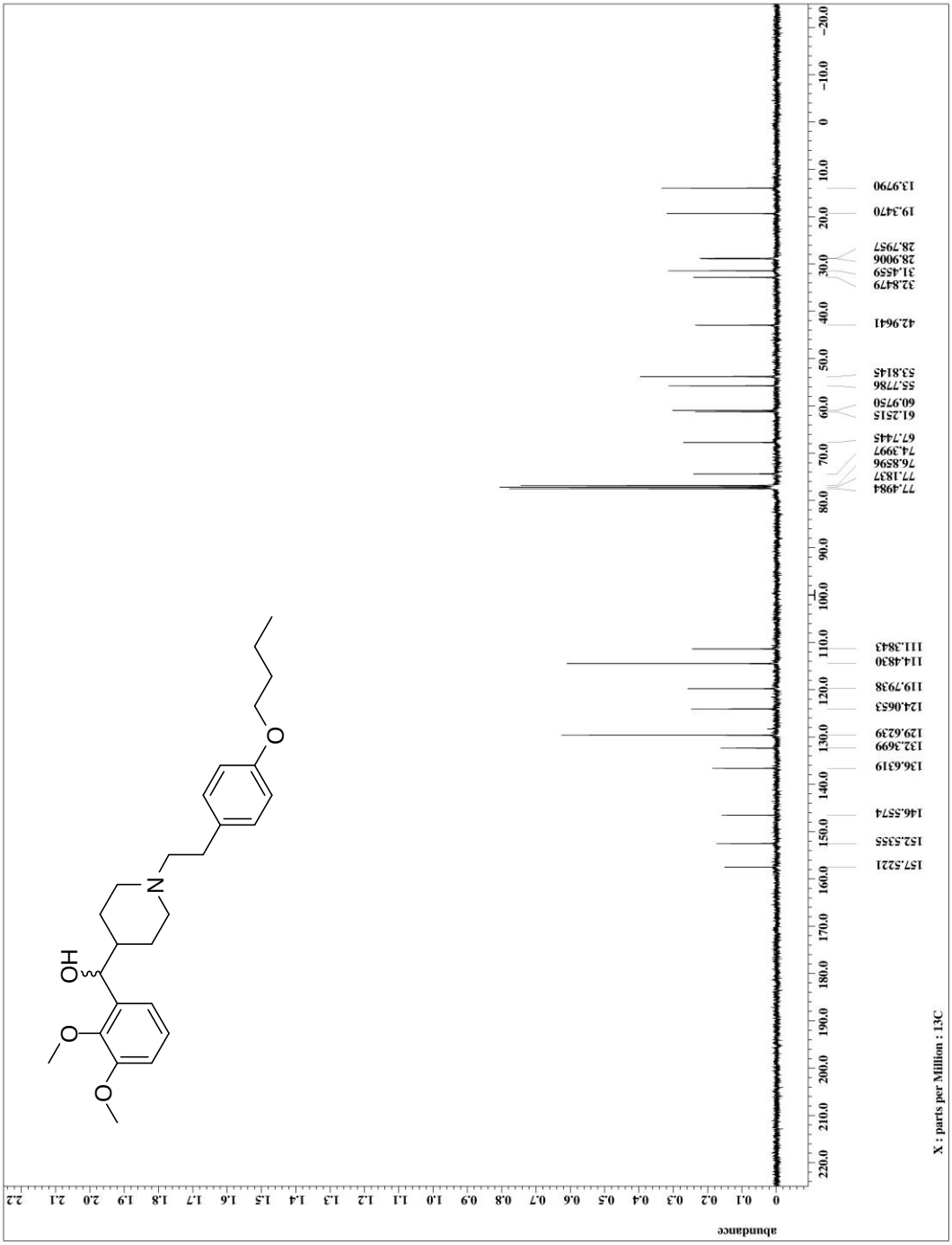


Figure B. 21: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(2,3-dimethoxyphenyl)(1-(4-(2-methoxyethoxy)phenethyl)piperidin-4-yl)methanol (3.30)

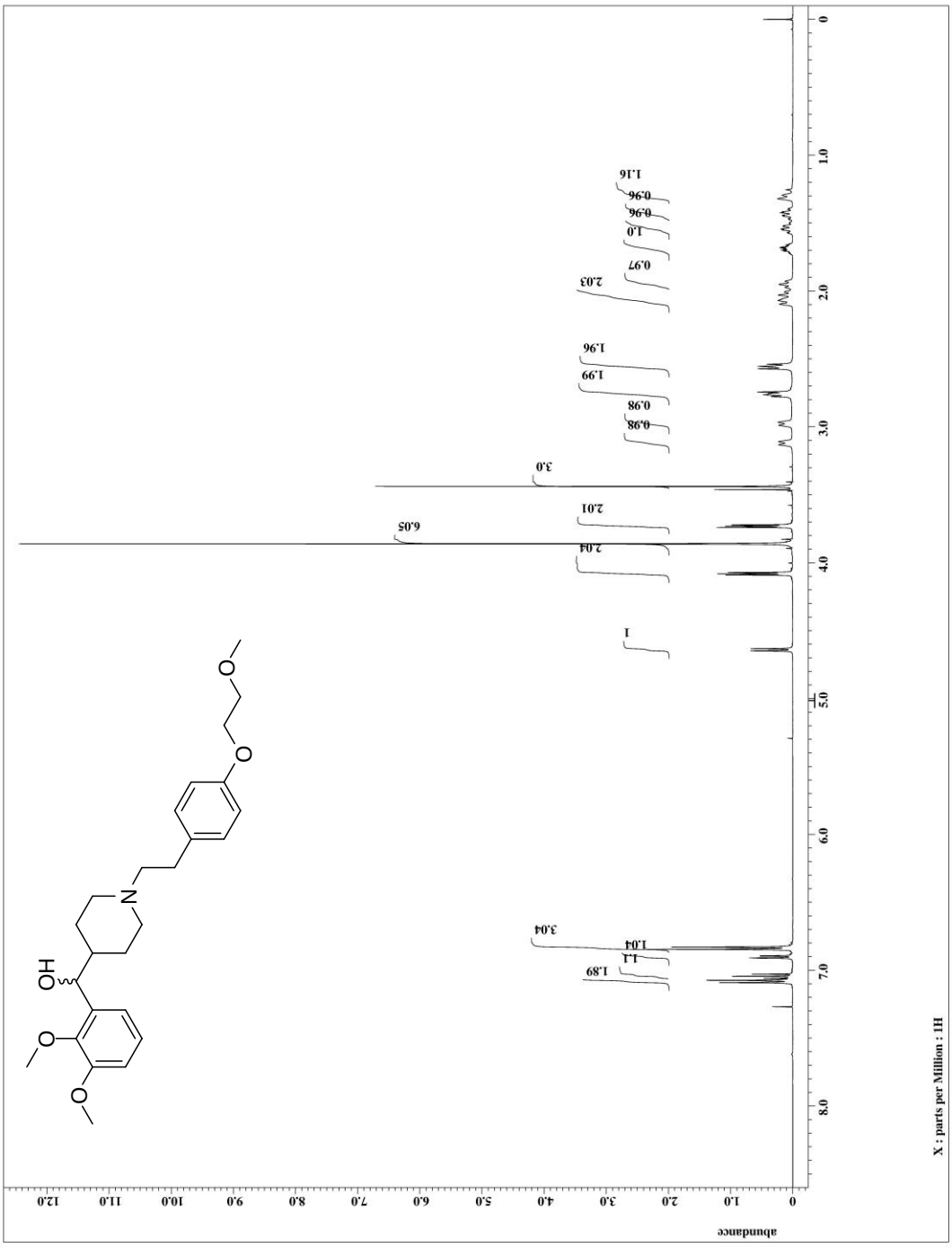


Figure B. 22: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(2,3-dimethoxyphenyl)(1-(4-(2-methoxyethoxy)phenethyl)piperidin-4-yl)methanol (3.30)

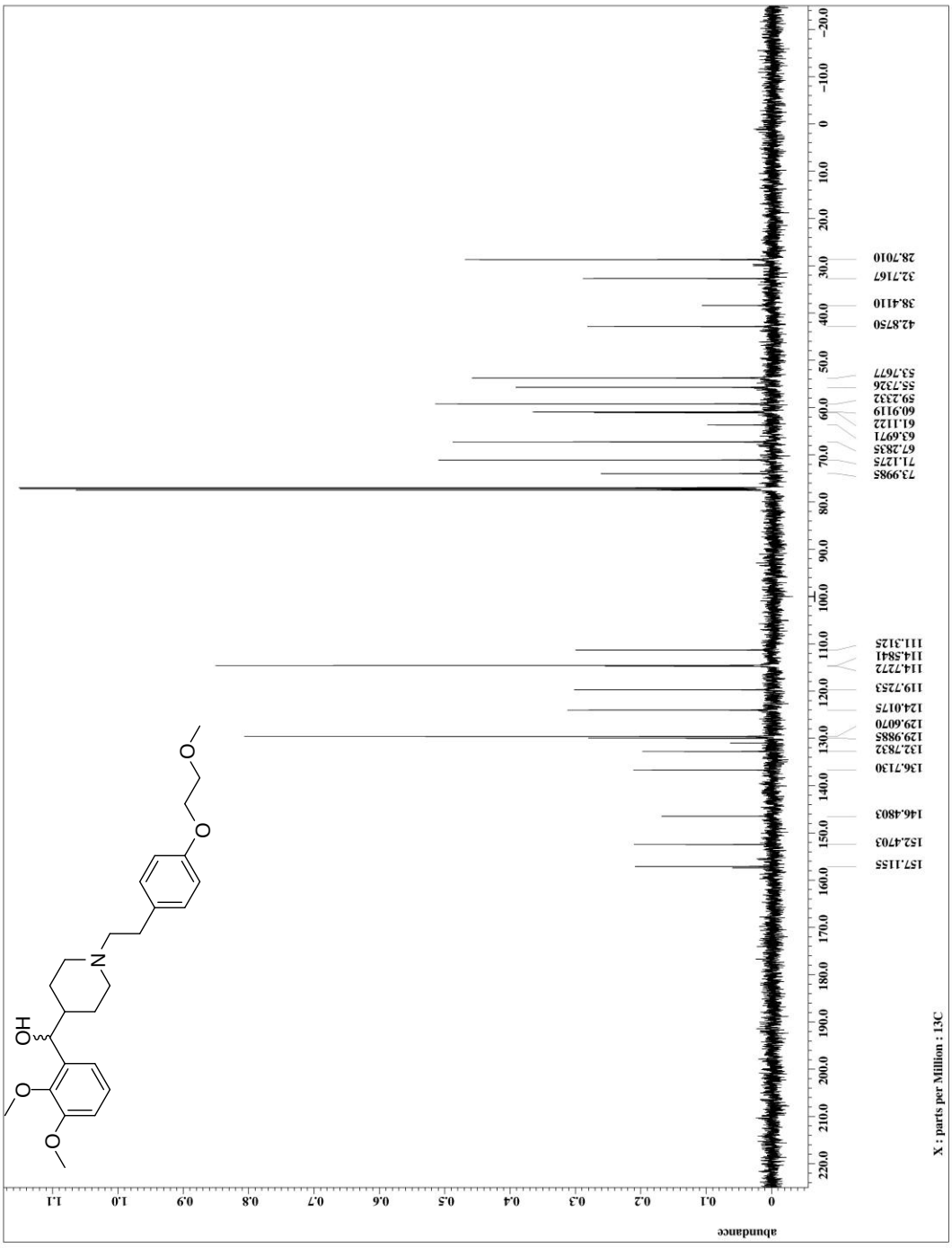


Figure B. 23: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(3-butoxy-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (3.31)

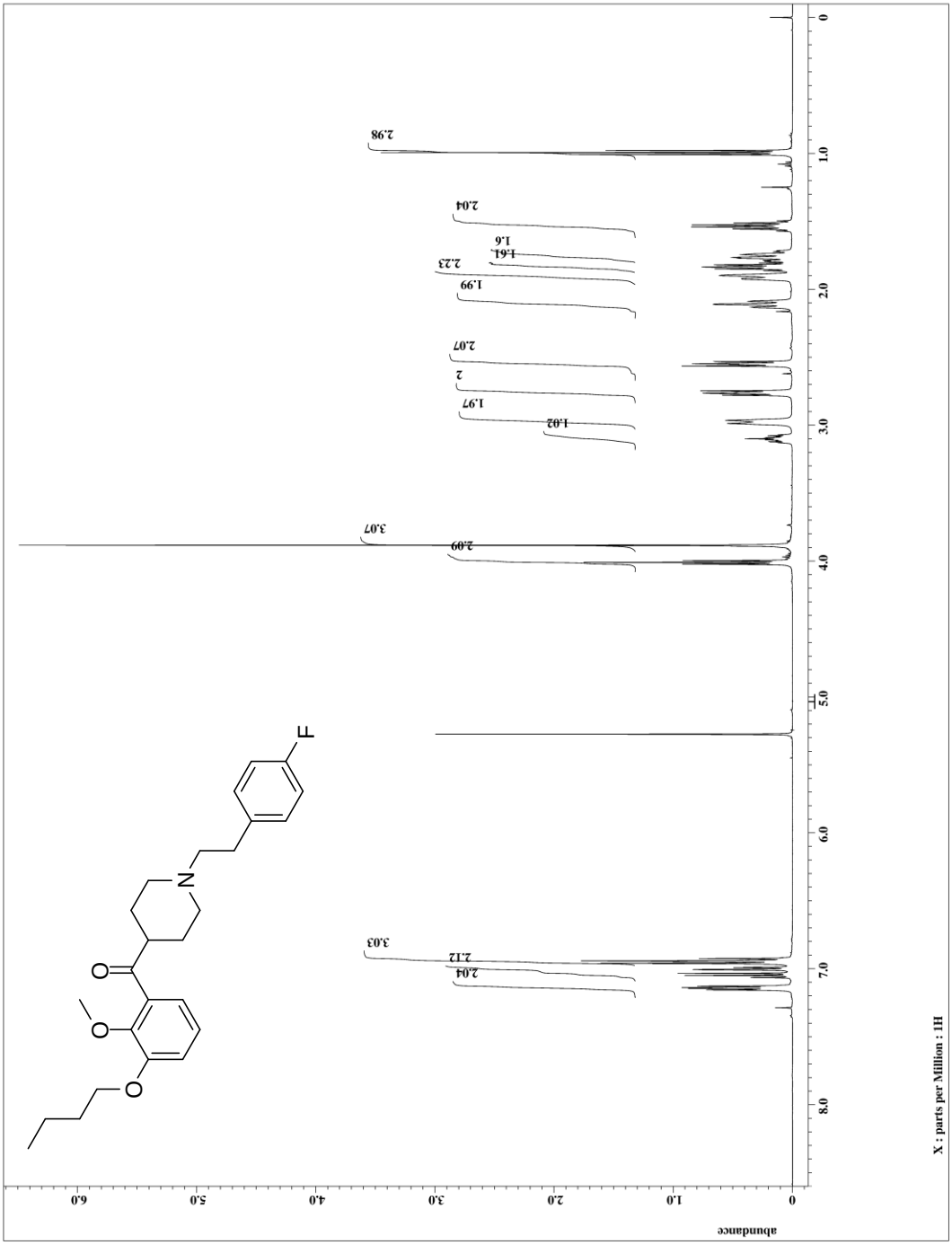


Figure B. 24: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3-butoxy-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (3.31)

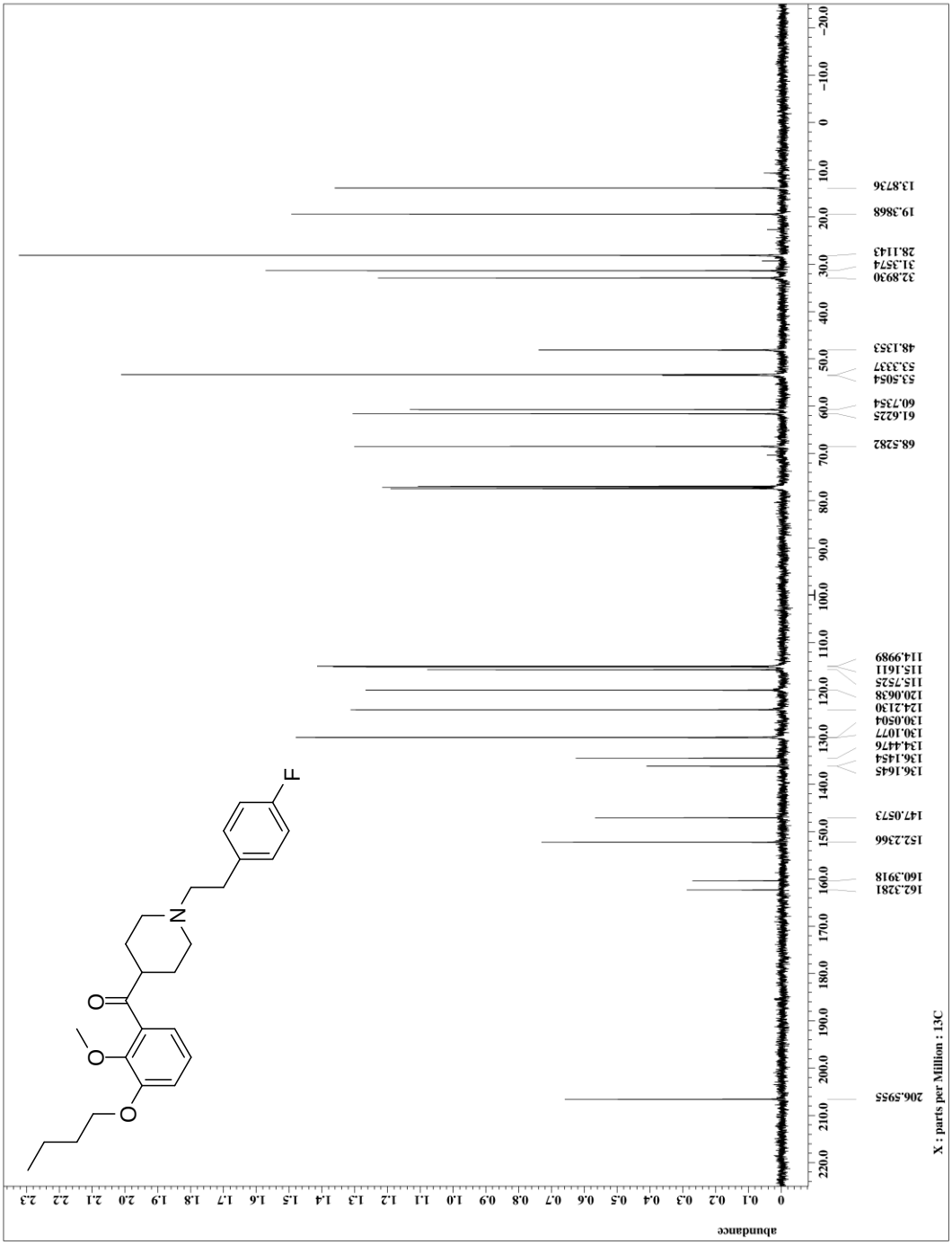


Figure B. 25: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
(1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(2-methoxyethoxy)phenyl) methanone (3.32)

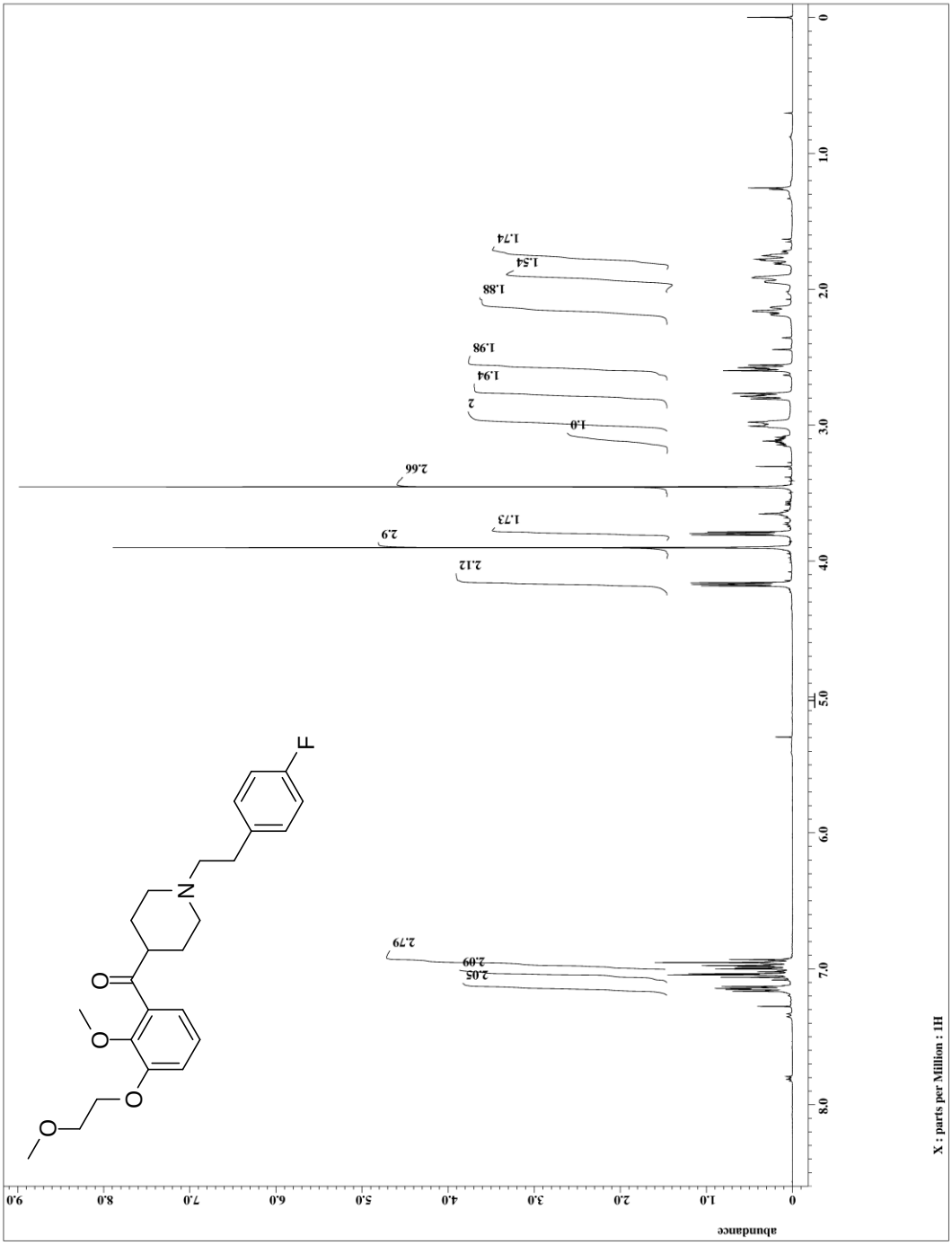


Figure B. 26: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
(1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(2-methoxyethoxy)phenyl) methanone (3.32)

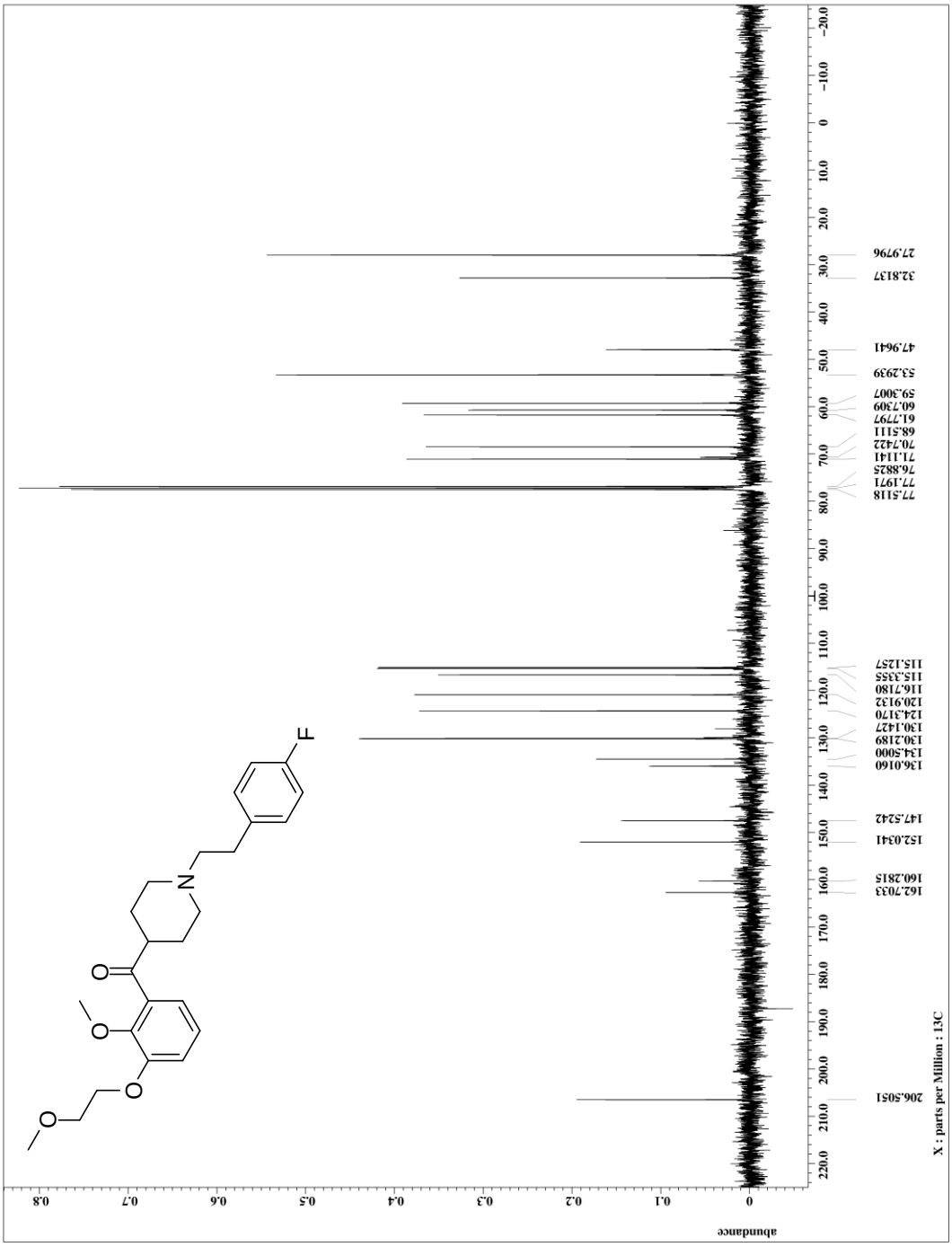


Figure B. 27: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
(3-butoxy-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanol (3.33)

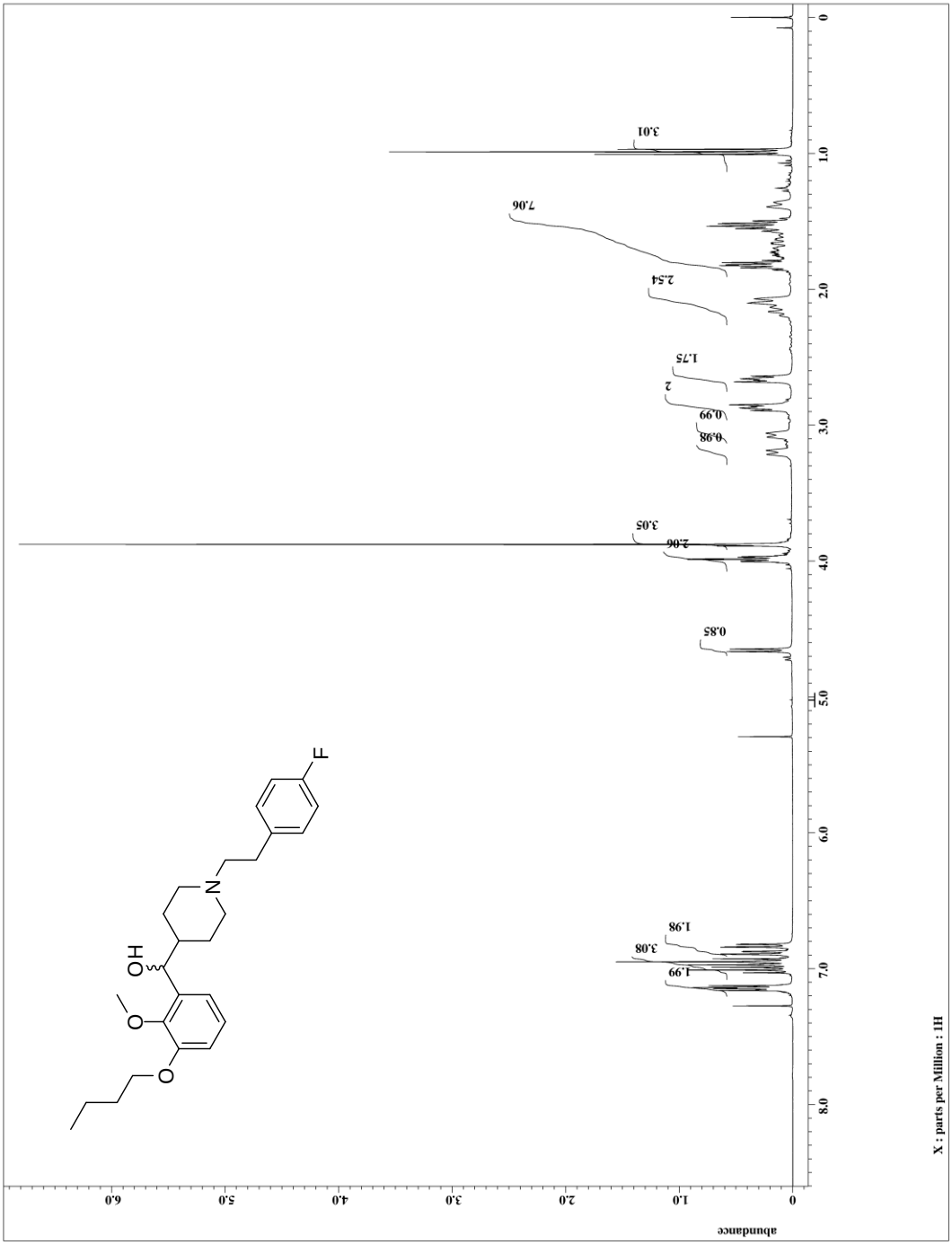


Figure B. 28: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
(3-butoxy-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanol (3.33)

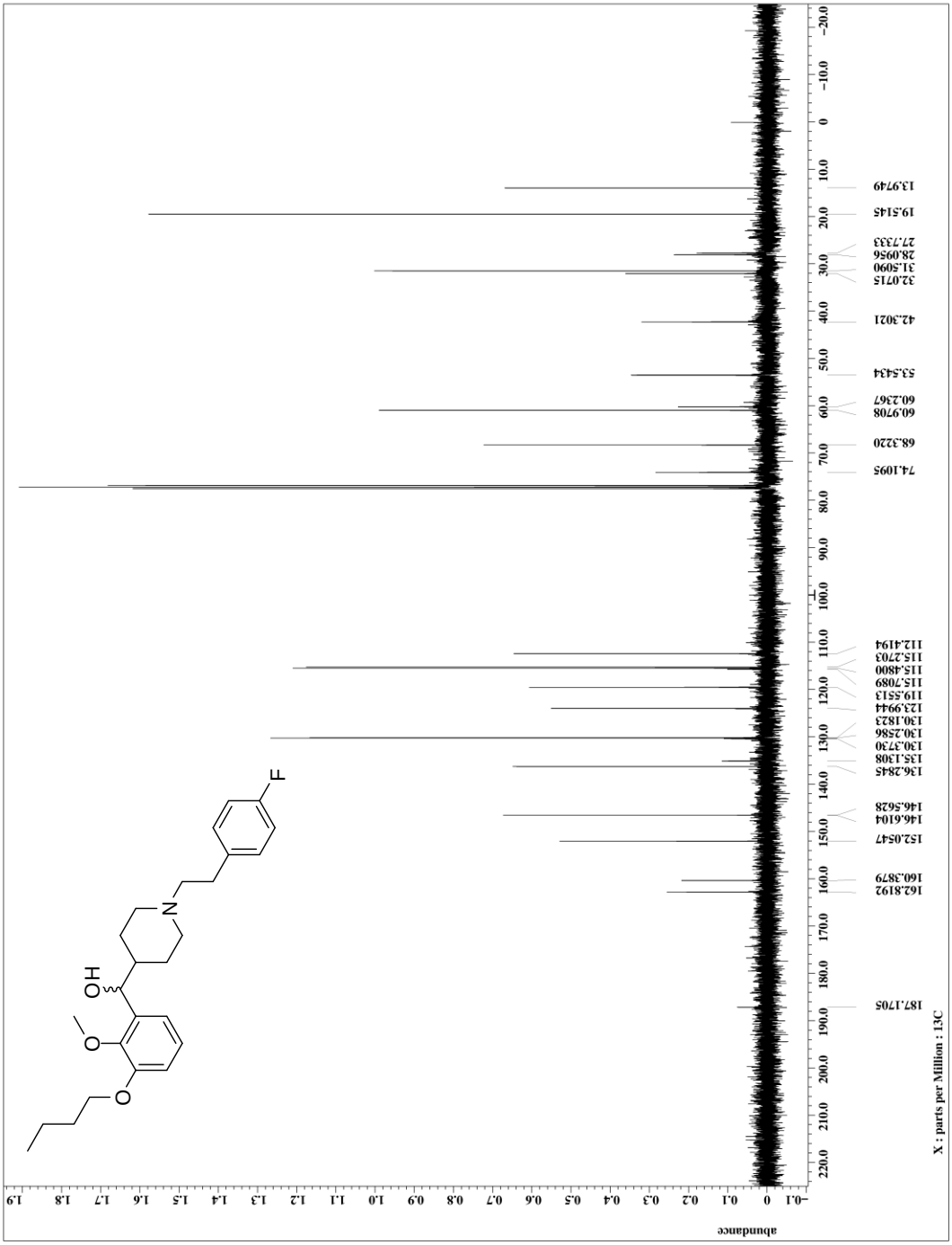


Figure B. 29: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(2-methoxyethoxy)phenyl) methanol (3.34)

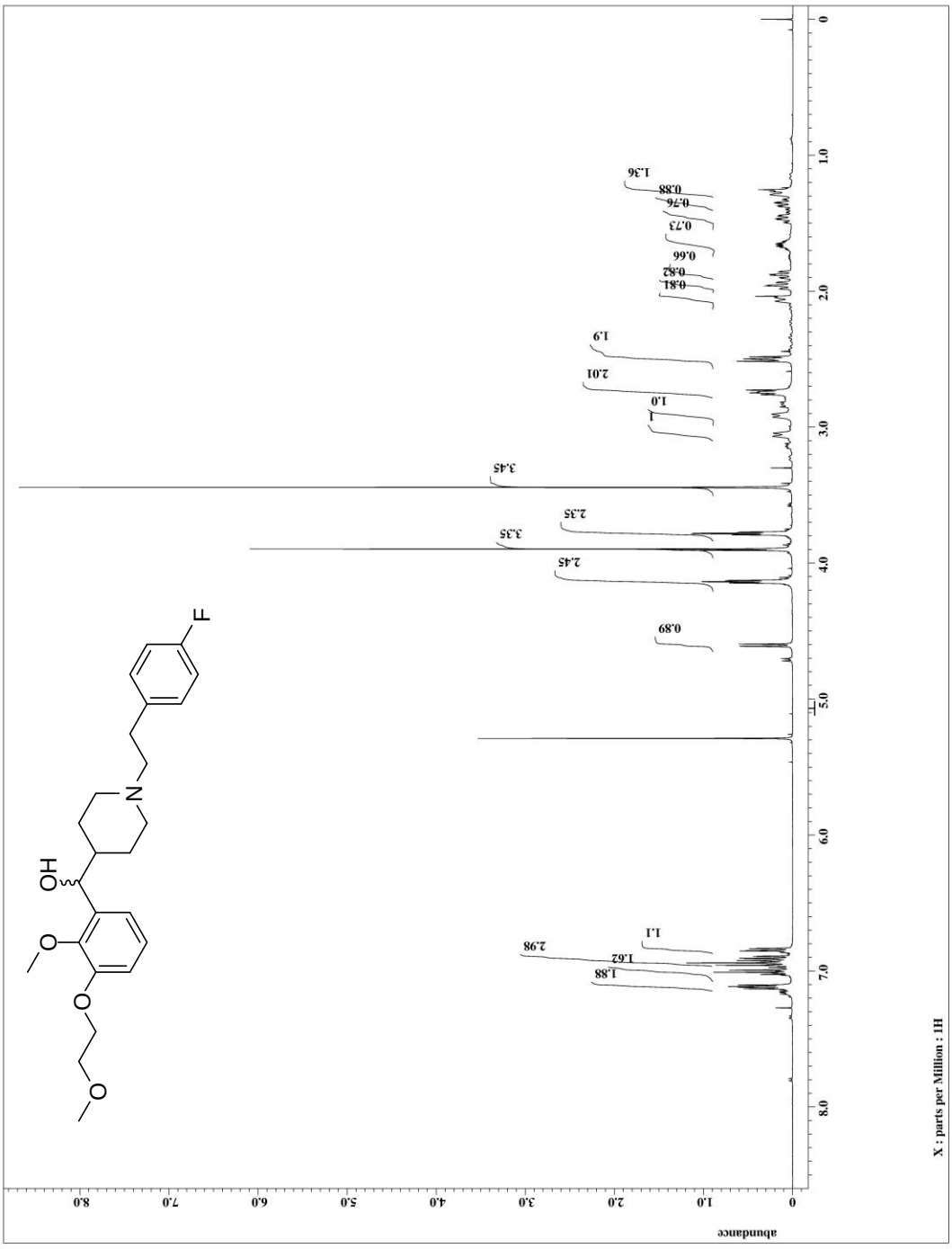


Figure B. 30: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(1-(4-fluorophenethyl)piperidin-4-yl)(2-methoxy-3-(2-methoxyethoxy)phenyl) methanol (3.34)

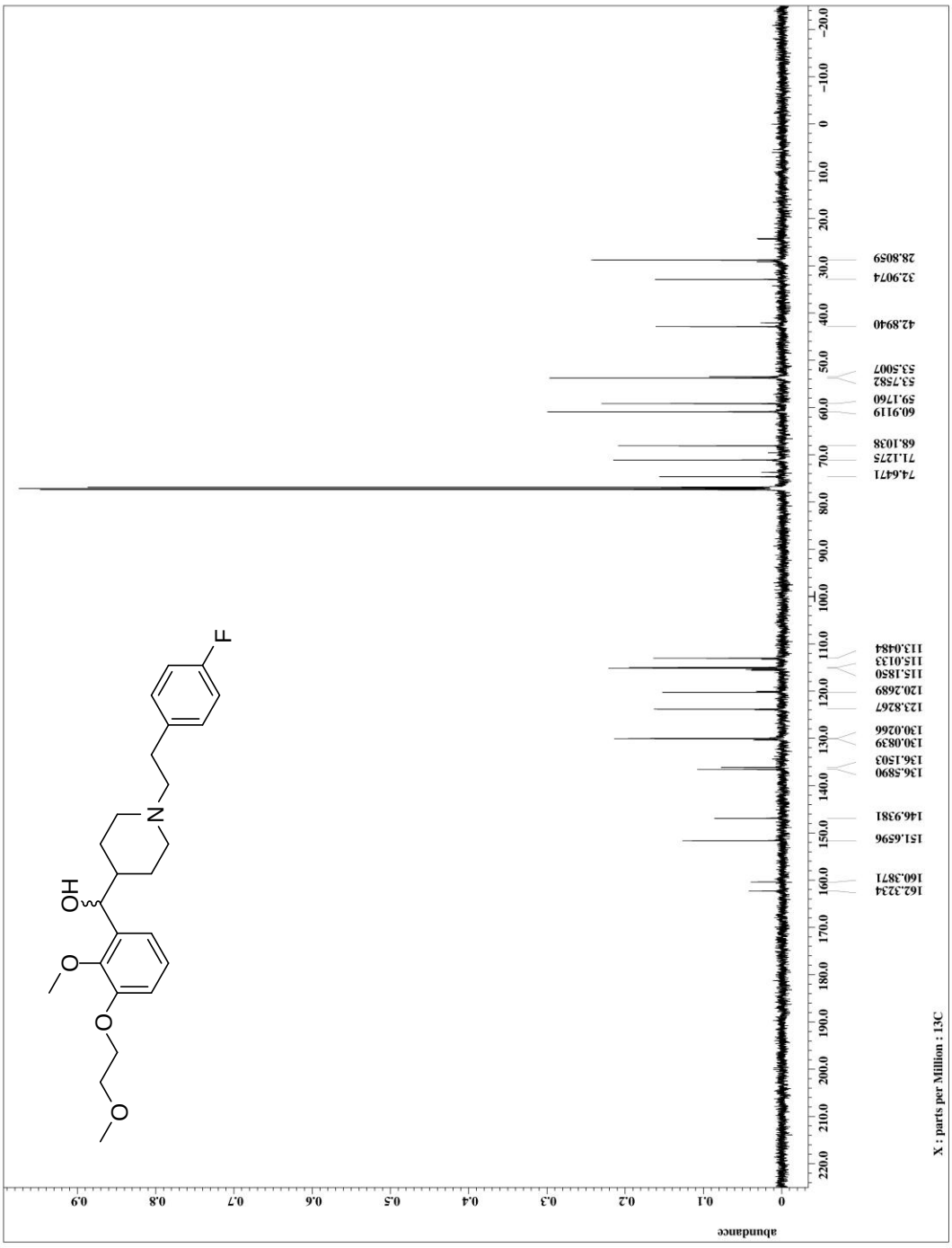


Figure B. 31: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(2,3-dimethoxyphenyl)(1-(4-hydroxyphenethyl)piperidin-4-yl)methanone (3.35)

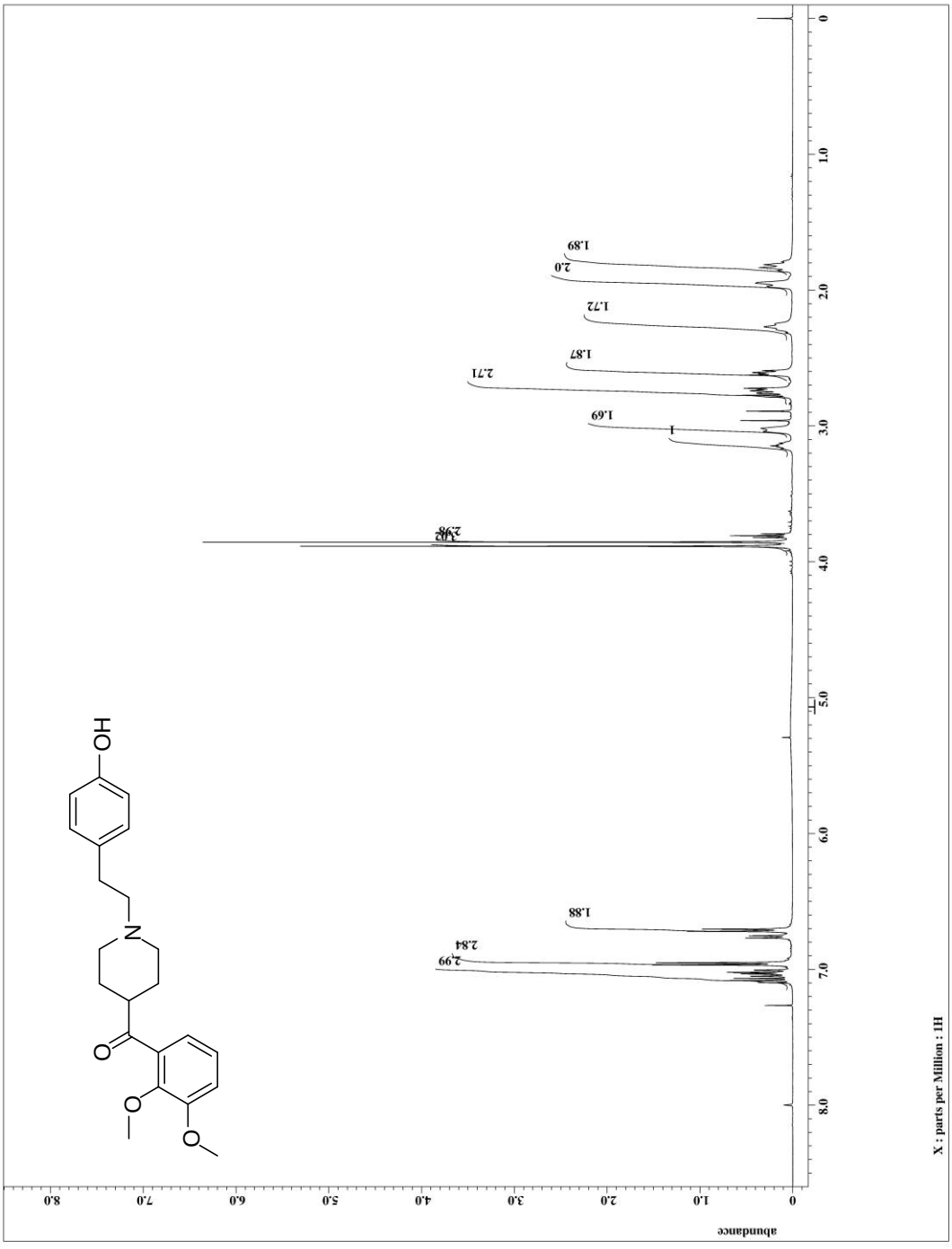


Figure B. 32: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(2,3-dimethoxyphenyl)(1-(4-hydroxyphenethyl)piperidin-4-yl)methanone (3.35)

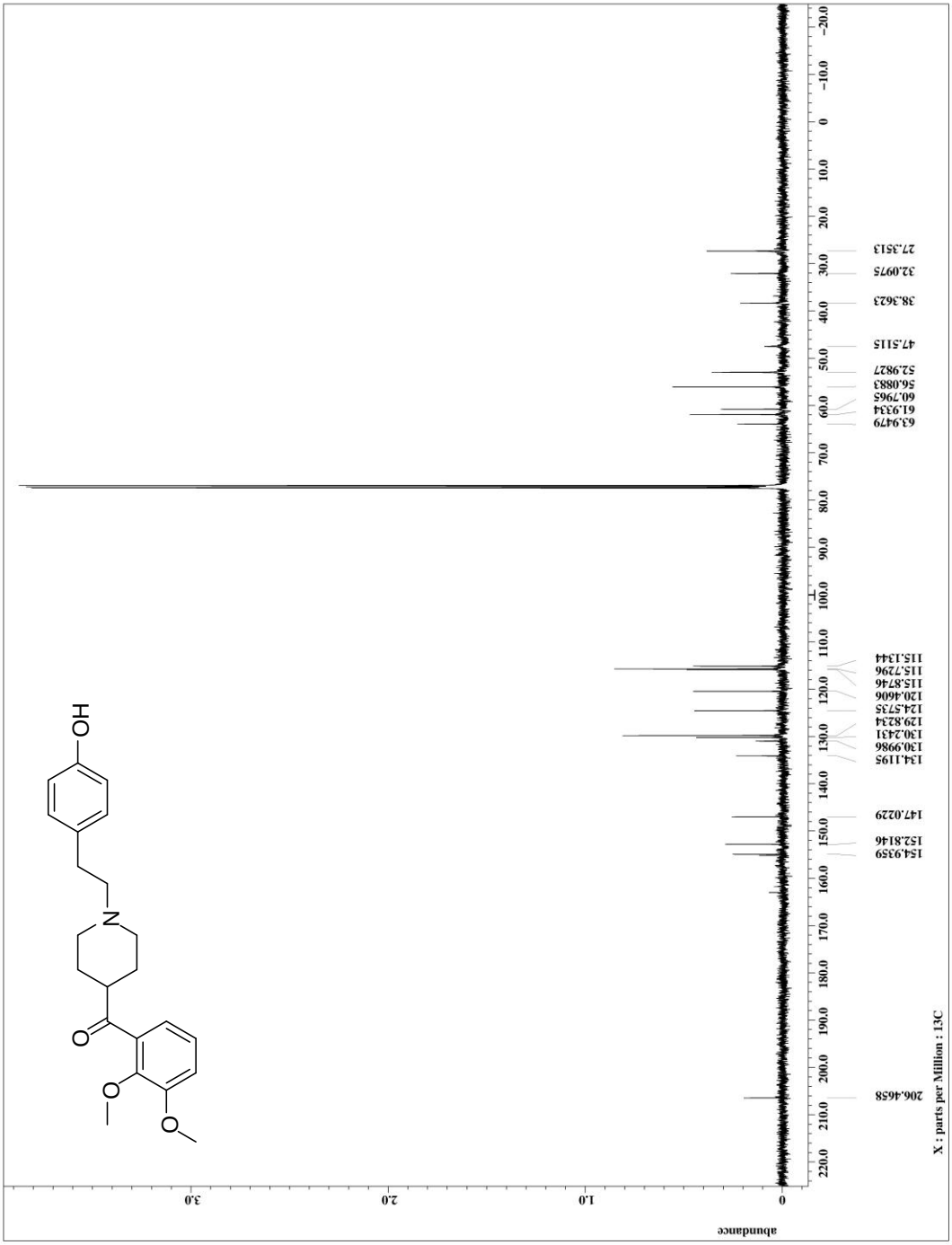


Figure B. 33: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
(1-(4-butoxyphenethyl)piperidin-4-yl)(2,3-dimethoxyphenyl)methanone (3.37)

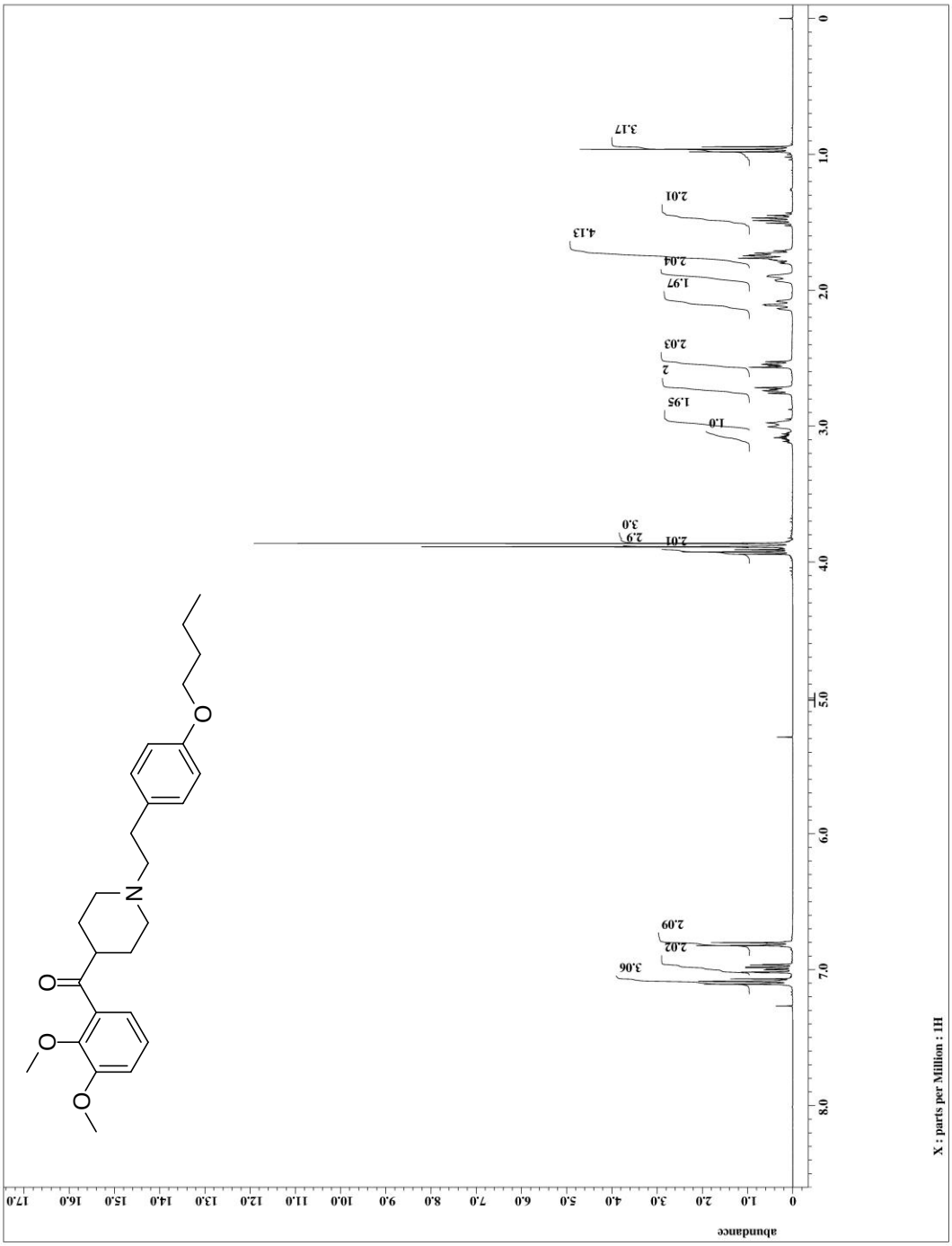


Figure B. 34: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
(1-(4-butoxyphenethyl)piperidin-4-yl)(2,3-dimethoxyphenyl)methanone (3.37)

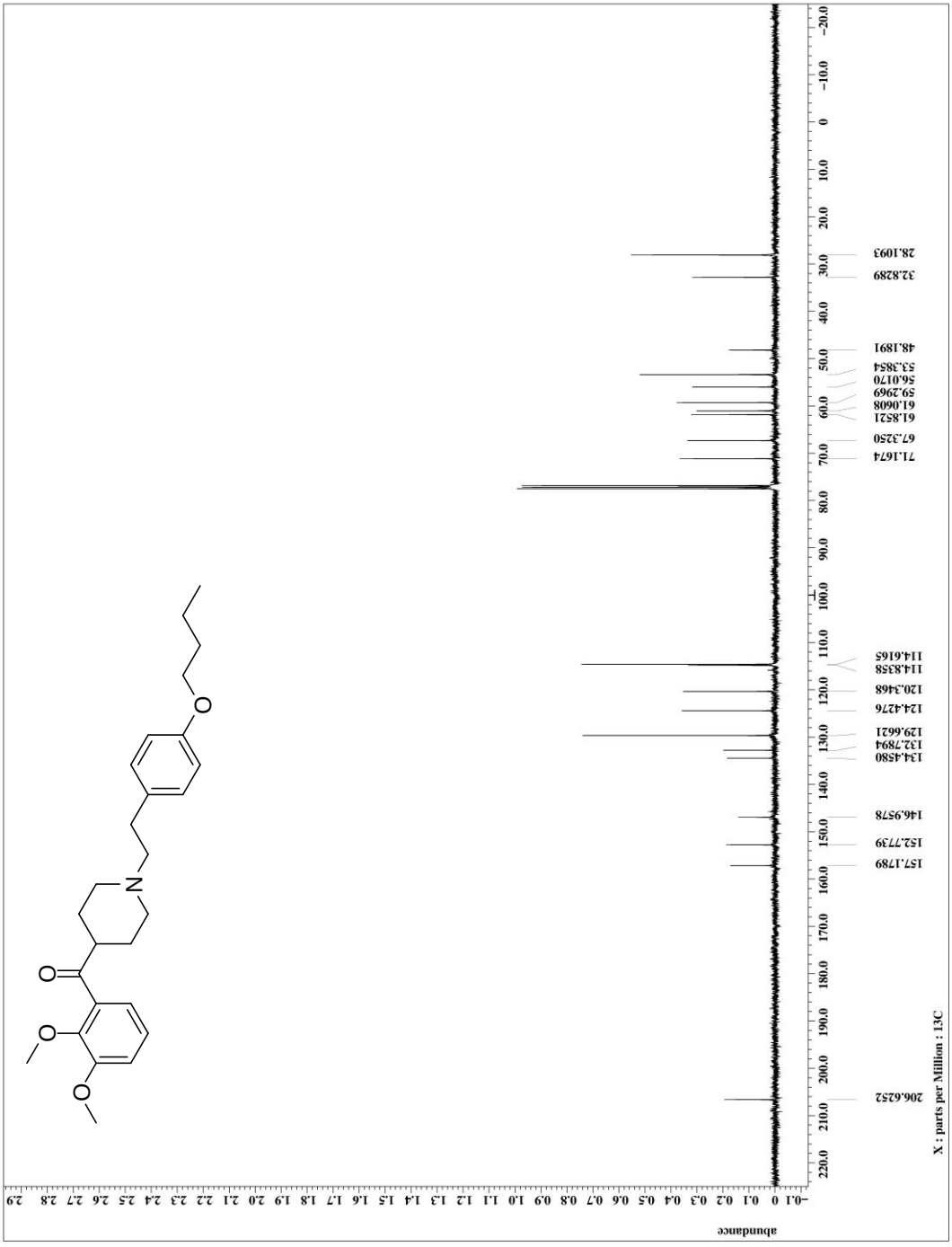


Figure B. 35: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
(2,3-dimethoxyphenyl)(1-(4-(2-methoxyethoxy)phenethyl)piperidin-4-yl)methanone (3.39)

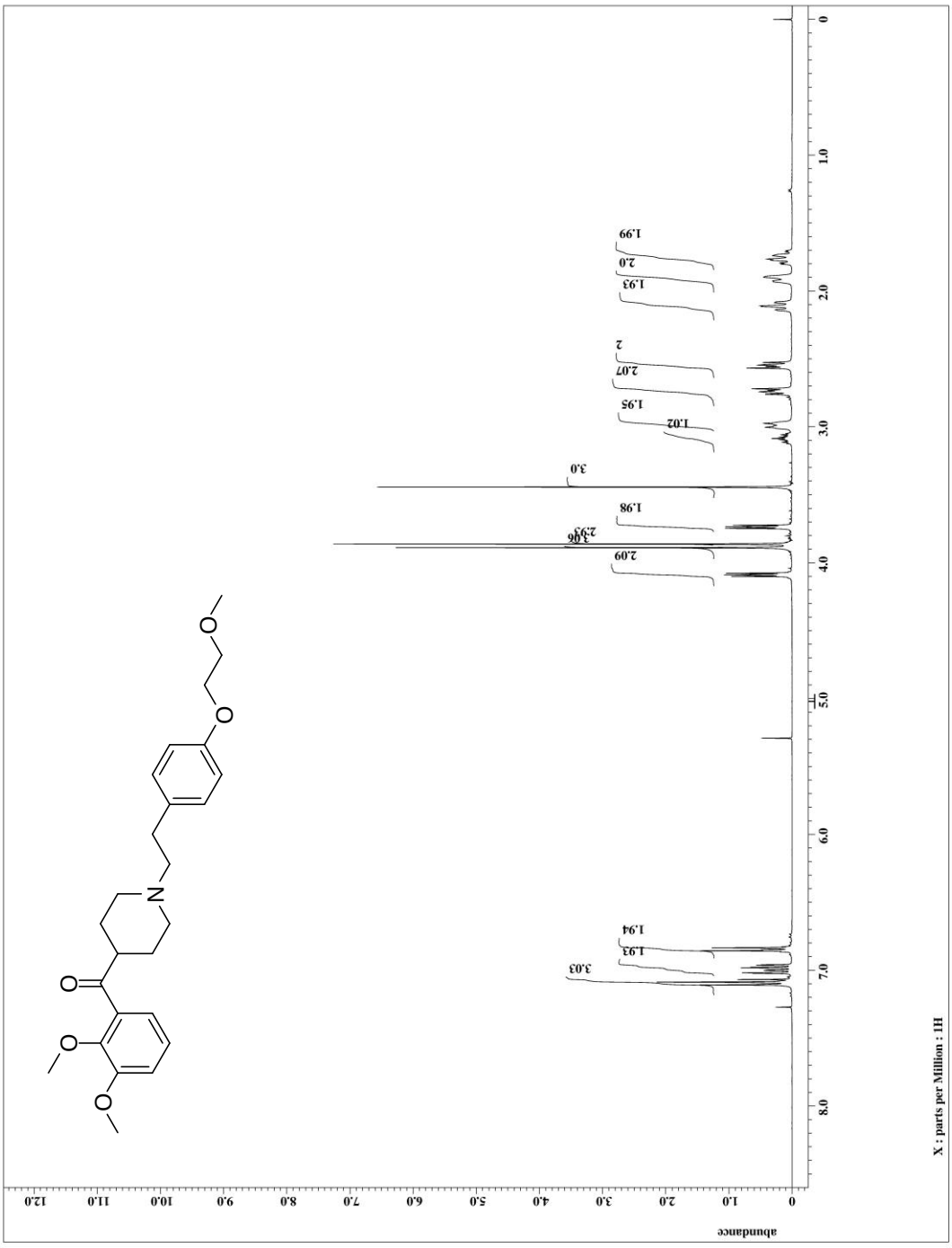


Figure B. 36: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
(2,3-dimethoxyphenyl)(1-(4-(2-methoxyethoxy)phenethyl)piperidin-4-yl)methanone (3.39)

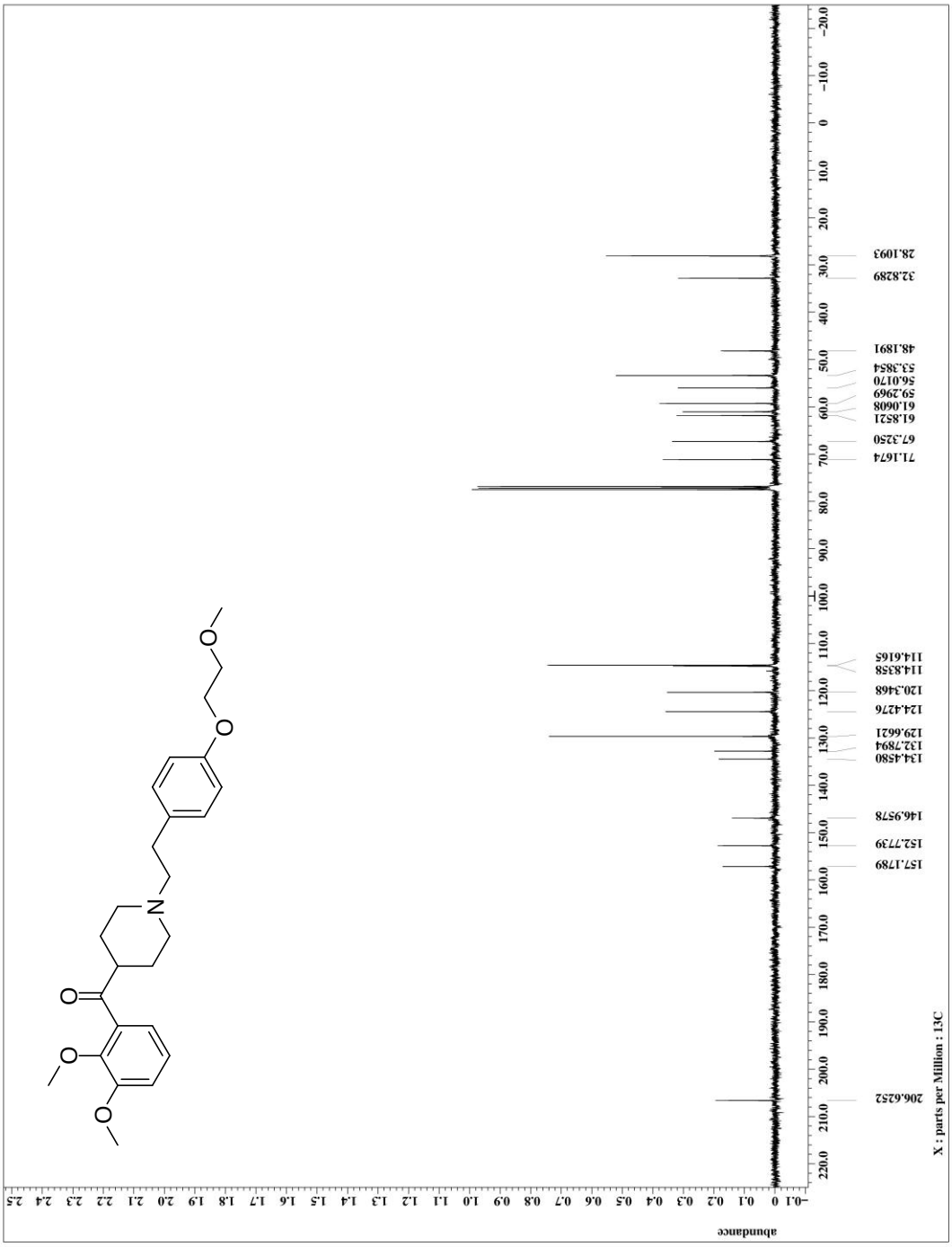


Figure B. 37: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(3-(2-(2-ethoxyethoxy)ethoxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (3.46)

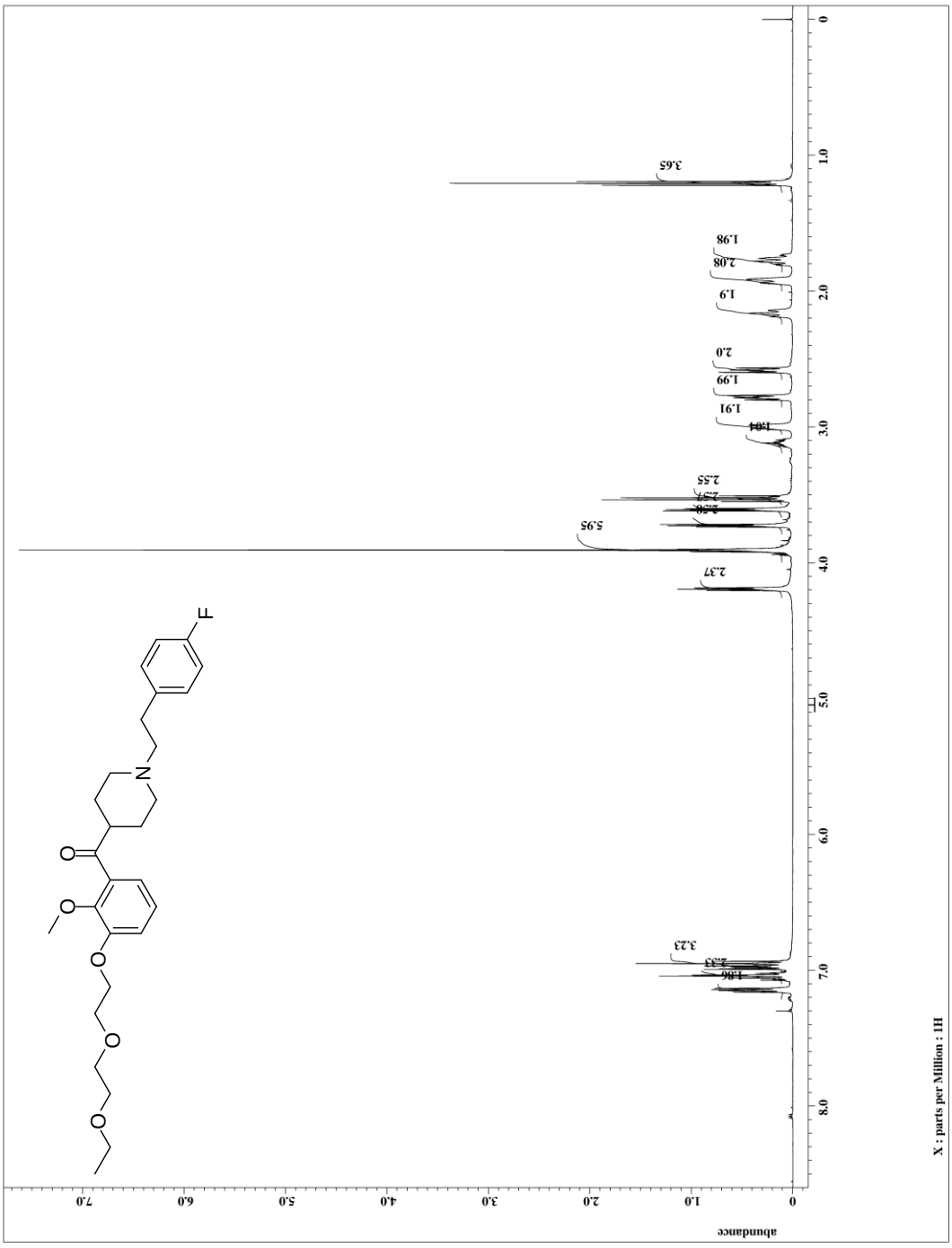


Figure B. 38: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3-(2-(2-ethoxyethoxy)ethoxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone (3.46)

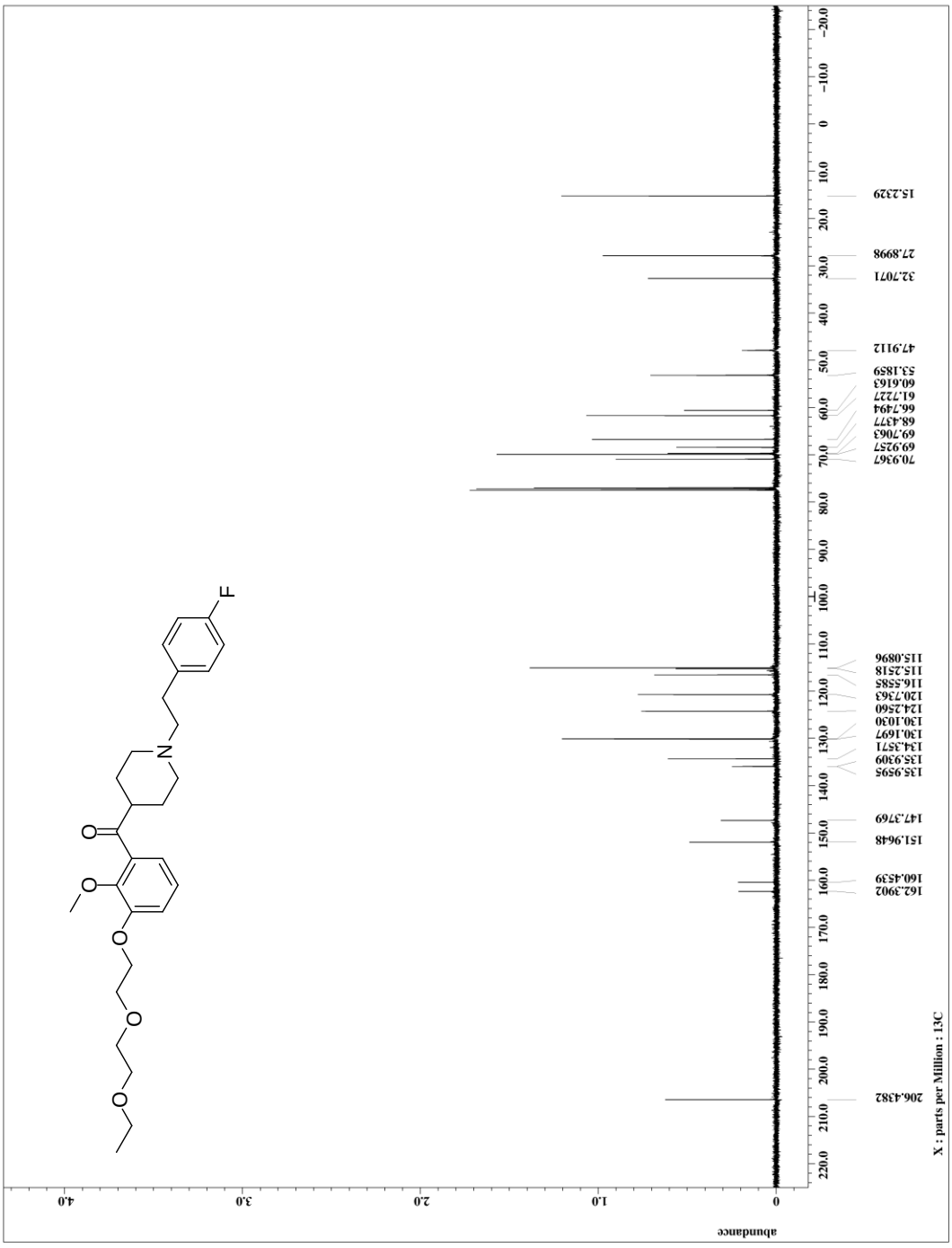


Figure B. 39: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(3-(2-(2-(2-ethoxyethoxy)ethoxy)ethoxy)-2-methoxyphenyl)(1-(4-fluorophenethyl) piperidin-4-yl)methanone (3.47)

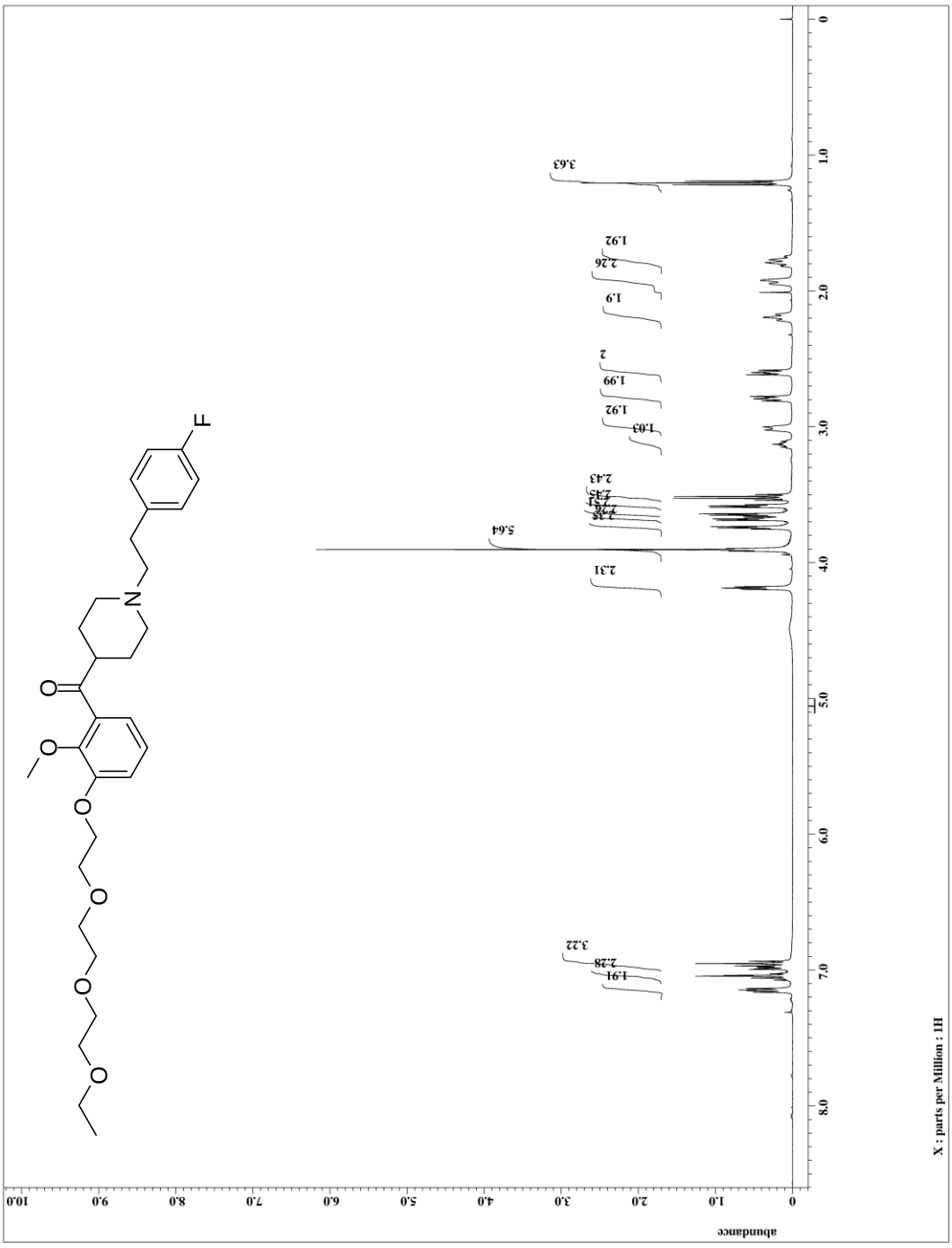


Figure B. 40: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3-(2-(2-(2-ethoxyethoxy)ethoxy)ethoxy)-2-methoxyphenyl)(1-(4-fluorophenethyl) piperidin-4-yl)methanone (3.47)

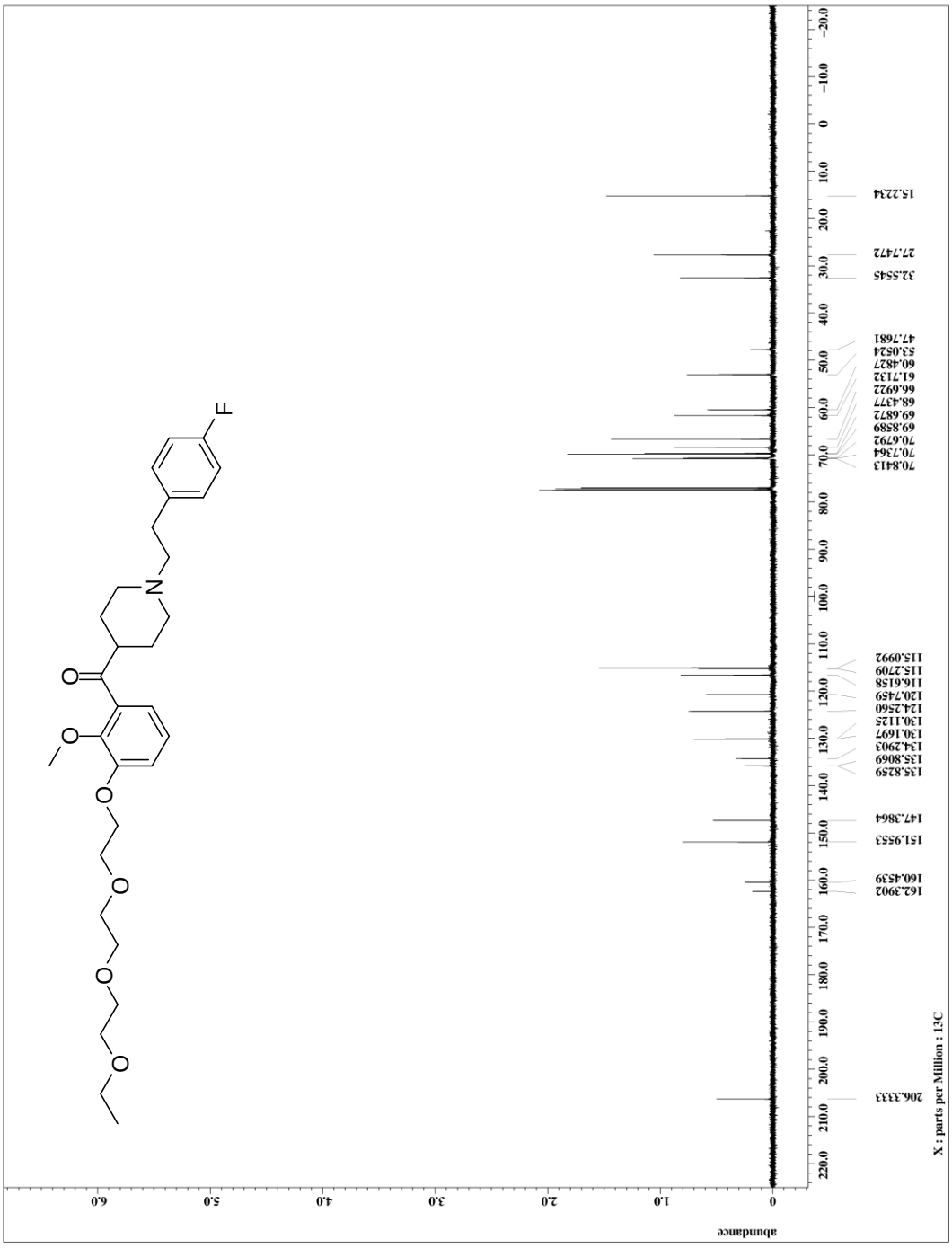


Figure B. 41: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(3-(2,5,8,11-tetraoxatridecan-13-yloxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone
(3.48)

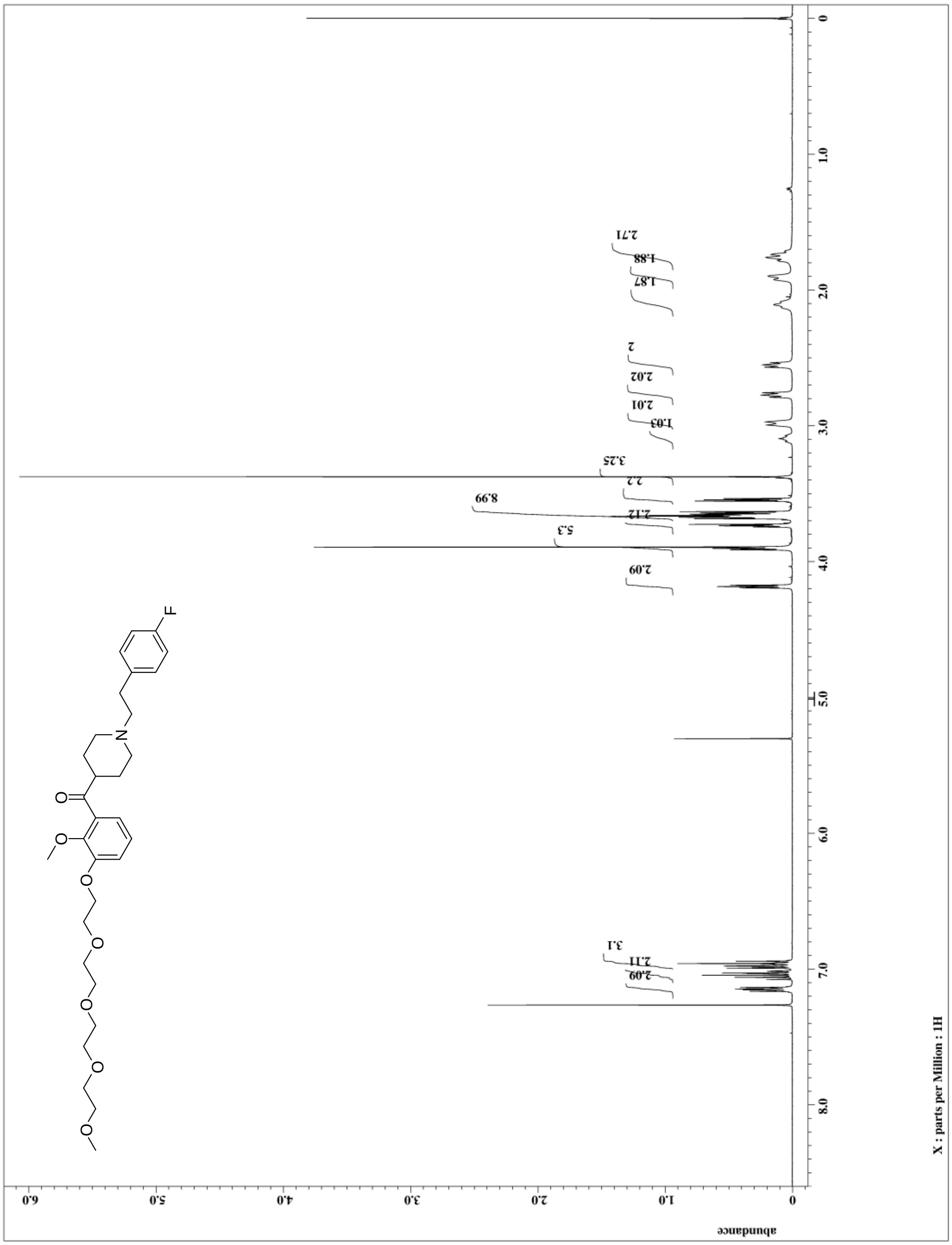


Figure B. 42: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3-(2,5,8,11-tetraoxatridecan-13-yloxy)-2-methoxyphenyl)(1-(4-fluorophenethyl)piperidin-4-yl)methanone
(3.48)

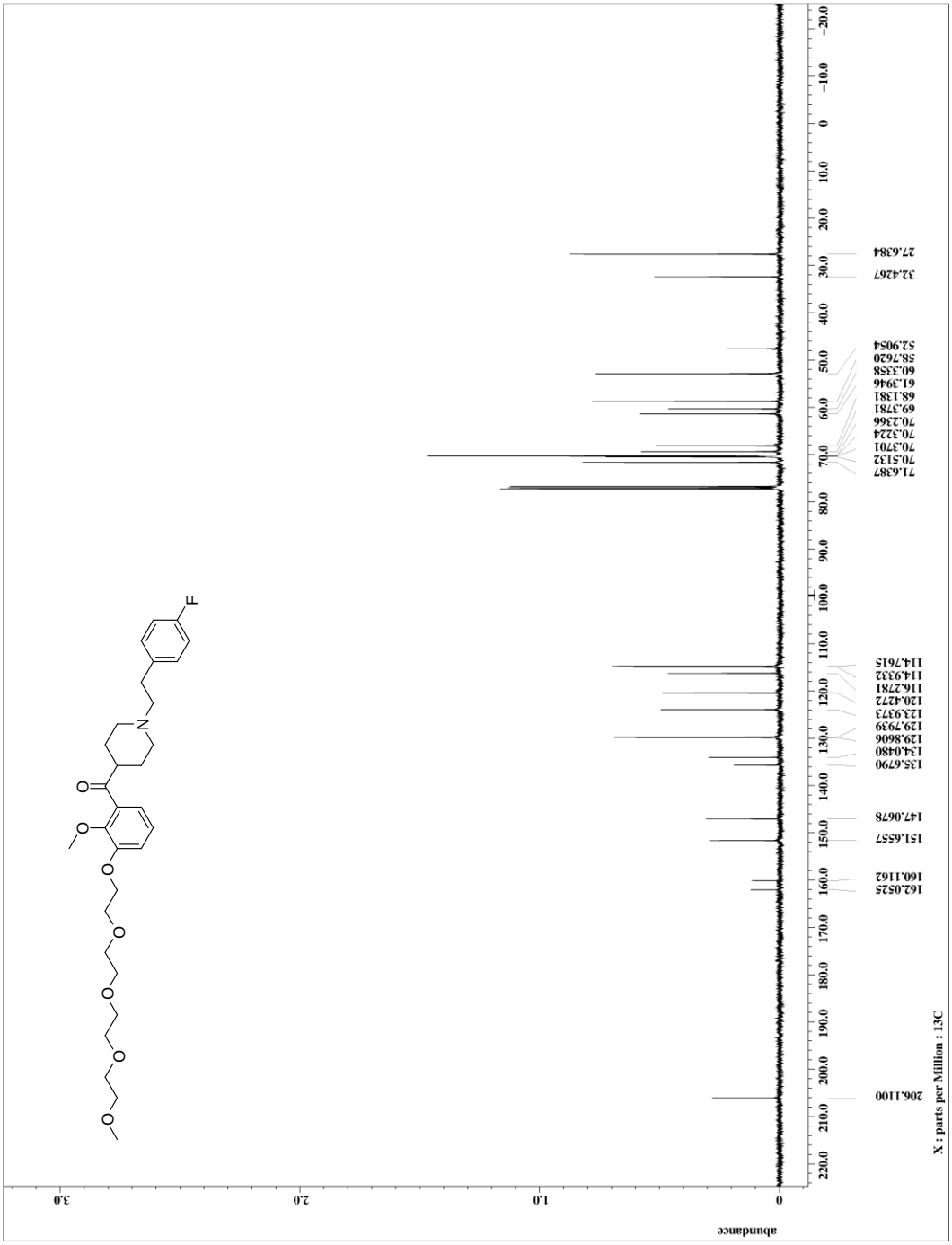


Figure B. 43: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)ethoxy) ethyl 4-methylbenzenesulfonate (3.68)

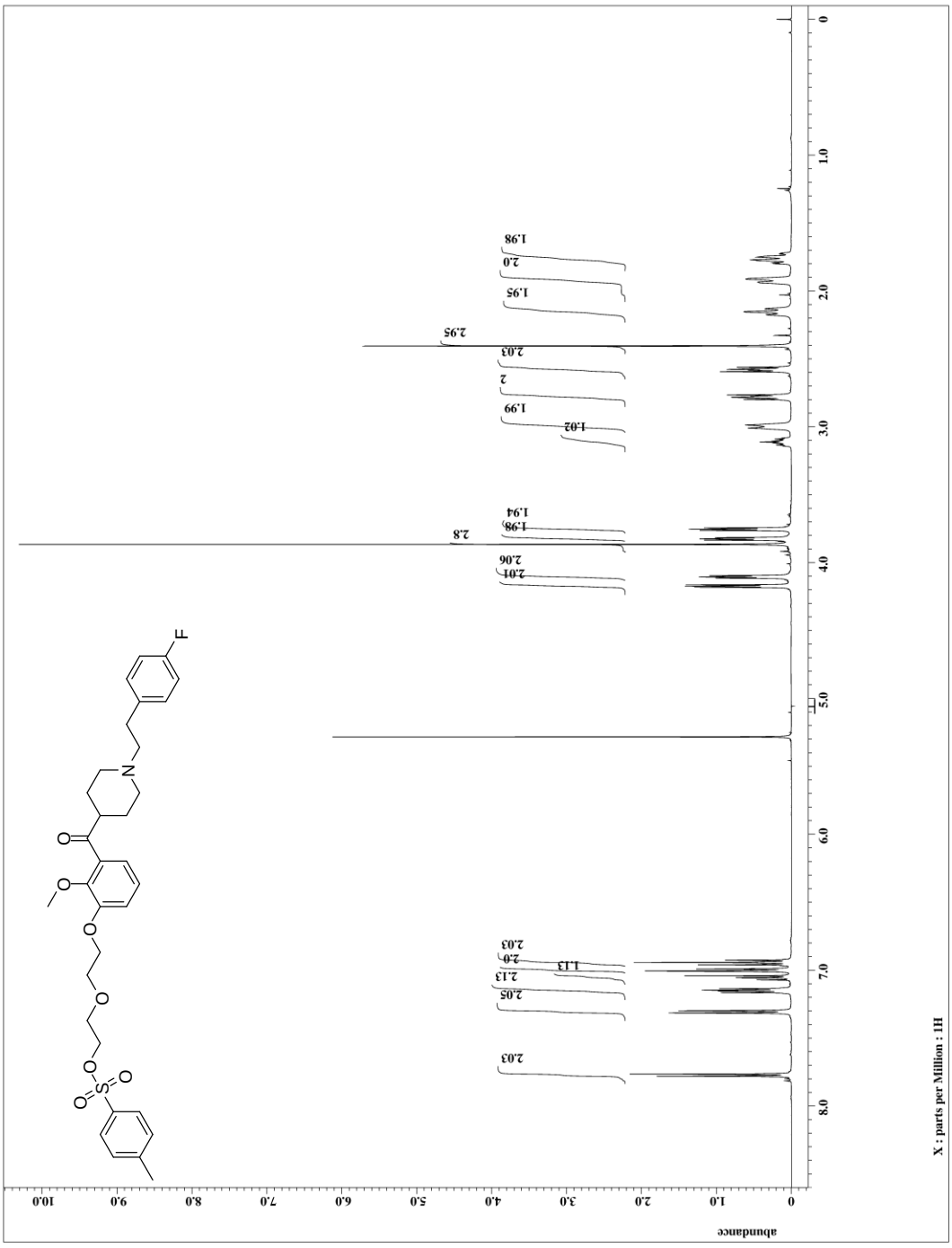


Figure B. 44: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)ethoxy) ethyl 4-methylbenzenesulfonate (3.68)

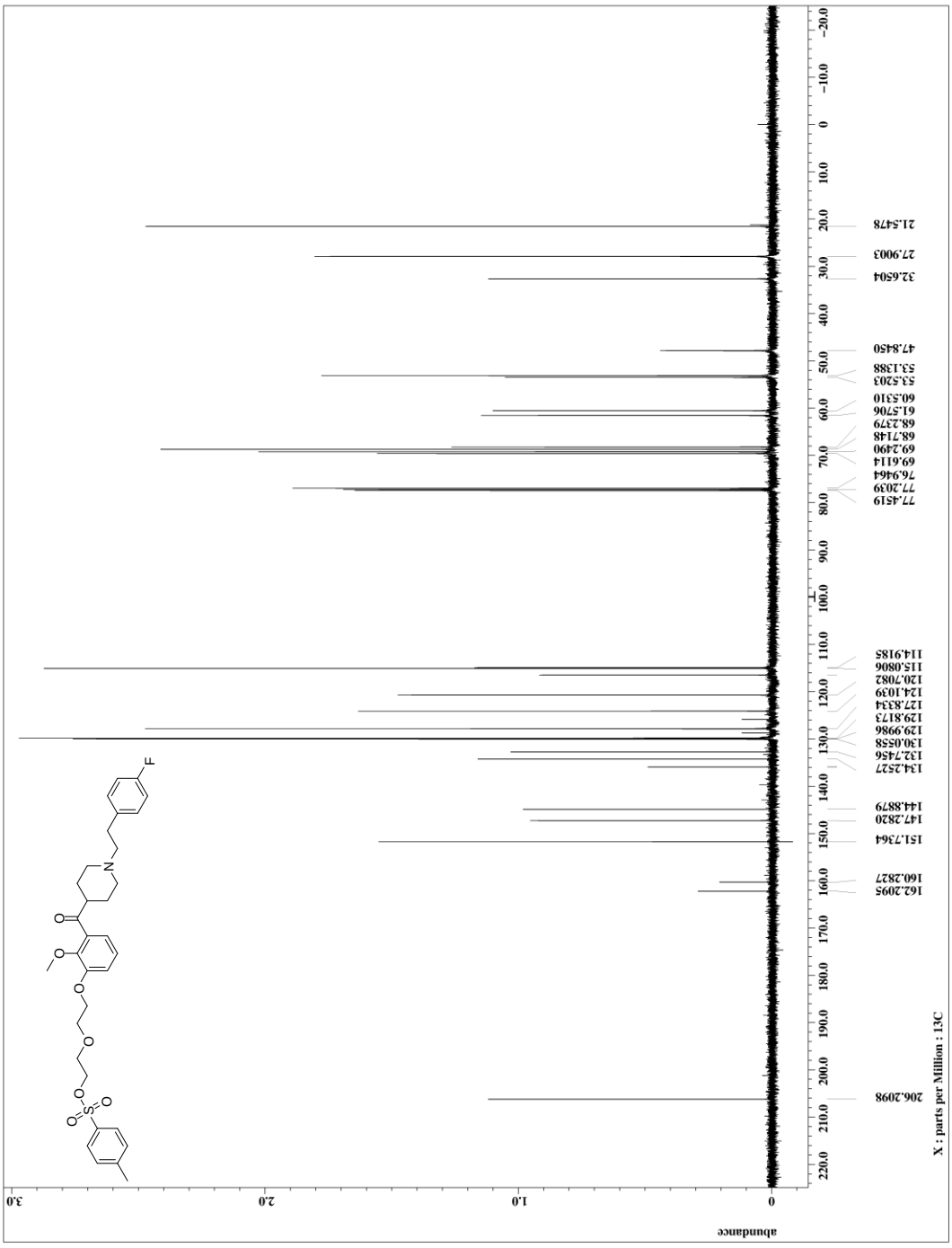


Figure B. 45: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
2-(2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)ethoxy) ethoxy)ethyl 4-methylbenzenesulfonate (3.69)

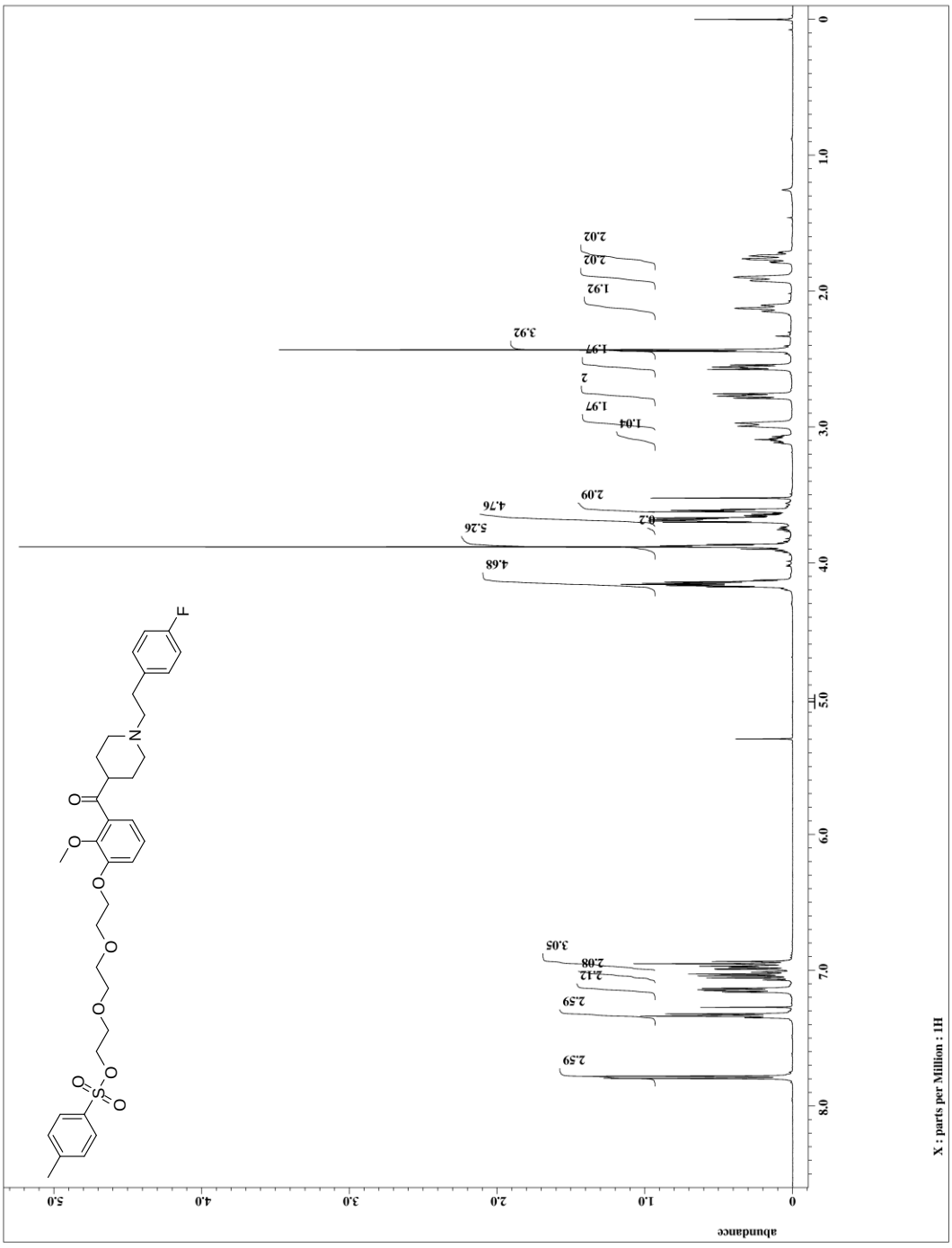


Figure B. 46: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
2-(2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)ethoxy) ethoxy)ethyl 4-methylbenzenesulfonate (3.69)

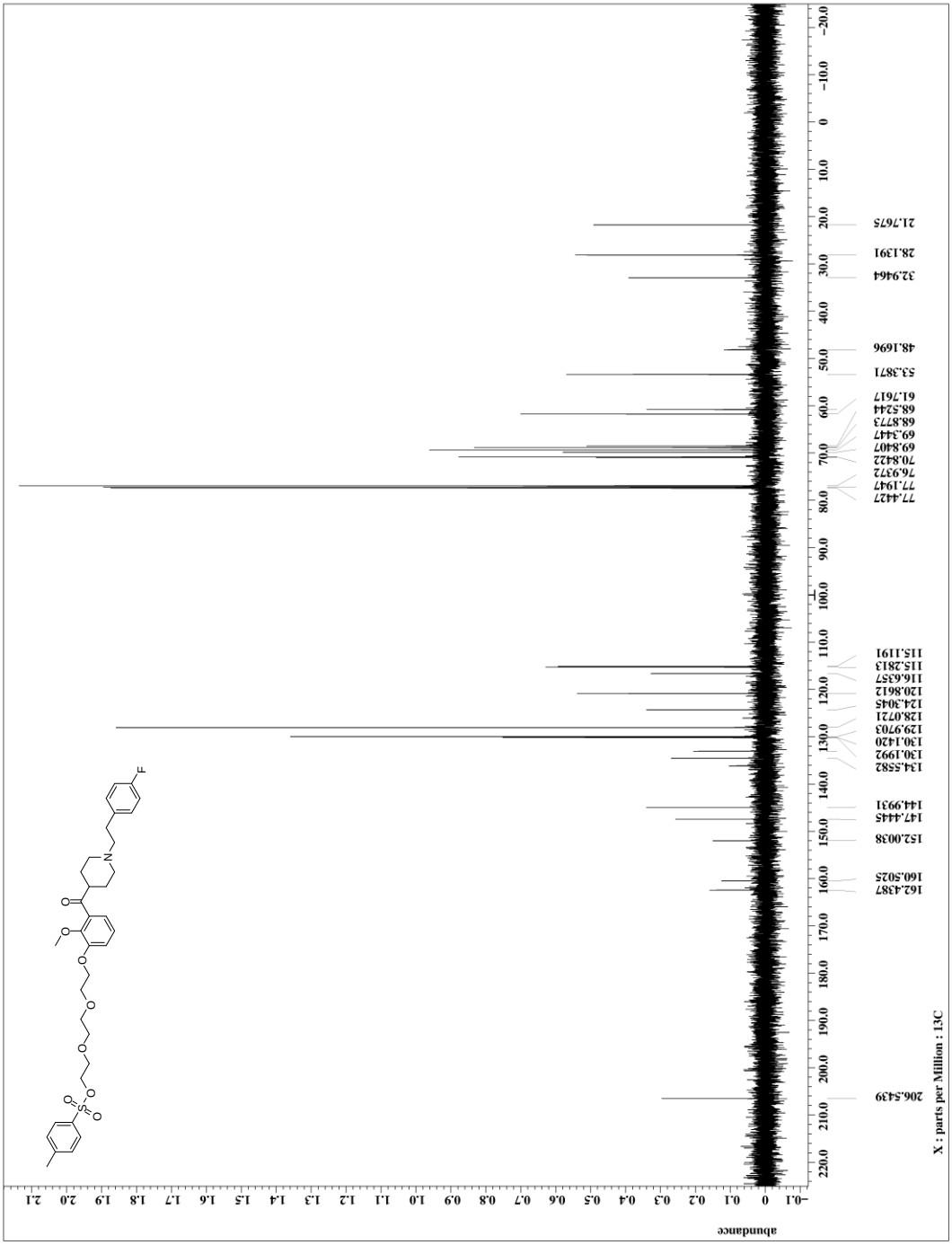


Figure B. 47: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
2-(2-(2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy) ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (3.70)

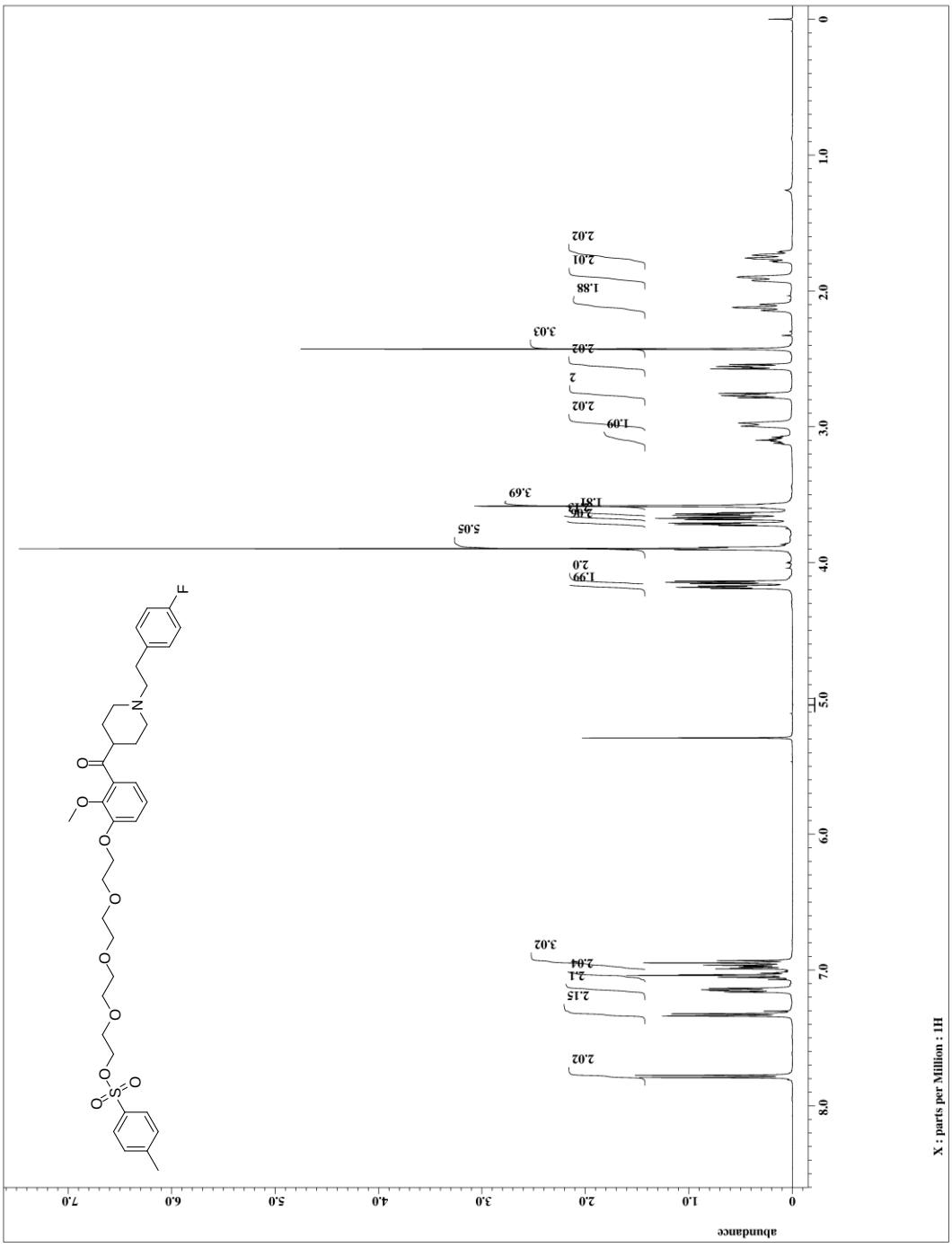


Figure B. 48: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
2-(2-(2-(2-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy) ethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (3.70)

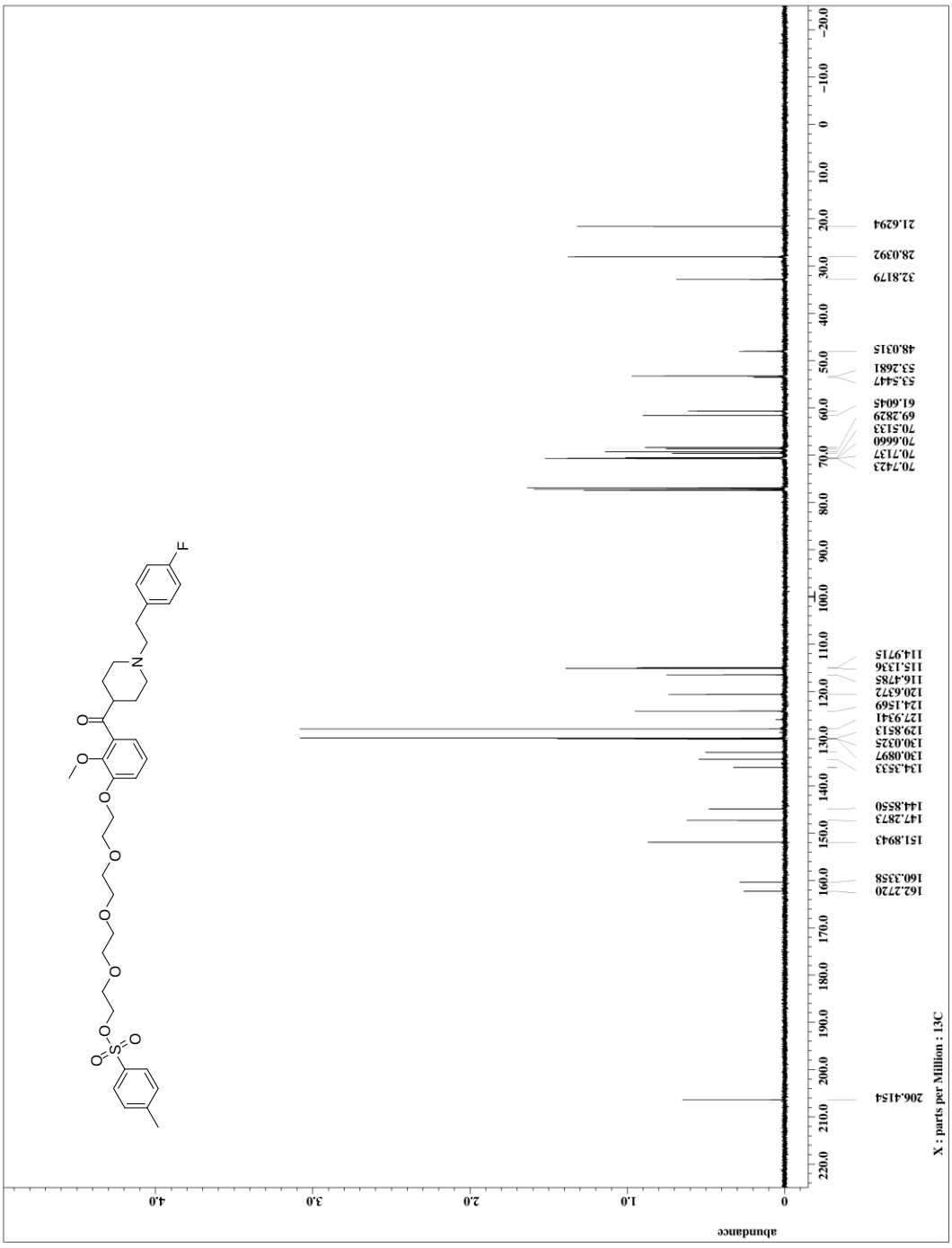


Figure B. 49: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
14-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate (3.71)

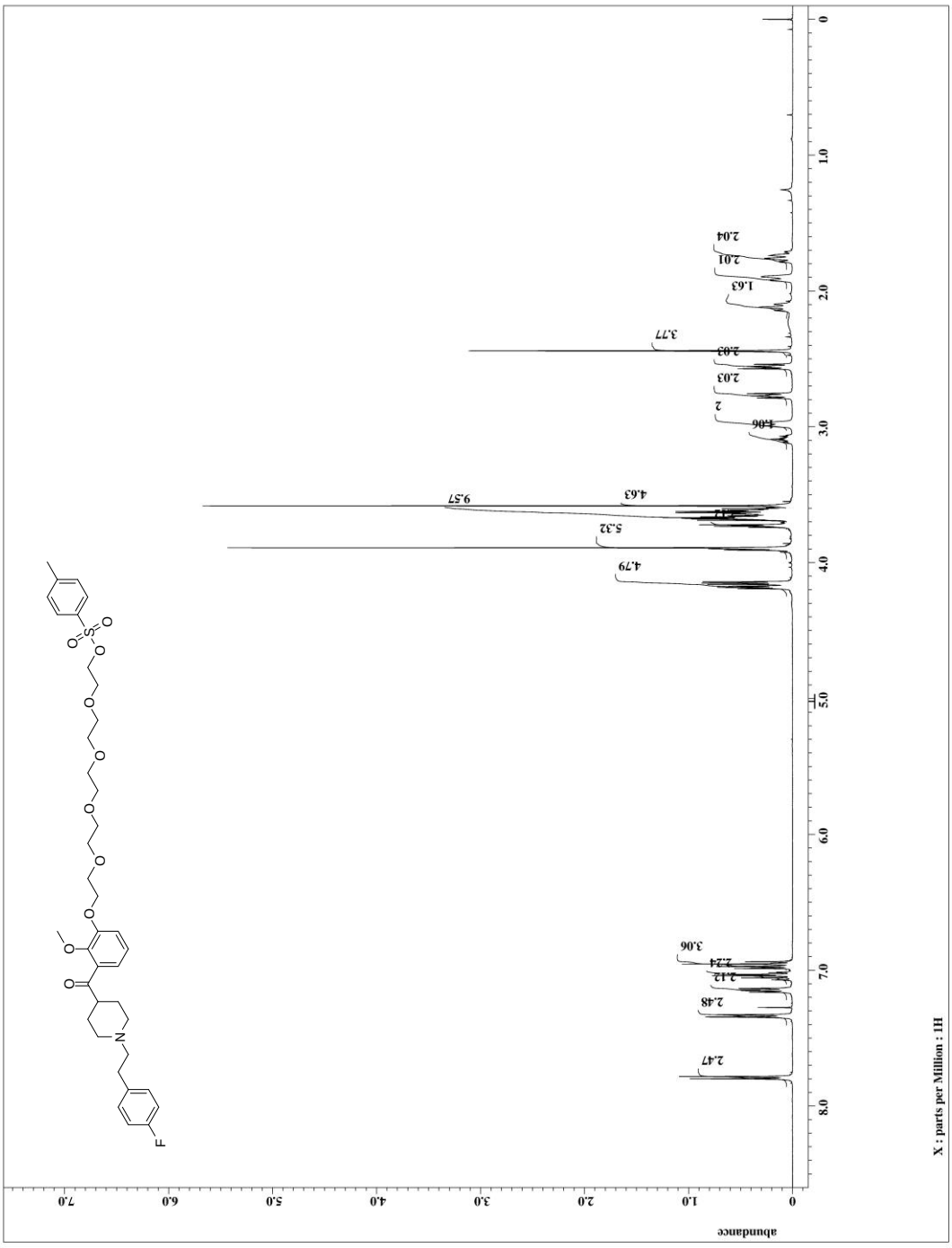


Figure B. 50: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)

14-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12-tetraoxatetradecyl 4-methylbenzenesulfonate (3.71)

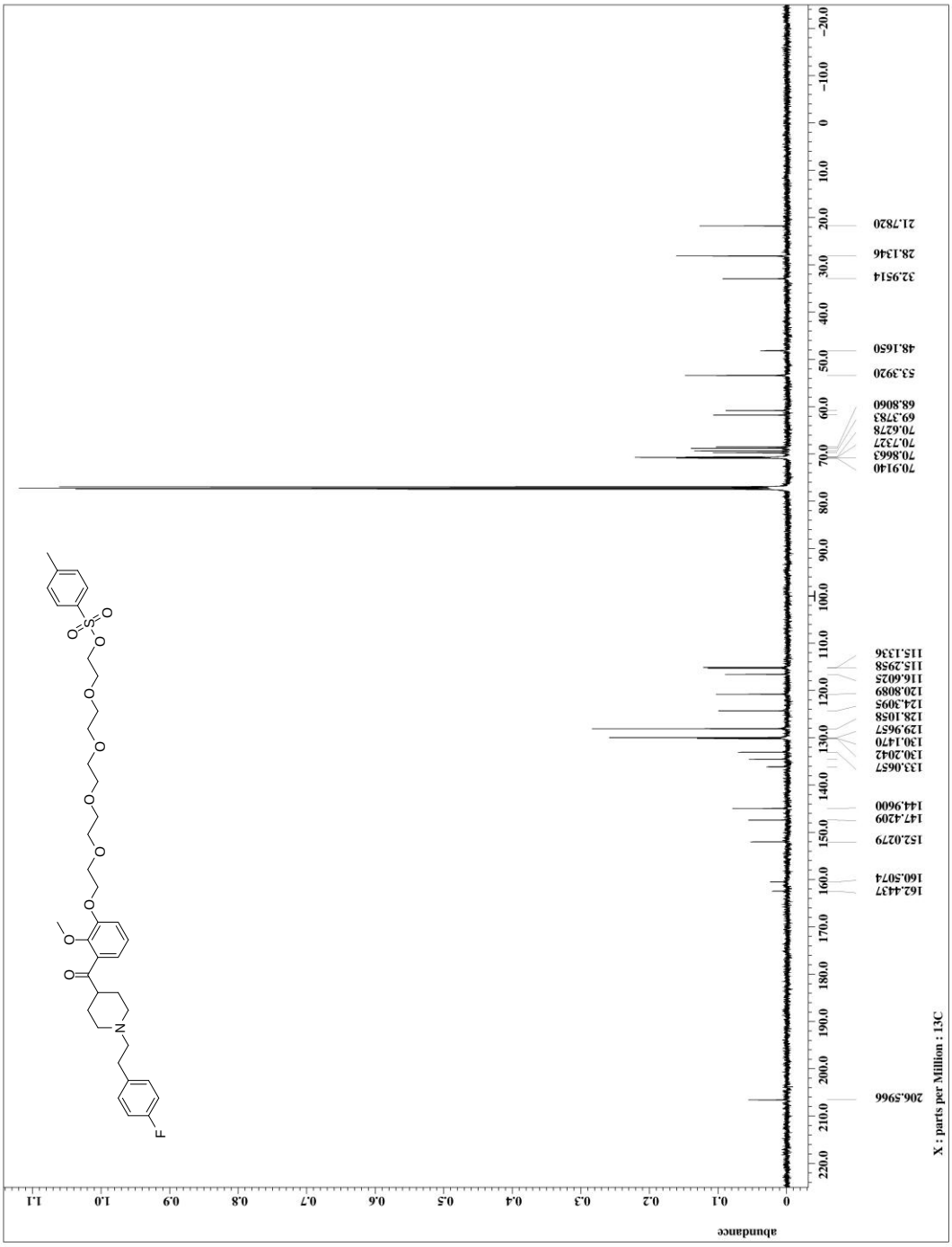


Figure B. 51: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
17-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (3.72)

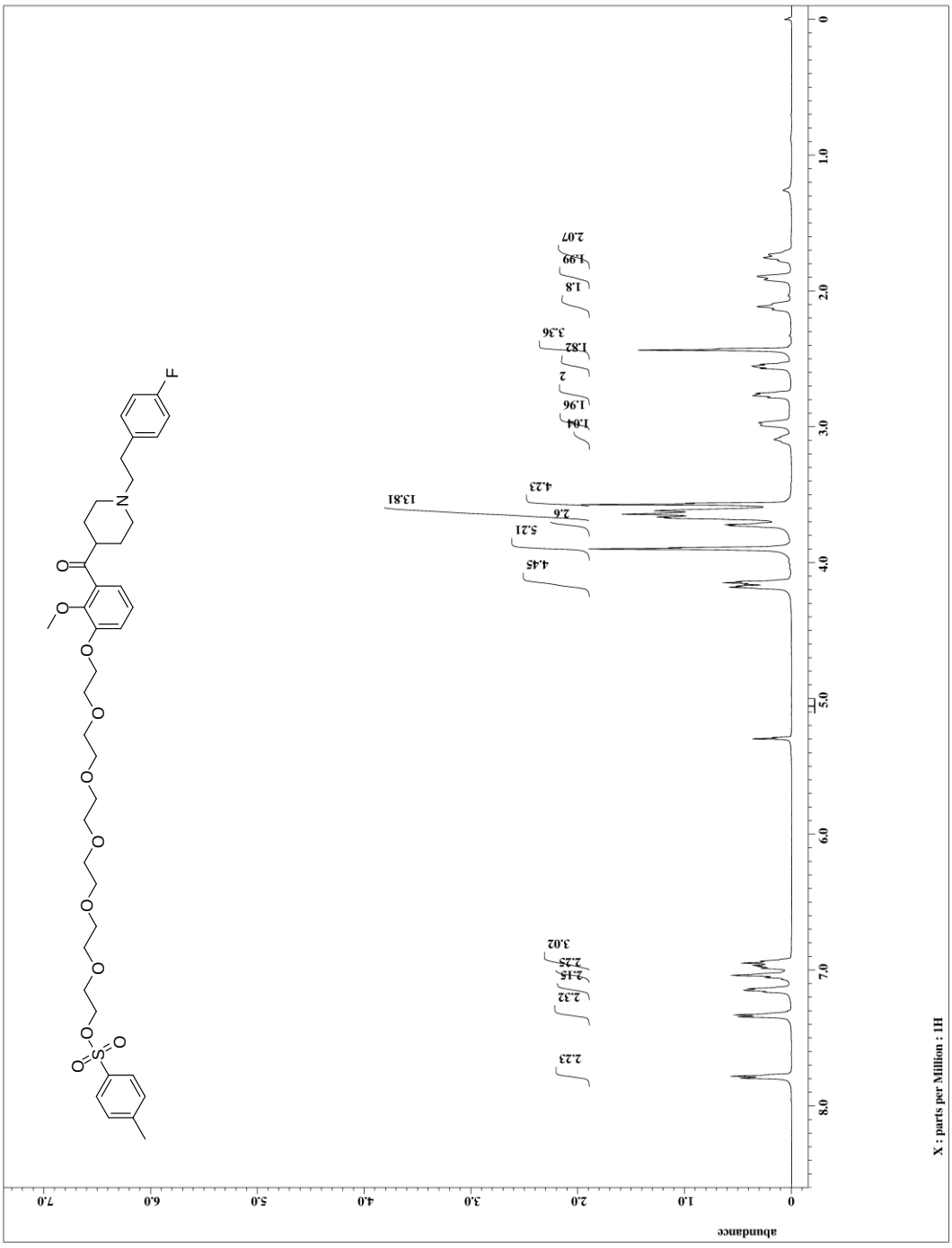


Figure B. 52: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
17-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15-pentaoxaheptadecyl 4-methylbenzenesulfonate (3.72)

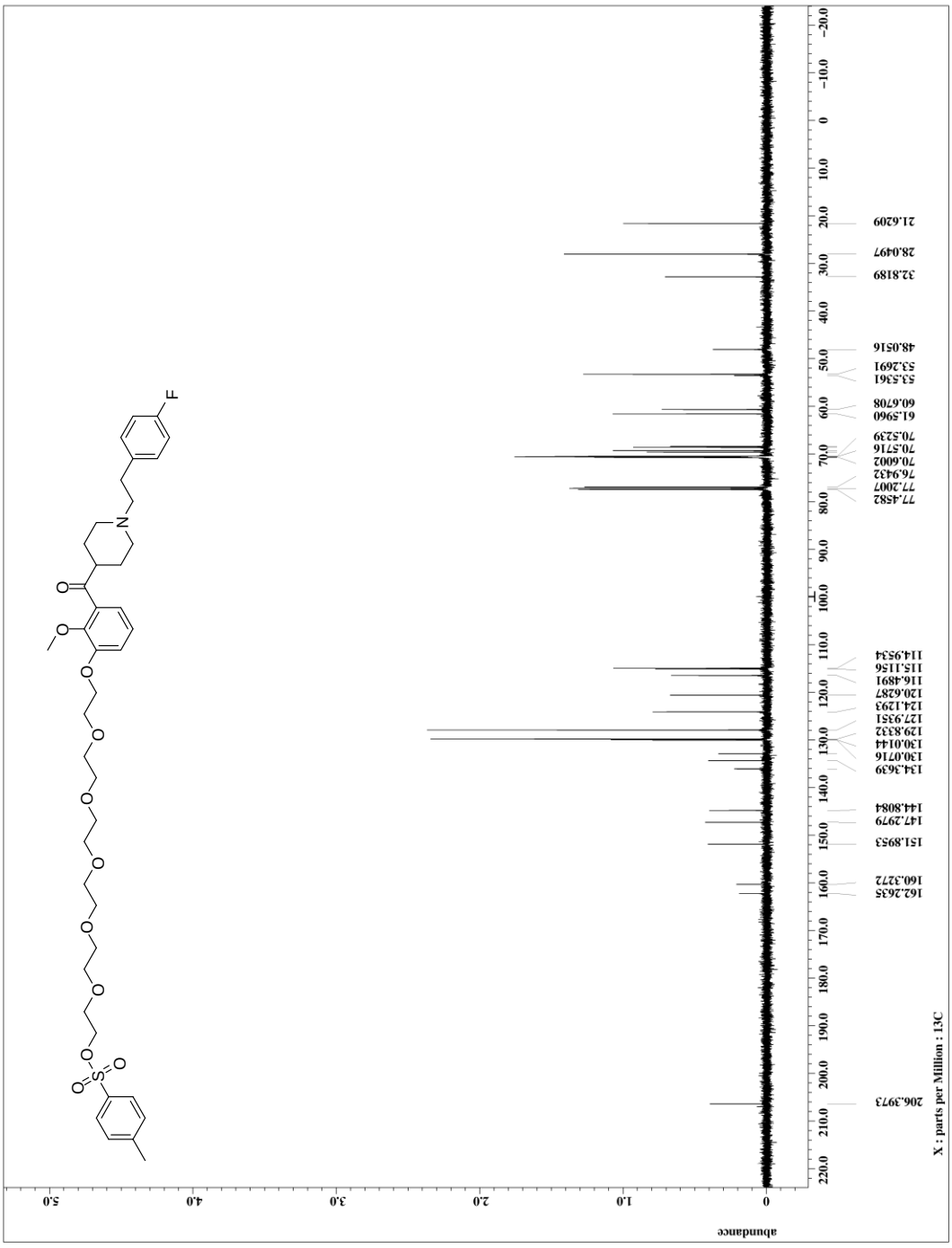


Figure B. 53: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
20-(3-((1-(4-fluorophenethyl)piperidin-4-yl)(hydroxy)methyl)-2-methoxyphenoxy)-3,6,9,12,15,18-hexaoxaicosyl 4-methylbenzenesulfonate (3.73)

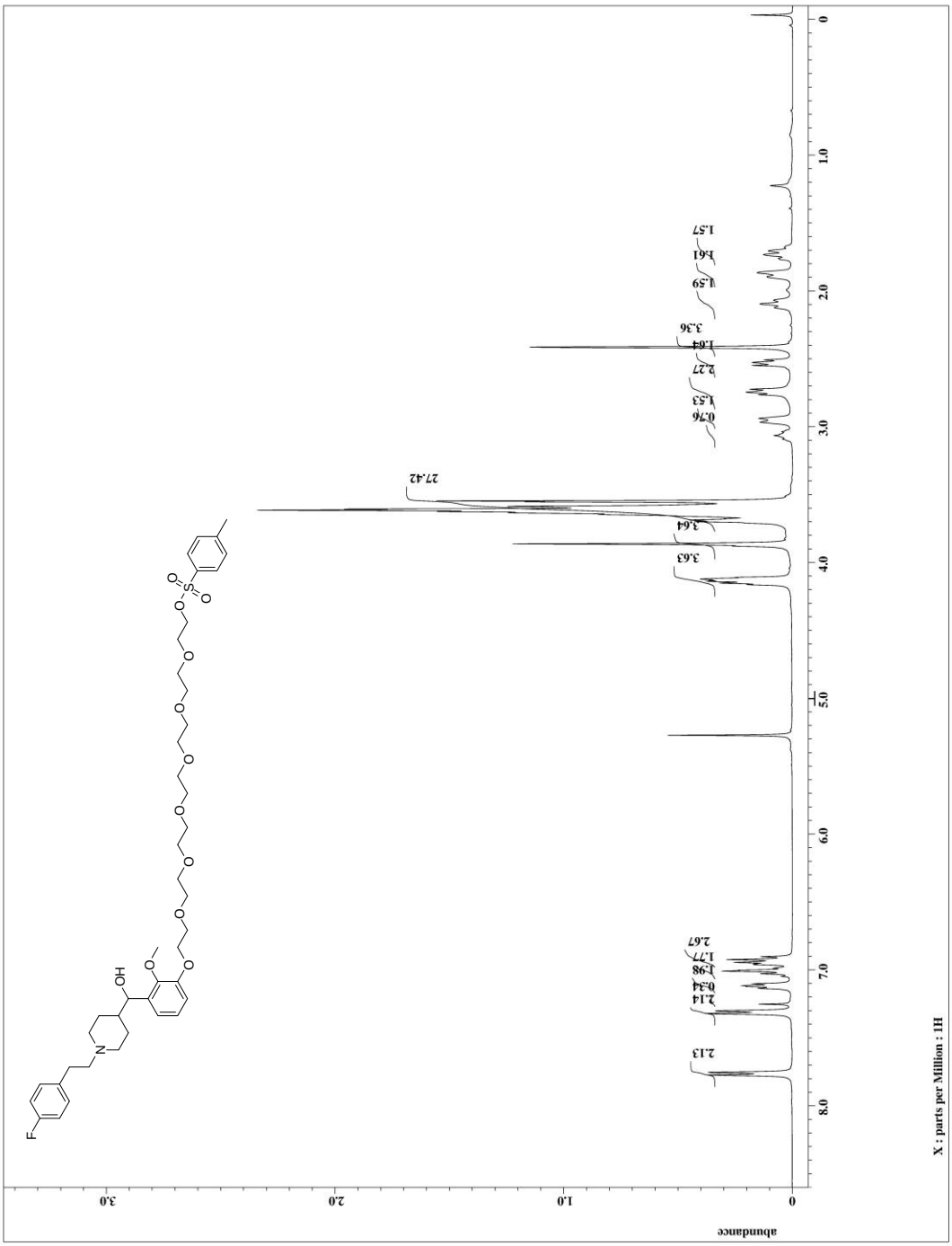


Figure B. 54: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
20-(3-((1-(4-fluorophenethyl)piperidin-4-yl)(hydroxy)methyl)-2-methoxyphenoxy)-3,6,9,12,15,18-hexaoxaicosyl 4-methylbenzenesulfonate (3.73)

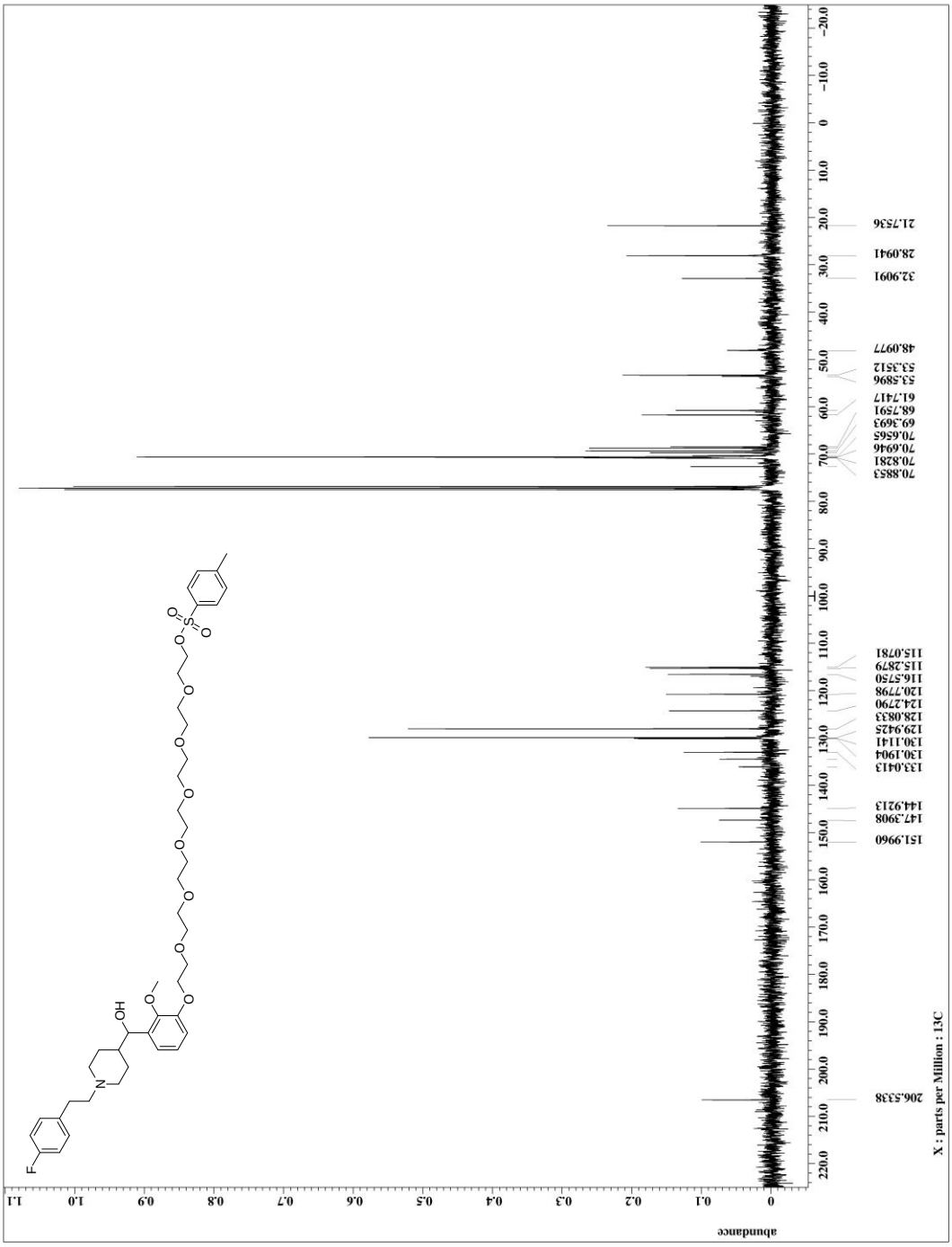


Figure B. 55: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
23-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15,18,21-heptaoxatricosyl 4-methylbenzenesulfonate (3.74)

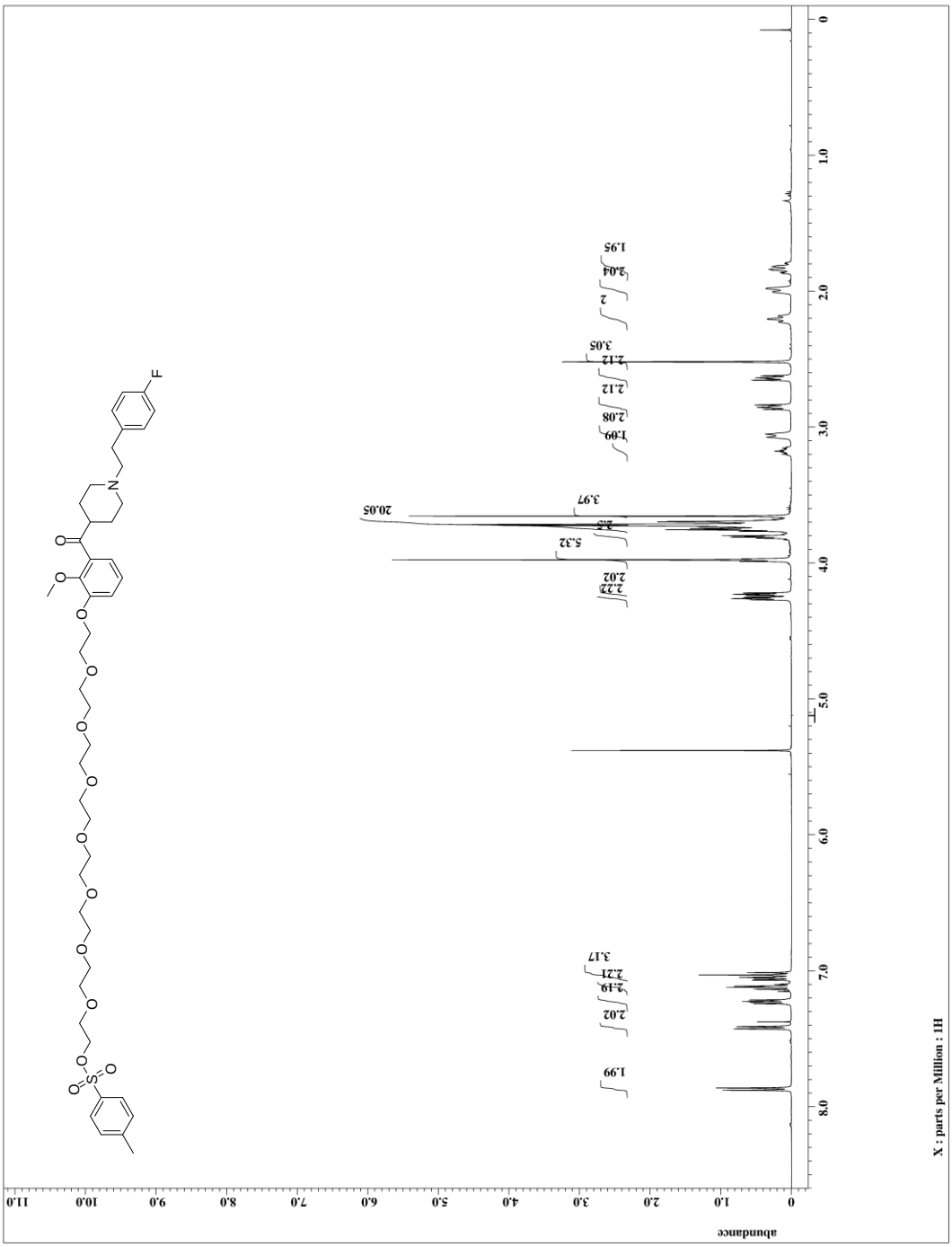


Figure B. 56: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
23-(3-(1-(4-fluorophenethyl)piperidine-4-carbonyl)-2-methoxyphenoxy)-3,6,9,12,15,18,21-heptaoxatricosyl 4-methylbenzenesulfonate (3.74)

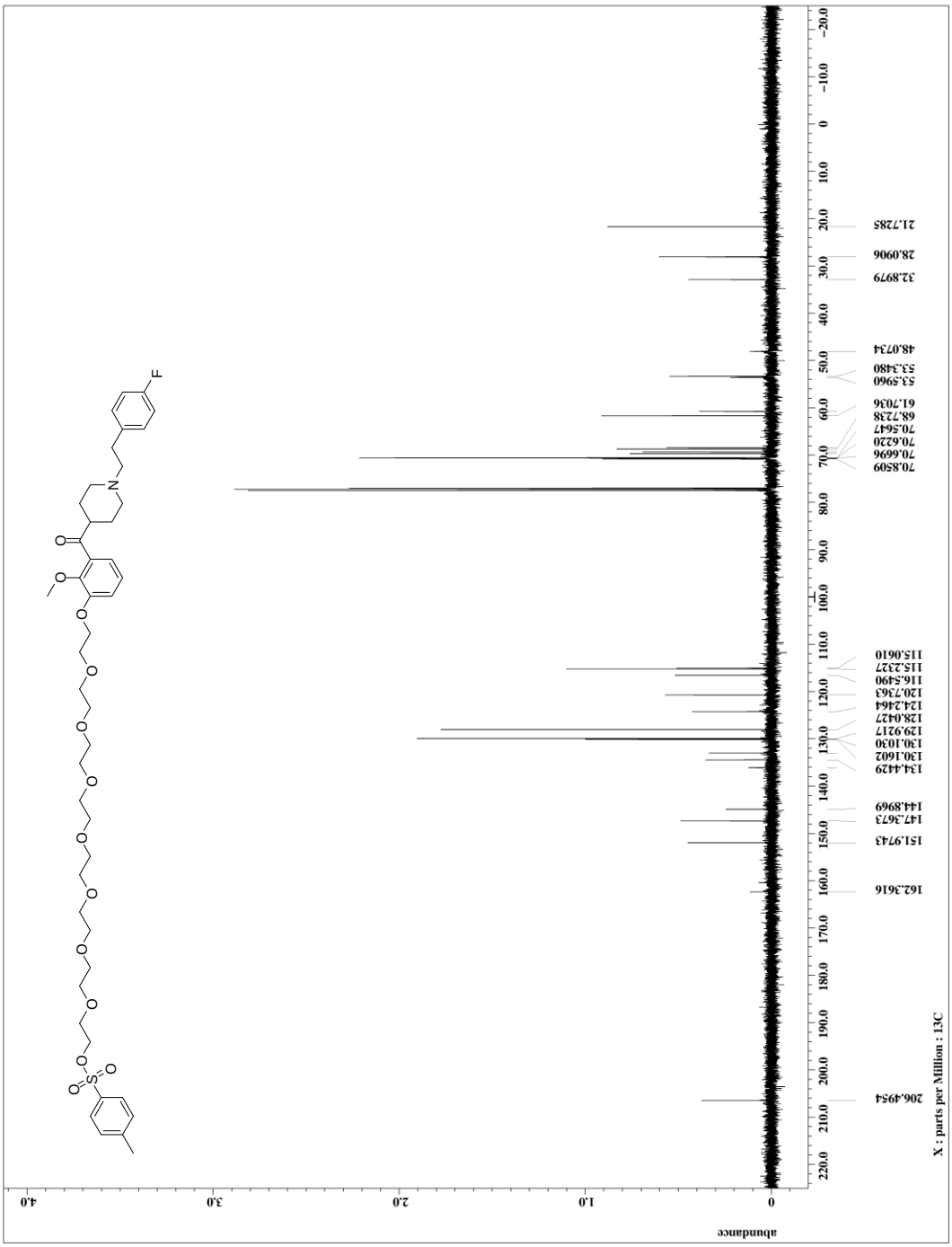


Figure B. 57: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
(3,3'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.75)

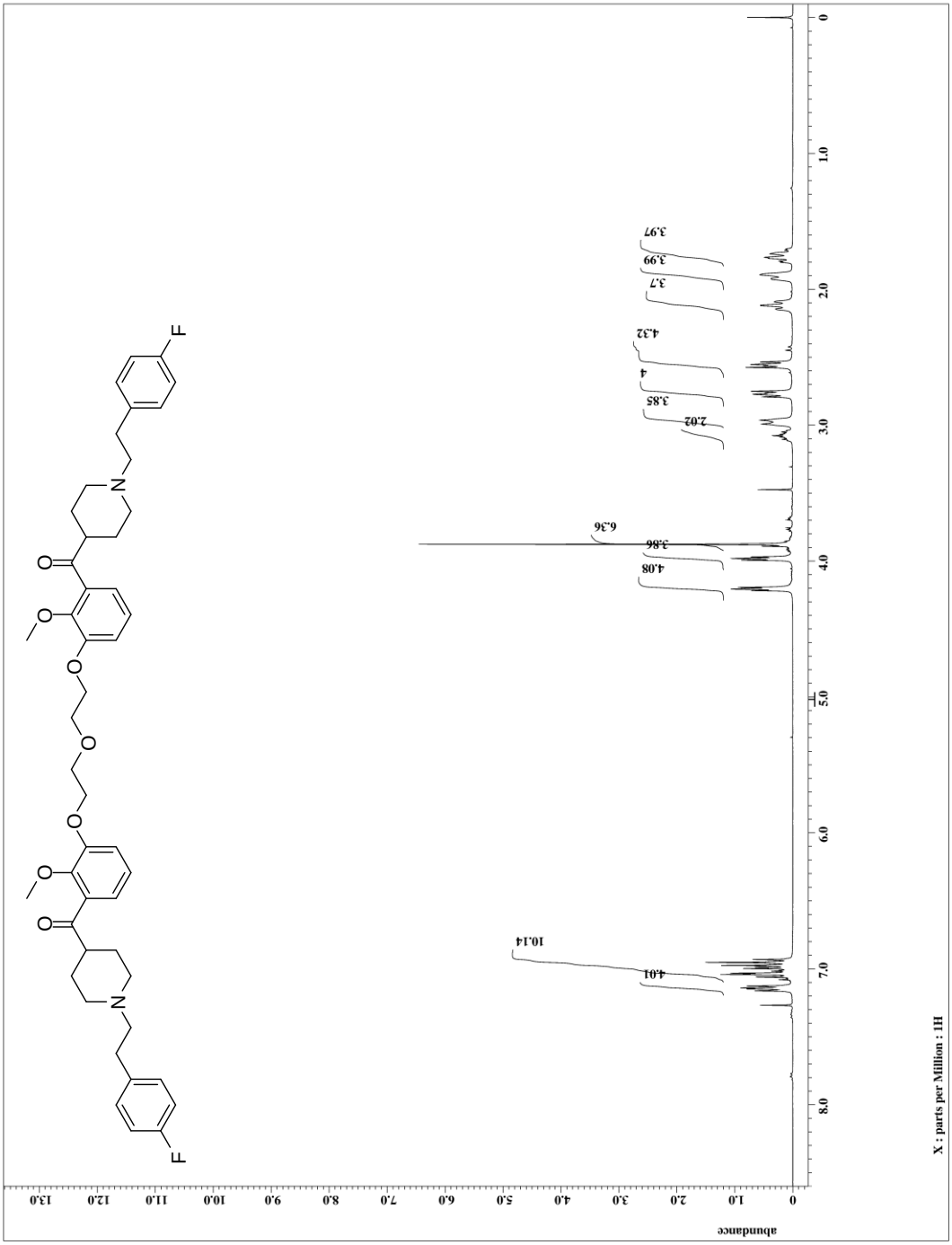


Figure B. 58: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
(3,3'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.75)

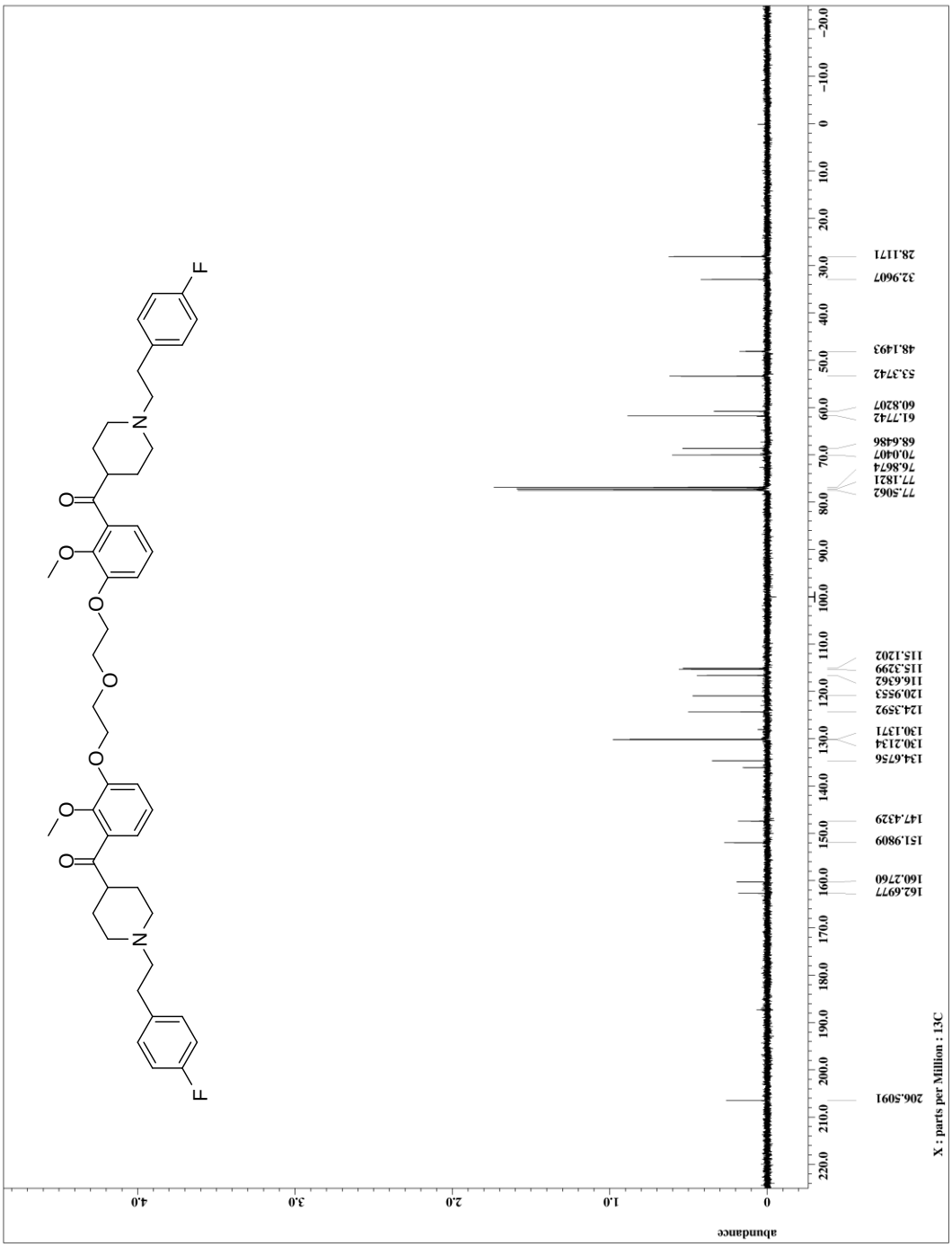


Figure B. 59: ^{1}H-NMR Spectra (400 MHz, $CDCl_3$)
(3,3'-(2,2'-(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.76)

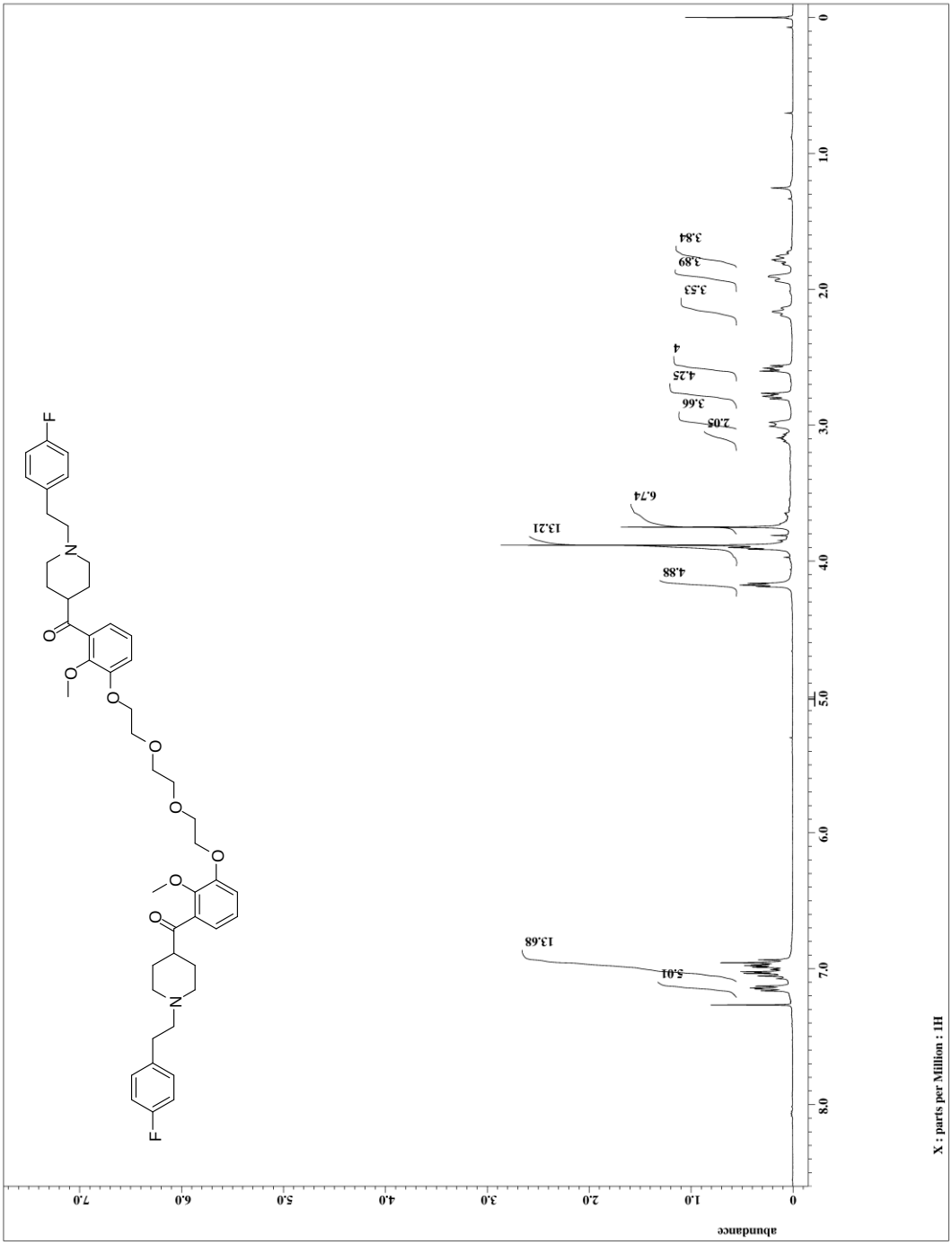


Figure B. 60: ^{13}C-NMR Spectra (100 MHz, $CDCl_3$)
(3,3'-(2,2'-(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.76)

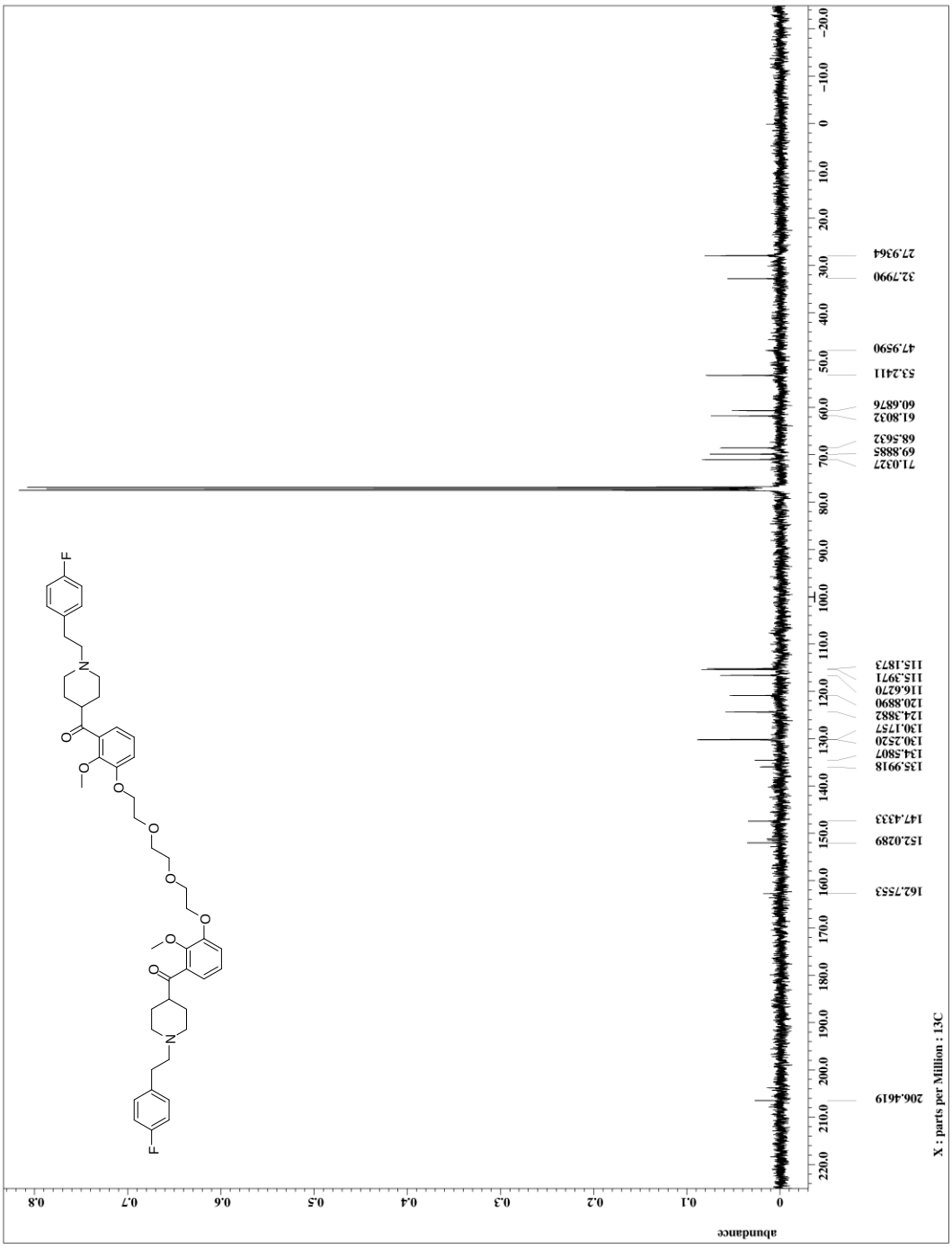


Figure B. 61: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(3,3'-(2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))bis(ethane-2,1-diyl))bis(oxy)bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.77)

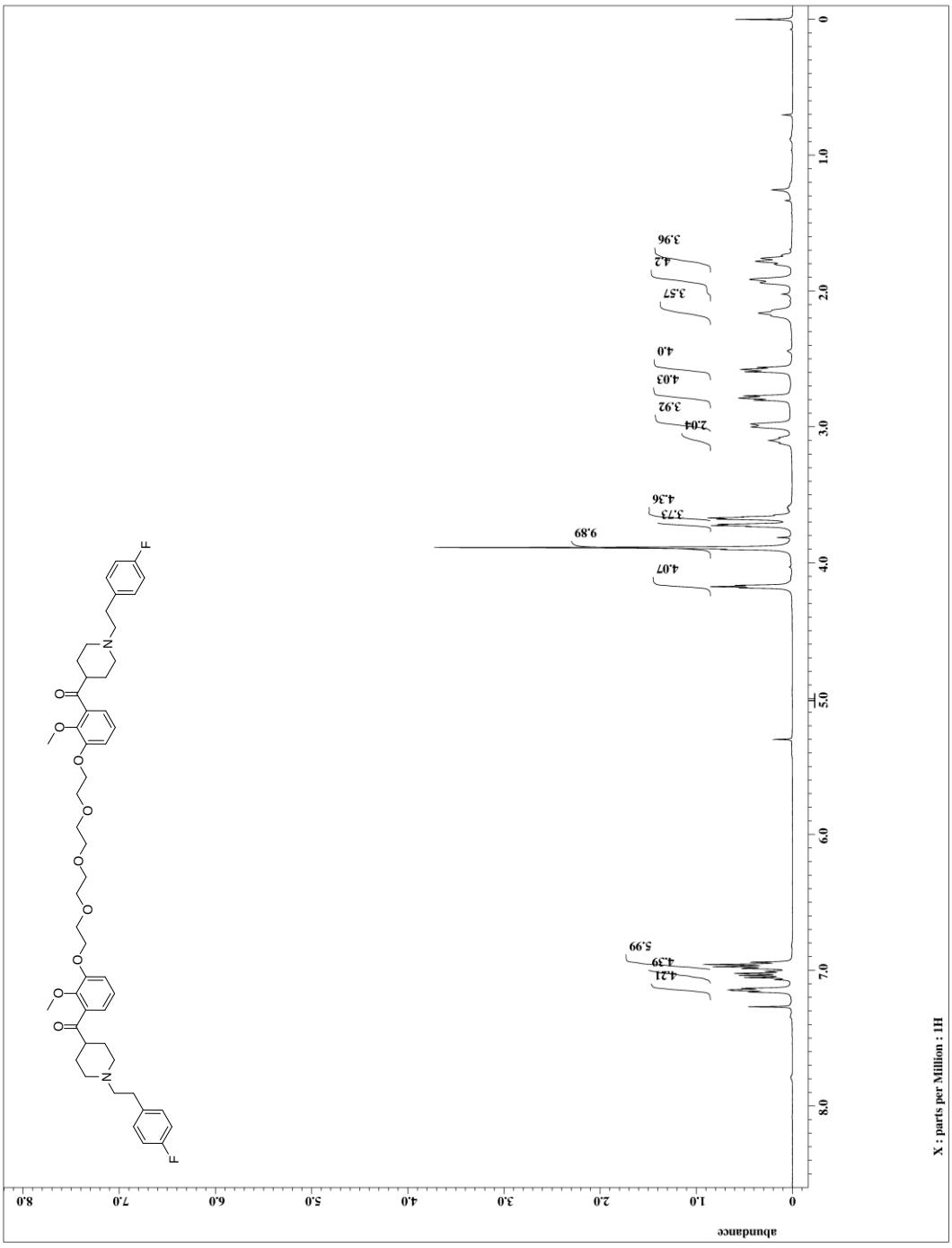


Figure B. 62: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3,3'-(3,6,9,12-tetraoxatetradecane-1,14-diylbis(oxy))bis(2-methoxy-3,1-phenylene)) bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.77)

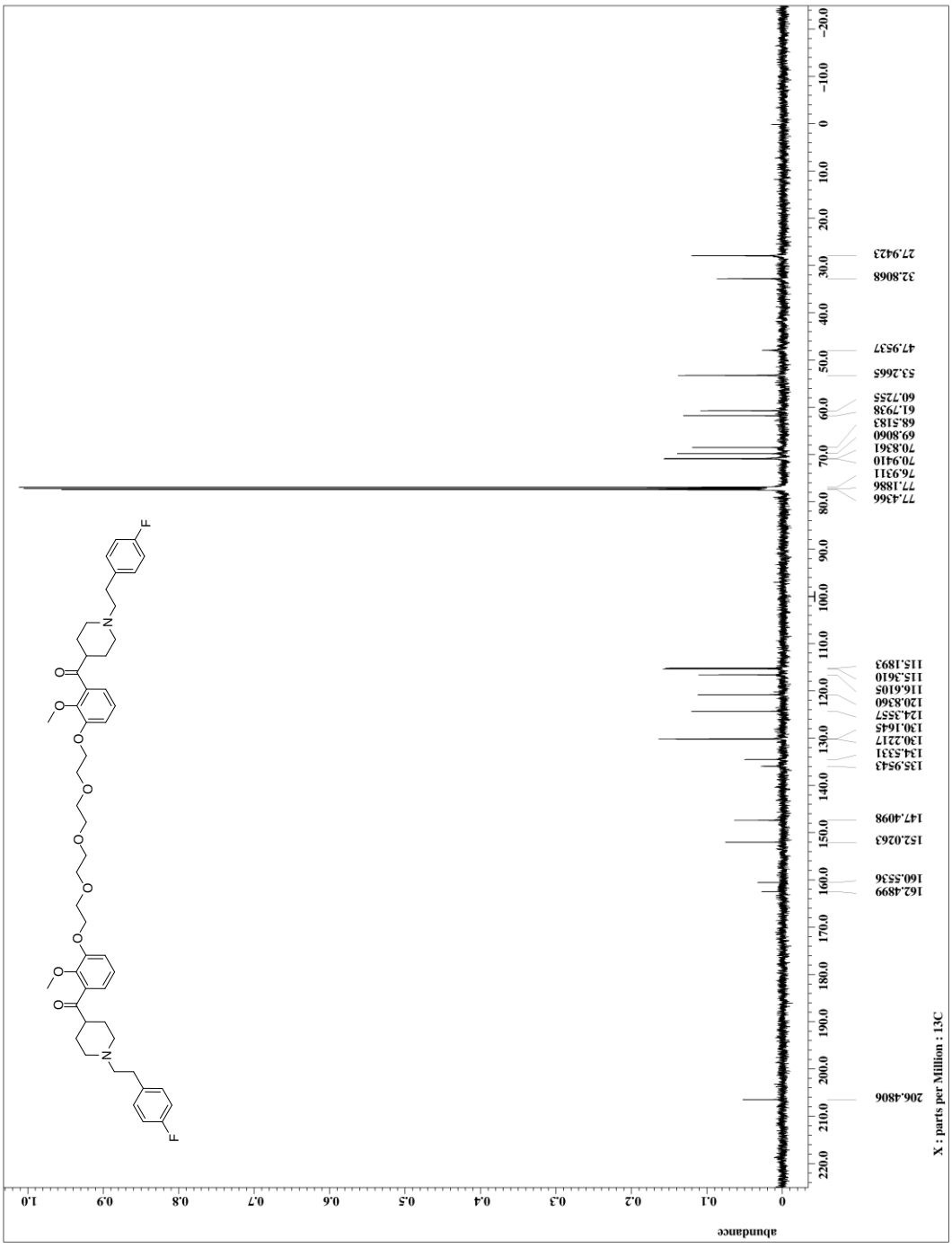


Figure B. 63: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(3,3'-(3,6,9,12-tetraoxatetradecane-1,14-diylbis(oxy))bis(2-methoxy-3,1-phenylene)) bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.78)

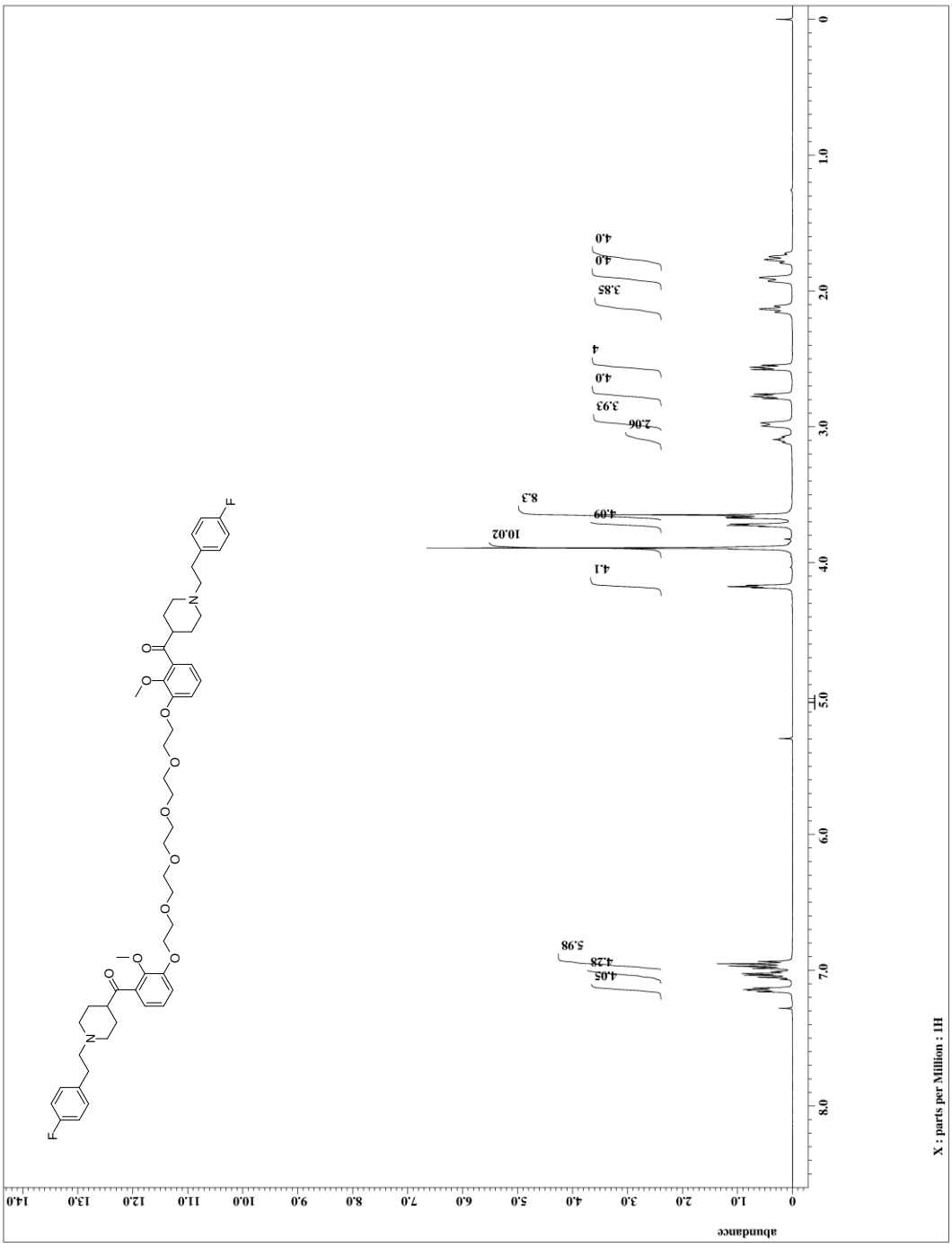


Figure B. 64: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3,3'-(3,6,9,12-tetraoxatetradecane-1,14-diylbis(oxy))bis(2-methoxy-3,1-phenylene)) bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.78)

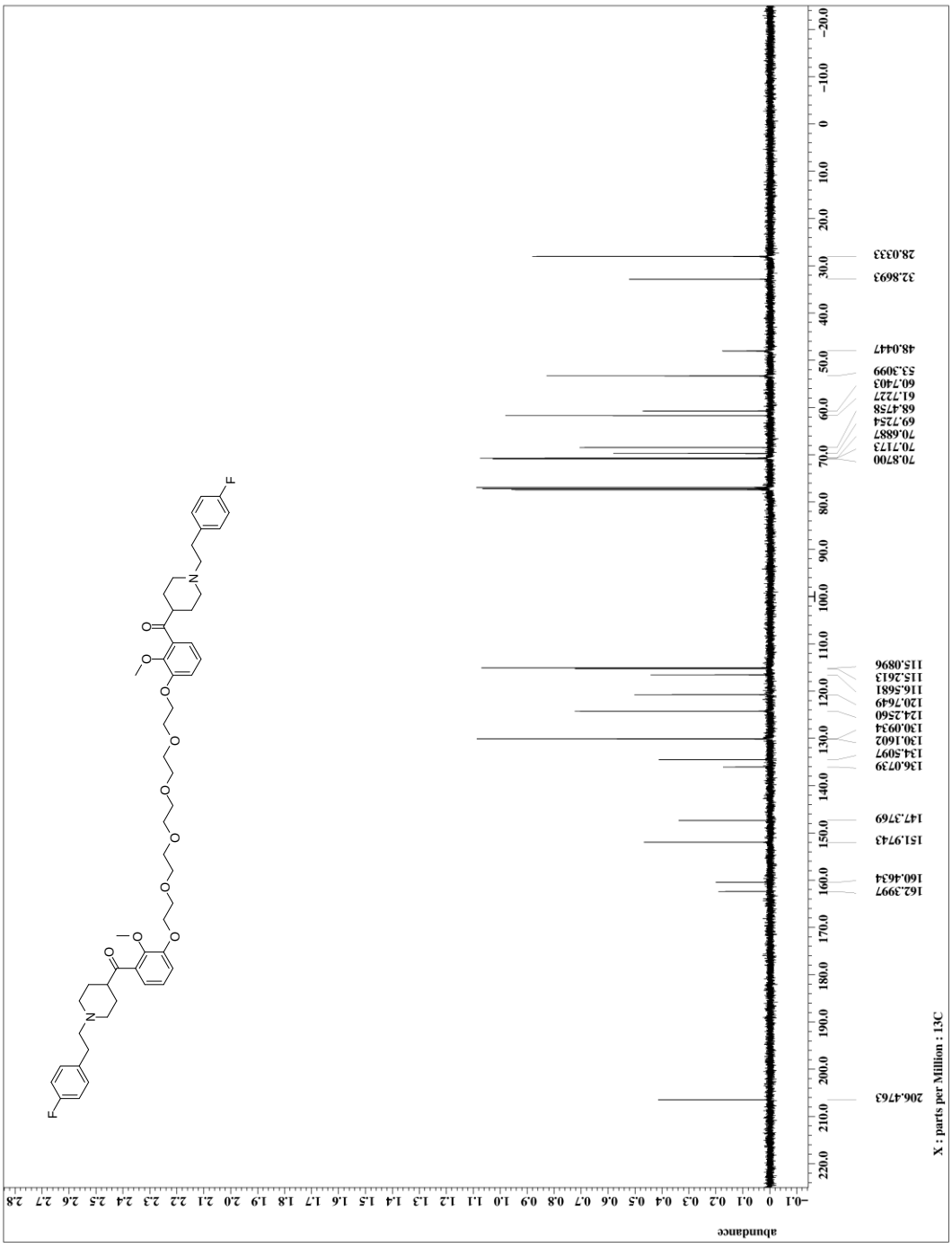


Figure B. 65: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(3,3'-(3,6,9,12,15-pentaoxaheptadecane-1,17-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.79)

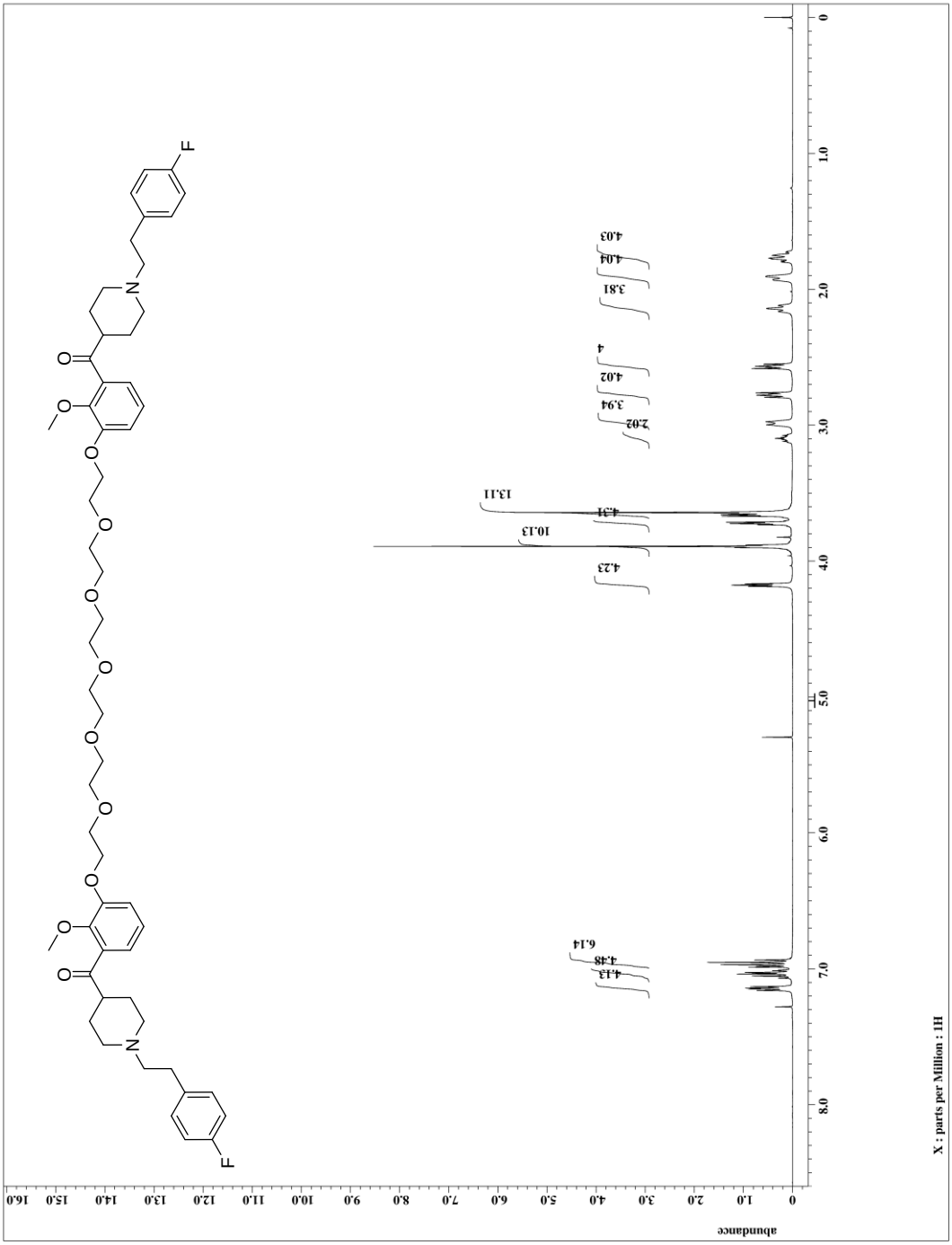


Figure B. 66: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3,3'-(3,6,9,12,15-pentaoxaheptadecane-1,17-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.79)

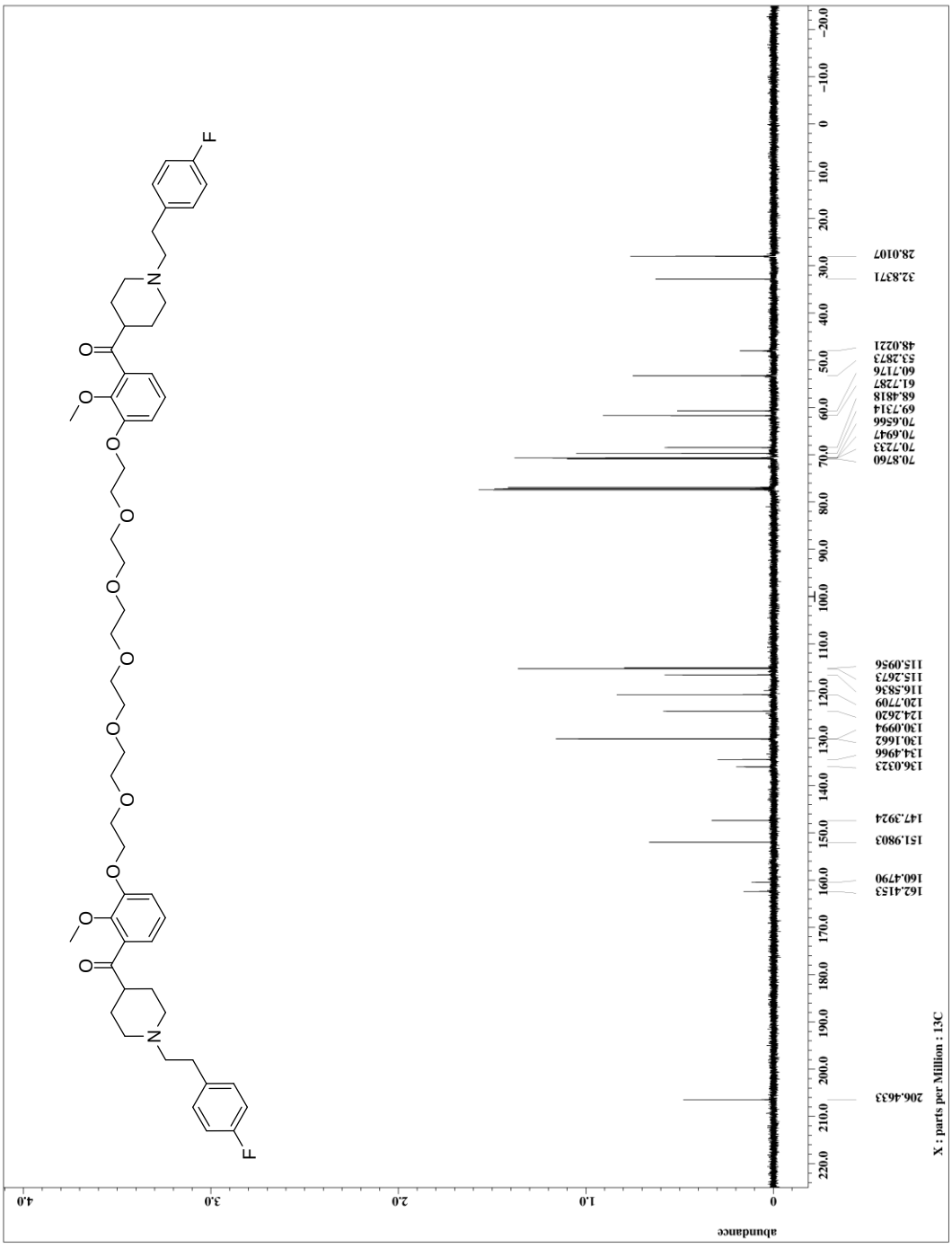


Figure B. 67: [1]H-NMR Spectra (500 MHz, $CDCl_3$)
(3,3'-(3,6,9,12,15,18-hexaoxaicosane-1,20-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.80)

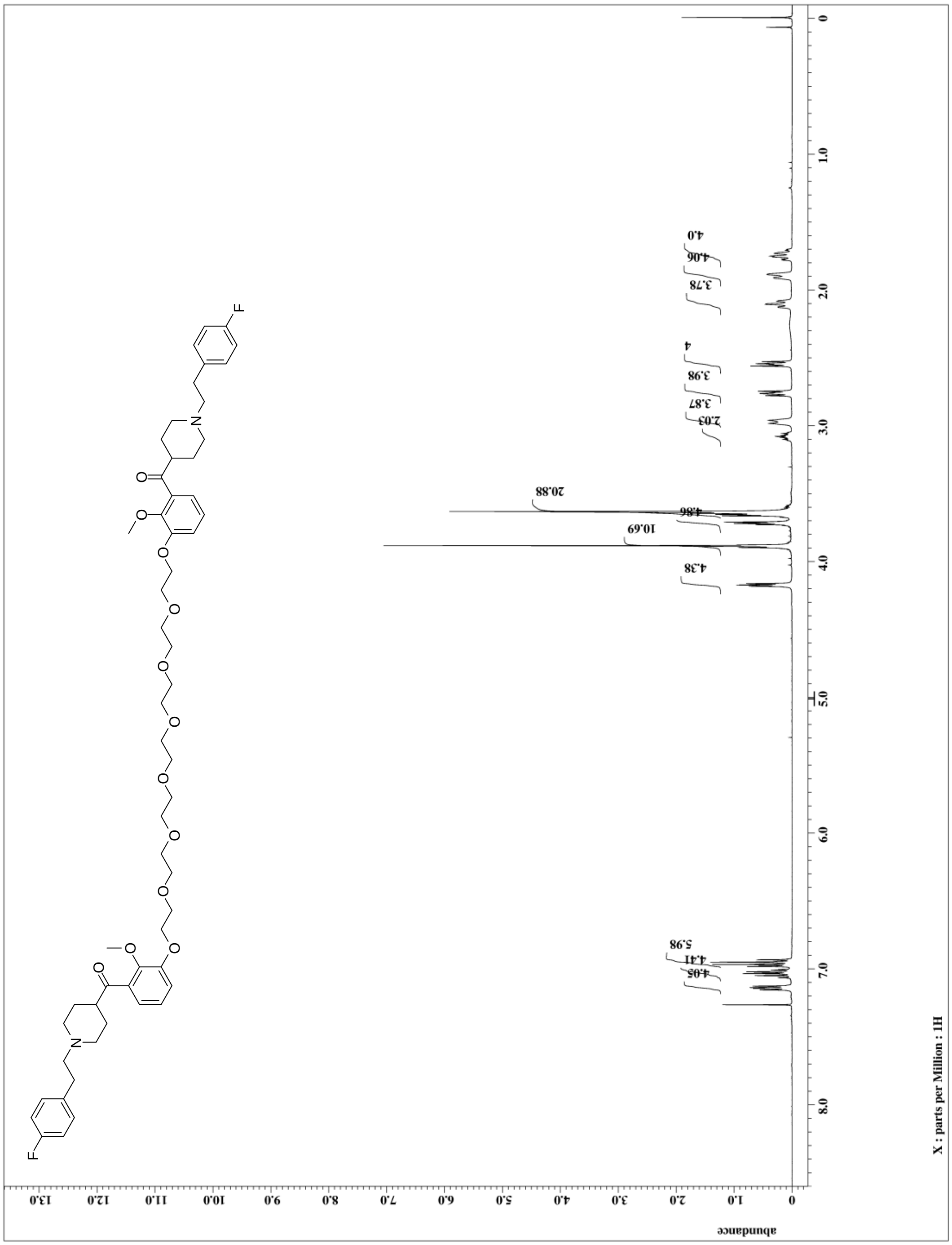


Figure B. 68: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3,3'-(3,6,9,12,15,18-hexaoxaicosane-1,20-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.80)

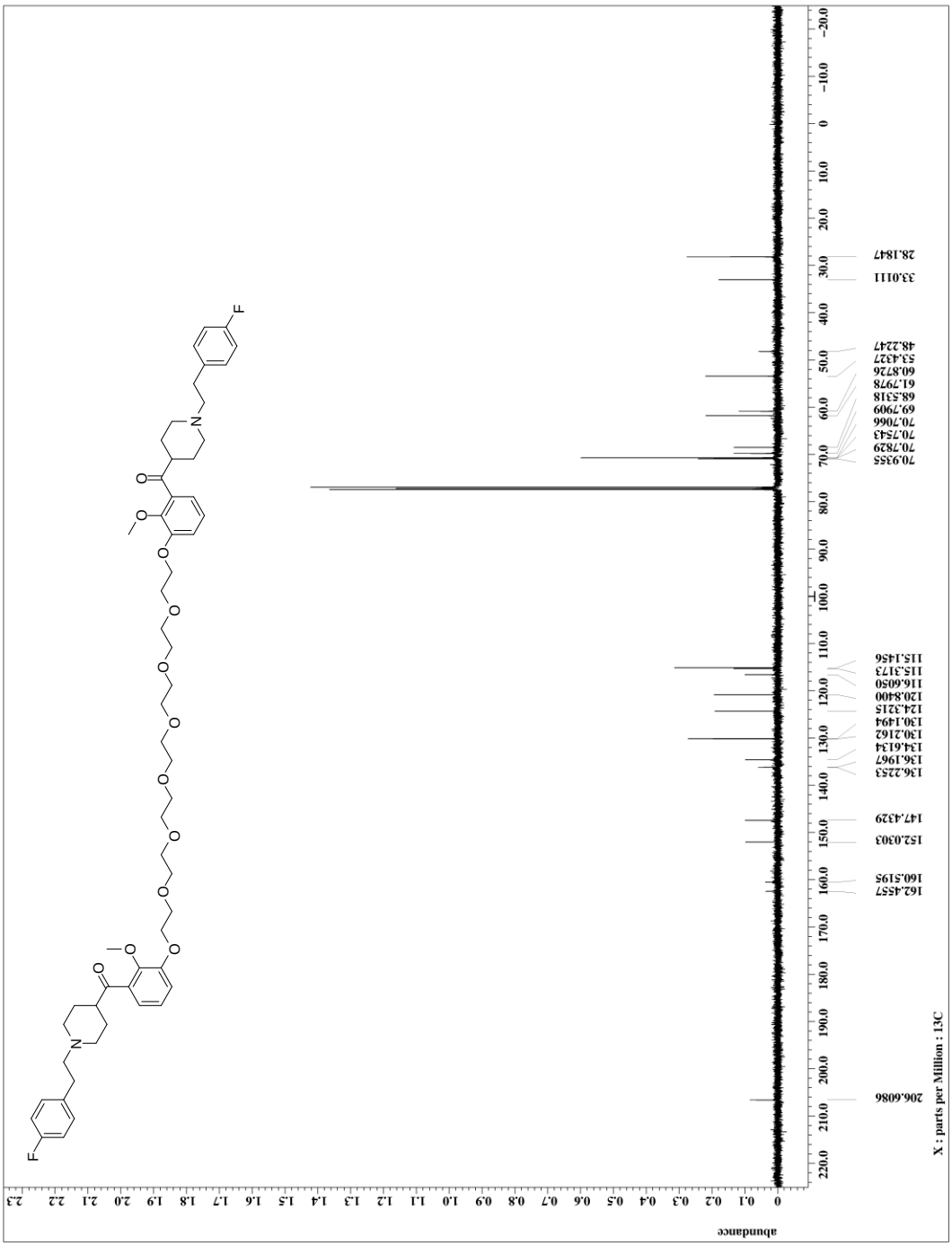


Figure B. 69: ^{1}H-NMR Spectra (500 MHz, $CDCl_3$)
(3,3'-(3,6,9,12,15,18,21-heptaoxatricosane-1,23-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.81)

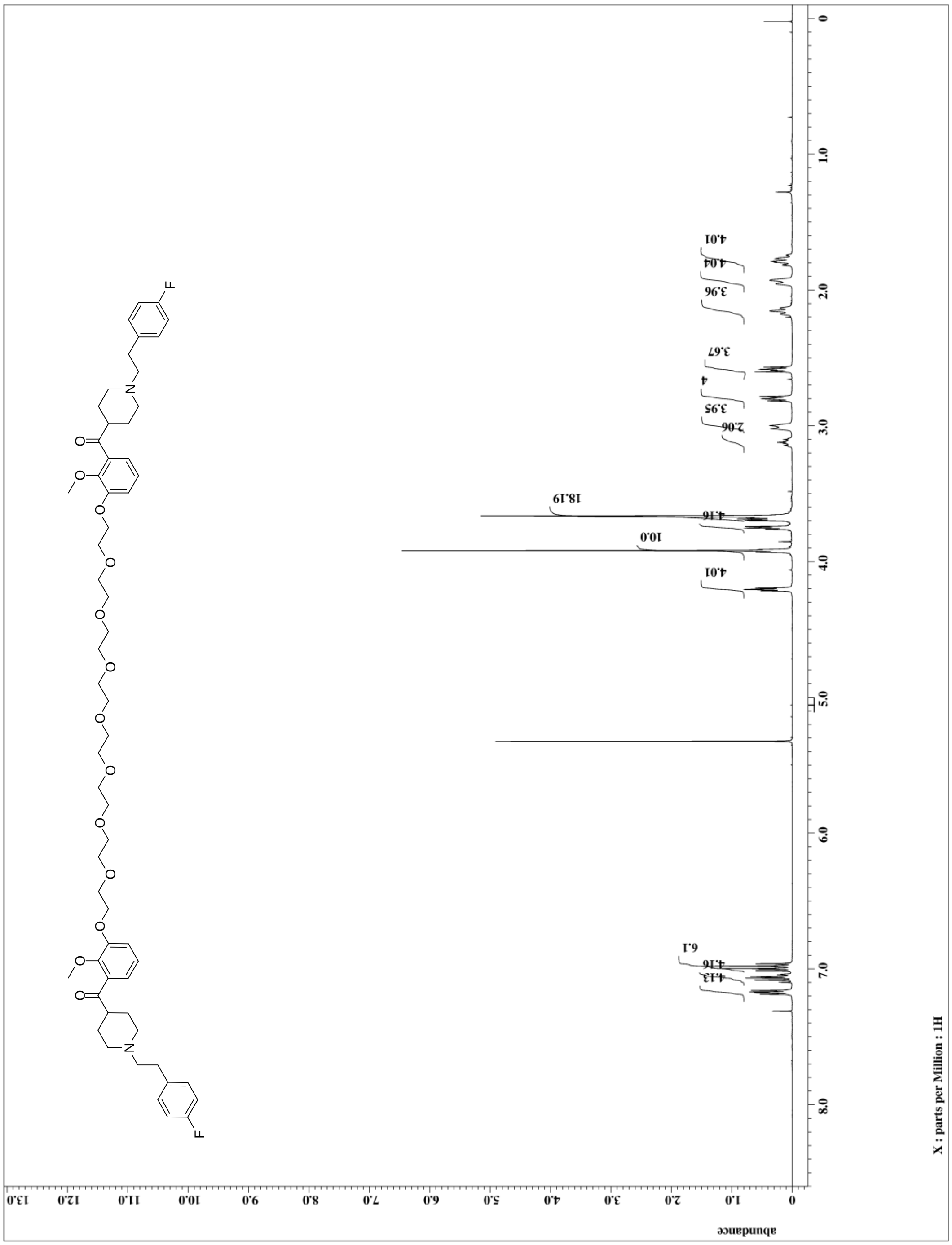
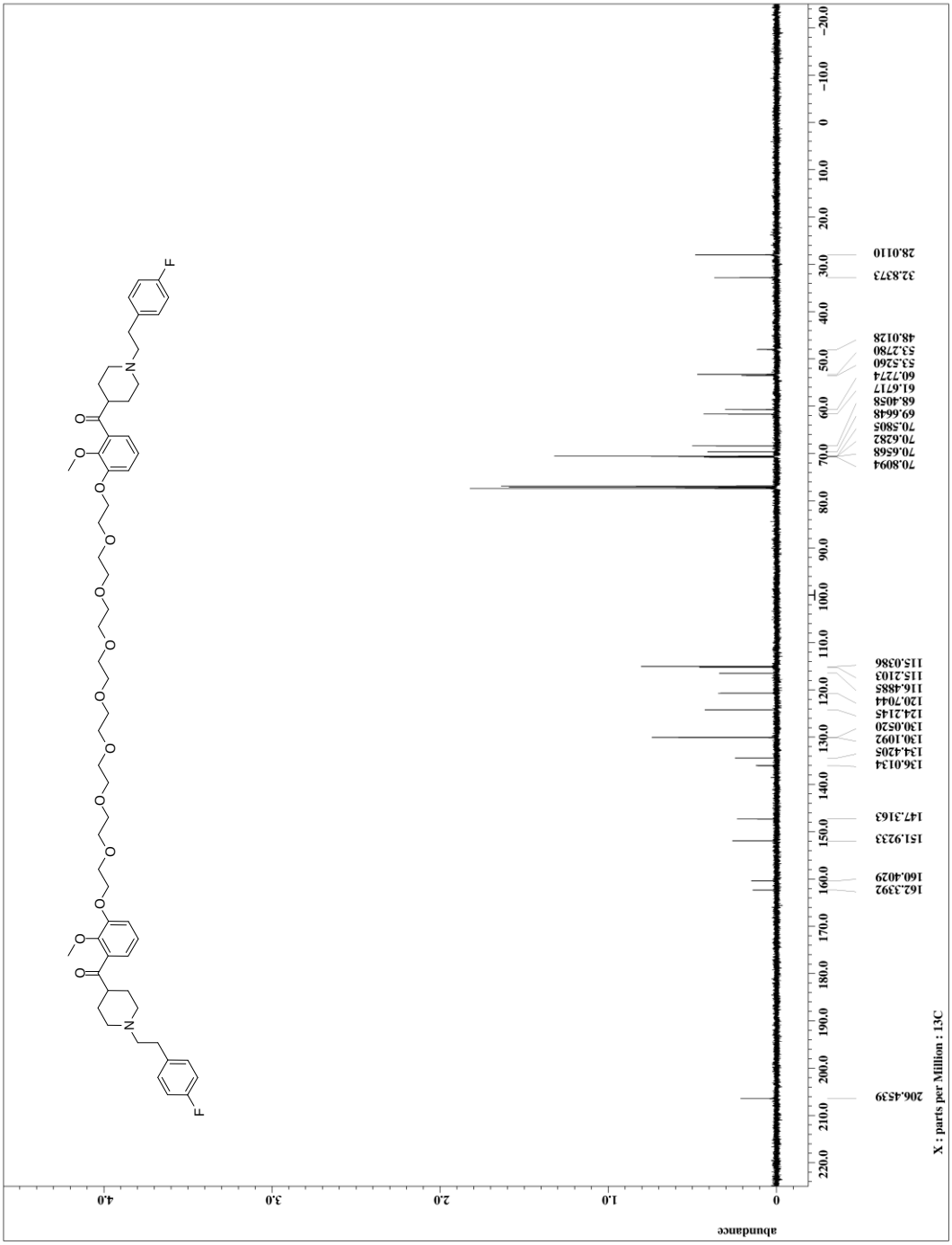


Figure B. 70: ^{13}C-NMR Spectra (125 MHz, $CDCl_3$)
(3,3'-(3,6,9,12,15,18,21-heptaoxatricosane-1,23-diylbis(oxy))bis(2-methoxy-3,1-phenylene))bis((1-(4-fluorophenethyl)piperidin-4-yl)methanone) (3.81)



References

1. Morphy, R.; Kay, C.; Rankovic, Z. *Drug Discovery Today*. **2004**, 9, 641-651.
2. Substance Abuse and Mental Health Services Administration. *Results from the 2009 National Survey on Drug Use and Health: Volume I. Summary of National Findings* (Office of Applied Studies, NSDUH Series H-38A, HHS Publication No. SMA 10-4586 Findings). Rockville, MD.
3. Baler, R.; Volkow, N. **2006**, 12¸ 559-566. Koob, G.; Sanna, P.; Bloom, F. *Neuron*. **1998**, 21, 467-476.
4. Goldstein, R.; DesLauriers, C.; Burda, A. *Disease a Month*. **2009**, 55, 6-38.
5. Das, G. *Journal of Clinical Pharmacology*. **1993**, 33, 296-310.
6. Afonso, L.; Mohammad, T.; Thatai, D. *The American Journal of Cardiology*. **2007**, 100, 1040-1043.
7. Warner, E. *Annals of Internal Medicine*. **1993**, 119, 226-235.
8. Shanti, C.; Lucas, C. *Critical Care Medicine*. **2003**, 31, 1851-1859.
9. Ritz, M.; Cone, E.; Kuhar, M. *Life Sciences*. **1990**, 46, 635-645.
10. Filip, M.; Bader, M. *Pharmacological Reports*. **2009**, 61, 761-777.
11. Halliday, G.; Tork, I.; *Brain Res. Bull*. **1989**, 22, 725-735.
12. Hoyer, D.; Hannon, J.; Martin, G. *Pharmacology Biochemistry Behavior.* **2002**, 7, 533-554.
13. Martin, G.; Humphrey, P. *Neuropharmacology*. **1994**, 33, 261-273.
14. Raymond, J.; Mukhin, Y.; Gelasco, A.; Turner, J.; Collinsworth, G.; Gettys, T.; Grewal, J.; Garnovskaya, M. *Pharmacology and Therapeutics.* **2001**, 92, 179-212.

15. Katsuki, H.; Okuda, S. *Prog. Neurobiology*. **1995**, 47, 607-636.

16. Fletcher, P.; Grottick, A. J.; Higgins, G. A. *Neuropsychopharmacology.* **2002**, 27, 576-586.

17. Felder, C.; Kanterman, R.; Ma, A.; Axelrod, J. *Proceedings of the National Academy of Science*. **1990**, 87, 2187-2191.

18. Bubar, M.; Cunningham, K. *Progress in Brain Research*. **2008**, 172, 319-345.

19. Bunney, E.; Appel, S.; Brodie, M. *The Journal of Pharmacology and Experimental Therapeutics*. **2001**, 297, 696-702.

20. Hyman, S.; Malenka, R.; Nestler, E. *Annual Reviewof Neuroscience*. **2006**, 29, 568-598.

21. Donna, M.; James, K.; Rogers, D. *Psychopharmacology*. **2002**, 163, 265-282.

22. Alex, K. ; Roth, B. *Pharmacology and Therapeutics*. **2007**, 113, 296-320.

23. Wise, R.; Rompre, P. *Annual Review of Psychology*. **1989**, 40, 191-225.

24. Nocjar, C.; Roth, B; Pehek, E. *Neuroscience*. **2002**, 111, 163-176.

25. Parsons, L.; Justice, J. *Brain Research*. **1993**, 606, 195-199.

26. Pehek, E.; Nocjar, C.; Roth, B.; Byrd, T.; Mabrouk, O. *Neuropsychopharmacology*. **2006**, 21, 265-277.

27. Berg, K.; Harvey, J.; Spampinato, U.; Clarke, W. *Trends in Pharmacological Sciences*. **2005**, 26, 625-630/

28. Wright, D.; Seroogy, K.; Lundgren, K.; Davis, B.; Jennes, L. *The Journal of Comparative Neurology*. **1995**, 35, 357-373.

29. DiMatteo, V.; Di Giovanni, G.; Di Mascio, M.; Esposito, E. *Neuropharmacology*. **1999**, 38, 1195-1205.

30. Gobert, A. ; Rivet, J. ; Lejeune, F. ; Newman-Tancredi, A. *Synapse*. **2000**, 36, 205-221.

31. Filip, M. ; Bubar, M. ; Cunningham, K. *Psychopharmacology*. **2006**, 183, 482-489.

32. Walsh, S.; Cunningham, K. *Psychopharmacology*. **1997**, 130, 41-58.

33. Filip, M.; Bubar, M.; Cunningham, K. *Pharmacology and Experimental Therapeutics*. **2004**, 310, 1246-1254.

34. Baumann, M.; Rothman, R. *Biological Psychiatry*. **1998,** 44, 578-591.

35. Liu, S.; Cunningham, K.; *Drug and Alcohol Dependence*. **2006**, 81, 275-282.

36. Gray, J.; Roth, B. *Brain Research Bulletin.* **2001**, 56, 441-451.

37. McCreary, A.; Cunningham, K. *Neuropsychopharmacology*. **1999**, 20, 556-564.

38. Nic Dhonnchadha, B.; Fox, B.; Stutz, S.; Rice, K.; Cunningham, K. *Behavioral Neuroscience*. **2009**, 123, 382-396.

39. Fletcher, P.; Chintoh, A.; Sinyard, J.; Higgins, G. *Neuropsychopharmacology*. **2004**, 29, 308-318.

40. Fletcher, P.; Rizos, Z.; Sinyard, J.; Tampakeras, M.; Higgins, G. *Neuropsychopharmacology*. **2008**, 33, 1402-1412.

41. Howard, A.; McAllister, G.; Feighner, S.; Liu, Q.; Nargund, R.; Van der Ploeg, L.; Patchett, A. *Trends in Pharmaccological Sciences*. **2001**, 22, 132-140.

42. Lagerstrom, M.; Schioth, H. *Nature Reiews Drug Discovery*. **2008**, 7, 339-357.

43. George, S.; O'Dowd, B.; Lee, S. *Nature Reviews Drug Discovery*. **2002**, 1, 808-820.

44. Tuteja, N. *Plant Signaling an Behavior*. **2009**, 4, 942-947.

45. Rana, B.; Shiina, T.; Insel, P. *Annual Review of Pharmacology and Toxicology*. **2001**, 41, 593-624.

46. Zhang, A.; Liu, Z.; Kan, Y. *Current Topics in Medicinal Chemistry*. **2007**, 7, 343-345.

47. Herrick-Davis, K.; Grinde, E.; Harrigan, T.; Mazurkiewicz, J. *The Journal of Biological Chemistry*. **2005**, 280, 40144-40151.

48. Bulenger, S.; Marullo, S.; Bouvier, M. *Trends in Pharmacological Sciences*. **2005**, 26, 131-137.

49. Hague, C. ; Uberti, M. ; Chen, Z. ; Hall, R. ; Minneman, K. *The Journal of Biological Chemistry*. **2004**, 279, 15541-15549.

50. AbdAlla, S.; Zaki, E.; Lother, H.; Quitterer, U. *The Journal of Biological Chemistry*. **1999**, 274, 26079-26084.

51. Rodriguez-Frade, J.; Vila-Coro, A.; Martin de Ana, A.; Albar, J.; Martinez-A., C.; Mellado, M. *Proceedings of the National Academy of Science*. **1999**, 96, 3628-3633.

52. Rocheville, M.; Lange, D.; Kumar, U.; Sasi, R.; Patel, R.; Patel, Y. *The Journal of Biological Chemistry*. **2000**, 275, 7862-7869.

53. Hovart, R.; Roess, D.; Nelson, S.; Barisas, G.; Clay, C. *Molecular Endocrinology*. **2001**, 15, 695-703.

54. Maggio, R.; Innamorati, G.; Parenti, M. *Journal of Neurochemistry*. **2007**, 103, 1741-1752.

55. Chinault, S. ; Overton, M. ; Blumer, K. *Journal of Biological Chemistry*. **2004**, 279, 16091-16100

56. Kniazeff, J.; Bessis, A.; Maurel, D.; Ansanay, H.; Prezeau, L.; Pin, J. *Nature Structural and Molecular Biology*. **2004**, 11, 706-713.

57. Nimchinsky, E.; Hof, P.; Janssen, W.; Morrison, J.; Schmauss, C. *The Journal of Biological Chemistry*. **1997**, 272, 29229-29237,

58. George, S.; Lee, S.; Varghese, G.; Zeman, P.; Seeman, P.; Ng, G.; O'Dowd, B. *The Journal of Biological Chemistry*. **1998**, 273, 30244-30248.

59. Jordan, B.; Devi, L. *Nature*. **1999**, 399, 697-700.

60. Mellado, M. ; Rodriguez-Frade, J. ; Vila-Coro, A. ; Fernadez, S. ; de Ana, A. ; Jones, D. ; Toran, J. ; Martinez, C. *The EMBO Journal*. **2001**, 20, 2497-2507.

61. Kunishia, N. ; Shimada, Y. ; Tsuji, Y. ; Sato, T.; Yamamoto, M.; Kumasaka, T.; Nakanishi, S.; Jingami, H.; Morikawa, K. *Nature*. **2000**, 407, 971-977.

62. Bennett, M.; Schlunegger, M.; Eisenberg, D. *Protein Science*. **1995**, 4, 2455-2468.

63. Salim, K.; Fenton, T.; Bacha, J.; Urien-Rodriguez, H. ; Bonnet, T. ; Skynner, H. ; Watts, E. ; Kerby, J. ; Heald, A. ; Beer, M.; McAllister, G.; Guest, P. *The Journal of Biological Chemistry*. **2002**, 277, 15482-15485.

64. Lee, S.; Xie, Z.; Varghese, G.; Nguyen, T.; O'Dowd, B.; George, S. *Neuropsychopharmacology*. **2000**, 23, S32-S40.

65. Mancia, F.; Assur, Z.; Herman, A.; Siegel, R.; Hendrickson, W. *EMBO Reports*. **2008**, 9, 363-369.

66. Morphy, R.; Rankovic, Z. *Journal of Medicinal Chemistry*. **2005**, 48, 6523-6543.

67. Edwards, I.; Aronson, J. *Lancet*. **2000**. 356, 1255-1259.

68. Portoghese, P. *Trends in Pharmacological Sciences*. **1989**. 10, 230-235.

69. Perez, M.; Pauwels, P.; Pallard-Sigogneaua, I.; Fourrier, C.; Chopin, P.; Palmier, C.; Colovray, V.; Halazy, S. *Bioorganic and Medicinal Chemistry Letters.* **1998.** 8, 3423-3428.

70. Heinrich, T.; Bottcher, H.; Schiemann, K.; Hoelzemann, G.; Schwarz, M.; Bartoszyk, G. D.; van Amsterdam, C.; Greiner, H. E.; Seyfried, C. A.; *Bioorganic and Medicinal Chemistry*. **2004**. 12, 4843-4852.

71. Choi, S.; Green, D.; Ho, A.; Klein, U.; Marquess, D.; Taylor, R.; Turner, D. *Journal of Medicinal Chemistry*. **2008**. 51, 3609-3616.

72. Tamiz, A.; Conti, P.; Zhang, M.; Johnson, K. M.; Kozikowski, A. P. *Bioorganic and Medicinal Chemistry Letters*. 2000, 10, 2741-2743.

73. Daniels, D.; Kulkarni, A.; Xie, Z.; Bhushan, R. G.; Portoghese, P. S. *Journal of Medicinal Chemistry.* **2005**. 48, 1713-1716.

74. Portoghese, P. *Journal of Medicinal Chemistry.* **2001**. 44, 2259-2269.

75. Perez, M.; Jorand-Lebrun, C.; Pauwels, P.; Pallard, I.; Halazy, S. *Bioorganic and Medicinal Chemistry Letters.***1998**. 8, 1407-1412.

76. Meltzer, P.; Kryatova, O.; Pham-Huu, D.; Donovan, P.; Janowsky, A. *Bioorganic and Medicinal Chemistry*. **2008**. 16, 1832-1841.

77. Halazy, S.; Perez, M.; Fourrier, C.; Pallard, I. ; Pauwels, P. ; Palmier, C. ; John, G. ; Valentin, J. ; Bonnafous, R. ; Martinez, J. *Journal of Medicinal Chemistry.*. **1996**. 39, 4920-4927.

78. Russo, O.; Bethouse, M.; Giner, M.; Soulier, J.; Rivail, L.; Sicsic, S.; Lezoualc'h, F.; Jockers, R.; Berque-Bestel, I. *Journal of Medicinal Chemistry*. **2007**. 50, 4482-4492.

79. Jacobson, K.; Xie, R.; Young, L.; Chang, L.; Liang, B. *The Journal of Biological Chemistry*. **2000**. 275, 30272-30279.

80. Soriano, A. ; Ventura, R. ; Molero, A. ; Hoen, R. ; Casado, V. ; Cortes, A. ; Fanelli, F. ; Albericio, F. ; Lluis, C. ; Franco, R. ; Royo, M. *Journal of Medicinal Chemistry*. **2009**. 52, 5590-5602.

81. Buijsman, R.; Basten, J.; van Dinther, T.; van der Marel, G.; van Boeckel, C.; van Boom, J. *Bioorganic and Medicinal Chemistry Letters*. **1999**. 9, 2013-2018.

82. Decker, M.; Lehmann, J.; *Current Topics in Medicinal Chemistry*.**2007**, 7, 347-353.

83. Messer, W. *Current Pharmaceutical Design.* **2004**, 10, 2015-2020.

84. Waldhoer, M.; Fong, J.; Jones, R.; Lunzer, M.; Sharma, S.; Kostenis, E.; Portoghese, P.; Whistler, J. *Proceedings of the National Academy of Science*. **2005**. 102, 9050-9055.

85. Zhang, S.; Yekkirala, A.; Tang, Y.; Portoghese, P. *Bioorganic and Medicinal Chemistry Letters*. **2009**. 19, 6978-6980.

86. Sabb, A.; Vogel, R. L.; Welmaker, G. S.; Sabalski, J. E.; Coupet, J.; Dunlop, J.; Rosenzweig-Lipson, S.; Harrison, B. *Bioorganic and Medicinal Chemistry Letters.* **2004**, 14, 2603-2607.

87. Dunlop, J.; Karen, M.L.; Lim, H.; Leung, L.; Kao, J.; Cheesman, C.; Rosenzweig-Lipson, S. *CNS Drug Review*. **2006**, 12, 167-177.

88. Sabb, A. L.; Vogel, R. L. ; Nelson, J. A. ; Rosenzweig-Lipson, S. J.; Welmaker, G.S.; Sabalski, J.E.; Preparation of cyclopenta[b][1,4] diazepino[6,7,1-hi] indoles as selective 5-HT_{2C} receptor agonists. *U.S. Patent 7,271,163 B2*, 2007.

89. Sabb, A. L.; Vogel, R. L. ; Welmaker, G.S.; Sabalski, J.E.; Cycloalkyl[b][1,4]diazepino[6,7,1-hi] indoles and derivatives. *WO 02/36596 A2,* 2002.

90. Jadidi, K.; Aryan, R.; Mehrdad, M.; Morteza, L.; Lugger, T.; Hanh, E. F.; Ng, S.W. *Journal of Molecular Structure*. **2004**, 692, 37-42.

91. Maryanoff, B.; Matthew, J.; Preaparation of benzodiazepines as vasopressin V2 receptor antagonists. *WO 2006/049984 A2*, 2006.

92. Haynes, J.; Doubleday, W.; Dyckman, A.; Godfrey, J.; Grosso, J.; Kiau, S.; Leftheris, K. *Journal of Organic Chemistry*. **2004**, 69, 1368-1371.

93. Entwistle, I.; Johnstone, R.; Wilby, A. *Tetrahedron*. **1982**, 38, 419-423.

94. HYDRAZINE INSTABILITY REFERENCE

95. Wessig, P.; Moellnitz, K.; Eiserbeck, C. *Chemistry A European Journal*. **2007**, 13, 4859-4872.

96. Chang, M.; Pai, C.; Kung, Y. *Tetrahedron Letters*. **2005**, 46, 8463-8465.

97. Woerpel, K. *Journal of Organic Chemistry*. **2006**, 71, 6851-6858.

98. Kaiho, T.; Sannohe, K.; Kajayi, S.; Suzuki, T.; Otsuka, K.; Ito, K.; Kamiya, K.; Maruyama, M. *Journal of Medicinal Chemistry*. **1989**, 32, 351-357.

99. Freedman, H.; Dubois, R. *Tetrahedron Letters*. **1975**, 38, 3251-3254.

100. Bajwa,N.; Jennings, M. *Tetrahedron Letters*. **2008**, 49, 390-393.

101. Berliner, M.; Belecki, K. *Organic Syntheses*. **2007**, 84, 102-110.

102. Aikins, J.; Haurez, M.; Rizzo, J.; Van Hoeck, J. ; Brione, W. ; Kestemont, J.; Stevens, C.; Lemair, X.; Stephenson, G.; Marlot ,E.; Frost, M.; Houpis, I. *Journal of Organic Chemistry*. **2005**, 70, 4695-4705.

103. Huber, V.; Dietz, M. *Tetrehedron Letters*. **2001**, 42, 2945-2948

104. Chan, A.; *U.S. Patent 6,916,922, B2*, 2005.

105. de Paulis, T. M-100907 Aventis. *Current Opinion in Investigational Drugs* **2001**, 2, 123-132.

106. http://clinicaltrials.gov/ct2/show/NCT00464243?term=volinanserin&rank=2

107. Kehne, J.; Baron, B.; Carr, A.; Chaney, S.; Elands, J.; Feldman, D.; Frank, R.; Van Giersbergen, P.; McCloskey, T.; Johnson, M.; McCarty, D.; Poirot, M.; Senyah, Y.; Siegel, B.; Widmaier, C. *The Journal of Pharmacology and Experimental Therapeutics*. **1996**. 277, 968-981.

108. Scott, D.; Heath. T. *Journal of Pharmaceutical and Biomedical Analysis*. **1998**. 17, 17-25.

109. Heinrich, T.; Bottcher, H.; Prucher, H.; Gottschlich, R.; Ackerman, K.; van Amsterdam, C. *ChemMedChem*. **2006**. 1, 245-255.

110. Ullrich, T.; Rice, K. *Bioorganic and Medicinal Chemistry*. **2000**. 8, 2427-2432.

111. a. Aslanian, R.; Lachowicz, J.; Berlin, M.; Hwa, J. Piperidine derivatives and methods of use thereof. WO 2008/108957 A2, February 27, 2008.

b. Misra, R.; Xiao, H.; Kim, K.; Lu, S.; Han, W.; Barbosa, S.; Hunt, J.; Rawlins, D.; Shan, W.; Ahmed, S.; Qian, L.; Chen, B.; Zhao, R.; Bednarz, M.; Kellar, K.; Mulheron, J.; Batorsky, R.; Roongta, U.; Kamath, A.; Marathe, P., Ranadive, S.; Sack, J.; Tokarski, J.; Pavletich, N.; Lee, F.; Webster, K.; Kimball, S. *Journal of Medicinal Chemistry*. **2004**. 47, 1719-1728.

112. Bernotas, R.; Brown, P.; Emmons, G.; King, C. Sulfuric acid mono-[3({1-[2-(4-fluoro-phenyl)-ethyl]-piperidin-4-yl}-hydroxy-methyl)-2-methoxy-phenyl]ester. US 6,465,490 B1, October 15, 2002.

113. Jiang, X.; Yang, X.; Zhao, C.; Sun, L. *Journal of Physical Organic Chemistry*. **2009**. 22, 1-8.

114. Keegstra, E.; Zwikker, J.; Roest, M.; Jenneskens, L. *The Journal of Organic Chemistry*. **1992**. 57, 6678-6680.

115. Brea, J.; Castro, M.; Giraldo, J.; Lopez-Gimenez, J.; Padin, J.; Quintian, F.; Cadavid, M.; Vilaro, M.; Mengod, G.; Berg, K.; Clarke, W.; Vilardaga, J.; Milligan, G.; Loza, M. *Molecular Pharmacology*. **2009**. 75, 1380-1391.

116. Britten, R.; Roberts, R. *Science*. **1960**. 131, 32-33.

117. Elia, G. *Proteomics*. **2008**. 8, 4012-4024.

Vita

Matthew Shashack was born on November 16, 1981 to Steve and Jamie Shashack in Maryville, Illinois. Mr. Shashack received his Bachelor of Science degree from Texas Lutheran University in 2004 where he doubled majored in biology and chemistry. Mr. Shashack came to the University of Texas Medical Branch to pursue his Ph.D. in the department of Pharmacology and Toxicology in August 2005. During the 2007-2008 academic year, he was chosen as a Bromberg Scholar which entailed serving as a scientific research mentor to a local high school student, Matthew Tramonte.

Education

B.S. 2004, Texas Lutheran University, Seguin TX

Publications

Lory, P. M. J.; Estrella-Jimenez, M. E.; **Shashack, M. J.**; Lokesh, G. L.; Natarajan, A.; Gilbertson, S. R. "Synthesis and screening of 3-substituted thioxanthen-9-one-10,10-dioxides." *Bioorg. Med. Chem. Lett.* **2007**, 17, 5940-5943.

Permanent address: 257 W. Convent, Seguin, TX 77539

This dissertation was typed by Matthew J. Shashack.