ABSTRACT

Design, Synthesis and Biological Evaluation of Novel Serotonin Reuptake Inhibitors and Novel Derivatives of a Nitrogen-Containing Combretastatin Analog

M. Graciela Miranda

Mentor: Kevin G. Pinney, Ph.D.

Depression is a common and serious illness that affects one out of every ten Americans each year. Since the 1980's, selective serotonin reuptake inhibitors (SSRIs) have been the pharmaceuticals of choice for the treatment of depression and related disorders. Despite their indisputable efficacy, there is still room for improvement in SSRIs, especially in regard to their onset of action and adverse reaction profile. The research presented herein focused on the design and synthesis of a library of thirty-three novel bivalent molecules that could combine into one molecular entity an enhanced antagonism towards the 5-HT_{2A} receptors while keeping a highly selective inhibition of the serotonin transporter (SERT). These bivalent molecules were constructed by covalently coupling two types of fluoxetine hydrochloride structural homologues (for SERT affinity) with a series of nine functionalized piperazines and piperidines (for 5-HT_{2A} antagonism). Preliminary biological evaluation shows that two of the synthesized molecules, **16b** and **17b**, exhibit the desired dual activity (K_i = 237 and 195 nM respectively for the 5-HT_{2A} receptor and K_i = 1.2 and1.8 μM respectively for SERT). The complete set of biological

data will outline the potential of the synthesized molecules as a new generation of improved antidepressants.

Although remarkable advances have been made in cancer pharmacotherapy, the American Cancer Society declared this disease as the top killer of Americans in January, 2005. Therefore, a second research project presented herein focused on the development of a bivalent drug candidate for the treatment of cancer, which could combine into one molecular entity two distinct forms of cancer treatment, vascular disruption and bioreduction. Although the desired target molecule was not achieved, two unexpected and structurally unique products were obtained, which were fully characterized in regards to their structure. Preliminary biological evaluation indicates that compound **73** shows significant inhibition of tubulin assembly (IC_{50} = 3.3 μM), while compound **74** shows potent and selective *in vitro* cytotoxicity towards three human cancer cell lines. Therefore, the continuation of this line of research aimed at an improved treatment option for cancer patients is strongly encouraged.

Design, Synthesis and Biological Evaluation of Novel Serotonin Reuptake Inhibitors and Novel Derivatives of a Nitrogen-Containing Combretastatin Analog

by

M. Graciela Miranda

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Marianna A. Busch, Ph.D., Chairperson

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Approved by the Dissertation Committee

Kevin G. Pinney, Ph.D., Chairperson

Robert R. Kane, Ph.D.

Charles M. Garner, Ph.D.

C. Kevin Chambliss, Ph.D.

N. Bradley Keele, Ph.D.

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J. Larry Lyon, Ph.D., Dean

TABLE OF CONTENTS

List of Figures vi

List of Schemes vii

List of Tables viii

Acknowledgments x

Dedication xi

Chapter One: Introduction 1

Chapter Two: Design, Synthesis and Biological Evaluation of Novel Serotonin Reuptake Inhibitors 3

Introduction 3

Background 5

Types and Symptoms of Depression 5

Causes of Depression 8

Diagnostic Evaluation and Therapeutic Strategies for Depression 9

Pharmacotherapy of Depression 10

The Role of Serotonin Reuptake Inhibitors 15

The Complex Serotonin System 22

Bivalent Approaches for Therapeutic Intervention 31

Rationale for Drug Design 34

Strategic Design 35

Homologues Type I and II 38

The Case for Autism 41

Chapter Three: Materials and Methods 43

General Section 43

Synthesis of 4-Fluorophenoxy Ethyl Bromide Intermediate 44

Synthesis of 4-Trifluoromethyl-phenoxy Ethyl Bromide Intermediate 47

Attempted Synthesis of Coupled Products from Bromide Intermediates 49

Synthesis of Homologues Type I – Compounds 1 to 7 49

Synthesis of Homologues Type I – Compounds 8 to 15 66

Synthesis of Homologues Type II – Compounds 16, 17, 24 and 25 80

Synthesis of Homologues Type II – Compounds 18 to 23, 26 and 27 89

Synthesis of Homologues Type II – Compounds 28 to 33 106

Biological Evaluation 118

Chapter Four: Results and Discussion 122

Synthesis of 4-Fluorophenoxy Ethyl Bromide Intermediate 122

Synthesis of 4-Trifluoromethyl-phenoxy Ethyl Bromide Intermediate 126

Attempted Synthesis of Coupled Products from Bromide Intermediates 127

Synthesis of Homologues Type I – Compounds 1 to 7 129

Synthesis of Homologues Type I – Compounds 8 to 15 132

Synthesis of Homologues Type II – Compounds 16, 17, 24 and 25 137

Synthesis of Homologues Type II – Compounds 18 to 23, 26 and 27 139

Synthesis of Homologues Type II – Compounds 28 to 33 142

Biological Evaluation 145

Chapter Five: Conclusions and Future Directions 149

Chapter Six: Synthesis of Two Novel 3,4,5-Trimethoxyphenyl Derivatives of a Nitrogen-Containing Combretastatin Analog 152

Introduction 152

Background 153

Design Rationale 158

Chapter Seven: Materials and Methods 159

General Section 159

Synthesis of *(Z)*-1-[(2',4'-dimethoxy-3'-nitro)phenyl]-2-[(3",4",5"-trimethoxy)phenyl]ethane 160

Synthesis of 2-amino-4-methyl-6-(3,4,5-trimethoxy-phenyl)pyrimidine 161

Biological Evaluation 162

Chapter Eight: Results and Discussion 164

Synthesis of *(Z)*-1-[(2',4'-dimethoxy-3'-nitro)phenyl]-2-[(3",4",5"-trimethoxy)phenyl]ethane 164

Synthesis of 2-amino-4-methyl-6-(3,4,5-trimethoxy-phenyl)pyrimidine 166

Biological Evaluation 169

Chapter Nine: Conclusions and Future Directions 171

Appendices 172

Appendix A: Selected NMR Spectra 173

Appendix B: Selected HPLC Spectra 367

References 402

LIST OF FIGURES

Figure		Page
1.	Structures of three neurotransmitters	8
2.	Structures of three monoamine oxidase inhibitors	11
3.	Structures of three tricyclic antidepressants	12
4.	Structure of four antidepressants with diverse mechanisms of action	13
5.	Mechanism of action of serotonin reuptake inhibitor Prozac®	16
6.	Structures of six currently prescribed SSRIs	18
7.	FDA recommended lowest effective dose of SSRIs for major depression	20
8.	Model of a serotonergic synapse	22
9.	Snake representation of hSERT	23
10.	Energy minimized SERT model viewed in the membrane plane	24
11.	Cocaine (A), *S*-citalopram (B) and *S*-amphetamine (C) docked into SERT model	25
12.	Structure of two 5-HT_{1A} ligands	27
13.	Structure of two 5-HT_{2A} antagonists effective in the treatment of SSRI-induced side effects	29
14.	Structure of four atypical antipsychotics	33
15.	Rationale for bivalent SSRI drug design	35
16.	Computer-generated ORTEP drawing with crystallographic numbering system	36
17.	Structures of nine aryl- and alkyl-piperazines and piperidines with demonstrated 5-HT_{2A} receptor antagonism	38
18.	Set up for 96-well plate for 5-HT Receptor and Transporter Binding Assays.	120
19.	Expansion of ^{1}H-NMR of compound **41**	128

20. Reagents and products of Mitsunobu coupling using DIAD and PPh_3 134

21. ^{1}H NMR of Compound **10a** contaminated with DCH 136

22. Expansion of ^{1}H-NMR of DCH 136

23. Reagents and products of Mitsunobu coupling using ADDP and Bu_3P 144

24. Structure of CA-4P 155

25. Structure of CA-1P 156

26. Structure of tirapazamine 157

27. Structure of target molecule XI 158

28. Expansion of ^{1}H-NMR of Compound **73** 165

29. X-ray Structure of compound **73** 166

30. X-ray Structure of compound **74** 167

LIST OF SCHEMES

Table		Page
1.	Synthesis of 4-fluorophenoxy ethyl bromide intermediate	123
2.	Mechanism of the Mitsunobu reaction	124
3.	Mechanism of conversion of ethyl amine to ethyl bromide using $TiBr_4$	125
4.	Synthesis of 4-trifluoromethyl-phenoxy ethyl bromide intermediate	126
5.	Attempted synthesis of 4-flurophenoxy-ethyl coupled products	127
6.	Attempted synthesis of a 4-trifluromethylphenoxy-ethyl coupled product	129
7.	Synthesis of homologues type I – compounds 1 to 7	130
8.	Mechanism of action of $NaBH_4$ reduction	131
9.	Synthesis of homologues type I – compounds 8 to 15	133
10.	Products 10a to 13a using DIAD and PPh_3, contaminated with DCH	135
11.	Synthesis of 1-[3-(4-fluorobenzoyl)propyl]piperazine (IX)	137
12.	Mechanism of the Mannich reaction	138
13.	Synthesis of homologues type II – compounds 16, 17, 24 and 25	139
14.	Synthesis of homologues type II – compounds 18 to 23, 26 and 27	141
15.	Products 19a to 21a using DIAD and PPh_3, contaminated with DCH	142
16.	Synthesis of homologues type II – compounds 28 to 33	143
17.	Products 26a to 29a, 32a and 33a using ADDP and Bu_3P, contaminated with Bu_3PO	145
18.	Synthesis of derivative 73	164
19.	A possible mechanism of formation for compound 73	166

20. Synthesis of derivative 74 167

21. A speculated mechanism of formation for compound 74 168

LIST OF TABLES

Table		Page
1.	DSM-IV diagnostic criteria for major depressive episode	5
2.	Approved FDA indications for the SSRIs in the United States	17
3.	Pharmacological similarities between SSRIs	19
4.	General structures of homologues type I and II	39
5.	Structures of studied compounds and identifying numbers assigned	40
6.	Ligands and conditions for 5-HT receptor binding assays	120
7.	Ligands and conditions for transporter binding assays	121
8.	Mitsunobu conditions and yields for homologues type I and II	146
9.	Mean % inhibition for compounds tested at receptor subtypes	147
10.	Ki values for compounds tested at receptor subtypes	147
11.	Inhibition of tubulin polymerization for compounds 73 and 74	169
12.	*In vitro* cytotoxicity (GI_{50} and ED_{50} in μM) for compounds 73 and 74	170

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DEDICATION

To my parents
Julio Cesar and Flor Maria

Parents are the bows from which children as living arrows are sent forth. The archer sees the mark upon the path of the infinite, and He bends the bow with His might that His arrows may go swift and far.

Gibran Khalil Gibran

CHAPTER ONE

Introduction

Rational drug design has revolutionized the process of drug discovery. From the historical "trial and error" methods for drug discovery, rational drug design begins the process with knowledge of specific chemical responses in the body or target organism, and then tailors combinations of these to fit a treatment profile. More and more successful examples of novel pharmacotherapies where rational drug design has played a key role are available every day. Ultimately, it is hoped that this rational approach of drug discovery will help alleviate the burden of disease worldwide.

Depression and cancer are very serious illnesses that affect millions of people every year. It is impossible to quantify the losses caused by these diseases because they cause pain and suffering not only to the individual, but also to those who care about them. These are illnesses that can destroy family life as well as the life of the ill person. Therefore, the importance of the availability of effective treatment options for these diseases cannot be overemphasized.

Herein, two rational drug design approaches for the treatment depression and cancer will be presented. Depression is a common and serious illness that affects approximately one out of every ten Americans each year. It is proposed to be caused by a decreased production of serotonin (5-HT) in the brain, combined with a decreased sensitivity of post synaptic 5-HT receptors to the available 5-HT. Since the 1980s, selective serotonin reuptake inhibitors (SSRIs) have been the leading pharmaceuticals prescribed to alleviate depression and several other psychiatric disorders. Fluoxetine

hydrochloride (Prozac ®) is probably the most representative of this medication group. However, despite their indisputable efficacy, there is still room for the improvement of SSRIs, especially with regards to their onset of action and adverse reaction profile. The research presented herein focused on the design, synthesis and biological evaluation of novel bivalent fluoxetine homologues that, by exhibiting an enhanced activity towards the 5-HT_{2A} receptors while keeping a highly selective inhibition of the serotonin transporter, can provide synergism in their potential efficacy over a wider variety of both depressive and anxiety disorders. A library of thirty-three novel compounds of this class were synthesized and characterized. Their full serotonergic and transporter binding profile is currently underway. However, preliminary biological evaluation has provided proof of principle showing that, in fact, some of the synthesized molecules exhibit the desired dual activity. Future directions in this area of research are limitless, and it is hoped that the knowledge obtained from this study will aid in the identification of novel therapeutics that can positively contribute to the battle against depressive disorders and disease in general.

The second project described herein also involved the design and synthesis of a novel bivalent molecule that could combine in one molecular entity two forms of cancer treatment, vascular disruption and bioreduction. During this process, two unexpected and structurally unique products were obtained from the synthetic approaches, which were fully characterized in regards to their structure and were also biologically evaluated to assess their potency as vascular disrupting agents. Preliminary biological results are very encouraging, therefore prompting the continuation of this line of research in the search for, if not a cure, an improved treatment option for patients suffering from cancer.

CHAPTER TWO

Design, Synthesis and Biological Evaluation of Novel Serotonin Reuptake Inhibitors

Introduction

Depression is a common and serious medical condition that negatively affects the way people feel, think and act. The National Institute of Mental Health (NIMH) estimates that in any given year almost 19 million Americans (nearly one in ten) suffer from a depressive disorder. Depression does not discriminate; it affects men and women, young and old, and people of all races, cultures, and incomes.[1,2] It is normal to develop feelings of sadness or grief in response to stressful situations such as the death of a loved one, the loss of a job, or the ending of a relationship. But sadness and depression are not the same. While feelings of sadness will lessen with time, depression can continue for weeks, months and even years.[3]

Depression is not just a state of mind; it is not a sign of personal weakness or a condition that can be willed or wished away. Depressed individuals are overcome by feelings of helplessness and regret, and the simple pleasures of life are no longer enjoyed. Depressive disorders cause pain and suffering not only to the individual, but also to those who care about them. Serious depression can destroy family life as well as the life of the ill person.[1-3] Rates of depression increase with the presence of a concomitant medical condition. The highest rates are reported for myocardial infarction survivors (40-65%), followed by diabetic patients (8-32%), cancer patients (25%) and stroke survivors (10-27%).[4-6] The overall prevalence of depression in the elderly is 15%, with much higher rates in subpopulations such as Alzheimer's patients (30-40%).[4-6]

Reported to be the leading cause of worldwide disability, depression also ranks second after ischemic heart disease as a burden to the established market economies.[2,7] Forty-three billion dollars are estimated to be lost annually by depression-related medical expenses, missed days at work and premature deaths in the United States alone.[3] Upon consideration that 59 to 87% of all suicide victims were diagnosed with major depression, and that depressed patients are 15 to 20 times more likely to attempt suicide, the importance of treating depression cannot be over-emphasized.[8,9]

Fortunately, depression is one of the most treatable mental illnesses. Thanks to years of fruitful research, 80 to 90% of all patients treated for depression experience significant improvement. Even those whose depression is extremely severe can be helped with proper treatment. Regretfully though, only an estimated 30% of patients with depression seek care.[1-4,9] Therefore, it is of critical importance that all health professionals play an active role in assuring those who do seek help receive optimal care.[4] Additionally, rational drug design continues to play a key role in the development of new therapeutic agents that are more efficacious, safer and better tolerated than older medications.[32]

In this chapter, the etiology of depression will be reviewed, as well as the history of antidepressant medications with focus on the selective serotonin reuptake inhibitors (SSRIs) family. The complexities of the serotonin system will also be addressed, as well as potentially new drug targets for depression. Finally, the design rationale for the novel compounds obtained within this research will be explained and supported.

Background

Types and Symptoms of Depression

The *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV),[10] the primary reference for psychiatric diagnoses, characterizes the two most common types of depression as major depression and dysthymia.[4,10] Major depression is manifested by a combination of symptoms (Table 1) that interfere with the individual's ability to work, study, sleep, eat and enjoy once pleasurable activities. For a major depression episode, symptoms should be present for two weeks or more and represent a change from a prior psychological state. At the same time, the episode must cause a significant distress that is not caused by substance abuse, bereavement, or another medical condition. Such a disabling episode of depression may occur only once but more commonly occurs several times in a lifetime.[1-4,10] Chronic major depression may require a person to continue treatment indefinitely.[2]

Table 1. DSM-IV diagnostic criteria for major depressive episode.[10]
(Reproduced directly from Ref. 10)

1. One of the following must be present:
    a. Depressed mood most of the day
    b. Markedly diminished interest or pleasure in nearly all life activities
2. Four or more of the following symptoms must be present:
    a. Significant weight loss or gain (more than 5% of body weight in a month)
    b. Insomnia or hypersomnia
    c. Fatigue or loss of energy
    d. Psychomotor agitation or retardation
    e. Feelings of worthlessness or excessive inappropriate guilt
    f. Diminished ability to think or concentrate, or indecisiveness
    g. Recurrent thoughts of death, recurrent suicidal ideation with or without a plan or a suicide attempt

Dysthymia or dysthymic disorder is the second most common form of depression. It is a milder, less severe type of depression. It involves long-term, chronic symptoms that do not cause as much impairment as major depression but still prevents the person from functioning well or from feeling good. The same symptoms and feelings of insomnia, fatigue, restlessness, and worthlessness are also common to dysthymia. Like major depression, dysthymia may be persistent if not properly treated.[1-4,10]

Depression can also appears in other psychiatric forms such as seasonal affective disorder (SAD), which affects 6% of Americans during the winter, and postpartum depression (PPD) that affects 10-20% of new mothers. Additionally, several other disorders can occur alongside depression which include post-traumatic stress disorder, obsessive-compulsive disorder (OCD), panic disorder, social phobia, and generalized anxiety disorder.[11]

Depression in women. Women experience depression about twice as often than men (about 1 in 4 women).[1,3] Hormonal factors may contribute to this increased rate, particularly menstrual cycle changes, pregnancy, miscarriage, postpartum time, pre-menopause and menopause. Other factors include additional stresses such as responsibilities both at work and home, single parenthood, and caring for children and for aging parents.[12-14]

Depression in men. It is estimated that 3 to 4 million men in the United States are affected by the depression (about 1 in 10 men).[1-3] Although men are less likely to admit to depression, the rate of suicide in men is four times that of women.[15] Additionally, depression can affect the physical health in men. A recent study shows that although

depression is associated with an increased risk of coronary heart disease in both men and women, men suffer a higher death rate.[16]

Depression in the elderly. Late life depression is a major public health problem. It is associated with increased morbidity, mortality, and functional impairment, as well as diminished quality of life.[47] Unfortunately, this kind of depression often goes unrecognized and untreated. The symptoms of depression in the elderly are often described as physical problems, because the person is reluctant to discuss feelings of hopelessness, sadness, lack of interest in normally pleasurable activities or extremely prolonged grief after a loss. However, efficacy studies have demonstrated that treatment with medication and/or psychotherapy will produce a significant improvement in the quality of life of the person.[17]

Depression in children and adolescents. Only in the past two decades has depression in children been taken very seriously. Anxiety disorders are the most prevalent psychiatric conditions among children[32] while depressive disorders are relatively common among adolescents. Adolescent depression is also highly recurrent (up to 75%).[80] Considering that suicide is the third leading cause of death among adolescents, and the serious short and long term implications of depression in this age group, it is critical that effective treatment options be available.[80] However, only a highly specialized psychiatrist can correctly diagnose the illness and determine the best course of treatment on a case by case basis.[18]

Causes of Depression

The exact cause of depression is still yet to be completely resolved, but scientists are in agreement that several factors such as brain biochemistry, inherited genes, social environment, and upbringing play important roles.[3,19] However, the monoamine hypothesis remains the most widely accepted basis for depression and it has been used to explain the efficacy of existing antidepressant therapies.[20,21] It assumes a deficit in the neurotransmitters, norepinephrine (NE), serotonin (5-HT), and dopamine (DA)[21] (Figure 1).

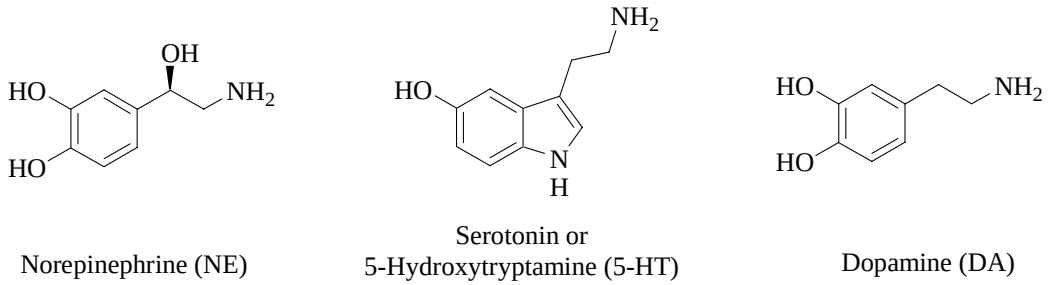


Figure 1. Structures of three neurotransmitters.

Under normal physiological conditions, these molecules are released from the presynaptic nerve into the synaptic cleft in response to an action potential. Then, the neurotransmitters interact with receptor proteins located on the postsynaptic nerve.[22] If enough neurotransmitters interact with these receptors, the postsynaptic nerve membrane is depolarized and an action potential is initiated. These molecules, moreover, upon their release are immediately reabsorbed by reuptake carrier proteins located on the presynaptic nerve and degraded by the enzyme monoamine oxidase.

According to the monoamine hypothesis, there is a deficient amount of neurotransmitters being released from presynaptic terminals in the limbic system (mood center) of the central nervous system (CNS) to initiate action potentials on the postsynaptic

nerve. This hypothesis also proposes that the postsynaptic nerves have become abnormally desensitized to these neurotransmitters. Thus, treatment for depression has been found effective by increasing the amount of DA, NE, and 5-HT released into the nerve terminal synapse favoring the propagation of an action potential.[22] Furthermore, the monoamine hypothesis is supported by evidence that certain drugs like reserpine, which block NE and 5-HT production, cause depression while other medications that increase their production alleviate it.[22,23]

Additionally, it has been theorized that the cause of this neurotransmitter disturbance in depressed individuals is the result of a loss in mass of the hippocampus; a part of the limbic system in the CNS involved in emotion and motivation.[11] One specific study supports this theory by illustrating that antidepressants protect against hippocampal volume loss during reoccurrent depression episodes.[24] The same study proposes that the delayed onset of action often observed in antidepressants represents the time necessary for new neurons in the hippocampus to be formed facilitating the antidepressant therapy.

Diagnostic Evaluation and Therapeutic Strategies for Depression

More than 80% of people suffering from depressive disorders improve when they receive appropriate treatment.[1-4,9] The first step to getting adequate treatment is a physical examination by a qualified physician to rule out other possible causes for the symptoms (i.e., other medical conditions and/or medications). Subsequently, the physician should refer the person to a mental health professional to conduct a diagnostic evaluation for depression. A good diagnostic evaluation will include a complete history of symptoms: when they started, how long they have lasted, how severe they are, whether the person has had them before and if so, whether the symptoms were treated and what

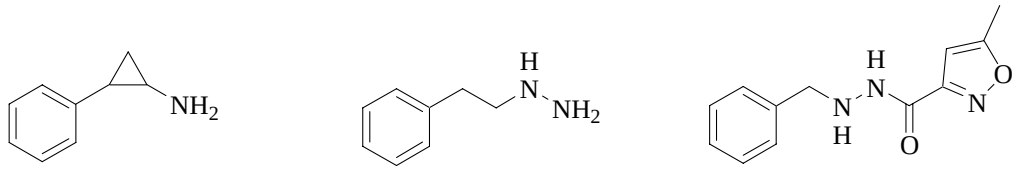
treatment was used. The psychiatrist should also ask about alcohol and drug use, and if the person has thoughts about death or suicide. Furthermore, the history should include questions about whether other family members have been treated for a depressive illness. Finally, the diagnostic evaluation should include a mental status examination to determine if speech or thought patterns or memory have been affected.[1-4,9]

The treatment of choice will depend on the person's diagnosis, severity of symptoms and preference. There are a variety of antidepressant medications and psychotherapies that can be used to treat depressive disorders. People with milder forms of depression may improve with psychotherapy alone. However, people with moderate to severe depression, especially those that are recurrent, most often benefit from a combined treatment (medication and psychotherapy), and respond very well to antidepressants.[1-4,6,9]

Pharmacotherapy of Depression

Monoamine oxidase inhibitors. MAOIs or monoamine oxidase inhibitors were one of the earliest antidepressants discovered.[25,26] Monoamine oxidase is an enzyme commonly found in presynaptic nerve terminals, and its responsible for the metabolism of the three neurotransmitters associated with depression: DA, 5-HT, and NET.[4,9] Monoamine oxidase A (MAO A) is primarily responsible for the breakdown of 5-HT and NE, while monoamine oxidase B (MAO B) primarily breaks down DA. MAOIs exert their mechanism of action by reversibly inhibiting both MAO A and MAO B, leading to an increase in the synaptic levels of all three neurotransmitters and ultimately alleviating depression.[4] Typical representatives of this class of antidepressants are tranylcypromine (Parnate®), phenelzine (Nardil®) and isocarboxazid (Marplan®).[9] MAOIs can be divided

into two groups on the basis of their chemical structure: the hydrazines (phenelzine and isocarboxazid) and the non-hydrazines (tranylcypromine) (Figure 2).[27]



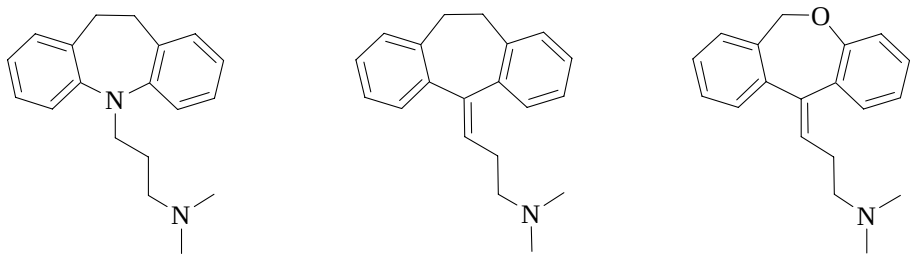
Tranylcypromine Phenelzine Isocarboxazid

Figure 2. Structures of three monoamine oxidase inhibitors.

Because these MAOIs bind irreversibly to the enzyme, inactivation of MAO persists even after the drugs are metabolized and removed from the body. Recovery from MAOIs requires the synthesis of new enzyme, and it can take weeks to return to normal functioning after treatment with a MAOI.[9] The overall efficacy of MAOIs was found contradictory and not clearly documented.[27] Moreover, their use was limited by a high occurrence of side-effects and severe toxicity. Side-effects include insomnia, tremor, vertigo, fatigue, sedation, GI distress and weight gain.[28] MAOIs are also characterized by serious food and drug interactions.[4,9] Furthermore, the most alarming toxic effect of MAOIs was the risk of a hypertensive crisis with potentially lethal consequences. Therefore, their use was soon discontinued for the treatment for depression.[9]

Tricyclic antidepressants. Together with MAOIs, tricyclic antidepressants (TCAs) are considered first-generation antidepressants.[9] They were discovered around the same time as MAOIs and became the preferred pharmaceuticals for the treatment of depression in the 1960s.[29] Receiving their name from their three fused cyclic ring structure, TCAs exert their mechanism of action by potently inhibiting NE reuptake and

mildly inhibiting 5-HT reuptake.[4,9] Due to this inhibition of reuptake, neurotransmitter levels remain elevated in the synaptic cleft for a prolonged period and therefore increased postsynaptic interaction occurs, which then leads to the alleviation of depression. Figure 3 depicts the structure of some of the most commonly used TCAs in the United States: imipramine (Tofranil®), amitriptyline (Elavil®), and doxepine (Adapin®).[9]



Imipramine Amitriptyline Doxepin

Figure 3. Structures of three tricyclic antidepressants.

The overall efficacy among TCAs is comparable, causing clinically significant improvement in 70 to 80% of patients[30,31] Nonetheless, TCAs produce an extensive range of side effects due to their narrow window of safe therapeutic concentrations and non-selective interaction with other CNS receptors such as the cholinergic, adrenergic and histamine receptors.[4,9,32] These interactions lead to dry mouth, blurred vision, weight gain, tachycardia, memory impairment, sedation and drowsiness, as well as severe cardiotoxicity at high concentrations.[4,9] Consequently TCAs, although very potent, fell out of preference with the emergence of more selective antidepressants with better adverse reaction profiles and lower cardiac risks.[32]

Other antidepressants. The following do not belong to any of the major classes of antidepressants due to their diverse mechanisms of action. They are approved by the

United States Food and Drug Administration (FDA) for the treatment of depression and related disorders, and are of much interest since they offer therapeutic advantages in very specific cases. These are, but are not limited to: bupropion, mirtazapine, venlafaxine and duloxetine (Figure 4).

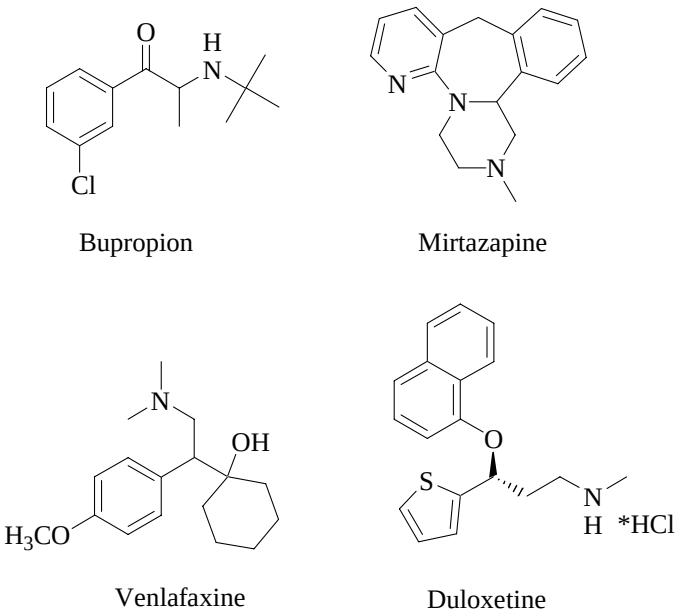


Figure 4. Structure of four antidepressants with diverse mechanisms of action.

Bupropion (Wellbutrin®) is an effective antidepressant that has no effect over 5-HT levels. It is a dual inhibitor of the reuptake of both NE and DA.[4,33] It is considered one of the most activating antidepressants, and can be particularly useful in the treatment of severe depression characterized by extreme fatigue, lethargy and psychomotor retardation.[33] Its ability to elevate dopamine levels is probably responsible for its positive effects in the treatment of attention deficit hyperactivity disorder (ADHD) and Parkinson's disease.[34] It has also been approved as an aid in smoking cessation.[4,33] Bupropion's most serious side effects are dose-dependent seizures.[35] Activating side effects include agitation, insomnia, restlessness, anxiety and nausea.[36,37] Other adverse

effects such as dry mouth, constipation, headache, nausea, excessive sweating, tremor and weight loss have also been reported.[4] It has few cardiovascular effects, few if any anticholinergic effects, causes little sedation and results in minimal or no sexual dysfunction. It has a very favorable drug interaction profile, with the exception of interaction with MAOIs.[4,33]

Mirtazapine (Remeron®) has been marketed in the United States since 1996 and has the most unique mechanism of action of all antidepressants.[33] It is a α_2-antagonist that produces an indirect increase in noradrenergic and serotonergic neurotransmission.[38] It does not block the reuptake of 5-HT, NE or DA, but binds to the 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptors.[38] Its tolerability is limited by its potent histamine-1 blockade, associated with sedation and weight gain.[39] A calming medication, it promotes sleep restoration and may be particularly effective in the treatment of agitated depression, mixed anxiety and depression and prominent insomnia.[40] Somnolence, increased appetite, weight gain, and dizziness are the most common adverse reactions reported. Anticholinergic effects are minor, with only dry mouth and constipation reported. Cardiovascular effects are minimal and no sexual dysfunction has been encountered. Alcohol and benzodiazepines produce increased sedative, psychomotor and cognitive effects when combined with mirtazapine. The use of MAOIs is also contraindicated in combination with mirtazapine.[4,33]

Venlafaxine (Effexor®) was the first non-ticyclic antidepressant to inhibit the reuptake of both 5-HT and NE.[41] It appears to provide a more rapid and possibly a superior response, especially in severe depression.[33] Venlafaxine is an activating antidepressant, and anxiety, nervousness and insomnia are associated with it.[37] The most

common side effect is nausea, but sleep disturbances, sexual dysfunction, anorexia, dizziness, dry mouth, sweating and tremor are also reported.[33] Venlafaxine is also associated with significant elevations in diastolic blood pressure in 3-13% of patients,[37] requiring blood pressure monitoring; therefore, its use is contraindicated in hypertensive patients. Although venlafaxine has a low potential for drug interactions, it should not be combined with MAOIs.[42]

Duloxetine (Cymbalta®)[43] was recently approved by the FDA for the treatment of major depression and related disorders, management of pain, and also for urinary incontinence.[44] Like venlafaxine, it blocks the reuptake of 5-HT and NE. Although data is still limited, adverse effects include nausea, dry mouth, dizziness, constipation and insomnia.[45,46] A significant incidence of hypertension has also been reported. As an inhibitor of both 5-HT and NE, it will probably also be advised not to combine with MAOIs.[33]

The Role of Serotonin Reuptake Inhibitors

The emergence of selective serotonin reuptake inhibitors (SSRIs) constituted a major breakthrough in psychopharmacology[32] and represents a key shift toward a different approach for treating depression that emphasizes the role of 5-HT.[4] Although considered second generation antidepressants,[9] SSRIs were the first class of psychiatric medications to be rationally designed using *in vitro* receptor binding affinity technology.[47]

Since the 1980s, SSRIs have become the pharmaceuticals of choice for the treatment of depression[48] and currently account for more than 80% of all antidepressants prescribed in the United States.[4] Having a general 50- to 100-fold selectivity for

inhibition of 5-HT uptake over NE or DA uptake *in vitro*,[49] SSRIs are well tolerated, easier to manage, and have fewer drug interactions than older antidepressants.[50]

Evidence indicates that serotonergic neurons in the limbic system of depressed patients fail to release an adequate amount of 5-HT to sustain normal postsynaptic receptor activation.[51,52] Consistent with this idea, SSRIs exert their therapeutic effect by selectively blocking the serotonin reuptake transporter (SERT) which removes 5-HT from the extracellular synaptic space, thereby increasing the synaptic concentration of 5-HT.[50] By increasing the 5-HT synaptic availability, SSRIs cause an increased interaction and activation of postsynaptic serotonin receptors, thus compensating for the diminished neurotransmitter release. Eli Lilly's fluoxetine hydrochloride (Prozac®) is one of the most representative SSRIs and is a highly selective serotonin reuptake inhibitor[32] both *in vitro* and *in vivo*[47] (Figure 5).

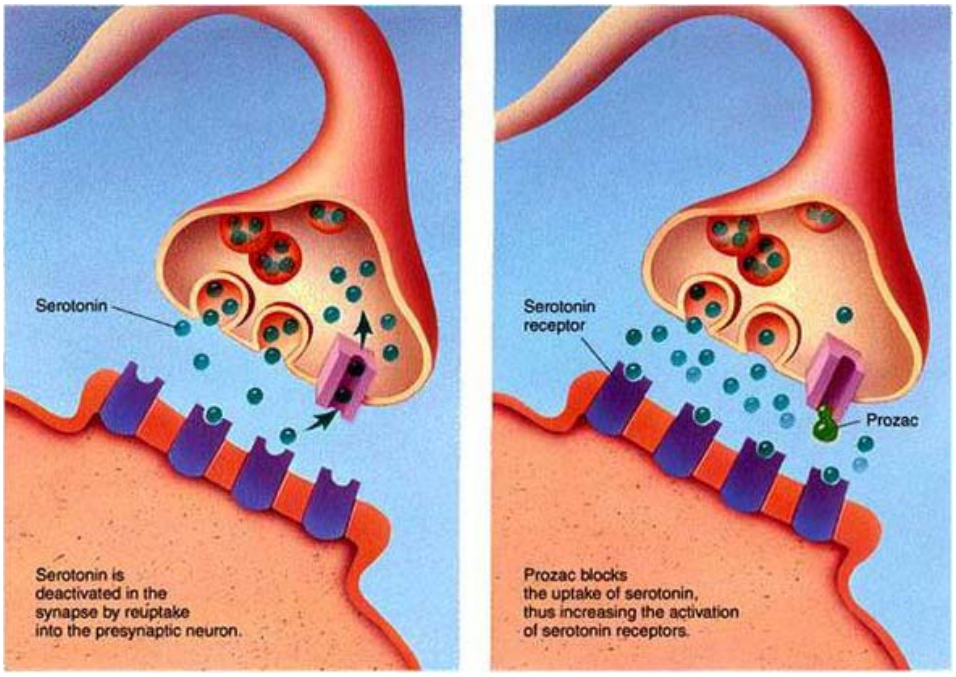


Figure 5. Mechanism of action of serotonin reuptake inhibitor Prozac®.[53] (Reproduced directly from Ref. 53).

This augmentation of neuronal signals by SSRIs has been found effective in the treatment of several other psychiatric ailments (many coexistent with depression) such as obsessive-compulsive disorder (OCD),[54] panic,[55] social anxiety,[56] and eating disorders[32] among others, in addition to depression[32] (Table 2). There are currently six SSRIs available in the United States. These are fluoxetine (Prozac®), sertraline (Zoloft®), paroxetine (Paxil®), fluvoxamine (Luvox®), citalopram (Celexa®), and escitalopram (Lexapro®)[47] (see Figure 6).

Table 2. Approved FDA indications for the SSRIs in the United States[47] (Adapted from Ref. 47).

SSRI/Indication	Fluoxetine	Sertraline	Paroxetine	Fluvoxamine	Citalopram	Escitalopram
Depression	X	X	X		X	X
Panic Disorder	X	X	X			
OCD	X	X	X	X		
Social Anxiety Disorder		X	X			
GAD			X			
PTSD		X	X			
Bulimia Nervosa	X			X		
PMDD	X	X		X		

OCD, obsessive-compulsive disorder; GAD, generalized anxiety disorder; PTSD, post-traumatic stress disorder; PMDD, premenstrual dysphoric disorder.

Moreover, the decreased adverse reaction profile of SSRIs compared to MAOIs and tricyclic antidepressants is hypothesized to be a result of their selective nature for only the serotonin reuptake transporter and their avoidance of the norepinephrine and dopamine catecholamine systems.[32,47]

Rational drug development. As mentioned before, SSRIs were the first rationally designed class of antidepressants, and their introduction marked a milestone in the history of drug development.[32,47] Researchers began investigating the pharmacology of TCAs and

MAOIs in the 1960s and 1970s. Although these early antidepressants were discovered serendipitously, they provided solid evidence that central serotonin agonism by inhibition of the SERT could be a means of obtaining an antidepressant response.[57] Carlsson and Lindqvist[58] were the first to report that imipramine (a TCA) was able to block the reuptake of 5-HT in serotonergic neurons. With this in mind, pharmaceutical developers in the 1970s began a research effort to rationally develop drug candidates that would inhibit the neuronal uptake of 5-HT without affecting the various other neuroreceptors and fast sodium channels responsible for the many adverse effects of TCAs and MAOIs.[59,60] These efforts resulted in the development of the first five SSRIs, fluoxetine, sertraline, paroxetine, fluvoxamine, and citalopram (Figure 6).

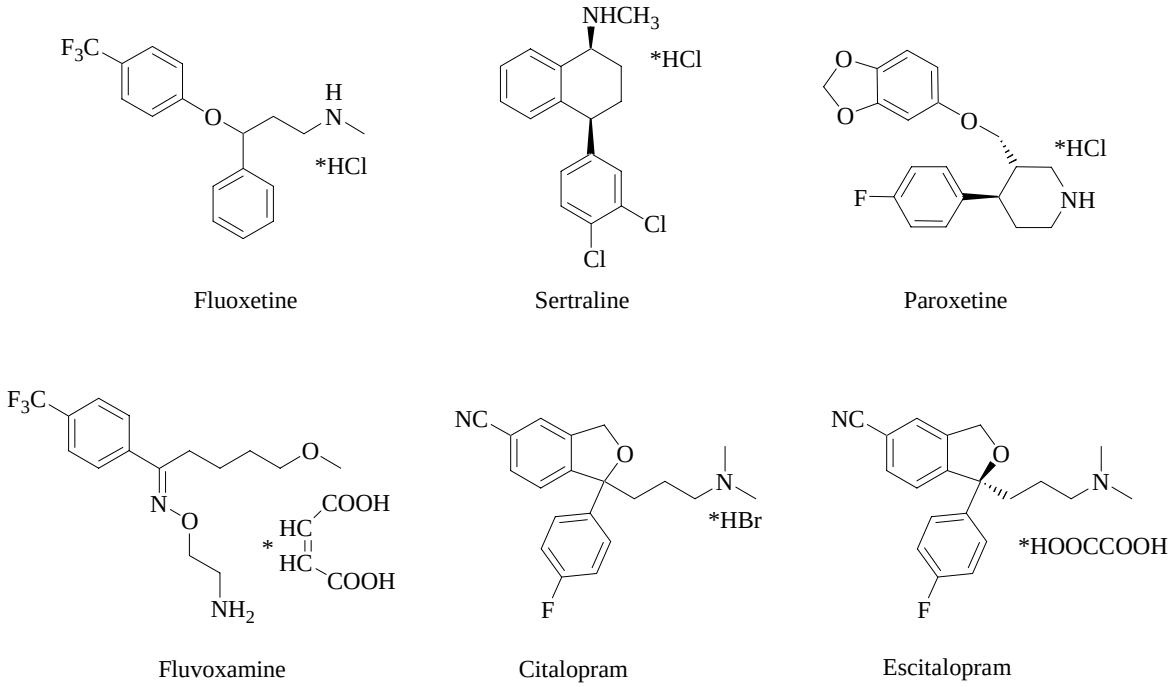


Figure 6. Structures of six currently prescribed SSRIs.

Chemistry, pharmacology and metabolism. All the SSRIs contain an asymmetric or chiral carbon, except fluvoxamine. However, only the pharmacologically active form of sertraline and paroxetine was marketed, while fluoxetine and citalopram were first marketed as racemates. Further development revealed that the *S*-enantiomer of citalopram appeared to have all the beneficial properties of citalopram, and is now available as escitalopram (Lexapro®) (Figure 6).[61,62] All the SSRIs selectively block the neuronal uptake pumps for 5-HT and, at the same time, act as indirect agonists at several 5-HT receptors: 5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3.[63,64] It is theorized that this agonistic activity over the 5-HT receptors mediates both the desired and undesired effects of SSRIs.[32] As a result of their selective inhibition of the SERT, SSRIs can reach concentrations that achieve almost complete inhibition without producing inhibition of the NE transporter (NET) or the DA transporter (DAT), and therefore share many similarities[65] (Table 3).

Table 3. Pharmacological similarities between SSRIs.[47,65]
(Adapted from Refs. 47 and 65).

1. Equivalent acute and maintenance antidepressant efficacy.
2. A flat dose-response curve for antidepressant efficacy.
3. An ascending dose-response curve for adverse effects.
4. An adverse effect profile consistent with excessive serotonin agonism.
5. 60 to 80% inhibition of serotonin uptake at their lowest, usually effective antidepressant dose
6. Efficacy in both depressive and anxiety disorders.

Because all the SSRIs have a flat dose-response curve, there is usually no advantage in using doses above the effective minimum dose, except under special circumstances. Figure 7 illustrates the FDA recommended lowest effective doses for the

treatment of depression for the six SSRIs.[47] These doses usually produce comparable effects on plasma 5-HT levels and the 5-HT uptake pump, regardless of the SSRI.[69]

The half-lives of the SSRIs under steady-state conditions are all close to 24 h: citalopram and escitalopram 1.5 days,[66] fluvoxamine 0.5-1 day[67], paroxetine 1 day (at 20 mg/day),[68] and sertraline 1 day.[68] For this reason, all of them except for fluvoxamine are recommended to be dosed once a day. The only antidepressant with a substantially longer half-life than 24 h is fluoxetine. It has a life time of 2 to 4 days, and its active metabolite, norfluoxetine, has a half life of 7 to 15 days.[69] For this reason, there may be a gradual development of adverse effects, sometimes making their detection difficult and can be persistent for a sustained period of time after the drug is discontinued.[69]

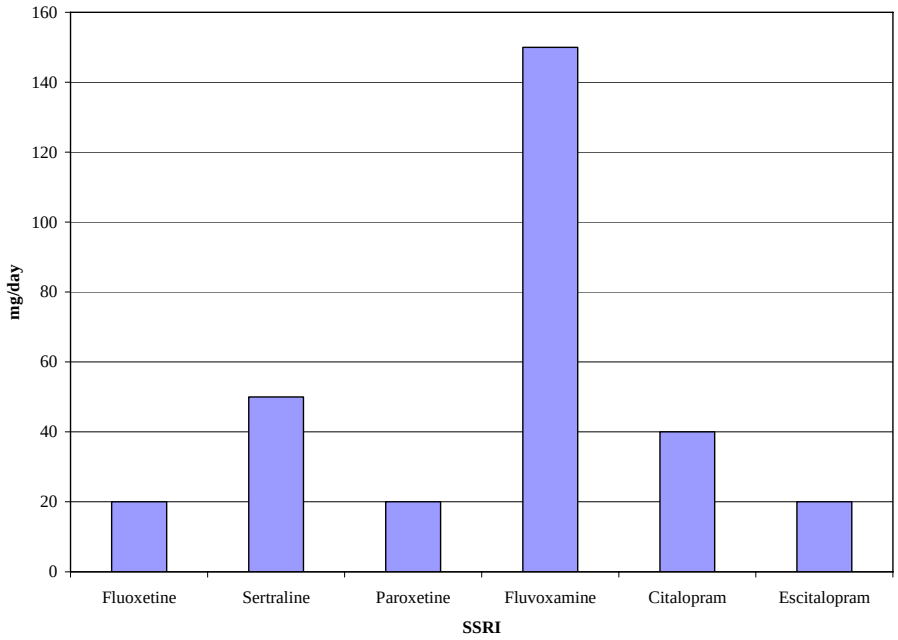


Figure 7. FDA recommended lowest effective dose of SSRIs for major depression.[33]

Safety and tolerability. SSRIs have good safety in terms of drug-drug interactions.[70] Also, as a group SSRIs have good tolerability, especially since they are

devoid of the cardiac toxicity present in older antidepressants. Also, there is almost no risk of overdose because of their wide therapeutic index, and no serious systemic toxicity has been demonstrated.[65,69] Finally, even though SSRIs are often taken on a long-term basis to prevent recurrence of depression, studies have found no evidence of long-term safety problems.[71] However, SSRIs should be avoided during pregnancy and lactation since studies have revealed that there is an increased incidence of miscarriage, premature birth, low birth weight and minor abnormalities in neonates exposed to SSRIs during the last trimester.[72] Additionally, it has been found that SSRIs are excreted through breast milk[73] and their long term effects on the newborn are yet to be documented.

Adverse Effects. Despite their indisputable efficacy, current SSRIs still exhibit some undesired side effects due to the complexity of the serotonin system in the brain.[47] Nausea is commonly associated with SSRIs (15-30%).[74] Other side effects include heightened states anxiety, agitation and restlessness, as well as headache, dizziness, sleep disturbances, sweating, tremor, and dry mouth.[71] Anorexia and weight loss have also been observed; however, in the long run there is a tendency to gain back the lost weight.[75] Additionally, sexual dysfunction occurs in 30-40% of the patients treated with SSRIs. Anorgasmia, decreased libido, and male erectile dysfunction are all commonly cited.[76-78] Another very important consideration is that patients treated with SSRIs experience a slow onset of action of at least 2-4 weeks, which can be higher in some cases depending on the SSRI.[4,47] Finally, in 2004 the FDA required manufacturers to include a “black box” warning label on antidepressants that alerts healthcare providers and consumers to a increased risk of suicidal thinking and behavior in children and adolescents treated with antidepressants, including the SSRIs.[79,80]

The Complex Serotonin System

Serotonin (5-HT) is one of the most important and studied neurotransmitters in the central and peripheral nervous systems.[81] Figure 8 depicts a model of a serotonergic synapse where the presynaptic and postsynaptic molecular entities involved in the synthesis, release, signaling, and reuptake of 5-HT are shown.[90] Tryptophan hydroxylase is transported to serotonegic synapses, where it initiates the synthesis of 5-HT. After 5-HT is synthesized and released, it activates as many as fourteen different receptors. The 5-HT_{1D} autoreceptor is located at the presynaptic side of the synapse. The other receptors, coupled to their respective G proteins, are located postsynaptically. The 5-HT_3 receptor, which is not G-protein coupled, functions as a receptor ionophore.[90]

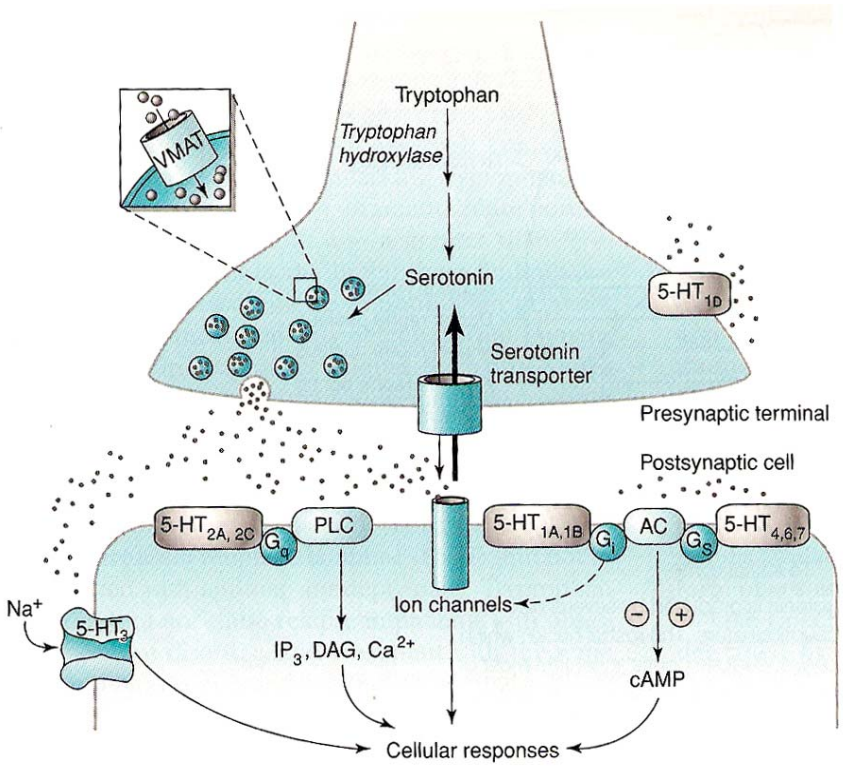


Figure 8. Model of a serotonergic synapse.[90] VMAT, vesicular monoamine transporter; PLC, phospholipase C; AC, adenyl cyclase; IP_3, inositol triphosphate; DAG, diacylglycerol. (Reproduced directly from Ref. 90).

The plasma membrane serotonin transporter (SERT) plays a very important role in the termination of serotonergic neurotransmission and is of much interest as the molecular target of SSRIs and other antidepressants.[82] The human SERT (hSERT), cloned in 1993,[83,84] is a 630-amino acid protein and is believed to have 12 transmembrane domains connected by alternating extracellular and intracellular loops, with N- and C-termini facing the intracellular compartment (Figure 9).[85]

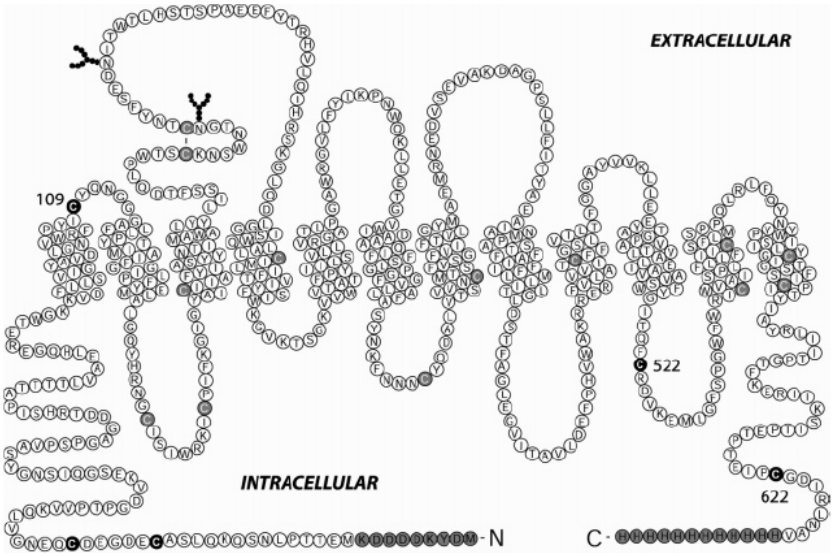


Figure 9. Snake representation of hSERT.[85] (Reproduced directly from Ref. 85).

SERT is closely related (40-90% sequence homology)[86] to the transporters of the other monoamines (DA and NE) and belongs to the family of the Na^+/Cl^--dependent membrane transporters.[82] Little is known about the tertiary structure of this class of transporters because a high-resolution structure is not yet available.[85] However, a very recent study by Ravna and coworkers[87] used the crystal structure of the lactose permease symporter (lac permease) as a template for molecular modeling of SERT, DAT and NET. These transporter models were based on the hypothesis that lac permease and the monoamine transporters belong to a common folding class due to their common functional

mechanism of using an ion gradient as energy source for translocation of molecules against a concentration gradient. The molecular modeling methods used in this study included amino acid sequence alignment, homology modeling and molecular mechanical energy calculations (Figure 10).[86]

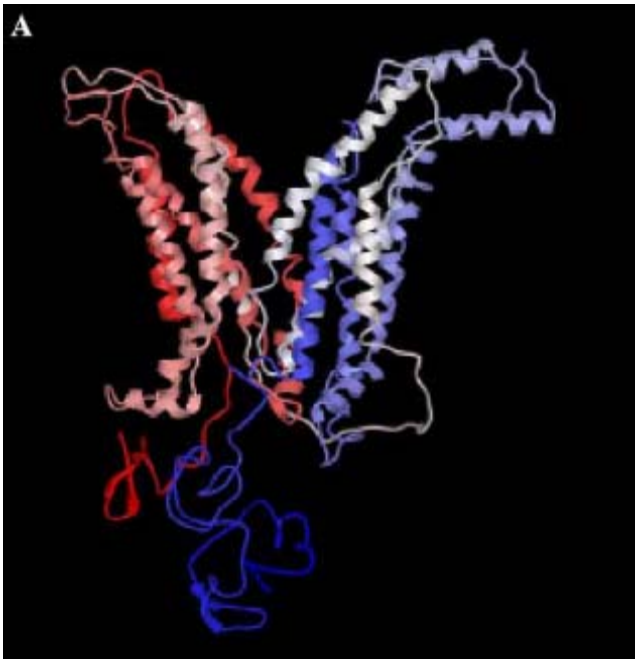


Figure 10. Energy minimized SERT model viewed in the membrane plane.[86]
(Reproduced directly from Ref. 86).

The models presented in this study were used to dock several known psychomotor stimulants (cocaine, *S*-citalopram and *S*-amphetamine) thus providing preliminary insight into their putative binding sites and the molecular mechanisms of their binding affinities. From these docking studies, several amino acids were suggested to be key for ligand interactions with the SERT (Asp98, Phe117, Tyr267, Phe551, among others) (Figure 11).[86] However, further research is needed to confirm the assumptions made in this study.

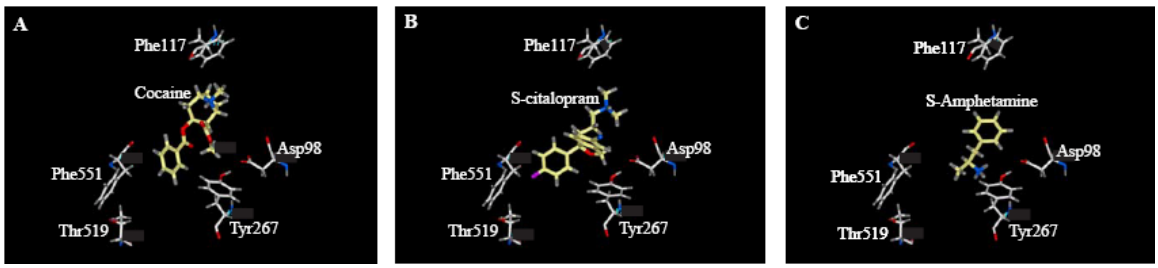


Figure 11. Cocaine (A), *S*-citalopram (B) and *S*-amphetamine (C) docked into SERT model.[86] (Reproduced directly from Ref. 86).

In an attempt to fully understand the different and complex physiological processes in which 5-HT is involved, over the last two decades a monumental research effort has yielded the characterization of multiple 5-HT receptor subtypes by a variety of molecular biologic, radioligand binding, and electrophysiologic studies.[88,89] Aside from the SERT, there are presently fourteen structurally and pharmacologically distinct receptor subtypes, classified into seven subfamilies, 5-HT_1 to 5-HT_7.[81,90] The extraordinary size of the 5-HT receptor family has many intriguing implications.[90] Even though much is still to be learned concerning the purpose of each 5-HT receptor, it is clear that they all play important physiological roles. A number of pharmacologic agents have aided researchers in the characterization and identification of the potential functional significance of each serotonin receptor subtype.[88]

Evidence indicates that the 5-HT_1 and 5-HT_2 receptor families are closely linked to mood disorders and psychoses, respectively. Particular attention has been set given to their role in depression[91-93] and generalized anxiety.[88,89,94] The 5-HT_3 receptors, on the other hand, are linked to vestibular control and have the potential to treat chemotherapy-induced nausea and vomiting, and irritable bowel syndrome.[81,96] The 5-HT_4 family is proposed to have mechanistic links to cardiac arrhythmias, neurological degenerative

diseases, and urinary incontinence.[97] Although the 5-HT_5 and 5-HT_6 receptor families remain poorly understood, the latter 5-HT_6 family has been recently identified as a potential target for therapeutics for Alzheimer's disease and other cognitive disorders.[95] Finally, the 5-HT_7 family is hypothesized to have a link to sleep disorders and cognitive deficits.[81,97]

5-HT_1 receptor class. 5-HT_1 receptors constitute the largest class of serotonin receptor subtypes. Based on conservation of structure, pharmacology and common signaling mechanisms,[96] the 5-HT_1 receptor class has been organized into five major subtypes: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}.[81,97] Among these subtypes, 5-HT_{1A} receptors have received a great deal of attention due to their role in generalized anxiety disorders and depression.[88,89]

5-HT_{1A} receptors were among the first 5-HT receptors to be cloned.[98,99] They belong to the family of G-protein coupled recetors and consist of seven transmembrane (TM) helices connected by intra- and extracellular loops; a comformation shared by all other 5-HT receptors except 5-HT_3.[89] 5-HT_{1A} receptors contain 421 amino acid residues,[90] and are largely distributed throughout the CNS. In the raphe nuclei, they are somatodendritic and act as autoreceptors to inhibit cell firing; postsynaptic 5-HT_{1A} receptors are present in a number of limbic structures, particularly in the hippocampus. Activation of 5-HT_{1A} receptors causes neuronal hyper-polarization.[97] Buspirone (Buspar®) (Figure 12), a 5-HT_{1A} partial agonist, was originally synthesized as a potential antipsychotic medication but was later found to be effective for the treatment of anxiety, with comparable anxiolytic effects as diazepam.[100] Later it was also found to be effective in the treatment of mixed anxious-depressive patients.[101] Buspirone's unique effect

profile has expanded the possible uses of anxiolytics, namely, buspirone has effectively been used to treat sexual dysfunction in patients with generalized anxiety.[102] Additionally, a study using 5-HT_{1A} receptor deficient mice demonstrated heightened levels of anxiety in several controlled rodent stress tests.[103] Interestingly, the 5-HT_{1A} receptor also plays a key role in enhancing therapeutic efficacy and shortening the onsets of action of SSRIs when pindolol (Visken®) (Figure 12), a 5-HT_{1A} receptor antagonist, was co-administered to depressed patients.[104] Finally, recent evidence suggests that 5-HT_{1A} antagonists may reverse the cognitive deficits in Alzheimer's disease.[105]

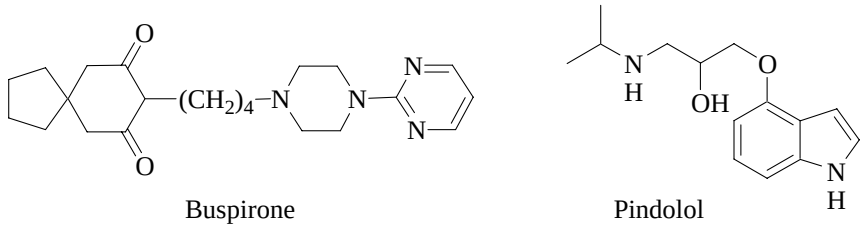


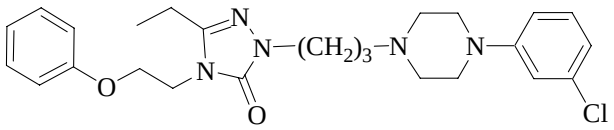
Figure 12. Structure of two 5-HT_{1A} ligands.

5-HT_{1B} and 5-HT_{1D} receptors have been the subject of extensive study due to their implication in migraine headaches. They contain 390 and 377 amino acid residues respectively.[90] Sumatriptan, a potent and selective agonist of 5-HT_{1D} receptors, has been used since 1993 as a highly effective treatment of migraines with minimal side-effects.[88,90] Since the year 2000, there has been much focus on mixed 5-HT_{1B}/5-HT_{1D} agonists as anti-migrane therapeutics with better pharmacokinetic profiles and reduced cardiovascular effects.[106] While progress in the area of the 5-HT_{1E} receptors has remained elusive, great efforts have been made in the past few years to identify potent 5-HT_{1F} receptor agonists, also for the treatment of migraines.[107]

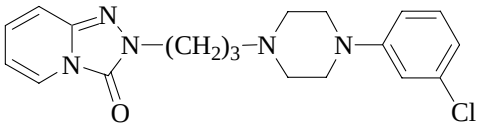
5-HT$_2$ receptor class. 5-HT$_2$ receptors are a very significant component of the 5-HT receptor family.[81] There are three distinct types of 5-HT$_2$ receptors: 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$. These subtypes share approximately 50% overall amino acid homology[108]

5-HT$_{2A}$ receptors, along with 5-HT$_{1A}$ receptors, were among the first members of the family to be identified, cloned[109] and extensively reviewed.[110,111] The 5-HT$_{2A}$ receptors contain 471 amino acid residues,[90] and are very widely distributed in peripheral and central tissues.[97] In the brain, the highest density occurs in the neocortex, claustrum and basal ganglia.[89,97] 5-HT$_{2A}$ receptors have been found to play an important role in anxiety, depression, anorexia nervosa, migraines, sleep, hypertension, and even certain cardiovascular disorders.[89,97,112] Consequently, 5-HT$_{2A}$ receptor antagonism has been found effective in restoring undesired serotonergic side effects. For example, 5-HT$_{2A}$ antagonism in several animal studies has illustrated decreased anxiety while stimulation of the 5-HT$_{2A}$ receptor has caused an anxiogenic effect.[113] Additionally, antagonism of the 5-HT$_{2A}$ receptor has been found to alleviate some of the SSRI-induced side effects such as sexual dysfunction. Studies have shown that a patient's SSRI induced sexual dysfunction can be treated with a nefazodone (Serozone®), an antidepressant with strong antagonism for 5-HT$_{2A}$.[114] Trazodone (Desyrel®), a related antidepressant and another strong antagonist of 5-HT$_{2A}$ receptors, has also proved effective in alleviating SSRI-induced side effects when prescribed simultaneously (Figure 13).[88] It is believed that these antagonistic molecules alleviate SSRI induced-side effects by reducing the heightened serotonergic activity at the 5-HT$_{2A}$ receptors. Interestingly, it has been established that all hallucinogenic drugs are partial agonists at 5-HT$_{2A}$ receptors: D-lysergic acid diethylamide (LSD), *N*,*N*-dimethyltryptamine (DMT), psilocybin, mescaline

and dimethoxymethylamphetamine (DOM). These findings stimulated interest in determining the role of 5-HT_{2A} receptors in psychotic disorders such as schizophrenia, and many of the most recently released modern antipsychotics (i.e., risperidone, clozapine, olanzapine) now exert potent antagonist activity in addition to their dopamine D_2 receptor antagonism.[90] This issue will be later discussed in detail.



Nefazodone



Trazodone

Figure 13. Structure of two 5-HT_{2A} antagonists effective in the treatment of SSRI-induced side effects.

5-HT_{2B} receptors have been implicated in the treatment of migraines and gastric motility,[115,116] while potential uses for 5-HT_{2C} ligands include anxiety, depression, obesity and cognitive dysfunction.[117-119] Recent studies have identified several 5-HT_{2C} agonists and partial agonists as potential anti-obesity therapeutics.[120,121]

5-HT_3 receptor class. The 5-HT_3 receptor is a ligand-gated ion channel, in contrast to all other known 5-HT receptors.[122] They are found in the periphery and also in the CNS, especially in the frontal cortex and hippocampus.[123] As mentioned before, 5-HT_3 antagonists are well known for the treatment of chemotherapy-induced nausea and vomiting, and irritable bowel syndrome.[81,96] There are also indications that they may be effective for the treatment of migraine and as enhancers of memory.[123] The chemistry

and pharmacology of 5-HT_3 agonists is less well understood and is currently under investigation.[81]

5-HT_4 receptor class. 5-HT_4 receptors have been located in several parts of the limbic system in the CNS and also in peripheral tissues.[124] 5-HT_4 receptors in the CNS appear to modulate neurotransmitter release and enhance synaptic transmission, and they may also play a role in memory enhancement.[97] As mentioned earlier, selective 5-HT_4 receptor agonists have been proposed to possess putative therapeutic utility in a number of disorders, including cardiac arrhythmia, neurodegenerative diseases and urinary incontinence.[97] Additionally, recent evidence suggests that 5-HT_4 agonists and partial agonists may also be useful in the treatment of irritable bowel syndrome.[125]

5-HT_5 receptor class. This class of receptors has remained poorly understood, mainly due to the lack of selective pharmacological agents to help elucidate their exact physiological function.[81,97] Two subtypes of the 5-HT_5 receptor (5-HT_A and 5-HT_B), sharing 70% overall sequence identity, have been found in rodents. However, only the 5-HT_{5A} subtype has been found in humans.[126] Nevertheless, potential roles have been proposed for this class of receptors, which include motor control, feeding, anxiety, depression and brain development.[127]

5-HT_6 receptor class. These receptors appear to be localized exclusively in the CNS and predominantly in the corpus striatum and in various limbic and cortical regions.[89] Interestingly, several antipsychotics and antidepressants have shown high affinity towards this receptor, a fact that has suggested its potential involvement in the pathogenesis of several neuropsychiatric disorders (bipolar disorders, Parkinson's

disease, among others).[128,129] Additional, recent evidence identifies 5-HT_6 receptors as a potential target for therapeutics for Alzheimer's disease and other cognitive disorders.[95]

5-HT_7 receptor class. This receptor class is the newest member of the 5-HT receptor family. They have an extensive vascular distribution but are also expressed in nonvascular smooth muscle and the CNS, in the thalamus, hypothalamus and cortical regions.[97] In addition to migraine, depression and schizophrenia, 5-HT_7 antagonists may be useful in the treatment of sleep disorders and cognitive deficits.[130] The lack of selective agonists has hindered the identification of the exact biological effects produced by stimulation of this receptor.[81,97]

Bivalent Approaches for Therapeutic Intervention

As mentioned before, SSRIs are effective in the treatment of major depression and related disorders, and are currently the pharmacological treatment of choice.[32,47,48] However, SSRIs still face challenges, especially concerning their onset of action and a series of SSRI-induced side effects which are believed to be caused by the non-selective interaction of current SSRIs with the large 5-HT receptor family.[47]

In order to address some of these problems, several strategies have been developed over the years to "potentiate" the antidepressant response of SSRIs.[93,104] The most notable of these strategies has been the use of pindolol (Figure 12), a 5-$HT_{1A/1B}$ and β-adrenoreceptor antagonist, to accelerate and in some cases enhance the onset of antidepressant effects when administered in combination with SSRIs.[93,104] The use of pindolol as an enhancer in depressed patients stemmed not from its β-adrenergic properties, but from its 5-$HT_{1A/1B}$ antagonistic activity.[104] SSRIs are thought to have a

delayed onset of action because of the need to overcome the inhibitory influence of 5-HT_{1A} somatodendritic autoreceptors, which inhibit the firing rate of serotonergic neurons. However, after chronic administration of SSRIs, a functional desensitization of 5-HT_{1A} autoreceptors occurs, resulting in an increased release of 5-HT in the prefrontal cortex of the brain. The time necessary to obtain these adaptative changes is consistent with the delayed onset of action of SSRIs in the treatment of major depression.[131,132]

The latter observations rapidly led to the first attempts to develop molecules that could concomitantly antagonize 5-HT_{1A} receptors while blocking the serotonin transporter in order to obtain a novel class of improved SSRIs with a faster onset of action.[133] Although the results of these studies were modest, a worldwide research effort was launched in pursuit of this goal and many examples can be found in the literature from both academia[131,134-136] and industry.[137-140] This idea continues to be of interest as evidenced by the continuous emergence of literature reporting advances in the subject.[141,142] However, therapeutic agents with this mechanism of action have not yet been marketed

Another example of enhancement of antidepressant efficacy by combining two mechanisms of action into one molecular entity is the case of dual 5-HT and NE reuptake inhibitors.[132,143] This approach has yielded a new class of antidepressants with a higher rate of efficacy and a lower dropout rate,[144] which include commercially available therapeutics such as venlafaxine (Effexor®)[41] and more recently, duloxetine (Cymbalta®)[43,44] (Figure 4). After the FDA-approval of Cymbalta® in 2004, and considering its well-tolerated profile and higher efficacy than venlafaxine itself, great research efforts continue in this area in the search for even more potent analogs.[143]

The process of *coupling* different structural moieties into one molecule with the purpose of improving efficacy has had a long history of success. The case of atypical antipsychotics with better adverse reaction profiles illustrates a good example. Although the complete mechanism of action has yet to be determined for the typical antipsychotics, it is known that these older medications posses affinity for dopaminergic neurons, particularly the D_2 dopamine receptor. They are used to treat chronic psychosis, including schizophrenia and manic states; however, their adverse effects include tardive dyskinesia, headache, vertigo, and a high risk of seizures.[145] Clinical evidence indicates that risperidone (Risperdal®) and clozapine (Clozaril ®) yield a better adverse reaction profile with equal if not better therapeutic effectiveness in controlled studies.[145] Other examples are olanzapine (Zyprexa®) and aripiprazole (Abilify®) (Figure 14). Notably, these new antipsychotics are known as atypical because they posses affinity for both the dopamine 2 (D_2) and the serotonin 2A (5-HT_{2A}) receptors. Interestingly, all these atypical antipsychotics possess a piperidine or piperazine 5-HT_{2A} antagonistic moiety in their structure.

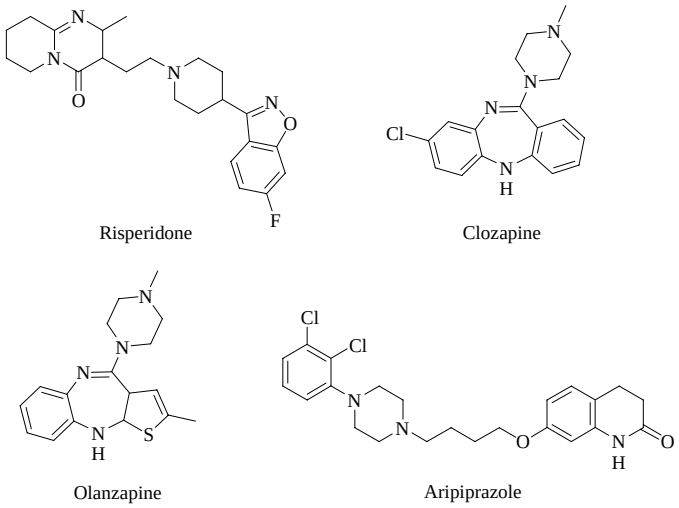


Figure 14. Structure of four atypical antipsychotics.

As evidenced by the previous examples, the advantages to combining two therapeutically active moieties into one molecular structure are several fold. First, with a bivalent therapeutic, only one pharmaceutical is administered as opposed to two, thus, decreasing the likelihood of drug-drug interactions and producing unwanted side effects. In addition, a bivalent pharmaceutical has the potential to decrease the cost of therapy since the patient will only be required to purchase one prescription. Finally, the *coupling* approach holds promise to obtain a more potent and efficient molecule for treating the target disease. It is also supportive that many research groups have now taken this "coupling" approach to create improved drugs and that combinatorial chemistry as well as rational drug design are largely responsible for the expanding pharmaceutical market.[112,132,146,147]

Rationale for Drug Design

It is clear that despite their indisputable efficacy, current SSRIs still face a variety of undesired side effects due to their non-selective interaction with the complex 5-HT receptor system.[47,48] Research efforts are currently underway to address some of these adverse effects, and have been explained previously. However, there are still important issues to consider. As mentioned before, patients treated with SSRIs reportedly suffer from heightened states of anxiety, agitation and restlessness during the first weeks of treatment, as well as headache, dizziness, anorexia, sleep disturbances, sweating, tremors, and dry mouth.[71] Additionally, a high incidence of sexual dysfunction is reported.[76-78]

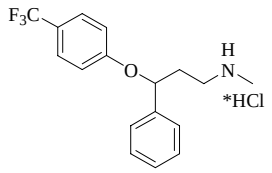
On the other hand, it has been established that activation of 5-HT_{2A} receptors plays an important role in anxiety, depression, anorexia nervosa, migraines, sleep, and hypertension, among others.[89,97,112] Moreover, 5-HT_{2A} receptor antagonistic agents have

been found effective in restoring some of these undesired serotonergic side effects when prescribed simultaneously with an SSRI (for example, nefazodone, trazodone, mirtazapine).[113,114,132]

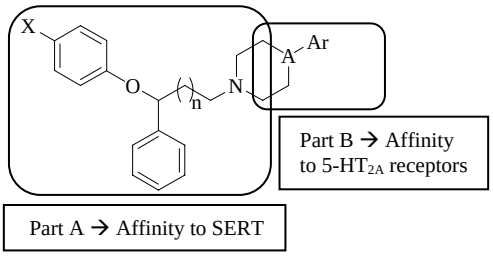
Therefore, it is our hypothesis that by merging into one molecular entity an enhanced antagonism towards 5-HT_{2A} receptors while maintaining a highly selective inhibition of the serotonin transporter (SERT), a superior psychotherapeutic agent may be achieved, with a decreased adverse reaction profile and potential applications in a wider variety of depressive and anxiety disorders. A molecule of such kind would have the potential to increase 5-HT on the synaptic cleft alleviating the symptoms of depression as well as blocking 5-HT interaction with the 5-HT_{2A} receptors minimizing the SSRI-induced side effects.

Strategic Design

Our chemical strategy for constructing this library of bivalent molecules was to covalently couple two types of fluoxetine structural homologues (for SERT affinity) with a series of nine functionalized piperazines and piperidines (for 5-HT_{2A} affinity) using the "overlapping type" approach (Figure 15).[133,134]



Fluoxetine hydrochloride (Prozac®)
Target: Inhibition of Serotonin Transporter



Novel Bivalent SSRIs
Targets: a) Inhibition of Serotonin Transporter and
b) Inhibition of 5-HT_{2A} receptors

Figure 15. Rationale for bivalent SSRI drug design.

Fluoxetine hydrochloride (Prozac®), one of the leading SSRIs prescribed, was chosen as a template for our bivalent SSRIs. A great deal of structure-activity relationship (SAR) studies has been done on fluoxetine hydrochloride to characterize its molecular structure. Using X-ray crystallography (Figure 16), Robertson and coworkers[148] established that the methyl propanamine moiety adopted a folded three-dimensional relationship to the trifluoromethylphenoxy moiety. It was proposed that this folded relationship was induced by the 3-phenyl group and modifications at this position demonstrated that removal of this group causes a 62-fold decrease in potency.[148] It was theorized that the 3-phenyl moiety possibly interacts with a hydrophobic pocket in the SERT and acts as a molecular anchor. Moreover, Robertson found that the two aromatic rings were skewed which excludes any aryl-aryl interaction and that the strong electronegative and hydrophobic trifluoromethyl substituent located in the *para* position of the phenoxy moiety was primarily responsible for the serotonin-uptake carrier affinity. Replacement of the *para*-trifluoromethyl substituent with an *ortho*-methoxy or methyl group altered the selectivity of the molecule from 5-HT to NE uptake inhibition.[148]

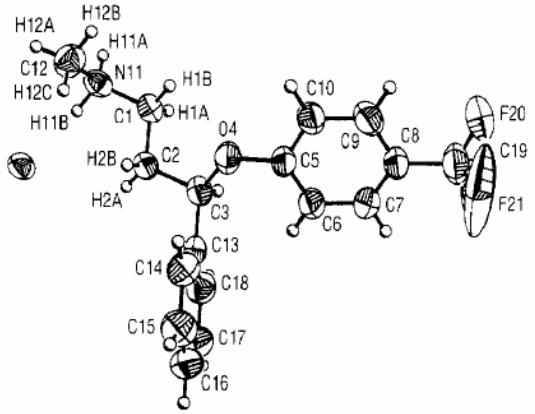


Figure 16. Computer-generated ORTEP drawing with crystallographic numbering system.[148] (Reproduced directly from Ref. 148).

It is important to note that fluoxetine hydrochloride, which contains one chiral center in its structure, is clinically prescribed as a racemic mixture. A different study by Robertson and coworkers[149] determined that the *S*- and *R*-enantiomers of fluoxetine have a very similar and potent affinity towards the serotonin transporter, 21 and 33 nM respectively, thereby justifying the clinical use of the racemate.

As mentioned before, numerous chemical agents have been used to investigate the physiologic functions of 5-HT receptors. Serotonin itself binds at most populations of 5-HT receptors with nanomolar affinity. Although no agent displays an absolute specificity for one 5-HT receptor subtype over another, certain generalizations have been made.[89] Specifically, studies have shown that alkyl- and aryl-piperazines (simple and long chain), and alkyl- and aryl-piperidines bind with high affinity and/or specificity to the 5-HT_{1A} and 5-HT_{2A} receptors in particular. The nature of the substituents has a great effect on their individual intrinsic activity.[89,150,151] Several examples can be found in previously mentioned commercially available medications such as mirtazapine (Figure 4), buspirone (Figure 12), nefazodone and trazodone (Figure 13), among many others. Based on these observations and an extensive review of the literature, nine functionalized piperazines and piperidines (Figure 17) where chosen as potential 5-HT_{2A} antagonistic moieties for the designed compounds and were incorporated into their structure: 1-phenyl-piperazine[112,141,152] (I), 1-(3-chlorophenyl)-piperazine[141,150,153] (II), 1-(3-trifluoromethyl-phenyl)-piperazine[141] (III), 1-(2-methoxyphenyl)-piperazine[112,141,154] (IV); 1-(2-pyrimidyl)-piperazine[112,141,154] (V), 1-methylpiperazine[152] (VI); 1-(4-fluorobenzoyl)-piperidine[112,154,155] (VII), 4-(6-fluoro-3-benzo[d]isoxazolyl)-piperidine[112,154,155] (VIII), and 1-[3-(4-fluoro-benzoyl)-propyl]-piperazine[112,154,155] (IX).

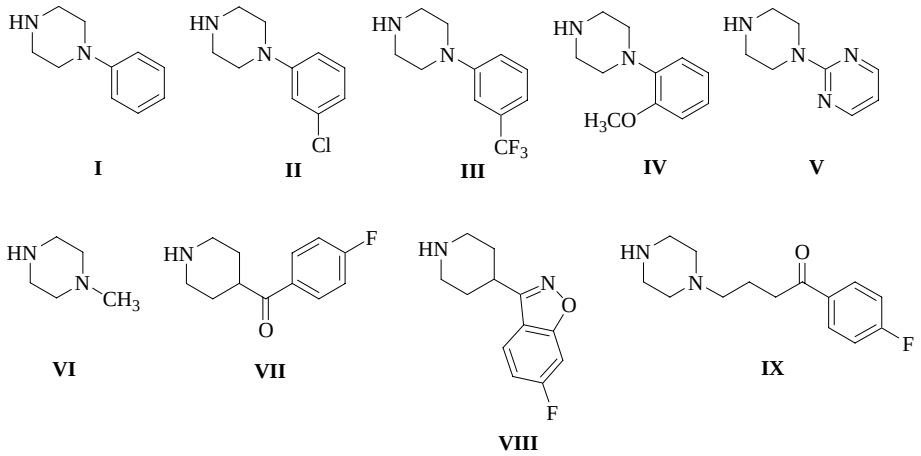


Figure 17. Structures of nine aryl- and alkyl-piperazines and piperidines with demonstrated 5-HT_{2A} receptor antagonism.

Homologues Type I and II

As mentioned previously, the chemical strategy for constructing the desired library of bivalent molecules was to covalently couple two types of fluoxetine structural homologues with a series of nine functionalized piperazines and piperidines. Taking into consideration the above mentioned SAR studies on fluoxetine hydrochloride, it was decided that for the library of bivalent molecules, the 3-phenyl moiety and the *para*-substitution at the phenoxy moiety would both be kept as key elements for binding at the SERT. Structural modifications would be introduced by having either an ethyl- or propyl-chain linker between the *para*-phenoxyphenyl moiety and the amine moiety. Additional structural variations would be introduced by alternating either a fluoro- or a trifluoromethyl substituent at the *para*-phenoxy position. These structural variations gave rise to a library of thirty-three novel compounds classified into two main groups: 1) The 2-[(4-aryloxy)-2-phenyl]-1-amino ethane derivatives or Homologues Type I; and 2) the 3-[(4-aryloxy)-3-phenyl]-1-amino propane derivatives or Homologues Type II (Table 4).

Table 4. General structures of homologues type I and II.

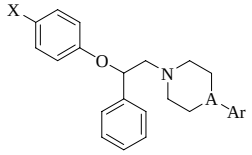
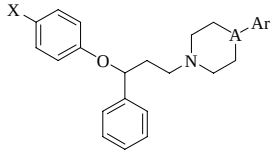
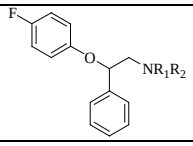
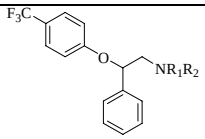
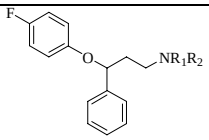
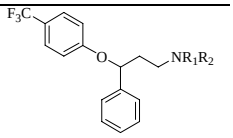
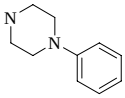
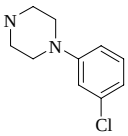
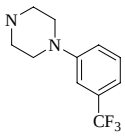
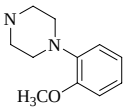
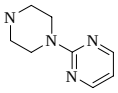
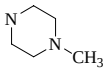
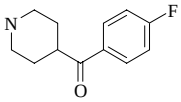
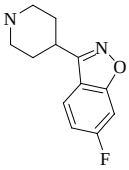
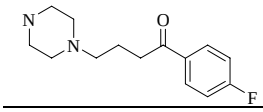
Homologues Type I Aminoethane Derivatives	Homologues Type II Aminopropane Derivatives
	
X = F or CF_3 A = N or C (piperazine or piperidine) Ar = variety of aryl or alkyl groups	X = F or CF_3 A = N or C (piperazine or piperidine) Ar = variety of aryl or alkyl groups

Table 5 depicts the structures of the novel compounds and the identifying numbers assigned to them. The synthetic design, thus, hopes to distinguish the importance of the nature of the *para*-substitution at the phenoxy moiety and the length of the alkyl-chain linker for a potent and selective affinity towards the SERT; as well as the nature of the cyclic amine moiety for affinity towards the 5-HT_{2A} receptor. Biological evaluation of the molecules at both molecular targets, and comparison to fluoxetine hydrochloride will clarify their potential as novel SSRI molecules with decreased adverse reaction profiles.

It is also important to mention that previous studies in the Pinney group by Dorsey and co-workers support the research described herein.[147] Under the same theory, although through different synthetic routes, three fluoxetine hydrochloride-like homologues were synthesized: A, B and C (Table 5). The three molecules were found to exhibit single-site binding at the SERT, although at the micromolar level (1.45, 9.56, 3.27 μM respectively)[147] as opposed to fluoxetine hydrochloride's documented average of 30 nM affinity.[132] Additionally, their potential activity over the 5-HT receptors was not addressed, thus holding areas for expansion. It is proposed that these new fluoxetine-like

homologues may hold the same single-site binding with better SERT affinity and specificity over the 5-HT_{2A} receptors.

Table 5. Structures of studied compounds and identifying numbers assigned.

Amino Fragment \ Alkyl Fragment	Homologues Type I		Homologues Type II	
				
	1	2	16	17
	A[a]	3	18	19
	B[a]	4	20	21
	C[a]	5	22	23
	6	7	24	25
	8	9	26	27
	10	11	28	29
	12	13	30	31
	14	15	32	33

[a] Compounds prepared by Dorsey and co-workers.[147]

Interestingly, fluoxetine hydrochloride (Figure 15) contains a secondary amine that is rapidly de-methylated in the liver. Its metabolite, norfluoxetine, has a long half-life of 7 to 15 days and is also biologically active.[32] This is positive in the sense that it minimizes the drug concentration changes when patients have poor compliance and miss a dose. However, this is also negative because it takes up to 4 weeks for the therapeutic levels to reach a steady state, and due to the long presence of norfluoxetine in the liver and plasma, fluoxetine hydrochloride cannot be prescribed to patients with liver or renal disorders.[32] Moreover, it has been proposed that this long half-life of fluoxetine and norfluoxetine contributes in some manner to the adverse side effects often reported.[146] Accordingly, these new homologues have the potential to avoid the de-methylation problem due to the cyclic tertiary nature of their amine moiety.

The Case for Autism

Finally, in the last decade it has been found that SSRIs have yet another application. Clinical evidence has shown that SSRIs are commonly used and proven effective in the treatment of some of the most important and incapacitating symptoms associated with autism spectrum disorders. Autism is defined as a "pervasive developmental disorder",[156] which involves impairments in three fundamental areas: a) social relations; b) communications and use of language; and c) restricted and recurrent compulsive patterns of behavior and activities.[157] Moreover, the latter are considered to be "integral and core components" of autistic disorder.[156] Persons with autistic disorder may present different clinical symptoms depending on their chronological age. Hyperactivity, stereotyped behaviors, irritability and temper tantrums may be the most prominent symptoms observed in patients in their early childhood, while tic-like behaviors, aggressiveness, and self-

injurious behavior may develop later in the child's life. In adolescence and adulthood, depression and obsessive-compulsive phenomena are often observed.[158]

At present time, there is general agreement between researchers that autism and related conditions are neurobiological disorders, although the specific biological cause has not yet been identified.[158,160,162] Therefore, no treatment specifically based on a cause has been developed in order to cure autism. Nevertheless, intensive research efforts have been made to establish the relationship between neurotransmitters and the clinical features of autism and other neuropsychiatric disorders, and preliminary findings strongly suggest that neurochemical factors play a major role in autism.[160]

SSRIs such as fluoxetine, fluvoxamine, sertraline and paroxetine (Figure 6) have been identified by researchers and patients' families as some of the most clinically useful agents, especially in targeting repetitive preoccupations, perseverative behaviors and anxiety-related symptoms.[159-161] At the same time, atypical antipsychotics such as risperidone and clozapine (Figure 14) have also proven effective in improving other kinds of symptoms in patients with autism, such as hyperactivity, and in reducing the frequency and intensity of temper outbursts and aggression in patients with autism.[162]

Based on these clinical results, it is our hypothesis that if SERT reuptake inhibition and 5-HT_{2A} receptor antagonism activities can be merged into one molecular entity, its clinical utility could potentially include the treatment of autistic spectrum disorders. A drug of this sort would not only combine the desired effects of two commonly used pharmacotherapies in the treatment of autistic symptomatology, but also provide an improved side-effect profile and a lower maintenance cost.

CHAPTER THREE

Materials and Methods

General Section

All chemicals used for synthesis were obtained from commercial sources such as 3B Medical Systems, Acros Chemicals, Aldrich Chemical Company, Alfa Aesar, Lancaster Chemicals and Fisher Scientific and used without further purification. Reactions which involved air or moisture sensitive reagents were performed in oven-dried glassware under nitrogen atmosphere using dried syringes, needles and cannulas to transfer solvents and reagents. Reactions were monitored by thin layer chromatography (TLC) using Merck Kieselgel 60 F_{254} glass backed plates. The plates were visualized using a multiband 254/365 nm UV lamp. In some cases, gas chromatography/mass spectrometry (Hewlett Packard GCD system with electron impact ionization) was also used to monitor reactions. Purification of intermediates and products was achieved by flash chromatography using silica gel (230-400 mesh) purchased from BODMAN industries. Solvents used for chromatography (hexane, ethyl acetate and methanol) were purchased from the above mentioned chemical companies and distilled prior to use. Removal of solvents was performed in a rotary evaporator under vacuum followed by further drying with a mechanical pump at a pressure lower than 0.5 Torr.

Structure elucidation of intermediates and products was carried out using NMR spectroscopy. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded with a Bruker DPX-300 spectrometer operating at 300 MHz for proton, 75 MHz for carbon and 282 MHz for fluorine. All NMR were recorded in $CDCl_3$ (0.03% of TMS as reference) unless stated

otherwise. Chemical shifts are expressed in ppm (δ). Peak patterns are reported as singlets (s), doublets (d), triplets (t), quartets (q), and multiplets (m). Coupling constants (*J*) are expressed in Hz. NMR processing was carried out using WinNMR and MestRec 4.8.6.0 software. Elemental analysis was performed by Atlantic Microlab, Inc. in Norcross, Georgia. Melting points were determined using a Thomas-Hoover melting point apparatus and are reported uncorrected. Reversed phase HPLC was carried out with a Beckman HPLC system running 32 Karat Software 5.0 with a model 126 solvent module, a model 168 diode array detector and a AquaSep C8 5 µm 100Å Column (ES Industries, Berlin, Germany). Ultrapure water and acetonitrile grade HPLC were obtained from Alfa Aesar. Two HPLC running buffers were used: A (0.1% TFA in HPLC H_2O) and B (CH_3CN:H_2O with 0.1% TFA (9:1)). The standard method was 1 mL/min with simple gradient of 5-95% buffer B over 17 min. Sample volume was 10 µL and peaks were detected between 168 and 254 nm.

Synthesis of 4-Fluorophenoxy Ethyl Bromide Intermediate

(2-Hydroxy-2-phenylethyl)carbamic acid tert-butyl ester **(34)**.[147]

To a well stirred solution of 2-amino-1-phenyl ethanol (5.07 g, 37.0 mmol) in anhydrous DMF (50 mL) under N_2 at 25 °C was added *tert*-butyl dicarbonate (t-boc anhydride) (12.30 g, 56.36 mmol). After complete dissolution, the mixture was heated at 50 °C for 15 min, and then cooled to 25 °C. After stirring for 16 h at 25 °C, EtOAc (100 mL) was added to the reaction mixture followed by extraction with H_2O (3 x 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The residue was re-suspended in a minimal amount of

EtOAc and allowed to crystallize. The product **34** was afforded as a white crystalline solid (6.85 g, 28.9 mmol) in 78% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34 (m, 5H), 4.96 (bs, 1H), 4.83 (dd, J = 4.94, 2.89 Hz, 1H), 3.48 (ddd, J = 13.81, 6.73, 3.23 Hz, 1H), 3.26 (m, 1H), 3.14 (bs, 1H), 1.45 (s, 9H).

Melting Point: 123-124 °C.

[2-(4-Fluorophenoxy)-2-phenylethyl]carbamic acid tert-butyl ester **(35)**.[147]

To a well stirred solution of (2-hydroxy-2-phenylethyl)carbamic acid *tert*-butyl ester **34** (3.10 g, 13.1 mmol) in anhydrous THF (20 mL) under N_2 at 25 °C was added 4-fluorophenol (2.93 g, 26.2 mmol) and PPh_3 (5.15 g, 19.6 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (3.88 mL, 19.6 mmol) was slowly added dropwise. After stirring at 25 °C for 24 h, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (5:95 EtOAc/hexane). The product **35** was afforded as a white solid (3.17 g, 9.58 mmol) in 73% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.33 (m, 5H), 6.88 (m, 2H), 6.77 (m, 2H), 5.16 (dd, J = 8.45, 3.39 Hz, 1H), 5.01 (s, 1H), 3.64 (ddd, J = 14.32, 7.85, 3.85 Hz, 1H), 3.38 (m, 1H), 1.44 (s, 9H).

Melting Point: 98-102 °C.

2-(4-Fluorophenoxy)-2-phenylethylamine **(36)**.[147]

To a well stirred solution of [2-(4-fluorophenoxy)-2-phenylethyl]carbamic acid tert-butyl ester **35** (4.22 g, 12.8 mmol) in 1,4-dioxane (10 mL) was added 4N HCl in dioxane (30 mL) dropwise for a period of 10 min. After stirring at 25 °C for 16 h, the reaction

mixture was quenched adding a saturated solution of $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 MeOH/ EtOAc). The product **36** was afforded as a yellow oil (2.38 g, 10.3 mmol) in 81% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.31 (m, 5H), 6.83 (m, 4H), 5.02 (dd, J = 7.18, 4.18 Hz, 1H), 3.08 (m, 2H), 1.55 (s, 2H).

2-(4-Fluorophenoxy)-2-phenyl ethyl bromide **(37)**.[147]

To a well stirred solution of $TiBr_4$ (1.88 g, 7.03 mmol) in anhydrous DMF (40 mL) under N_2 at 25 °C was added dropwise a solution of *t*-butyl nitrite (0.60mL, 5.01 mmol) in anhydrous DMF (5 mL). A solution of 2-(4-fluorophenoxy)-2-phenylethylamine **36** (1.01 g, 4.37 mmol) in anhydrous DMF (5 mL) was added dropwise to the reaction mixture for a period of 20 min. After gas evolution ceased, the reaction mixture was quenched with 20% aq. HCl (150 mL) and extracted with Et_2O (4 x 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (100% hexane). The product **37** was afforded as a clear oil (0.20 g, 0.68 mmol) in 16% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.37 (m, 5H), 6.87 (m, 4H), 5.22 (dd, J = 8.33, 4.00 Hz, 1H), 3.73 (dd, J = 10.85, 8.36 Hz, 1H), 3.61 (dd, J = 10.87, 4.02 Hz, 1H).

Synthesis of 4-Trifluoromethylphenoxy Ethyl Bromide Intermediate

[2-Phenyl-2-(4-trifluoromethylphenoxy)ethyl]carbamic acid tert-butyl ester **(38)**.

To a well stirred solution of (2-hydroxy-2-phenylethyl)carbamic acid *tert*-butyl ester **34** (3.09 g, 13.0 mmol) in anhydrous THF (20 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (4.23 g, 26.1 mmol) and PPh_3 (5.13 g, 19.6 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (3.86 mL, 19.6 mmol) was slowly added dropwise. After stirring the reaction mixture for 24 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (5:95 EtOAc/hexane). The product **38** was afforded as a yellow oil (2.66 g, 6.98 mmol) in 53% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.51 (d, J = 8.31 Hz, 2H), 7.36 (m, 5H), 6.91 (d, J = 8.25 Hz, 2H), 5.29 (dd, J = 5.74, 2.96 Hz, 1H), 5.04 (m, 1H), 3.67 (ddd, J = 11.64, 7.44, 3.60 Hz, 1H), 3.43 (m, 1H), 1.44 (s, 9H).

2-Phenyl-2-(4-trifluoromethylphenoxy)ethylamine **(39)**.

To a well stirred solution of [2-phenyl-ethyl -2-(4-trifluoromethylphenoxy)ethyl] carbamic acid *tert*-butyl ester **38** (5.14 g, 13.5 mmol) in 1,4-dioxane (20 mL) was added 4N HCl in dioxane (30 mL) dropwise for a period of 10 min. After stirring for 16 h at 25 °C, the reaction mixture was quenched adding a saturated solution of $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 MeOH/ EtOAc). The product **39** was afforded as a yellow oil (1.55 g, 5.52 mmol) in 41% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.44 (d, *J* = 8.58 Hz, 2H), 7.34 (m, 5H), 6.92 (d, *J* = 8.49 Hz, 2H), 5.17 (dd, *J* = 7.32, 4.12 Hz, 1H), 3.16 (dd, *J* = 13.60, 7.33 Hz, 1H), 3.08 (dd, *J* = 13.60, 4.11 Hz, 1H), 1.70 (s, 2H).

2-Phenyl-2-(4-trifluoromethylphenoxy)ethyl bromide **(40)**.

To a well stirred solution of $TiBr_4$ (1.60 g, 4.35 mmol) in anhydrous DMF (40 mL) under N_2 at 25 °C was added dropwise a solution of *t*-butyl nitrite (0.51 mL, 4.23 mmol) in anhydrous DMF (5 mL). A solution of 2-phenyl-2-(4-trifluoromethylphenoxy)ethylamine **39** (1.09 g, 3.88 mmol) in anhydrous DMF (5 mL) was added dropwise to the reaction mixture for a period of 20 min. After gas evolution ceased, the reaction mixture was quenched with 20% aq. HCl (150 mL) and extracted with Et_2O (4 x 100 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (100% hexane). The product **40** was afforded as a yellow oil (0.32 g, 0.93 mmol) in 24% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.46 (d, *J* = 8.92 Hz, 2H), 7.37 (m, 5H), 6.94 (d, *J* = 8.96 Hz, 2H), 5.37 (dd, *J* = 8.39, 3.97 Hz, 1H), 3.75 (dd, *J* = 10.96, 8.41 Hz, 1H), 3.64 (dd, *J* = 10.95, 3.97 Hz, 1H).

Attempted Synthesis of Coupled Products from Bromide Intermediates

1-(4-Fluorophenoxy)-1-phenylethene **(41).**

To a well stirred solution of 1-methylpiperazine **VI** (0.50 mL, 4.51 mmol) in DMF (20 mL), at 25 °C under N_2, was added K_2CO_3 (0.53 g, 3.83 mmol) and stirred for 15 min. A solution of 2-(4-fluorophenoxy)-2-phenylethyl bromide **37** (0.41 g, 1.39 mmol) in DMF

(5 mL) was added to the reaction mixture. After refluxing for 16 h, the reaction was quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (100% hexane). The unexpected product **41** was afforded as a clear oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.68 (m, 2H), 7.38 (m, 3H), 7.05 (m, 4H), 5.00 (d, *J* = 2.55 Hz, 1H), 4.34 (d, *J* = 2.54 Hz, 1H).

Synthesis of Homologues Type I – Compounds 1 to 7

1-Phenyl-2-(4-phenylpiperazinyl)ethanone **(43).** [163]

To a well stirred solution of 1-phenylpiperazine **Ia** (0.84 g, 5.19 mmol) in acetonitrile (25 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (0.84 g, 6.10 mmol). After stirring for 15 min, 2-chloroacetophenone (0.80 g, 5.19 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was quenched with H_2O (50 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **43** was afforded as a yellow solid (0.87 g, 3.11 mmol) in 60% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.03 (ddd, *J* = 8.47, 3.05, 1.68 Hz, 2H), 7.59 (t, *J* = 7.36 Hz, 1H), 7.47 (m, 2H), 7.28 (m, 2H), 6.95 (d, *J* = 7.36 Hz, 2H), 6.87 (t, *J* = 7.28 Hz, 1H), 3.91 (s, 2H), 3.30 (m, 4H), 2.80 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 196.03, 151.21, 135.98, 133.38, 129.14, 128.62, 128.13, 119.88, 116.20, 64.27, 53.50, 49.01.

Melting Point: 104-106°C.

1-Phenyl-2-(4-phenylpiperazinyl)ethanol **(44).**[163]

To a well stirred solution of 1-phenyl-2-(4-phenylpiperazinyl)ethanone **43** (0.87 g, 3.11 mmol) in methanol (25 mL) under N_2 at 0 °C was added sodium borohydride (0.29 g, 7.77 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice, treated with glacial acetic acid (0.2 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was dried under vacuum. The product **44** was afforded as a white crystalline solid (0.84 g, 2.98 mmol) in 96% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34 (m, 7H), 6.95 (dd, J = 8.75, 0.91 Hz, 2H), 6.88 (tt, J = 7.29, 0.89 Hz, 1H), 4.80 (dd, J = 9.33, 4.67 Hz, 1H), 3.99 (bs, 1H), 3.25 (td, J = 6.23, 3.79 Hz, 4H), 2.93 (m, 2H), 2.60 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 151.17, 141.92, 129.17, 128.42, 127.61, 125.87, 119.96, 116.20, 68.79, 66.21, 53.07, 49.34.

Melting Point: 107-109°C.

1-[2-(4-Fluorophenoxy)-2-phenylethyl]-4-phenylpiperazine **(1a).**[163]

To a well stirred solution of 1-phenyl-2-(4-phenylpiperazinyl)ethanol **44** (0.71 g, 2.52 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-fluorophenol (0.56

g, 5.03 mmol) and PPh_3 (1.00 g, 4.57 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.75 mL, 4.57 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (20:80 EtOAc/hexane). The product **1a** was afforded as a white solid (0.80 g, 2.13 mmol) in 36% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.36 (m, 4H), 7.226 (m, 3H), 6.92 (dd, J = 8.76, 0.91 Hz, 2H), 6.83 (m, 5H), 5.29 (dd, J = 8.45, 3.10 Hz, 1H), 3.21 (t, J = 5.02 Hz, 4H), 3.06 (dd, J = 13.75, 8.48 Hz, 1H),2.85 (td, J = 10.24, 4.99 Hz, 2H), 2.74 (td, J = 10.30, 3.64 Hz, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 157.31 (d, $J_{C\text{-}F}$ = 238.5 Hz), 153.94 (d, $J_{C\text{-}F}$ = 2.3 Hz), 151.32, 140.36, 129.11, 128.68, 127.86, 126.14, 119.72, 117.24 (d, $J_{C\text{-}F}$ = 8.3 Hz), 116.06, 115.71 (d, $J_{C\text{-}F}$ = 23.4 Hz), 79.72, 65.40, 53.64, 49.25.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -123.53.

Melting Point: 80-82°C

Elemental Analysis (%) calculated for $C_{24}H_{25}ON_2F$: C, 76.60; H, 6.65; N, 7.45. Found: C, 76.58; H, 6.75; N, 7.46.

1-[2-(4-Fluorophenoxy)-2-phenylethyl]-4-phenylpiperazine hydrochloride **(1b).**[163]

To a well stirred solution of 1-[2-(4-fluorophenoxy)-2-phenylethyl]-4-phenylpiperazine **1a** (0.80 g, 2.13 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (11.0 mL, 21.3 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous EtOH. The resulting solution was refluxed for 15 min

and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **1b** was afforded as a white solid (0.66 g, 1.60 mmol) in 75% yield.

HPLC (standard method) t_R = 8.82 min, purity > 99%.

Melting Point: 195-198°C.

1-Phenyl-4-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine **(2a).**[163]

To a well stirred solution of 1-phenyl-2-(4-phenylpiperazinyl)ethanol **44**(0.84 g, 2.98 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.97 g, 5.99 mmol) and PPh_3 (1.17 g, 4.47 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.88 mL, 4.47 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (20:80 EtOAc/hexane). The product **2a** was afforded as a yellow oil (0.51 g, 1.20 mmol) in 40% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.44 (m, 2H), 7.31 (m, 7H), 6.93 (m, 4H), 6.85 (m, 1H), 5.42 (dd, J = 8.33, 3.21 Hz, 1H), 3.19 (t, J = 5.02 Hz, 4H), 3.08 (dd, J = 13.85, 8.35 Hz, 1H), 2.79 (m, 5H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.26, 151.30, 139.74, 129.12, 128.82, 128.07, 126.81 (q, $J_{C\text{-}F}$ = 3.8 Hz), 126.01, 124.36 (q, $J_{C\text{-}F}$ = 271.0 Hz), 123.02 (q, $J_{C\text{-}F}$ = 32.5 Hz), 119.76, 116.08, 115.94, 79.23, 65.31, 53.67, 49.28.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.53.

Elemental Analysis (%) calculated for $C_{25}H_{25}ON_2F_3$: C, 70.42; H, 5.87; N, 6.57. Found: C, 70.51; H, 5.94; N, 6.57.

1-Phenyl-4-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine hydrochloride **(2b).**[163]

To a well stirred solution of 1-phenyl-4-[2-phenyl-2-(4-trifluoromethylphenoxy) ethyl]piperazine **2a** (0.96 g, 2.25 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (12.0 mL, 22.5 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **2b** was afforded as a blue-green solid (0.85 g, 1.84 mmol) in 82% yield.

HPLC (standard method) t_R = 9.00 min, purity > 99%.

Melting Point: 161 - 163°C.

2-[4-(3-Chlorophenyl)piperazinyl]-1-phenylethanone **(45)**.

To a well stirred solution of 1-(3-chlorophenyl)-piperazine **II** (0.40 mL, 2.43 mmol) in acetonitrile (25 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (0.38 g, 2.75 mmol). After stirring for 15 min, 2-chloroacetophenone (0.38 g, 2.46 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **45** was afforded as a white solid (0.59 g, 1.88 mmol) in 77% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.02 (d, *J* = 7.28 Hz, 2H), 7.59 (t, *J* = 7.69 Hz, 1H), 7.47 (t, *J* = 7.76 Hz, 2H), 7.16 (t, *J* = 8.10, 1H), 6.88 (m, 1H), 6.80 (ddd, *J* = 8.02, 3.94, 1.58 Hz, 2H), 3.89 (s, 2H), 3.28 (m, 4H), 2.76 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 196.07, 152.27, 135.98, 134.97, 133.39, 130.04, 128.63, 128.12, 119.39, 115.86, 113.94, 64.26, 53.29, 48.57.

Melting Point: 103 – 106 °C.

2-[4-(3-Chlorophenyl)piperazinyl]-1-phenylethanol **(46)**.

To a well stirred solution of 2-[4-(3-chlorophenyl)piperazinyl]-1-phenylethanone **45** (0.59 g, 1.64 mmol) in methanol (25 mL) under N_2 at 0 °C was added sodium borohydride (0.16 g, 4.20 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice, treated with glacial acetic acid (0.1 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was dried under vacuum. The product **46** was afforded as a white solid (0.47 g, 1.30 mmol) in 79% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.35 (m, 5H), 7.17 (t, *J* = 8.10, 1H), 6.89 (m, 1H), 6.81 (t, *J* = 8.14 Hz, 2H), 4.79 (dd, *J* = 8.80, 5.02 Hz, 1H), 3.88 (bs, 1H), 3.25 (m, 4H), 2.90 (m, 2H), 2.58 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 152.20, 141.86, 135.01, 130.07, 128.42, 127.63, 125.85, 119.52, 115.92, 113.99, 68.85, 66.17, 52.88, 48.88.

Melting Point: 98 – 100 °C.

1-(3-Chlorophenyl)-4-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine **(3a)**.

To a well stirred solution of 2-[4-(3-chlorophenyl)piperazinyl]-1-phenylethanol **46** (0.47 g, 1.30 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.43 g, 2.65 mmol) and PPh_3 (0.51 g, 1.94 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.39 mL, 1.97 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (20:80 EtOAc/hexane). The product **3a** was afforded as a yellow oil (0.58 g, 1.26 mmol) in 97% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42 (d, J = 8.84 Hz, 2H), 7.30 (m, 5H), 7.12 (t, J = 8.10 Hz, 1H), 6.92 (d, J = 8.77 Hz, 2H), 6.85 (m, 1H), 6.76 (m, 2H), 5.39 (dd, J = 8.22, 3.21 Hz, 1H), 3.15 (t, J = 4.99 Hz, 4H), 3.06 (dd, J = 13.84, 8.28 Hz, 1H), 2.74 (m, 5H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.31, 152.40, 139.70, 134.99, 130.09, 128.88, 128.15, 126.88 (q, $J_{C\text{-}F}$ = 3.8 Hz), 126.08, 124.46 (q, $J_{C\text{-}F}$ = 271.0 Hz), 123.06 (q, $J_{C\text{-}F}$ = 32.5), 119.30, 116.00, 115.77, 113.91, 79.28, 65.22, 53.49, 48.78.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.33.

Elemental Analysis (%) calculated for $C_{25}H_{24}ClF_3N_2O$: C, 65.15; H, 5.25; N, 6.08. Found: C, 65.19; H, 5.27; N, 6.11.

1-(3-Chlorophenyl)-4-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine hydrochloride **(3b)**.

To a well stirred solution of 1-(3-chlorophenyl)-4-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine **3a** (0.74 g, 1.61 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (8.04 mL, 16.09

mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **3b** was afforded as a white solid (0.47 g, 0.95 mmol) in 59% yield.

HPLC (standard method) t_R = 9.30 min, purity > 99%.

Melting Point: 167-170°C.

1-Phenyl-2-[4-(3-trifluoromethylphenyl)piperazinyl]ethanone **(47)**.

To a well stirred solution of 1-(3-trifluoromethylphenyl)piperazine **III** (0.45 mL, 2.40 mmol) in acetonitrile (25 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (0.38 g, 2.75 mmol). After stirring for 15 min, 2-chloroacetophenone (0.38 g, 2.46 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was then quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **47** was afforded as a yellow oil (0.84 g, 2.41 mmol) in 99% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.02 (d, J = 7.12 Hz, 2H), 7.59 (t, J = 7.36 Hz, 1H), 7.48 (t, J = 7.48 Hz, 2H), 7.35 (t, J = 8.00 Hz, 1H), 7.09 (m, 3H), 3.90 (s, 2H), 3.33 (m, 4H), 2.79 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 202.72, 163.14, 156.16, 151.30, 145.02, 135.95, 133.42, 131.67, 129.56, 128.64, 128.13, 118.75, 64.24, 53.27, 48.57. ($J_{C\text{-}F}$ could not be detected due to the low concentration of the sample).

^{19}F-NMR (282 MHz, $CDCl_3$): δ -62.70.

1-Phenyl-2-[4-(3-trifluoromethylphenyl)piperazinyl]ethanol **(48)**.

To a well stirred solution of 1-phenyl-2-[4-(3-trifluoromethylphenyl)piperazinyl] ethanone **46** (0.84 g, 2.41 mmol) in methanol (25 mL) under N_2 at 0 °C was added sodium borohydride (0.24 g, 6.30 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice, treated with glacial acetic acid (0.1 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was dried under vacuum. The product **48** was afforded as a yellow solid (0.74 g, 2.11 mmol) in 88% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.37 (m, 6H), 7.10 (m, 3H), 4.92 (t, J = 6.84 Hz, 1H), 3.36 (m, 4H), 3.02 (m, 2H), 2.79 (m, 2H), 2.69 (d, J = 6.73 Hz, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 151.14, 141.70, 131.52 (q, JC-F = 31.9 Hz), 129.64, 128.48, 127.72, 125.87, 124.30 (q, $J_{C\text{-}F}$ = 272.5 Hz), 118.95, 116.25 (q, $J_{C\text{-}F}$ = 3.8 Hz), 112.44 (q, $J_{C\text{-}F}$ = 3.8 Hz), 68.84, 66.08, 52.92, 48.66.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -62.70.

Melting Point: 72 – 75 °C.

1-[2-Phenyl-2-(4-trifluoromethylphenoxy)ethyl]-4-(3-trifluoromethylphenyl)piperazine **(4a)**.

To a well stirred solution of 1-phenyl-2-[4-(3-trifluoromethylphenyl)piperazinyl] ethanol **47** (0.74 g, 2.11 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.68 g, 4.19 mmol) and PPh_3 (0.85 g, 3.24 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.63 mL, 3.19 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **4a** was afforded as a yellow oil (0.31 g, 0.63 mmol) in 30% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.44 (d, J = 8.77 Hz, 2H), 7.33 (m, 6H), 7.06 (m, 3H), 6.93 (d, J = 8.66 Hz, 2H), 5.41 (dd, J = 8.24, 3.25 Hz, 1H), 3.23 (t, J = 5.01 Hz, 4H), 3.08 (dd, J = 13.85, 8.28 Hz, 1H), 2.79 (m, 5H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.24, 151.37, 139.63, 131.44 (q, $J_{C\text{-}F}$ = 31.8 Hz), 129.54, 128.83, 128.11, 126.83 (q, $J_{C\text{-}F}$ = 3.8 Hz), 126.01, 124.36 (q, $J_{C\text{-}F}$ = 271.1 Hz), 123.08 (q, $J_{C\text{-}F}$ = 32.5 Hz), 120.74 (q, $J_{C\text{-}F}$ = 272.6 Hz), 118.68, 115.92, 115.82 (q, $J_{C\text{-}F}$ = 3.8 Hz), 112.17 (q, $J_{C\text{-}F}$ = 3.8 Hz), 79.29, 65.16, 53.45, 48.79.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.55, -62.68.

Elemental Analysis (%) calculated for $C_{26}H_{24}F_6N_2O$: C, 63.15; H, 4.89; N, 5.66. Found: C, 63.31; H, 4.95; N, 5.60.

1-[2-Phenyl-2-(4-trifluoromethylphenoxy)ethyl]-4-(3-trifluoromethylphenyl)piperazine hydrochloride **(4b)**.

To a well stirred solution of 1-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]-4-(3-trifluoromethylphenyl)piperazine **4a** (0.31 g, 0.63 mmol) in anhydrous THF (25 mL) under

N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (3.14 mL, 6.28 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **4b** was afforded as a cream solid (0.12 g, 0.23 mmol) in 36% yield.

HPLC (standard method) t_R = 9.33 min, purity > 99%.

Melting Point: 177-180°C.

2-[4-(2-Methoxyphenyl)piperazinyl]-1-phenylethanone **(49)**.

To a well stirred solution of 1-(2-methoxyphenyl)piperazine **IV** (0.67 g, 3.49 mmol) in acetonitrile (75 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (0.60 g, 4.34 mmol). After stirring for 15 min, 2-chloroacetophenone (0.54 g, 3.49 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was then quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **49** was afforded as an orange oil (0.93 g, 3.00 mmol) in 86% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.03 (d, *J* = 7.10 Hz, 2H), 7.58 (t, *J* = 7.35 Hz, 1H), 7.47 (t, *J* = 7.35 Hz, 2H), 7.00 (m, 1H), 6.93 (m, 2H), 6.86 (dd, *J* = 7.80, 1.23 Hz, 1H), 3.90 (s, 2H), 3.87 (s, 3H), 3.17 (m, 4H), 2.83 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 196.34, 152.29, 141.28, 136.15, 133.25, 128.57, 128.16, 122.98, 121.03, 118.30, 111.21, 64.60, 55.38, 53.87, 50.51.

2-[4-(2-Methoxyphenyl)piperazinyl]-1-phenylethanol **(50)**.

To a well stirred solution of 2-[4-(3-methoxyphenyl)piperazinyl]-1-phenylethanone **48** (0.93 g, 3.00 mmol) in methanol (25 mL) under N_2 at 0 °C was added sodium borohydride (0.29 g, 7.67 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was dried under vacuum. The product **50** was afforded as a yellow solid (0.72 g, 2.31 mmol) in 77% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34 (m, 5H), 6.96 (m, 4H), 4.79 (dd, J = 10.16, 3.55 Hz, 1H), 3.87 (s, 3H), 3.14 (s, 4H), 2.98 (dd, J = 9.56, 4.52 Hz, 2H), 2.66 (dd, J = 10.71, 5.27 Hz, 2H), 2.55 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 152.29, 142.14, 141.18, 128.39, 127.54, 125.89, 123.09, 121.04, 118.27, 111.26, 68.77, 66.31, 55.40, 53.29, 50.81.

Melting Point: 80 – 83 °C.

1-(2-Methoxyphenyl)-4-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine **(5a)**.

To a well stirred solution of 2-[4-(2-methoxyphenyl)piperazinyl]-1-phenylethanol **49** (0.72 g, 2.31 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.77 g, 4.75 mmol) and PPh_3 (0.93 g, 3.55 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.68 mL, 3.44 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and

purified by flash chromatography (20:80 EtOAc/hexane). The product **5a** was afforded as a yellow oil (0.63 g, 1.38 mmol) in 60% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42 (d, *J* = 5.27 Hz, 2H), 7.30 (m, 5H), 6.95 (m, 5H), 6.84 (d, *J* = 7.69 Hz, 1H), 5.43 (dd, *J* = 8.29, 3.01 Hz, 1H), 3.83 (s, 3H), 3.10 (m, 5H), 2.88 (td, *J* = 9.22, 4.54 Hz, 2H), 2.78 (td, *J* = 6.81, 4.31 Hz, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.37, 152.32, 141.37, 139.91, 128.84, 128.04, 126.81 (q, $J_{C\text{-}F}$ = 3.8 Hz), 126.06, 124.46 (q, $J_{C\text{-}F}$ = 271.1 Hz), 122.98, 122.96 (q, $J_{C\text{-}F}$ = 32.5 Hz), 121.05, 118.24, 116.03, 111.25, 79.21, 65.54, 55.37, 53.93, 50.80.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.40.

Elemental Analysis (%) calculated for $C_{26}H_{27}F_3N_2O_2$: C, 68.41; H, 5.96; N, 6.14. Found: C, 68.39; H, 5.99; N, 6.15.

1-(2-Methoxyphenyl)-4-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine hydrochloride **(5b)**.

To a well stirred solution of 1-(2-methoxyphenyl)-4-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]-piperazine **5a** (0.63 g, 1.38 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (6.90 mL, 13.82 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **5b** was afforded as a cream solid (0.60 g, 1.22 mmol) in 88% yield.

HPLC (standard method) t_R = 9.05 min, purity > 98%.

Melting Point: 193-196°C.

1-Phenyl-2-[4-(2-pyrimidinyl)piperazinyl]-1-ethanone **(51).**

To a well stirred solution of 1-(2-pyrimidyl)piperazine **V** (0.34 g, 2.07 mmol) in acetonitrile (75 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (0.33 g, 2.39 mmol). After stirring for 15 min, 2-chloroacetophenone (0.33 g, 2.13 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was then quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (50:50 EtOAc/hexane). The product **51** was afforded as a yellow oil (0.42 g, 1.49 mmol) in 72% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.31 (d, J = 4.75 Hz, 2H), 8.02 (dd, J = 8.36, 1.29 Hz, 2H), 7.59 (t, J = 7.35 Hz, 1H), 7.48 (t, J = 7.46 Hz, 2H), 6.49 (t, J = 4.74 Hz, 1H), 3.93 (m, 4H), 3.89 (s, 2H), 2.70 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 196.14, 157.72, 136.00, 133.35, 129.90, 128.61, 128.12, 109.90, 64.40, 53.36, 43.54.

1-Phenyl-2-[4-(2-pyrimidinyl-piperazinyl]-1-ethanol **(52)**.

To a well stirred solution of 1-phenyl-2-[4-(2-pyrimidinyl)piperazinyl]-1-ethanone **51** (0.42 g, 1.49 mmol) in methanol (25 mL) under N_2 at 0 °C was added sodium borohydride (0.14 g, 3.70 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and the solvent

was evaporated under reduced pressure. The residue was dried under vacuum. The product **51** was afforded as a clear oil (0.38 g, 1.33 mmol) in 90% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.32 (d, *J* = 4.74 Hz, 2H), 7.38 (m, 5H), 6.51 (t, *J* = 4.75 Hz, 1H), 4.83 (t, *J* = 6.97 Hz, 1H), 3.91 (m, 4H), 2.84 (m, 2H), 2.56 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.64, 157.74, 141.83, 128.42, 127.63, 125.88, 110.09, 68.80, 66.37, 52.98, 43.72.

1-[2-(4-Fluorophenoxy)-2-phenylethyl]-4-(2-pyrimidyl)piperazine **(6a)**.

To a well stirred solution of 1-phenyl-2-[4-(2-pyrimidinyl)piperazinyl]-1-ethanol **52** (0.38 g, 1.34 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-fluorophenol (0.27 g, 2.39 mmol) and PPh_3 (0.47 g, 1.80 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.35 mL, 1.80 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (50:50 EtOAc/hexane). The product **6a** was afforded as a yellow oil (0.22 g, 0.58 mmol) in 48% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.29 (d, *J* = 4.74 Hz, 2H), 7.32 (m, 5H), 6.83 (m, 4H), 6.45 (t, *J* = 4.74 Hz, 1H), 5.28 (dd, *J* = 8.39, 3.22 Hz, 1H), 3.83 (t, *J* = 5.11 Hz, 4H), 3.04 (dd, *J* = 13.80, 8.42 Hz, 1H), 2.73 (td, *J* = 7.37, 4.22, 4.22 Hz, 3H), 2.64 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 158.88, 158.71 (d, $J_{C\text{-}F}$ = 238.5 Hz), 157.70, 153.97 (d, $J_{C\text{-}F}$ = 2.3 Hz), 140.35, 128.67, 127.86, 126.13, 117.24 (d, $J_{C\text{-}F}$ = 8.3 Hz), 115.71 (d, $J_{C\text{-}F}$ = 23.4 Hz), 109.83, 79.65, 65.55, 53.48, 43.80.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -123.47.

1-[2-(4-Fluorophenoxy)-2-phenylethyl]-4-(2-pyrimidyl)piperazine hydrochloride **(6b)**.

To a well stirred solution of 1-[2-(4-fluorophenoxy)-2-phenylethyl]-4-(2-pyrimidyl)piperazine **6a** (0.21 g, 0.55 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (2.77 mL, 5.55 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **6b** was afforded as a cream solid (0.16 g, 0.38 mmol) in 70% yield.

HPLC (standard method) t_R = 8.50 min, purity > 98%.

Melting Point: 175-178°C.

1-[2-Phenyl-2-(4-trifluoromethylphenoxy)ethyl]-4-(2-pyrimidyl)piperazine **(7a)**.

To a well stirred solution of 1-phenyl-2-[4-(2-pyrimidinyl)piperazinyl]-1-ethanol **52** (0.38 g, 1.34 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.44 g, 2.71 mmol) and PPh_3 (0.53 g, 2.01 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.40 mL, 2.02 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (50:50 EtOAc/hexane). The product **7a** was afforded as a yellow oil (0.44 g, 1.03 mmol) in 78% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.29 (d, J = 4.73 Hz, 2H), 7.43 (d, J = 8.70 Hz, 2H), 7.32 (m, 5H), 6.92 (d, J = 4.73 Hz, 2H), 6.47 (t, J = 4.74 Hz, 1H), 5.43 (dd, J = 8.31, 3.16

Hz, 1H), 3.83 (t, J = 5.07 Hz, 4H), 3.08 (dd, J = 13.89, 8.35 Hz, 1H), 2.76 (ddd, J = 13.75, 8.28, 4.15 Hz, 3H), 2.65 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.64, 160.21, 157.71, 139.64, 128.82, 128.07, 126.80 (q, $J_{C\text{-}F}$ = 3.8 Hz), 125.99, 124.36 (q, $J_{C\text{-}F}$ = 271.0 Hz), 123.02 (q, $J_{C\text{-}F}$ = 32.5 Hz), 115.93, 109.88, 79.04, 65.39, 53.47, 43.72.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.52.

1-[2-Phenyl-2-(4-trifluoromethylphenoxy)ethyl]-4-(2-pyrimidyl)piperazine hydrochloride **(7b)**.

To a well stirred solution of 1-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]-4-(2-pyrimidyl)piperazine **7a** (0.10 g, 0.23 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (1.17 mL, 2.33 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **7b** was afforded as a cream solid (0.05 g, 0.11 mmol) in 47% yield.

HPLC (standard method) t_R = 8.80 min, purity > 96%.

Melting Point: 203 – 206 °C.

Synthesis of Homologues Type I – Compounds 8 to 15

2-(4-Methylpiperazinyl)-1-phenylethanol **(53)**.

To a well stirred solution of 1-methylpiperazine **VI** (2.8 mL, 25.2 mmol) in anhydrous CH_2Cl_2 (50 mL) under N_2 was added anhydrous K_2CO_3 (3.45g, 24.96 mmol).

After stirring for 15 min, styrene oxide (0.95 mL, 8.30 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was quenched with H_2O (100mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 MeOH/EtOAc). The combined fractions were evaporated and the residue was recrystallized from EtOAc. The product **53** was afforded as a white solid (1.32g, 6.0 mmol) in 72% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.33 (m, 5H), 4.73 (dd, *J* = 9.98, 4.07 Hz, 1H), 2.79 (bs, 2H), 2.51 (m, 8H), 2.31 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 142.12, 128.34, 127.49, 125.83, 68.74, 66.14, 55.24, 46.06.

Melting Point: 98 – 100 °C.

1-[2-(4-Fluorophenoxy)-2-phenylethyl]-4-methylpiperazine **(8a)**.

To a well stirred solution of 2-(4-methylpiperazinyl)-1-phenylethanol **53** (0.20g, 0.91 mmol) in anhydrous THF (20 mL) under N_2 at 25 °C was added 4-fluorophenol (0.20 g, 1.78 mmol) and PPh_3 (0.36 g, 1.37 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.27 mL, 1.37 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 MeOH/EtOAc). The product **8a** was afforded as an orange oil (0.25 g, 0.65 mmol) in 72% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.30 (m, 5H), 6.82 (m, 4H), 5.24 (dd, *J* = 8.46, 3.08 Hz, 1H), 3.00 (dd, *J* = 13.78, 8.49 Hz, 1H), 2.69 (m, 5H), 2.45 (s, 4H), 2.27 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 157.27 (d, J_{C-F} = 238.5 Hz), 153.98 (d, J_{C-F} = 2.3 Hz), 140.48, 128.63, 127.78, 126.11, 117.22 (d, J_{C-F} = 7.5 Hz), 115.64 (d, J_{C-F} = 22.6 Hz), 79.61, 65.43, 55.22, 53.52, 46.07.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -123.64.

1-[2-(4-Fluorophenoxy)-2-phenylethyl]-4-methylpiperazine hydrochloride **(8b)**.

To a well stirred solution of 1-[2-(4-fluorophenoxy)-2-phenylethyl]-4-methylpiperazine **8a** (0.20 g, 0.64 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (3.2 mL, 6.4 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **8b** was afforded as a cream solid (0.17 g, 0.48 mmol) in 75% yield.

HPLC (standard method) t_R = 7.37 min, purity > 98%.

Melting Point: 213 – 215 °C.

1-Methyl-4-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine **(9a)**.

To a well stirred solution of 2-(4-methylpiperazinyl)-1-phenylethanol **53** (0.20g, 0.91 mmol) in anhydrous THF (20 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.29 g, 1.79 mmol) and PPh_3 (0.36 g, 1.37 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.27 mL, 1.37 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and

purified by flash chromatography (30:70 MeOH/EtOAc). The product **9a** was afforded as a yellow oil (0.22 g, 0.60 mmol) in 66% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42 (d, J = 8.61 Hz, 2H), 7.30 (m, 5H), 6.91 (d, J = 8.58 Hz, 2H), 5.37 (dd, J = 8.36, 3.11 Hz, 1H), 3.03 (dd, J = 13.87, 8.38 Hz, 1H), 2.68 (m, 5H), 2.44 (s, 4H), 2.27 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.31, 139.82, 128.76, 127.97, 126.74 (q, $J_{C\text{-}F}$ = 3.8 Hz), 125.97, 124.38 (q, $J_{C\text{-}F}$ = 271.0), 122.93 (q, $J_{C\text{-}F}$ = 32.5 Hz), 115.93, 79.12, 65.30, 55.17, 53.52, 46.02.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.52.

1-Methyl-4-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine hydrochloride **(9b)**.

To a well stirred solution of 1-methyl-4-[2-phenyl-2-(4-trifluoromethylphenoxy) ethyl]piperazine **9a** (0.20 g, 0.55 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (2.7 mL, 5.5 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **9b** was afforded as a light yellow solid (0.17 g, 0.41 mmol) in 75% yield.

HPLC (standard method) t_R = 7.68 min, purity > 99%.

Melting Point: 220 – 222 °C.

2-[4-(4-Fluorobenzoyl)piperidinyl]-1-phenyl-1-ethanol **(54)**.

To a well stirred solution of 4-(4-fluorobenzoyl)piperidine **VII** (4.13 g, 20.0 mmol) in anhydrous CH_2Cl_2 (75 mL) under N_2 was added anhydrous K_2CO_3 (2.78 g, 20.1 mmol). After stirring for 15 min, styrene oxide (0.95 mL, 8.30 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was quenched with H_2O (100mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (70:30 EtOAc/hexane). The product **54** was afforded as a white solid (3.52 g, 10.76 mmol) in 62% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.97 (dd, J = 8.91, 5.41 Hz, 2H), 7.33 (m, 5H), 7.14 (t, J = 8.65 Hz, 2H), 4.75 (dd, J = 10.18, 3.77 Hz, 1H), 3.26 (m, 2H), 2.92 (dt, J = 5.41 Hz, 2.89 1H), 2.52 (m, 3H), 2.21 (dt, J = 11.31, 3.81 Hz, 1H), 1.89 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 200.84, 165.70 (d, $J_{C\text{-}F}$ = 255.1 Hz), 142.15, 132.40 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.89 (d, $J_{C\text{-}F}$ = 9.1 Hz), 128.36, 127.51, 125.86, 115.84 (d, $J_{C\text{-}F}$ = 21.9 Hz), 68.86, 66.43, 54.64, 51.63, 43.38, 28.98, 28.68.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -105.24.

Melting Point: 145 – 147 °C.

4-(4-Fluorobenzoyl)-1-[2-(4-fluorophenoxy)-2-phenylethyl]piperidine **(10a)**.

To a well stirred solution of 2-[4-(4-fluorobenzoyl)piperidinyl]-1-phenyl-1-ethanol **54** (0.47 g, 1.44 mmol) in anhydrous benzene (50 mL) under N_2 at 25 °C was added 4-fluorophenol (0.18 g, 1.60 mmol) and PPh_3 (0.41 g, 1.56 mmol). After complete dissolution, a solution of ADDP (0.40 g, 1.59 mmol) in anhydrous benzene (10 mL) was

slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **10a** was afforded as a clear oil (0.39 g, 0.93 mmol) in 65% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.95 (dd, J = 8.85, 5.43 Hz, 2H), 7.31 (m, 5H), 7.12 (t, J = 8.62 Hz, 2H), 6.83 (m, 4H), 5.24 (dd, J = 8.15, 3.47 Hz, 1H), 3.19 (m, 2H), 3.02 (m, 2H), 2.71 (dd, J = 13.80, 3.51 Hz, 1H), 2.37 (m, 2H), 1.84 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 201.04, 165.63 (d, $J_{C\text{-}F}$ = 254.4 Hz), 157.29 (d, $J_{C\text{-}F}$ = 238.5 Hz), 154.05 (d, $J_{C\text{-}F}$ = 1.51 Hz), 140.53, 132.48 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.88 (d, $J_{C\text{-}F}$ = 9.1 Hz), 128.63, 127.78, 126.13, 117.25 (d, $J_{C\text{-}F}$ = 7.5 Hz), 115.77 (d, $J_{C\text{-}F}$ = 21.9 Hz), 115.70 (d, $J_{C\text{-}F}$ = 23.4 Hz), 79.70, 65.57, 53.67, 53.50, 43.52, 28.85.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -105.45, -123.54.

4-(4-fluorobenzoyl)-1-[2-(4-fluorophenoxy)-2-phenylethyl]piperidine hydrochloride **(10b)**.

To a well stirred solution of 4-(4-fluorobenzoyl)-1-[2-(4-fluorophenoxy)-2-phenyl ethyl]piperidine **10a** (0.29 g, 0.70 mmol)in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.70 mL, 1.40 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **10b** was afforded as a pale orange solid (0.29 g, 0.64 mmol) in 93% yield.

HPLC (standard method) t_R = 8.91 min, purity > 98%.

Melting Point: 89 – 91 °C.

4-(4-fluorobenzoyl)-1-[2- phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperidine **(11a).**

To a well stirred solution of 2-[4-(4-fluorobenzoyl)piperidinyl]-1-phenyl-1-ethanol **54** (0.30 g, 0.92 mmol) in anhydrous benzene (50 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.16 g, 0.99 mmol) and PPh_3 (0.26 g, 0.99 mmol). After complete dissolution, a solution of ADDP (0.25 g, 0.99 mmol) in anhydrous benzene (10 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **11a** was afforded as a clear oil (0.39 g, 0.93 mmol) in 38% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.95 (dd, J = 8.89, 5.42 Hz, 2H), 7.43 (d, J = 8.59 Hz, 2H), 7.31 (m, 5H), 7.12 (t, J = 8.64 Hz, 2H), 6.92 (d, J = 8.58 Hz, 2H), 5.38 (dd, J = 8.04, 3.47 Hz, 1H), 3.19 (m, 2H), 3.06 (dd, J = 13.91, 8.09 Hz, 1H), 2.99 (d, J = 11.27 Hz, 1H), 2.76 (dd, J = 13.90, 3.51 Hz, 1H), 2.39 (m, 2H), 1.83 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 200.98, 165.64 (d, $J_{C\text{-}F}$ = 255.1 Hz), 160.30, 139.84, 132.44 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.86 (d, $J_{C\text{-}F}$ = 9.1 Hz), 128.76, 127.97, 126.78 (q, $J_{C\text{-}F}$ = 3.8 Hz), 125.99, 124.38 (q, $J_{C\text{-}F}$ = 271.7 Hz), 122.95 (q, $J_{C\text{-}F}$ = 32.5), 115.93, 115.61, 79.08, 65.41, 53.68, 53.40, 43.41, 28.79, 28.77.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.51, -105.43.

4-(4-fluorobenzoyl)-1-[2- phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperidine hydrochloride **(11b).**

To a well stirred solution of 4-(4-fluorobenzoyl)-1-[2- phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperidine **11a** (0.13 g, 0.27 mmol)in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.27 mL, 0.55 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **11b** was afforded as a pale brown solid (0.13 g, 0.26 mmol) in 95% yield.

HPLC (standard method) t_R = 9.10 min, purity > 99%.

Melting Point: 92 – 95 °C.

2-[4-(6-Fluoro-3-benzo[d]isoxazolyl)piperidinyl]-1-phenyl-1-ethanol **(55)**.

To a well stirred solution of 4-(6-fluoro-3-benzo[d]isoxazolyl)piperidine **VIII** (1.70 g, 7.70 mmol) in anhydrous CH_2Cl_2 (75 mL) under N_2 was added anhydrous K_2CO_3 (1.06 g, 7.70 mmol). After stirring for 15 min, styrene oxide (0.80 mL, 7.00 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was quenched with H_2O (100mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (70:30 EtOAc/hexane). The product **55** was afforded as a white solid (1.29 g, 3.80 mmol) in 54% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.68 (dd, *J* = 8.73, 5.10 Hz, 1H), 7.38 (m, 5H), 7.29 (m, 1H), 7.08 (dt, *J* = 8.85, 2.15 Hz, 1H), 4.78 (dd, *J* = 10.20, 3.76 Hz, 1H), 3.33 (dd, *J* = 11.32, 1.26 Hz, 1H), 3.13 (ddd, *J* = 15.53, 10.54, 5.25 Hz, 1H), 2.99 (dd, *J* = 11.53, 1.39 Hz, 1H), 2.56 (m, 3H), 2.28 (dt, *J* = 11.21, 3.46 Hz, 1H), 2.13 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.15 (d, $J_{C\text{-}F}$ = 250.6 Hz), 163.92 (d, $J_{C\text{-}F}$ = 15.8 Hz), 160.88, 142.08, 128.90, 128.40, 127.55, 125.86, 122.43 (d, $J_{C\text{-}F}$ = 11.3 Hz), 112.44 (d, $J_{C\text{-}F}$ = 24.9 Hz), 97.53 (d, $J_{C\text{-}F}$ = 27.2 Hz), 68.91, 66.51, 55.00, 51.91, 34.33, 30.82, 30.54.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -109.42.

Melting Point: 127 – 130 °C.

4-(6-Fluoro-3-benzo[d]isoxazolyl)-1-[2-(4-fluorophenoxy)-2-phenylethyl]piperidine **(12a)**.

To a well stirred solution of 2-[4-(6-fluoro-3-benzo[d]isoxazolyl)piperidinyl]-1-phenyl-1-ethanol **55** (0.27 g, 0.79 mmol) in anhydrous benzene (50 mL) under N_2 at 25 °C was added 4-fluorophenol (0.10 g, 0.89 mmol) and PPh_3 (0.23 g, 0.87 mmol). After complete dissolution, a solution of ADDP (0.22 g, 0.89 mmol) in anhydrous benzene (10 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **12a** was afforded as a pale yellow oil (0.13 g, 0.30 mmol) in 38% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.68 (dd, *J* = 8.71, 5.14 Hz, 1H), 7.33 (m, 5H), 7.24 (dd, *J* = 8.63, 2.15 Hz, 1H), 7.05 (dt, *J* = 8.81, 2.01 Hz, 1H), 6.85 (m, 4H), 5.27 (dd, *J* = 8.31, 3.31 Hz, 1H), 3.24 (d, *J* = 11.40 Hz, 1H), 3.06 (m, 3H), 2.74 (dd, *J* = 13.81, 3.33 Hz, 1H), 2.42 (dt, *J* = 11.19, 3.85 Hz, 2H), 2.09 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.10 (d, J_{C-F} = 250.6 Hz), 163.89 (d, J_{C-F} = 13.6 Hz), 161.11, 157.30 (d, J_{C-F} = 238.5 Hz), 154.07 (d, J_{C-F} = 1.5 Hz), 140.48, 128.65, 127.81, 126.13, 122.57 (d, J_{C-F} = 10.7 Hz), 117.24 (d, J_{C-F} = 7.5 Hz), 115.70 (d, J_{C-F} = 22.6 Hz), 112.31 (d, J_{C-F} = 24.9 Hz), 97.45 (d, J_{C-F} = 27.3 Hz), 79.69, 65.66, 53.92, 53.88, 34.40, 30.70, 30.66.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -109.64, -123.58.

Elemental Analysis (%) calculated for $C_{26}H_{24}F_2N_2O_2$: C, 71.89; H, 5.53; N, 6.45. Found: C, 71.86; H, 5.52; N, 6.37.

4-(6-Fluoro-3-benzo[d]isoxazolyl)-1-[2-(4-fluorophenoxy)-2-phenylethyl]piperidine hydrochloride **(12b)**.

To a well stirred solution of 4-(6-fluoro-3-benzo[d]isoxazolyl)-1-[2-(4-fluorophenoxy)-2-phenylethyl]piperidine **12a** (0.13 g, 0.29 mmol)in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.59 mL, 1.18 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **12b** was afforded as a white crystalline solid (0.073 g, 0.16 mmol) in 53% yield.

HPLC (standard method) t_R = 8.92 min, purity > 97%.

Melting Point: 208 – 211 °C.

4-(6-Fluoro-3-benzo[d]isoxazolyl)-1-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl] piperidine **(13a)**.

To a well stirred solution of 2-[4-(6-fluoro-3-benzo[d]isoxazolyl)piperidinyl]-1-phenyl-1-ethanol **55** (0.56 g, 1.65 mmol) in anhydrous benzene (50 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.29 g, 1.79 mmol) and PPh_3 (0.48 g, 1.82 mmol). After complete dissolution, a solution of ADDP (0.46 g, 1.82 mmol) in anhydrous benzene (10 mL) was slowly added dropwise to the reaction mixture. After stirred for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **13a** was afforded as a pale yellow oil (0.33 g, 0.67 mmol) in 41% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.66 (dd, J = 8.72, 5.07 Hz, 1H), 7.44 (d, J = 8.56 Hz, 2H), 7.34 (m, 5H), 7.21 (dd, J = 8.52, 1.92 Hz, 1H), 7.02 (dt, J = 8.86, 2.14 Hz, 1H), 6.95 (d, J = 8.52 Hz, 2H), 5.42 (dd, J = 8.15, 3.35 Hz, 1H), 3.23 (dd, J = 11.41, 1.06 Hz, 1H), 3.07 (m, 3H), 2.78 (dd, J = 13.90, 3.42 Hz, 1H), 2.43 (dt, J = 10.93, 3.71 Hz, 2H), 2.09 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.11 (d, $J_{C\text{-}F}$ = 250.6 Hz), 168.88 (d, $J_{C\text{-}F}$ = 13.6 Hz), 161.09, 160.41, 139.85, 128.81, 128.03, 126.81 (q, $J_{C\text{-}F}$ = 3.8 Hz), 126.03, 124.42 (q, $J_{C\text{-}F}$ = 271.0 Hz), 122.94 (q, $J_{C\text{-}F}$ = 32.5 Hz), 122.59 (d, $J_{C\text{-}F}$ = 11.3 Hz), 117.34, 115.96, 112.32 (d, $J_{C\text{-}F}$ = 25.7 Hz), 97.43 (d, $J_{C\text{-}F}$ = 26.4 Hz), 79.13, 65.56, 53.97, 53.86, 34.34, 30.69, 30.64.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.55, -105.66.

Elemental Analysis (%) calculated for $C_{27}H_{24}F_4N_2O_2$: C, 66.94; H, 4.99; N, 5.78. Found: C, 67.07; H, 5.03; N, 5.66.

4-(6-Fluoro-3-benzo[d]isoxazolyl)-1-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]-piperidine hydrochloride **(13b)**.

To a well stirred solution of 4-(6-fluoro-3-benzo[d]isoxazolyl)-1-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperidine **13a** (0.27 g, 0.55 mmol)in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.55 mL, 1.10 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **13b** was afforded as a white crystalline solid (0.26 g, 0.50 mmol) in 91% yield.

HPLC (standard method) t_R = 9.13 min, purity > 99%.

Melting Point: 112 – 115 °C.

1-[3-(4-fluorobenzoyl)propyl]piperazine **(IX).**

To a well stirred solution of piperazine (2.58 g, 29.95 mmol) and TBAI (0.018 g, 0.049 mmol) in MIBK (50 mL) under N_2 was added 4-chloro-4'-fluoro-butyrophenone (0.82 mL, 4.98 mmol) dropwise. After refluxing for 16 h, the reaction was poured onto with H_2O (300mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 MeOH/EtOAc). The product **IX** was afforded as a yellow solid (0.79 g, 3.16 mmol) in 63% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.01 (dd, *J* = 8.57, 5.44 Hz, 1H), 7.87 (d, *J* = 8.78 Hz, 1H), 7.13 (t, *J* = 8.51 Hz, 1H), 6.84 (d, *J* = 8.91 Hz, 1H), 3.30 (m, 2H), 2.96 (m, 4H), 2.58 (s, 4H), 2.46 (dd, *J* = 16.18, 7.22 Hz, 2H), 1.96 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 198.08, 165.65 (d, $J_{C\text{-}F}$ = 254.4 Hz), 133.62 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.64 (d, $J_{C\text{-}F}$ = 9.1 Hz), 115.65 (d, $J_{C\text{-}F}$ = 21.9 Hz), 57.60, 52.73, 51.55, 47.24, 44.19.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -105.51.

Melting Point: 95 – 98 °C.

2-{[4-(4-fluorobenzoyl)propyl]piperazinyl}-1-phenyl-1-ethanol **(56).**

To a well stirred solution of 1-[3-(4-fluorobenzoyl)propyl]piperazine **IX** (1.79 g, 7.16 mmol) in anhydrous CH_2Cl_2 (75 mL) under N_2 was added anhydrous K_2CO_3 (1.09 g, 7.89 mmol). After stirring for 15 min, styrene oxide (0.82 mL, 7.16 mmol) was added to the the reaction mixture. After refluxing for 16 h, the reaction was quenched with H_2O (100mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 MeOH/EtOAc). The product **55** was afforded as a yellow solid (0.50 g, 1.35 mmol) in 19% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.00 (dd, *J* = 8.88, 5.43 Hz, 2H), 7.36 (m, 4H), 7.30 (m, 1H), 7.13 (t, *J* = 8.65 Hz, 2H), 4.72 (dd, *J* = 9.15, 4.85 Hz, 1H), 2.98 (t, *J* = 7.10 Hz, 1H), 2.72 (s, 2H), 2.48 (m, 11H), 2.04 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 198.40, 165.64 (d, J_{C-F} = 254.4 Hz), 142.08, 133.62 (d, J_{C-F} = 3.0 Hz), 130.68 (d, J_{C-F} = 9.1 Hz), 128.36, 127.51, 125.86, 115.62 (d, J_{C-F} = 21.9 Hz), 77.27, 68.71, 66.16, 57.65, 53.19, 36.17, 21.65.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -105.66.

Melting Point: 110 – 113 °C.

4-[3-(4-fluoro-benzoyl)propyl]-1-[2-(4-fluorophenoxy)-2-phenylethyl]piperazine **(14a)**.

To a well stirred solution of 2-{[4-(4-fluorobenzoyl)propyl]piperazinyl}-1-phenyl-1-ethanol **56** (0.20 g, 0.53 mmol) in anhydrous benzene (25 mL) under N_2 at 25 °C was added 4-fluorophenol (0.07 g, 0.62 mmol) and PPh_3 (0.15 g, 0.57 mmol). After complete dissolution, a solution of ADDP (0.15 g, 0.59 mmol) in anhydrous benzene (10 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (100% EtOAc). The product **14a** was afforded as a yellow oil (0.084 g, 0.18 mmol) in 34% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.99 (dd, J = 8.81, 5.44 Hz, 2H), 7.31 (m, 5H), 7.11 (t, J = 8.61 Hz, 2H), 6.82 (m, 4H), 5.22 (dd, J = 8.45, 3.06 Hz, 1H), 2.97 (m, 3H), 2.55 (m, 11H), 1.93 (td, J = 14.15, 7.08 Hz, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 198.42, 165.63 (d, J_{C-F} = 254.4 Hz), 157.27 (d, J_{C-F} = 239.2 Hz), 153.97 (d, J_{C-F} = 2.2 Hz), 133.62 (d, J_{C-F} = 3.0 Hz), 130.68 (d, J_{C-F} = 9.1 Hz), 128.62, 127.77, 126.10, 125.03, 117.21 (d, J_{C-F} = 8.3 Hz), 115.65 (d, J_{C-F} = 23.4 Hz), 115.59 (d, J_{C-F} = 21.9 Hz), 79.61, 65.46, 57.69, 53.48, 53.16, 36.22, 32.22.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -105.68, -123.69.

4-[3-(4-fluorobenzoyl)propyl]-1-[2-(4-fluorophenoxy)-2-phenylethyl]piperazine hydrochloride **(14b)**.

To a well stirred solution of 4-[3-(4-fluorobenzoyl)propyl]-1-[2-(4-fluorophenoxy)-2-phenylethyl]piperazine **14a** (0.04 g, 0.10 mmol)in anhydrous Et_2O (10 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.10 mL, 0.19 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **14b** was afforded as a white solid (0.033 g, 0.067 mmol) in 70% yield.

HPLC (standard method) t_R = 8.37 min, purity > 98%.

Melting Point: 207 – 210 °C.

4-[3-(4-fluorobenzoyl)propyl]-1-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine **(15a).**

To a well stirred solution of 2-{[4-(4-fluorobenzoyl)propyl]piperazinyl}-1-phenyl-1-ethanol **56** (0.21 g, 0.55 mmol) in anhydrous benzene (25 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.10 g, 0.62 mmol) and PPh_3 (0.16 g, 0.61 mmol). After complete dissolution, a solution of ADDP (0.15 g, 0.59 mmol) in anhydrous benzene (10 mL) was slowly added dropwise to the reaction mixture.. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (100% EtOAc). The product **15a** was afforded as a yellow oil (0.085 g, 0.17 mmol) in 30% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.99 (dd, *J* = 8.90, 5.43 Hz, 2H), 7.43 (d, *J* = 8.59 Hz, 2H), 7.32 (m, 5H), 7.11 (t, *J* = 8.65 Hz, 2H), 6.90 (d, *J* = 8.54 Hz, 2H), 5.35 (dd, *J* = 8.37, 3.08 Hz, 1H), 2.99 (m, 3H), 2.54 (m, 11H), 1.93 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 198.41, 165.63 (d, $J_{C\text{-}F}$ = 253.6 Hz), 160.27, 133.62 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.67 (d, $J_{C\text{-}F}$ = 9.0 Hz), 128.77, 127.98, 126.75 (q, $J_{C\text{-}F}$ = 3.8 Hz), 125.96, 125.04, 124.36 (q, $J_{C\text{-}F}$ = 271.0 Hz), 122.94 (q, $J_{C\text{-}F}$ = 32.5), 115.93, 115.59 (d, $J_{C\text{-}F}$ = 21.9 Hz), 79.11, 65.35, 57.67, 53.49, 53.13, 36.20, 32.22.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.55, -105.66.

4-[3-(4-fluorobenzoyl)propyl]-1-[2-phenyl-2-(4-trifluoromethylphenoxy)ethyl]piperazine hydrochloride **(15b).**

To a well stirred solution of 4-[3-(4-fluorobenzoyl)propyl]-1-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine **15a** (0.06 g, 0.11 mmol) in anhydrous Et_2O (10 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.11 mL, 0.22 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **15b** was afforded as a white solid (0.034 g, 0.062 mmol) in 56% yield.

HPLC (standard method) t_R = 8.70 min, purity > 97%.

Melting Point: 220 – 222 °C.

Synthesis of Homologues Type II – Compounds 16, 17, 24 and 25

1-Phenyl-3-(4-phenylpiperazinyl)-1-propanone **(57)**.[146,163]

A well-stirred mixture of acetophenone (3.40 mL, 29.0 mmol), 1-phenylpiperazine hydrochloride **Ib** (4.48g, 22.5 mmol), and concentrated HCl (0.05mL) in absolute ethanol (35mL) under N_2 was heated at reflux conditions. Paraformaldehyde (1.00g, 33.3 mmol) was added in four equal portions over 40 minutes. After refluxing for 16 h, the solution was cooled and poured onto ice. A crude solid was separated by filtration, dried, and recrystallized in a small amount of aqueous ethanol (<10mL). The resulting solid was collected by filtration and dried under vacuum. The product **57** was afforded as a white solid (5.32 g, 18.1 mmol) in 62% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.03 (d, J = 7.24 Hz, 2H), 7.63 (t, J = 7.39 Hz, 1H), 7.50 (t, J = 7.62 Hz, 2H), 7.31 (m, 2H), 6.96 (m, 3H), 3.89 (t, J = 6.80 Hz, 2H), 3.63 (m, 8H), 3.11 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 195.96, 149.25, 135.39, 134.23, 129.49, 128.94, 128.36, 121.99, 117.42, 52.35, 51.97, 46.90, 33.14.

Melting Point: 182 – 185 °C.

1-Phenyl-3-(4-phenylpiperazinyl)-1-propanol **(58).**[146,163]

To a well stirred solution 1-phenyl-3-(4-phenylpiperazinyl)-1-propanone **57** (5.32g, 18.1 mmol) in methanol (25 mL) under N_2 at 0 °C was added sodium borohydride (1.72 g, 45.5 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice, treated with glacial acetic acid (0.2 mL) and extracted with EtOAc (3 x 100 mL). The

combined organic layers were washed with H_20 (3 x 100 mL) and brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was triturated with methanol (3 mL), filtered and dried under vacuum. The product **58** was afforded as a white crystalline solid (3.57 g, 12.0 mmol) in 67% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.36 (m, 5H), 7.24 (m, 1H), 6.90 (m, 4H), 4.97 (m, 1H), 3.26 (t, *J* = 4.99 Hz, 4H), 2.81 (m, 2H), 2.67 (m, 4H), 1.92 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 151.09, 144.78, 129.18, 128.27, 127.00, 125.55, 120.06, 116.29, 75.53, 57.11, 53.29, 49.30, 33.73.

Melting Point: 85 – 87 °C.

1-[3-(4-Fluorophenoxy)-3-phenylpropyl]-4-phenylpiperazine **(16a)**.[163]

To a well stirred solution of 1-phenyl-3-(4-phenylpiperazinyl)-1-propanol **58** (1.84g, 6.22 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-fluorophenol (1.40g, 12.4 mmol) and PPh_3 (2.49g, 9.32 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (1.85 mL, 9.32 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (20:80 EtOAc/hexane). The product **16a** was afforded as a yellow oil (0.70 g, 1.80 mmol) in 29% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34 (m, 4H), 7.26 (m, 3H), 6.93 (d, *J* = 7.91 Hz, 2H), 6.82 (m, 5H), 5.16 (dd, *J* = 8.09, 4.98 Hz, 1H), 3.20 (m, 4H), 2.59 (m, 6H), 2.23 (m, 1H), 2.01 (m, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 157.21 (d, J_{C-F} = 238.5 Hz), 154.33 (d, J_{C-F} = 2.3 Hz), 151.29, 141.61, 129.11, 128.63, 127.69, 126.03, 119.73, 117.11 (d, J_{C-F} = 8.3 Hz), 116.04, 115.66 (d, J_{C-F} = 23.4 Hz), 79.35, 54.63, 53.27, 49.16, 35.97.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -123.80.

Elemental Analysis (%) calculated for $C_{25}H_{27}ON_2F$: C, 76.92; H, 6.92; N, 7.18. Found: C, 77.17; H, 7.28; N, 6.85.

1-[3-(4-Fluorophenoxy)-3-phenylpropyl]-4-phenylpiperazine hydrochloride **(16b).**[163]

To a well stirred solution of 1-[3-(4-fluorophenoxy)-3-phenylpropyl]-4-phenylpiperazine **16a** (0.16 g, 0.40 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (2.0 mL, 4.0 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **16b** was afforded as a blue-green solid (0.083 g, 0.19 mmol) in 46% yield.

HPLC (standard method) t_R = 8.85 min, purity > 98%.

Melting Point: 176 – 179 °C.

1-Phenyl-4-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine **(17a).**[163]

To a well stirred solution of 1-phenyl-3-(4-phenylpiperazinyl)-1-propanol **58** (1.71 g, 5.78 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-fluorophenol (1.87 g, 11.6 mmol) and PPh_3 (2.29 g, 8.67 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (1.70 mL, 8.67 mmol) was slowly added dropwise.

After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (20:80 EtOAc/hexane). The product **17a** was afforded as an orange oil (1.38 g, 3.17 mmol) in 54% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.43 (d, *J* = 8.54 Hz, 2H), 7.33 (m, 5H), 7.26 (m, 2H), 6.92 (dd, *J* = 8.11, 5.20 Hz, 4H), 6.86 (t, *J* = 7.29 Hz, 1H), 5.31 (dd, *J* = 8.02, 5.01 Hz, 1H), 3.36 (m, 4H), 2.64 (m, 4H), 2.56 (t, *J* = 7.30 Hz, 2H), 2.27 (dt, *J* = 14.90, 14.64, 7.35 Hz, 1H), 2.05 (dt, *J* = 12.96, 12.81, 6.86 Hz, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.57, 158.70, 151.13, 129.16, 128.81, 127.95, 126.77 (q, $J_{C\text{-}F}$ = 3.8 Hz), 125.85, 124.38 (q, $J_{C\text{-}F}$ = 271.0 Hz), 122.88 (q, $J_{C\text{-}F}$ = 32.5 Hz), 119.97, 116.17, 115.77, 78.63, 54.51, 53.22, 49.04, 35.73.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -64.70.

Elemental Analysis (%) calculated for $C_{26}H_{27}ON_2F_3$: C, 70.91; H, 6.14; N, 6.36. Found: C, 70.94; H, 6.33; N, 6.17.

1-Phenyl-4-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine hydrochloride **(17b).**[163]

To a well stirred solution of 1-phenyl-4-[3-phenyl-3-(4-trifluoromethylphenoxy) propyl]piperazine **17a** (0.17 g, 0.40 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (2.0 mL, 4.0 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried

under vacuum. The product **17b** was afforded as a white solid (0.077 g, 0.26 mmol) in 42% yield.

HPLC (standard method) t_R = 9.05 min, purity > 99%.

Melting Point: 180 – 183 °C.

1-Phenyl-3-[4-(2-pyrimidinyl)piperazinyl]-1-propanone **(59)**.[134]

A well-stirred mixture of acetophenone (3.40 mL, 29.0 mmol), 1-(2-pyrimidyl) piperazine dihydrochloride **Vb** (4.48g, 22.5 mmol), and concentrated HCl (0.05mL) in absolute ethanol (35mL) under N_2 was heated at reflux conditions. Paraformaldehyde (1.00g, 33.3 mmol) was added in four equal portions over 40 minutes. After refluxing for 16 h, the solution was cooled, poured onto ice and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous K_2CO_3 and the solvent was evaporated under reduced pressure. The residue was recrystallized in a small amount of anhydrous CH_2Cl_2. The resulting solid was collected by filtration and dried under vacuum. The product **59** was afforded as a white solid (1.49 g, 5.03 mmol) in 20% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.35 (d, *J* = 4.76 Hz, 2H), 8.02 (d, *J* = 7.26 Hz, 2H), 7.63 (t, *J* = 7.39 Hz, 1H), 7.50 (t, *J* = 7.82 Hz, 2H), 6.64 (t, *J* = 4.78 Hz, 1H), 4.88 (d, *J* = 13.90 Hz, 2H), 3.84 (m, 4H), 3.52 (dd, *J* = 14.72, 9.46 Hz, 4H), 2.88 (dd, *J* = 20.48, 9.98 Hz, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 195.92, 160.83, 157.99, 135.40, 134.22, 128.93, 128.35, 111.60, 52.27, 52.10, 40.71, 33.11.

Melting Point: 201 – 204 °C.

1-Phenyl-3-[4-(2-pyrimidinyl)piperazinyl]-1-propanol **(60)**.[134]

To a well stirred solution 1-phenyl-3-[4-(2-pyrimidinyl)piperazinyl]-1-propanone **59** (1.46 g, 4.93 mmol) in methanol (25 mL) under N_2 at 0 °C was added sodium borohydride (0.47 g, 12.42 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was dried under vacuum. The product **60** was afforded as a cream solid (1.12 g, 3.76 mmol) in 76% yield.

^{1}H -NMR (300 MHz, $CDCl_3$): δ 8.31 (d, *J* = 4.74 Hz, 2H), 7.37 (m, 4H), 7.26 (m, 1H), 6.50 (t, *J* = 4.73 Hz, 1H), 4.98 (t, *J* = 5.71 Hz, 1H), 3.89 (t, *J* = 5.03 Hz, 4H), 2.70 (m, 4H), 2.56 (m, 2H), 1.92 (dd, *J* = 11.06, 6.00 Hz, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.58, 157.73, 144.76, 128.27, 127.00, 125.55, 110.07, 75.59, 57.27, 53.19, 43.67, 33.72.

Melting Point: 90 – 93 °C.

1-[3-(4-Fluorophenoxy)-3-phenylpropyl]-4-(2-pyrimidyl)piperazine **(24a)**.

To a well stirred solution of 1-Phenyl-3-[4-(2-pyrimidinyl)piperazinyl]-1-propanol **60** (0.76 g, 2.55 mmol) in anhydrous benzene (100 mL) under N_2 at 25 °C was added 4-fluorophenol (0.31 g, 2.77 mmol) and ADDP (0.71 g, 2.81 mmol). After complete dissolution, Bu_3P (0.70 mL, 2.83 mmol) was carefully added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, PBu_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel

and purified by flash chromatography (50:50 EtOAc/hexane). The product **24a** was afforded as a pale yellow oil (0.83 g, 2.12 mmol) in 83% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.30 (d, *J* = 4.73 Hz, 2H), 7.33 (m, 4H), 7.27 (m, 1H), 6.90-6.73 (m, 4H), 6.48 (t, *J* = 4.73 Hz, 1H), 5.16 (dd, *J* = 8.05, 4.98 Hz, 1H), 3.82 (m, 4H), 2.52 (m, 6H), 2.23 (dt, *J* = 14.66, 7.21 Hz, 1H), 2.02 (m, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 157.20 (d, $J_{C\text{-}F}$ = 238.5 Hz), 154.34 (d, $J_{C\text{-}F}$ = 2.3 Hz), 141.64, 128.63, 127.68, 126.03, 117.09 (d, $J_{C\text{-}F}$ = 7.5 Hz), 115.65 (d, $J_{C\text{-}F}$ = 22.6 Hz), 109.84, 79.33, 54.72, 53.14, 43.77, 36.03.

^{19}-NMR (282 MHz, $CDCl_3$): δ -123.81.

1-[3-(4-Fluorophenoxy)-3-phenylpropyl]-4-(2-pyrimidyl)piperazine hydrochloride **(24b)**.

To a well stirred solution of 1-[3-(4-fluorophenoxy)-3-phenylpropyl]-4-(2-pyrimidyl)piperazine **24a** (0.22 g, 0.56 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (2.80 mL, 5.60 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **24b** was afforded as an off-white solid (0.22 g, 0.50 mmol) in 90% yield.

HPLC (standard method) t_R = 8.57 min, purity > 98%.

Melting Point: 183 – 186 °C.

1-[3-Phenyl-3-(4-trifluoromethylphenoxy)propyl]-4-(2-pyrimidyl)piperazine **(25a)**.[134]

To a well stirred solution of 1-phenyl-3-[4-(2-pyrimidinyl)piperazinyl]-1-propanol **60** (1.12 g, 3.76 mmol) in anhydrous benzene (100 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.67 g, 4.13 mmol) and ADDP (1.04 g, 4.12 mmol). After complete dissolution, Bu_3P (1.02 mL, 4.13 mmol) was carefully added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, PBu_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (50:50 EtOAc/hexane). The product **25a** was afforded as a pale yellow oil (1.36 g, 3.08 mmol) in 82% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.30 (d, J = 4.72 Hz, 2H), 7.43 (d, J = 8.91 Hz, 2H), 7.32 (m, 5H), 6.91 (d, J = 8.83 Hz, 2H), 6.48 (t, J = 4.71 Hz, 1H), 5.31 (dd, J = 7.97, 5.01 Hz, 1H), 3.83 (m, 4H), 2.51 (m, 6H), 2.26 (dt, J = 14.85, 7.27 Hz, 1H), 2.05 (ddd, J = 16.66, 9.85, 6.39 Hz, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.70, 160.65, 157.70, 141.02, 128.77, 127.88, 126.75 (q, $J_{C\text{-}F}$ = 3.8 Hz), 125.90, 124.39 (q, $J_{C\text{-}F}$ = 271.0 Hz), 122.80 (q, $J_{C\text{-}F}$ = 32.5 Hz), 115.78, 109.87, 78.66, 54.59, 53.15, 43.76, 36.00.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.52.

1-[3-Phenyl-3-(4-trifluoromethylphenoxy)propyl]-4-(2-pyrimidyl)piperazine hydrochloride **(25b)**.

To a well stirred solution of 1-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]-4-(2-pyrimidyl)piperazine **25a** (0.22 g, 0.50 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (2.50 mL, 5.00 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining

solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **24b** was afforded as an off-white solid (0.13 g, 0.27 mmol) in 54% yield.

HPLC (standard method) t_R = 8.82 min, purity > 98%.

Melting Point: 180 – 183 °C.

Synthesis of Homologues Type II – Compounds 18 to 23, 26 and 27

3-[4-(3-Chlorophenyl)piperazinyl]-1-phenyl-1-propanone **(61)**.

To a well stirred solution of 1-(3-chlorophenyl)piperazine **II** (0.98 mL, 5.94 mmol) in acetonitrile (25 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (0.96 g, 6.95 mmol). After stirring for 15 min, 3-chloropropiophenone (1.00 g, 5.93 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **61** was afforded as a yellow oil (1.26 g, 3.82 mmol) in 64% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.98 (dd, *J* = 8.38, 1.36 Hz, 2H), 7.58 (t, *J* = 7.33 Hz, 1H), 7.47 (t, *J* = 7.39 Hz, 2H), 7.15 (t, *J* = 8.11 Hz, 1H), 6.87 (t, *J* = 2.14 Hz, 1H), 6.79 (ddt, *J* = 8.25, 2.15, 0.76 Hz, 2H), 3.21 (m, 6H), 2.90 (dd, *J* = 7.84, 6.77 Hz, 2H), 2.66 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 198.99, 152.28, 136.92, 134.95, 133.19, 130.02, 128.67, 128.05, 119.30, 115.75, 113.88, 53.10, 48.68, 36.29.

3-[4-(3-Chlorophenyl)piperazinyl]-1-phenyl-1-propanol **(62)**.

To a well stirred solution of 3-[4-(3-chlorophenyl)piperazinyl]-1-phenyl-1-propanone **61** (1.25 g, 3.80 mmol) in methanol (50 mL) under N_2 at 0 °C was added sodium borohydride (0.36 g, 9.52 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was dried under vacuum. The product **62** was afforded as a white solid (1.2 g, 3.6 mmol) in 95% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34 (m, 5H), 7.17 (t, J = 8.10 Hz, 1H), 6.88 (t, J = 2.14 Hz, 1H), 6.80 (dddd, J = 12.49, 8.34, 2.14, 0.76 Hz, 2H), 4.96 (m, 1H), 3.25 (t, J = 5.04 Hz, 4H), 2.77 (m, 3H), 2.63 (m, 3H), 1.91 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 152.11, 144.71, 135.00, 130.08, 128.28, 127.02, 125.53, 119.61, 116.01, 114.00, 75.51, 57.06, 53.06, 48.81, 33.77.

Melting point: 98 – 101 °C.

1-(3-Chlorophenyl)-4-[3-(4-fluorophenoxy)-3-phenyl-propyl]piperazine **(18a).**

To a well stirred solution of 3-[4-(3-chlorophenyl)piperazinyl]-1-phenyl-1-propanol **62** (1.1 g, 3.3 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-fluorophenol (0.75 g, 6.69 mmol) and PPh_3 (1.31 g, 5.00 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.99 mL, 5.01 mmol) was

slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure and the residue was re-dissolved in CH_2Cl_2 (100 mL). The new organic layer was washed with H_2O (3 x 100 mL) and brine (1 x 100 mL), dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (10:90 EtOAc/hexane). The product **18a** was afforded as a clear oil (0.74 g, 1.75 mmol) in 53% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34 (m, 5H), 7.15 (t, J = 8.11 Hz, 1H), 6.83 (m, 7H), 5.16 (dd, J = 8.07, 4.99 Hz, 1H), 3.20 (m, 4H), 2.57 (m, 6H), 2.22 (m, 1H), 2.01 (ddd, J = 21.35, 7.27, 5.05 Hz, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 157.21 (d, $J_{C\text{-}F}$ = 238.5 Hz), 154.33 (d, $J_{C\text{-}F}$ = 2.3 Hz), 152.34, 141.61, 134.96, 130.01, 128.65, 127.71, 126.02, 119.24, 117.10 (d, $J_{C\text{-}F}$ = 8.3 Hz), 115.70, 115.67 (d, $J_{C\text{-}F}$ = 23.4 Hz), 113.82, 79.29, 54.56, 53.06, 48.73, 36.02.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -123.76.

Elemental Analysis (%) calculated for $C_{25}H_{26}ClFN_2O$: C, 70.66; H, 6.17; N, 6.59. Found: C, 70.62; H, 6.22; N, 6.53.

1-(3-Chlorophenyl)-4-[3-(4-fluorophenoxy)-3-phenylpropyl]piperazine hydrochloride **(18b).**

To a well stirred solution of 1-(3-chlorophenyl)-4-[3-(4-fluorophenoxy)-3-phenyl propyl]piperazine **18a** (0.33 g, 0.78 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (1.56 mL, 3.11 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15

min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **18b** was afforded as a white solid (0.28 g, 0.62 mmol) in 80% yield.

HPLC (standard method) t_R = 9.13 min, purity > 97%.

Melting Point: 147 – 150 °C.

1-(3-Chlorophenyl)-4-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine **(19a).**

To a well stirred solution of 3-[4-(3-chlorophenyl)piperazinyl]-1-phenyl-1-propanol **62** (0.75 g, 2.27 mmol) in anhydrous benzene (100 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.40 g, 2.47 mmol) and PPh_3 (0.66 g, 2.51 mmol). After complete dissolution, a solution of ADDP (0.63 g, 2.50 mmol) in anhydrous benzene (20 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (20:80 EtOAc/hexane). The product **19a** was afforded as a clear oil (0.29 g, 0.60 mmol) in 27% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42 (d, *J* = 8.54 Hz, 2H), 7.30 (m, 5H), 7.14 (t, *J* = 8.11 1H), 6.91 (d, *J* = 8.52 Hz, 2H), 6.86 (t, *J* = 2.11 Hz, 1H), 6.78 (ddd, *J* = 8.56, 8.16, 1.45 Hz, 2H), 5.30 (dd, *J* = 7.96, 5.06 Hz, 1H), 3.18 (m, 4H), 2.56 (m, 6H), 2.25 (dt, *J* = 14.34, 7.56 Hz, 1H), 2.03 (dtd, *J* = 12.39, 7.22, 5.20 Hz, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.70, 152.36, 141.04, 134.99, 129.84, 128.83, 127.94, 126.80 (d, $J_{C\text{-}F}$ = 3.0 Hz), 124.51 (d, $J_{C\text{-}F}$ = 271.0 Hz), 122.83 (d, $J_{C\text{-}F}$ = 122.83 Hz), 119.28, 115.83, 115.73, 113.87, 78.65, 54.43, 53.08, 48.73, 36.00.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.38.

Elemental Analysis (%) calculated for $C_{26}H_{26}ClF_3N_2O$: C, 65.75; H, 5.52; N, 5.90. Found: C, 65.49; H, 5.52, N, 5.90.

1-(3-Chlorophenyl)-4-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine hydrochloride **(19b).**

To a well stirred solution of 1-(3-chloro-phenyl)-4-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine **19a** (0.25 g, 0.53 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.53 mL, 1.06 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **19b** was afforded as a cream solid (0.26 g, 0.52 mmol) in 97% yield.

HPLC (standard method) t_R = 9.25 min, purity > 99%.

Melting Point: 167 – 170 °C.

1-Phenyl-3-[4-(3-trifluoromethylphenyl)piperazinyl]-1-propanone **(63)**.

To a well stirred solution of 1-(3-trifluoromethylphenyl)piperazine **III** (1.11 mL, 5.91 mmol) in acetonitrile (25 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (0.96 g, 6.95 mmol). After stirring for 15 min, 3-chloropropiophenone (1.00 g, 5.93 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was then quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified

by flash chromatography (30:70 EtOAc/hexane). The product **63** was afforded as a yellow oil (1.26 g, 3.82 mmol) in 69% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.98 (dd, J = 8.38, 1.33 Hz, 2H), 7.58 (t, J = 7.33 Hz, 1H), 7.48 (t, J = 7.38 Hz, 2H), 7.34 (t, J = 7.92 Hz, 1H), 7.07 (m, 3H), 3.25 (m, 6H), 2.91 (t, J = 7.30 Hz, 2H), 2.69 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 198.98, 151.33, 136.92, 133.20, 131.4 (q, $J_{C\text{-}F}$ = 31.7 Hz), 129.55, 128.68, 128.05, 124.4 (q, $J_{C\text{-}F}$ = 272.5 Hz), 118.68, 115.83 (d, $J_{C\text{-}F}$ = 3.8 Hz), 112.14 (d, $J_{C\text{-}F}$ = 3.8 Hz), 53.08, 48.64, 36.27.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -62.68.

1-Phenyl-3-[4-(3-trifluoromethylphenyl)piperazinyl]-1-propanol **(64)**.

To a well stirred solution of 1-phenyl-3-[4-(3-trifluoromethylphenyl)piperazinyl]-1-propanone **62** (1.48 g, 4.09 mmol) in methanol (50 mL) under N_2 at 0 °C was added sodium borohydride (0.39 g, 10.31 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was dried under vacuum. The product **64** was afforded as a light yellow solid (1.2 g, 3.0 mmol) in 81% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34 (m, 5H), 7.26 (m, 1H), 7.09 (m, 3H), 4.97 (m, 1H), 3.30 (t, J = 5.04 Hz, 4H), 2.78 (ddd, J = 12.93, 11.37, 5.47 Hz, 3H), 2.65 (m, 3H), 1.92 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 151.17, 144.67, 131.49 (q, J_{C-F} = 31.7 Hz), 129.62, 128.30, 127.05, 125.53, 124.30 (q, J_{C-F} = 272.5 Hz), 118.86, 116.19 (q, J_{C-F} = 3.0 Hz), 112.44 (q, J_{C-F} = 3.8 Hz), 75.50, 57.03, 53.04, 48.82, 33.77.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -62.71.

Melting Point: 80 – 83 °C.

1-[3-(4-Fluorophenoxy)-3-phenylpropyl]-4-(3-trifluoromethylphenyl)piperazine **(20a).**

To a well stirred solution of 1-phenyl-3-[4-(3-trifluoromethylphenyl)piperazinyl]-1-propanol **64** (1.1 g, 3.0 mmol) in anhydrous THF (50 mL) under N_2 at 25 °C was added 4-fluorophenol (0.68 g, 6.07 mmol) and PPh_3 (1.19 g, 4.53 mmol). After complete dissolution, the reaction mixture was cooled to 0 °C and DIAD (0.90 mL, 4.56 mmol) was slowly added dropwise. After stirring for 48 h at 25 °C, the solvent was removed from the reaction mixture under reduced pressure and the residue was re-dissolved in CH_2Cl_2 (100 mL). The new organic layer was washed with H_2O (3 x 100 mL) and brine (1 x 100 mL), dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (10:90 EtOAc/hexane). The product **20a** was afforded as a clear oil (0.53 g, 1.16 mmol) in 39% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34 (m, 5H), 7.29 (m, 1H), 7.08 (m, 3H), 6.82 (m, 4H), 5.16 (dd, J = 8.09, 4.99 Hz, 1H), 3.24 (m, 4H), 2.58 (m, 6H), 2.23 (dt, J = 14.33, 7.45 Hz, 1H), 2.01 (dtd, J = 12.47, 7.43, 5.03 Hz, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 157.21 (d, J_{C-F} = 238.5 Hz), 154.31 (d, J_{C-F} = 1.5 Hz), 151.37, 141.59, 131.42 (q, J_{C-F} = 31.7 Hz), 129.53, 128.66, 127.72, 126.03, 124.34 (q,

$J_{C\text{-}F}$ = 272.5 Hz), 118.65, 117.08 (d, $J_{C\text{-}F}$ = 7.6 Hz), 115.76 (q, $J_{C\text{-}F}$ = 3.8 Hz), 115.68 (d, $J_{C\text{-}F}$ = 22.6 Hz), 112.11 (q, $J_{C\text{-}F}$ = 3.8 Hz), 79.26, 54.55, 53.05, 48.72, 36.03.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -62.68, -123.75.

Elemental Analysis (%) calculated for $C_{26}H_{26}F_4N_2O$: C, 68.11; H, 5.72; N, 6.11. Found: C, 68.09; H, 5.72; N, 6.03.

1-[3-(4-Fluorophenoxy)-3-phenylpropyl]-4-(3-trifluoromethylphenyl)piperazine hydrochloride **(20b).**

To a well stirred solution of 1-[3-(4-fluorophenoxy)-3-phenyl-propyl]-4-(3-trifluoromethylphenyl)piperazine **20a** (0.26 g, 0.58 mmol) in anhydrous THF (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (1.16 mL, 2.31 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **20b** was afforded as a cream crystalline solid (0.29 g, 0.58 mmol) in quantitative yield.

HPLC (standard method) t_R = 9.17 min, purity > 99%.

Melting Point: 155 – 158 °C.

1-[3-Phenyl-3-(4-trifluoromethylphenoxy)propyl]-4-(3-trifluoromethylphenyl)piperazine **(21a)**.

To a well stirred solution of 1-phenyl-3-[4-(3-trifluoromethylphenyl)piperazinyl]-1-propanol **64** (0.64 g, 1.76 mmol) in anhydrous benzene (100 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.31 g, 1.91 mmol) and PPh_3 (0.51 g, 1.94 mmol). After complete dissolution, a solution of ADDP (0.49 g, 1.94 mmol) in anhydrous benzene (20

mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (20:80 EtOAc/hexane). The product **21a** was afforded as a clear oil (0.20 g, 0.40 mmol) in 23% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42 (d, J = 8.54 Hz, 2H), 7.31 (m, 6H), 7.07 (m, 3H), 6.92 (d, J = 8.51 Hz, 2H), 5.32 (dd, J = 7.94, 5.07 Hz, 1H), 3.23 (m, 4H), 2.58 (m, 6H), 2.26 (tt, J = 14.13, 6.90 Hz, 1H), 2.04 (dtd, J = 12.30, 7.19, 5.28 Hz, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.75, 151.44, 141.07, 131.46 (q, $J_{C\text{-}F}$ = 31.7 Hz), 129.58, 128.83, 127.95, 126.81 (q, $J_{C\text{-}F}$ = 3.8 Hz), 125.96, 124.47 (q, $J_{C\text{-}F}$ = 271.0 Hz), 122.84 (q, $J_{C\text{-}F}$ = 32.5 Hz), 122.64 (q, $J_{C\text{-}F}$ = 274.7 Hz), 118.70, 115.84, 115.76 (q, $J_{C\text{-}F}$ = 3.8 Hz), 112.12 ($J_{C\text{-}F}$ = 3.8 Hz), 78.64, 54.39, 53.06, 48.74, 36.01.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.39, -62.56.

Elemental Analysis (%) calculated for $C_{27}H_{26}F_6N_2O$: C, 63.77; H, 5.51; N, 5.51. Found: C, 63.68; H, 5.15; N, 5.51.

1-[3-Phenyl-3-(4-trifluoromethylphenoxy)propyl]-4-(3-trifluoromethylphenyl)piperazine hydrochloride **(21b).**

To a well stirred solution of 1-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]-4-(3-trifluoromethylphenyl)piperazine **21a** (0.20 g, 0.34 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.40 mL, 0.80 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure.

The remaining solid was dried under vacuum. The product **19b** was afforded as a white solid (0.18 g, 0.33 mmol) in 97% yield.

HPLC (standard method) t_R = 9.37 min, purity > 98%.

Melting Point: 208 – 210 °C.

3-[4-(2-Methoxyphenyl)piperazinyl]-1-phenyl-1-propanone **(65)**.[134]

To a well stirred solution of 1-(2-methoxyphenyl)piperazine **IV** (5.70 mL, 29.65 mmol) in acetonitrile (50 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (4.80 g, 34.73 mmol). After stirring for 15 min, 3-chloropropiophenone (5.00 g, 29.7 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated. The residue was adsorbed onto silica gel and purified by flash chromatography (50:50 EtOAc/hexane). The product **65** was afforded as a yellow oil (7.48 g, 23.08 mmol) in 78% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.98 (d, *J* = 7.10 Hz, 2H), 7.57 (t, *J* = 7.32 Hz, 1H), 7.47 (t, *J* = 7.41 Hz, 2H), 6.94 (m, 4H), 3.86 (s, 3H), 3.24 (m, 2H), 3.11 (bs, 4H), 2.93 (t, *J* = 7.45 Hz, 2H), 2.74 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 199.15, 152.30, 141.30, 137.00, 133.10, 128.64, 128.06, 122.95, 121.00, 118.22, 111.23, 55.39, 53.47, 50.66, 36.24.

3-[4-(2-Methoxyphenyl)piperazinyl]-1-phenyl-1-propanol **(66)**.[134]

To a well stirred solution of 3-[4-(2-methoxyphenyl)piperazinyl]-1-phenyl-1-propanone **65** (7.08 g, 21.9 mmol) in methanol (50 mL) under N_2 at 0 °C was added

sodium borohydride (2.07 g, 54.72 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was dried under vacuum. The product **66** was afforded as a pale yellow solid (6.09 g, 18.68 mmol) in 86% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.37 (m, 4H), 7.25 (m, 1H), 7.02 (m, 1H), 6.94 (m, 2H), 6.87 (d, *J* = 7.55 Hz, 1H), 4.97 (t, *J* = 5.69 Hz, 1H), 3.87 (s, 3H), 3.14 (bs, 4H), 2.78 (m, 6H), 1.91 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 152.28, 144.93, 141.03, 128.24, 126.92, 125.55, 123.14, 121.08, 118.33, 111.23, 75.68, 57.21, 55.39, 53.50, 50.72, 33.65.

Melting Point: 79 – 82 °C.

1-[3-(4-Fluorophenoxy)-3-phenylpropyl]-4-(2-methoxyphenyl)-piperazine **(22a)**.

To a well stirred solution of 3-[4-(2-methoxyphenyl)piperazinyl]-1-phenyl-1-propanol **66** (1.00 g, 3.07 mmol) in anhydrous benzene (100 mL) under N_2 at 25 °C was added 4-fluorophenol (0.38 g, 3.39 mmol) and ADDP (0.85 g, 3.37 mmol). After complete dissolution, Bu_3P (0.83 mL, 3.37 mmol) was carefully added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PBu_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (20:80 EtOAc/hexane). The product **22a** was afforded as a clear oil (1.05 g, 2.50 mmol) in 81% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.35 (m, 4H), 7.29 (m, 1H), 6.97 (m, 3H), 6.83 (m, 5H), 5.16 (dd, *J* = 8.08, 5.00 Hz, 1H), 3.86 (s, 3H), 3.10 (bs, 4H), 2.65 (bs, 4H), 2.56 (m, 2H), 2.23 (dt, *J* = 14.35, 7.89 Hz, 1H), 2.02 (m, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 157.20 (d, $J_{C\text{-}F}$ = 238.5 Hz), 154.40 (d, $J_{C\text{-}F}$ = 1.5 Hz), 152.30, 141.74, 141.39, 128.61, 127.64, 126.05, 122.89, 121.00, 118.20, 117.15 (d, $J_{C\text{-}F}$ = 8.3 Hz), 115.64 (d, $J_{C\text{-}F}$ = 22.6 Hz), 111.24, 79.46, 55.38, 54.72, 53.51, 50.76, 36.09.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -123.86.

Elemental Analysis (%) calculated for $C_{26}H_{29}FN_2O_2$: C, 74.26; H, 6.95; N, 6.66. Found: C, 74.13; H, 7.04; N, 6.71.

1-[3-(4-Fluorophenoxy)-3-phenylpropyl]-4-(2-methoxyphenyl)piperazine hydrochloride **(22b)**.

To a well stirred solution of 1-[3-(4-fluorophenoxy)-3-phenylpropyl]-4-(2-methoxyphenyl)piperazine **22a** (0.25 g, 0.59 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (2.98 mL, 5.96 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **22b** was afforded as a cream crystalline solid (0.11 g, 0.24 mmol) in 41% yield.

HPLC (standard method) t_R = 8.98 min, purity > 99%.

Melting Point: 171 – 174 °C.

1-(2-Methoxyphenyl)-4-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine **(23a)**.[134]

To a well stirred solution of 3-[4-(2-methoxyphenyl)piperazinyl]-1-phenyl-1-propanol **66** (1.00 g, 3.07 mmol) in anhydrous benzene (100 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.55 g, 3.39 mmol) and ADDP (0.85 g, 3.37 mmol). After complete dissolution, Bu_3P (0.83 mL, 3.37 mmol) was carefully added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PBu_3O salts were removed by filtration and the solvent was evaporated. The residue was adsorbed onto silica gel and purified by flash chromatography (20:80 EtOAc/hexane). The product **23a** was afforded as a yellow oil (1.11 g, 2.36 mmol) in 77% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.43 (d, J = 8.51 Hz, 2H), 7.33 (m, 5H), 6.97 (m, 5H), 6.86 (dd, J = 7.81, 1.19 Hz, 1H), 5.31 (dd, J = 8.01, 5.04 Hz, 1H), 3.86 (s, 3H), 3.10 (bs, 4H), 2.65 (bs, 4H), 2.56 (t, J = 6.96 Hz, 2H), 2.26 (dt, J = 14.60, 7.11 Hz, 1H), 2.04 (m, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.72, 152.29, 141.36, 141.13, 128.74, 127.84, 126.74 (q, $J_{C\text{-}F}$ = 3.8 Hz), 125.92, 124.41 (q, $J_{C\text{-}F}$ = 271.0 Hz), 122.76 (q, $J_{C\text{-}F}$ = 32.5 Hz), 121.00, 118.20, 115.81, 111.24, 78.77, 55.37, 54.56, 53.51, 50.75, 36.04.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.51.

Elemental Analysis (%) calculated for $C_{27}H_{29}F_3N_2O_2$: C, 68.92; H, 6.21; N, 5.95. Found: C, 68.62; H, 6.30; N, 5.86.

1-(2-Methoxyphenyl)-4-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine hydrochloride **(23b)**.

To a well stirred solution of 1-(2-methoxyphenyl)-4-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine **23a** (0.25 g, 0.53 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (2.66 mL, 5.32 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **22b** was afforded as a cream crystalline solid (0.21 g, 0.41 mmol) in 78% yield.

HPLC (standard method) t_R = 9.15 min, purity > 99%.

Melting Point: 186 – 188 °C.

3-(4-Methylpiperazinyl)-1-phenyl-1-propanone **(67)**.

To a well stirred solution of 1-methylpiperazine **VI** (3.33 mL, 29.75 mmol) in acetonitrile (50 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (4.80 g, 34.73 mmol). After stirring for 15 min, 3-chloropropiophenone (5.00 g, 29.7 mmol) was added to the reaction mixture. After refluxing for 6 h, the reaction was quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated. The residue was adsorbed onto silica gel and purified by flash chromatography (10:90 MeOH/EtOAc). The product **67** was afforded as an orange oil (4.50 g, 19.40 mmol) in 65% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.96 (d, *J* = 7.07 Hz, 2H), 7.57 (t, *J* = 7.34 Hz, 1H), 7.46 (t, *J* = 7.45 Hz, 2H), 3.20 (m, 2H), 2.86 (m, 2H), 2.51 (d, *J* = 29.08 Hz, 8H), 2.29 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 199.10, 136.97, 133.08, 128.61, 128.02, 55.12, 53.22, 53.14, 46.05, 36.25.

3-(4-Methylpiperazinyl)-1-phenyl-1-propanol **(68)**.

To a well stirred solution of 3-(4-methylpiperazinyl)-1-phenyl-1-propanone **67** (4.50 g, 19.4 mmol) in methanol (50 mL) under N_2 at 0 °C was added sodium borohydride (1.83 g, 48.37 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice and extracted with EtOAc (3 x 100 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was dried under vacuum. The product **68** was afforded as a yellow oil (3.80 g, 16.24 mmol) in 84% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.36 (m, 4H), 7.24 (m, 1H), 6.72 (bs, 1H), 4.93 (m, 1H), 2.77 (m, 10H), 2.30 (s, 3H), 1.86 (m, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 144.92, 128.20, 126.89, 125.52, 75.60, 57.03, 55.15, 53.24, 45.99, 33.66.

1-[3-(4-Fluorophenoxy)-3-phenylpropyl]-4-methylpiperazine **(26a)**.

To a well stirred solution of 3-(4-methylpiperazinyl)-1-phenyl-1-propanol **68** (0.65 g, 2.78 mmol) in anhydrous benzene (100 mL) under N_2 at 25 °C was added 4-fluorophenol (0.34 g, 3.03 mmol) and PPh_3 (0.80 g, 3.05 mmol). After complete

dissolution, a solution of ADDP (0.77 g, 3.05 mmol) in anhydrous benzene (20 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 MeOH/EtOAc). The product **26a** was afforded as a yellow oil (0.20 g, 0.40 mmol) in 38% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.29 (m, 5H), 6.82 (m, 4H), 5.12 (dd, J = 8.05, 5.02 Hz, 1H), 2.50 (m, 10H), 2.29 (s, 3H), 2.18 (td, J = 14.37, 7.47 Hz, 1H), 1.97 (ddd, J = 19.54, 10.85, 6.71 Hz, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 157.18 (d, $J_{C\text{-}F}$ = 238.5 Hz), 154.38 (d, $J_{C\text{-}F}$ = 2.3 Hz), 141.70, 128.59, 127.62, 126.02, 117.12 (d, $J_{C\text{-}F}$ = 7.5 Hz), 115.62 (d, $J_{C\text{-}F}$ = 23.4 Hz), 79.42, 55.24, 54.58, 53.23, 46.08, 36.08.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -123.88.

Elemental Analysis (%) calculated for $C_{20}H_{25}FN_2O$: C, 73.14; H, 7.67; N, 8.53. Found: C, 73.00; H, 7.74; N, 8.44.

1-[3-(4-Fluorophenoxy)-3-phenylpropyl]-4-methylpiperazine hydrochloride **(26b)**.

To a well stirred solution of 1-[3-(4-fluorophenoxy)-3-phenylpropyl]-4-methyl piperazine **26a** (0.32 g, 0.97 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.97 mL, 1.94 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **26b** was afforded as a white solid (0.15 g, 0.42 mmol) in 43% yield.

HPLC (standard method) t_R = 7.28 min, purity > 96%.

Melting Point: 207 – 210 °C.

1-Methyl-4-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine **(27a)**.

To a well stirred solution of 3-(4-methylpiperazinyl)-1-phenyl-1-propanol **68** (0.65 g, 2.78 mmol) in anhydrous benzene (100 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.50 g, 3.08 mmol) and PPh_3 (0.80 g, 3.05 mmol). After complete dissolution, a solution of ADDP (0.77 g, 3.05 mmol) in anhydrous benzene (20 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 MeOH/EtOAc). The product **27a** was afforded as a yellow oil (0.24 g, 0.64 mmol) in 23% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42 (d, J = 8.90 Hz, 2H), 7.30 (m, 5H), 6.90 (d, J = 8.82 Hz, 2H), 5.27 (dd, J = 7.97, 5.10 Hz, 1H), 2.49 (m, 10H), 2.29 (s, 3H), 2.20 (td, J = 14.58, 7.22 Hz, 1H), 2.00 (m, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.70, 141.09, 128.73, 127.82, 126.73 (q, $J_{C\text{-}F}$ = 3.8 Hz), 125.89, 124.40 (q, $J_{C\text{-}F}$ = 271.7 Hz), 122.79 (q, $J_{C\text{-}F}$ = 32.5 Hz), 122.52, 122.09, 115.79, 78.73, 55.23, 54.42, 53.22, 46.07, 36.03.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.51

Elemental Analysis (%) calculated for $C_{21}H_{25}F_3N_2O$: C, 66.67; H, 6.61; N, 7.41. Found: C, 66.81; H, 6.71; N, 7.33.

1-Methyl-4-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine hydrochloride **(27b)**.

To a well stirred solution of 1-methyl-4-[3-phenyl-3-(4-trifluoromethylphenoxy) propyl]piperazine **27a** (0.14 g, 0.36 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.72 mL, 1.44 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **27b** was afforded as a white solid (0.13 g, 0.42 mmol) in 83% yield.

HPLC (standard method) t_R = 7.53 min, purity > 98%.

Melting Point: 218 – 221 °C.

Synthesis of Homologues Type II – Compounds 28 to 33

3-Chloro-1-phenyl-1-propanol **(69)**.

To a well stirred solution of 3-chloropropiophenone (10.0 g, 59.3 mmol) in methanol (100 mL) under N_2 at 0 °C was added sodium borohydride (5.6 g, 148.0 mmol) in portions over a period of 15 min. After bubbling ceased, the reaction mixture was stirred at room temperature for 8 h. The reaction was then poured onto ice and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was dried under vacuum. The product **69** was afforded as a white solid (9.62 g, 56.38 mmol) in 95% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.31 (m, 5H), 4.90 (s, 1H), 3.70 (ddd, *J* = 10.87, 8.11, 5.74 Hz, 1H), 3.53 (m, 1H), 2.23 (m, 2H), 2.05 (m, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 143.71, 128.68, 127.94, 125.81, 71.32, 41.75, 41.45.

Melting Point: 30 – 33 °C.

3-[4-(4-fluorobenzoyl)piperidinyl]-1-phenyl-1-propanol **(70)**.

To a well stirred solution of 4-(4-fluorobenzoyl)piperidine **VII** (1.27 g, 6.14 mmol) in acetonitrile (25 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (0.99 g, 7.16 mmol). After stirring for 15 min, 3-chloro-1-phenyl-propanol **69** (1.05g, 6.15 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was then quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (70:30 EtOAc/hexane). The product **70** was afforded as a white solid (1.05 g, 3.08 mmol) in 50% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.96 (dd, *J* = 8.57, 5.44 Hz, 2H), 7.36 (m, 4H), 7.24 (m, 1H), 7.14 (t, *J* = 8.46 Hz, 2H), 6.59 (s, 1H), 4.94 (dd, *J* = 7.13, 4.11 Hz, 1H), 3.19 (m, 3H), 2.70 (ddd, *J* = 12.83, 8.26, 4.63 Hz, 1H), 2.58 (td, *J* = 9.36, 4.35 Hz, 1H), 2.21 (m, 1H), 2.06 (m, 1H), 1.89 (m, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 200.57, 165.67 (d, $J_{C\text{-}F}$ = 255.1 Hz),144.95, 132.41 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.86 (d, $J_{C\text{-}F}$ = 9.1 Hz), 128.21, 126.87, 125.53, 115.82 (d, $J_{C\text{-}F}$ = 21.1 Hz), 75.46, 57.27, 53.90, 52.59, 43.29, 33.89, 28.78, 28.56.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -105.33.

Melting Point: 106 – 108 °C.

4-(4-fluorobenzoyl)-1-[3-(4-fluorophenoxy)-3-phenylpropyl]piperidine **(28a).**

To a well stirred solution of 3-[4-(4-fluorobenzoyl)piperidinyl]-1-phenyl-1-propanol **70** (0.89 g, 2.61 mmol) in anhydrous benzene (100 mL) under N_2 at 25 °C was added 4-fluorophenol (0.32 g, 2.85 mmol) and PPh_3 (0.75 g, 2.85 mmol). After complete dissolution, a solution of ADDP (0.72 g, 2.85 mmol) in anhydrous benzene (20 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **28a** was afforded as a yellow oil (0.68 g, 1.56 mmol) in 60% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.96 (dd, J = 8.78, 5.44 Hz, 2H), 7.33 (m, 4H), 7.25 (m, 1H), 7.13 (t, J = 8.58 Hz, 2H), 6.83 (m, 4H), 5.16 (dd, J = 7.98, 5.12 Hz, 1H), 3.19 (m, 1H), 2.98 (d, J = 11.20 Hz, 2H), 2.50 (t, J = 6.78 Hz, 2H), 2.09 (m, 4H), 1.84 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 201.10, 165.64 (d, $J_{C\text{-}F}$ = 255.1 Hz), 157.19 (d, $J_{C\text{-}F}$ = 237.7 Hz), 154.38 (d, $J_{C\text{-}F}$ = 1.5 Hz), 141.72, 132.50 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.86 (d, $J_{C\text{-}F}$ = 9.0 Hz), 128.59, 127.62, 126.07, 117.11 (d, $J_{C\text{-}F}$ = 8.3 Hz), 115.77 (d, $J_{C\text{-}F}$ = 21.9 Hz), 115.64 (d, $J_{C\text{-}F}$ = 22.6 Hz), 79.32, 54.71, 53.39, 53.19, 43.80, 36.13, 28.92, 28.84.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -105.47, -123.86.

Elemental analysis (%) calculated for $C_{27}H_{27}F_2NO_2$: C, 74.48; H, 6.21; N, 3.22. Found: C, 74.29; H, 6.20; N, 3.32.

4-(4-fluorobenzoyl)-1-[3-(4-fluorophenoxy)-3-phenylpropyl]piperidine hydrochloride **(28b).**

To a well stirred solution of 4-(4-fluorobenzoyl)-1-[3-(4-fluorophenoxy)-3-phenyl propyl]piperidine **28a** (0.50 g, 1.16 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (1.16 mL, 3.20 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **28b** was afforded as a light orange solid (0.50 g, 1.07 mmol) in 92% yield.

HPLC (standard method) t_R = 8.97 min, purity > 99%.

Melting Point: 78 – 81 °C.

4-(4-fluorobenzoyl)-1-[3- phenyl-3-(4-trifluoromethylphenoxy)propyl]piperidine **(29a).**

To a well stirred solution of 3-[4-(4-fluorobenzoyl)piperidinyl]-1-phenyl-1-propanol **70** (0.46 g, 1.35 mmol) in anhydrous benzene (50 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.24 g, 1.48 mmol) and PPh_3 (0.39 g, 1.48 mmol). After complete dissolution, a solution of ADDP (0.37 g, 1.48 mmol) in anhydrous benzene (10 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **29a** was afforded as a dark yellow oil (0.22 g, 0.45 mmol) in 33% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.96 (dd, J = 8.92, 5.42 Hz, 2H), 7.42 (d, J = 8.54 Hz, 2H), 7.31 (m, 5H), 7.12 (t, J = 8.65 Hz, 2H), 6.92 (d, J = 8.51 Hz, 2H), 5.32 (dd, J =

7.88, 5.17 Hz, 1H), 3.21 (m, 1H), 2.98 (d, J = 11.43 Hz, 2H), 2.50 (t, J = 7.36 Hz, 2H), 2.24 (dt, J = 14.04, 7.74 Hz, 1H), 2.01 (m, 3H), 1.85 (m, 4H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 201.08, 165.65 (d, $J_{C\text{-}F}$ = 255.1 Hz), 160.73, 141.12, 132.49 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.87 (d, $J_{C\text{-}F}$ = 9.1 Hz), 128.74, 127.83, 126.75 (q, $J_{C\text{-}F}$ = 3.0 Hz), 124.44 (q, $J_{C\text{-}F}$ = 270.1 Hz), 125.95, 122.72 (q, $J_{C\text{-}F}$ = 32.5 Hz), 115.80, 115.78 (d, $J_{C\text{-}F}$ = 21.9 Hz), 78.65, 54.57, 53.36, 53.22, 43.75, 36.10, 28.92, 28.84.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.44, -105.41

Elemental Analysis (%) calculated for $C_{28}H_{27}F_4NO_2$: C, 69.27; H, 5.60; N, 2.88. Found: C, 69.43; H: 5.73; N, 2.98.

4-(4-fluorobenzoyl)-1-[3- phenyl-3-(4-trifluoromethylphenoxy)propyl]piperidine hydrochloride **(29b).**

To a well stirred solution of 4-(4-fluorobenzoyl)-1-[3- phenyl-3-(4-trifluoromethylphenoxy)propyl]piperidine **29a** (0.13 g, 0.26 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.26 mL, 0.52 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **29b** was afforded as a crystalline white solid (0.12 g, 0.23 mmol) in 88% yield.

HPLC (standard method) t_R = 9.15 min, purity > 98%.

Melting Point: 80 – 83 °C.

3-[4-(6-Fluoro-3-benzo[d]isoxazolyl)piperidinyl]-1-phenyl-1-propanol **(71)**.

To a well stirred solution of 4-(6-fluoro-3-benzo[d]isoxazolyl)piperidine **VIII** (2.64 g, 12.01 mmol) in acetonitrile (50 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (1.94 g, 14.04 mmol). After stirring for 15 min, 3-chloro-1-phenyl-propanol **69** (2.05g, 12.01 mmol) was added to the reaction mixture. After refluxing for 16 h, the reaction was quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (70:30 EtOAc/hexane). The product **71** was afforded as a white solid (1.88 g, 5.31 mmol) in 44% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.68 (dd, J = 8.65, 5.15 Hz, 1H), 7.37 (m, 4H), 7.25 (m, 2H), 7.06 (dt, J = 8.77, 1.45 Hz, 1H), 6.75 (s, 1H), 4.98 (t, J = 5.67 Hz, 1H), 3.19 (m, 3H), 2.76 (td, J = 12.95, 6.37 Hz, 1H), 2.64 (td, J = 9.43, 4.51 Hz, 1H), 2.33 (dt, J = 11.30, 7.15 Hz, 1H), 2.13 (m, 5H), 1.91 (dd, J = 10.79, 5.93 Hz, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.14 (d, $J_{C\text{-}F}$ = 251.3 Hz), 163.97 (d, $J_{C\text{-}F}$ = 13.6 Hz), 160.71, 144.86, 128.26, 126.97, 125.56, 122.58 (d, $J_{C\text{-}F}$ = 11.3 Hz), 117.15, 112.46 (d, $J_{C\text{-}F}$ = 24.9 Hz), 97.51 (d, $J_{C\text{-}F}$ = 27.2 Hz), 75.61, 57.38, 54.29, 52.75, 34.31, 33.89, 30.69, 30.43.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -109.41

Melting Point: 133 – 135 °C.

4-(6-Fluoro-3-benzo[d]isoxazolyl)-1-[3-(4-fluorophenoxy)-3-phenylpropyl]piperidine **(30a)**.

To a well stirred solution of 3-[4-(6-fluoro-3-benzo[d]isoxazolyl)piperidinyl]-1-phenyl-1-propanol **71** (0.80 g, 2.26 mmol) in anhydrous benzene (100 mL) under N_2 at 25

°C was added 4-fluorophenol (0.28 g, 2.50 mmol) and PPh_3 (0.65 g, 2.48 mmol). After complete dissolution, a solution of ADDP (0.63 g, 2.50 mmol) in anhydrous benzene (20 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **30a** was afforded as a pale yellow oil (0.42 g, 0.94 mmol) in 41% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): 7.67 (dd, J = 8.72, 5.13 Hz, 1H), 7.34 (m, 4H), 7.26 (m, 3H), 7.05 (dt, J = 8.85, 2.11 Hz, 1H), 6.83 (m, 4H), 5.17 (dd, J = 8.03, 5.05 Hz, 1H), 3.06 (m, 3H), 2.55 (t, J = 7.21 Hz, 2H), 2.12 (m, 8H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.09 (d, $J_{C\text{-}F}$ = 250.6 Hz), 162.86 (d, $J_{C\text{-}F}$ = 13.6 Hz), 161.14, 157.19 (d, $J_{C\text{-}F}$ = 238.5 Hz), 154.44 (d, $J_{C\text{-}F}$ = 2.3 Hz), 141.74, 128.66, 127.70, 126.08, 122.64 ($J_{C\text{-}F}$ = 10.6 Hz), 117.38, 117.16 (d, $J_{C\text{-}F}$ = 8.3 Hz), 115.68 (d, $J_{C\text{-}F}$ = 22.6 Hz), 112.33 (d, $J_{C\text{-}F}$ = 24.9 Hz), 97.40 (d, $J_{C\text{-}F}$ = 27.2 Hz), 79.42, 54.87, 53.68, 53.50, 36.15, 34.58, 30.65, 30.63

^{19}F-NMR (282 MHz, $CDCl_3$): δ -109.62, -123.82.

Elemental Analysis (%) calculated for $C_{27}H_{26}F_2N_2O_2$: C, 72.30; H, 5.84; N, 6.25. Found: C, 72.19; H, 5.92; N, 6.30.

4-(6-Fluoro-3-benzo[d]isoxazolyl)-1-[3-(4-fluorophenoxy)-3-phenylpropyl]piperidine hydrochloride **(30b)**.

To a well stirred solution of 4-(6-fluoro-3-benzo[d]isoxazolyl)-1-[3-(4-fluorophenoxy)-3-phenylpropyl]piperidine **30a** (0.42 g, 0.94 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.94 mL, 1.88

mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **30b** was afforded as a pale yellow crystalline solid (0.31 g, 0.64 mmol) in 69% yield.

HPLC (standard method) t_R = 9.00 min, purity > 99%.

Melting Point: 74 – 77 °C.

4-(6-Fluoro-3-benzo[d]isoxazolyl)-1-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl] piperidine **(31a)**.

To a well stirred solution of 3-[4-(6-fluoro-3-benzo[d]isoxazolyl)piperidinyl]-1-phenyl-1-propanol **71** (0.80 g, 2.26 mmol) in anhydrous benzene (100 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.40 g, 2.47 mmol) and PPh_3 (0.65 g, 2.48 mmol). After complete dissolution, a solution of ADDP (0.63 g, 2.50 mmol) in anhydrous benzene (20 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (30:70 EtOAc/hexane). The product **31a** was afforded as a yellow oil (0.40 g, 0.79 mmol) in 35% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.66 (dd, *J* = 8.70, 5.09 Hz, 1H), 7.43 (d, *J* = 8.63 Hz, 2H), 7.35 (m, 3H), 7.24 (m, 2H), 7.03 (dt, *J* = 8.86, 2.10 Hz, 1H), 6.93 (d, *J* = 8.57 Hz, 2H), 5.33 (dd, *J* = 7.81, 5.08 Hz, 1H), 3.07 (m, 3H), 2.55 (t, *J* = 7.09 Hz, 2H), 2.18 (m, 8H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 164.11 (d, J_{C-F} = 250.6 Hz), 163, 87 (d, J_{C-F} = 13.6 Hz), 161.13, 160.78, 141.14, 128.78, 127.87, 126.76 (q, J_{C-F} = 3.0 Hz), 125.95, 124.45 (q, J_{C-F} = 270.1 Hz), 122.73 (q, J_{C-F} = 32.5 Hz), 122.56 (d, J_{C-F} = 10.7 Hz), 117.37, 115.83, 112.33 (d, J_{C-F} = 24.9 Hz), 97.42 (d, J_{C-F} = 26.2 Hz), 78.77, 54.71, 53.68, 53.53, 36.14, 34.59, 30.69, 30.66.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.50, -109.58.

4-(6-Fluoro-3-benzo[d]isoxazolyl)-1-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl] piperidine hydrochloride **(31b)**.

To a well stirred solution of 4-(6-Ffluoro-3-benzo[d]isoxazolyl)-1-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperidine **31a** (0.23 g, 0.47 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.47 mL, 0.93 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and then the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **31b** was afforded as a pale yellow crystalline solid (0.22 g, 0.41 mmol) in 88% yield.

HPLC (standard method) t_R = 9.20 min, purity > 98%.

Melting Point: 94 – 97 °C.

3-{[4-(4-fluorobenzoyl)-propyl]piperazinyl}-1-phenyl-1-propanol **(72)**

To a well stirred solution of 1-[3-(4-fluorobenzoyl)propyl]piperazine **IX** (1.70 g, 6.79 mmol) in acetonitrile (25 mL) under N_2 at 25 °C was added anhydrous K_2CO_3 (1.10 g, 7.96 mmol). After stirring for 15 min, 3-chloro-1-phenyl-propanol **69** (1.16g, 6.79) was added to the reaction mixture. After refluxing for 16 h, the reaction was quenched with

H_2O (100 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated. The residue was adsorbed onto silica gel and purified by flash chromatography (10:90 MeOH/EtOAc). The product **72** was afforded as a yellow oil (1.00 g, 2.31 mmol) in 34% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.00 (dd, J = 8.72, 5.44 Hz, 2H), 7.35 (m, 5H), 7.13 (t, J = 8.64 Hz, 2H), 4.93 (dd, J = 6.69, 4.69 Hz, 1H), 2.97 (t, J = 7.10 Hz, 2H), 2.62 (m, 10H), 2.42 (t, J = 7.11 Hz, 2H), 1.94 (p, J = 7.07 Hz, 2H), 1.84 (td, J = 8.56, 4.45 Hz, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 198.23, 165.54 (d, $J_{C\text{-}F}$ = 254.4 Hz), 160.64, 144.98, 133.63 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.65 (d, $J_{C\text{-}F}$ = 9.1 Hz), 128.15, 126.84, 125.52, 115.54 (d, $J_{C\text{-}F}$ = 21.9 Hz), 75.26, 57.49, 56.84, 53.01, 36.08, 33.79, 21.60.

^{19}F-NMR (282 MHz, $CDCl_3$): -105.65.

4-[3-(4-fluorobenzoyl)propyl]-1-[3-(4-fluorophenoxy)-3-phenylpropyl]piperazine **(32a)**.

To a well stirred solution of 3-{[4-(4-fluorobenzoyl)propyl]piperazinyl}-1-phenyl-1-propanol **72** (0.50 g, 1.17 mmol) in anhydrous benzene (50 mL) under N_2 at 25 °C was added 4-fluorophenol (0.14 g, 1.25 mmol) and PPh_3 (0.34 g, 1.29 mmol). After complete dissolution, a solution of ADDP (0.32 g, 1.27 mmol) in anhydrous benzene (10 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated. The residue was adsorbed onto silica gel and purified by flash chromatography (100% EtOAc). The product **32a** was afforded as a yellow oil (0.25 g, 0.52 mmol) in 44% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.99 (dd, *J* = 8.87, 5.44 Hz, 2H), 7.28 (m, 5H), 7.10 (t, *J* = 8.64 Hz, 2H), 6.82 (m, 4H), 5.12 (dd, *J* = 8.04, 5.03 Hz, 1H), 2.96 (t, *J* = 7.11 Hz, 2H), 2.45 (m, 12H), 2.17 (dt, *J* = 14.43, 7.82 Hz, 1H), 1.95 (m, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 198.42, 165.61 (d, $J_{C\text{-}F}$ = 254.4 Hz), 157.17 (d, $J_{C\text{-}F}$ = 238.5 Hz), 154.38 (d, $J_{C\text{-}F}$ = 2.3 Hz), 141.70, 133.65 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.69 (d, $J_{C\text{-}F}$ = 9.1 Hz), 128.59, 127.62, 126.02, 117.12 (d, $J_{C\text{-}F}$ = 7.5 Hz), 115.62 (d, $J_{C\text{-}F}$ = 23.4 Hz), 115.6 (d, $J_{C\text{-}F}$ = 21.9 Hz), 79.41, 57.68, 54.60, 53.17, 36.19, 36.01, 21.68.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -105.67, -123.79.

Elemental Analysis (%) calculated for $C_{29}H_{32}F_2N_2O_2$: C, 72.78; H, 6.74; N. 5.85. Found: C, 72.36; H, 6.68; N, 5.76.

4-[3-(4-fluorobenzoyl)propyl]-1-[3-(4-fluorophenoxy)-3-phenyl-propyl]piperazine hydrochloride **(32b)**.

To a well stirred solution of 4-[3-(4-fluorobenzoyl)propyl]-1-[3-(4-fluorophenoxy)-3-phenyl-propyl]piperazine **(32a)** (0.14 g, 0.29 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.29 mL, 0.58 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **32b** was afforded as a white solid (0.11 g, 0.21 mmol) in 71% yield.

HPLC (standard method) t_R = 8.13 min, purity > 99%.

Melting Point: 222 – 225 °C.

4-[3-(4-fluorobenzoyl)propyl]-1-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine **(33a).**

To a well stirred solution of 3-{[4-(4-fluorobenzoyl)propyl]piperazinyl}-1-phenyl-1-propanol **72** (0.56 g, 1.29 mmol) in anhydrous benzene (50 mL) under N_2 at 25 °C was added 4-trifluoromethylphenol (0.23 g, 1.42 mmol) and PPh_3 (0.37 g, 1.41 mmol). After complete dissolution, a solution of ADDP (0.36 g, 1.42 mmol) in anhydrous benzene (10 mL) was slowly added dropwise to the reaction mixture. After stirring for 48 h at 25 °C that time, hexane (100 mL) was added to the reaction mixture. PPh_3O salts were removed by filtration and the solvent was evaporated under reduced pressure. The residue was adsorbed onto silica gel and purified by flash chromatography (100% EtOAc). The product **33a** was afforded as a yellow oil (0.27 g, 0.52 mmol) in 40% yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.99 (dd, J = 8.26, 5.52 Hz, 2H), 7.42 (d, J = 8.79 Hz, 2H), 7.30 (m, 5H), 7.11 (t, J = 8.35 Hz, 2H), 6.90 (d, J = 8.75 Hz, 2H), 5.27 (dd, J = 7.86, 5.08 Hz, 1H), 2.96 (t, J = 7.07 Hz, 2H), 2.55 (m, 12H), 2.20 (dt, J = 21.37, 7.35 Hz, 1H), 1.97 (m, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 198.42, 165.62 (d, $J_{C\text{-}F}$ = 254.4 Hz), 160.70, 141.09, 133.64 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.69 (d, $J_{C\text{-}F}$ = 9.8 Hz), 128.73, 127.82, 126.72 (q, $J_{C\text{-}F}$ = 3.0 Hz), 126.20, 125.89, 122. 71 (q, $J_{C\text{-}F}$ = 32.5 Hz), 122.61, 115.79, 115.58 (q, $J_{C\text{-}F}$ = 21.9 Hz), 78.74, 57.68, 54.45, 53.16, 36.19, 35.96, 21.66.

^{19}F-NMR (282 MHz, $CDCl_3$): δ -61.46, -105.68.

Elemental Analysis (%) calculated for $C_{30}H_{32}F_4N_2O_2$: C, 68.17; H, 6.10; N, 5.30. Found: C, 68.19; H, 6.08; N, 5.23.

4-[3-(4-fluorobenzoyl)propyl]-1-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl] piperazine hydrochloride **(33b).**

To a well stirred solution of 4-[3-(4'-fluorobenzoyl)propyl]-1-[3-phenyl-3-(4-trifluoromethylphenoxy)propyl]piperazine **(33a)** (0.32 g, 0.61 mmol) in anhydrous Et_2O (25 mL) under N_2 at 25 °C was added a solution of 2M HCl in anhydrous Et_2O (0.61 mL, 1.21 mmol) and stirred for 2 h at 25 °C. Upon completion, the reaction mixture was filtered and the remaining solid was re-dissolved in 10 mL of anhydrous ethanol. The resulting solution was refluxed for 15 min and the solvent was evaporated under reduced pressure. The remaining solid was dried under vacuum. The product **33b** was afforded as a light orange solid (0.34 g, 0.61 mmol) in quantitative yield.

HPLC (standard method) t_R = 8.40 min, purity > 99%.

Melting Point: 220 – 222 °C.

Biological Evaluation

Biological Evaluation of the synthesized compounds was carried out through the National Institutes of Health Psychoactive Drug Screening Program (NIH-PDSP). This service provides screening of novel psychoactive compounds for pharmacological and functional activity at cloned human or rodent CNS receptors, channels, and transporters. The laboratory of Bryan Roth MD, PhD (Case Western Reserve University), director of the NIH-PDSP, performs such screenings as a contractor to the National Institutes of Mental Health (NIMH). Full serotonergic and monoamine transporter characterization of compounds **1b** to **33b** is currently underway (serotonin receptors: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-HT_{5A}, 5-HT_6, and 5-HT_7; monoamine transporters:

SERT, NET, and DAT). The procedures detailed herein are PDSP-Roth Lab Standard Protocols.[203-205]

5-HT Receptor Subtype Binding Assays[204]

All binding assays are carried out with an incubation time of 60 min/RT (RT=room temp). All binding assays are performed in a similar manner Table 6 gives the (1) radioligand; (2) assay buffer; (3) unlabelled reference ligand used for each receptor subtype assay.[204] A 96-well plate is set up as described in Figure 18 and the following protocol is used:

1. For ^{3}H-ligands use 1-2 nM final concentration of radioligand; for ^{125}I-radioligands, use 0.05-0.1 nM final concentration.
2. Pipette in following order: a) binding buffer; b) radioligand; c) cold unknown ligand; d) cold reference ligand; e) membranes.
3. Incubate at RT as listed in binding assay table above.
4. At the end of the incubation, harvest onto pre-soaked (0.3% polyethyleneimine) GF/C filters using 96-well harvester. Wash with 3 quick washes with ice-cold harvest buffer.
5. Remove filters and air dry overnight.
6. Count in 96-well counter using 100 μL of scintillant/well.
7. Calculate Ki value of reference compound using LIGAND Program.
8. Calculate % inhibition using following formula:

$$\% \text{ Inhibition} = \frac{\text{Counts bound at 10 } \mu\text{M concentration of unknown compound}}{\text{Total specific counts}} \times 100$$

Table 6. Ligands and conditions for 5-HT receptor binding assays.[204]

Receptor	Radioligand	Assay Buffer	Unlabelled Reference Ligand
5-HT_{1A}	^{3}H-8-OH-DPAT	A	5-HT
5-HT_{2A}	^{3}H-GR125743	A	Ergotamine
5-HT_{Da}	^{3}H-GR125743	A	Ergotamine
5-HT_{Db}	^{3}H-GR125743	A	Ergotamine
5-HT_{1E}	^{3}H-5HT	A	5-HT
5-HT_{1F}	^{3}H-5HT	A	5-HT
5-HT_{2A}	^{3}H-ketanserin	A	Chlorpromazine
5-HT_{2B}	^{3}H-LSD	A	Norphenfluramine
5-HT_{2C}	^{3}H-mesulergine	A	Chlorpromazine
5-HT_3	^{3}H-zacopride	A	LY-278,584
5-HT_4	^{3}H-5HT	A	SDZ-205557
5-HT_{5a}	^{3}H-LSD	A	Ergotamine
5-HT_6	^{3}H-LSD	A	Chlorpromazine
5-HT_7	^{3}H-LSD	A	Chlorpromazine

Assay Buffers. A=50 mM Tris-Cl, 0.1 mM EDTA, 10 mM $MgCl_2$, pH=7.4.

Transporter Binding Assays[205]

All transporter binding assays are performed in a similar manner, although the buffers may differ from assay to assay. Table 7 gives the (1) radioligand; (2) assay buffer; (3) unlabelled reference ligand, (4) incubation time, and (5) unlabelled reference for each transporter assay.[205] A 96-well plate is set up as described in Figure 18 and the following protocol is used:

1. For ^{3}H-ligands use 1-2 nM final concentration of radioligand; for ^{125}I-radioligands, use 0.05-0.1 nM final concentration.
2. Pipette in following order: a) binding buffer; b) radioligand; c) cold unknown ligand; d) cold reference ligand; e) membranes.
3. Incubate at RT as listed in binding assay table above.

4. At the end of the incubation, harvest onto pre-soaked (0.3% polyethyleneimine) GF/C filters using 96-well harvester. Wash with 3 quick washes with ice-cold harvest buffer.
5. Remove filters and air dry overnight.
6. Count in 96-well counter using 100 μL of scintillant/well.
7. Calculate Ki value of reference compound using LIGAND Program.
8. Calculate % inhibition using following formula:

$$\% \text{ Inhibition} = \frac{\text{Counts bound at 10 } \mu\text{M concentration of unknown compound}}{\text{Total specific counts}} \times 100$$

9. Down-load all raw data for quality control studies.

UNK #1	UNK #1	UNK #2	UNK #2	UNK #3	UNK #3	UNK #4	UNK #4
UNK #5	UNK #5	UNK #6	UNK #6	UNK #7	UNK #7	UNK #8	UNK #8
UNK #9	UNK #9	UNK #10	UNK #10	UNK #11	UNK #11	UNK #12	UNK #12
STANDARD #1	STANDARD #1	STANDARD #2	STANDARD #2	TOTAL BINDING	TOTAL BINDING	TOTAL BINDING	TOTAL BINDING
STANDARD #1 AT 1 nM	STANDARD #1 AT 1 nM	STANDARD #1 AT 3 nM	STANDARD #1 AT 3 nM	STANDARD #1 AT 10 nM	STANDARD #1 AT 10 nM	STANDARD #1 AT 30 nM	STANDARD #1 AT 30 nM
STANDARD #1 AT 100 nM	STANDARD #1 AT 100 nM	STANDARD #1 AT 300 nM	STANDARD #1 AT 300 nM	STANDARD #1 AT 1000 nM	STANDARD #1 AT 1000 nM	TOTAL COUNTS ADDED / PLATE	TOTAL COUNTS ADDED / PLATE

Figure 18. Set up for 96-well plate for 5-HT receptor and transporter binding assays.[204,205]

Table 7. Ligands and conditions for transporter binding assays.[205]

Transporter	Radioligand	Assay Buffer	Min/RT	Unlabelled Reference Ligand
SERT	^{3}H-Citalopram	D	60	Fluoxetine
NET	^{3}H-Nisoxitine	H	60	Nortriptiline
DAT	^{3}H-WIN35428	H	60	4',4"-Diflouro-3a-(diphenylmethoxy)tropane HCL

Assay Buffers. D=50 mM Tris-Cl, pH=7.4; H=50 mM Tris-HCl, 150 nM NaCl, 5mM KCl, pH=7.4.

CHAPTER FOUR

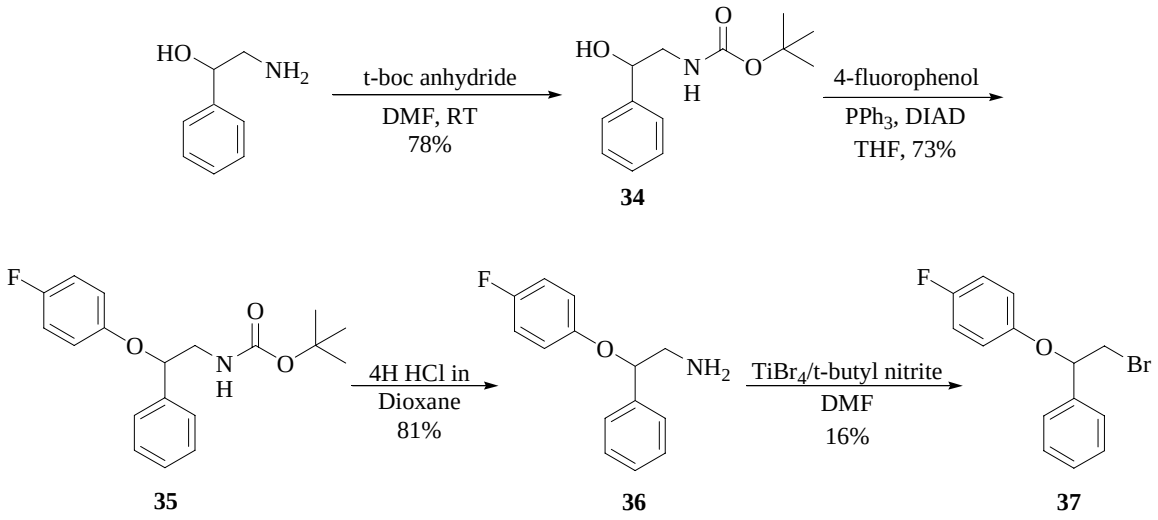
Results and Discussion

In this chapter, the synthetic procedures for the construction of a library of thirty-three novel potential SSRIs will be discussed, as well as the preliminary biological data available. The discussion will begin by addressing the first synthetic attempts to obtain the desired compounds utilizing the procedures from Dorsey and co-workers.[147] The challenges faced with this procedure, as well as alternative routes used for the complete synthesis of Homologues Type I and II will be explained. The biological results obtained from preliminary evaluation will be addressed, and finally some structure-activity relationship inferences will be made.

Synthesis of 4-Fluorophenoxy Ethyl Bromide Intermediate

Scheme 1 depicts the general synthesis of the 4-fluorophenoxy-ethyl bromide intermediate **37**. This procedure was initially developed by Dorsey and co-workers[147] in the Pinney Research group. The first step is the protection of the free amine moiety of commercially available 2-amino-1-phenyl-ethanol with di-*tert*-butyl-dicarbonate (*t*-boc anhydride). This reaction proceeds in a straight-forward manner and provides the protected alcohol **34** in good yield, 78%. The second step is a Mitsunobu coupling between protected alcohol **34** and 4-fluorophenol. The Mitsunobu reaction[164] offers an extremely useful and versatile synthetic pathway for a large range of products.[165] It has traditionally been used to convert alcohols to halides; however, there are other useful variations of these reaction conditions to convert alcohols to amines, esters, and

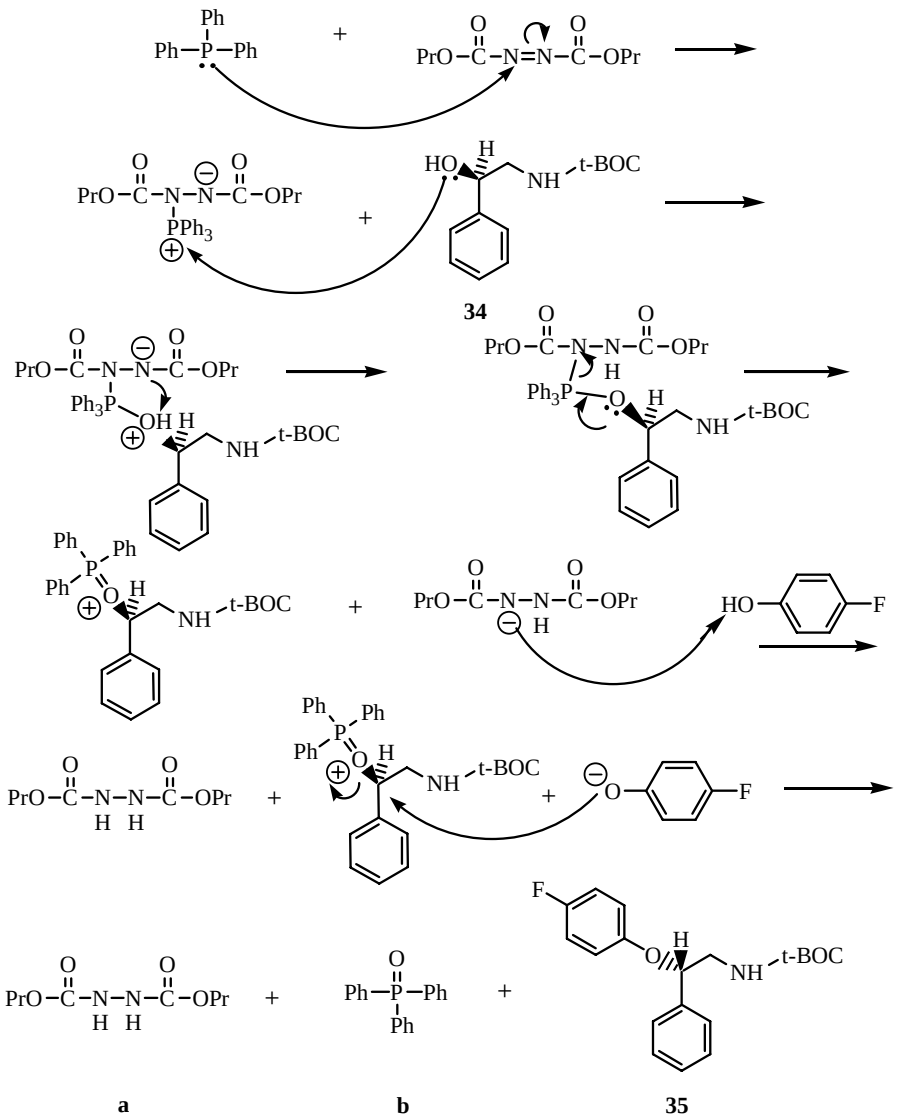
tosylates.[166] When a chiral center is present in the substrate, the Mitsunobu reaction occurs with inversion of configuration; thus, it is widely sustained that the reaction works via an S_N2 mechanism. However, in this and all subsequent Mitsunobu reactions performed in this study, a racemic mixture of alcohols was used and therefore inversion of stereochemistry was not an issue.



Scheme 1. Synthesis of 4-fluorophenoxy ethyl bromide intermediate.[147]

The Mitsunobu reaction uses a combination of triphenylphosphine (PPh_3) and an azodicarboxylic derivative (DEAD, DIAD, ADDP, among others) to activate the alcohol substituent on the substrate, thereby making it susceptible to nucleophilic attack by another reagent.[167] Specifically, in our case diisopropyl azodicarboxylate (DIAD) activated PPh_3 to nucleophilic attack by protected alcohol **34** thus forming an alkoxyphosphonium intermediate. This intermediate finally reacts as an electrophile in an S_N2 type reaction with a phenoxide ion (from 4-fluorophenol in this case), producing the desired coupled product **35** in good yield, 73% (Scheme 2). Alternatively, the phenolic lone pair of electrons may serve the nucleophilic role with proton transfer taking

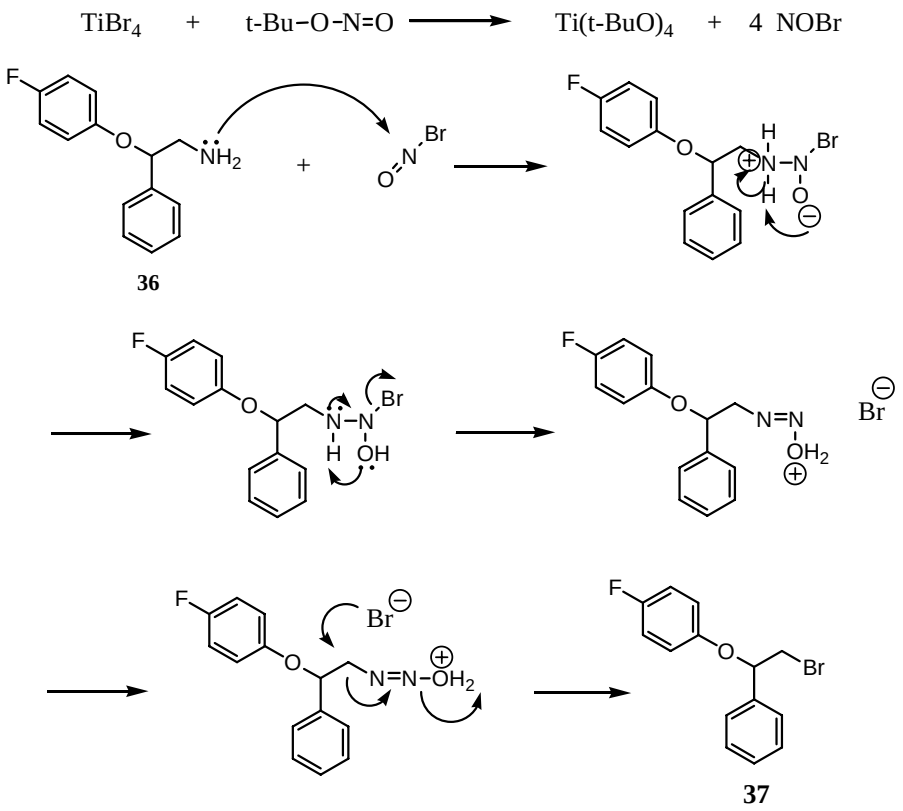
place as a subsequent step. Although the reaction employed a racemic starting material, stereogenic centers are noted in the mechanism of the reaction in order to denote the inversion of stereochemistry that takes place under Mitsunobu conditions.



Scheme 2. Mechanism of the Mitsunobu reaction.

The third step of the synthesis consists of the deprotection of the 4-fluorophenoxy protected intermediate **35** using a 4N HCl solution in dioxane. The reaction proceeded smoothly to provide the desired 4-fluorophenoxyethyl amine **36** in good yield, 81%

(Scheme 1). The final step involved the conversion of ethyl amine **36** into ethyl bromide **37**. This was achieved by a diazotization reaction using titanium (IV) bromide ($TiBr_4$): A primary aliphatic amine can be converted to a corresponding bromide via *in situ* generation of a nitrosyl bromide in the presence of $TiBr_4$ and *t*-butyl nitrite[168] (Scheme 3). This reaction is analogous to the well-known Sandmeyer reaction.

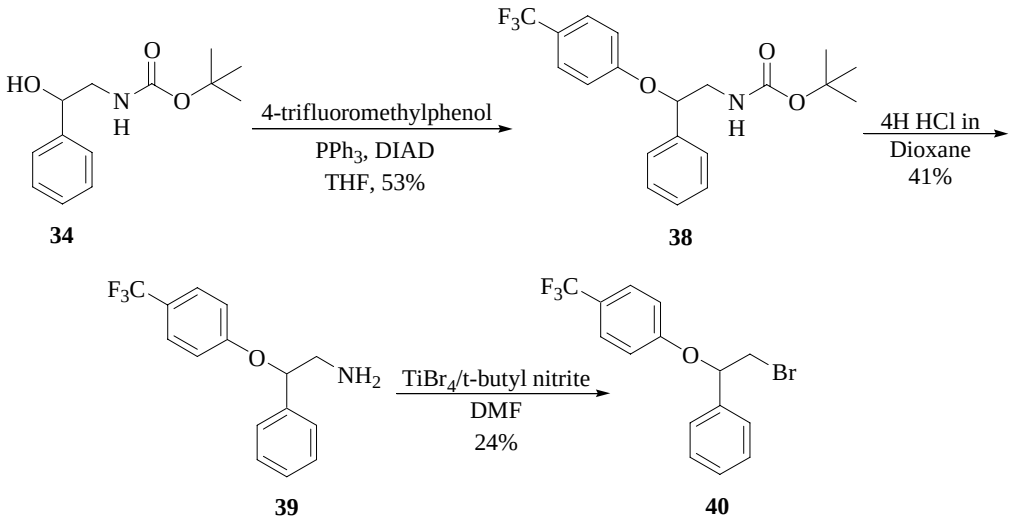


Scheme 3. Mechanism of conversion of ethyl amine to ethyl bromide using $TiBr_4$.

4-Fluorophenoxy ethyl bromide intermediate **37** was afforded in low yield (16%), and was characterized by NMR spectroscopy. Additional confirmation was obtained by GC/mass spectrometry, which showed the M^+ (295) and M+2 (297) peaks in a 1:1 ratio characteristic of a mono-brominated compound.[169]

Synthesis of 4-Trifluoromethylphenoxy Ethyl Bromide Intermediate

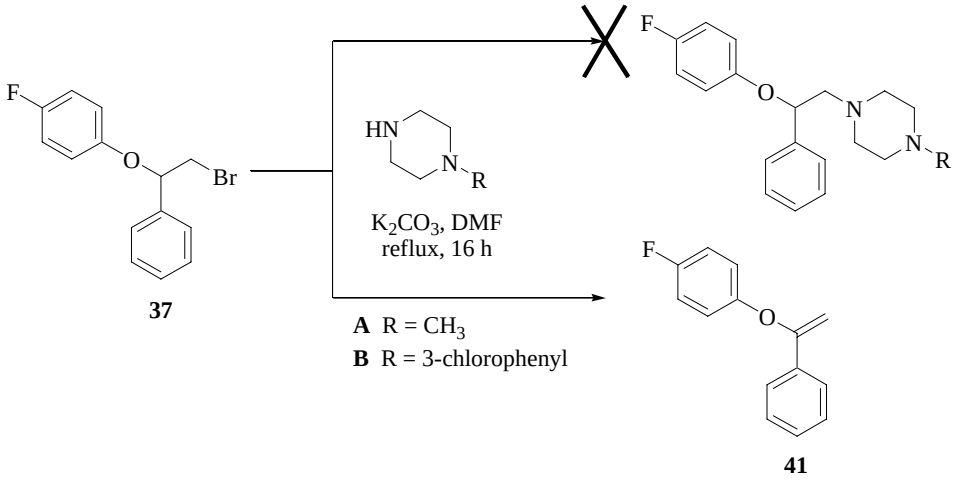
Scheme 3 depicts the general synthesis of the 4-trifluoromethylphenoxy ethyl bromide intermediate **40**, which was obtained in a similar manner as the 4-fluorophenoxy ethyl bromide intermediate previously discussed. Mitsunobu coupling between protected alcohol **34** and 4-trifluoromethyl-phenol in the presence of PPh_3 and DIAD produced the desired coupled product **38** in moderate yield, 53%. Subsequent deprotection with 4N HCl in dioxane afforded 4-trifluoromethyl-phenyl-ethyl amine **39** in moderate yield, 41%. Finally, ethyl amine **39** was then converted to its corresponding ethyl bromide **40** using $TiBr_4$ and *t*-butyl nitrite, affording the product in low yield, 24%. Bromide **40** was characterized by NMR spectroscopy and additional confirmation was again obtained by GC/mass spectrometry, which showed the M^+ (345) and M+2 (347) peaks in a 1:1 ratio as is characteristic of a mono-brominated compound.[169]



Scheme 4. Synthesis of 4-trifluoromethylphenoxy ethyl bromide intermediate.

Attempted Synthesis of Coupled Products from Bromide Intermediates

The purpose of synthesizing 4-fluorophenoxy ethyl bromide **37** and 4-trifluorophenoxy ethyl bromide **40** was to use them as key intermediates for the coupling reactions with the previously chosen functionalized piperazine and piperidine moieties. As stated by Dorsey and co-workers, this could be done by a straightforward N-alkylation of the cyclic amines with each of the alkyl bromides in the presence of base (K_2CO_3).[147,170] However, several attempts were made to obtain the N-alkylation of two different piperazine moieties, 1-methylpiperazine (VI) and 1-(3-chlorophenyl)-piperazine (II), with 4-fluorophenoxy ethyl bromide **37**. In every case the reaction failed to proceed, and instead of the desired substitution products, the elimination product 1-(4-fluorophenoxy)-1-phenyl-ethene **41** was obtained (Scheme 5).



Scheme 5. Attempted synthesis of 4-flurophenoxyethyl coupled products.

NMR spectroscopy of **41** indicated the presence of the nine aromatic protons as well as the two geminal protons on the double bond. They appear as two distinct doublets, each with a *J* value of 2.5 Hz, which is characteristic of a terminal methylene

group (0 to 3 Hz)[169] (Figure 19). Additionally, GC/mass spectrometry confirmed the identity of the product showing the M^+ peak (214).

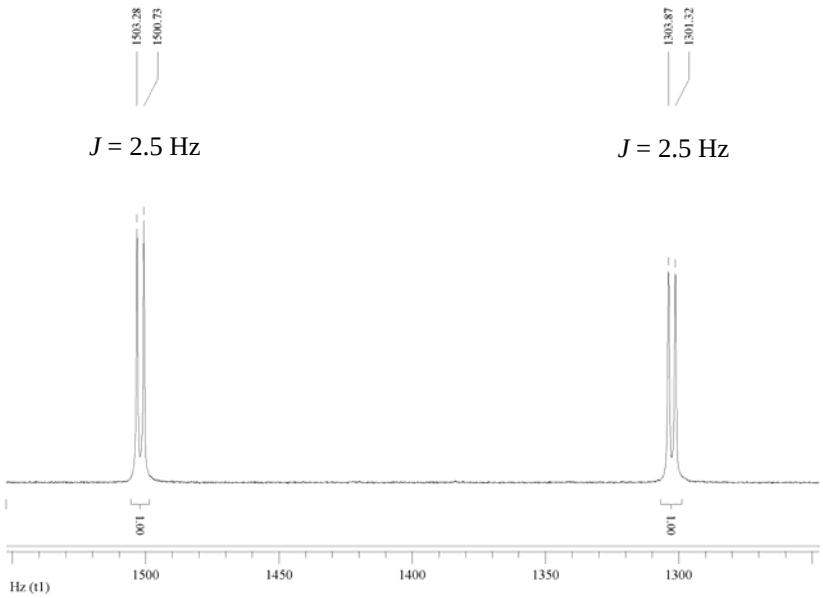
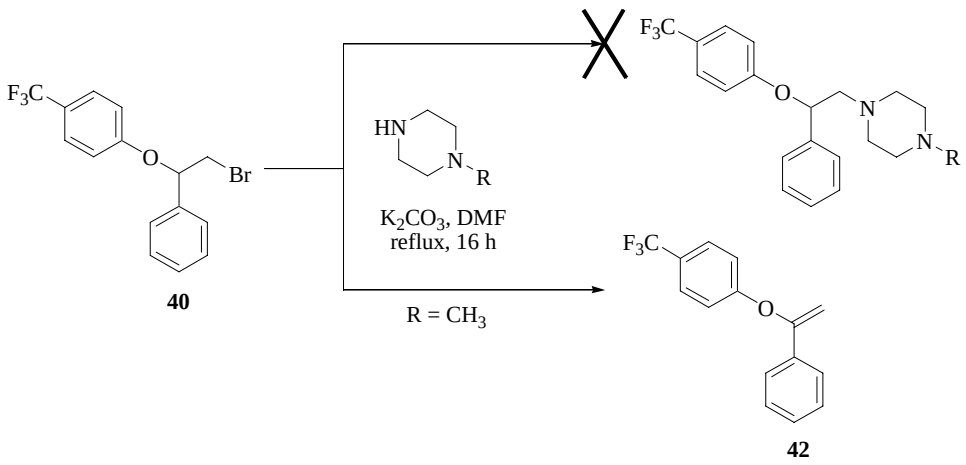


Figure 19. Expansion of ^{1}H-NMR of compound **41**.

The reaction conditions in all cases involved reflux in DMF for 16 h in the presence of a non-nucleophilic base (K_2CO_3). It is believed that even though a primary halide is being used, the high temperatures applied to the reaction (~150 °C) and the bulky nature of the piperazine moieties favored the formation of the elimination product instead of the substitution product. A similar situation was encountered when N-alkylation of 1-methyl piperazine (VI) with 4-trifluorophenoxy ethyl bromide **40** in the presence of K_2CO_3 was attempted under the same reaction conditions (Scheme 6). Instead of the desired substitution product, the elimination product 1-phenyl-1-(trifluoromethyl-phenyl)ethene **42** was obtained. GC/mass spectrometry confirmed the identity of the product showing the M^+ peak (264).



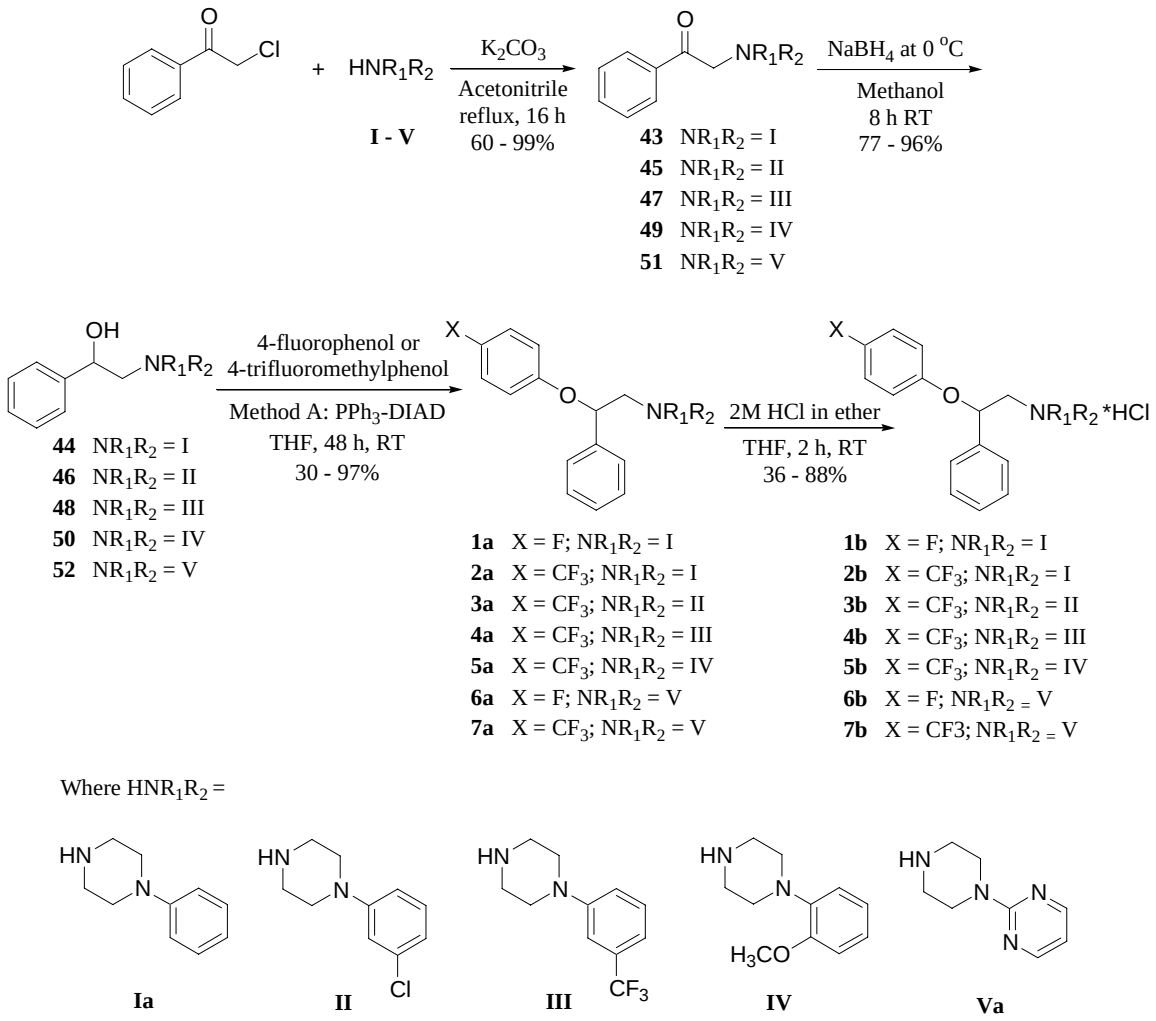
Scheme 6. Attempted synthesis of a 4-trifluromethylphenoxy-ethyl coupled product.

Synthesis of Homologues Type I – Compounds 1 to 7

While the results of the above mentioned reactions were being studied and understood, a new search of the literature was started in order to find a more convenient and efficient route towards the proposed Homologues Type I and II. Following in the steps of a publication by Orjales and co-workers,[132] which used a similar substrate for their own purposes, it was decided to use commercially available 2-chloro-acetophenone as a starting material, and attempt an N-alkylation of our cyclic amines with that alkyl halide. This in fact was feasible and it gave rise to a whole new synthetic approach for the initial seven members of our Homologues Type I family (Scheme 7).

The first step of this synthetic route was the N-alkylation of piperazines I-V with 2-chloroacetophenone in the presence of K_2CO_3 to afford the desired ethanones **43**, **45**, **47**, **49**, and **51** in excellent yields ranging from 60 to 99% (Scheme 7). These reactions proceeded smoothly, and all the products were purified by flash chromatography in silica gel. It is important to mention that piperazines II, III, and IV were commercially available as free amines, and piperazines I and V were available as hydrochloride salts.

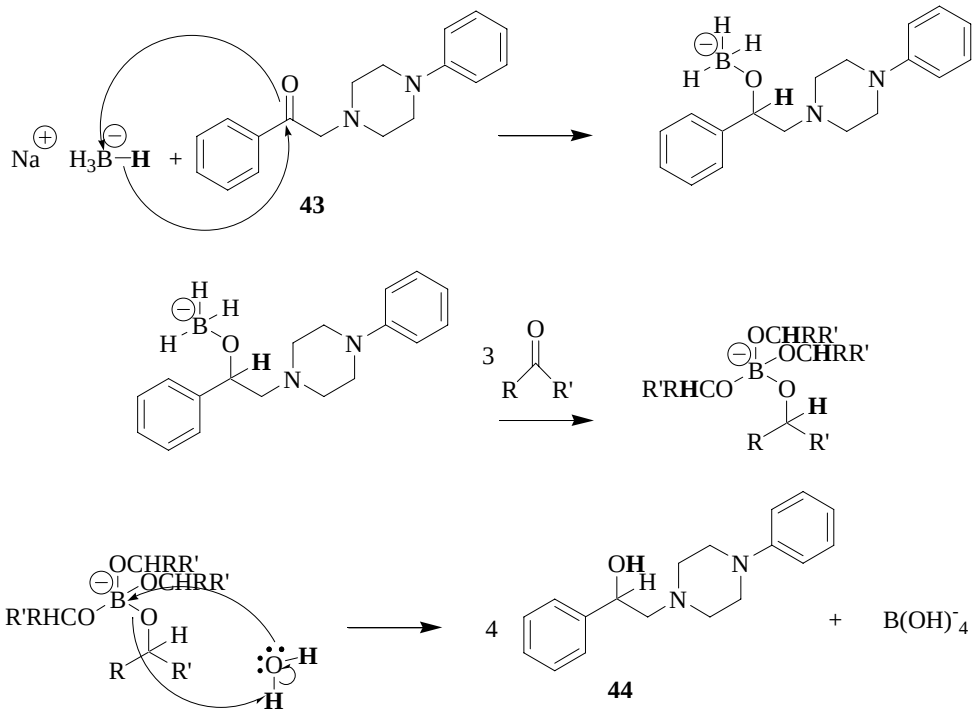
For these two cases, initial de-salting of each piperazine was carried out using an excess of aq. 2M NaOH and extracting with Et_2O afforded the free amines **Ia** and **Va**.



Scheme 7. Synthesis of homologues type I – compounds 1 to 7.

The second step of this synthetic route was the reduction of the ketone moieties with $NaBH_4$ to afford compounds **44**, **46**, **48**, **50**, and **52** in excellent yields ranging from 77 to 96%. $NaBH_4$ is a very common and efficient reducing agent. Its mechanism of action starts with nucleophilic attack on the carbonyl by a hydride ion, H^- (Scheme 8). The alkoxyborohydride formed then donates three more hydrides to other carbonyl

carbons forming a tetraalkoxyborate. Upon workup of the reaction with H_2O, the tetraalkoxyborate undergoes spontaneous hydrolysis yielding four reduced products and a borate ion, $B(OH)_4^-$.[171]



Scheme 8. Mechanism of action of $NaBH_4$ reduction.

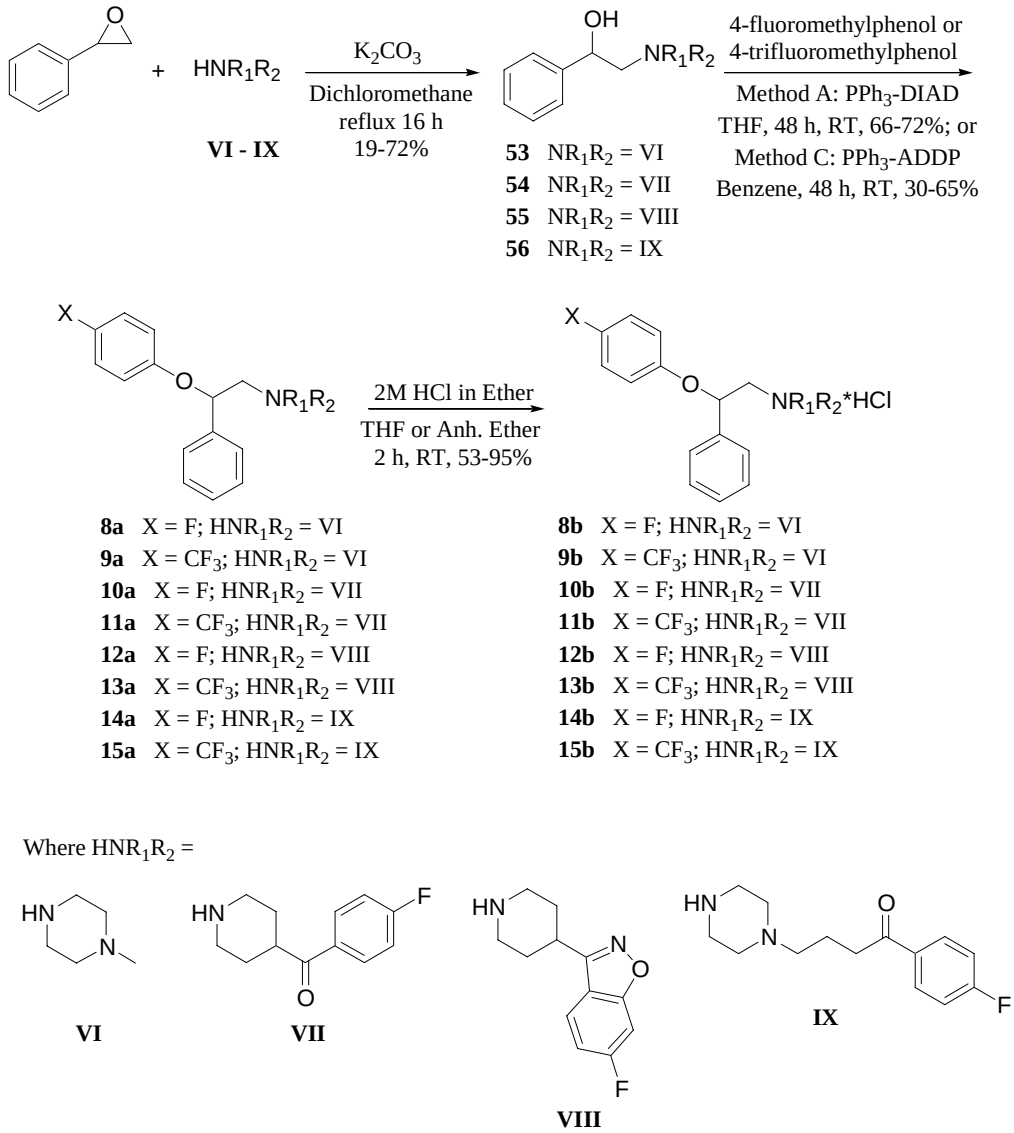
The third step of this synthetic route was a Mitsunobu coupling between the ethyl alcohols and 4-fluorophenol or 4-trifluoromethyl-phenol using PPh_3 and DIAD to afford final ethyl ethers **1a** to **7a** in good yields ranging from 30 to 96%. The mechanism of action of this reaction has been discussed previously. These reactions proceeded smoothly and all the products were purified by column chromatography in silica gel. Many side products are formed during the course of this reaction, and much care was taken in identifying and separating them from the desired products **1a** to **7a**. The purity of this products was confirmed by NMR spectroscopy (^1H, ^{13}C and ^{19}F) and elemental

analysis. The fourth and final step of this synthetic route was the formation of the hydrochloride salts of products **1a** to **7a**, to afford salts **1b** to **7b** in good yields ranging from 36 to 88%. Solubility under physiological conditions is extremely important because pharmaceuticals have to be permeable within the gastrointestinal tract and favorable aqueous solubility is important for oral availability. Therefore, each free amine product was converted into its hydrochloride salt, which is one of the most common halide salt forms. The synthetic reaction, ultimately, involves the relatively basic amine homologue reacting with an excess of strong acid (2M HCl) (Scheme 7).[147] The purity of all the hydrochloride salts was determined by HPLC.

Synthesis of Homologues Type I – Compounds 8 to 15

A modification to the previously discussed synthetic route was needed in order to overcome the presence of carbonyl functional groups in some of the cyclic amines (VI and VII) without the use of protecting groups. Evidence was found in the literature that non-symmetric epoxides can be regioselectively attacked at their least substituted position by nucleophiles such as amines[172] in the presence of base[173] to obtain the corresponding secondary alcohols. It was decided to try this possibility starting with commercially available styrene oxide, using 1-methylpiperazine (VI) as the nucleophile and K_2CO_3 as the base in the reaction mixture (Scheme 9). The good results obtained in this first reaction (72% yield for ethyl alcohol **53**) encouraged the synthesis of compounds **8** to **15** through this new synthetic route, which interestingly involved one less step than the previously described route. The first step, as already mentioned, was the regioselective nucleophilic attack by cyclic amines VI to IX at the least substituted

position of styrene oxide in the presence of base to afford the corresponding ethyl alcohols **53** to **56** in yields ranging from 19 to 72%.



Scheme 9. Synthesis of homologues type I – compounds 8 to 15.

The second step of this synthetic route was the coupling between ethyl alcohols **53-56** with 4-fluorophenol or 4-trifluoromethyl-phenol using Mitsunobu conditions. For compounds **8** and **9**, the Mitsunobu coupling was carried out using the traditional reagents, which has been denominated as Method A. These reactions proceeded smoothly and the

products were obtained in pure form and in with excellent yields (72% for **8a** and 66% for **9a**). However, challenges were encountered when attempting the same conditions with 4-fluorobenzoyl-piperidinyl ethyl alcohol **54**. Figure 20 depicts in detail the reagents and products of our Mitsunobu reaction when promoted by the traditional reagents DIAD and PPh_3.

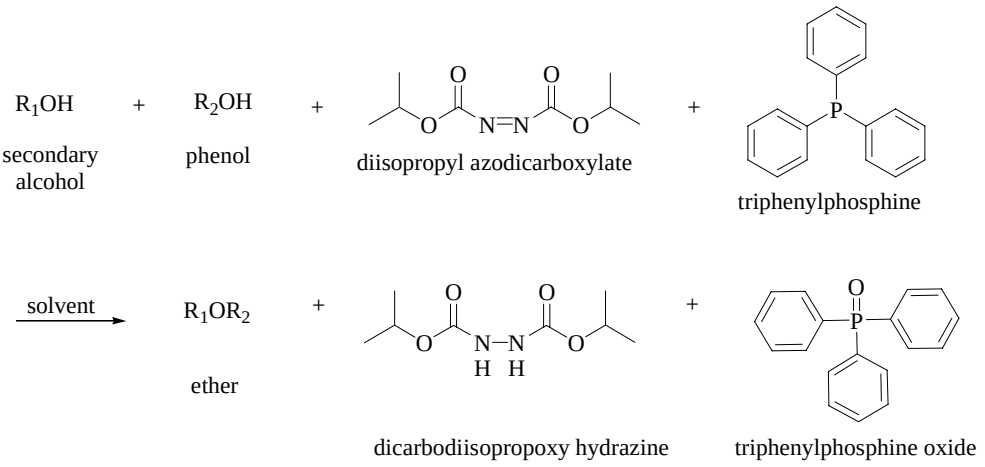
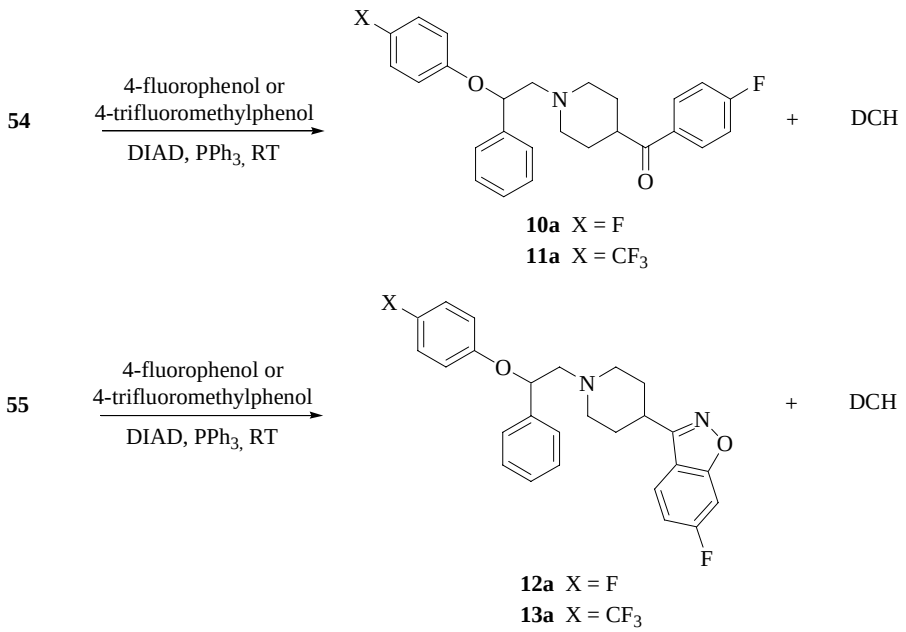


Figure 20. Reagents and products of Mitsunobu coupling using DIAD and PPh_3.

The Mitsunobu reaction is an excellent tool in organic synthesis and medicinal chemistry because of its scope, stereospecificity and mild reaction conditions,[174,175] and the reaction has been extensively reviewed.[176,177] However, it is well documented in the literature that many side products are formed during this reaction and that separation can be very difficult.[165,174,175,178] This was proven true for products **10a**, **11a**,**12a** and **13a**, where the co-elution from column chromatography of dicarbodiisopropoxy hydrazine (DCH) along with the desired products became an issue, and purification was not possible under the conditions used. Products **10a** to **13a** were formed as expected (Scheme 10) and this was clearly observed through NMR spectroscopy; however the presence of DCH was also evident (Figure 21). Many methods of purification were

attempted, such as second and third columns, crystallization, simple filtration and filtration through a polar agents (celite and florisil), but none seemed to provide acceptable purity and significant amounts of product were lost with every attempt. The arrows in Figure 21 denote the peaks corresponding to the protons in DCH.



Scheme 10. Products **10a** to **13a** using DIAD and PPh_3, contaminated with DCH.

A great deal of time was spent trying to overcome this issue, and the reactions were repeated many times trying to eliminate the side products during the workup process (Scheme 10). However, this was largely unsuccessful. Partial crystallization of DCH was achieved several times, and NMR showed that its relative concentration in the mixture decreased although it did not disappear completely. Moreover, NMR was done on a sample of DCH, which crystallized as a white solid, further confirming its identity (Figure 22).

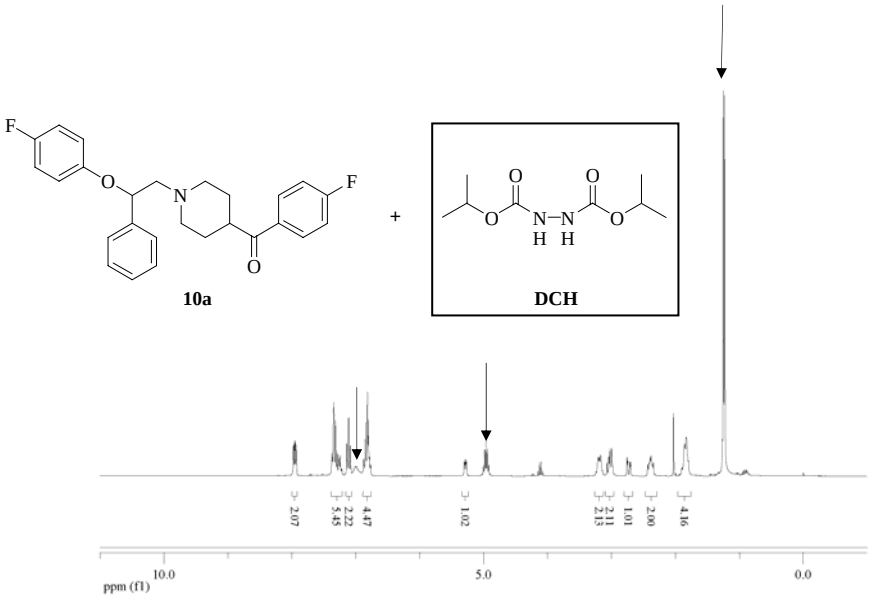


Figure 21. ^{1}H-NMR of compound **10a** contaminated with DCH. Arrows denote the peaks corresponding to DCH.

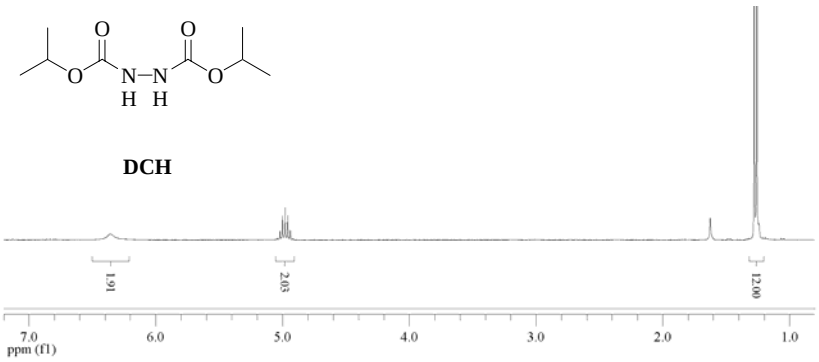
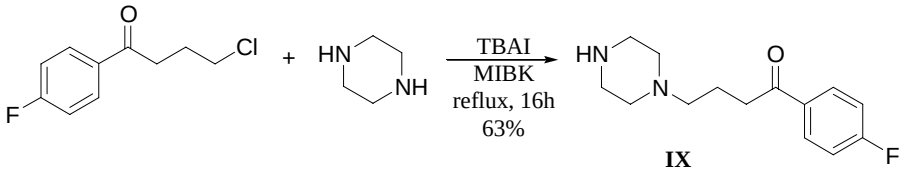


Figure 22. Expansion of ^{1}H-NMR of DCH.

In light of this situation, a new review of the literature was done in an effort to search for other reagents for the Mitsunobu reaction. The reason it was so important to keep this reaction in the synthesis was because of its specificity for the formation of the desired ethers and its lack of interference with other functional groups in the molecules. After exploring a different set of reagents (Method B) whose results will be later discussed in detail, a third set of reagents was found to be the most effective in overcoming the problem

of co-elution of side products. This new set of reagents, Method C, involved the use of 1,1'-(azodicarbonyl)-dipiperidide (ADDP) and PPh_3[179,180] under the same mild reaction conditions. The yields obtained using these reagents were comparable to those obtained with Method A (average of 40%). Additionally, the removal of the side products from these reagents was possible solely through the workup process. Therefore, compounds **10a** to **15a** where obtained through this procedure. The final step of the synthetic route was the formation of hydrochloride salts **10b** to **15b** by reaction with a strong acid (2M HCl), as described previously.

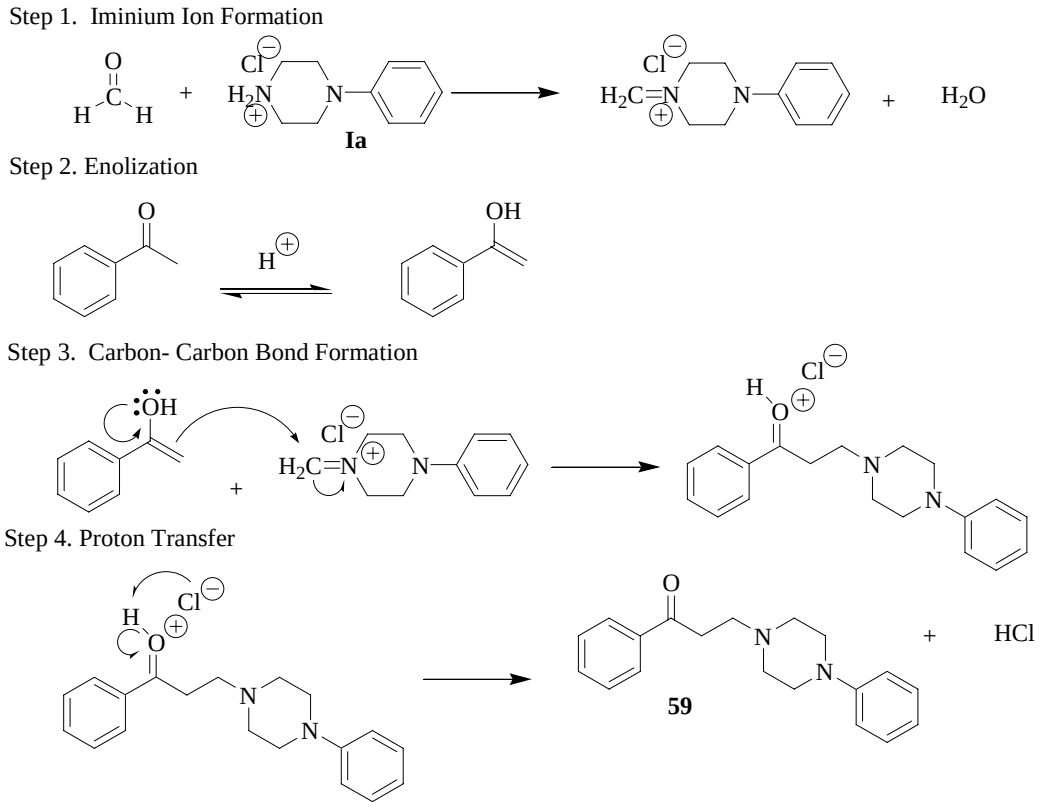
It is important to note that cyclic amines VI, VII and VIII were commercially available, while piperazine IX had to be synthesized in the laboratory (Scheme 11).[181] This was achieved by mono-N-alkylation of piperazine with 4-chloro-4'-fluoro-butyrophenone using catalytic amounts of tetra-butyl ammonium iodide (TBAI) and methyl isobutyl ketone (MIBK) as solvent, obtaining the desired product in 63% yield (Scheme 11). Interestingly, for this particular reaction it was observed that changing the solvent to a more apolar option such as CH_2Cl_2 favored the formation of the di-substituted product, which otherwise would only be obtained in a very small percentage (~10%).



Scheme 11. Synthesis of 1-[3-(4-fluoro-benzoyl)-propyl]-piperazine (IX).

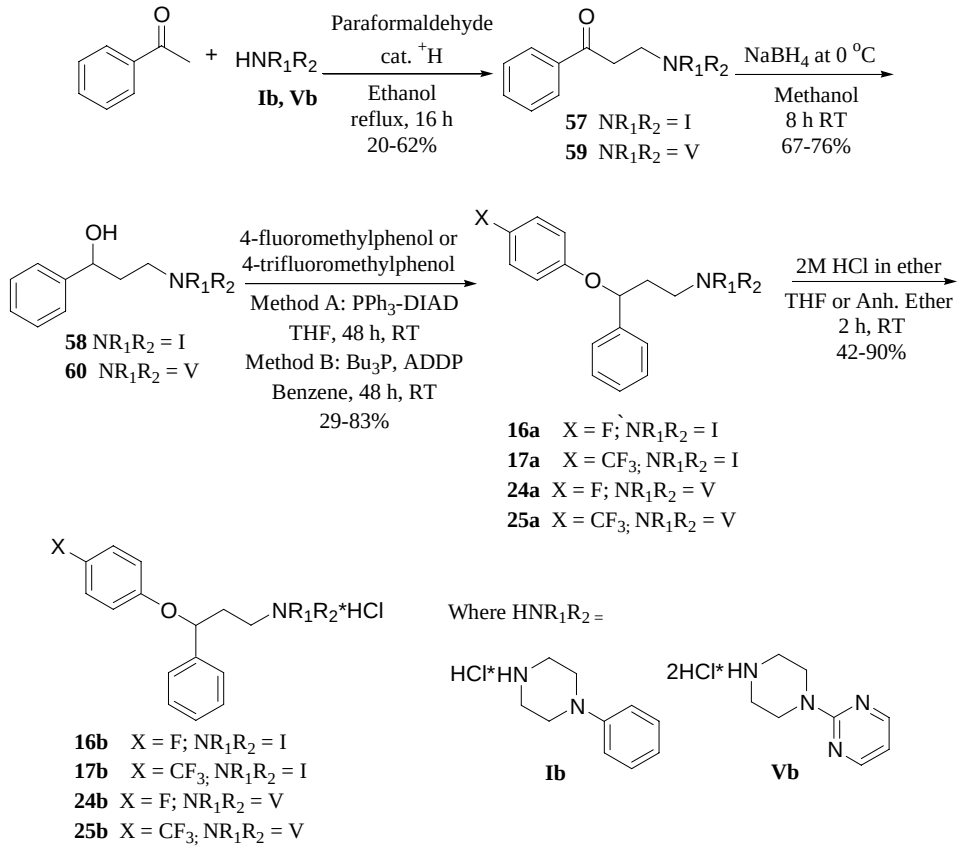
Synthesis of Homologues Type II – Compounds 16, 17, 24 and 25

Scheme 13 depicts the general synthesis of compounds **16**, **17**, **24** and **25**, which represent some the first compounds in the series of Homologues Type II. The first step in the synthetic route involved a Mannich reaction between the hydrochloride salts of two of the cyclic amines (**Ib** and **Vb**), acetophenone and paraformaldehyde in the presence of catalytic amounts of acid to obtain propanones **57** and **59** in good yields (20-62%). The product of the Mannich reaction results from an enol functioning as a nucleophile. Following workup, the coupled compound is termed the Mannich base (Scheme 12).[171] The second step of this synthetic route was the reduction of the ketone moieties to afford alcohols **58** and **60** in good yields ranging from 67to 76%, using $NaBH_4$ as previously discussed.



Scheme 12.. Mechanism of the Mannich reaction.

The third step was the Mitsunobu coupling of the obtained alcohols **58** and **60** with 4-fluorophenol or 4-trifluoromethylphenol. For compounds **16a** and **17a** this was achieved using the traditional set of reagents, Method A. These reactions proceeded smoothly and in good yields ranging from 29 to 54%. However, for compounds **24a** and **25a** a different set of reagents was used, ADDP and tributylphosphine (Bu_3P),[179,182] which we have termed Method B. The literature indicates that this is a highly reactive and very efficient combination of reagents,[179] which was evidenced by excellent yields > 80%. This method will be further discussed in the next sections. The final step of the synthetic route was the formation of hydrochloride salts **16b**, **17b, 24b** and **25b** by reaction with a strong acid (2M HCl), as described previously.



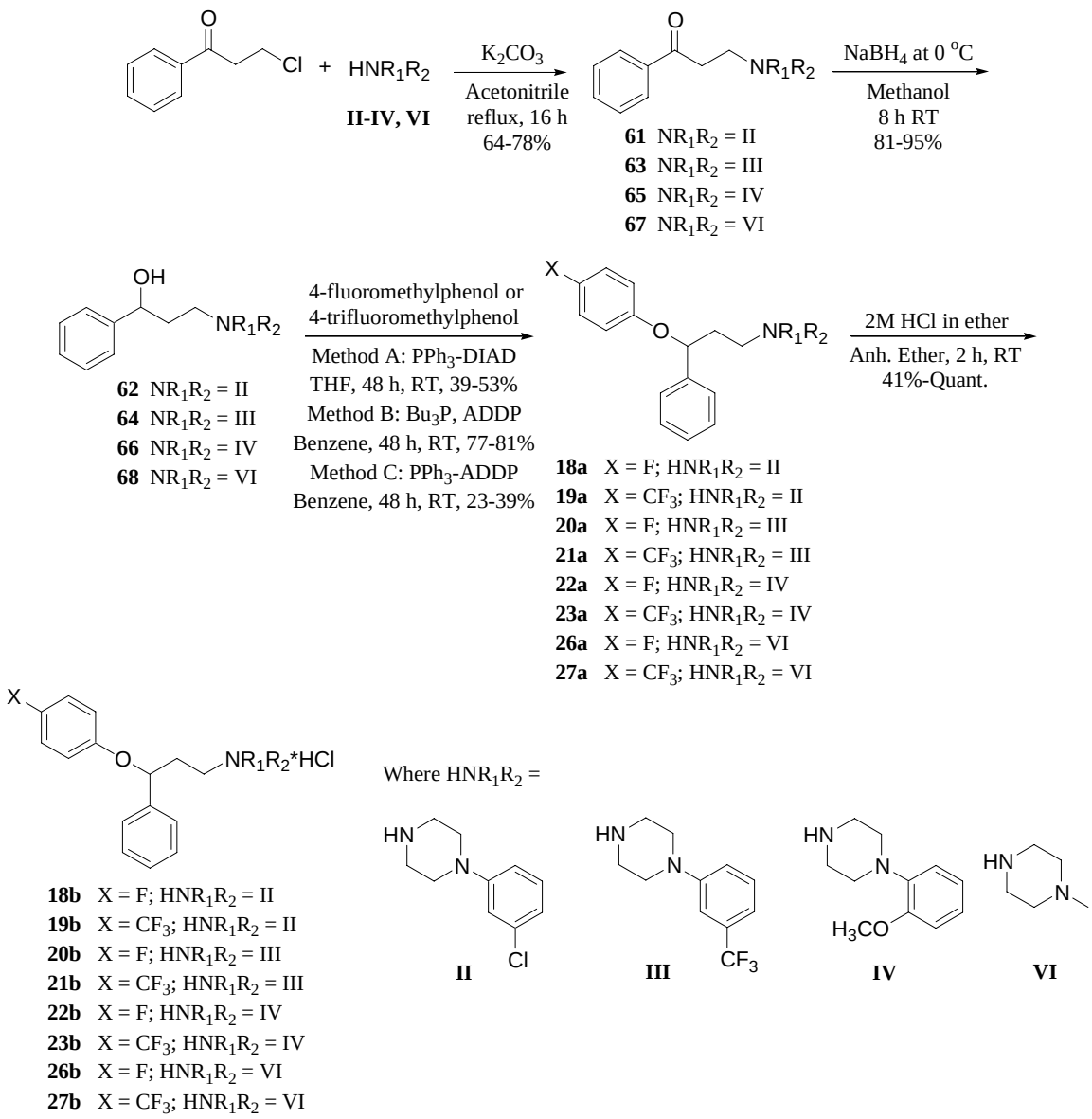
Scheme 13. Synthesis of homologues type II – compounds 16, 17, 24 and 25.

Synthesis of Homologues Type II – Compounds 18 to 23, 26 and 27

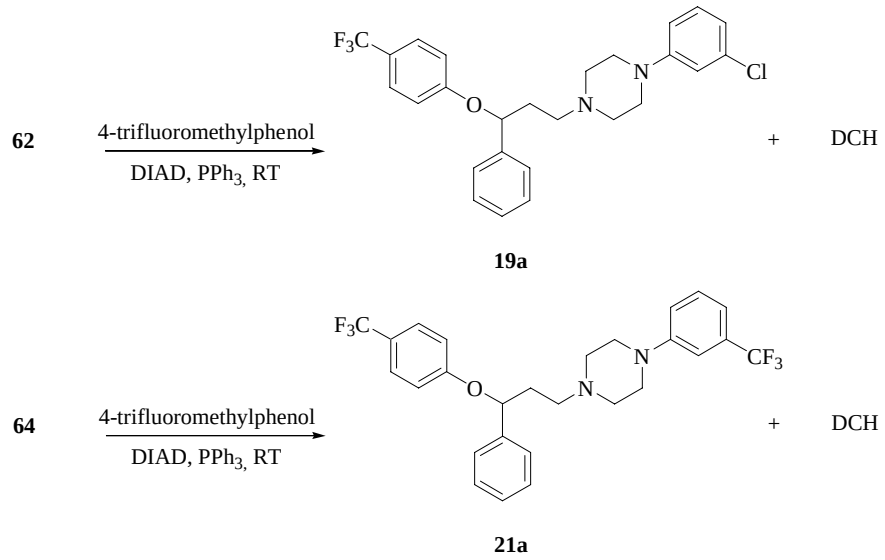
Scheme 14 depicts the general synthesis of eight Homologues Type II, compounds **18** to **23**, and **26** and **27**. The first step consisted in a straightforward N-alkylation of piperazines II, III, IV and VI with 3-chloropropiophenone in the presence of K_2CO_3 to afford propiophenones **61**, **63**, **65**, and **67** in good yields, ranging from 64 to 78%. The second step was the reduction of the ketone moieties using $NaBH_4$ as discussed previously to afford propanols **62**, **64**, **66**, and **68** in excellent yields, 81-95%. The third step was the coupling of the obtained propanols to 4-fluorophenol or 4-trifluoromethylphenol using Mitsunobu conditions. Compounds **18a** and **20a** were synthesized using Method A, DIAD and PPh_3, in good yields (53% and 39%, respectively). These reactions proceeded smoothly and presented no problems. Considering this, the synthesis of compounds **19a** and **21a** was attempted through the same method. However, although both products were formed as expected and their identity was demonstrated by NMR spectroscopy, they both presented the problem of co-elution of DCH during column chromatography (Scheme 15). Crystallization and filtration of DCH was attempted, but again its complete removal was not possible under the conditions used.

This situation prompted a new review of the literature, in the search not only of reported ways to remove the unwanted side products but also of other combinations of reagents where this problem could somehow be avoided. This is when Method B was found, which involved the use of ADDP and Bu_3P. Compounds **22a** and **23a** were obtained through this method with excellent yields, 77-81% (Scheme 14). Encouraged by these results, the synthesis of other final compounds was attempted using the reagents in Method B with mixed results which will be discussed in the next section. Ultimately,

compounds **19a**, **21a**, **26a** and **27a** were synthesized using Method C (ADDP and PPh_3) with yields ranging between 23 and 53% with avoidance of side product co-elution. The final step of the synthetic route was the formation of hydrochloride salts **18b** to **23b**, **26b** and **27b** by reaction with a strong acid (2M HCl), as described previously (Scheme 14).



Scheme 14. Synthesis of homologues type II – compounds 18 to 23, 26 and 27.

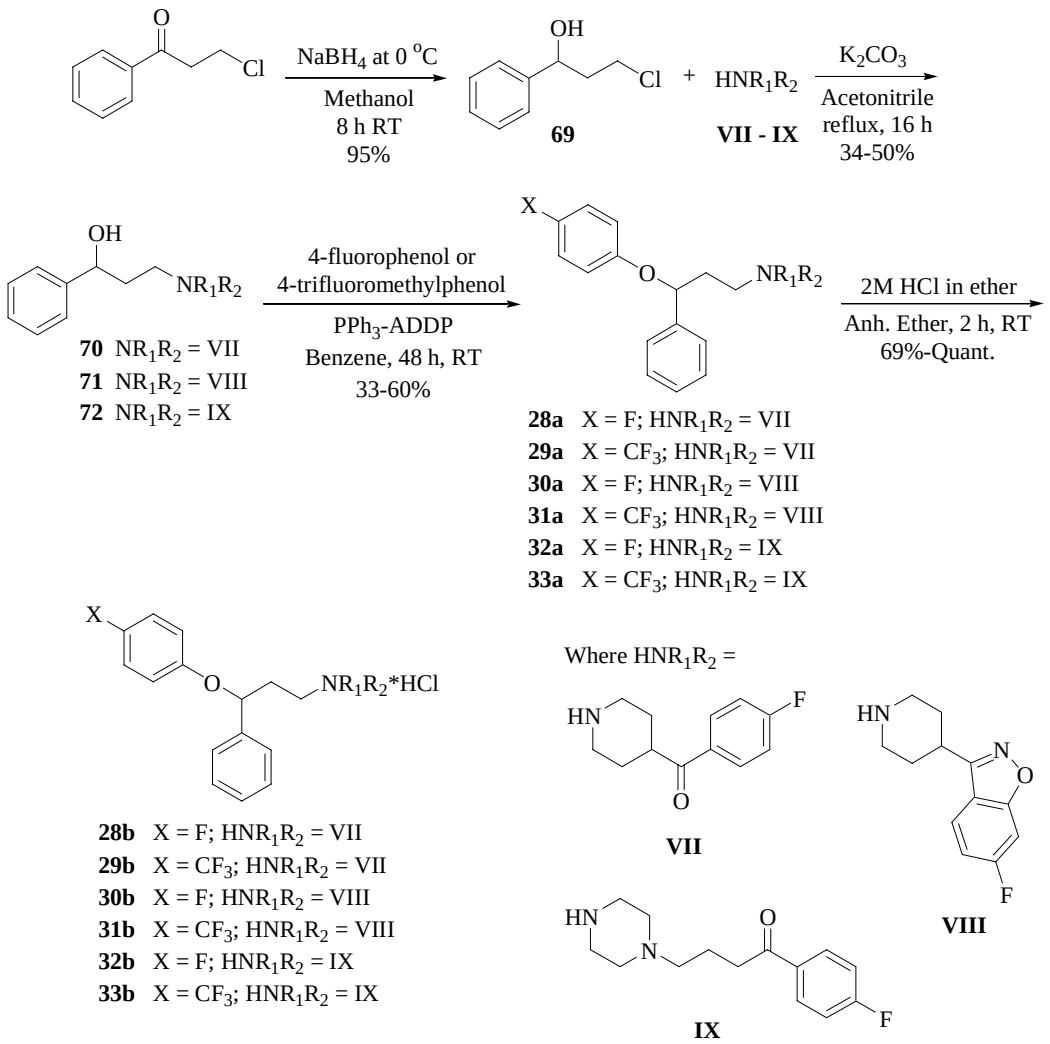


Scheme 15. Products **19a** to **21a** using DIAD and PPh_3, contaminated with DCH.

Synthesis of Homologues Type II – Compounds 28 to 33

Scheme 16 depicts the general synthesis of the final six members of the Homologues Type II series, compounds **28** to **33**. To obtain these compounds, once again a modification of the synthetic route was necessary in order to overcome the presence of carbonyl functional groups in some of our cyclic amines (VI and VII) without the use of protecting groups. Inspired by examples found in the literature, [132] a rather simple but highly efficient method was used. Commercially available 3-chloropropiophenone, previously utilized as starting material in Scheme 14, was reduced to the corresponding chloropropanol **69**, in 95% yield (Scheme 16). Compound **69** was used as a starting material for the three subsequent reactions, which consisted of N-alkylation of cyclic amines VII, VIII and IX in the presence of K_2CO_3 to obtain propanols **70** to **72** in good yields ranging from 34 to 50%. The next step of the synthetic route was the coupling of the obtained propanols with 4-fluorophenol or 4-trifluoromethyl phenol using Mitsunobu conditions. Encouraged by the high yields previously obtained with Method B, these

conditions were attempted for the synthesis of compounds **26a** to **29a**, **32a** and **33a**. Figure 23 depicts in detail the reagents and products of the Mitsunobu reaction when promoted by ADDP and Bu_3P.



Scheme 16. Synthesis of homologues type II – compounds 28 to 33.

Once again, although the desired products were formed as expected (Scheme 17), difficulties were faced during purification due to co-elution of side products, specifically, tributylphospine oxide (Bu_3PO). Although much of this compound was precipitated out of the reaction mixture by crashing with pure hexanes, a significant amount remained in the

organic layer and was not retained by silica gel during chromatography. Moreover, due to its lack of UV absorption, monitoring was not possible under the conditions used.

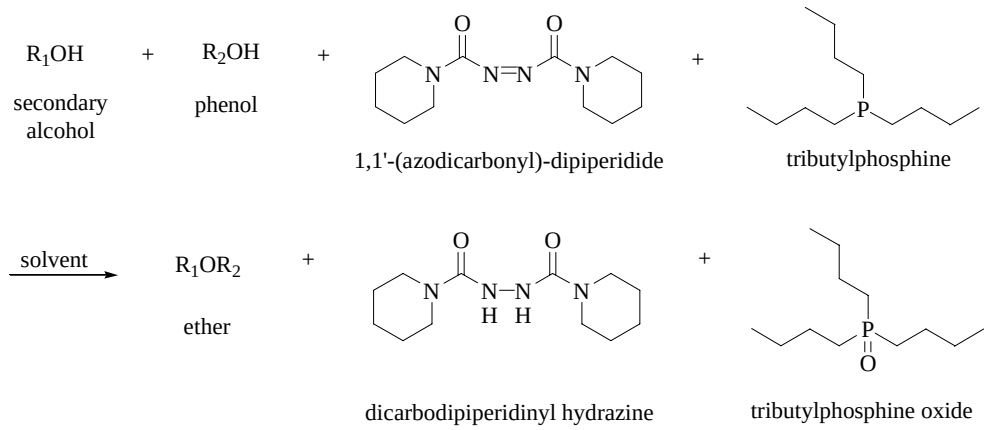
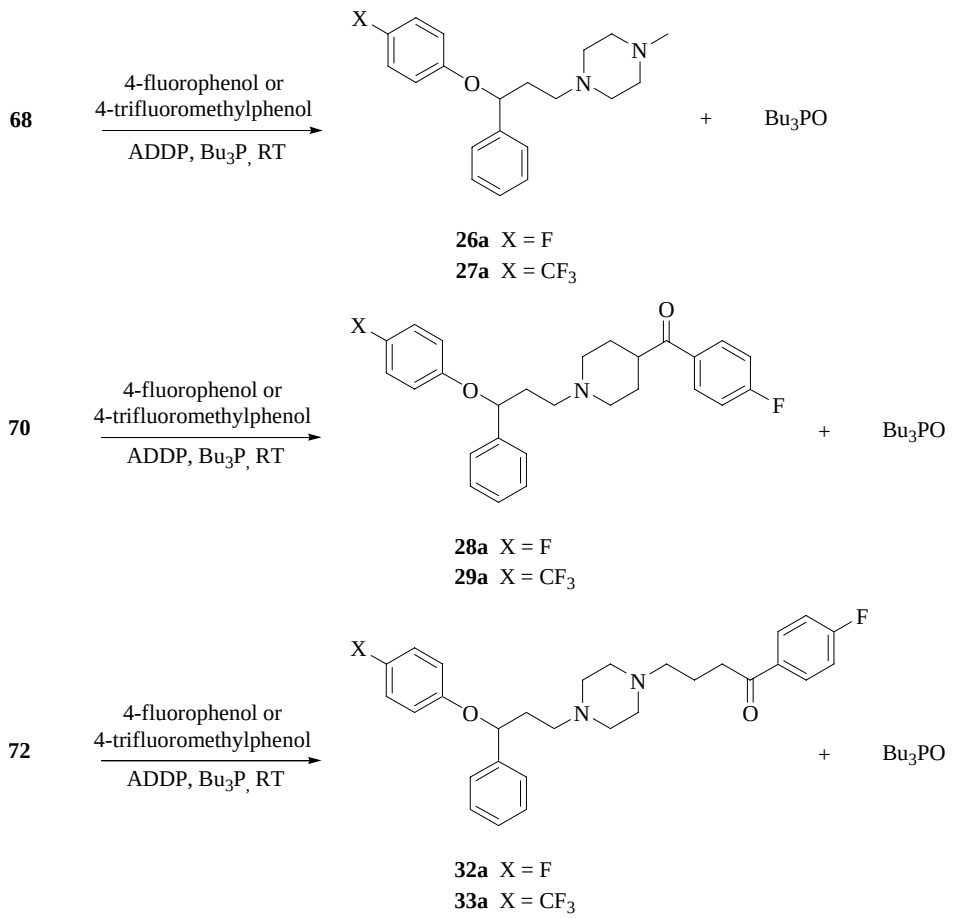


Figure 23. Reagents and products of Mitsunobu coupling using ADDP and Bu_3P.

Many methods of purification where attempted, such as second and third columns, crystallization, simple filtration and filtration through a polar agents (celite and florisil), but none seemed to provide acceptable purity as discussed previously. This demonstrated that although the combination of reagents in Method B afforded some of best yields (~80%) for some of the final compounds, this was counterbalanced by the difficulties in purification, a fact that prompted the utilization of a third combination, Method C, which used ADDP and PPh_3. Ultimately, compounds **26a** to **33a** were synthesized using this method, which afforded the desired products in good yields, ranging from 33 to 60%. Table 8 presents a comparison between the three methods and the yields obtained for the 33 members of our library. Bolded compounds in Table 8 the highest yields obtained using the different Mitsunobu conditions.



Scheme 17. Products **26a** to **29a**, **32a** and **33a** using ADDP and Bu_3P, contaminated with Bu_3PO.

Biological Evaluation

Biological Evaluation of the synthesized compounds was done through the National Institutes of Health Psychoactive Drug Screening Program (NIH-PDSP). This service provides screening of novel psychoactive compounds for pharmacological and functional activity at cloned human or rodent CNS receptors, channels, and transporters. The laboratory of Bryan Roth MD, PhD (Case Western Reserve University), director of the NIH-PDSP, performs such screenings as a contractor to the National Institutes of Mental Health (NIMH).[203-205]

Table 8. Mitsunobu conditions and yields for homologues type I and II.

Homologues Type I			Homologues Type II		
Compound	Method	Yield (%)	Compound	Method	Yield
1a	A	36	16a	A	29
2a	A	40	17a	A	54
3a	A	**97**	18a	A	53
4a	A	30	19a	C	27
5a	A	60	20a	A	39
6a	A	48	21a	C	23
7a	A	78	22a	B	**81**
8a	A	72	23a	B	77
9a	A	66	24a	B	**83**
10a	C	65	25a	B	**82**
11a	C	38	26a	C	38
12a	C	38	27a	C	23
13a	C	41	28a	C	60
14a	C	34	29a	C	33
15a	C	30	30a	C	41
			31a	C	35
			32a	C	44
			33a	C	40

Method A: DIAD and PPh_3, THF, 48 h, RT; Method B: ADDP and Bu_3P, Benzene, 48 h, RT; Method C: ADDP and PPh_3, Benzene, 48 h, RT.

Full serotonergic and monoamine transporter characterization of the complete library of thirty-three compounds is currently underway (serotonin receptors: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-HT_{5A}, 5-HT_6, and 5-HT_7; monoamine transporters: SERT, NET, and DAT). However, preliminary affinity data is available for seven of the thirty-three compounds synthesized. Compounds

1b through **5b**, **16b** and **17b** were analyzed for their affinity towards the serotonin 5-HT_{1A} receptor as well as their affinity towards the 5-HT_{2A} receptor and the SERT.

Table 9. Mean % inhibition for compounds tested at receptor subtypes.

Compound	5-HT_{1A}	5-HT_{2A}	SERT
1b	98.0	61.0	12.0[a]
2b	93.0	7.0[a]	16.0[a]
3b	102.0	11.0[a]	-1.0[a]
4b	71.0	17.0[a]	22.0[a]
5b	76.0	-2.0[a]	16.0[a]
16b	100.0	96.0	62.0
17b	91.0	81.0	67.0

[a] % Inhibition considered significant when > 50%.

Data in Table 9 represents mean % inhibition (N = 4 determinations) for the compounds tested at each receptor subtypes. Significant inhibition is considered > 50%. In cases where negative inhibition (-) is seen, this represents a stimulation of binding. Occasionally, compounds at high concentrations will non-specifically increase binding. Ki values were determined only for compounds with % inhibition > 50%.

Table 10. K_i values for compounds tested at receptor subtypes.

	K_i (nM)		
Compound	5-HT_{1A}	5-HT_{2A}	SERT
1b	257.9	3,493.0	-[a]
2b	375.3	-[a]	-[a]
3b	712.5	-[a]	-[a]
4b	724.4	-[a]	-[a]
5b	88.7	-[a]	-[a]
16b	102.0	237.1	1,242.0
17b	716.1	195.2	1,871.0
8-OH-DPAT	1.2[b]	> 1000[b]	> 1000[b]
mirtazapine	> 1000[b]	14.8[b]	> 1000[b]
fluoxetine	> 1000[b]	> 1000[b]	30.8[b]

[a] Significant inhibition is considered when > 50%. Since result was < 50% (see Table 1), NIH-PDSP does not provide this information.
[b] Ref. 132.

As can be seen in Table 10, the seven compounds show low to moderate affinity for 5-HT_{1A}, and for compounds **2b** to **5b** there is selectivity for the 5-HT_{1A} over the 5-HT_{2A} receptors. Compound **5b** shows the highest affinity for 5-HT_{1A} (88.7 nM), suggesting that the *o*-methoxy substituent in the phenyl-piperazine ring could be determinant in the activity towards that receptor. Potent and selective affinity towards the 5-HT_{1A} receptor could potentially represent novel applications for these types of compounds. Interestingly, compounds **2b** to **5b** all belong to the family of Homologues Type I. Compounds **16b** and **17b** exhibit the desired dual activity toward the 5-HT_{2A} receptor and the SERT, although at the micromolar level. Further functional assays will determine if the affinity of the compounds toward the 5-HT_{2A} receptor is agonistic or antagonistic. None of the compounds has exhibited significant affinity for SERT when compared to fluoxetine hydrochloride (30.8 nM); however, compounds **16b** and **17b** show micromolar affinity (1.24 and 1.87 μM, respectively) which is still comparable to the affinity for the SERT showed by compounds previously synthesized by the Pinney group (lowest, 1.45 μM.).[147] The fact that compounds **16b** and **17b** belong to the family of Homologues Type II suggests that the aminopropane chain may be determinant for affinity towards the SERT. From the complete set of biological data it will be possible to correctly establish the structure-activity relationship features necessary for activity and/or selectivity towards each of the serotonin receptors and the monoamine transporters.

CHAPTER FIVE

Conclusions and Future Directions

The research presented herein focuses on the development of novel bivalent molecules that, by exhibiting an enhanced antagonism towards the 5-HT_{2A} receptors while keeping a highly selective inhibition of the serotonin transporter, can potentially provide an enhanced efficacy over a wider variety of both depressive and anxiety disorders. In order to develop this idea a library of novel molecules which judiciously combine portions of known 5-HT_{2A} receptor antagonistic agents with two distinct fluoxetine structural homologues have designed and prepared. Homologues I or amino-ethane derivatives, and Homologues Type II or amino-propane derivatives constitute the general classification of the two new kinds of molecules. From the synthetic organic chemistry point of view, several conclusions can be made:

1. A library of thirty-three novel molecules was successfully constructed using synthetic organic chemistry procedures and techniques.
2. The synthetic routes chosen were fairly straightforward, avoiding extra steps of protection and deprotection, thereby improving the overall atom economy.
3. N-alkylation of nine cyclic secondary amines (I to XI) with diverse primary alkyl halides was successful in the presence of base, K_2CO_3.
4. Nucleophilic attack of styrene oxide at its least substituted position by four cyclic secondary amines (VI to IX) was successful in the presence of base, K_2CO_3, to provide the corresponding benzylic secondary alcohols.

5. Mitsunobu coupling between thirty-three benzylic secondary alcohols and two different phenols proved to be an extremely useful synthetic tool in the construction of this library of compounds for its specificity and lack of interference with additional functional groups.
6. Three different methods for the Mitsunobu reaction were explored, each one presenting a different set of advantages and disadvantages.
7. The main disadvantage encountered with the use of the Mitsunobu reaction was the co-elution of the side products of the promoting reagents, making purification extremely difficult.
8. Method C was the most efficient method in avoiding the co-elution of side products, although its yields are only moderate.
9. In every case moderate to high yields were obtained with the Mitsunobu reaction. With the exception of a 97% yield obtained with Method A, Method B provided some of the best yields (> 80%).
10. The final thirty-three compounds were obtained in high purity, which was assessed by NMR spectroscopy, elemental analysis and HPLC studies.

So far, the hypothesis proposed in this study has proven to be feasible since in preliminary binding studies at the NIH-PDSP program compounds **16b,** and **17b** have shown the desired dual activity towards the SERT and the 5-HT_{2A} receptors. This suggests that the aminopropane moiety may be necessary for the affinity to the SERT. At the same time, compound **5b** has show a moderate affinity to the 5-HT_{1A} receptor (88 μM) suggesting that the *o*-methoxy moiety in the phenyl-piperazine ring could be determinant in the activity towards that receptor. None of the compounds has exhibited

significant affinity for SERT when compared to fluoxetine hydrochloride (30.8 nM); however, compounds **16b** and **17b** show micromolar affinity (1.24 and 1.87 μM, respectively) which is comparable to the affinity for the SERT showed by three compounds previously synthesized by the Pinney group (lowest, 1.45 μM).[147] The complete set of biological data is needed to correctly establish the structure-activity relationship features necessary for activity and/or selectivity towards each of the serotonin receptors and the monoamine transporters. This study is currently underway.

Further development of this project can be approached in many directions. The suggested next steps are:

1. Identification of the most potent compounds at the desired receptor and monoamine transporter targets.
2. Separation of the enantiomers of those compounds using preparative reversed-phase HPLC.
3. Biological evaluation of each enantiomer to determine the most potent, and, to determine if one enantiomer maintains the desired dual activity or if there is separation of biological activity between the enantiomers.
4. Construction of a new library of improved compounds using the structure-activity information obtained from the present study.

The possibilities are essentially endless. It is hoped that the knowledge obtained from this study will aid in the identification of novel therapeutics that can positively contribute to the battle against depressive disorders and disease in general.

CHAPTER SIX

Synthesis and Biological Evaluation of Novel Derivatives of a Nitrogen-Containing Combretastatin Analog

Introduction

Although remarkable advances have been made in cancer chemotherapy, this disease still remains among the leading causes of death in the United States.[183] Two forms of therapy currently receiving a great deal of attention in research and clinical trials are vascular disruption and bioreduction. Vascular disrupting agents, or specifically, tubulin-binding molecules, work by disrupting the tubulin cytoskeleton of the endothelial cells that line the blood vessels in the tumor. This disruption leads to a clogging of the vessels and eventual death of the tumor from suffocation and starvation.[184,206] On the other hand, bioreductive molecules become activated in the tumor microenvironment by a one-electron reduction.[185] This reduction forms radicals that react with the DNA of the tumor cells causing chromosome breaks and aberrations, which stop the tumor's growth and lead to its death.[186]

These two forms of therapy have shown promising results in biological evaluations and clinical trials, but there is still much room for improvement. For instance, while the vascular disrupting molecules demonstrate much potency against the tumors, some still leave a viable rim of tumor cells that can re-grow. These agents have shown more promising results when combined with other forms of therapy, such as radiation.[183] Also, some tubulin-binding agents can succeed when administered alone, but tumor re-growth has been shown after administration has ceased.[187]

The work described herein illustrates an attempt to create a molecule that combines the described two forms of therapy into one molecular unit. The reasoning for such an attempt lies in the hypothesis that such a molecule can prove to be a more potent and effective anti-cancer agent. Through the attempts, two unexpected, but novel molecules were synthesized and biologically evaluated as potential vascular disrupting agents.

Background

Vascular Disruption

A tumor's survival depends on its vasculature. Blood vessels supply the cells with oxygen and nutrients and remove the toxic wastes produced by cell metabolism. It follows, then, that if treatment can block or constrict the vessels, the tumor cells will die. The basis of vascular targeting rests on this conclusion.[183,206]

One of the greatest advantages of vascular disrupting agents (VDAs) is that they specifically target only the tumor vasculature and do not harm normal body tissue or vessels. VDAs also have several other advantages over other cancer treatment methods. For instance, damage to a single vessel creates much damage to the tumor since one vessel potentially feeds thousands of cells with nutrients and removes their waste products, and only one point of the vessel has to be damaged. Also, instead of killing the endothelial cells, this treatment requires local blood coagulation due to shape change of the endothelial cells, and since the endothelial cells are adjacent to the blood stream, drug delivery is ensured. Drug resistance is improbable because the target, a normal diploid cell, is unlikely to develop genetic mutations. The convenience of this cancer therapy is

also desirable because the selected markers of biological activity can be measured in the clinic. Studies show that only temporary damage to the vascular function may be necessary, and the VDAs will probably only require administration intermittently, unlike antiangiogenic drugs, which prevent the formation of new blood vessels in the tumor.[188]

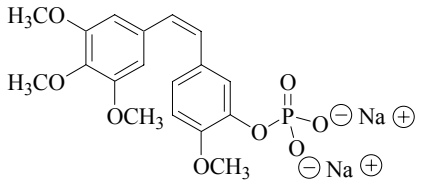
Tubulin-Binding Agents

Tubulin is the cytoskeleton protein that gives a cell its shape and structure and also plays an important role in mitosis. During mitosis, microtubules attach to the kinetochores of the chromosomes and pull the chromatids apart to opposite ends of the cell. Daughter cells can then reform around the separated chromosomes. Disruption of the tubulin protein leads to structural changes and an inhibition of cell division by preventing the chromosomes from being pulled apart. Tubulin is made up of α and β subunits that provide the attachment sites of tubulin-binding drugs.[189]

Tubulin-binding agents, which are one of the most important types of VDAs, are currently receiving much attention both in research and clinical trials. These agents disrupt vessels by causing depolymerization of the tubulin cytoskeleton in the newly forming endothelial cells of the vessels.[184] Unlike the cells in normal vasculature which have a well-defined actin cytoskeleton, these newly forming cells in the immature vasculature rely on the tubulin cytoskeleton for the maintenance of their three-dimensional, elongated shape.[190] The depolymerization leads to a shape change, a rounding-up, in the endothelial cells and probably several other effects, such as a decreased production of vasodilators and increased vasoconstrictors, an increase in vascular permeability, and an increased interaction with blood cells by the endothelial

cells. Each of these disruptions then leads to several other problems for the vessels and, eventually, stops blood flow causing tumor cell death.[184]

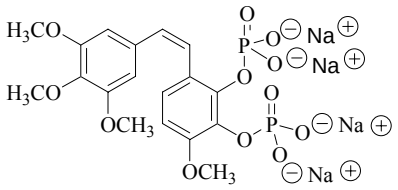
One group of tubulin-binding agents is the combretastatins. They were isolated by Professor George R. Pettit from the South African tree *Combretum caffrum* about twenty years ago.[191] Combretastatin A4 phosphate (CA-4P) has shown promising results in clinical trials. This drug greatly disrupts the tubulin cytoskeleton of proliferating endothelial cells and has little effect on more mature cells. The drug discriminates between new and mature cells most likely because the older cells have a more developed cytoskeleton and can maintain their shape in the face of tubulin depolymerization. Consequently, CA-4P has a high potency against cancer vasculature, but it does not hurt the normal body tissue.[183] Also, because of CA-4P's action against neovasculature, it is also useful against angioproliferative diseases.[192] Although CA-4P has demonstrated significant activity against cancer vasculature, it has not shown substantial tumor growth slowdown. Expansive cell kill occurs after vasculature shutdown, but the drug leaves a viable, well-oxygenated rim around the edge of the tumor, and these cells can cause rapid re-growth of the tumor. However, the combination of this drug with other treatments can alleviate this problem. Other therapies, such as radiation, cisplatin, and 5-fluorouracil attack the cells at the rim of the tumor while CA-4P attacks the inner cells, and this combination can bring the desired growth retardation.[183]



CA-4P

Figure 24. Structure of CA-4P

A newer member of the combretastatin family, Oxi4503, has also shown promising results in tubulin binding and disruption. Oxi4503 is the diphosphate prodrug of CA-1 (originally referred to as CA-1P). This compounds has proven to have effects like those of CA-4P but is even more effective than CA-4P in reducing tumor size.[187] Although many tumors studied were able to re-grow after treatment ceased, the drug was able to target the vessels in the tumor periphery. This characteristic shows that the drug can possibly be used as a single agent instead of requiring a combination with other treatments to be most effective in reducing the tumor.[187]



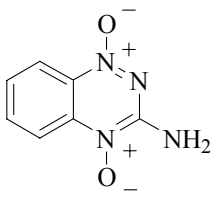
CA-1P

Figure 25. Structure of CA-1P.

Bioreduction

Bioreductive drugs are anticancer prodrugs that utilize the hypoxic conditions of tumors to become activated by a one-electron reduction.[185] The characteristic hypoxia that exists in many solid tumors presents a problem for radiation and chemotherapy because these tumors are known to be two to three times more resistant to radiotherapy and less sensitive to many chemotherapeutic agents.[185] The hypoxia results from the jumbled and sometimes incomplete development of tumor vasculature. This chaos disrupts the oxygen delivery and creates subpopulations of hypoxic cells.[193] As a result, bioreductive drugs have begun to prove very beneficial since they thrive in this environment.[193]

Tirapazamine (TPZ) (3-amino-1,2,4-benzotriazine-1,4-dioxide) is an anticancer bioreductive drug currently in phases II and III in clinical trials.[186] The cytotoxicity of TPZ causes single-strand and double-strand breaks in DNA and chromosome aberrations. The one-electron reduction of the parent compound creates a radical intermediate. This radical then creates deoxyribose radicals by removing hydrogen atoms from the DNA backbone. Further reaction of TPZ with these radicals then produces the strand breaks and aberrations. The radical produced is easily reoxygenated in aerobic conditions, which is why TPZ is selective for hypoxic conditions.[186]



TPZ

Figure 26. Structure of tirapazamine[194]

TPZ can also be seen as an all-in-one bioreductive drug because it increases its own toxicity by inhibiting nitric oxide synthase (NOS) and the production of nitric oxide (NO). Tirapazamine apparently diverts electrons from the reductase domain of NOS and therefore inhibits the enzyme. Since TPZ works in the hypoxic environment of the tumor, this action facilitates the drug by maintaining the low-oxygen environment.[185]

Design Rationale

The idea behind this project was to combine the modes of cancer therapy, specifically tubulin binding and bioreduction. The structure of the target molecule reflected a combination of the structure of a combretastatin family analog (believed to be responsible for tubulin binding and depolymerization activity), and the structure of tirapazamine (a bioreductive drug). The combined structure could possibly create a selective delivery mechanism for the bioreductive component into the tumor microenvironment.

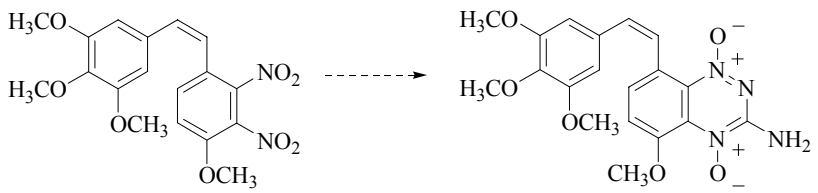


Figure 27. Structure of target molecule XI.

Taking advantage of a 2,3-di-nitro analog of CA-1 (**X**) already synthesized by Dr. Mallinath Hadimani,[189] a member of the Pinney Research Group at Baylor University, it was decided to synthetically close the third ring, a benzo-triazine through a series of synthetic organic transformations, and then biologically evaluate the resulting compound for both of its targets, with the hope that by combining the two forms of therapy into one molecule, a more potent anti-cancer agent can be created and used in the fight against cancer.

CHAPTER SEVEN

Materials and Methods

General Section

All chemicals used for synthesis were obtained from commercial sources such as Acros Chemicals, Aldrich Chemical Company, and Fisher Scientific and used without further purification. Reactions which involved air or moisture sensitive reagents were performed in oven-dried glassware under nitrogen atmosphere using dried syringes, needles and cannulas to transfer solvents and reagents.

Reactions were monitored by thin layer chromatography (TLC) using Merck Kieselgel 60 F_{254} glass backed plates. The plates were visualized using a multiband 254/365 nm UV lamp. Purification of intermediates and products was achieved by flash chromatography using silica gel (230-400 mesh) purchased from BODMAN industries. Solvents used for chromatography (hexane, ethyl acetate and methanol) were purchased from the above mentioned chemical companies and distilled prior to use. Removal of solvents was performed in a rotary evaporator under vacuum followed by further drying with a mechanical pump at a pressure lower than 0.5 Torr.

Structure elucidation of intermediates and products was carried out using NMR spectroscopy. ^{1}H, and ^{13}C spectra were recorded with a Bruker DPX-300 spectrometer operating at 300 MHz for proton, and 75 MHz for carbon. All NMR were recorded in $CDCl_3$ (0.03% of TMS as reference) unless stated otherwise. Chemical shifts are expressed in ppm (δ). Peak patterns are reported as singlets (s), doublets (d), triplets (t),

quartets (q), and multiplets (m). Coupling constants (*J*) are expressed in Hz. NMR processing was carried out using WinNMR and MestRec 4.8.6.0 software.

Elemental analysis was performed by Atlantic Microlab, Inc. in Norcross, Georgia. Melting points were determined using a Thomas-Hoover melting point apparatus and are reported uncorrected.

X-ray crystallography was done on a Bruker X8 APEX diffractometer using graphitemonochromated Mo Kα X-radiation. Structures were solved by direct methods which gave the positions of all non-hydrogen atoms using SHELXTL ver. 6.10.[195]

Synthesis of (Z)-1-[(2',4'-dimethoxy-3'-nitro)phenyl]-2-[(3",4",5"-trimethoxy)-phenyl]ethane **(73).**[196]

To a solution of sodium methoxide in methanol (1.2 mL, 5.1 mmol) was added guanidine hydrochloride (0.49 g, 5.1 mmol). The mixture was heated at reflux temperature for 30 min. Sodium chloride was filtered from the solution and the resulting free guanidine was added to the starting material (Z)-1-[(4'-methoxy-2',3'-dinitro)-phenyl]-2-[(3",4",5"-trimethoxy)-phenyl]ethene (1.0 g, 2.6 mmol) dissolved in 5 mL of methanol. After heating the mixture at reflux temperature for 2 h, potassium hydroxide (0.28 g, 5.1 mmol) was added and reflux was continued for 12 h. The reaction mixture was quenched with 50 mL of water and extracted with dichloromethane (3×50 mL). The combined organic layers were washed with brine and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the residue was adsorbed onto silica gel and purified via flash column chromatography (30:70 EtOAc/Hexanes). Ethene **73** was afforded as a pale yellow solid (0.16 g, 0.43 mmol, 17%yield). Recrystallization (hexanes-ethyl acetate) afforded an analytically pure sample.

^{1}H-NMR ($CDCl_3$): δ 7.28 (d, J = 8.87 Hz, 1H), 6.63 (dd, J = 10.50, 4.20 Hz, 2H), 6.53 (d, J = 12.12 Hz, 1H), 6.46 (s, 2H), 3.92 (s, 3H), 3.87-3.85 (m, 3H), 3.84 (s, 3H), 3.71 (s, 6H).

^{13}C-NMR ($CDCl_3$):153.06, 150.69, 150.65, 137.64, 132.28, 131.76, 131.70, 124.04, 122.94, 107.23, 105.97, 62.76, 60.94, 56.60, 56.00.

Elemental Analysis (%) calculated for $C_{25}H_{25}ON_2F_3$: C, 60.79; H, H, 5.64; N, 3.73. Found: C, 60.65; H, 5.53; N, 3.71.

Synthesis of 2-amino-4-methyl-6-(3,4,5-trimethoxy-phenyl)-pyrimidine **(74).**[196]

A mixture of guanidine hydrochloride (0.17 g, 1.8 mmol) and sodium methoxide (0.09 g, 1.8 mmol) in 20 mL of isopropanol was heated at reflux temperature for 30 min. Sodium chloride was filtered from the solution and the resulting free guanidine was added to the staring material (Z)-1-[(4'-methoxy-2',3'-dinitro)-phenyl]-2-[(3",4",5"-trimethoxy)-phenyl]ethane (0.3569 g, 0.9144 mmol) (0.35 g, 0.91 mmol). After heating at reflux temperature for 2 h, potassium hydroxide (0.10 g, 1.8 mmol) was added and reflux was continued for 12 h. The reaction mixture was quenched with water (50 mL) and extracted with dichloromethane (3×50 mL). The combined organic layers were washed with brine and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the residue was adsorbed onto silica gel and purified by flash column chromatography (100% EtOAc). Pyrimidine **74** was afforded as a yellow solid (0.02 g, 0.06 mmol, 6% yield). Recrystallization (hexanes-ethyl acetate) afforded an analytically pure sample.

^{1}H-NMR ($CDCl_3$): δ 7.24 (s, 2H), 6.88 (s, 1H), 5.04 (s, 1H), 3.96 (s, 6H), 3.90 (s, 3H), 2.43 (s, 3H).

^{13}C-NMR ($CDCl_3$): δ 168.54, 164.90, 162.93, 153.40, 140.20, 132.80, 107.08, 104.26, 60.94, 59.51, 56.25.

Biological Evaluation

Biological evaluation of these compounds was possible through collaborative efforts with Professor Mary Lynn Trawick, at Baylor University, for the tubulin polymerization assay; and Professor George R. Pettit, at Arizona State University, for the *in vitro* cytotoxicity studies.

Tubulin Polymerization Assay[197,198]

This assay measures microtubule assembly by turbidity with spectrophotometry. The assay begins with preincubation of serial dilutions of the drug with purified bovine brain tubulin. The samples are incubated on ice and GTP is added to induce assembly. The samples are placed in a spectrophotometer, the temperature is raised to 30 °C (or 37 °C), and the extent of assembly is monitored by turbidimetry at 350 nm. The IC_{50} values for tubulin assembly are then determined graphically from a standard assay.

In Vitro Cytotoxicity Studies[199]

This assay evaluates the cancer cell growth inhibitory activity of drugs against selected human cancer cell lines, which in this case included: pancreas adenocarcinoma (BXPC-3), breast adenocarcinoma (MCF-7), CNS glioblastoma (SF268), non-small cell lung carcinoma (H640), Colon (KM20L2), prostate (DU-145), and P388 leukemia cells. This is done using the National Cancer Institute's standard sulforhodamine B (SRB) assay, a colorimetric cell viability assay. SRB is a protein-binding dye that binds to the basic amino acids of cellular components. The cell lines are incubated with serial

dilutions of the drug for a certain time period, in 96-well microtitre plates. After 48 h, the plates are fixed *in situ* with trichloroacetic acid and stained with SRB for 10 min. Unbound SRB is removed with 1% acetic acid. The solubilized stain is then measured spectrophotometrically at 515 nm and the IC_{50} or GI_{50} is calculated from the optical density data.

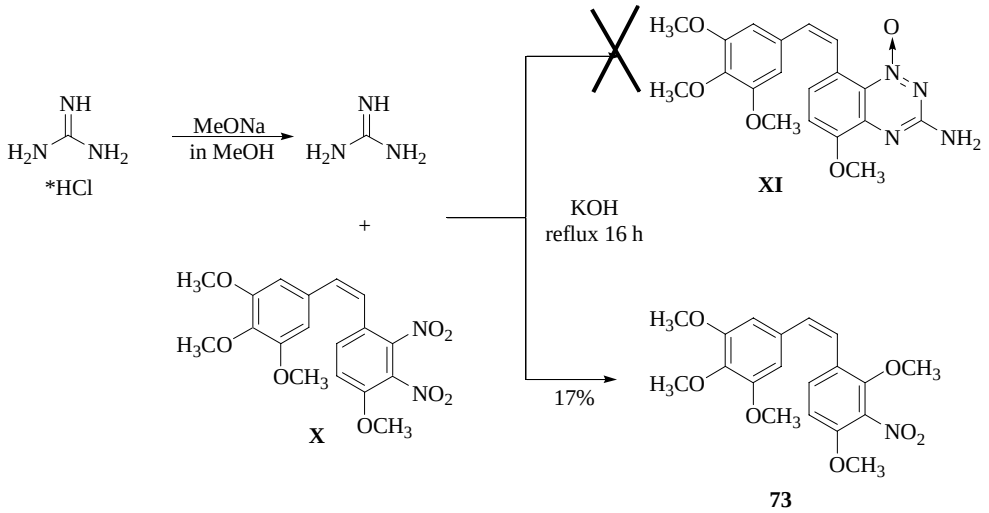
CHAPTER EIGHT

Results and Discussion

In this chapter, the synthetic procedures that afforded unexpected compounds **73** and **74** will be discussed, as well as their characterization by NMR spectroscopy and X-ray crystallography. A plausible mechanism for the formation of compounds **73** and **74** will be proposed and finally, results from *in vitro* biological evaluation will be addressed.

Synthesis of (Z)-1-[(2',4'-dimethoxy-3'-nitro)phenyl]-2-[(3",4",5"-trimethoxy)-phenyl]ethane **(73).**

In an attempt to further functionalize the B ring of *(Z)*-1-[(4'-methoxy-2',3'-dinitro)phenyl]-2-[(3",4",5"-trimethoxy)phenyl]ethane (**X**), a dinitro derivative of CA-4 previously synthesized in the Pinney group by Dr. Mallinath Hadimani,[189] two interesting products were obtained unexpectedly. Scheme 18 depicts the reaction conditions by which the first one of those, compound **73,** was obtained.



Scheme 18. Synthesis of derivative **73.**

Following a procedure found in the literature,[200] it was proposed that reaction between di-nitro stilbenoid **X** and guanidine in methanol, under reflux conditions and in the presence of KOH, could promote ring closure to afford benzotriazine N-oxide moiety **XI**. However, this did not occur and instead, a structurally similar synthetic byproduct **73** was formed in 17% yield. A great deal of effort was put into determining the identity of this unexpected product, which was achieved by NMR spectroscopy and ultimately confirmed by X-ray crystallography (Figure 28). ^{1}H-NMR showed the characteristic doublet of doublets for the vinylic protons, coupling with a J = 12.1 Hz, as expected for Z isomers (Figure 29).

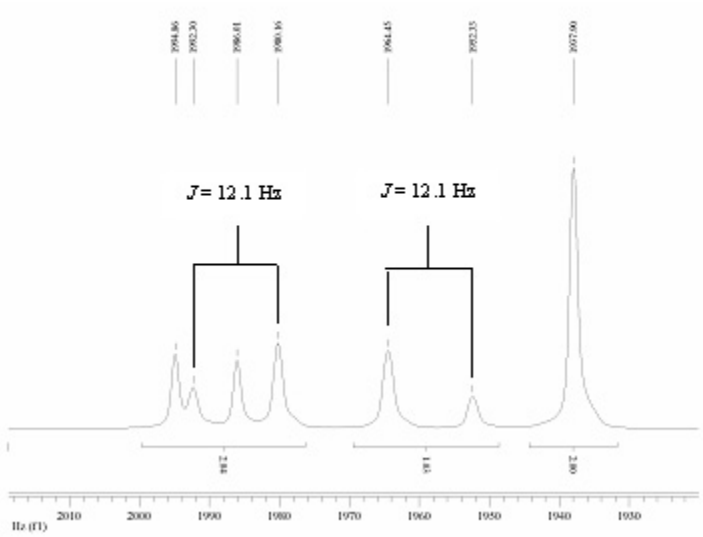
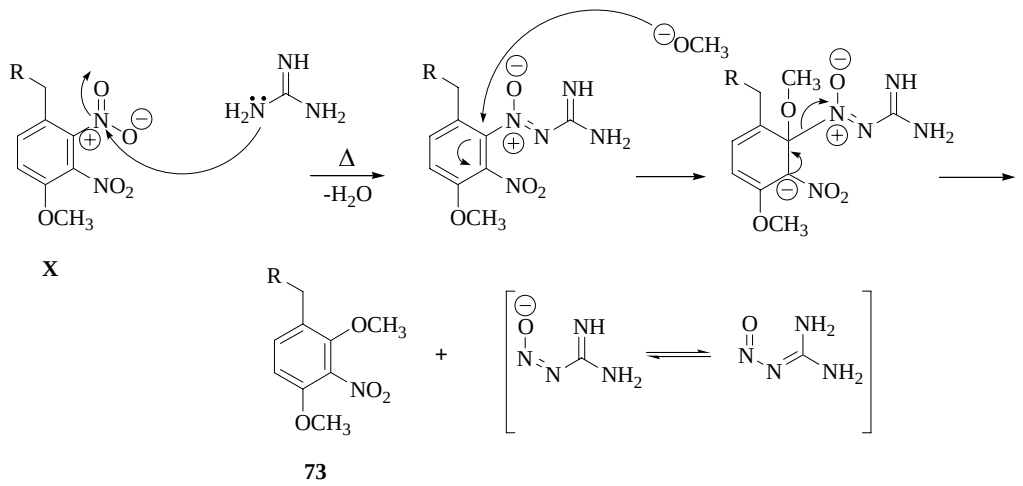


Figure 29. Expansion of ^{1}H-NMR of Compound **73**.

From the reaction conditions, the presence of methoxide ions is suggested, available from the initial NaOMe in MeOH used for de-salting of guanidine. It is proposed that the mechanism of formation of this product results from the nucleophilic

addition of guanidine to the nitro group in position 2 of the B ring, followed by nucleophilic aromatic substitution of a methoxide ion, with the leaving group being a more stable guanidine-modified nitro moiety (Scheme 19).



Scheme 19. A possible mechanism of formation for compound **73**.

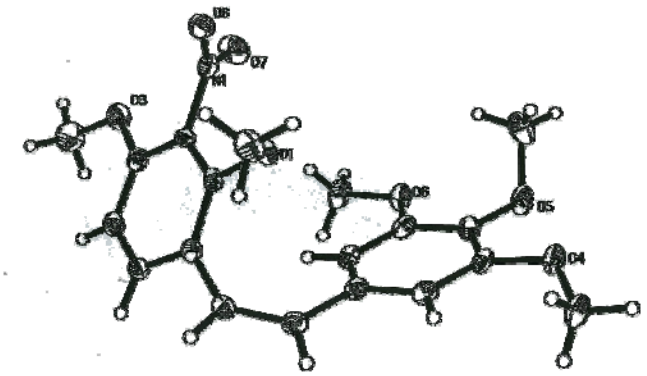
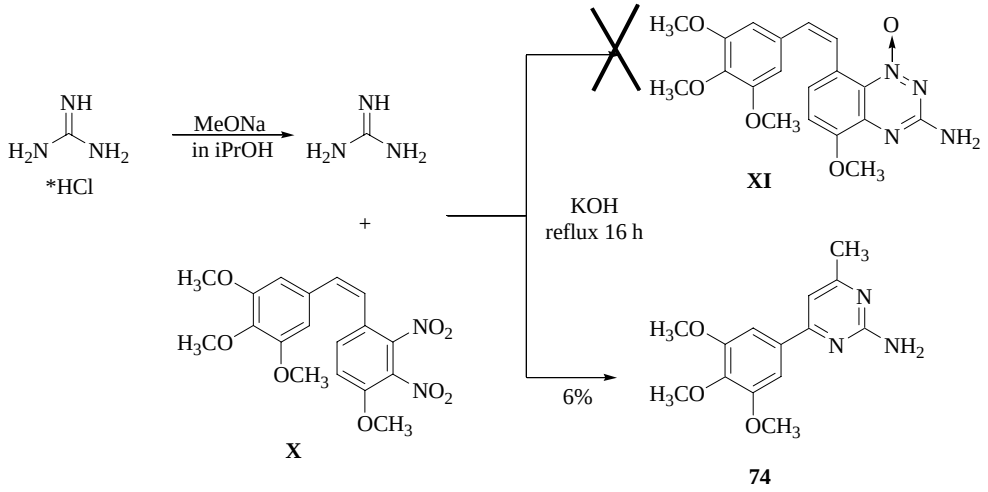


Figure 28. X-ray structure of compound **73**.[196,201]

Synthesis of 2-amino-4-methyl-6-(3,4,5-trimethoxy-phenyl)-pyrimidine **(74)**

In a separate reaction, stilbenoid **X** was treated under similar reaction conditions except for a change in solvents (isopropanol instead of methanol), in another attempt to

obtain desired product **XI**. Once again, **XI** was not obtained and instead the interesting bi-aryl analog **74** was obtained in 6% yield (Scheme 20). The identity of this compound was again studied by NMR spectroscopy and ultimately confirmed by X-ray crystallography (Figure 30).



Scheme 20. Synthesis of derivative **74.**

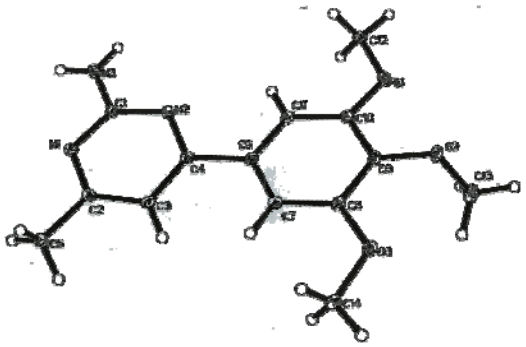
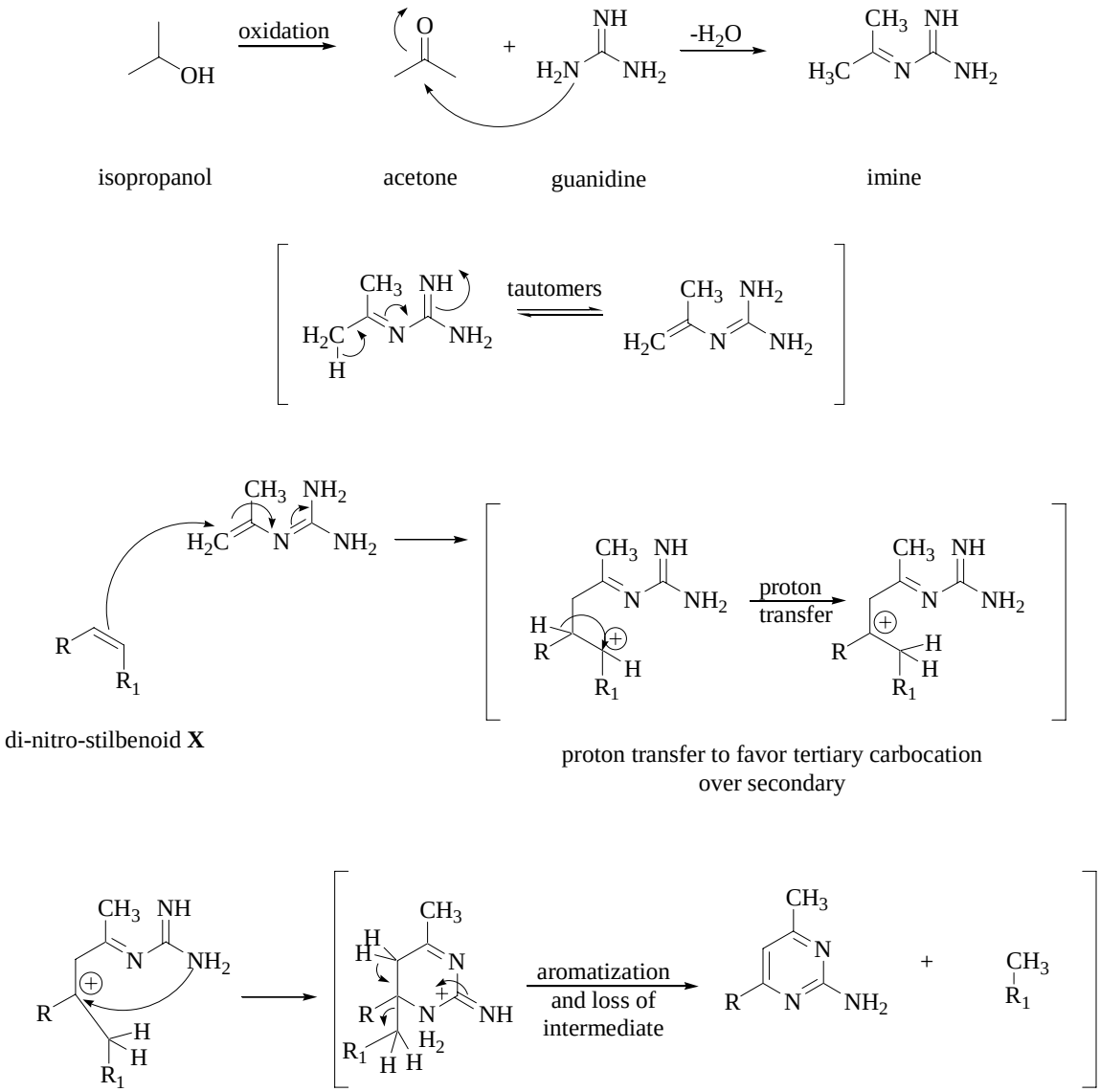


Figure 30. X-ray structure of compound **74**.[196,201]

Because of the interesting structural features of compound of biaryl compound **74**, a great deal of time was devoted to understanding the mechanism of its formation. It is believed that oxidation of isopropanol due to contact with air produces acetone. Reaction

of these acetone molecules with guanidine to produce the corresponding imine may be the first step. (Scheme 21). This may be followed by nucleophilic attack of the di-nitro stilbenoid **X** to a tautomer of the imine as a second step. Proton transfer to favor a tertiary carbocation over a secondary carbocation followed by nucleophilic attack on that position by the amine moiety may be the third step. Subsequent aromatization and loss of an intermediate may be the final steps of this mechanism of formation.



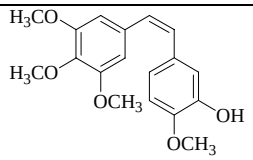
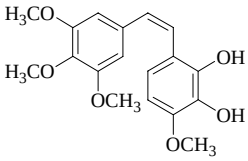
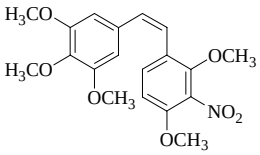
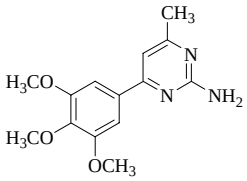
Scheme 21. Speculated mechanism of formation for compound **74**.

Biological Evaluation

Biological evaluation of our compounds was possible through collaborative efforts with Professor Mary Lynn Trawick, at Baylor University, for the tubulin polymerization assay; and Professor George R. Pettit, at Arizona State University, for the *in vitro* cytotoxicity studies.

Table 11 indicates the IC_{50} (μM) values for inhibition of tubulin polymerization of compounds **73** and **74**, and CA-4 and CA1 for comparison purposes. As can be observed, compound **73** showed a low IC_{50} while compound **74** produced no significant inhibition. It is surmised that this activity is related to the stilbenoid structure of **73**, as compared to that of CA-4 and CA-1.

Table 11. Inhibition of tubulin polymerization for compounds **73** and **74.**

Compound	Structure	IC_{50} Tubulin Inhibition (μM)
CA-4		1.2[a]
CA-1		1.9[a]
73		**3.3**
74		> 40

[a] Ref. 202.

Additionally, Table 12 indicates the *in vitro* cytotoxicity data against six human cancer cell lines of compounds **73** and **74**, and Oxi8006 for comparison purposes. As can be observed, compound **73** displayed no significant cytotoxicity while compound **74** showed interesting results. On one hand, it showed no significant cytotoxicity against pancreas, breast or CNS carcinomas. However, it showed surprisingly potent and selective cytotoxicity against lung, colon and prostate cell lines. These data, combined with the fact that compound **74** exhibited no significant inhibition of tubulin polymerization, suggests that this compound may have a different mechanism of action against cancerous cells, and this should be explored further. In the case of compound **73**, considering its encouraging IC_{50} inhibition of tubulin, further *in vivo* blood flow reduction assays should be performed to fully evaluate its utility as a vascular disrupting agent.

Table 12. *In vitro* cytotoxicity (GI_{50} and ED_{50} in μM) for compounds **73** and **74**.

Compound	P388 (ED_{50})	BXPC-3	MCF-7	SF268	NCI-H460	KM20L2	DU-145
Oxi8006[a]		0.099	0.005	0.012	0.0058	0.0093	< 0.0022
73	> 10	0.61	0.43	0.72	0.85	0.48	0.88
74	> 10	> 10	> 10	> 10	**0.09**	**0.09**	**0.10**

[a] Ref. 202.

Furthermore, exploration of the bioreductive assays and evaluation of the two molecules for this novel application is strongly recommended, especially for compound **74**.

CHAPTER NINE

Conclusions and Future Directions

The research presented herein focused on the development of a novel anticancer agent that could combine into one molecular entity two different anti-cancer therapies, vascular disruption and bioreduction. Although the synthesis of the originally selected target molecule was not achieved, very important findings were made with this project. From the synthetic organic chemistry point of view, several conclusions can be made:

1. Two unexpected but structurally unique molecules, **73** and **74**, were obtained while attempting the synthesis of the target molecule.
2. Interestingly, the only difference in reaction conditions for the synthesis of one or the other is a simple change of solvents (MeOH or *i*PrOH).
3. X-ray crystallography provided ultimate confirmation of the structures of the unexpected compounds.
4. A plausible mechanism for the formation of both products has been suggested, where each solvent plays a determinant role.

From the biological activity point of view, several conclusions can be made as well.

5. Both molecules were evaluated for their inhibition of tubulin polymerization and for their *in vitro* cytotoxicity against six human cancer cell lines.
6. Compound **73** showed a very potent IC_{50} value (3.3 μM), while compound **74** showed no significant inhibition of tubulin assembly.
7. The activity of compound **73** is suggested to rely on its structural features, very similar to those of CA-1 and CA-4.

8. Compound **74** showed a very potent and selective *in vitro* cytotoxicity towards lung, colon and prostate cancer cell lines. However, its lack of inhibition of tubulin assembly suggests that it might work through a different mechanism.

Further development of this project can be pursued in many directions. The suggested next steps are:

1. Re-synthesis of both compounds in order to have sufficient amounts of sample available for future biological evaluation.
2. Determination of *in vivo* blood flow reduction assays for both compounds to fully evaluate their utility as a vascular disrupting agents.
3. Exploration of the bioreductive pathway and evaluation of the two molecules for this novel application.

APPENDICES

APPENDIX A

Selected NMR Spectra

Spectrum		Page
1.	^{1}H-NMR of compound 34	183
2.	^{1}H-NMR of compound 35	184
3.	^{1}H-NMR of compound 36	185
4.	^{1}H-NMR of compound 37	186
5.	^{1}H-NMR of compound 38	187
6.	^{1}H-NMR of compound 39	188
7.	^{1}H-NMR of compound 40	189
8.	^{1}H-NMR of compound 41	190
9.	^{1}H-NMR of compound 43	191
10.	^{13}C-NMR of compound 43	192
11.	^{1}H-NMR of compound 44	193
12.	^{13}C-NMR of compound 44	194
13.	^{1}H-NMR of compound 1a	195
14.	^{13}C-NMR of compound 1a	196
15.	^{19}F-NMR of compound 1a	197
16.	^{1}H-NMR of compound 2a	198
17.	^{13}C-NMR of compound 2a	199
18.	^{19}F-NMR of compound 2a	200

19.	^{1}H-NMR of compound 45	201
20.	^{13}C-NMR of compound 45	202
21.	^{1}H-NMR of compound 46	203
22.	^{13}C-NMR of compound 46	204
23.	^{1}H-NMR of compound 3a	205
24.	^{13}C-NMR of compound 3a	206
25.	^{19}F-NMR of compound 3a	207
26.	^{1}H-NMR of compound 47	208
27.	^{13}C-NMR of compound 47	209
28.	^{19}F-NMR of compound 47	210
29.	^{1}H-NMR of compound 48	211
30.	^{13}C-NMR of compound 48	212
31.	^{19}F-NMR of compound 48	213
32.	^{1}H-NMR of compound 4a	214
33.	^{13}C-NMR of compound 4a	215
34.	^{19}F-NMR of compound 4a	216
35.	^{1}H-NMR of compound 49	217
36.	^{13}C-NMR of compound 49	218
37.	^{1}H-NMR of compound 5a	219
38.	^{13}C-NMR of compound 5a	220
39.	^{19}F-NMR of compound 5a	221
40.	^{1}H-NMR of compound 50	222
41.	^{13}C-NMR of compound 50	223

42. ^{1}H-NMR of compound 51 224

43. ^{13}C-NMR of compound 51 225

44. ^{1}H-NMR of compound 52 226

45. ^{13}C-NMR of compound 52 227

46. ^{1}H-NMR of compound 6a 228

47. ^{13}C-NMR of compound 6a 229

48. ^{19}F-NMR of compound 6a 230

49. ^{1}H-NMR of compound 7a 231

50. ^{13}C-NMR of compound 7a 232

51. ^{19}F-NMR of compound 7a 233

52. ^{1}H-NMR of compound 53 234

53. ^{13}C-NMR of compound 53 235

54. ^{1}H-NMR of compound 8a 236

55. ^{13}C-NMR of compound 8a 237

56. ^{19}F-NMR of compound 8a 238

57. ^{1}H-NMR of compound 9a 239

58. ^{13}C-NMR of compound 9a 240

59. ^{19}F-NMR of compound 9a 241

60. ^{1}H-NMR of compound 54 242

61. ^{13}C-NMR of compound 54 243

62. ^{19}F-NMR of compound 54 244

63. ^{1}H-NMR of compound 10a 245

64. ^{13}C-NMR of compound 10a 246

65. ^{19}F-NMR of compound 10a 247

66. ^{1}H-NMR of compound 11a 248

67. ^{13}C-NMR of compound 11a 249

68. ^{19}F-NMR of compound 11a 250

69. ^{1}H-NMR of compound 55 251

70. ^{13}C-NMR of compound 55 252

71. ^{19}F-NMR of compound 55 253

72. ^{1}H-NMR of compound 12a 254

73. ^{13}C-NMR of compound 12a 255

74. ^{19}F-NMR of compound 12a 256

75. ^{1}H-NMR of compound 13a 257

76. ^{13}C-NMR of compound 13a 258

77. ^{19}F-NMR of compound 13a 259

78. ^{1}H-NMR of compound IX 260

79. ^{13}C-NMR of compound IX 261

80. ^{19}F-NMR of compound IX 262

81. ^{1}H-NMR of compound 56 263

82. ^{13}C-NMR of compound 56 264

83. ^{19}F-NMR of compound 56 265

84. ^{1}H-NMR of compound 14a 266

85. ^{13}C-NMR of compound 14a 267

86. ^{19}F-NMR of compound 14a 268

87. ^{1}H-NMR of compound 15a 269

88. ^{13}C-NMR of compound 15a 270

89. ^{19}F-NMR of compound 15a 271

90. ^{1}H-NMR of compound 57 272

91. ^{13}C-NMR of compound 57 273

92. ^{1}H-NMR of compound 58 274

93. ^{13}C-NMR of compound 58 275

94. ^{1}H-NMR of compound 16a 276

95. ^{13}C-NMR of compound 16a 277

96. ^{19}F-NMR of compound 16a 278

97. ^{1}H-NMR of compound 17a 279

98. ^{13}C-NMR of compound 17a 280

99. ^{19}F-NMR of compound 17a 281

100. ^{1}H-NMR of compound 61 282

101. ^{13}C-NMR of compound 61 283

102. ^{1}H-NMR of compound 62 284

103. ^{13}C-NMR of compound 62 285

104. ^{1}H-NMR of compound 18a 286

105. ^{13}C-NMR of compound 18a 287

106. ^{19}F-NMR of compound 18a 288

107. ^{1}H-NMR of compound 19a 289

108. ^{13}C-NMR of compound 19a 290

109. ^{19}F-NMR of compound 19a 291

110. ^{1}H-NMR of compound 63 292

111. ^{13}C-NMR of compound 63 293

112. ^{19}F-NMR of compound 63 294

113. ^{1}H-NMR of compound 64 295

114. ^{13}C-NMR of compound 64 296

115. ^{19}F-NMR of compound 64 297

116. ^{1}H-NMR of compound 20a 298

117. ^{13}C-NMR of compound 20a 299

118. ^{19}F-NMR of compound 20a 300

119. ^{1}H-NMR of compound 21a 301

120. ^{13}C-NMR of compound 21a 302

121. ^{19}F-NMR of compound 21a 303

122. ^{1}H-NMR of compound 65 304

123. ^{13}C-NMR of compound 65 305

124. ^{1}H-NMR of compound 66 306

125. ^{13}C-NMR of compound 66 307

126. ^{1}H-NMR of compound 22a 308

127. ^{13}C-NMR of compound 22a 309

128. ^{19}F-NMR of compound 22a 310

129. ^{1}H-NMR of compound 23a 311

130. ^{13}C-NMR of compound 23a 312

131. ^{19}F-NMR of compound 23a 313

132. ^{1}H-NMR of compound 59 314

133. ^{13}C-NMR of compound 59 315

134. ^{1}H-NMR of compound 60 316

135. ^{13}C-NMR of compound 60 317

136. ^{1}H-NMR of compound 24a 318

137. ^{13}C-NMR of compound 24a 319

138. ^{19}F-NMR of compound 24a 320

139. ^{1}H-NMR of compound 25a 321

140. ^{13}C-NMR of compound 25a 322

141. ^{19}F-NMR of compound 25a 323

142. ^{1}H-NMR of compound 67 324

143. ^{13}C-NMR of compound 67 325

144. ^{1}H-NMR of compound 68 326

145. ^{13}C-NMR of compound 68 327

146. ^{1}H-NMR of compound 26a 328

147. ^{13}C-NMR of compound 26a 329

148. ^{19}F-NMR of compound 26a 330

149. ^{1}H-NMR of compound 27a 331

150. ^{13}C-NMR of compound 27a 332

151. ^{19}F-NMR of compound 27a 333

152. ^{1}H-NMR of compound 69 334

153. ^{13}C-NMR of compound 69 335

154. ^{1}H-NMR of compound 70 336

155. ^{13}C-NMR of compound 70 337

156. ^{19}F-NMR of compound 70 338

157. ^{1}H-NMR of compound 28a 339

158. ^{13}C-NMR of compound 28a 340

159. ^{19}F-NMR of compound 28a 341

160. ^{1}H-NMR of compound 29a 342

161. ^{13}C-NMR of compound 29a 343

162. ^{19}F-NMR of compound 29a 344

163. ^{1}H-NMR of compound 71 345

164. ^{13}C-NMR of compound 71 346

165. ^{19}F-NMR of compound 71 347

166. ^{1}H-NMR of compound 30a 348

167. ^{13}C-NMR of compound 30a 349

168. ^{19}F-NMR of compound 30a 350

169. ^{1}H-NMR of compound 31a 351

170. ^{13}C-NMR of compound 31a 352

171. ^{19}F-NMR of compound 31a 353

172. ^{1}H-NMR of compound 72 354

173. ^{13}C-NMR of compound 72 355

174. ^{19}F-NMR of compound 72 356

175. ^{1}H-NMR of compound 32a 357

176. ^{13}C-NMR of compound 32a 358

177. ^{19}F-NMR of compound 32a 359

178. ^{1}H-NMR of compound 33a 360

179. ^{13}C-NMR of compound 33a 361

180. ^{19}F-NMR of compound 33a 362

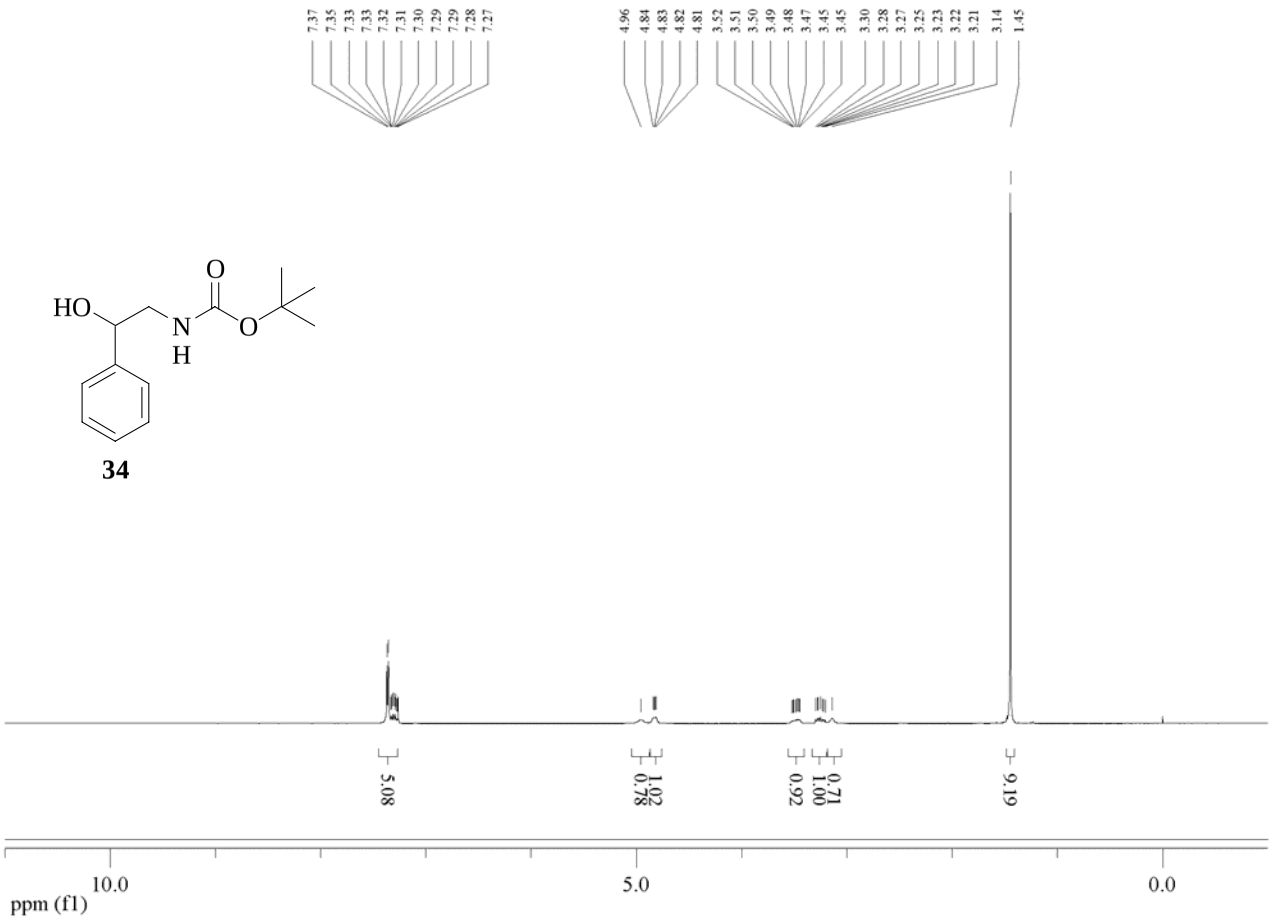
181. ^{1}H-NMR of compound 73 363

182. ^{13}C-NMR of compound 73 364

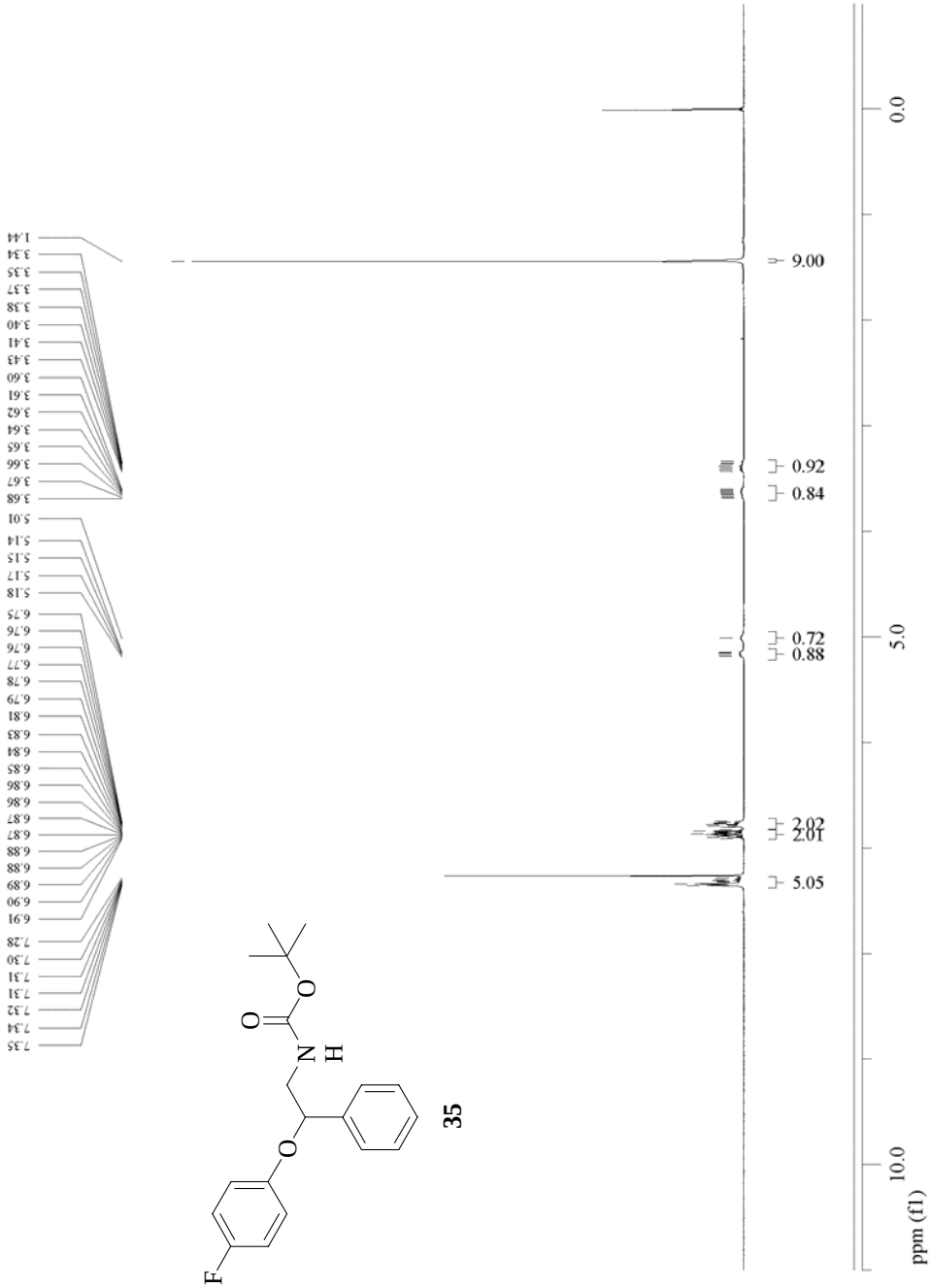
183. ^{1}H-NMR of compound 74 365

184. ^{13}C-NMR of compound 74 366

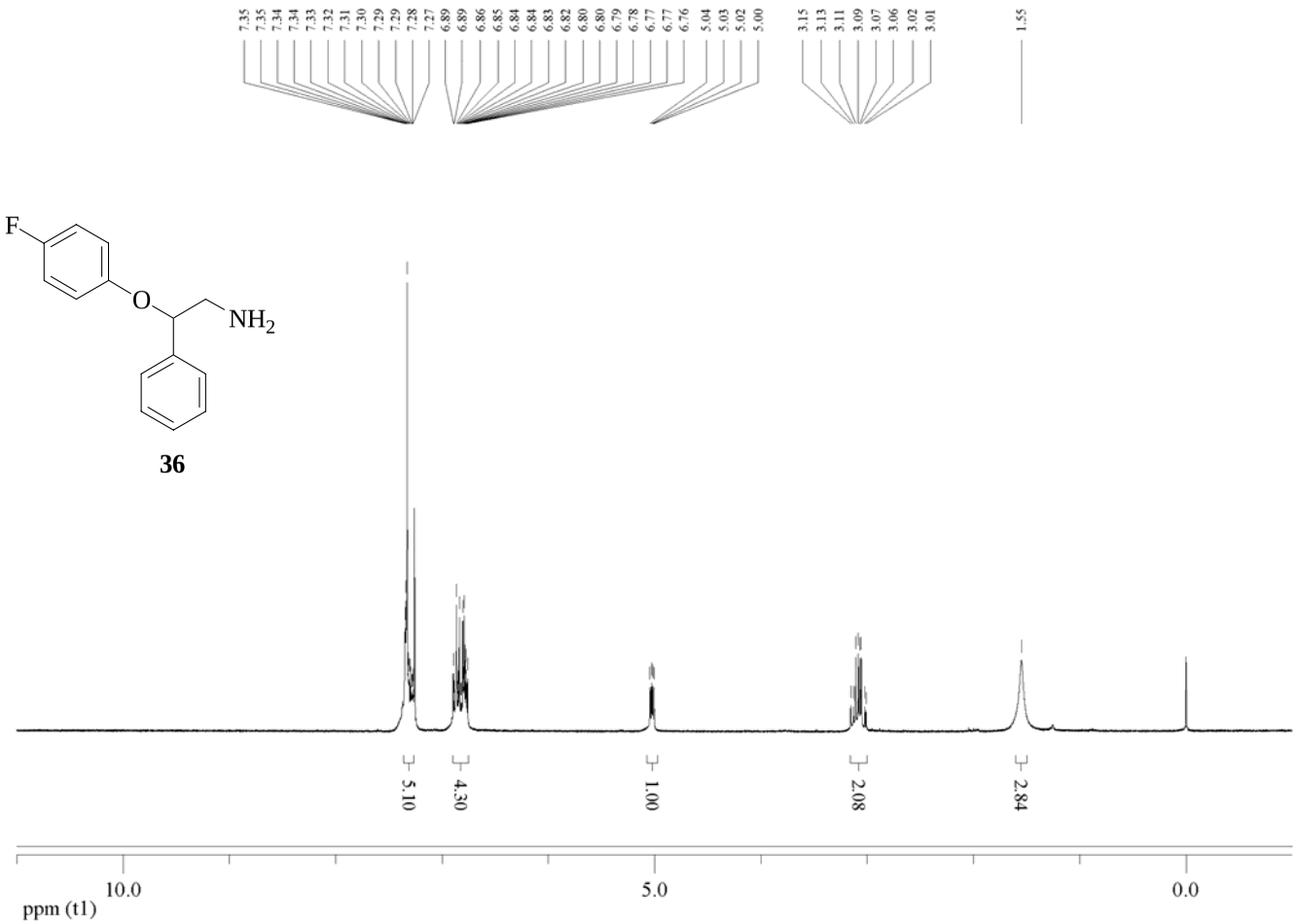
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **34**



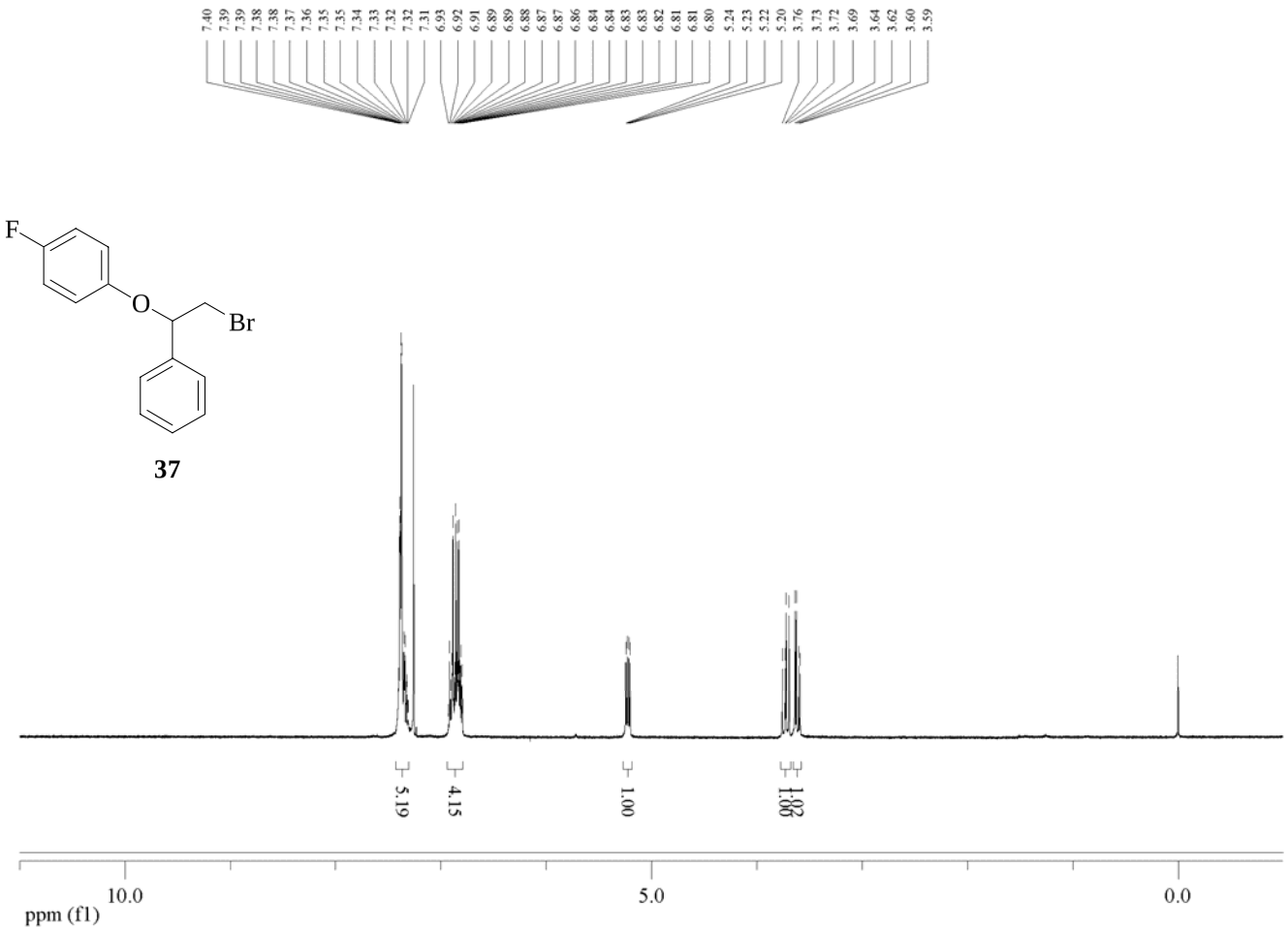
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **35**



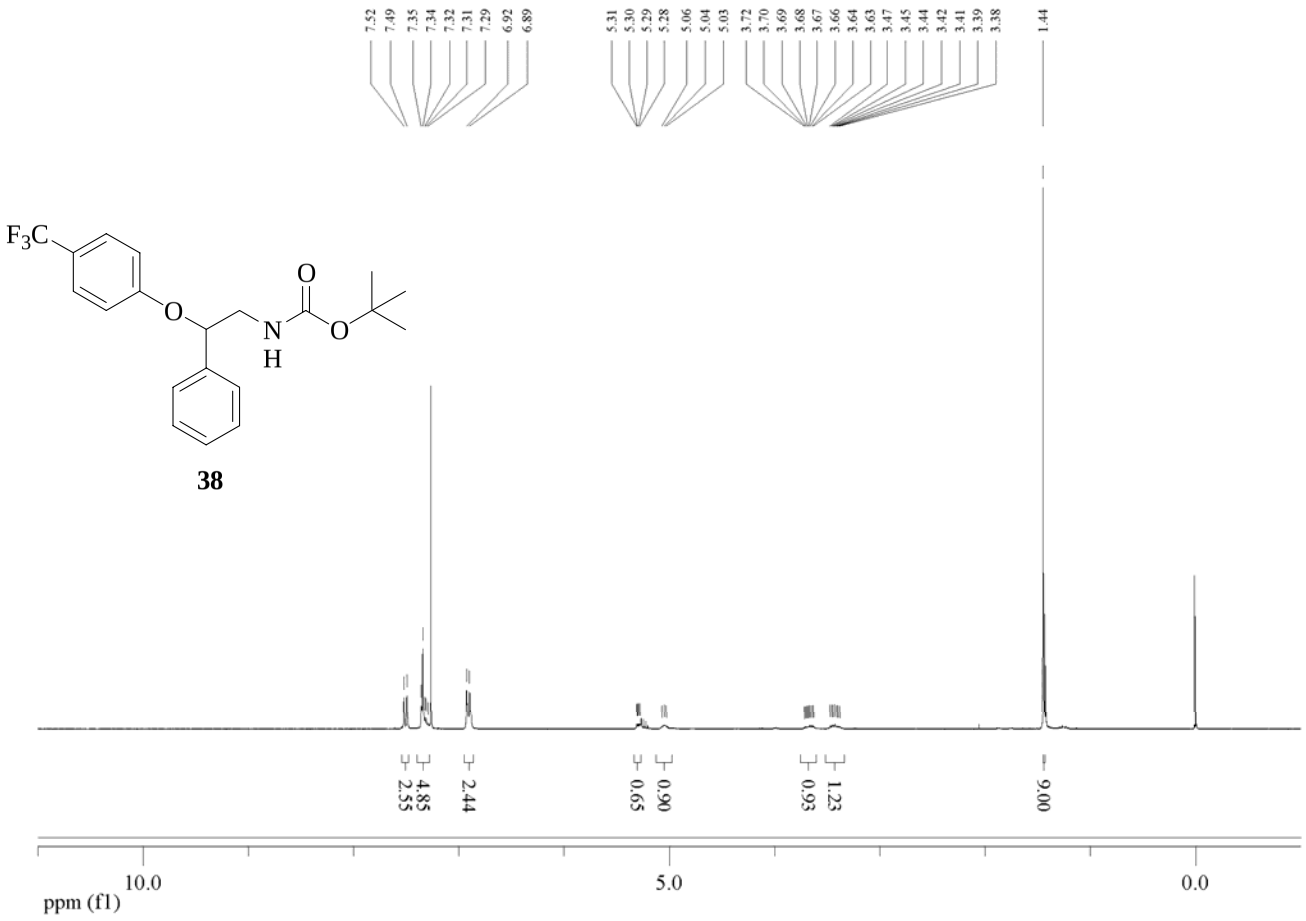
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **36**



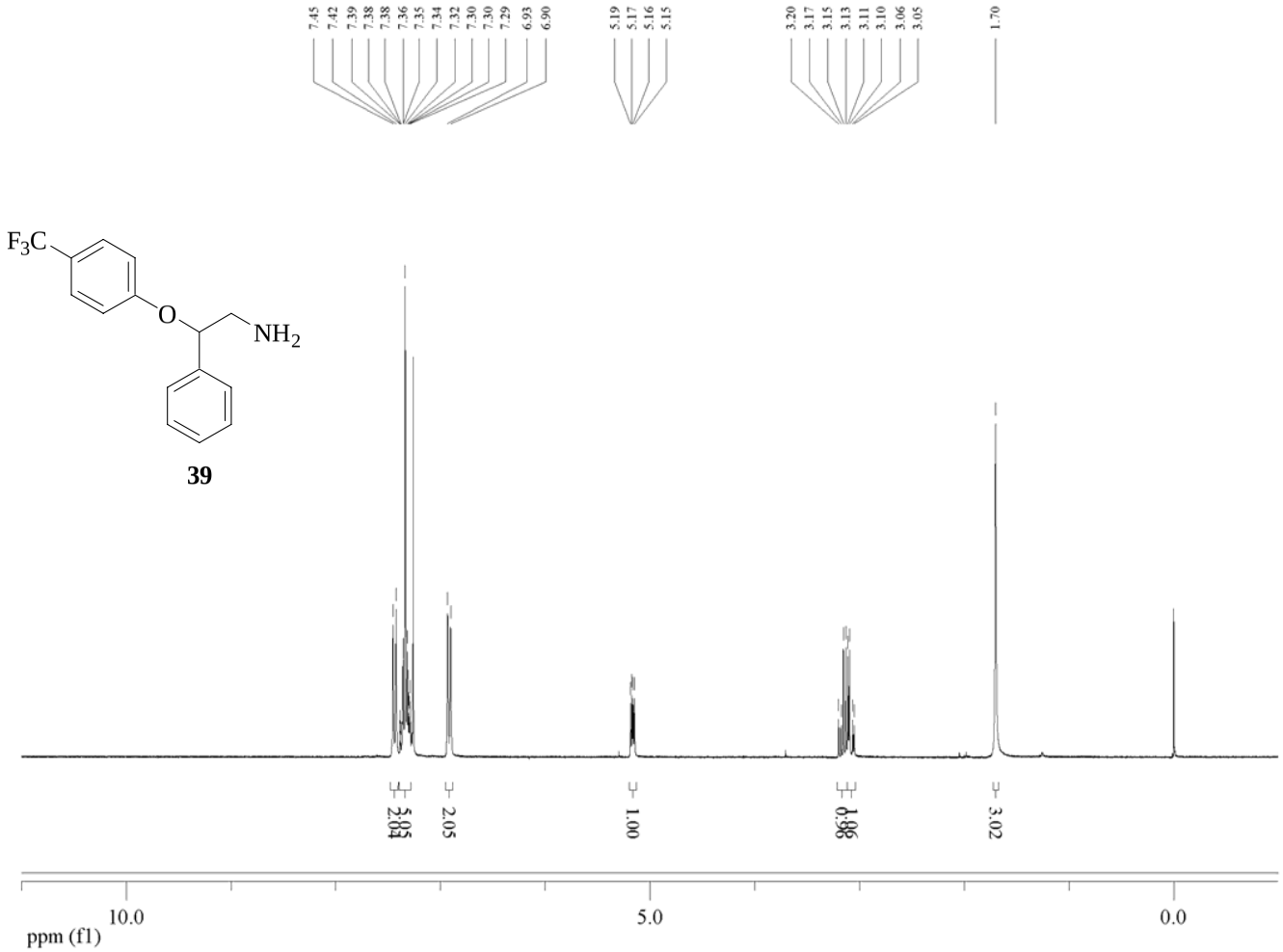
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **37**



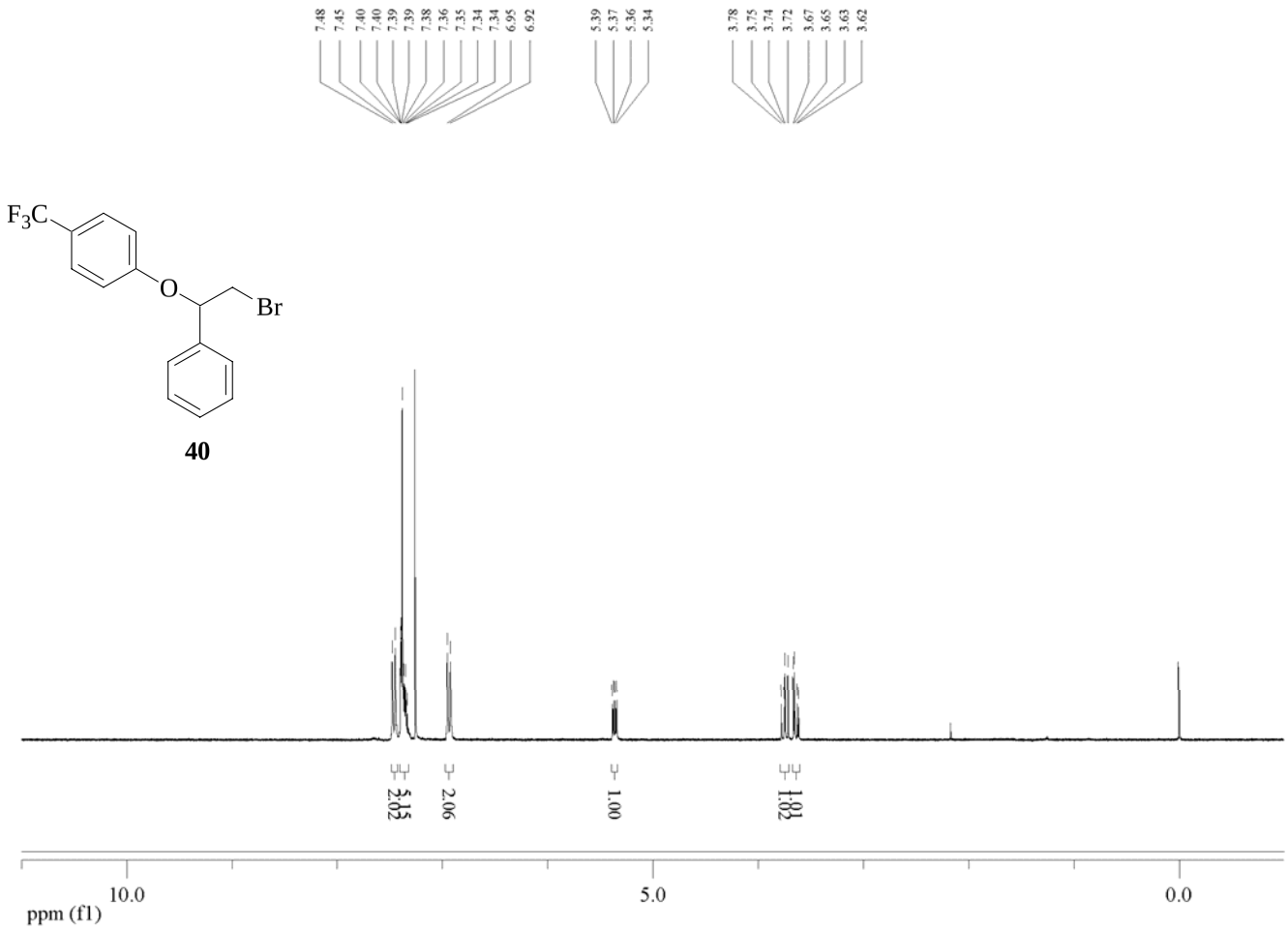
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **38**



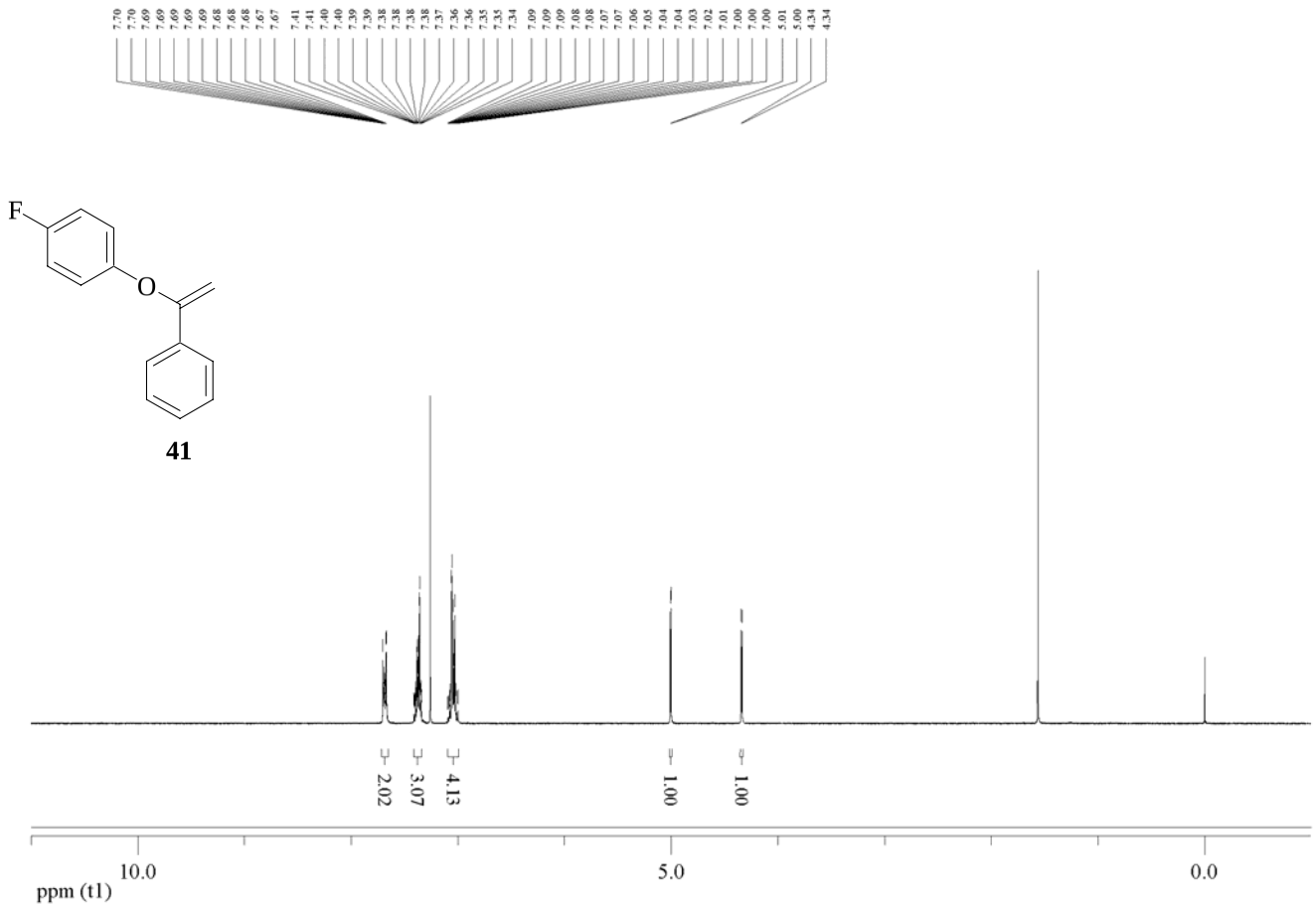
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **39**



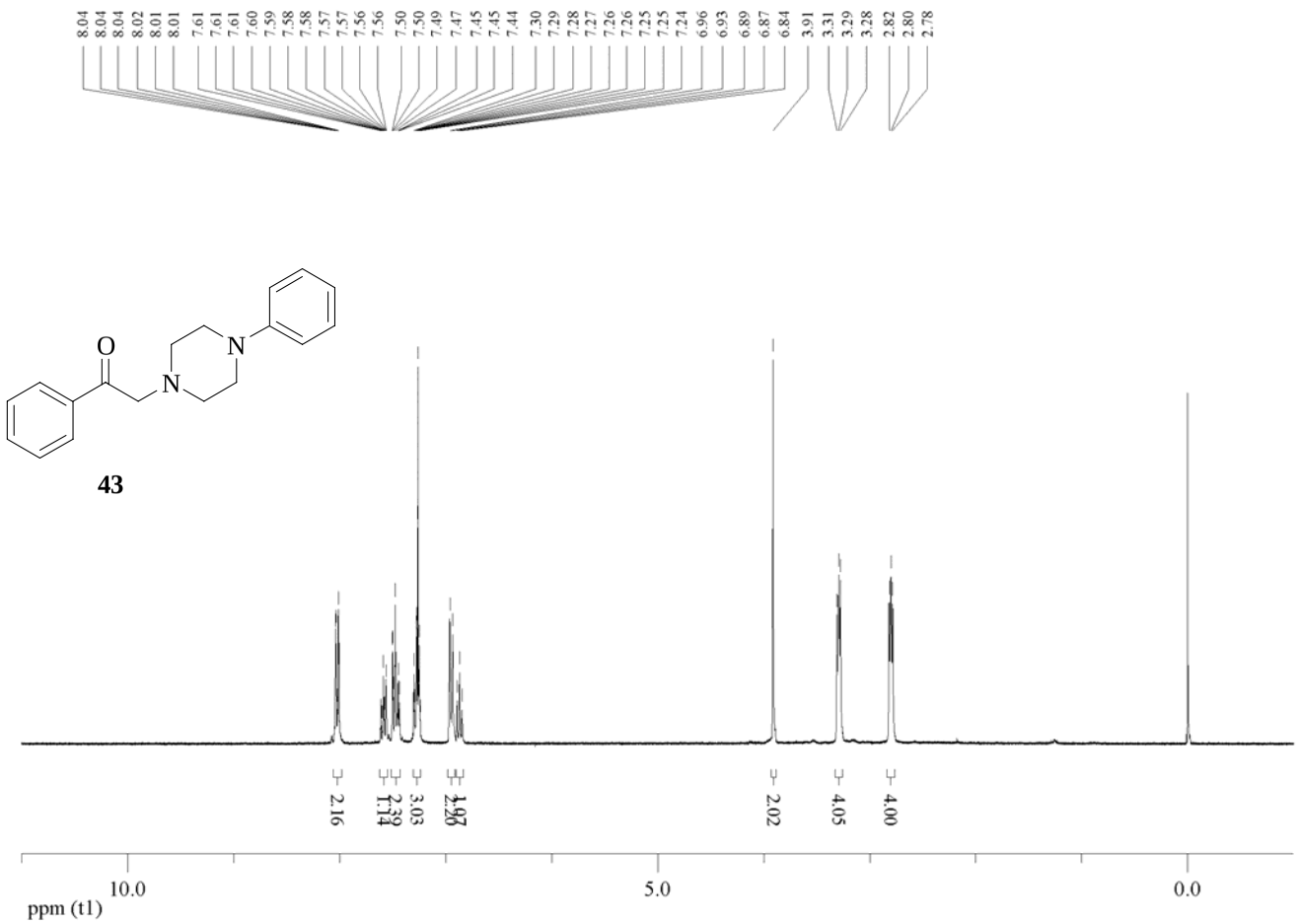
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **40**



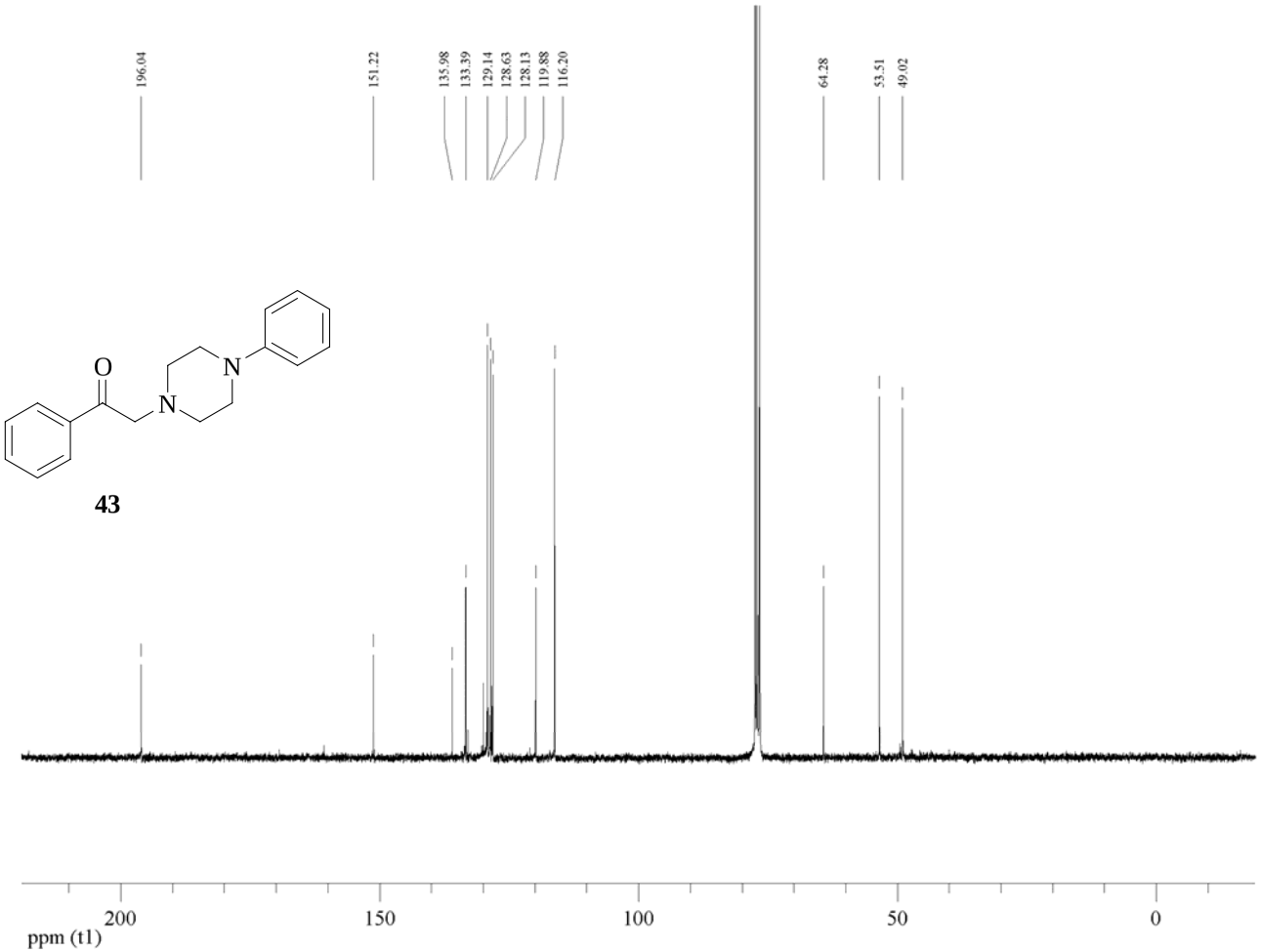
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **41**



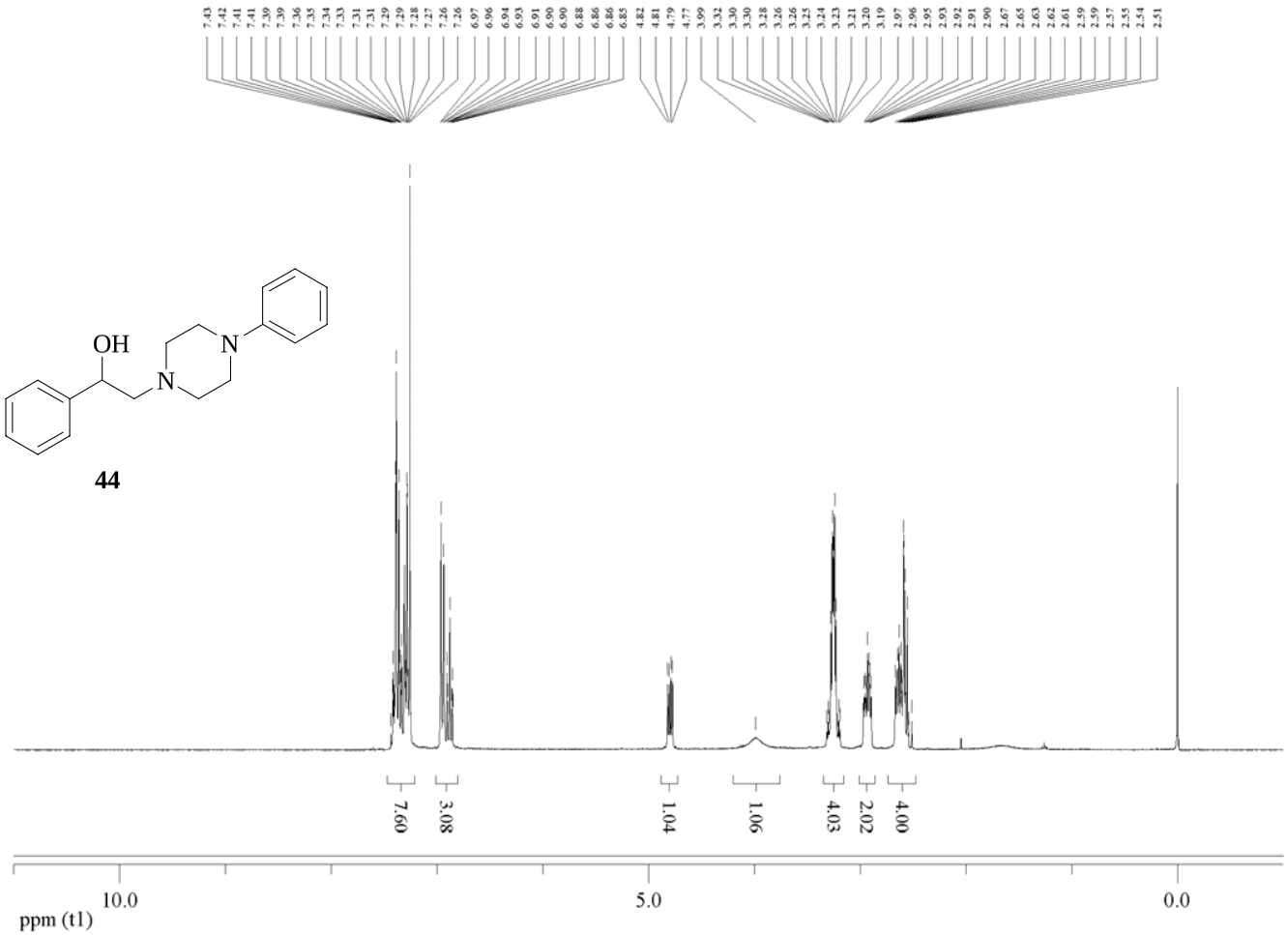
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **43**



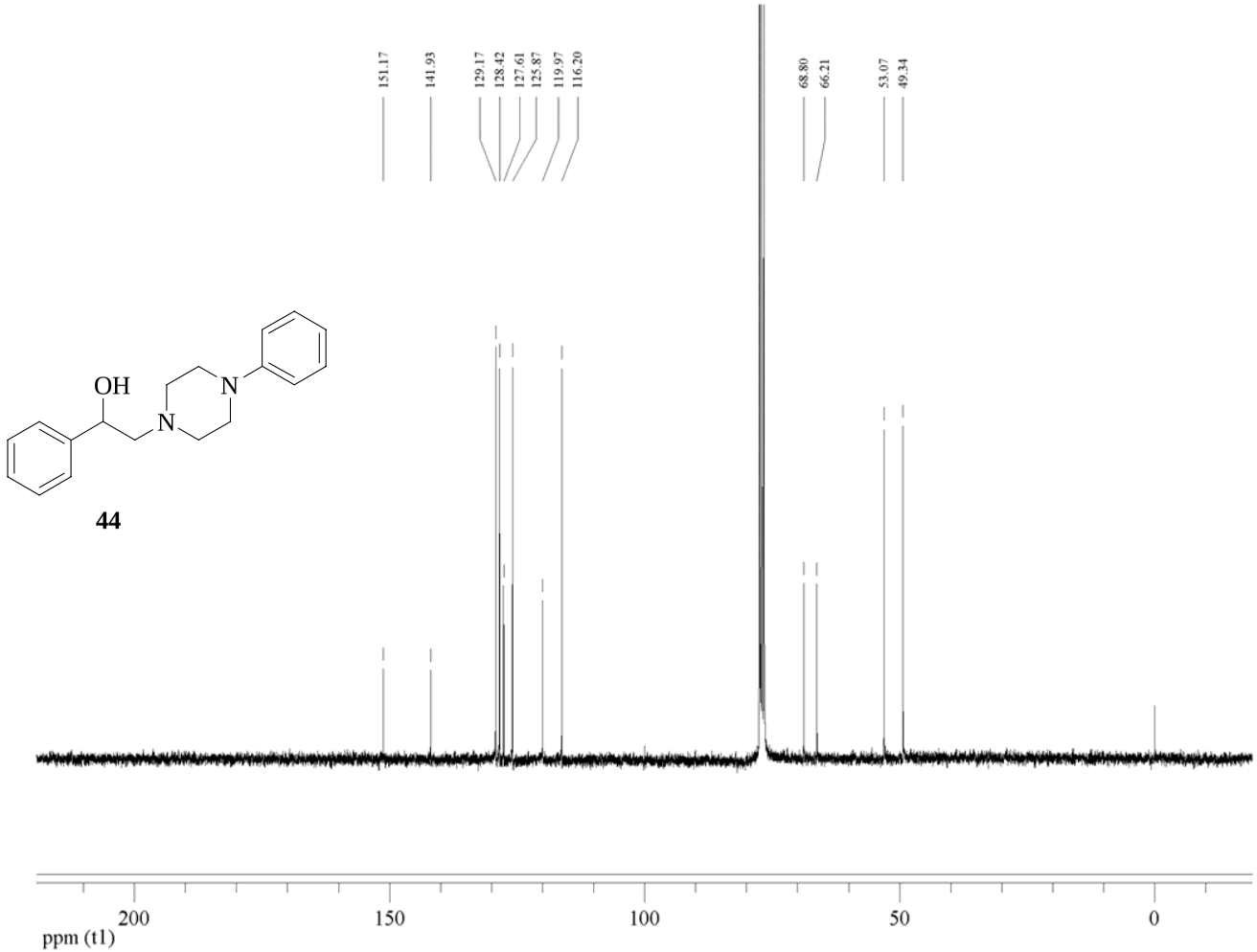
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **43**



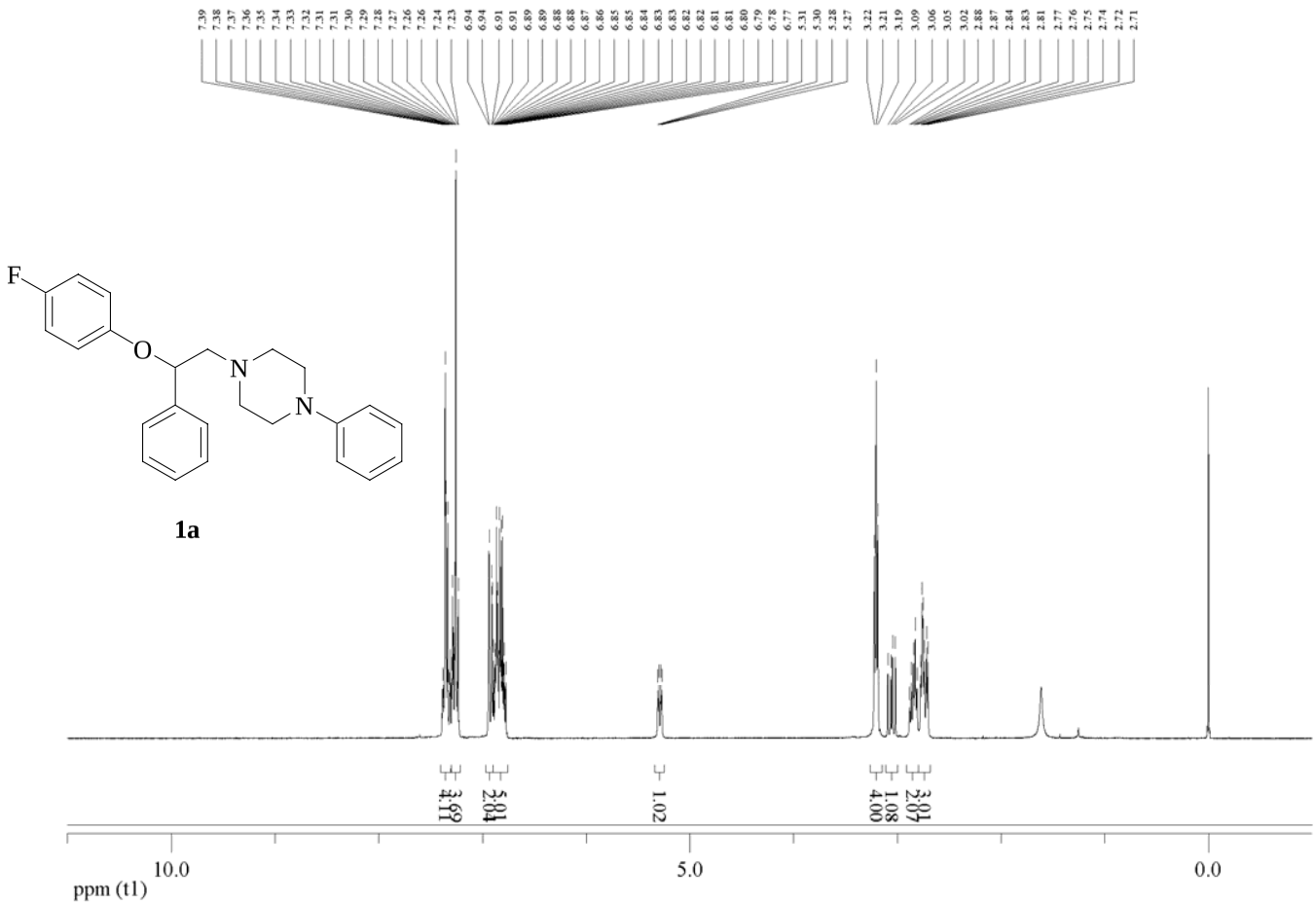
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **44**



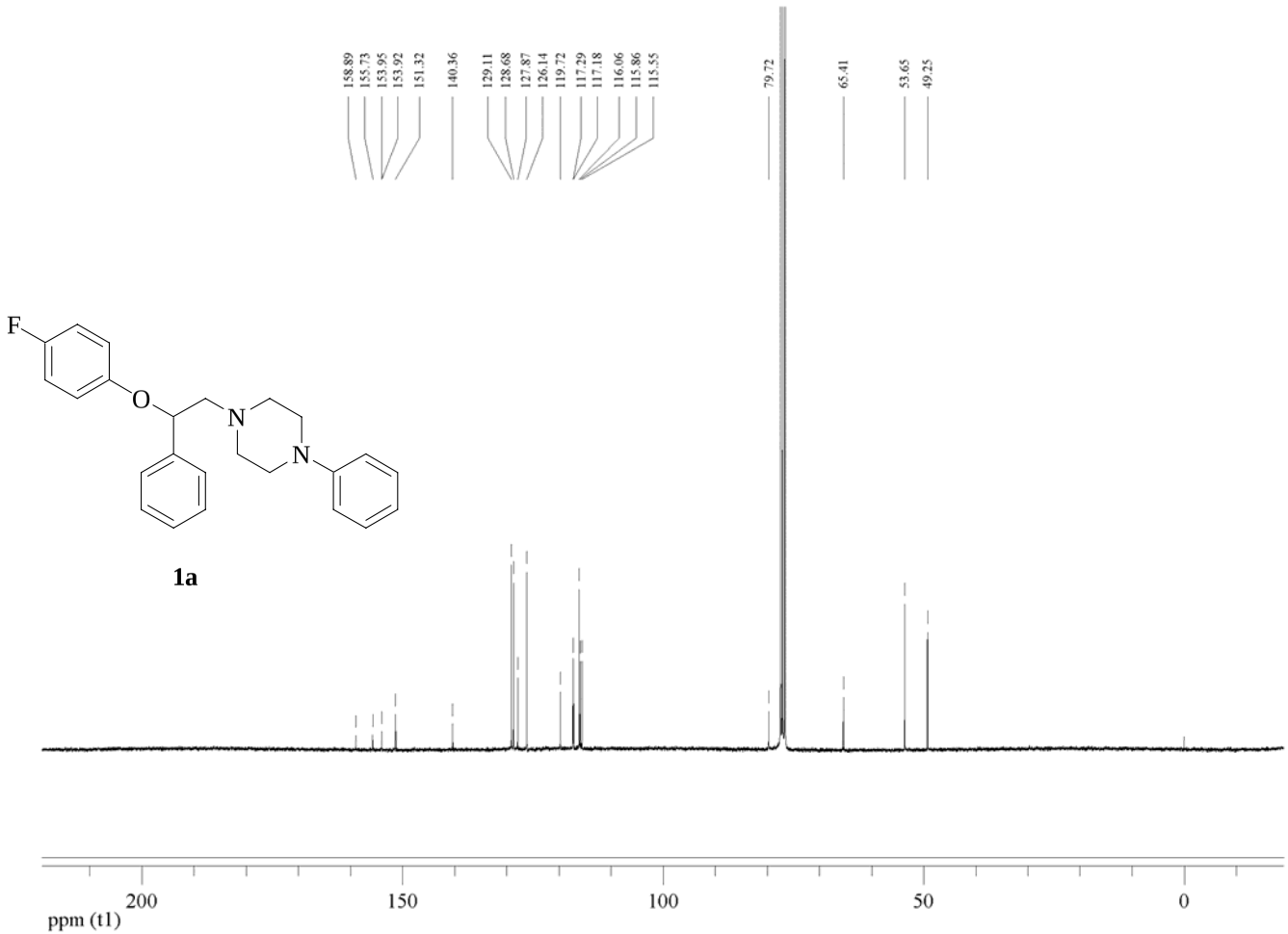
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **44**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **1a**

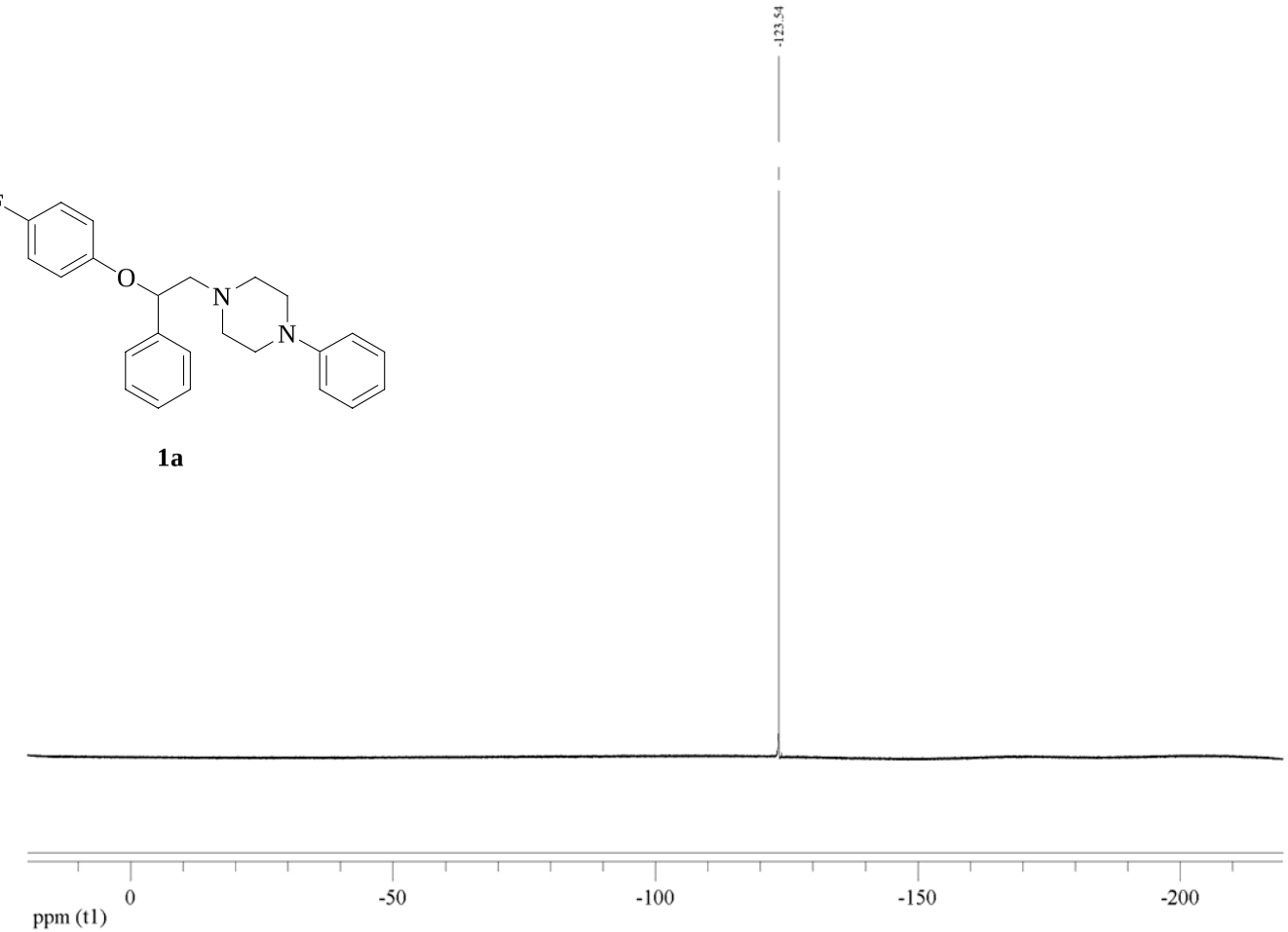


^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **1a**

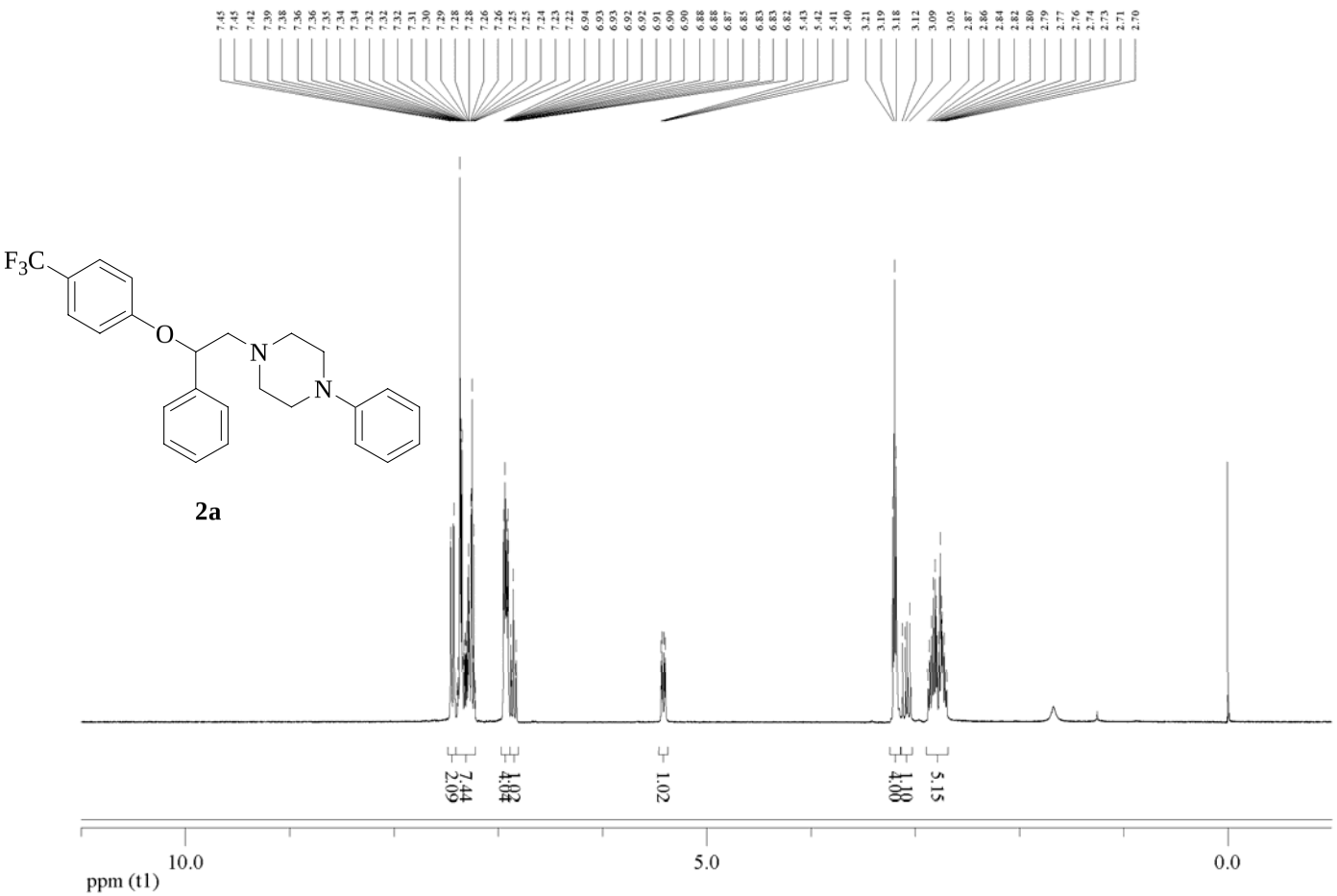


^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **1a**

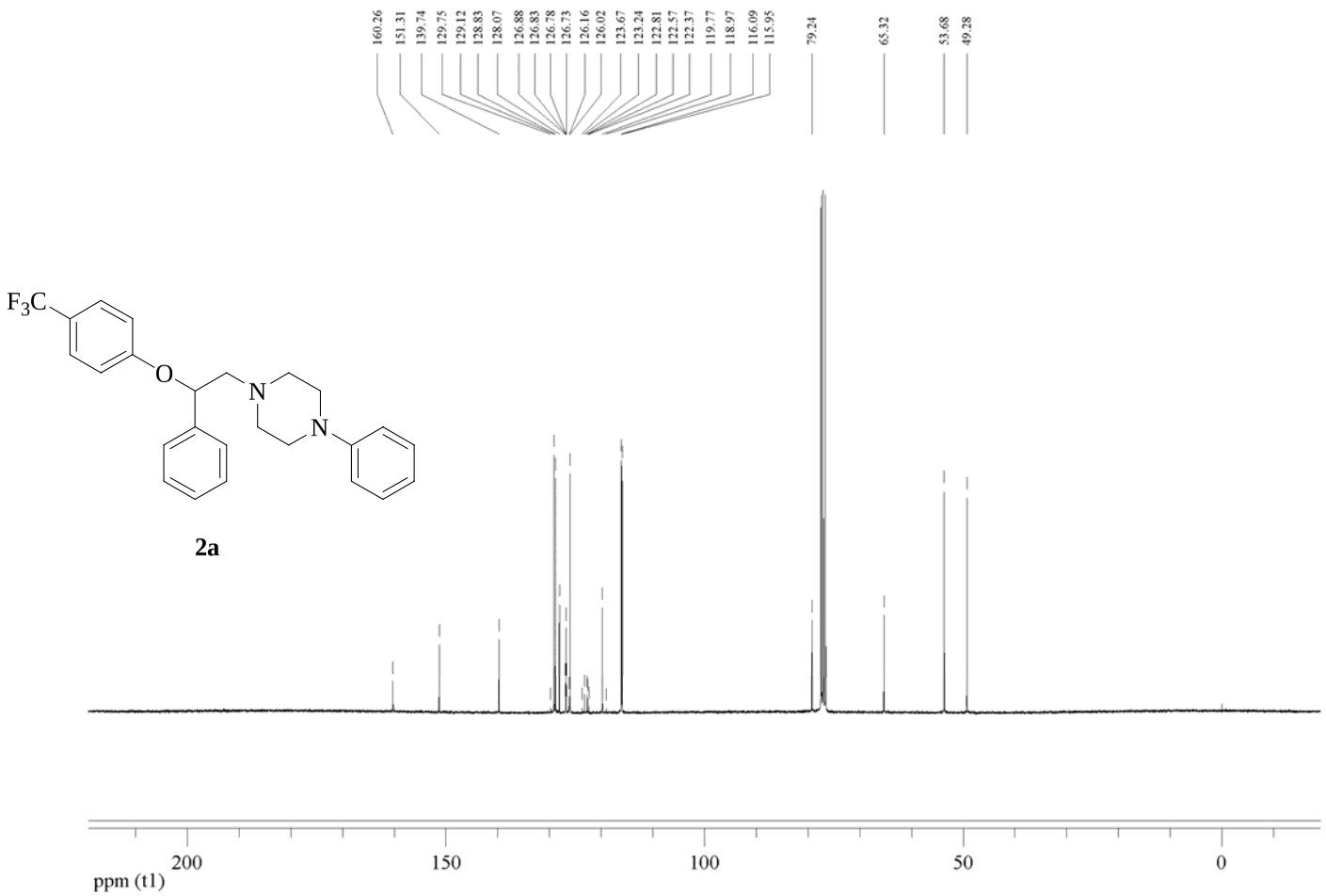
1a



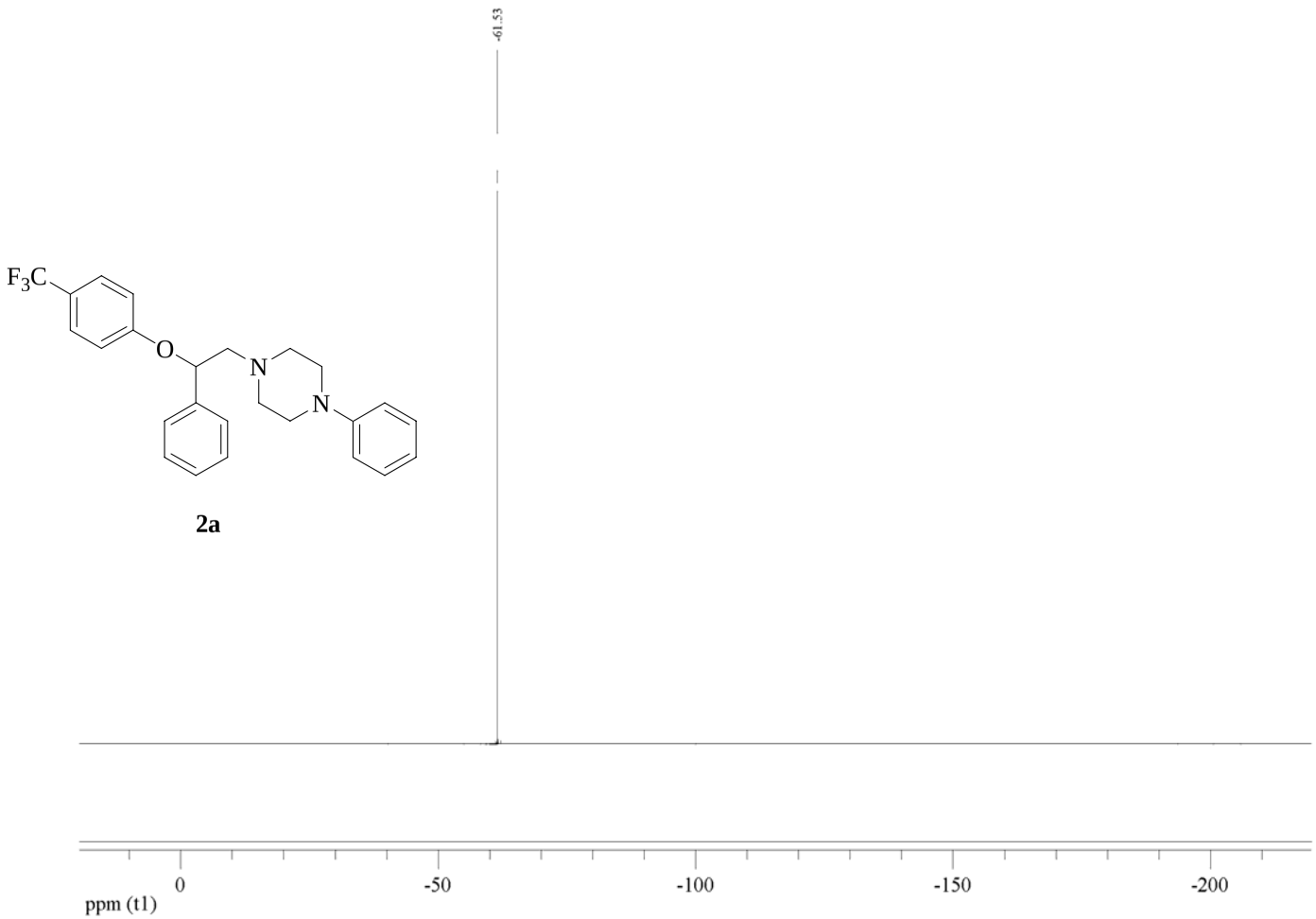
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **2a**



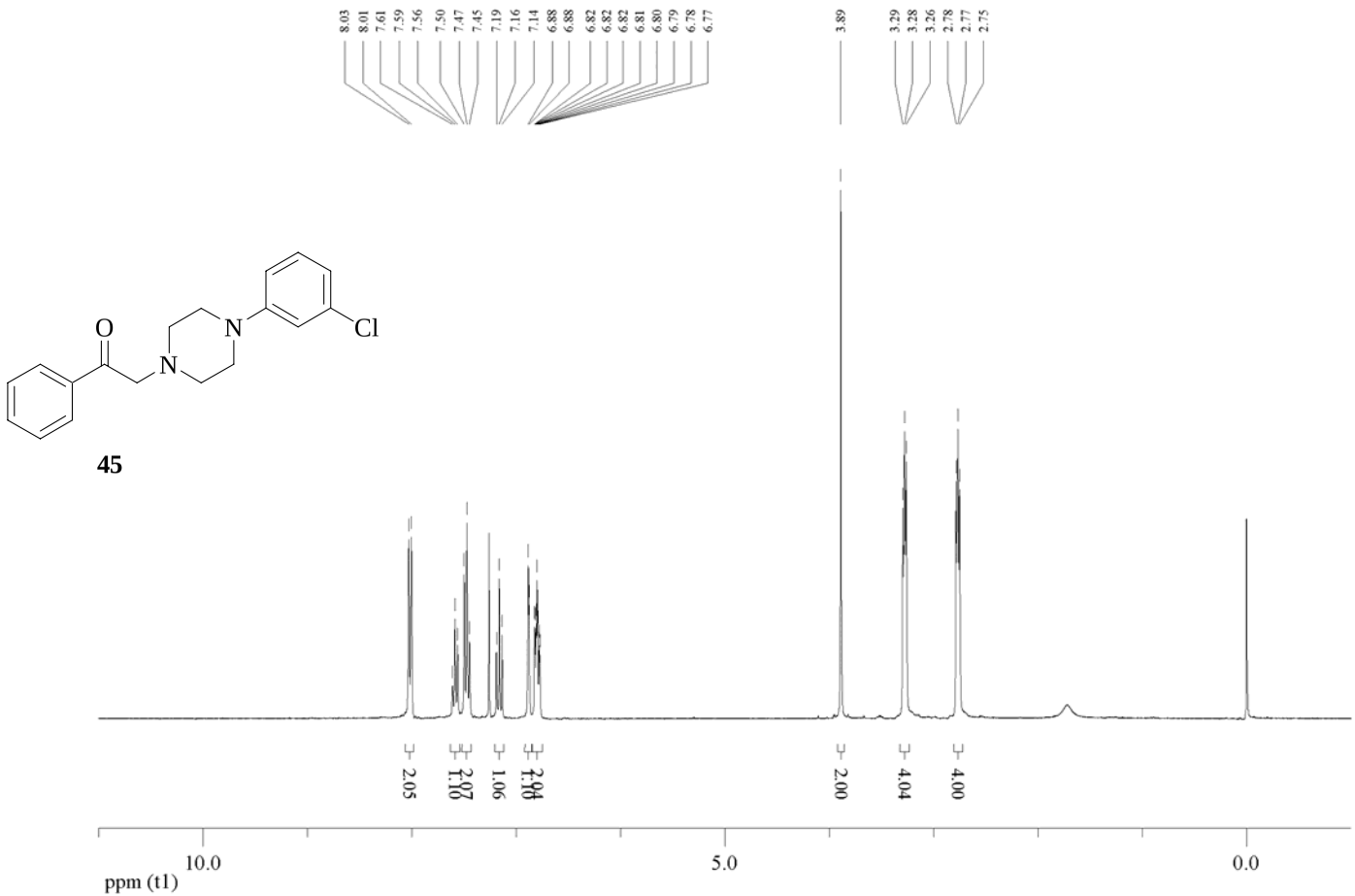
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **2a**



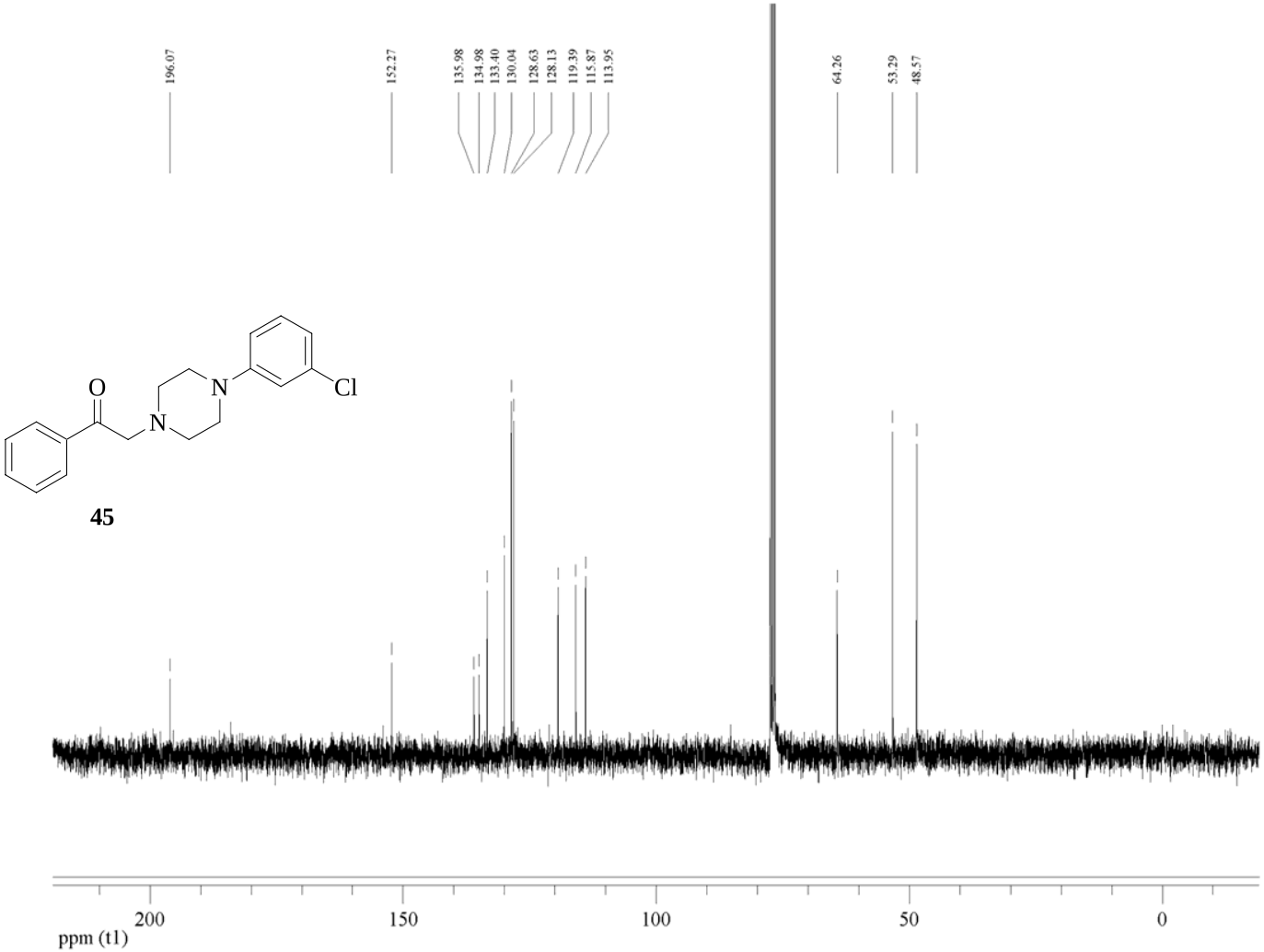
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **2a**



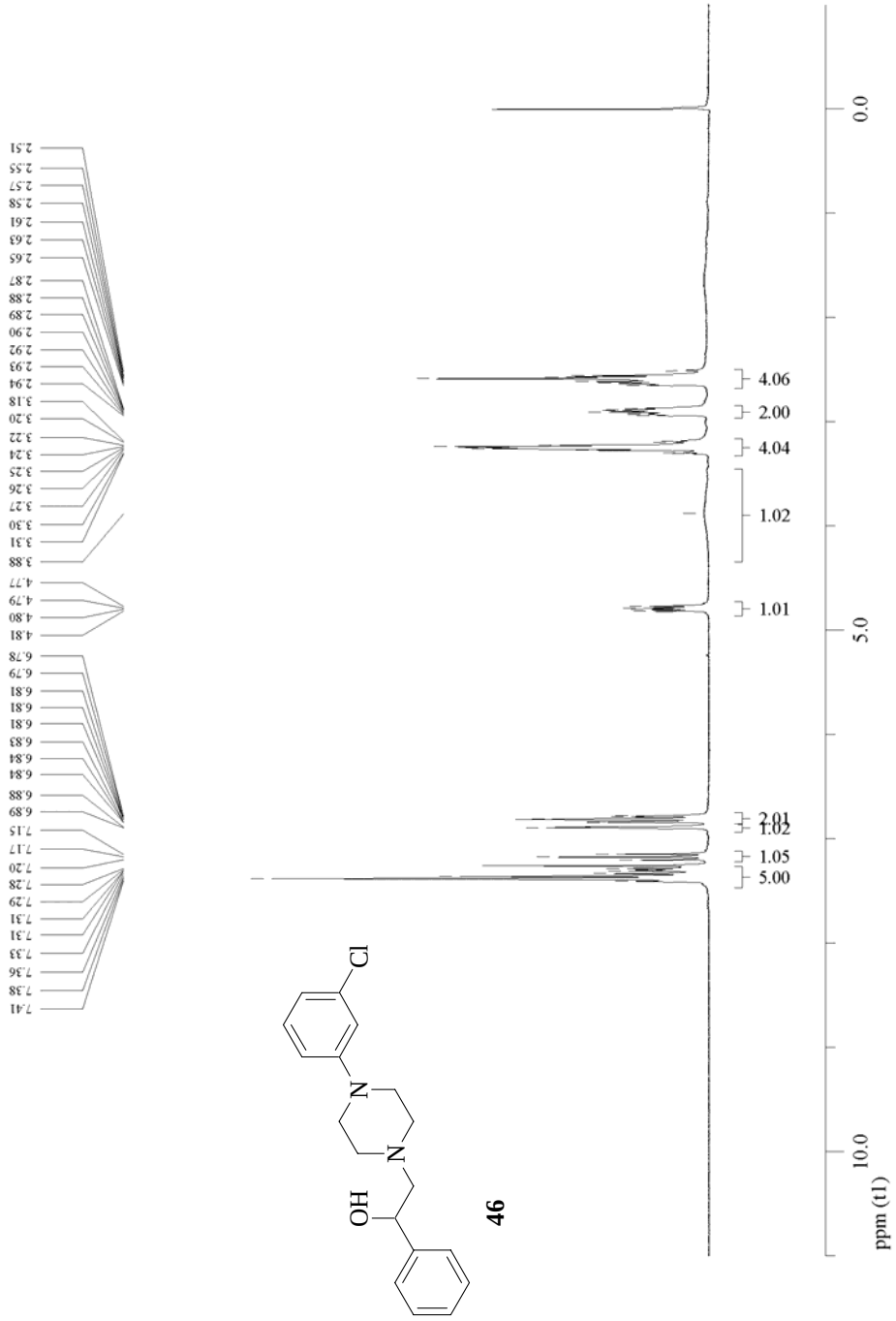
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **45**



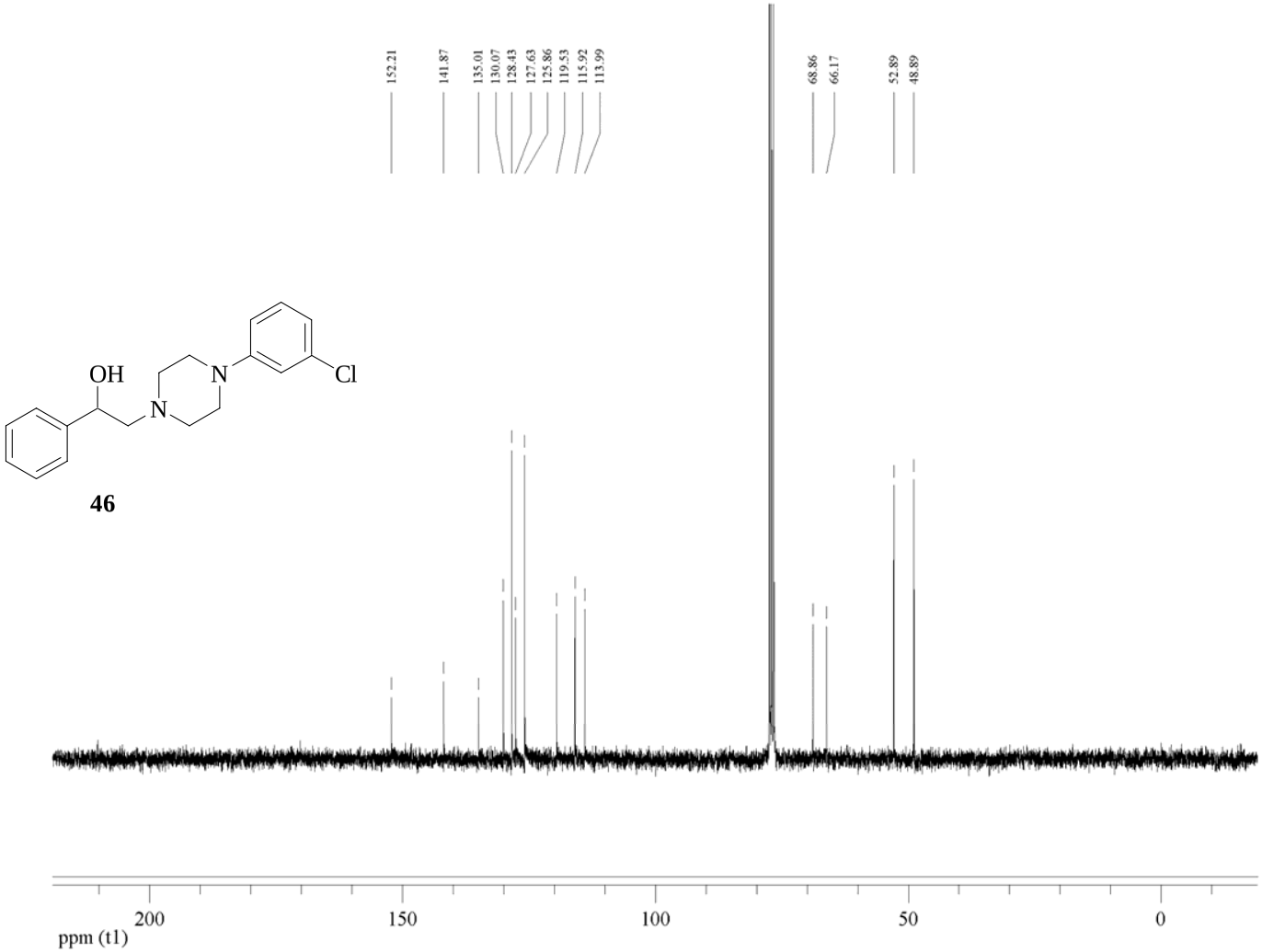
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **45**



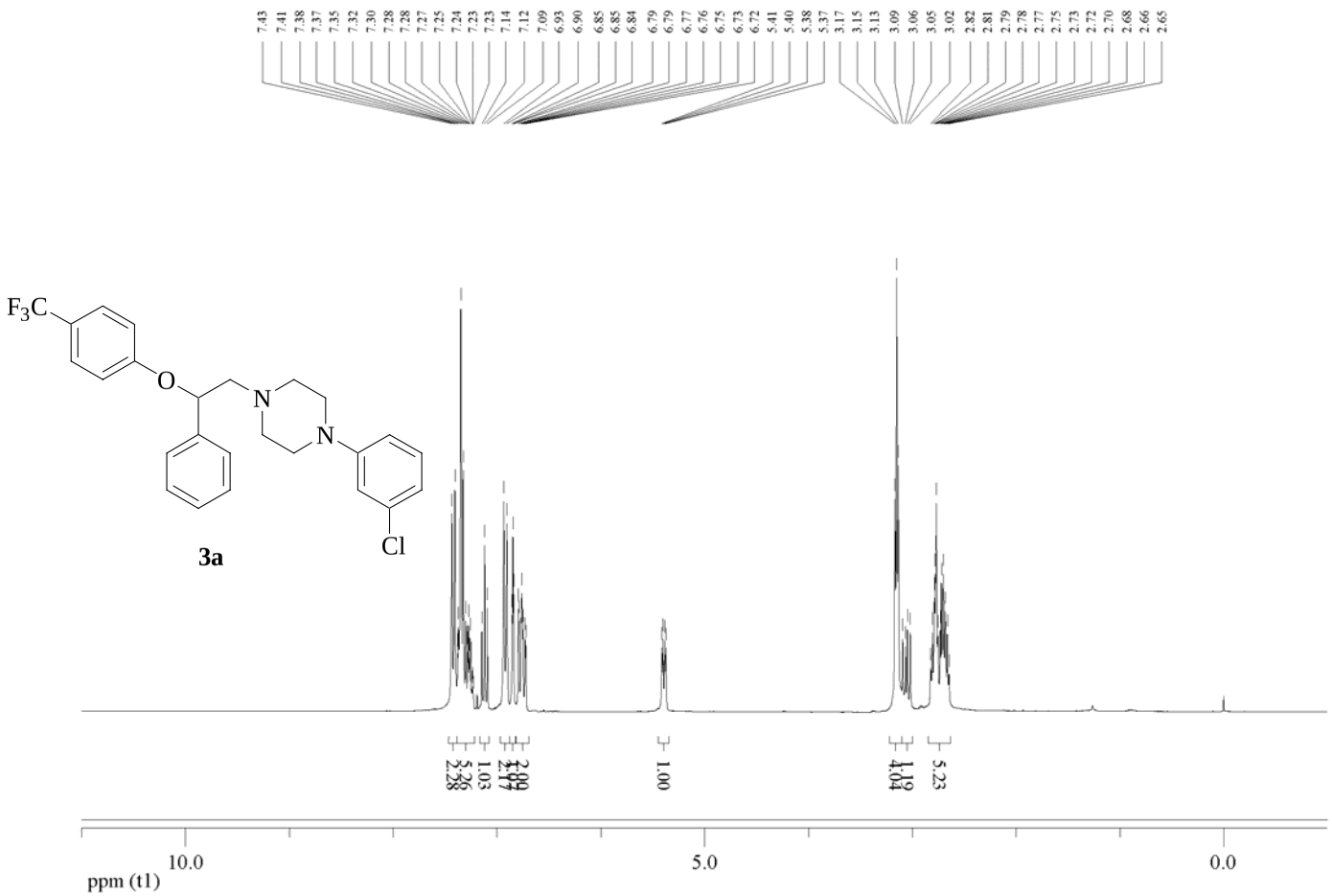
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **46**



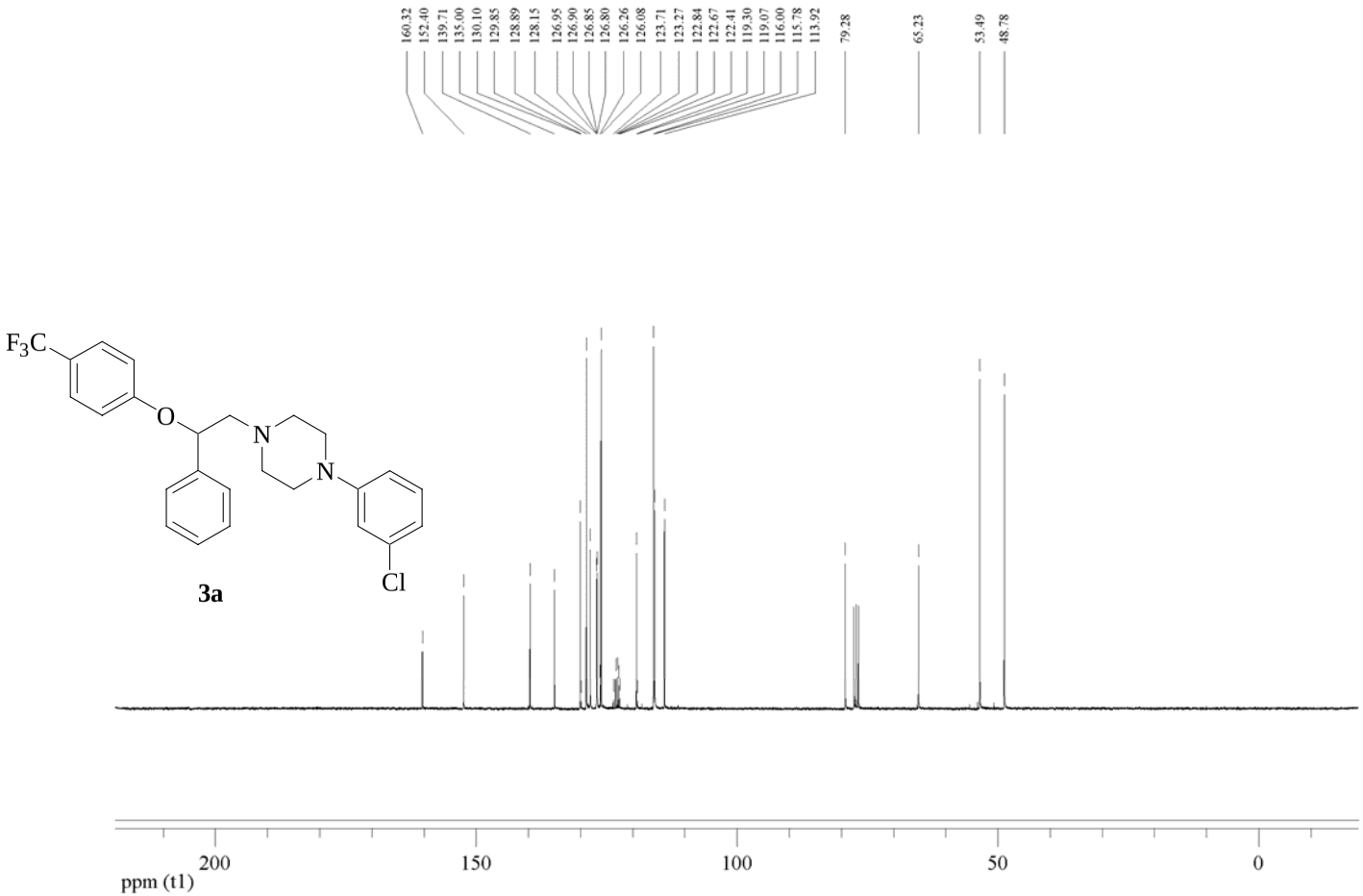
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **46**



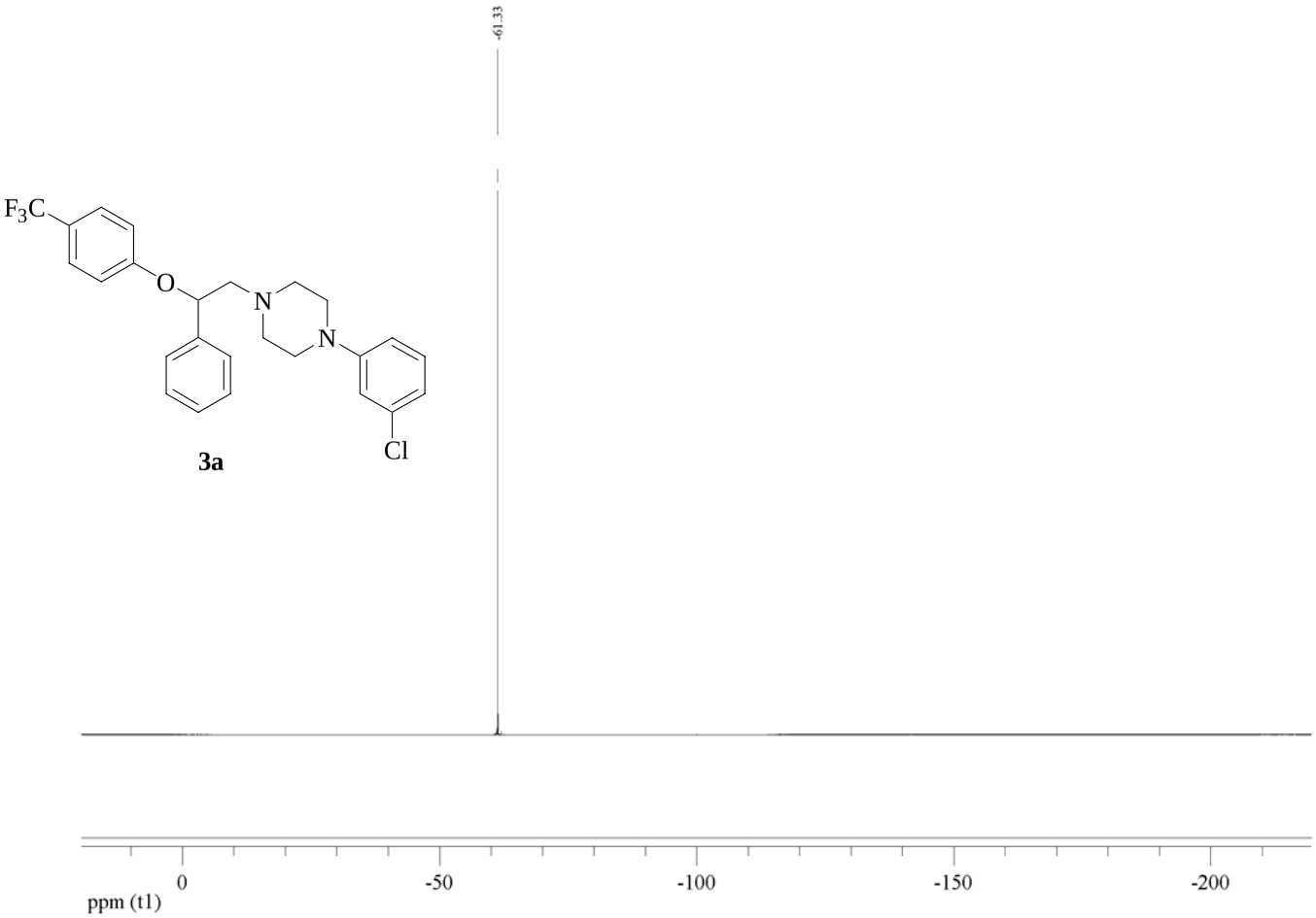
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **3a**



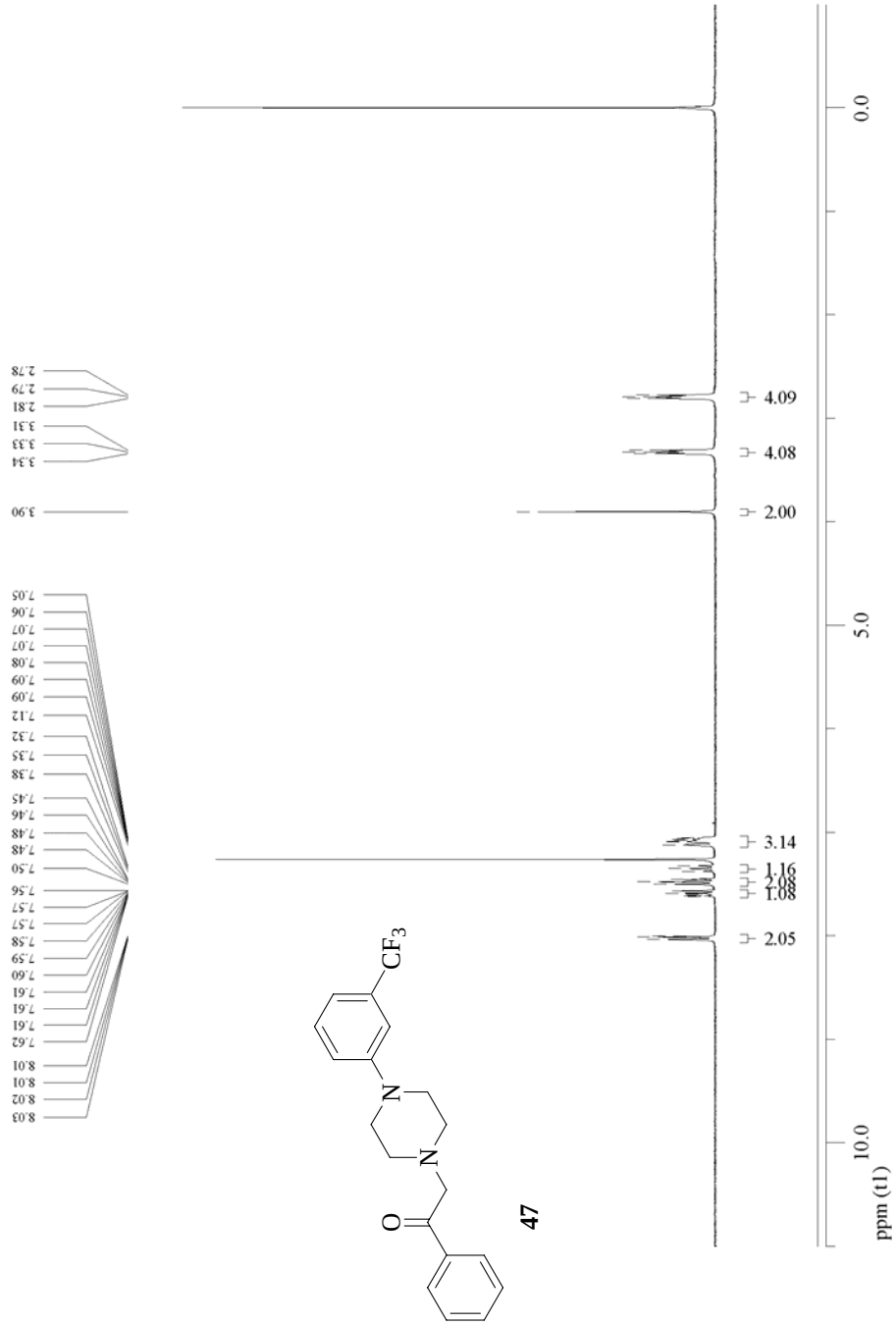
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **3a**



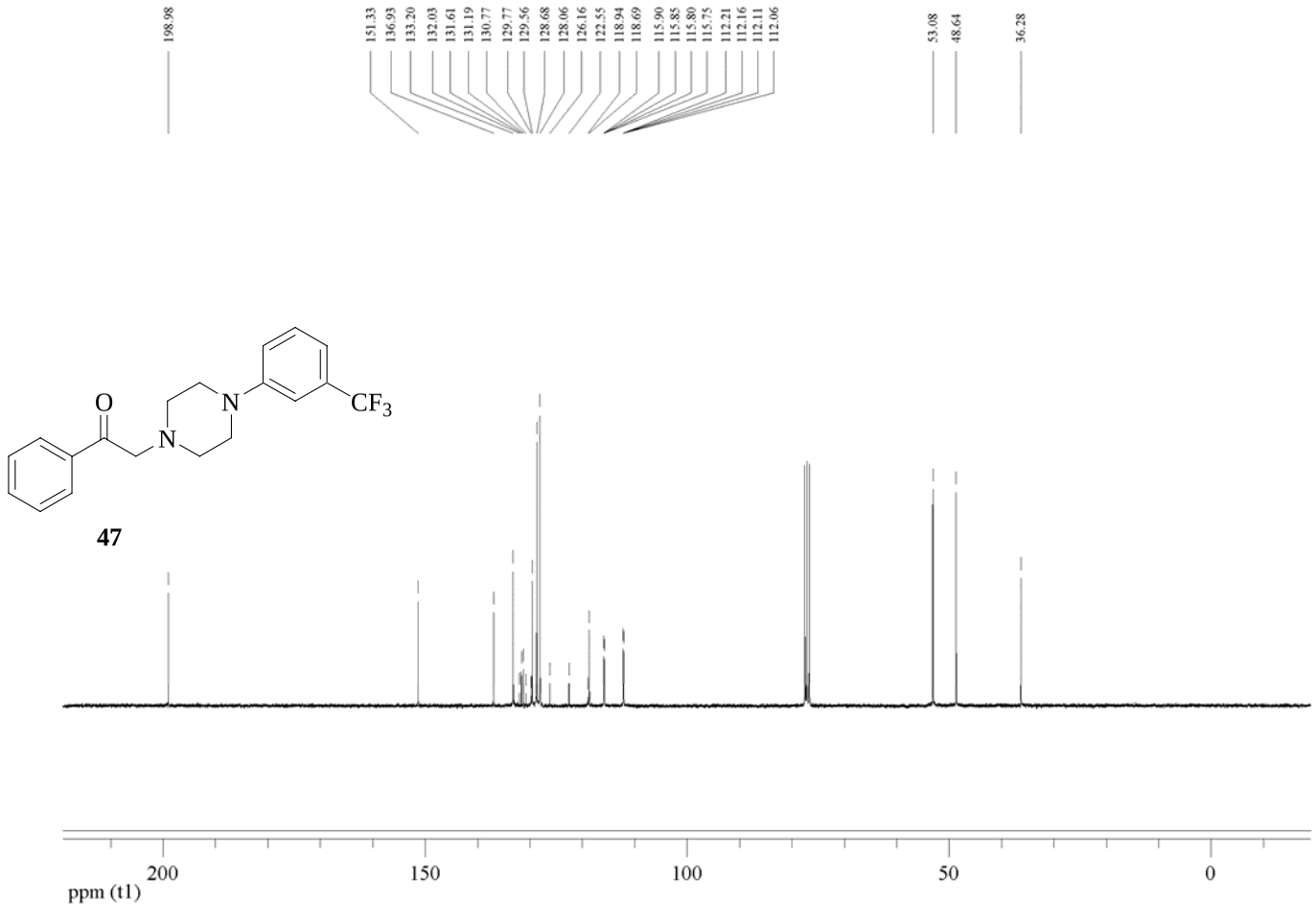
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **3a**



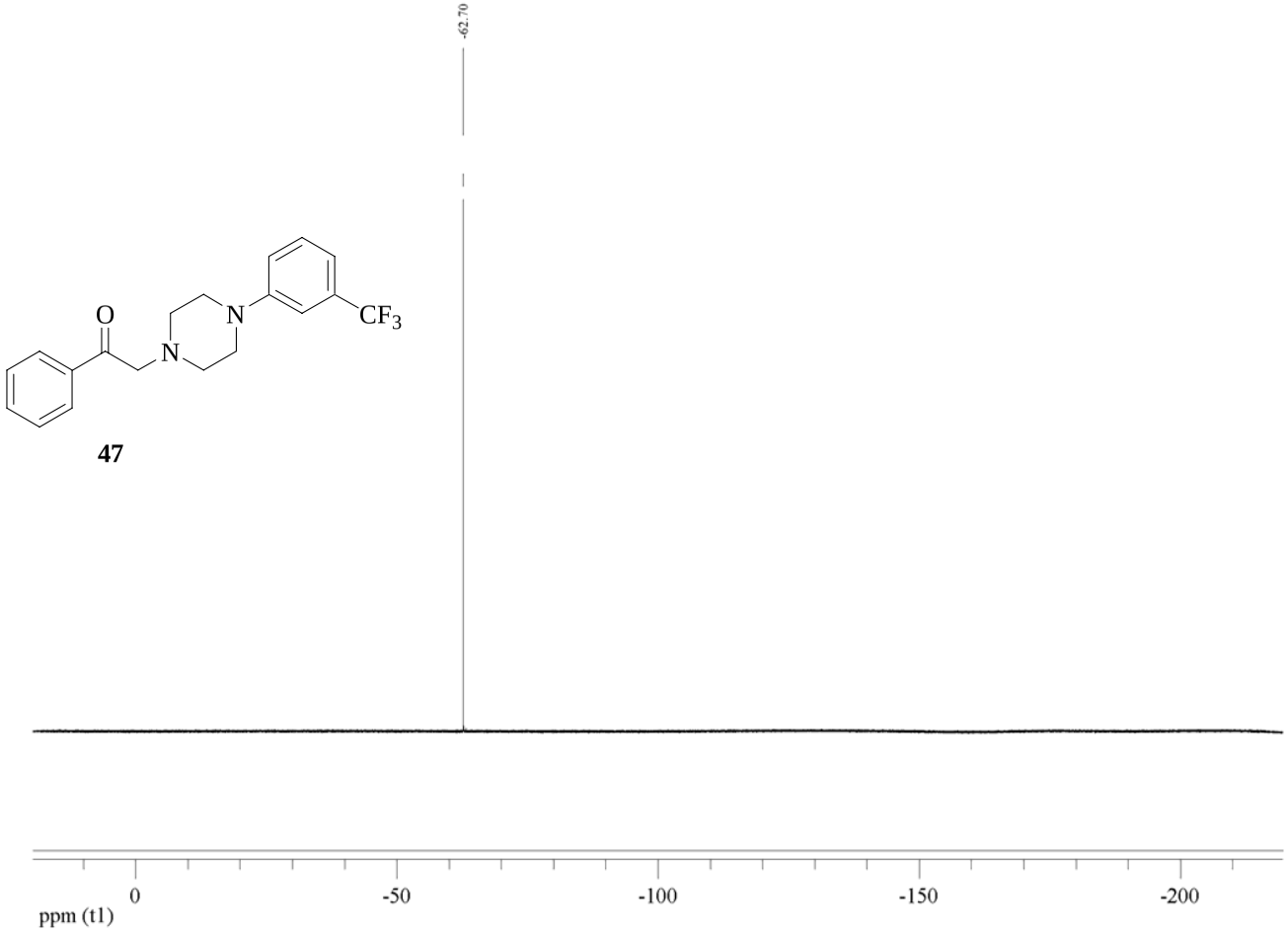
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **47**



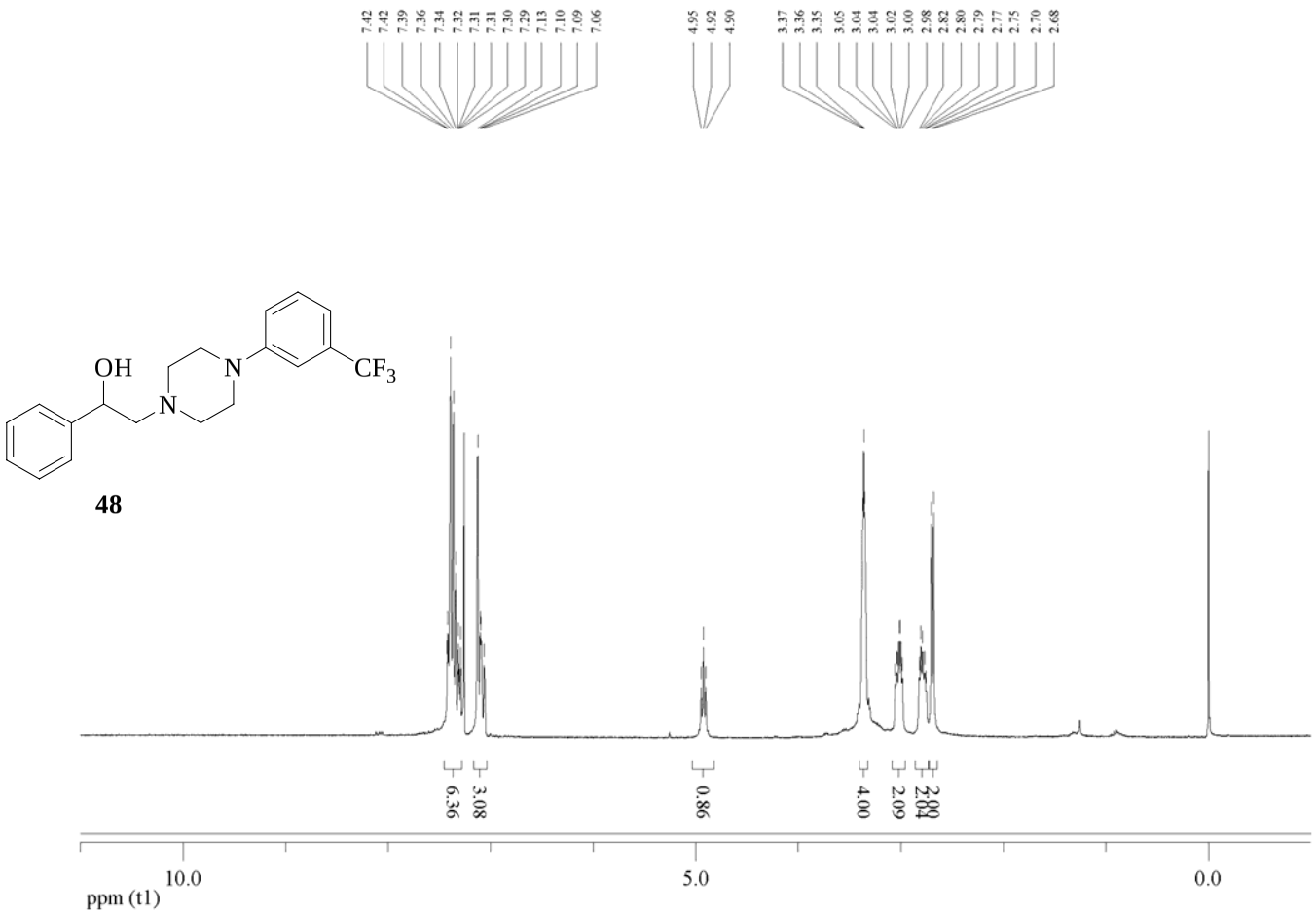
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **47**



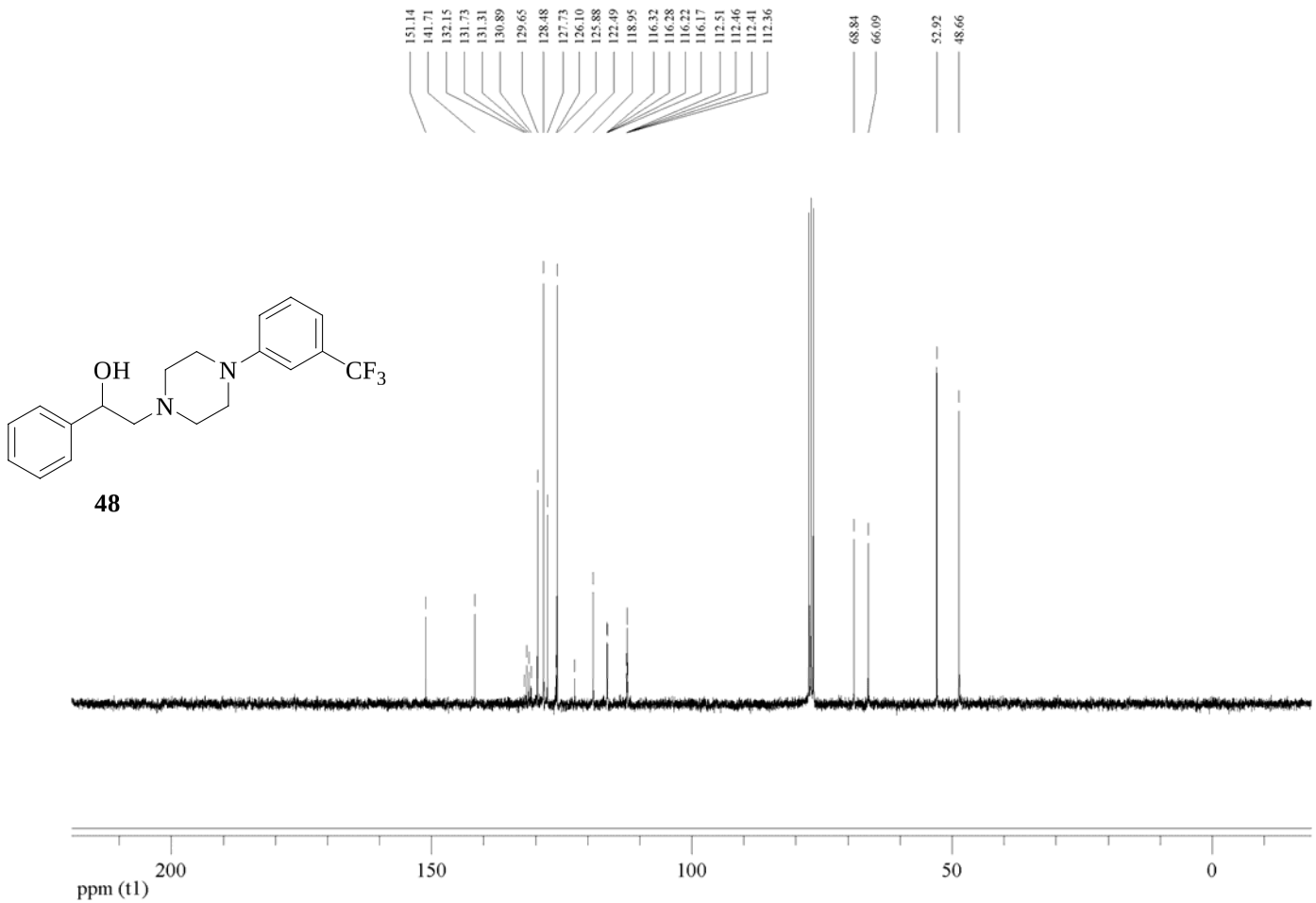
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **47**



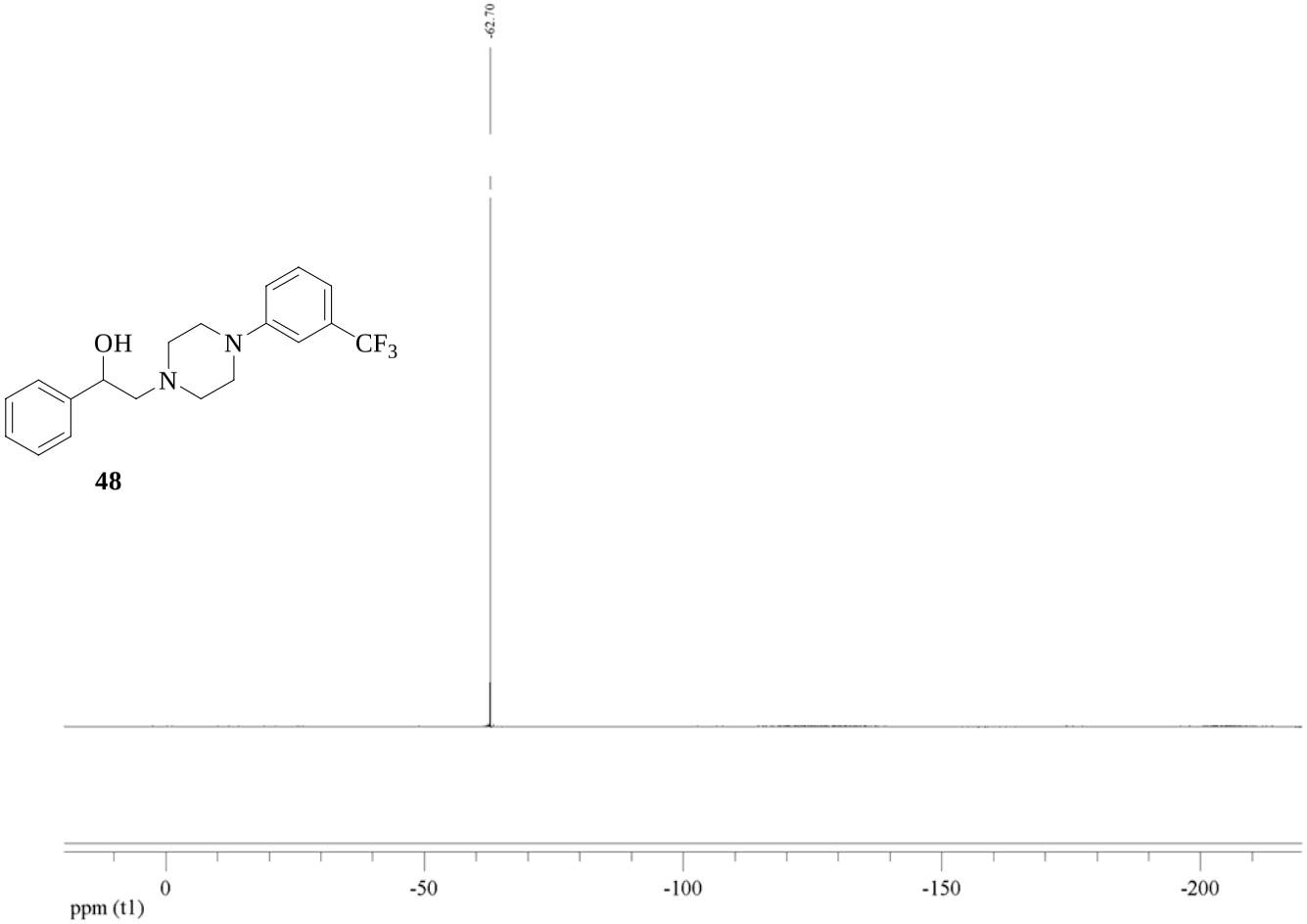
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **48**



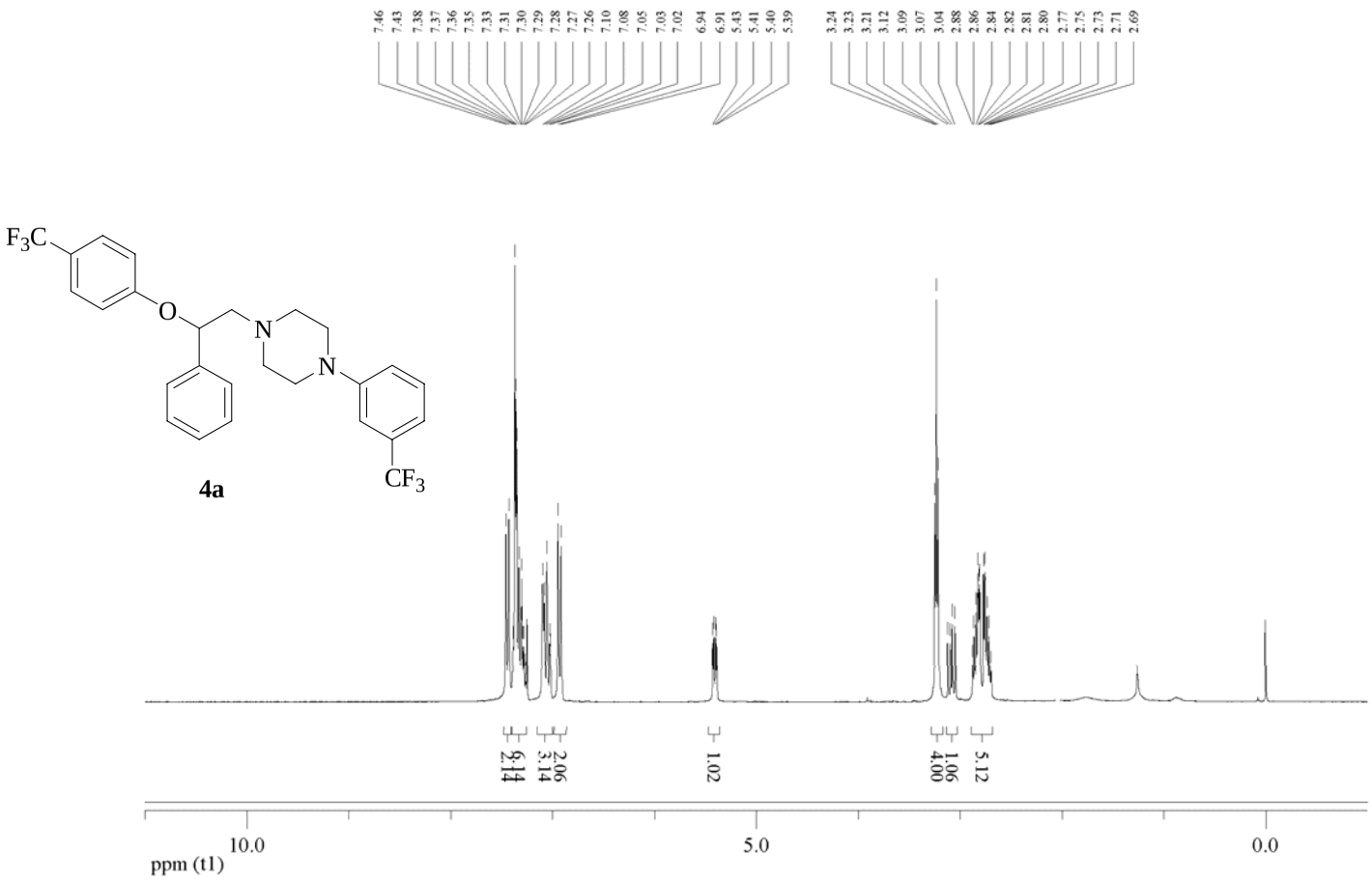
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **48**



^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **48**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **4a**



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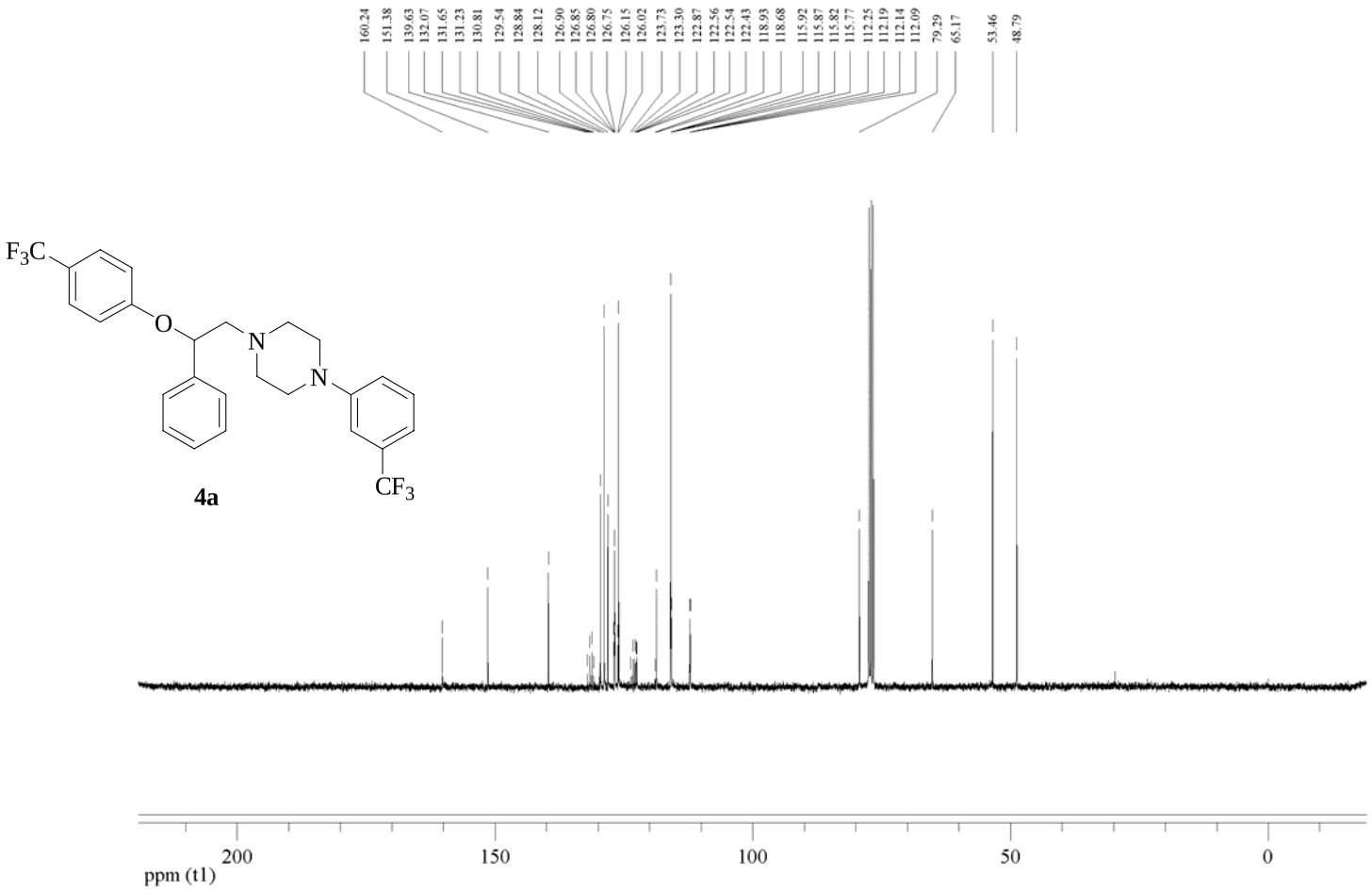
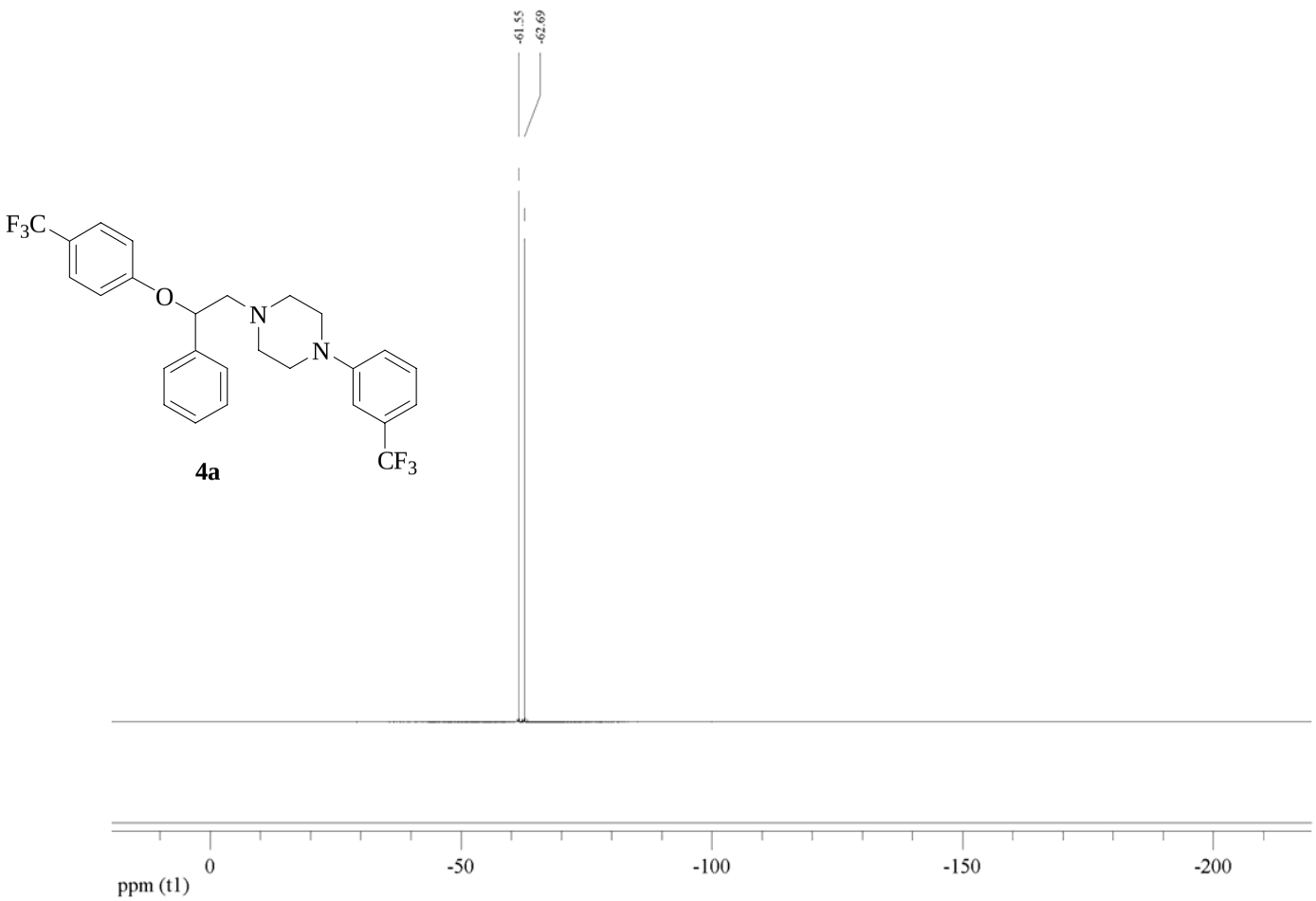
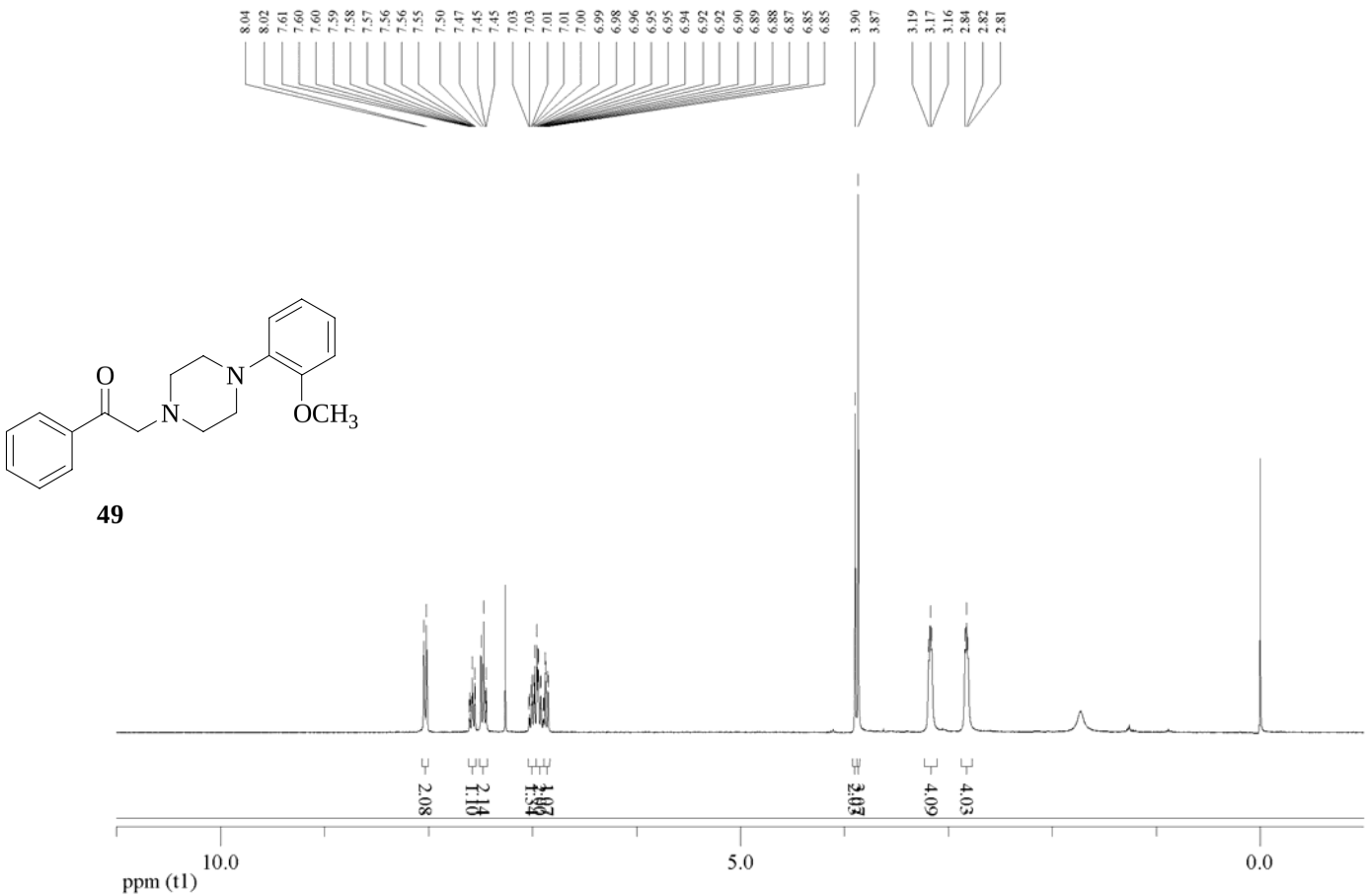


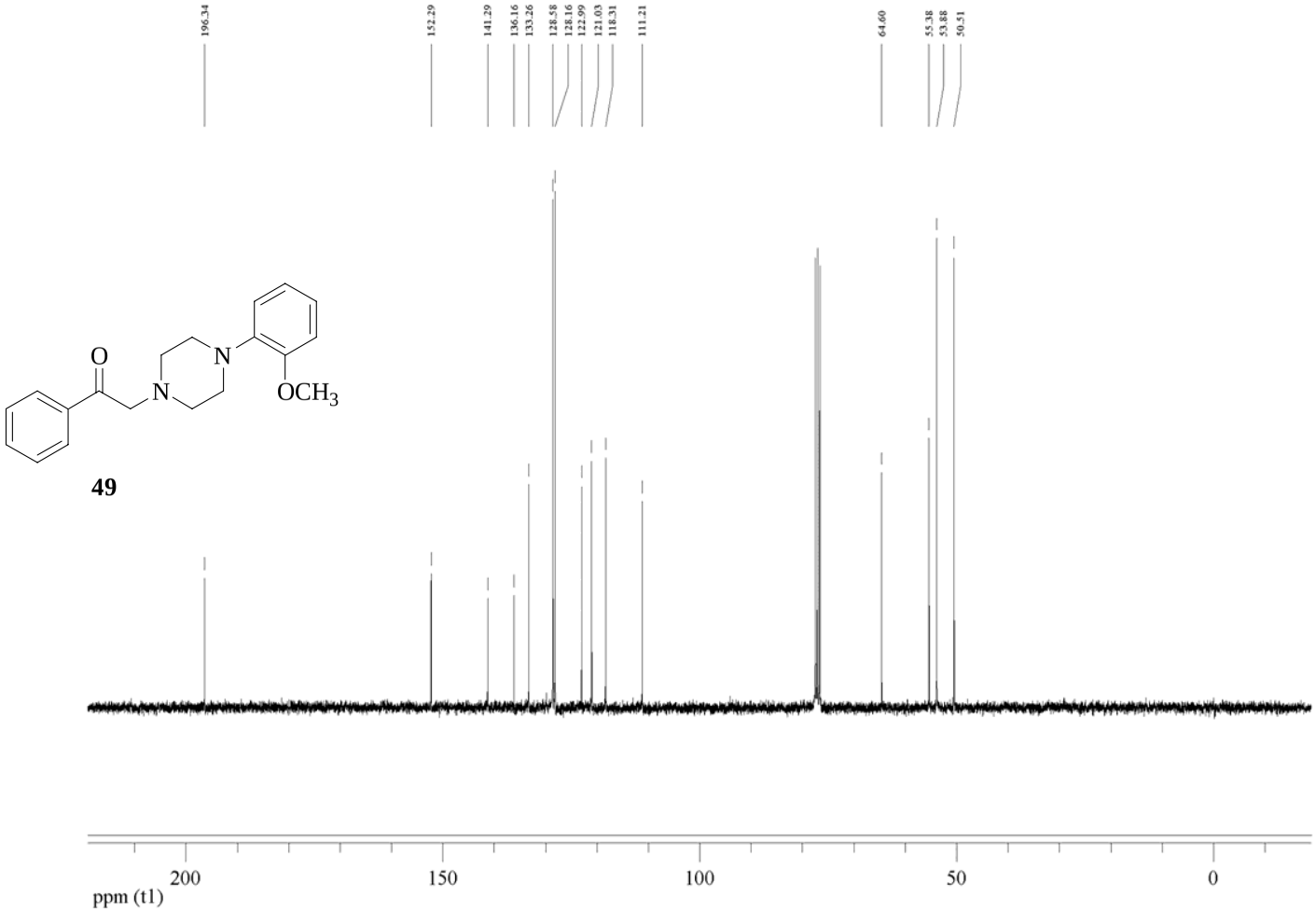
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **4a**



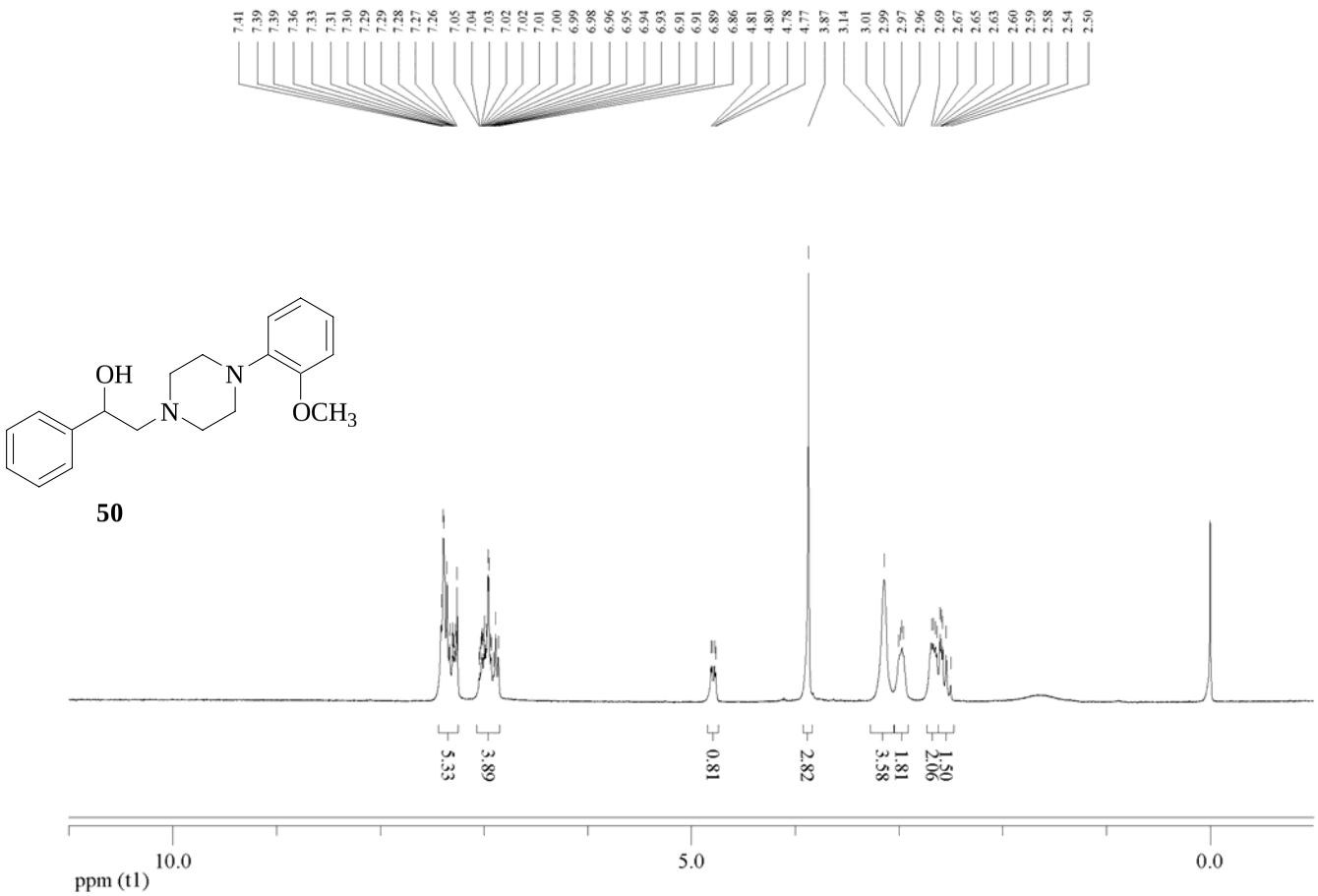
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **49**



^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **49**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **50**



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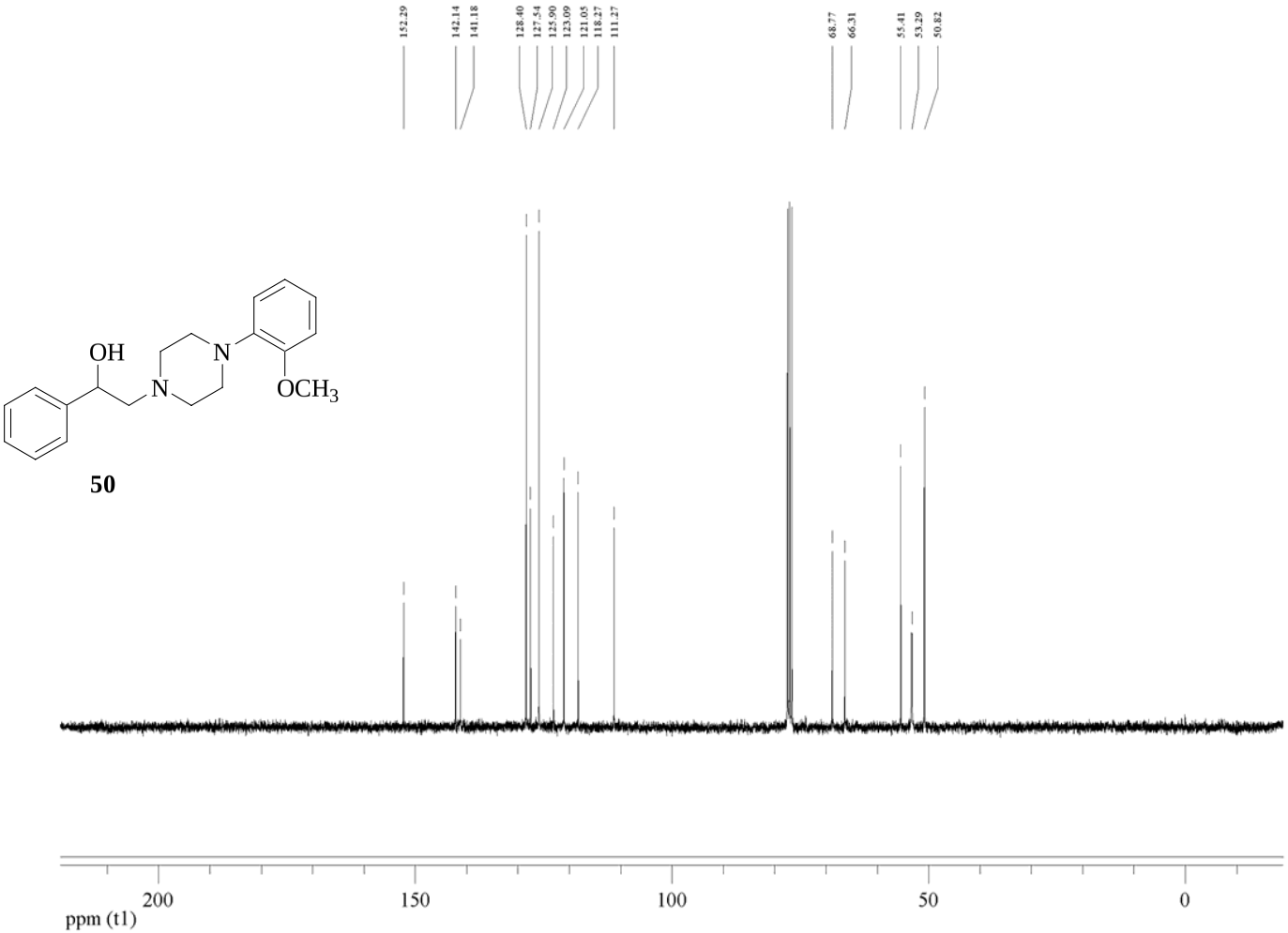
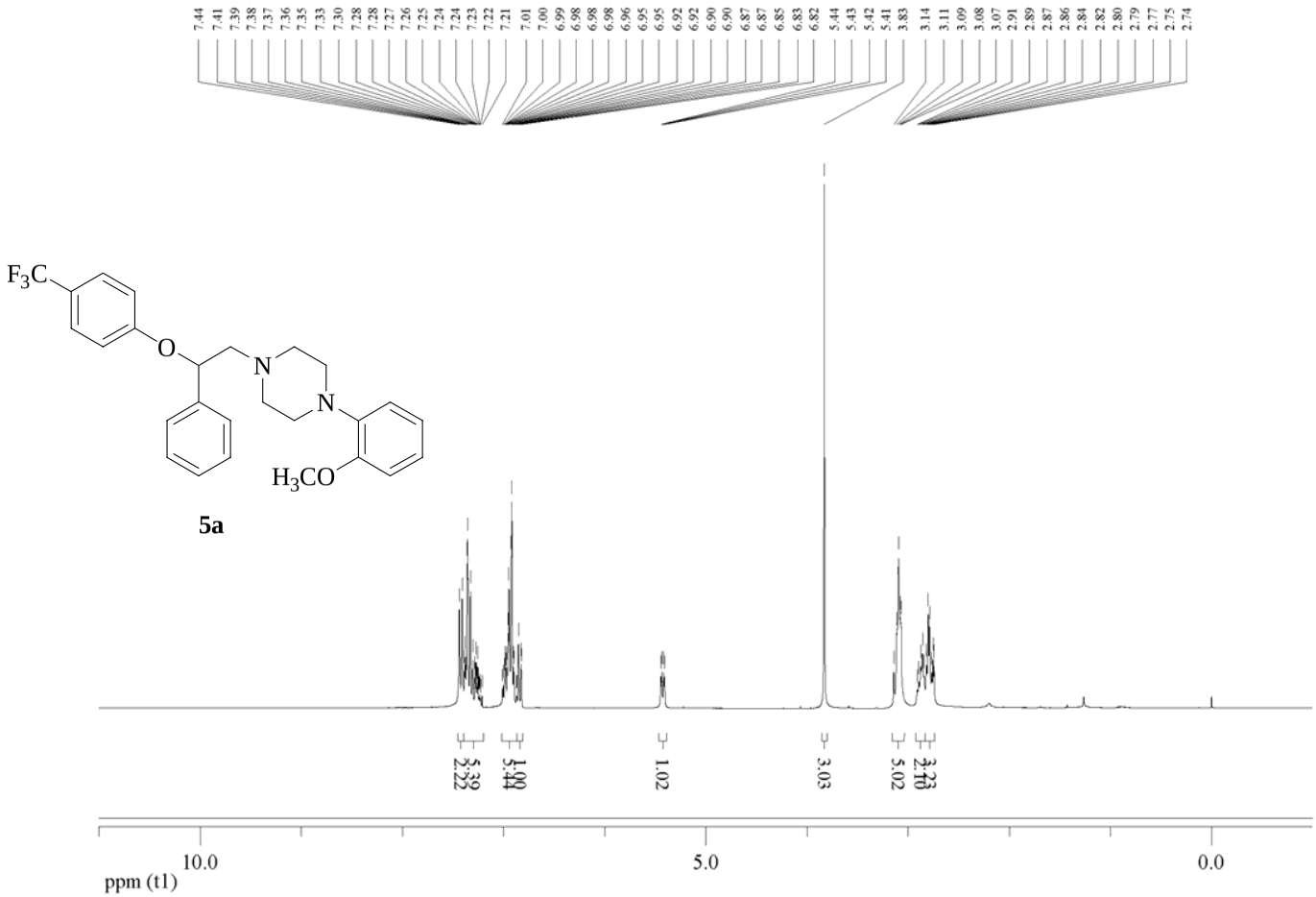
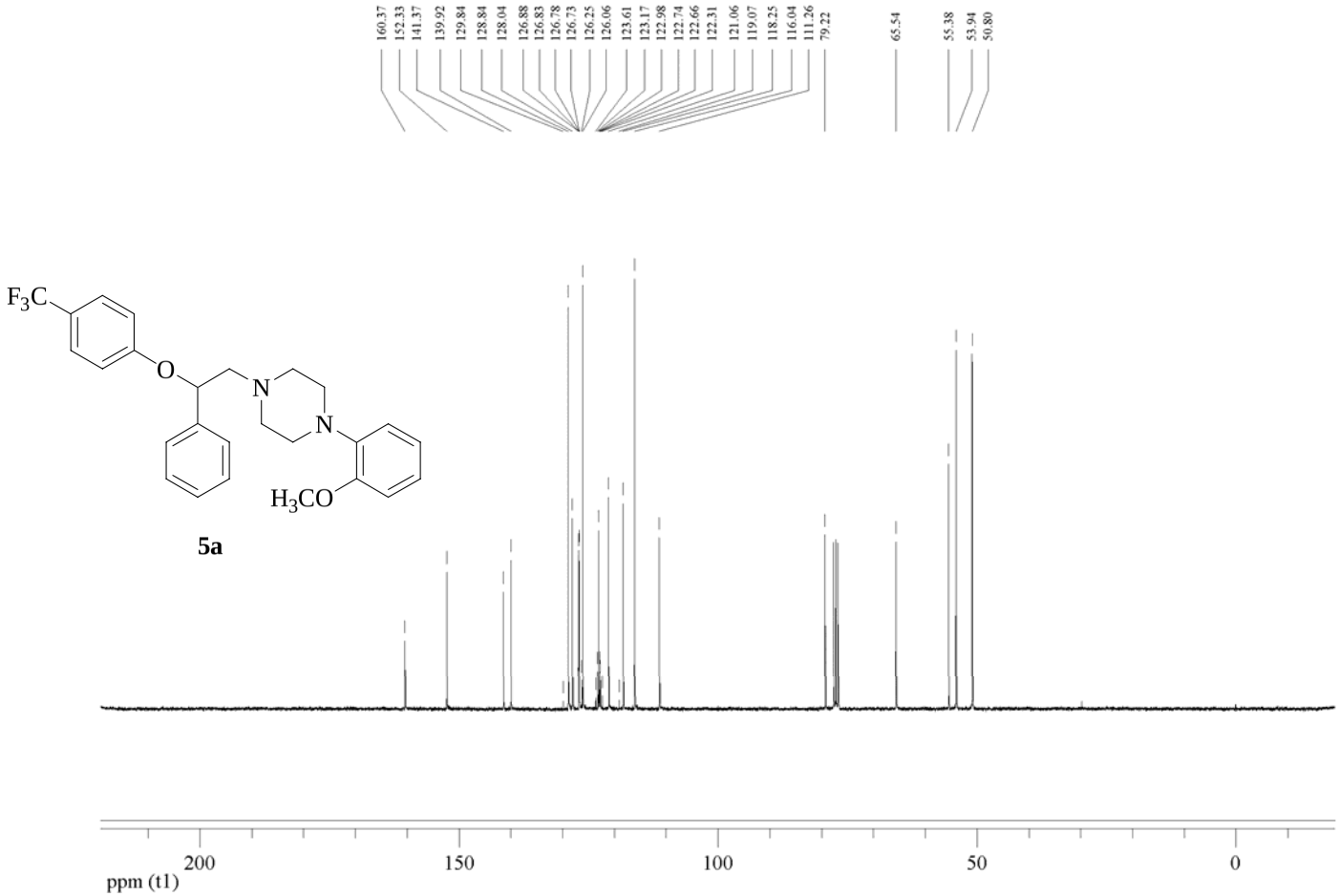


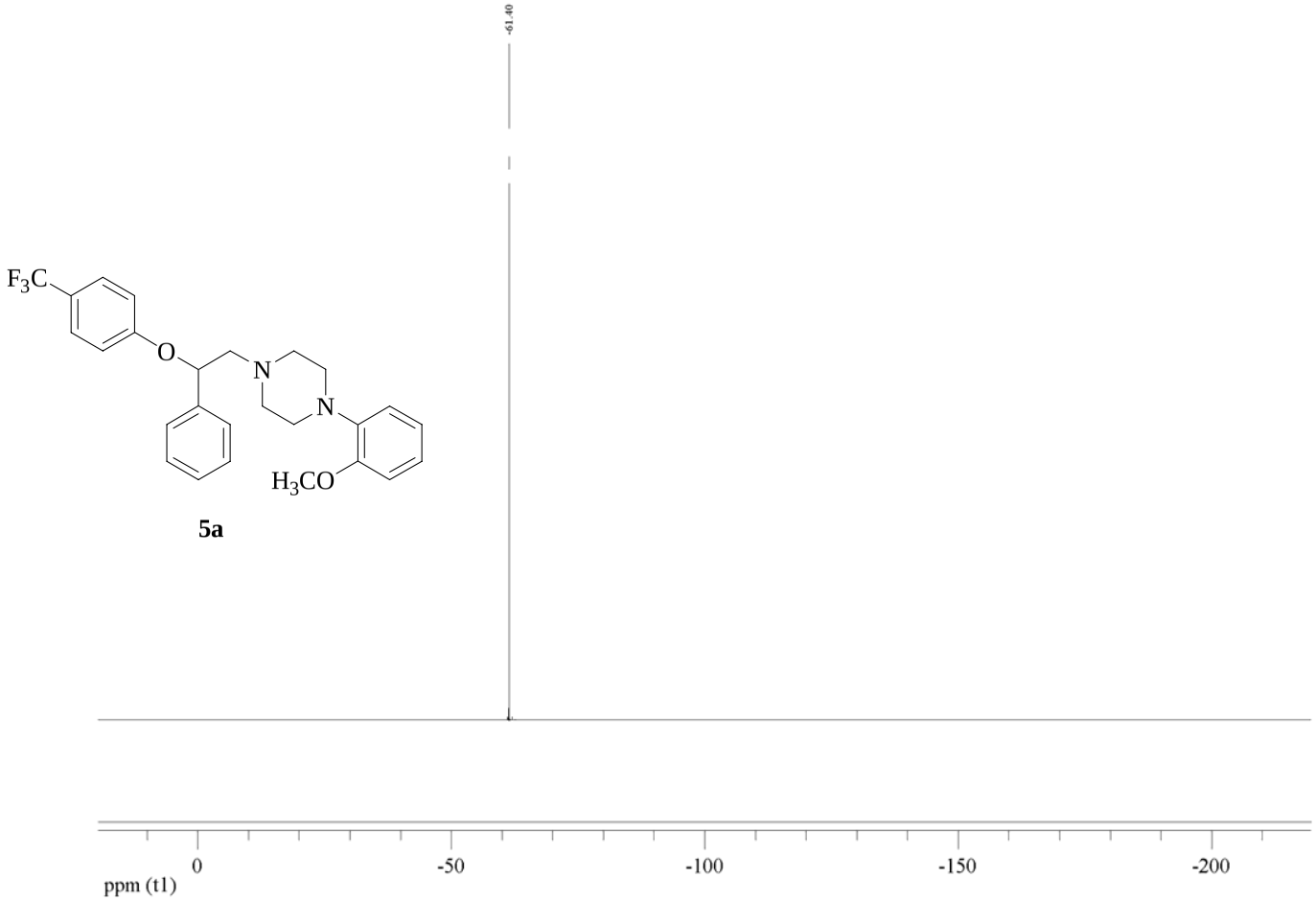
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **5a**



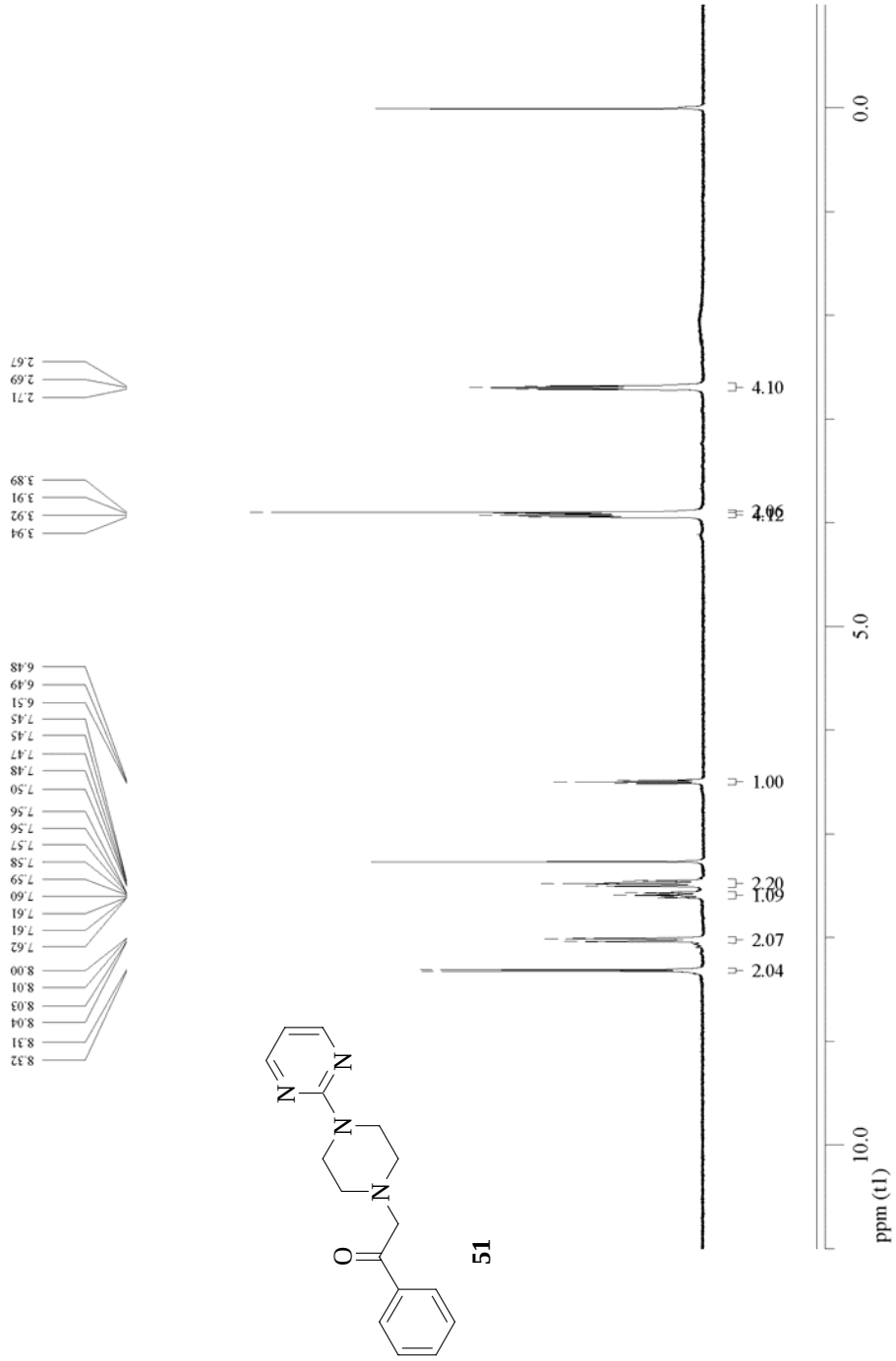
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **5a**



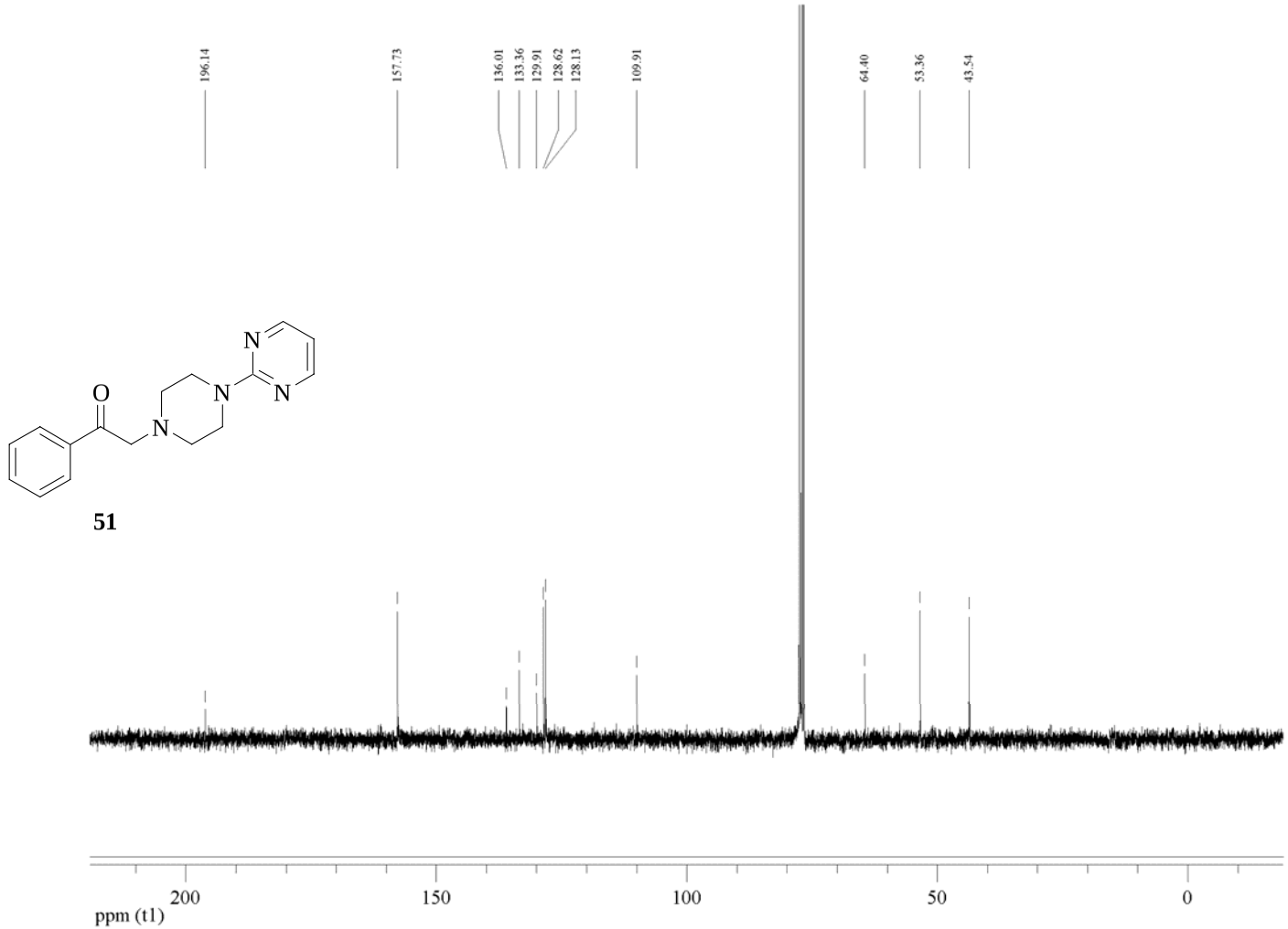
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **5a**



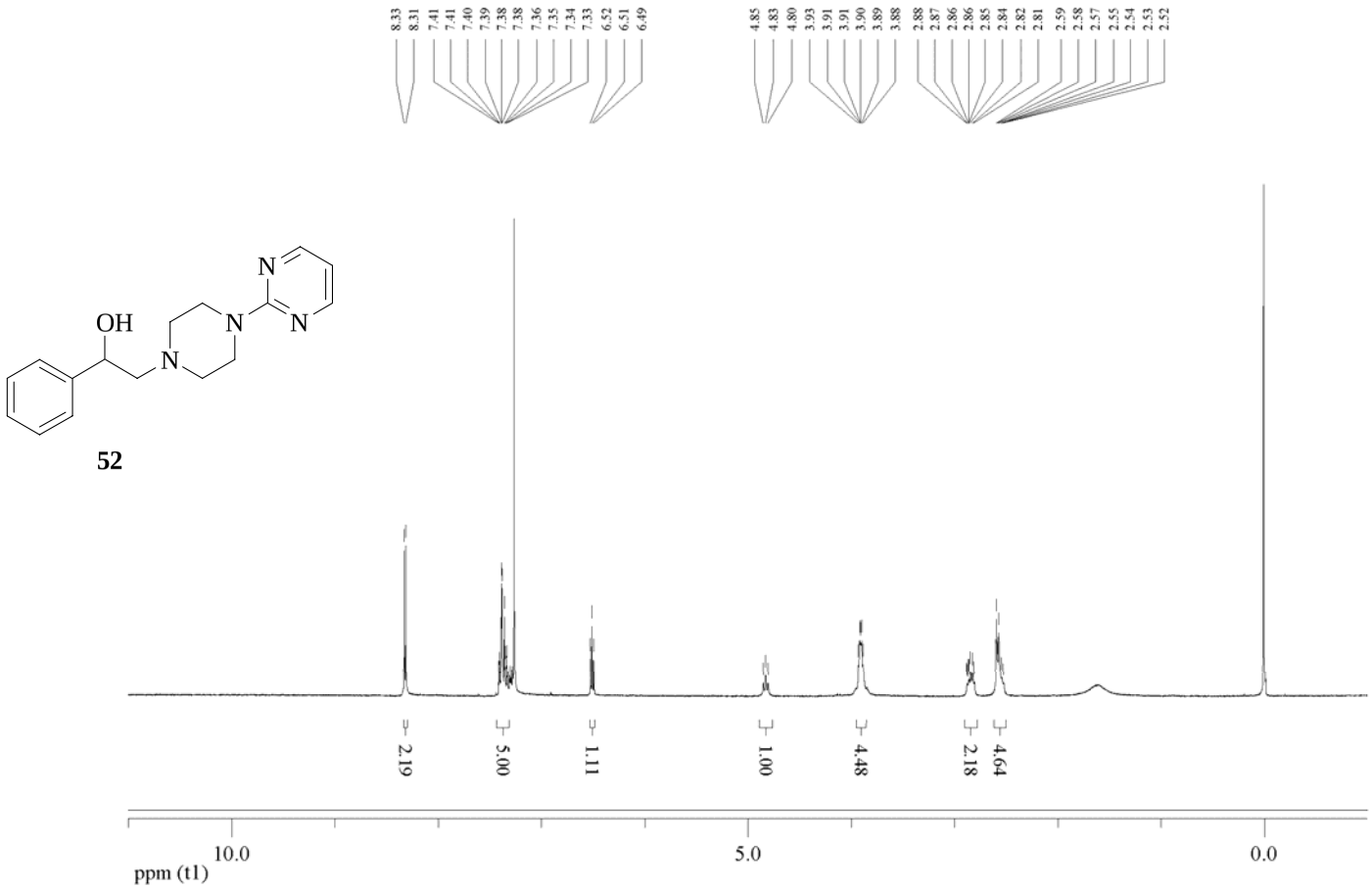
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **51**



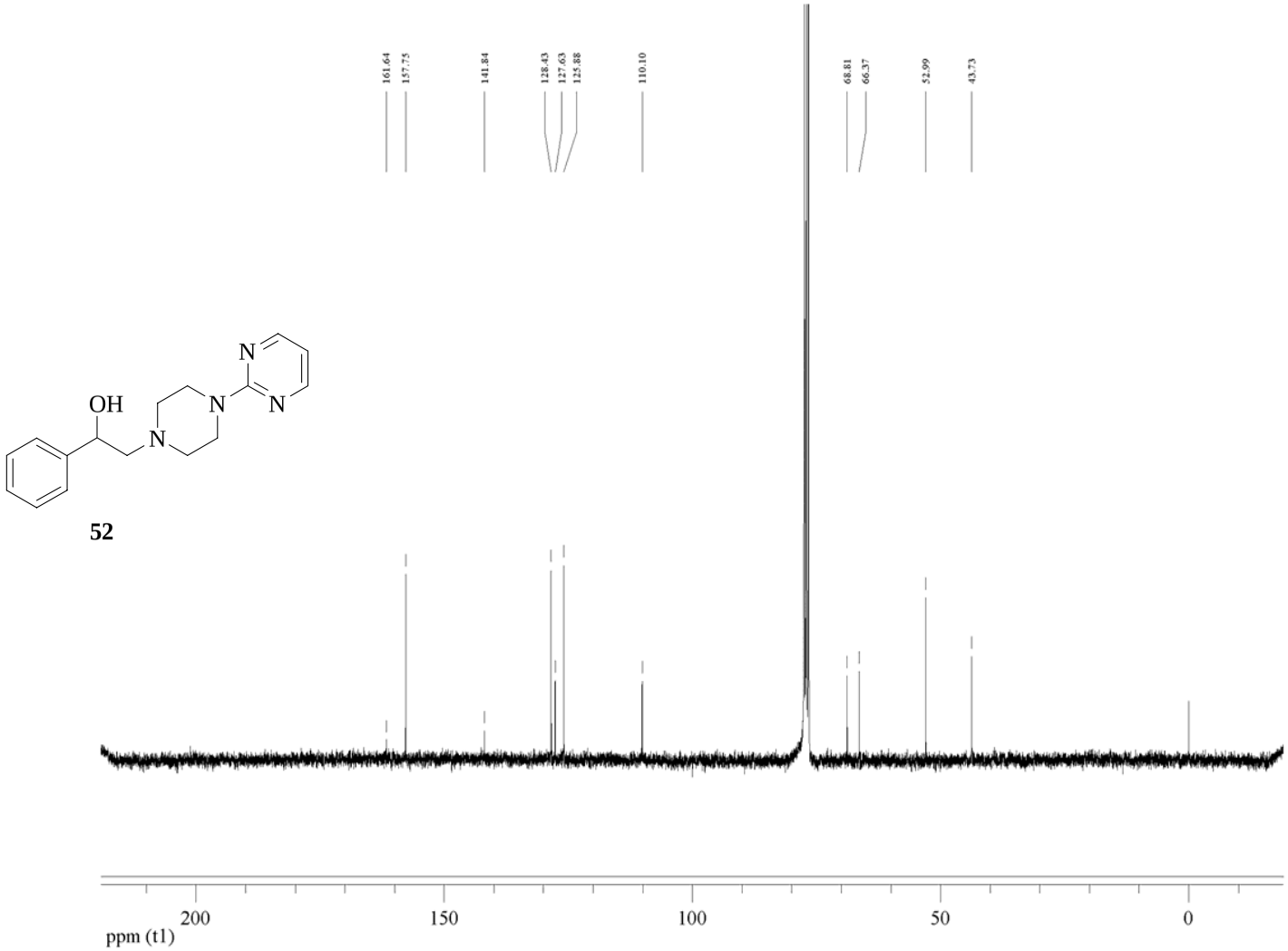
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **51**



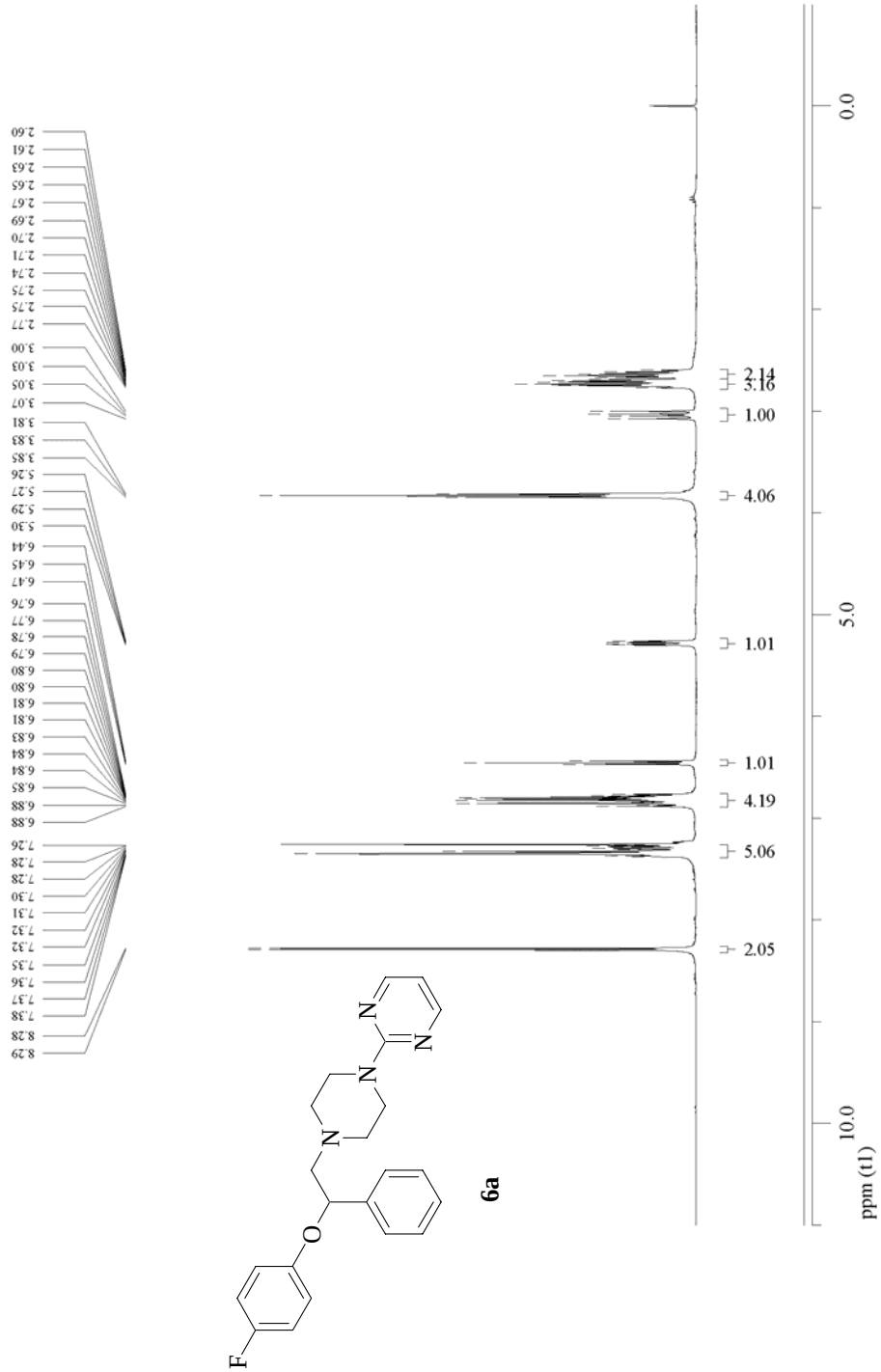
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **52**



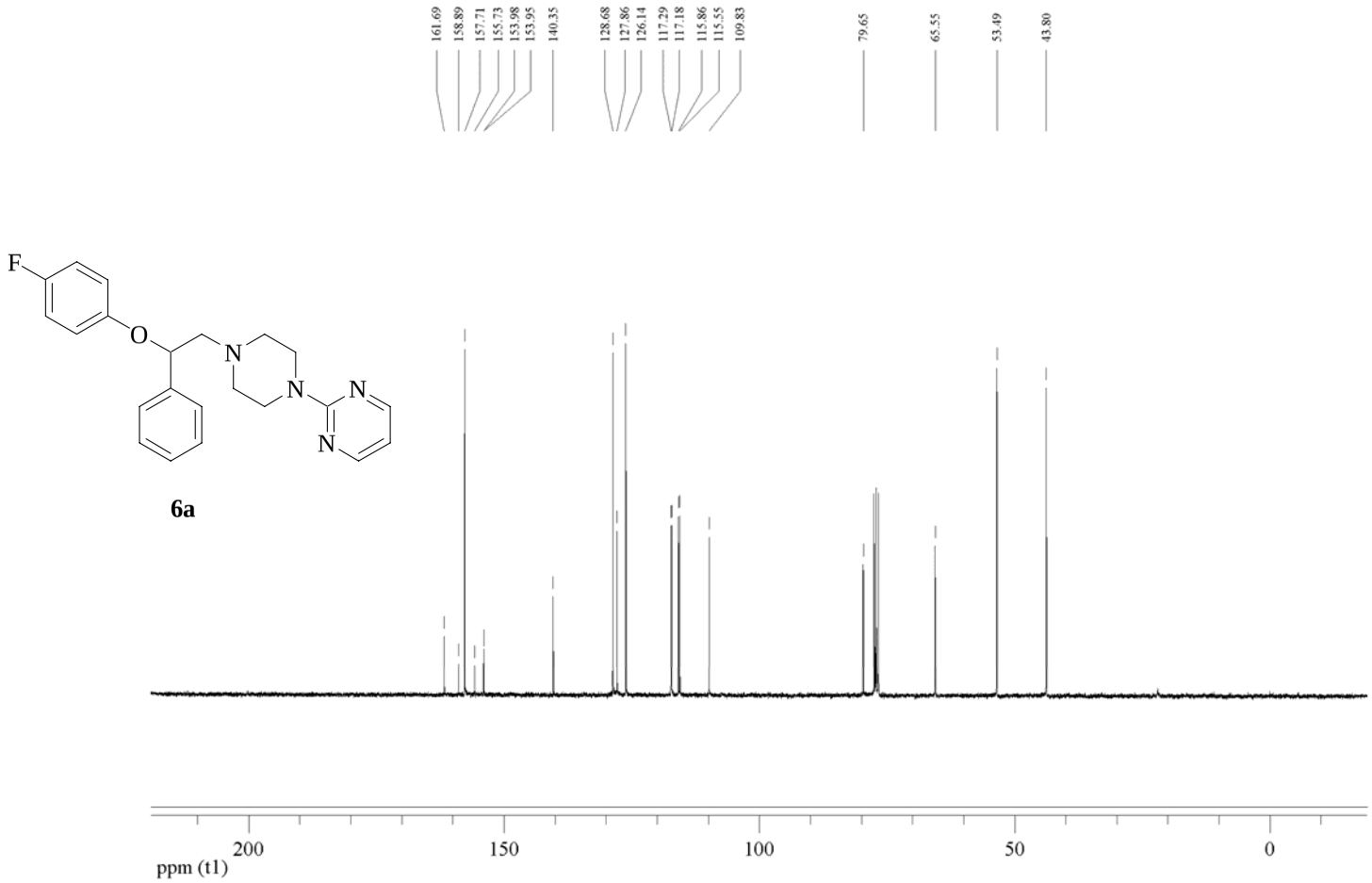
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **52**



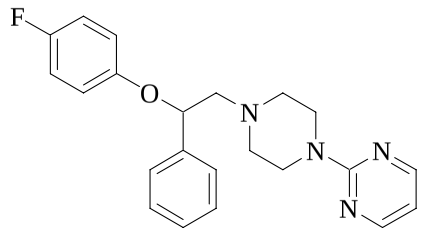
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **6a**



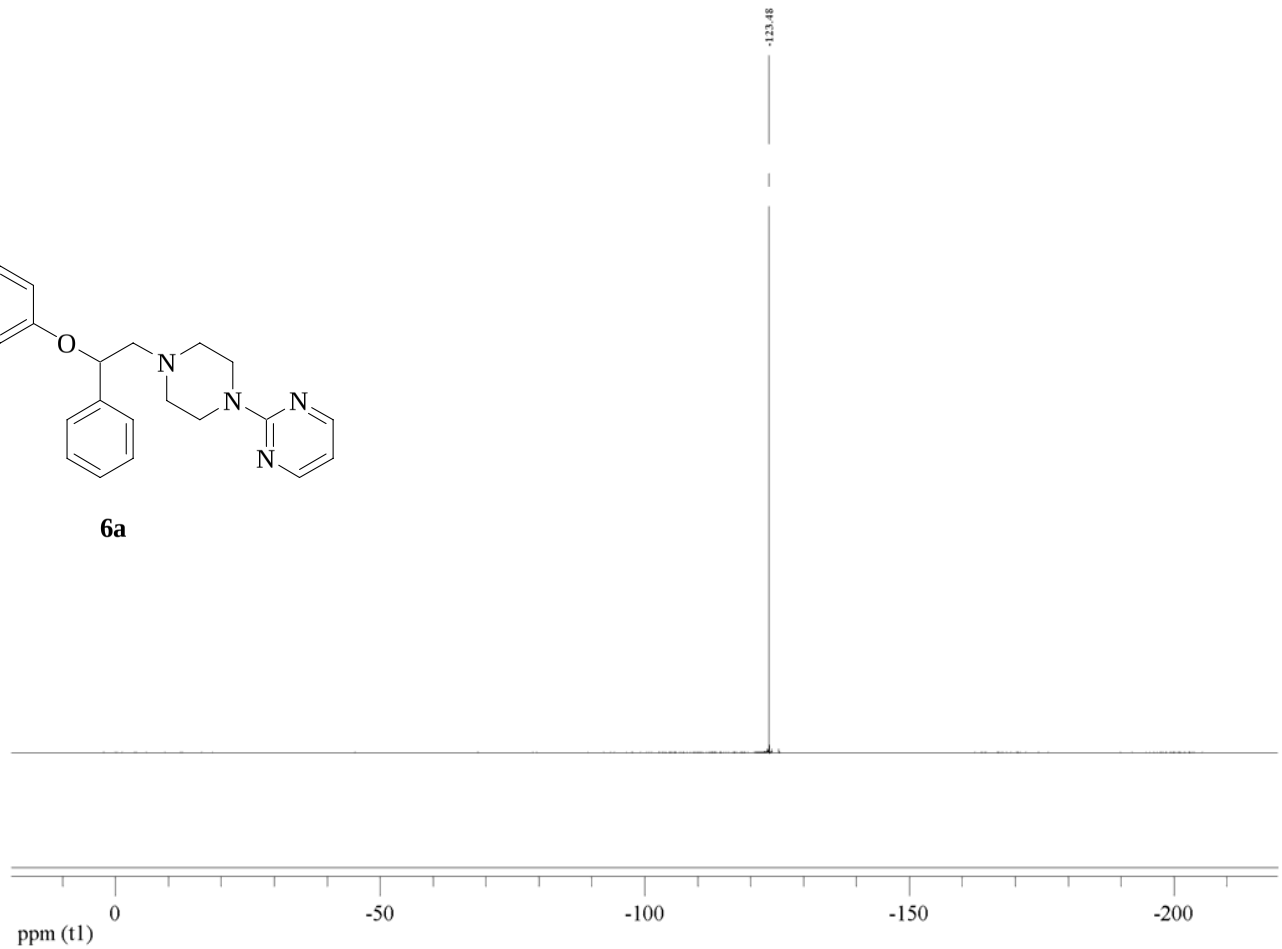
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **6a**



^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **6a**



6a



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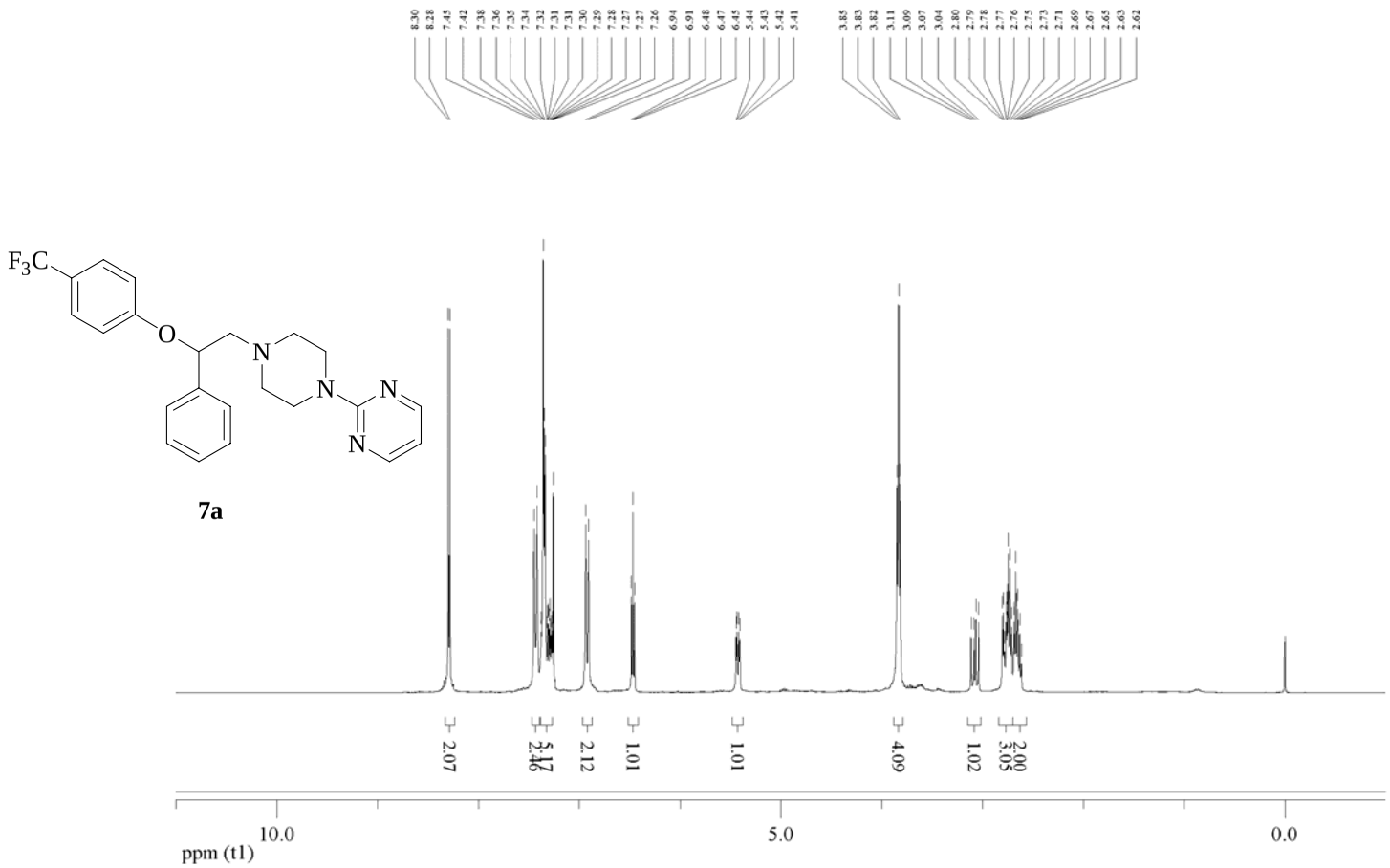
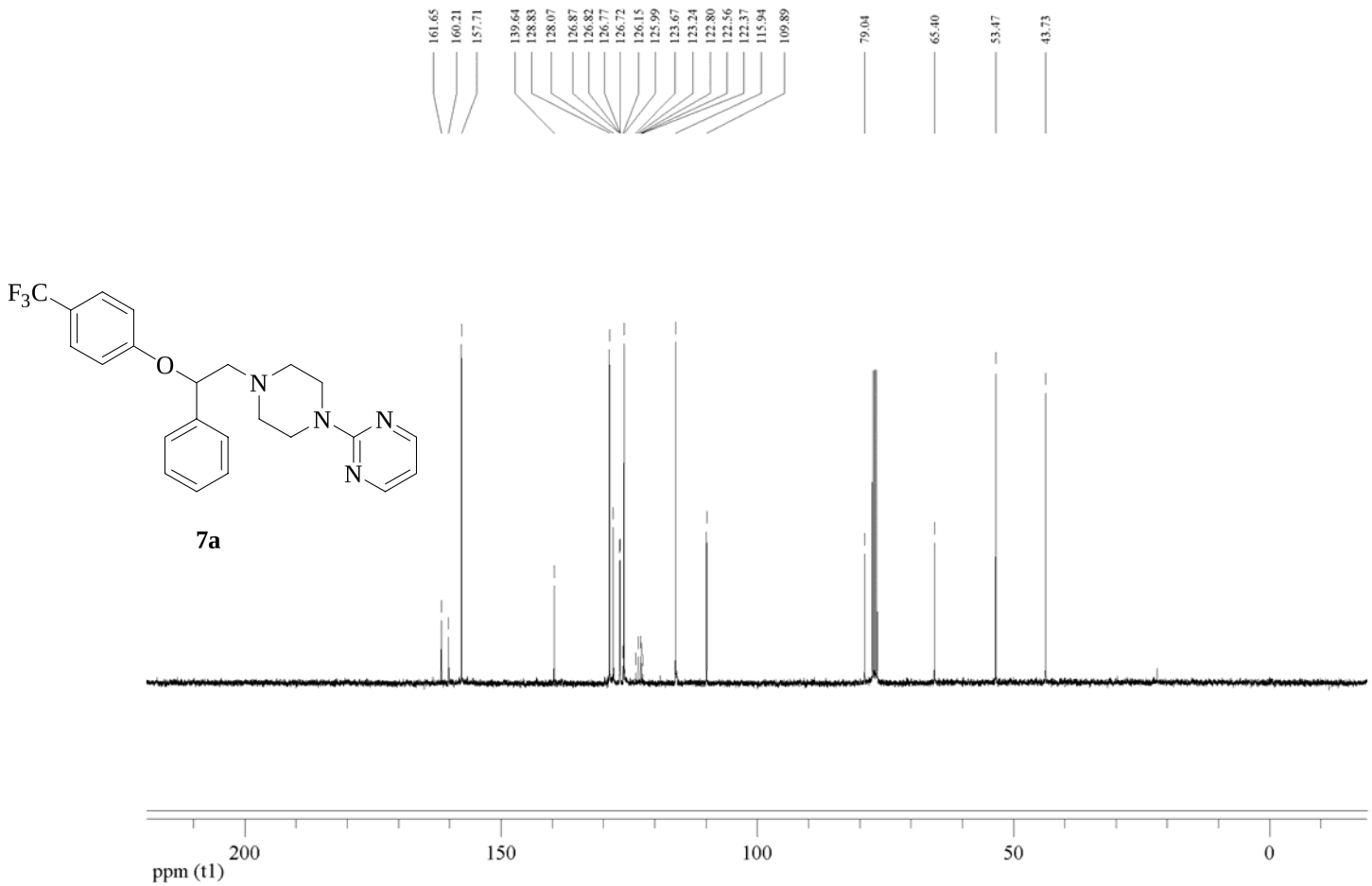
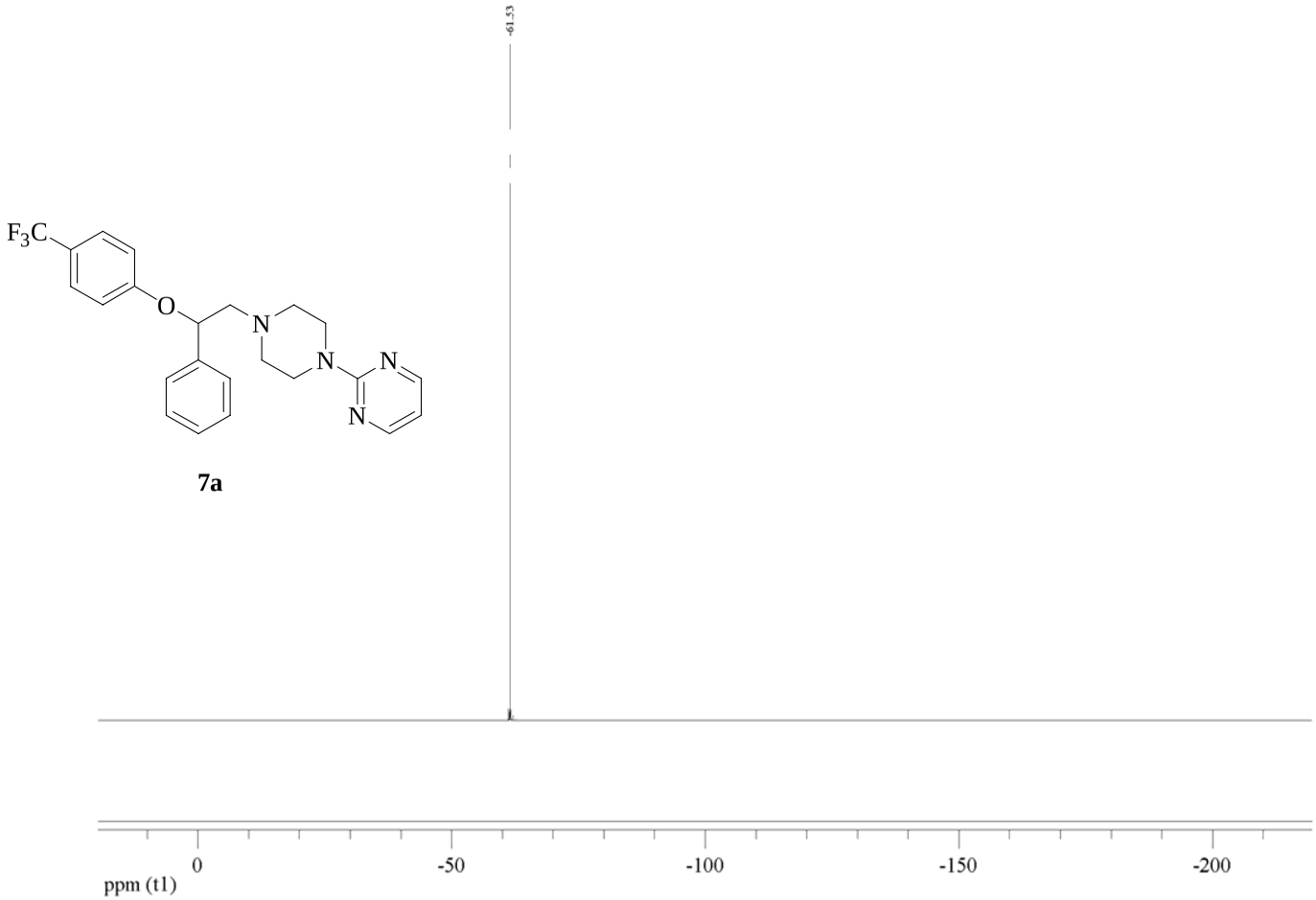


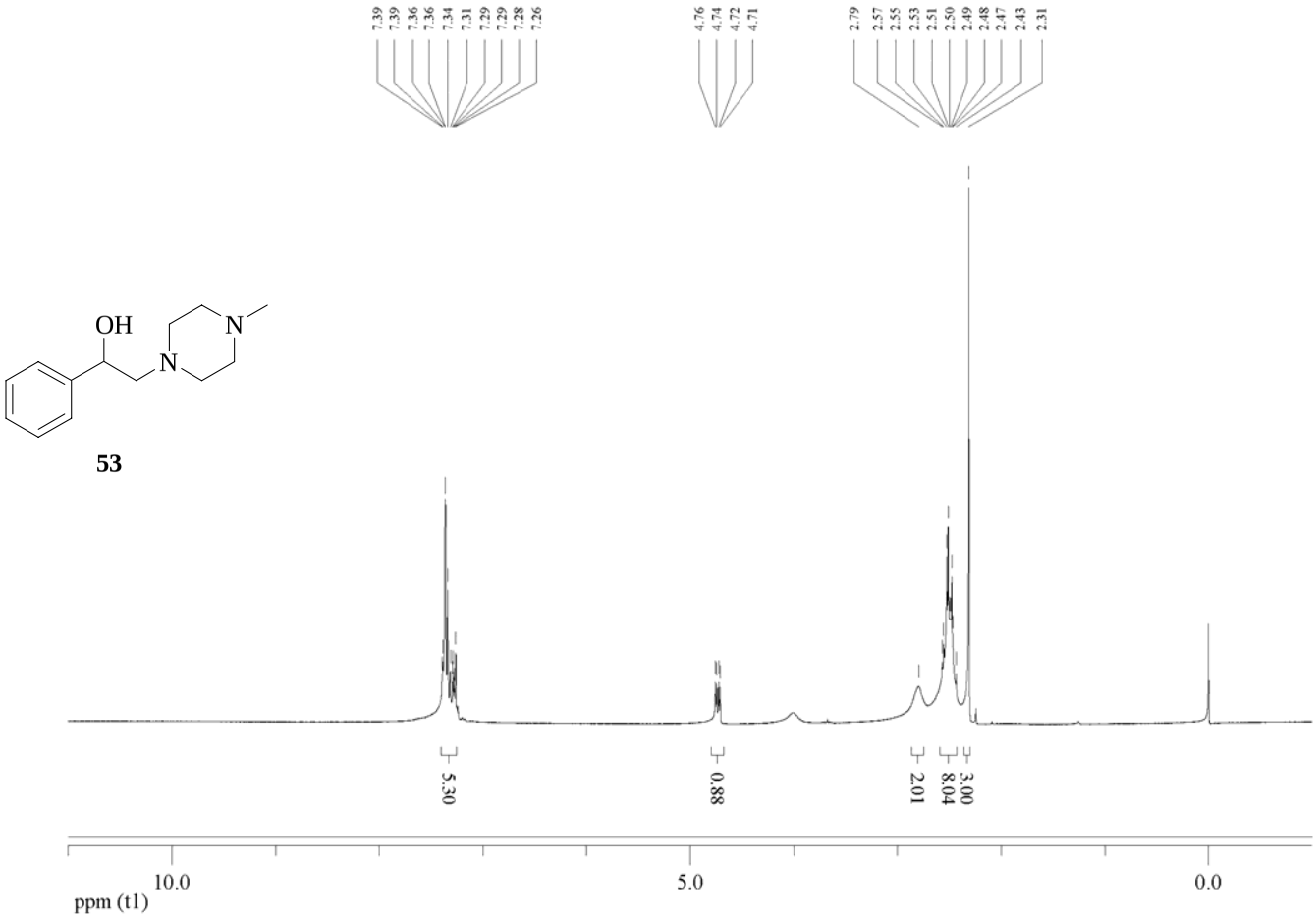
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **7a**



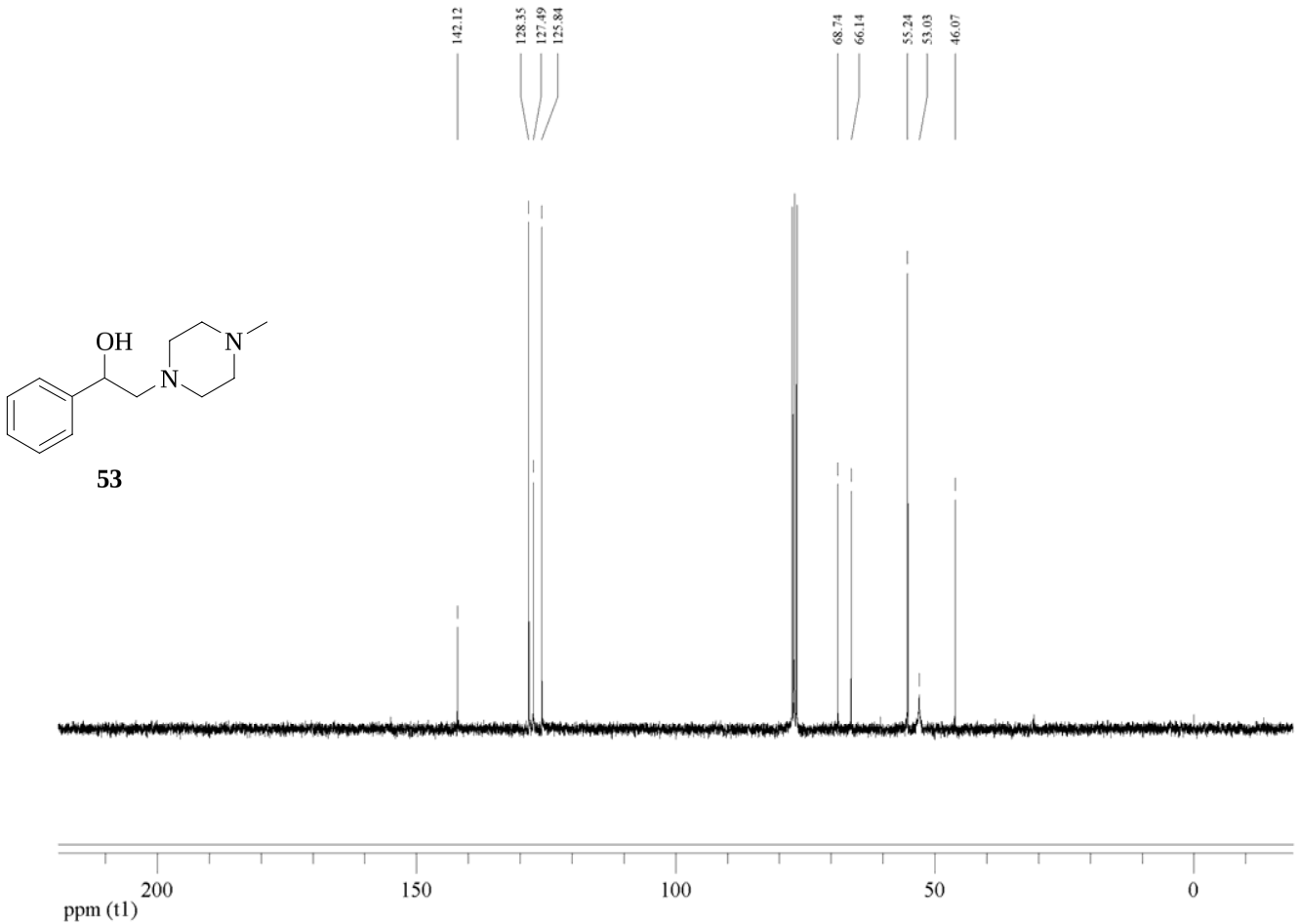
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **7a**



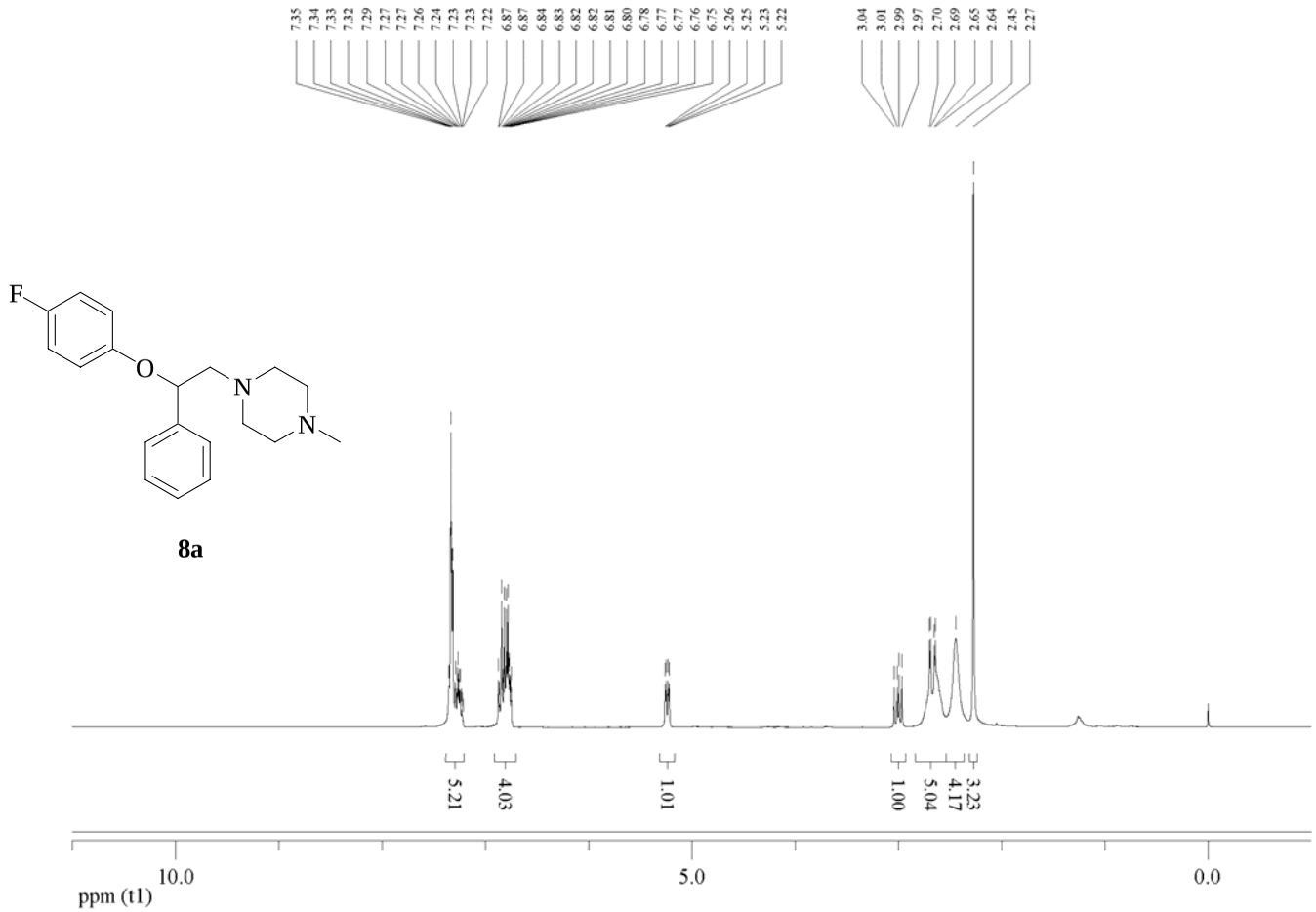
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **53**



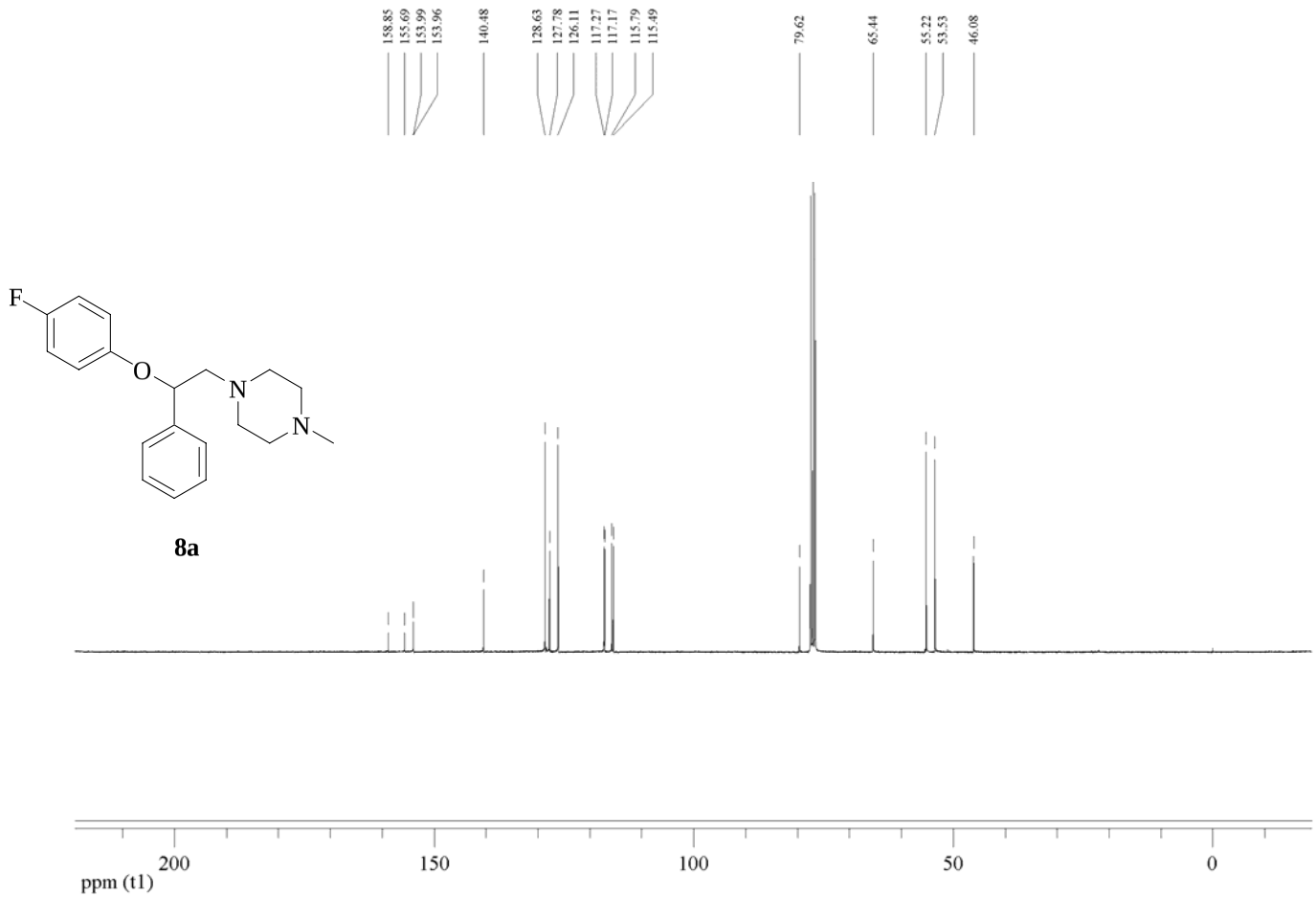
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **53**



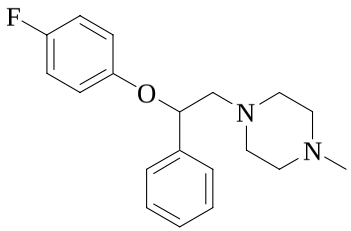
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **8a**



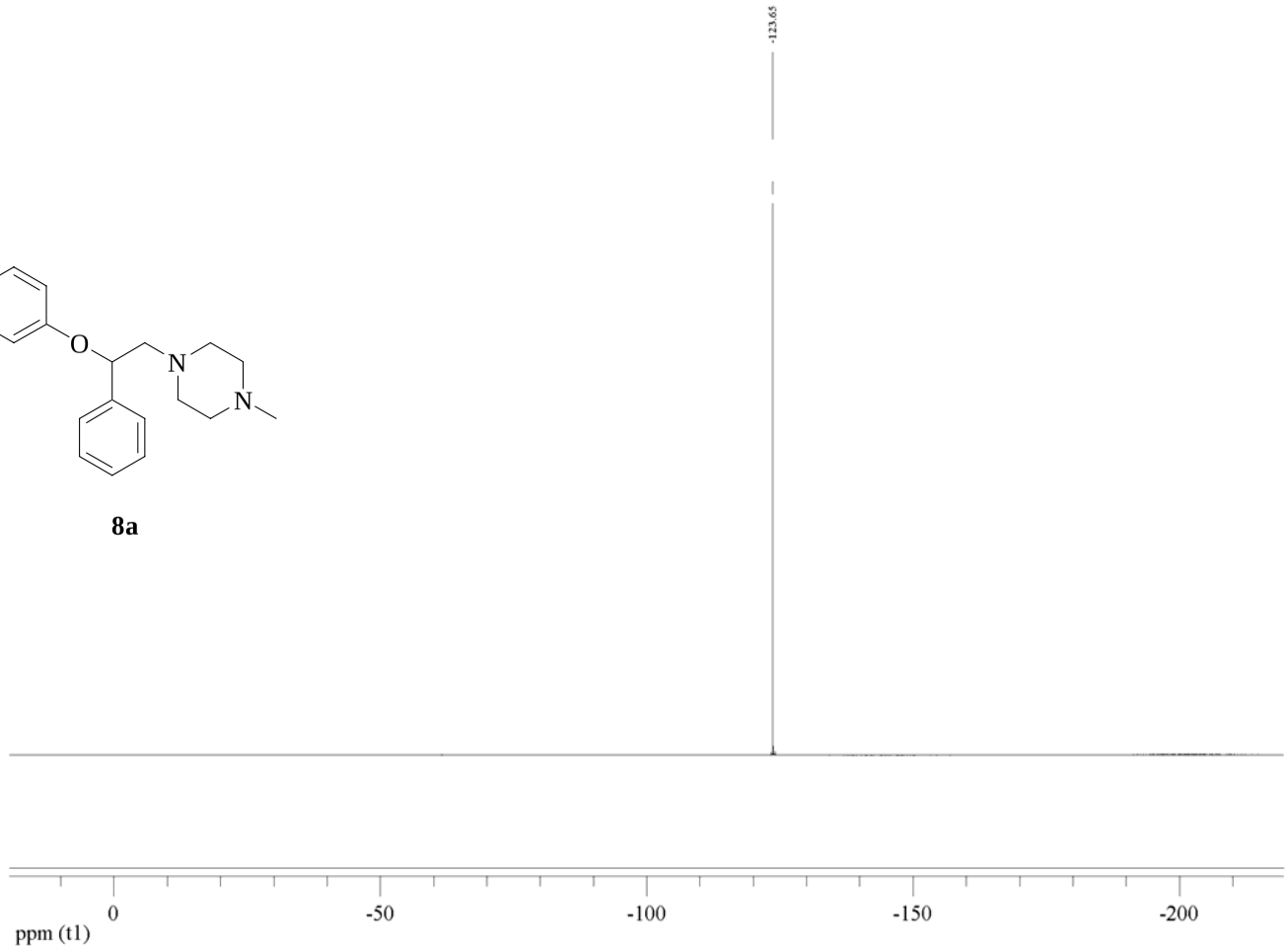
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **8a**



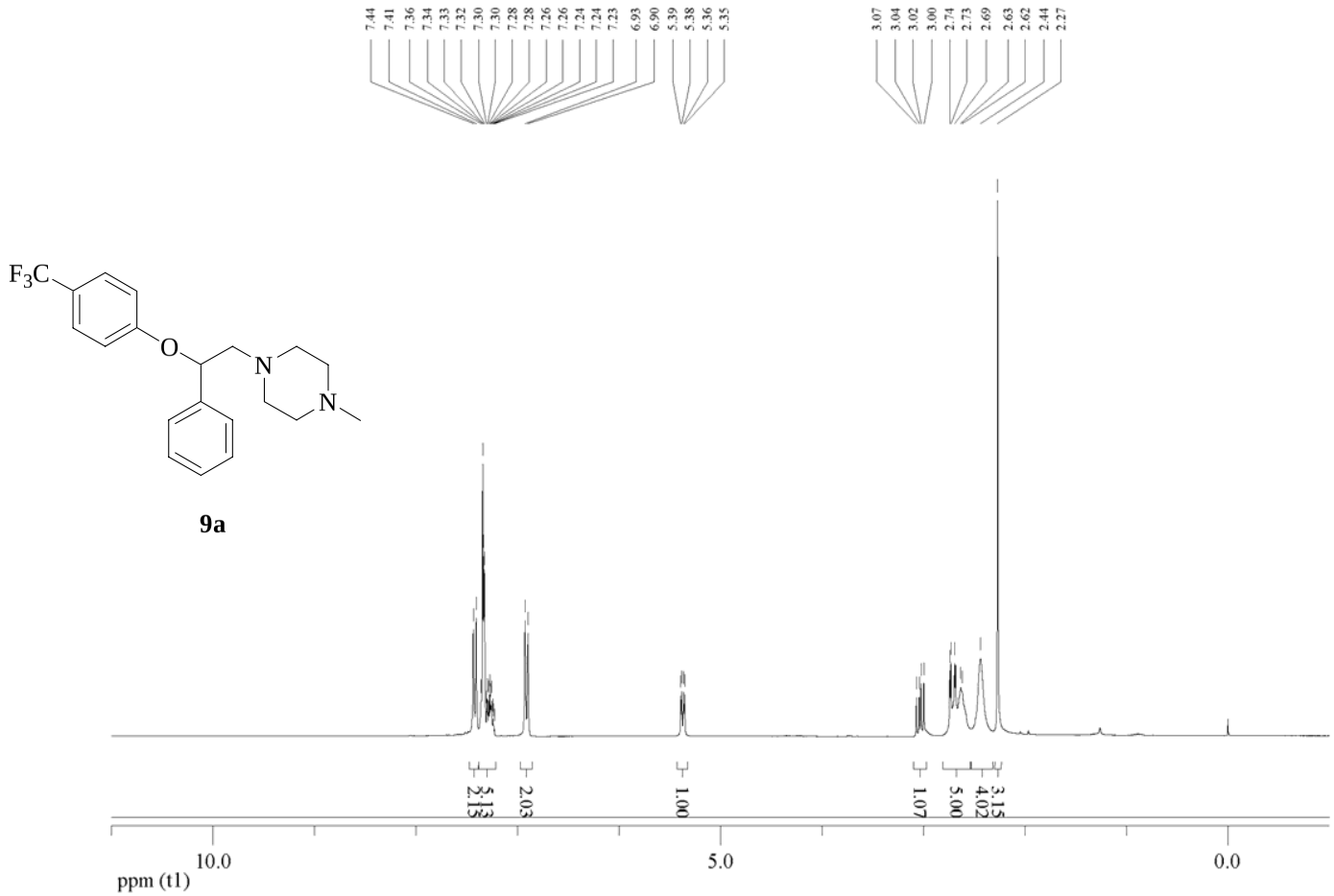
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **8a**



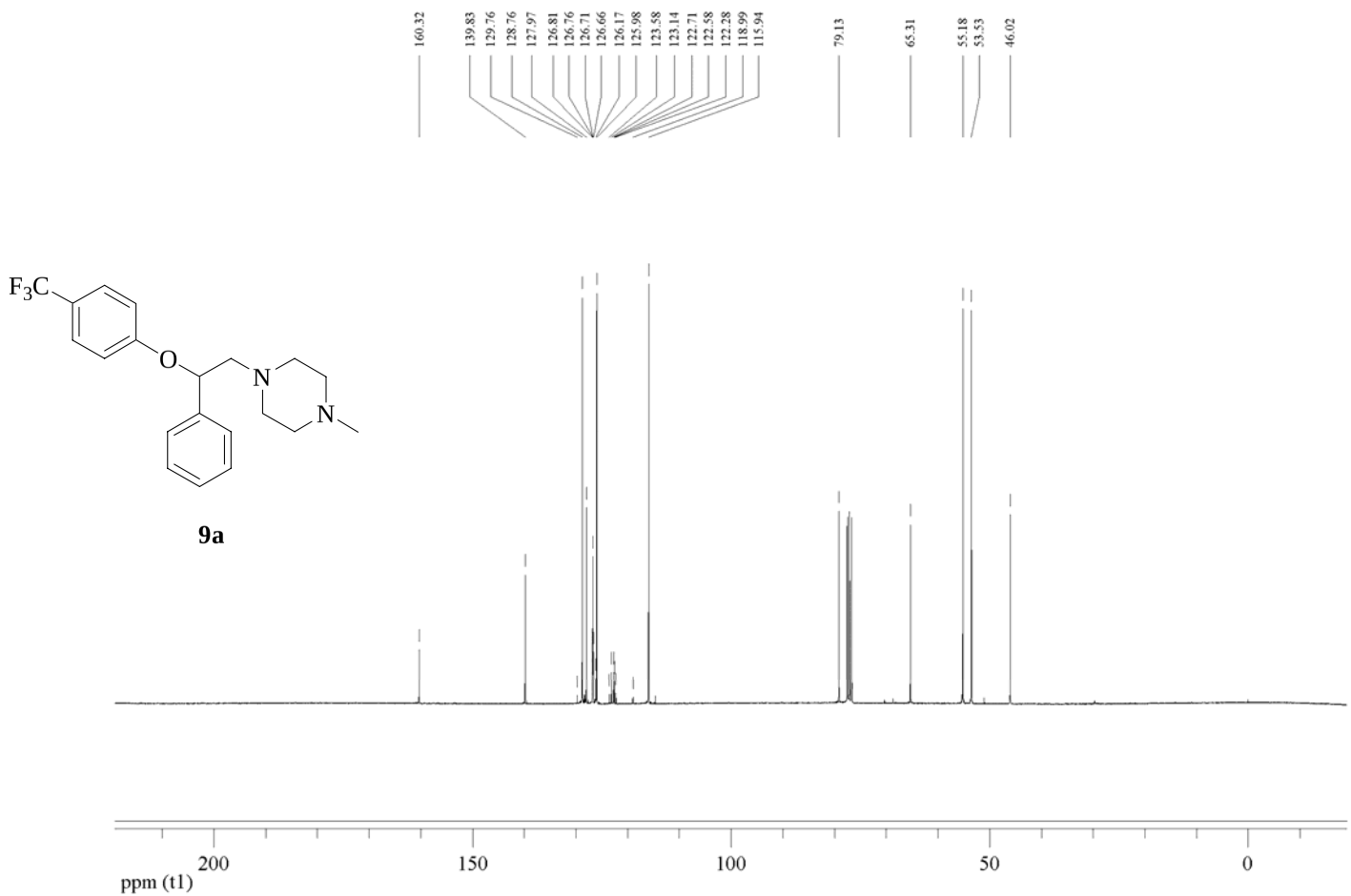
8a



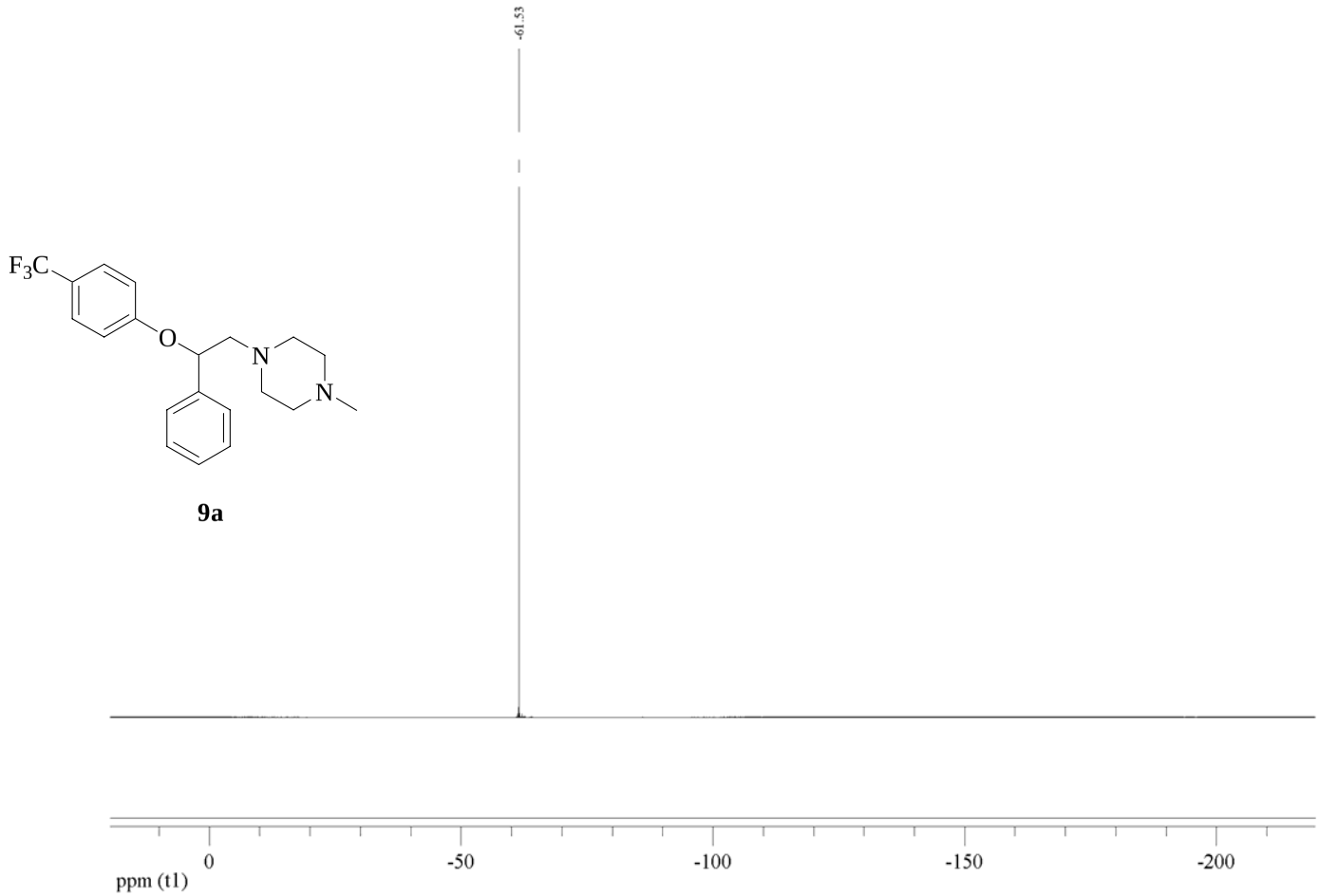
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **9a**



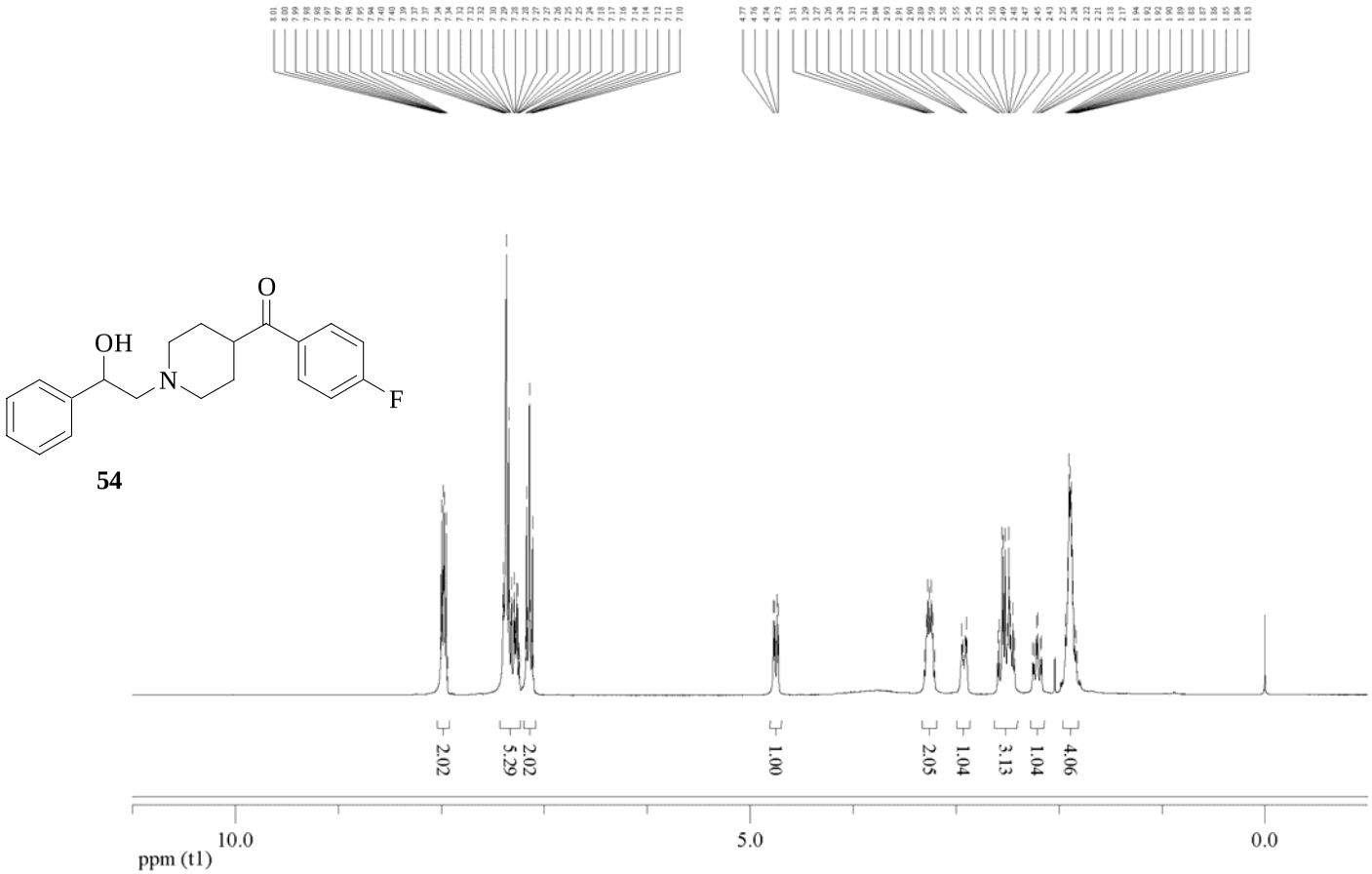
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **9a**



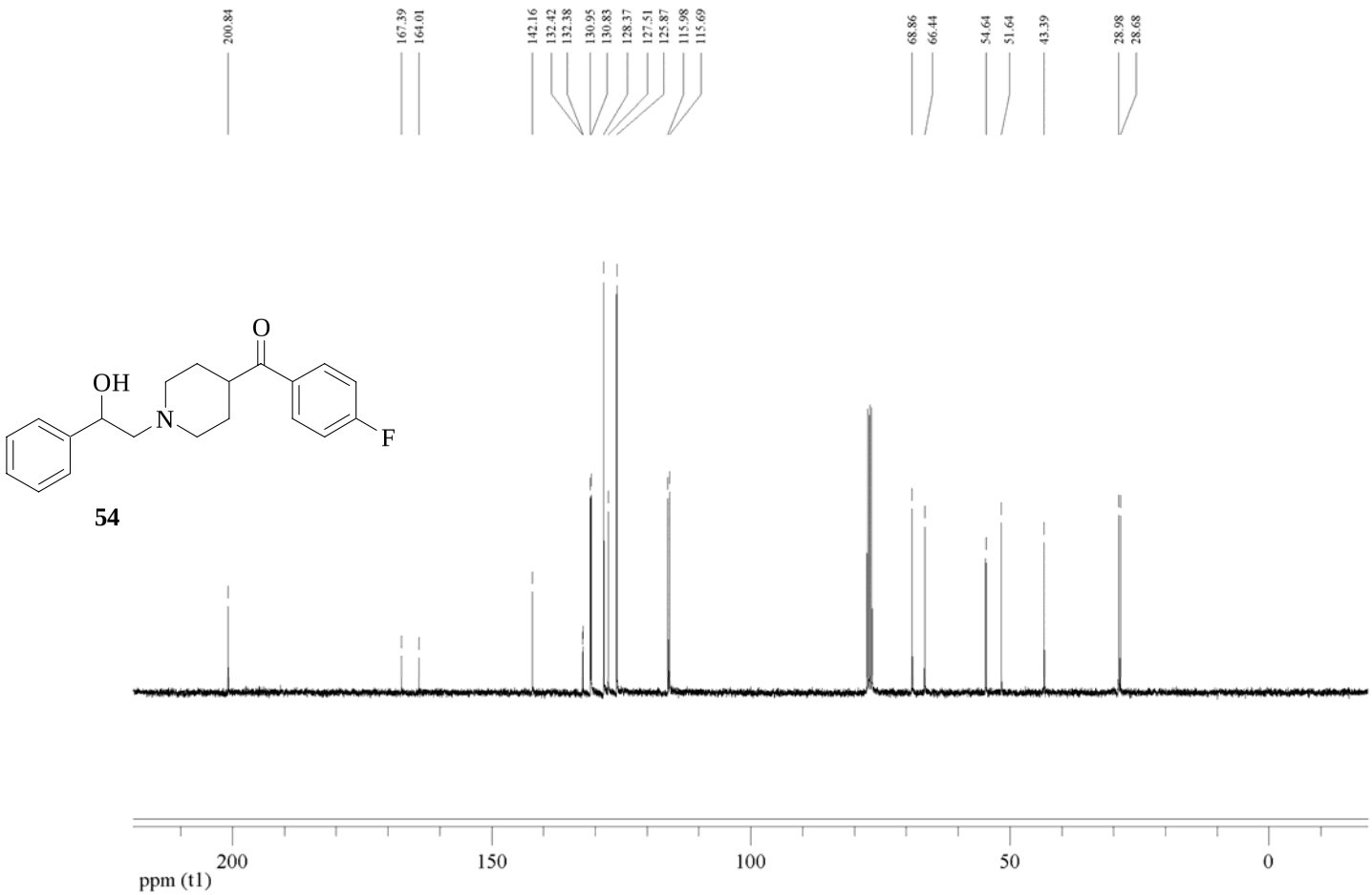
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **9a**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **54**

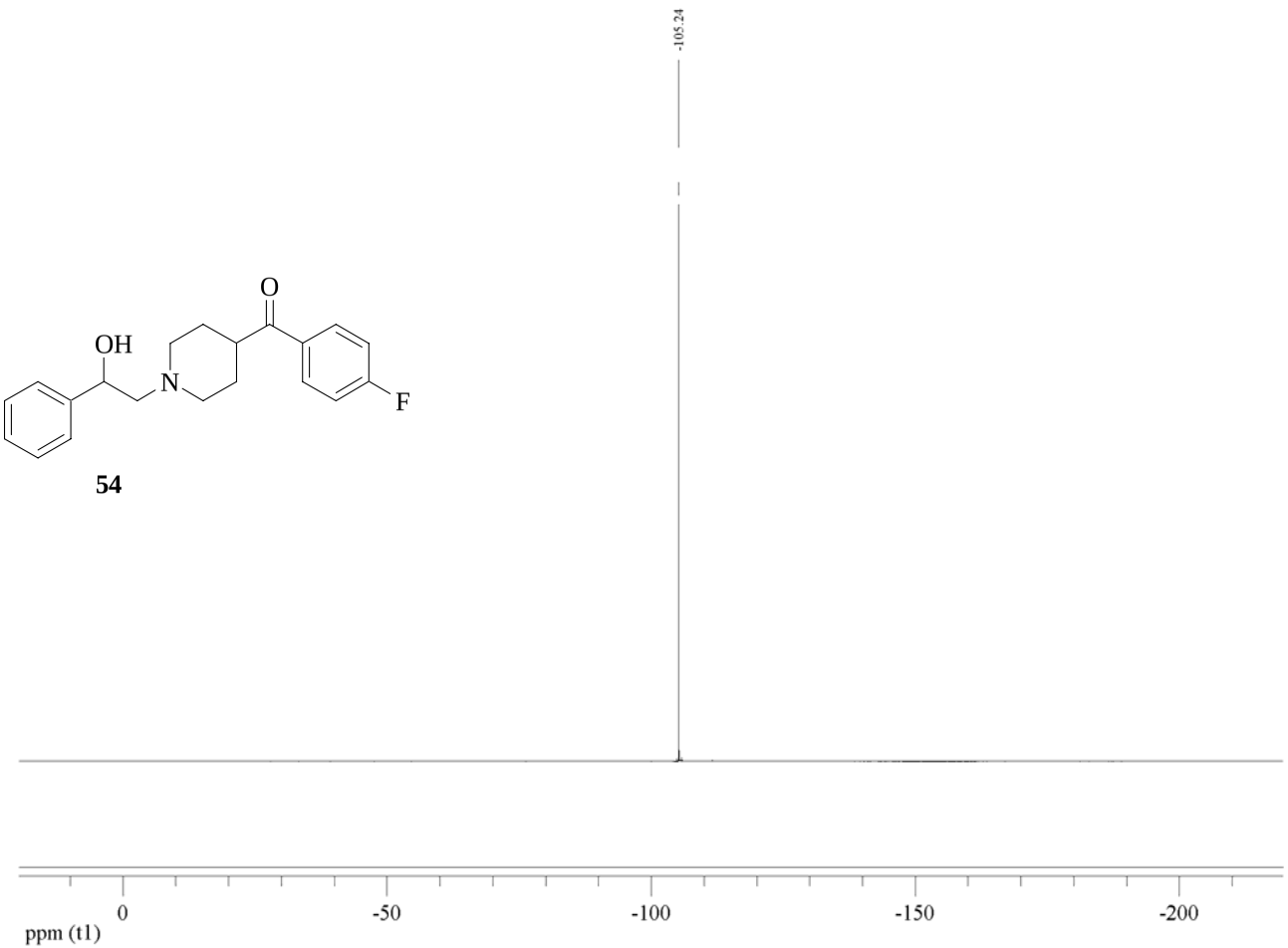


^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **54**

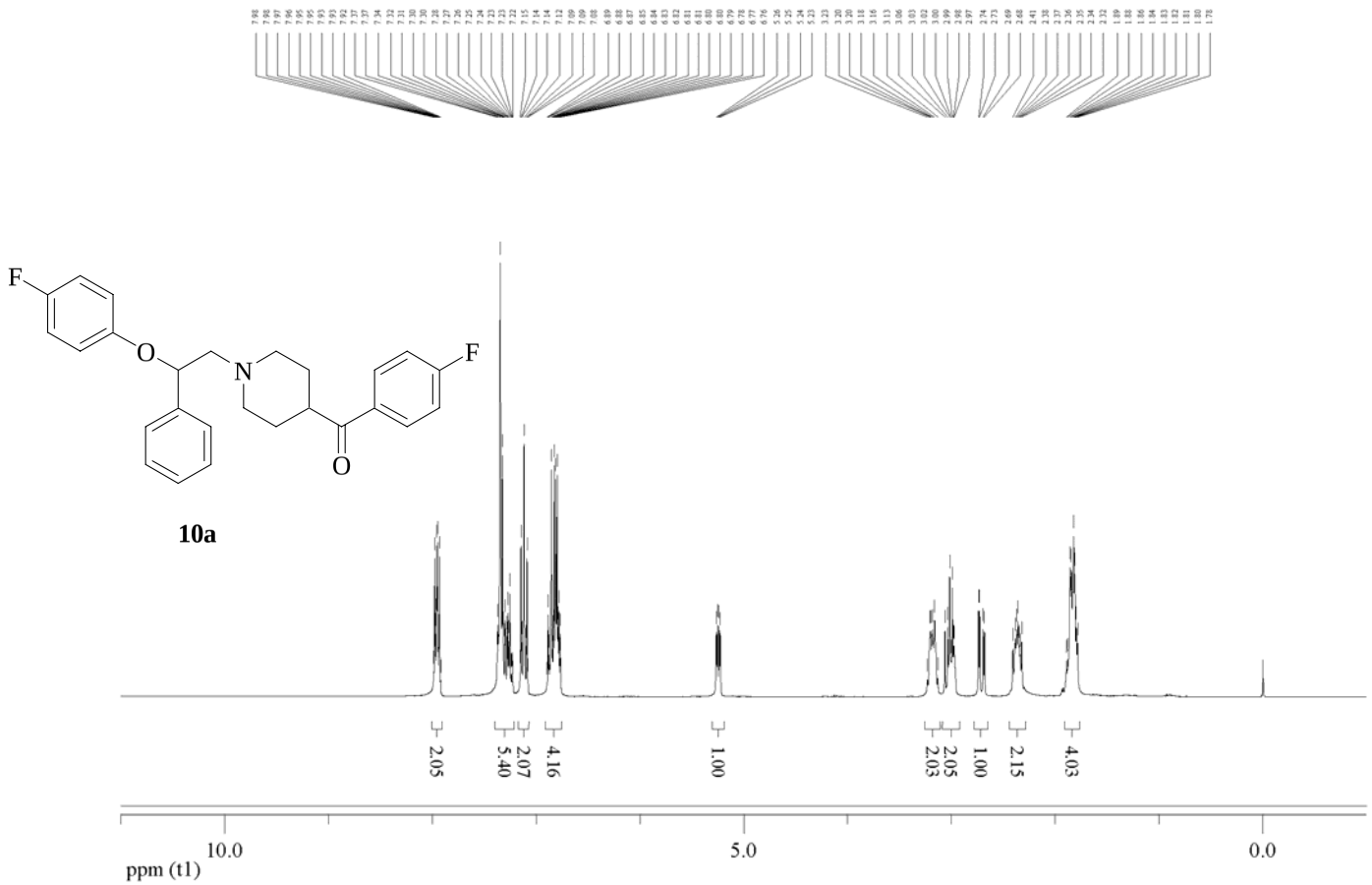


^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **54**

54



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **10a**



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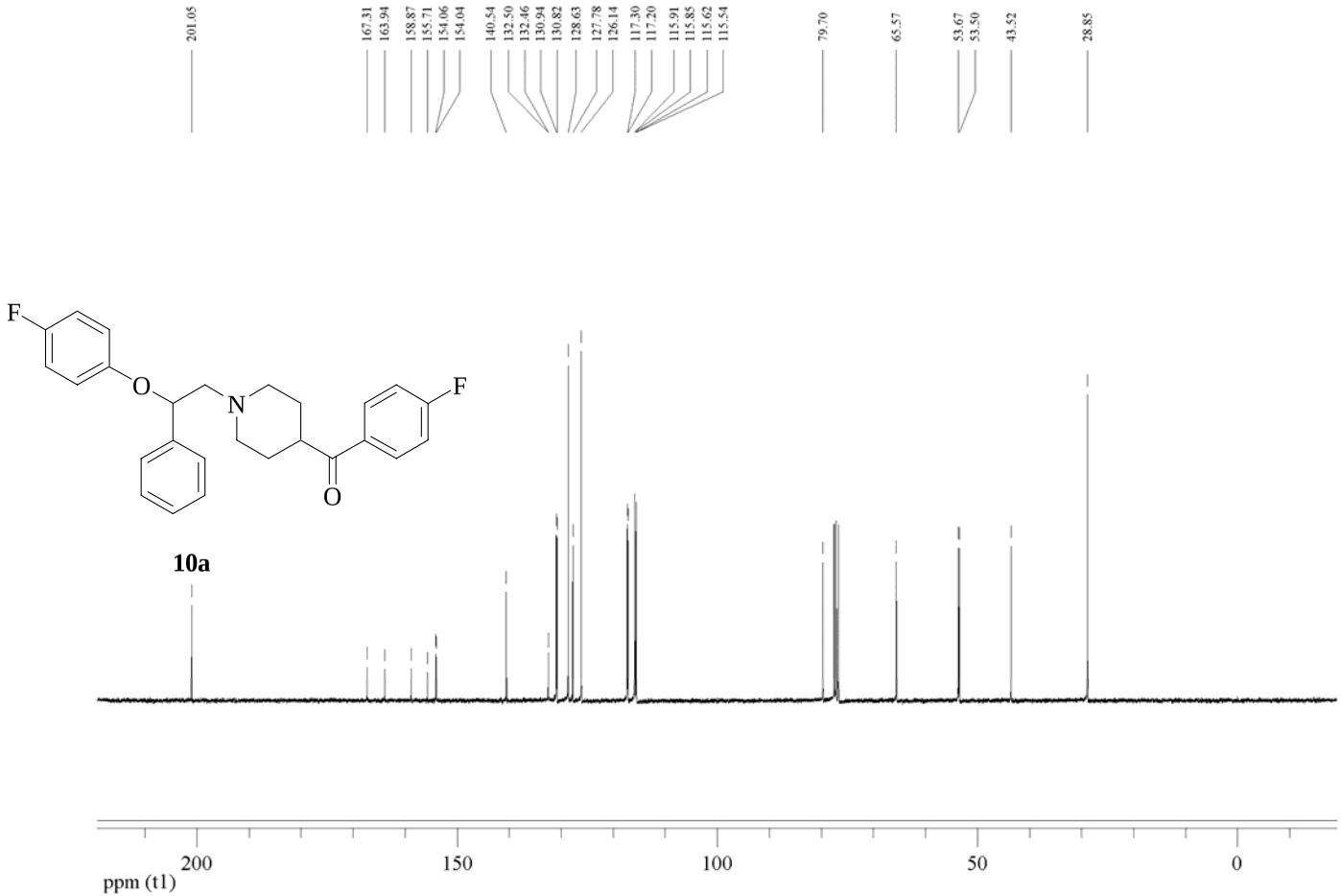

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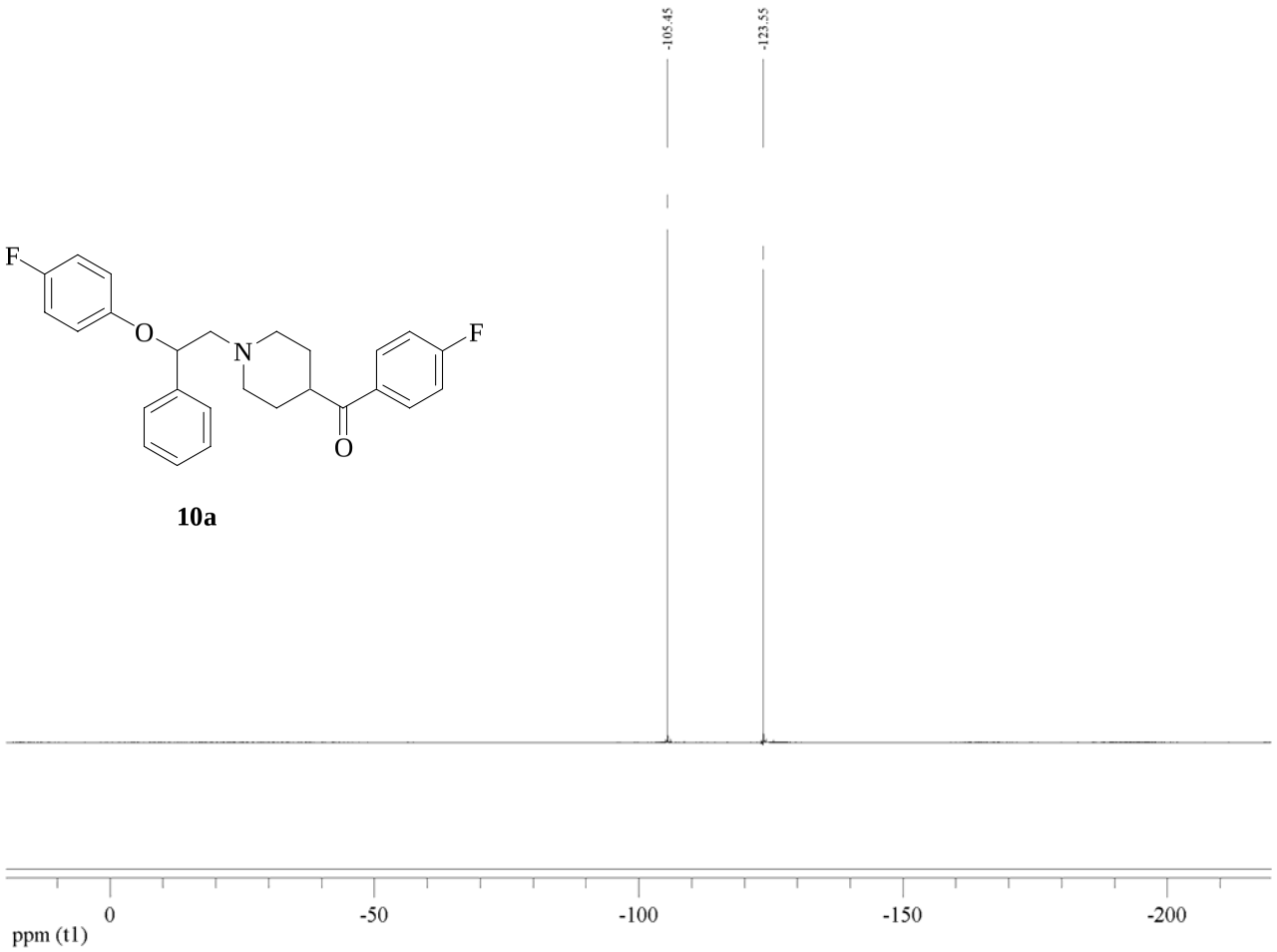
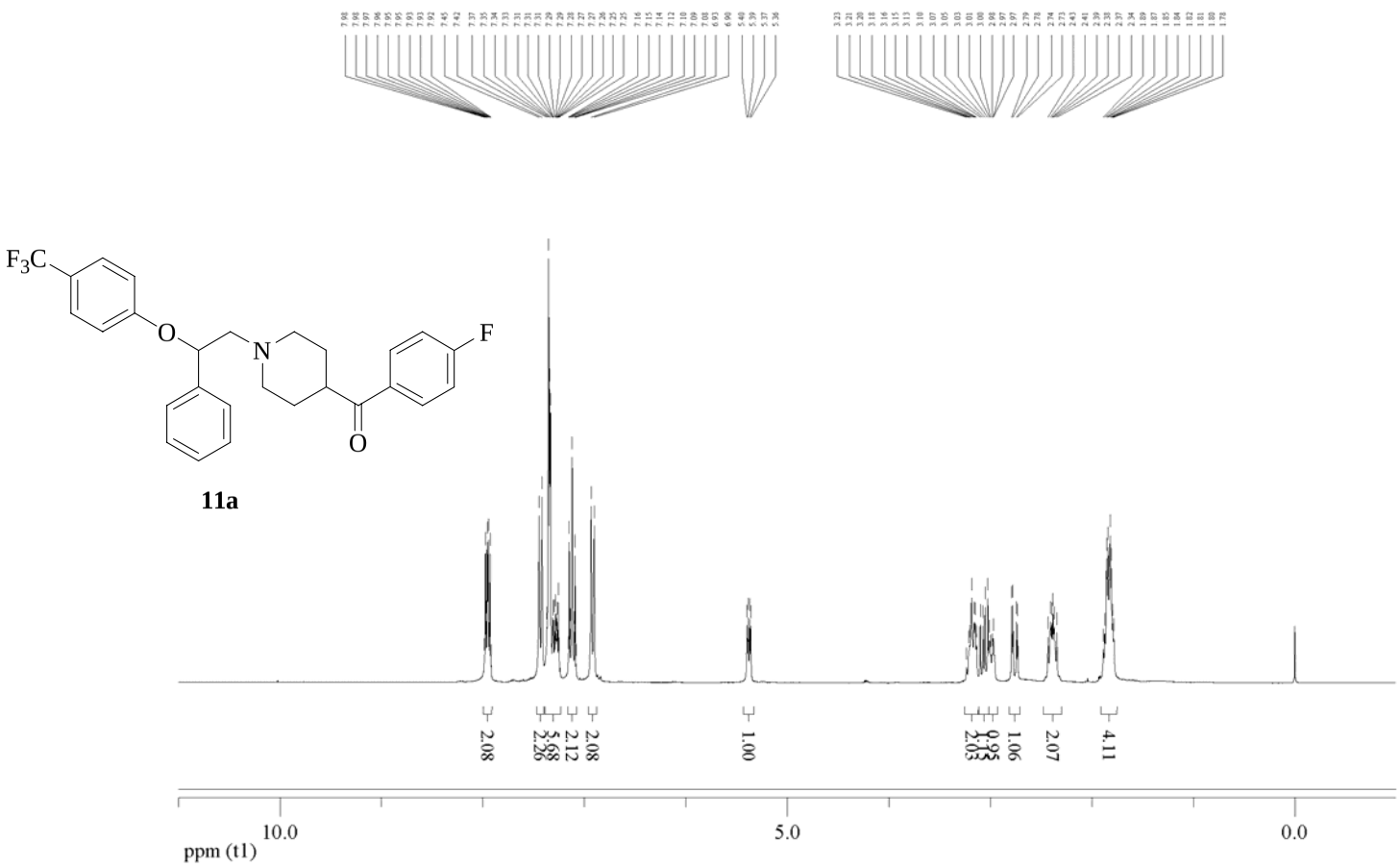


^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **11a**



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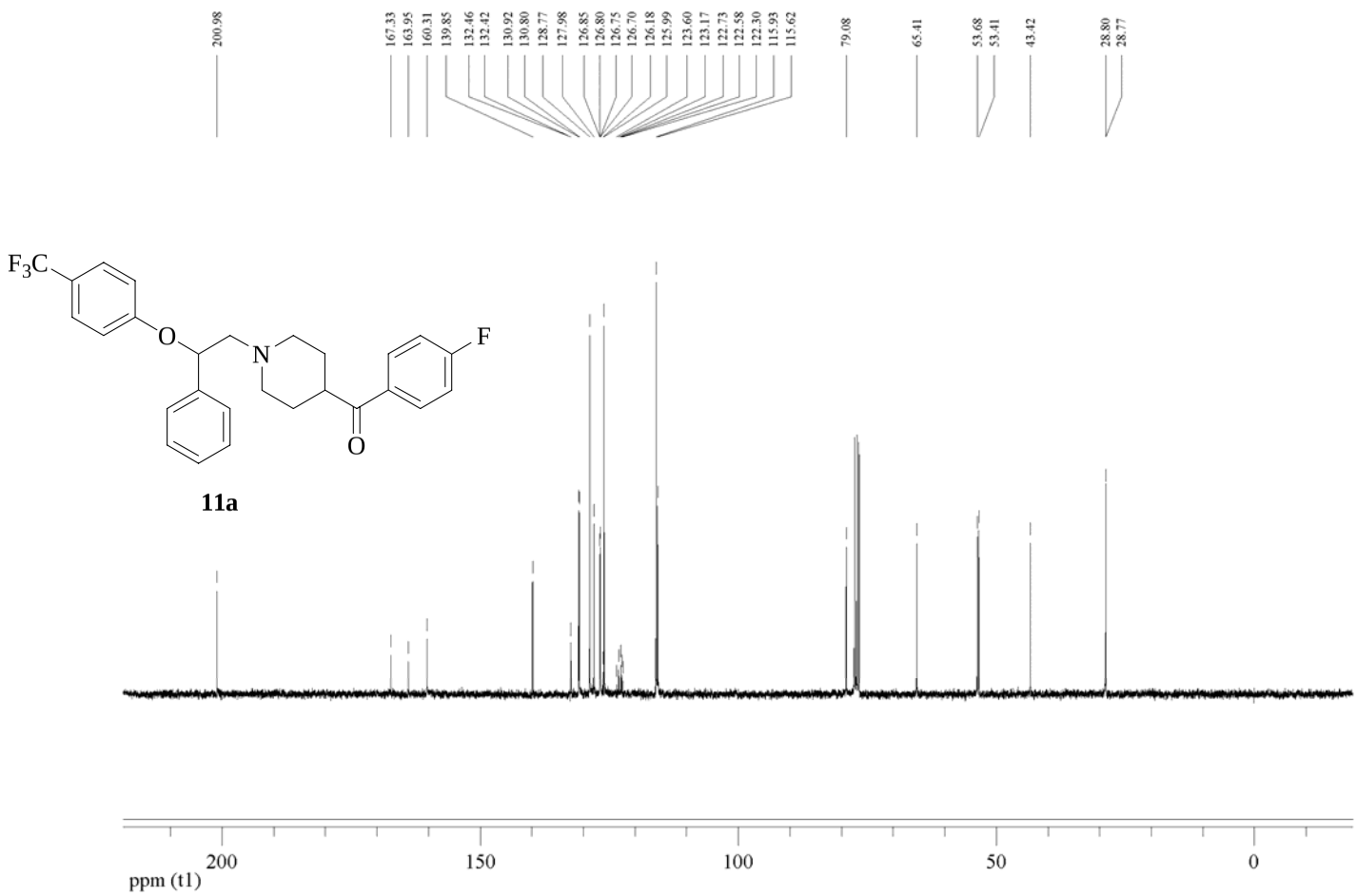

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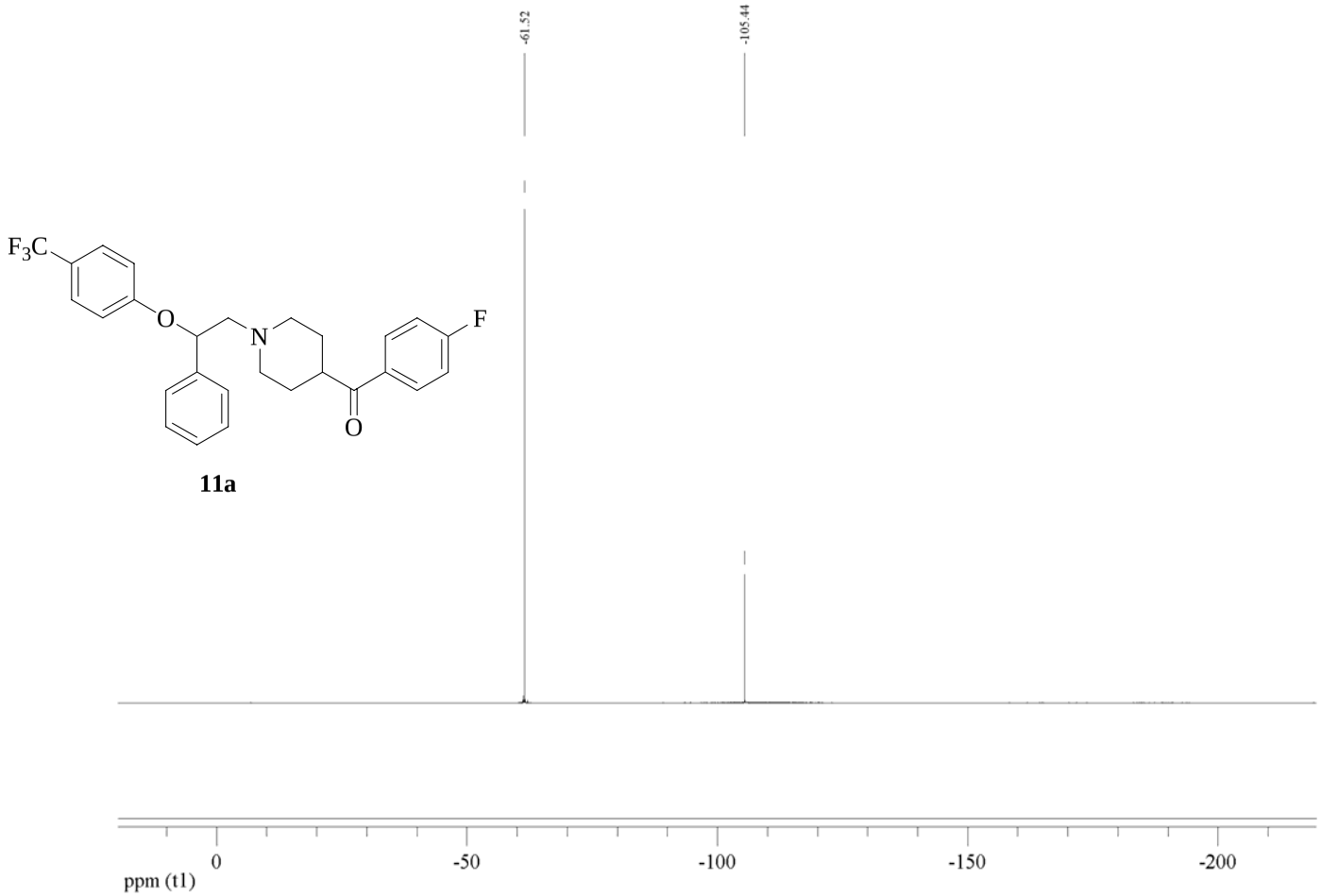
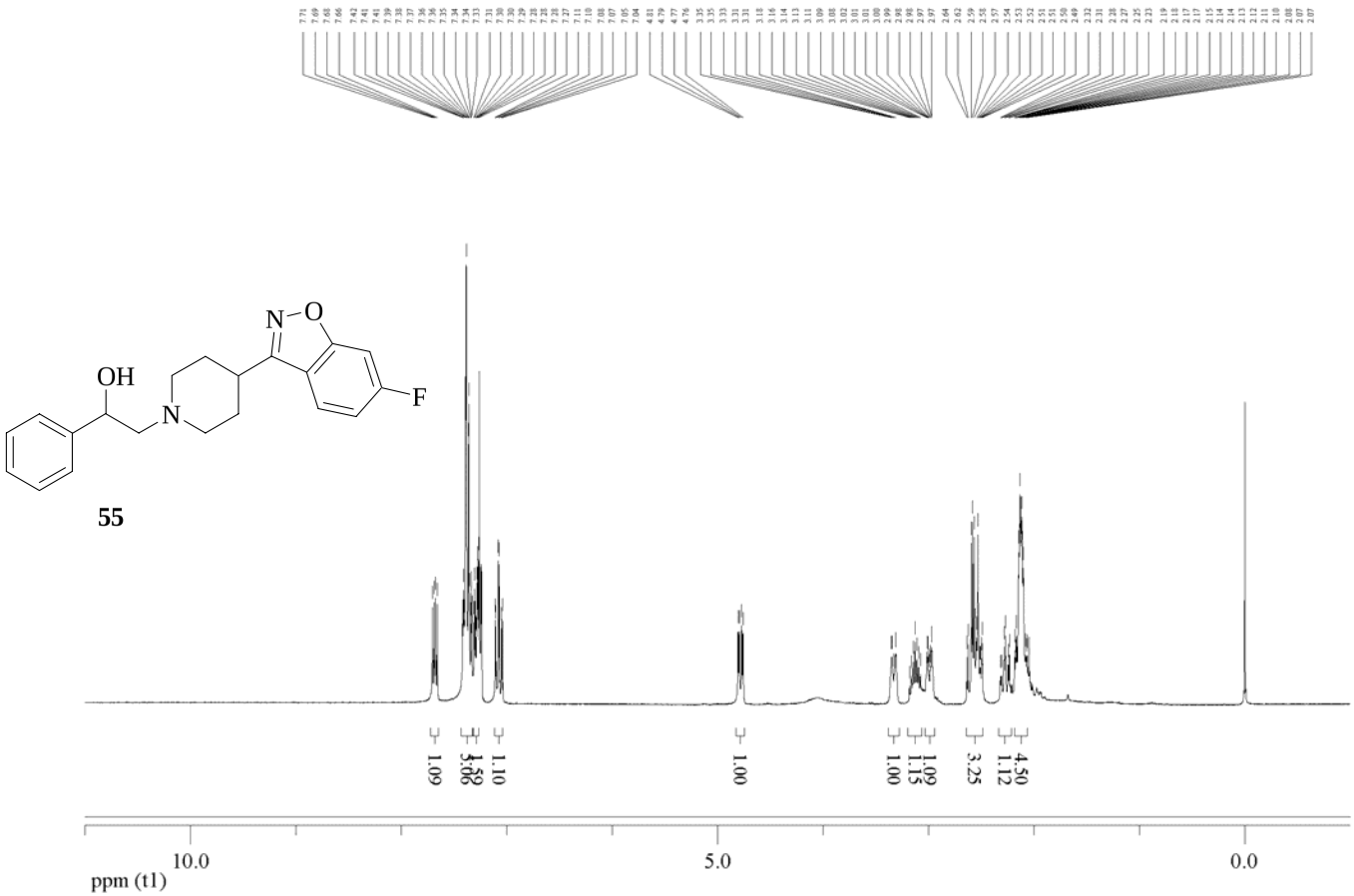
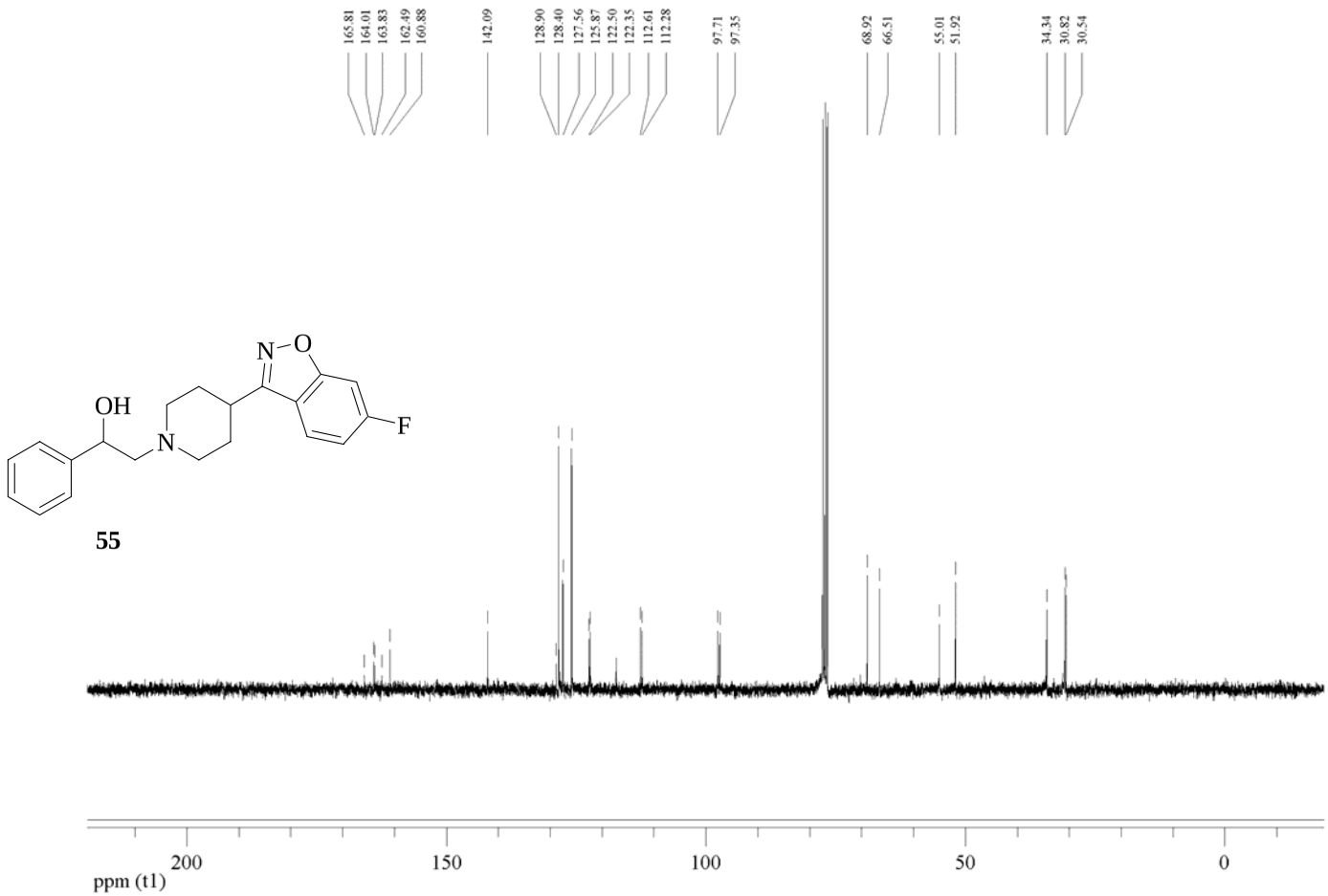


^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **55**



^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **55**



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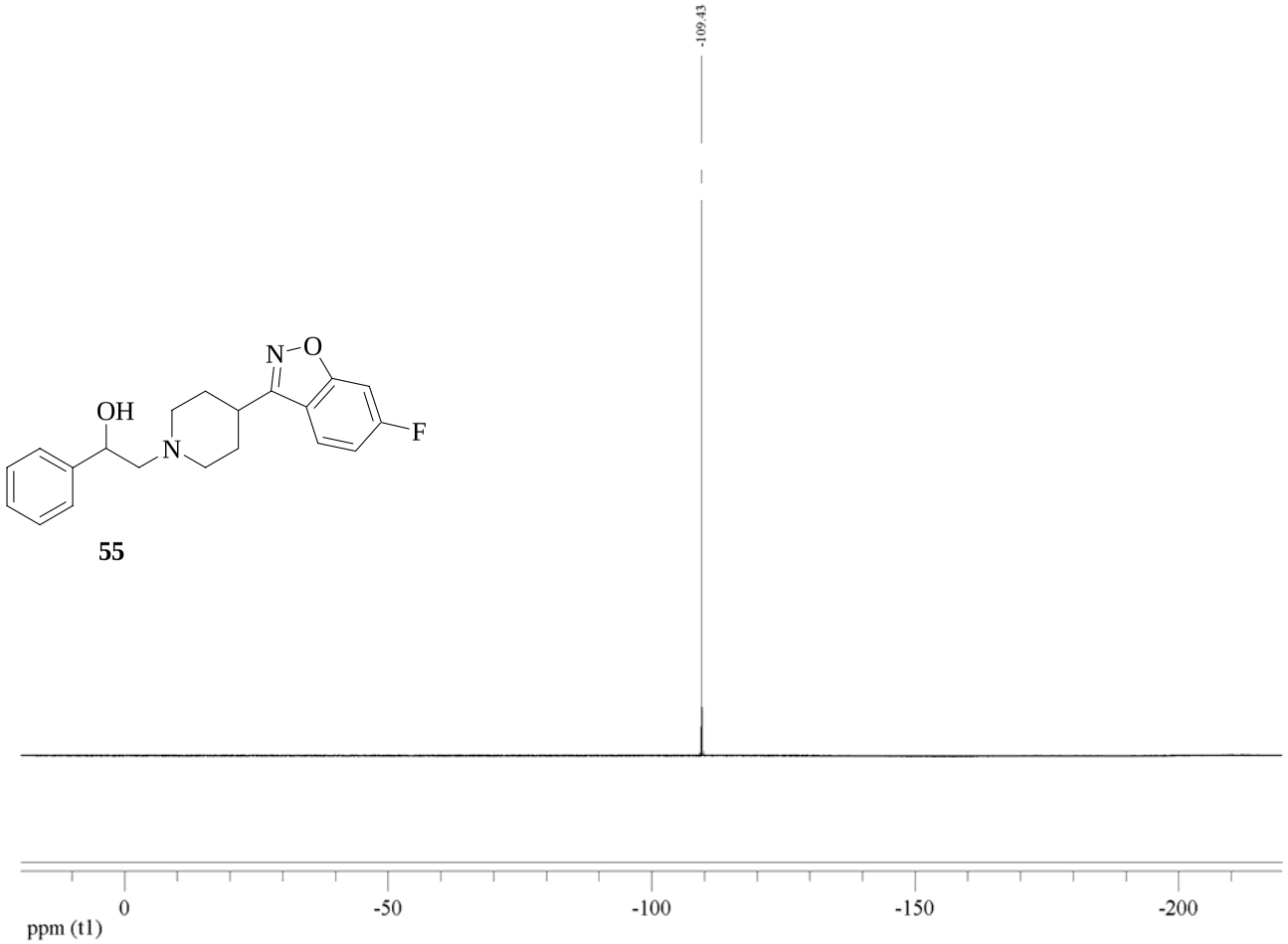
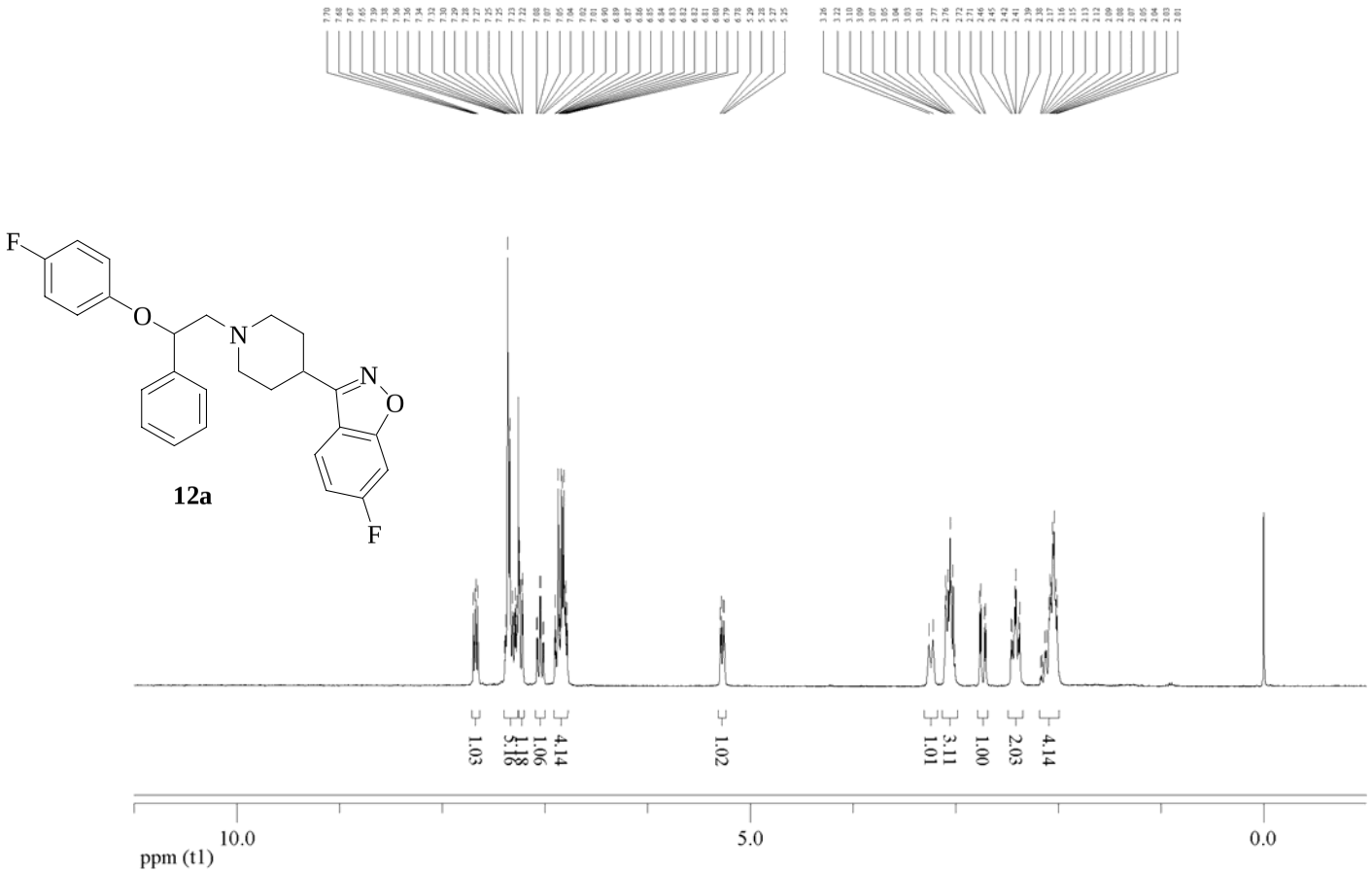
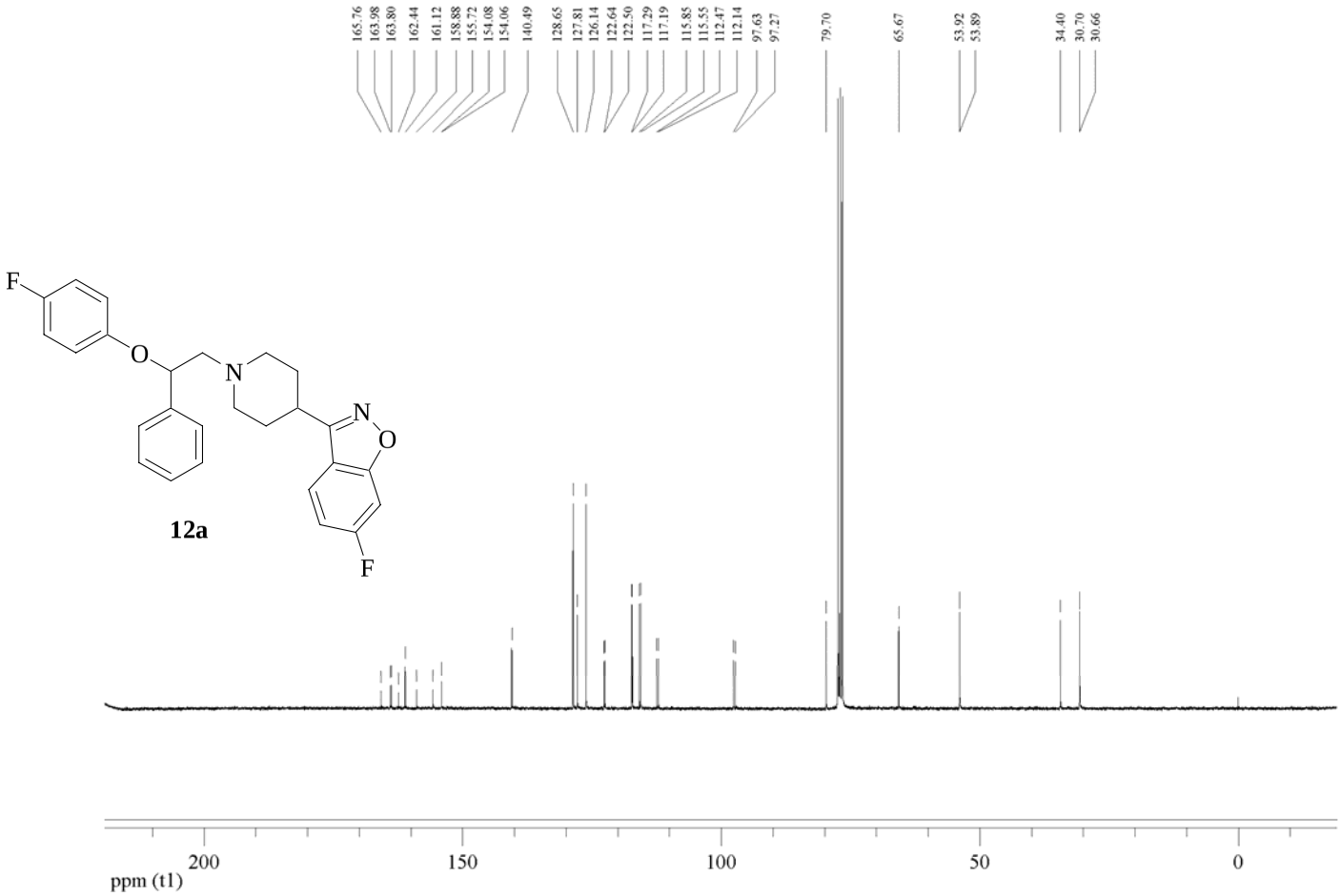


^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **12a**

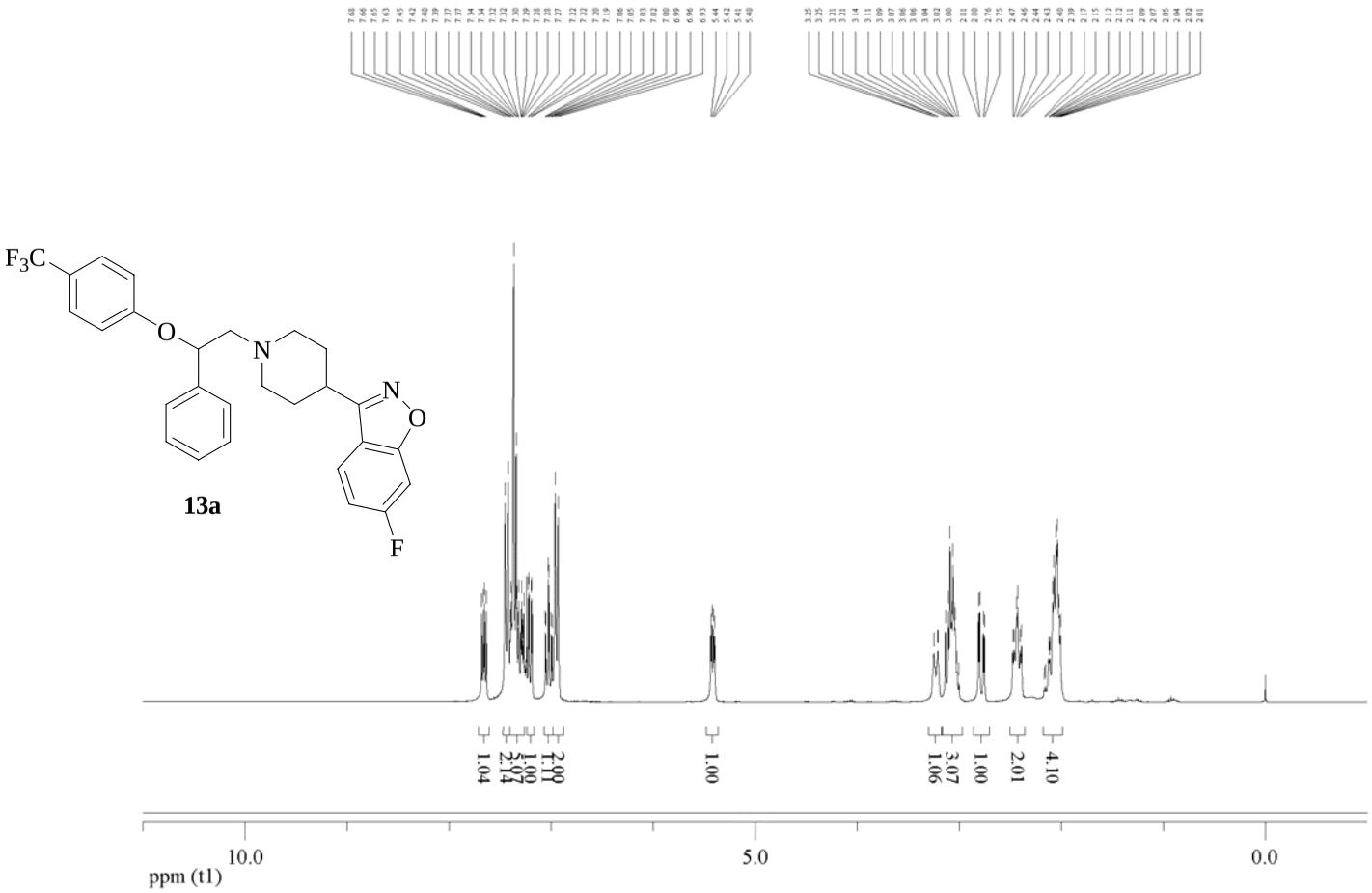


^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **12a**

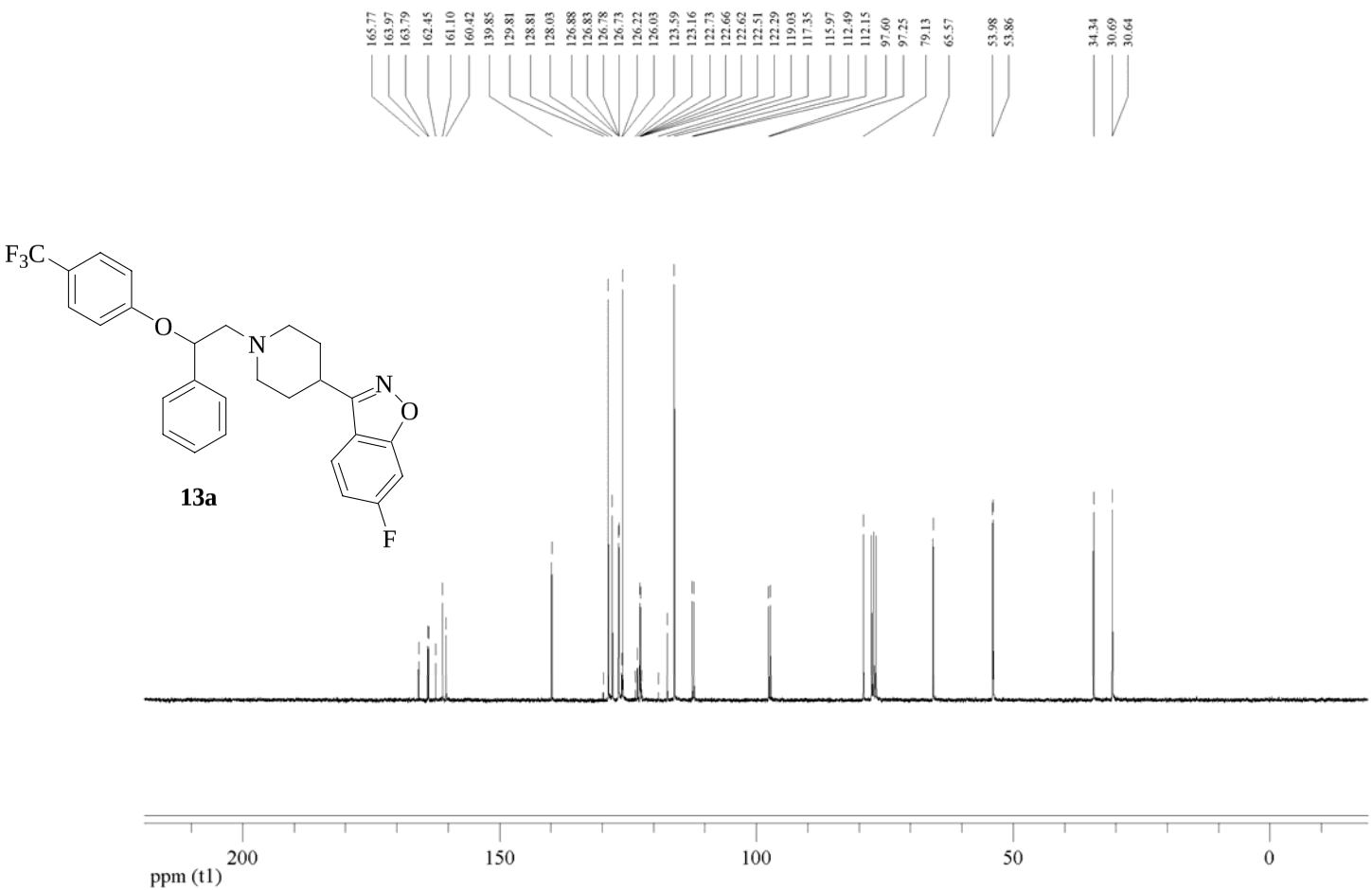


^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **12a**

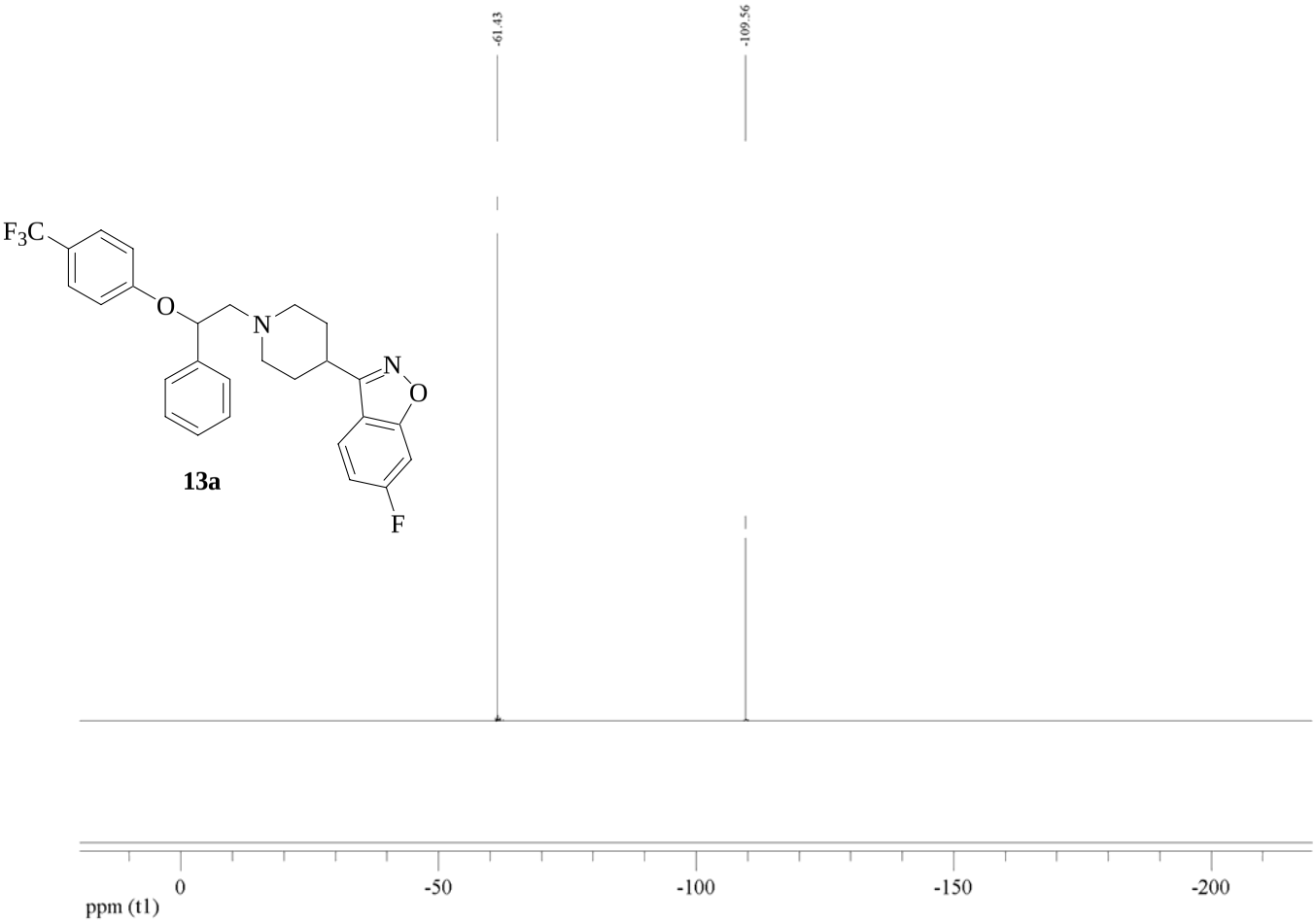
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **13a**



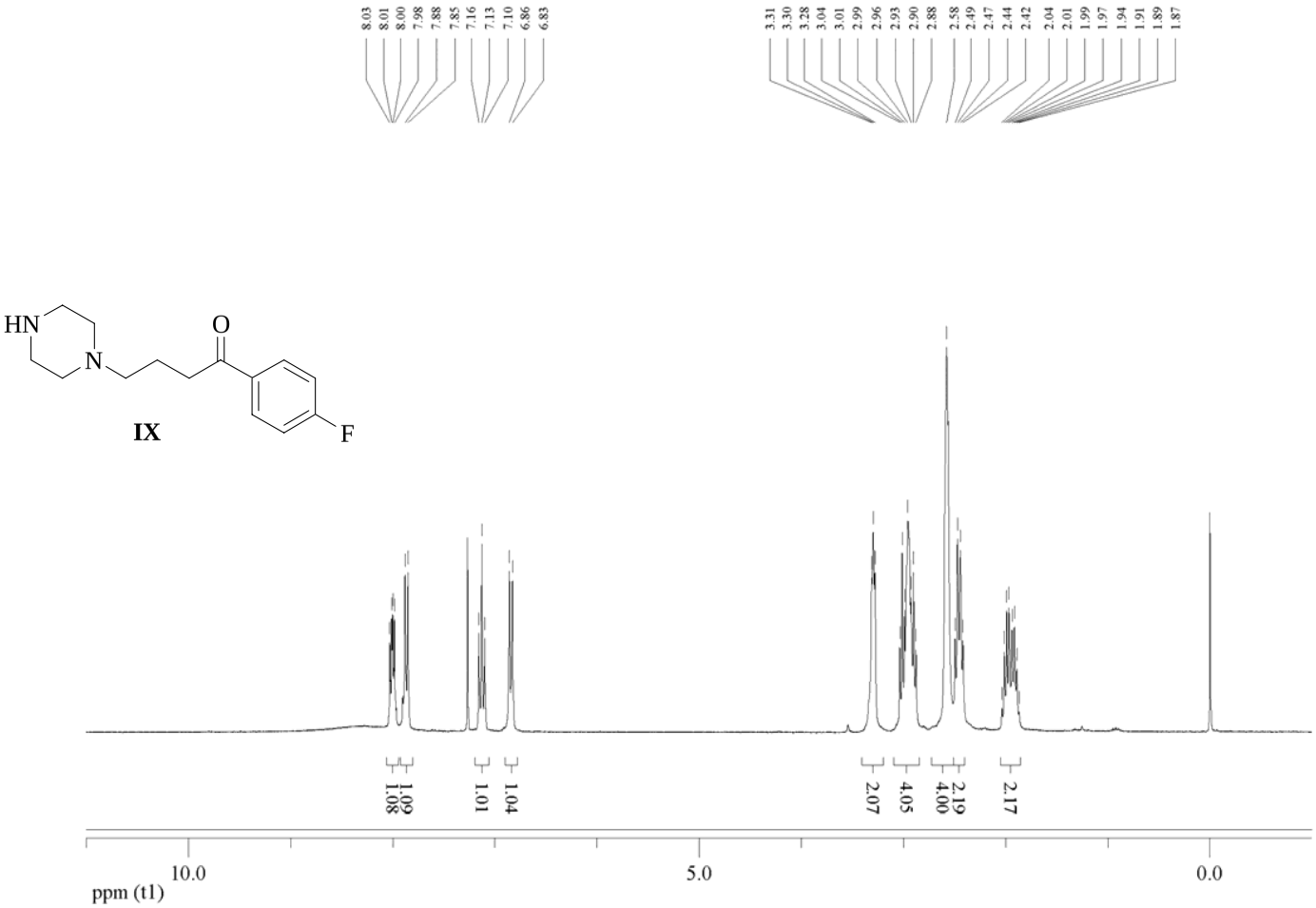
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **13a**



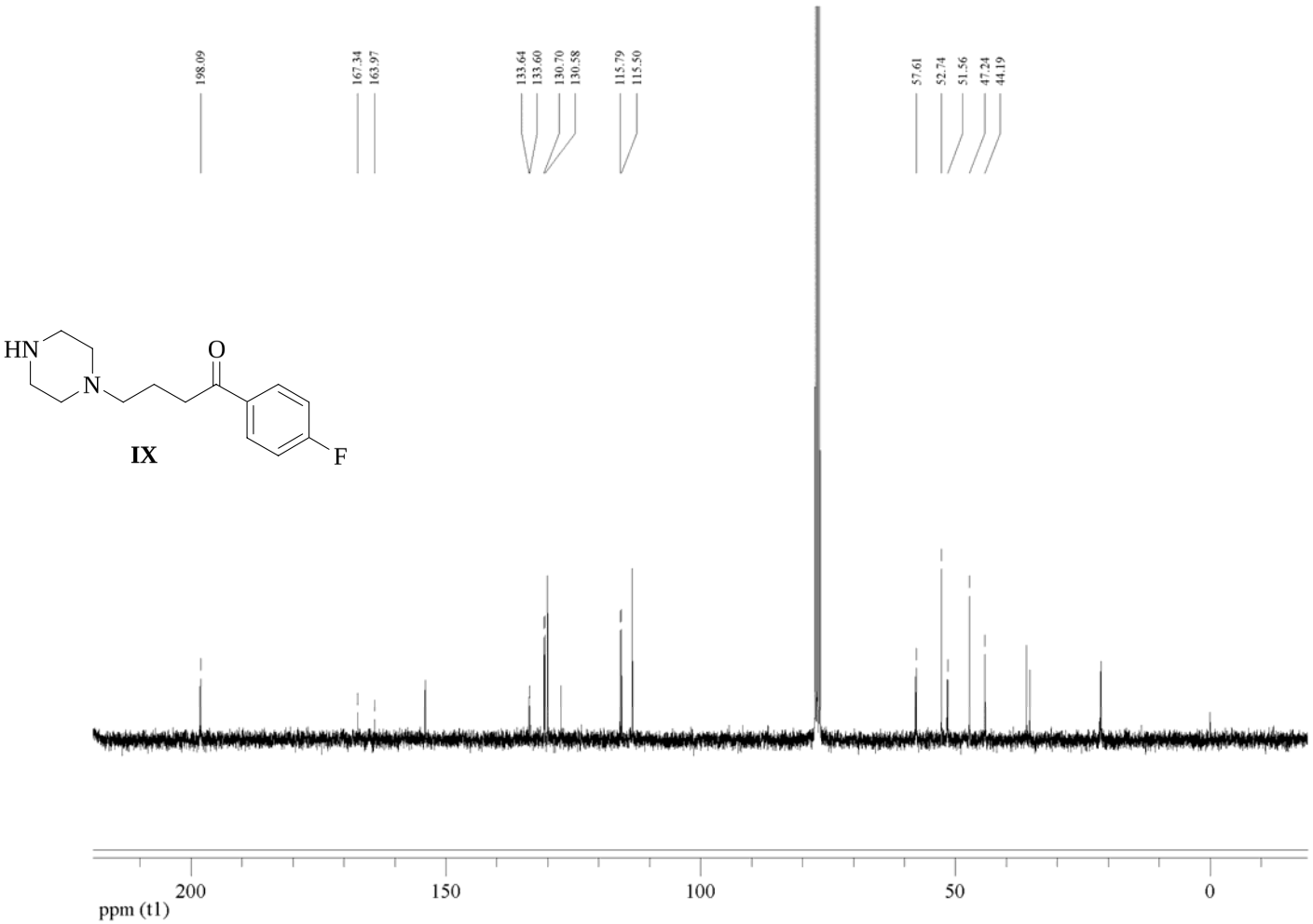
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **13a**



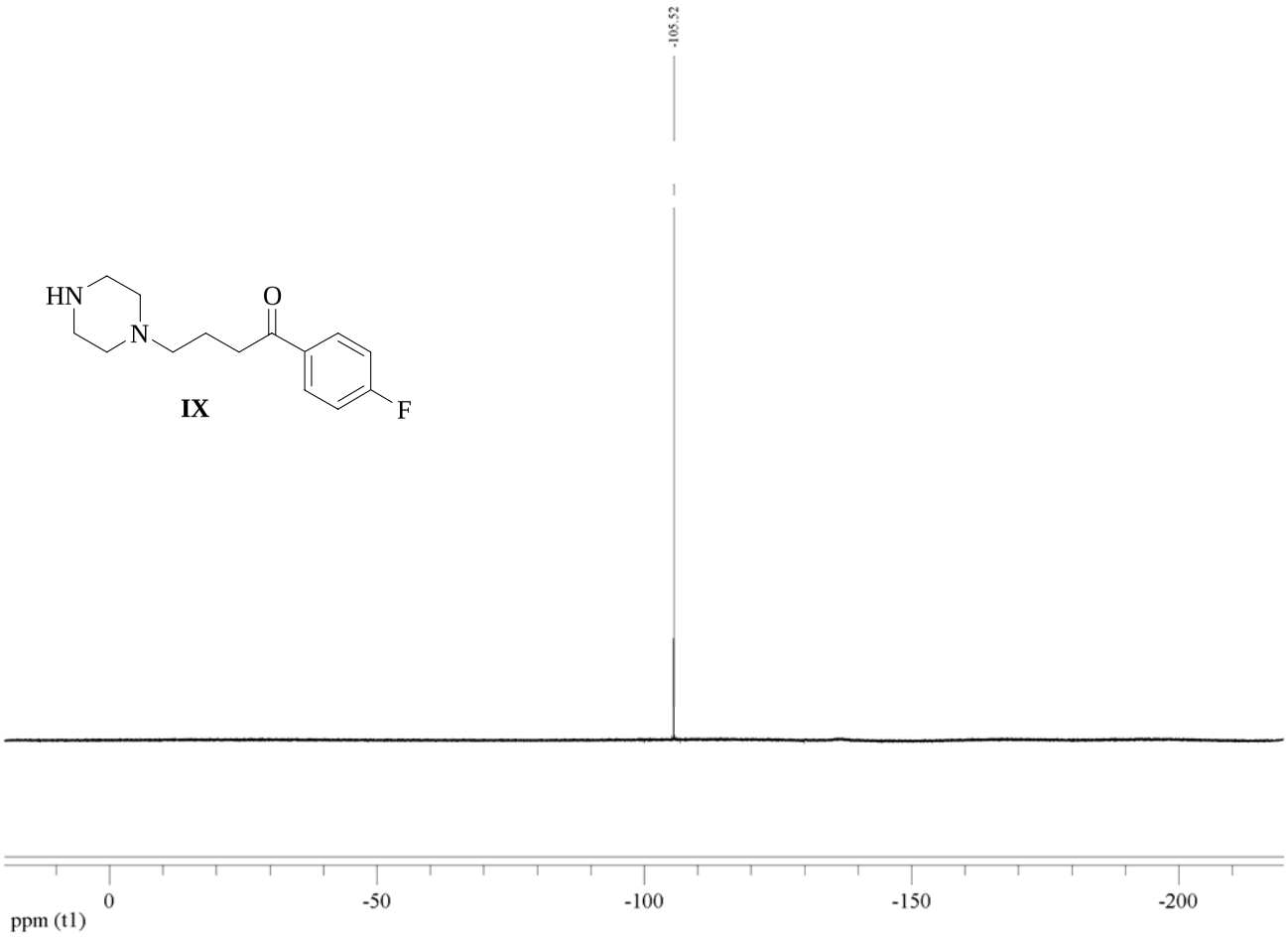
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **IX**



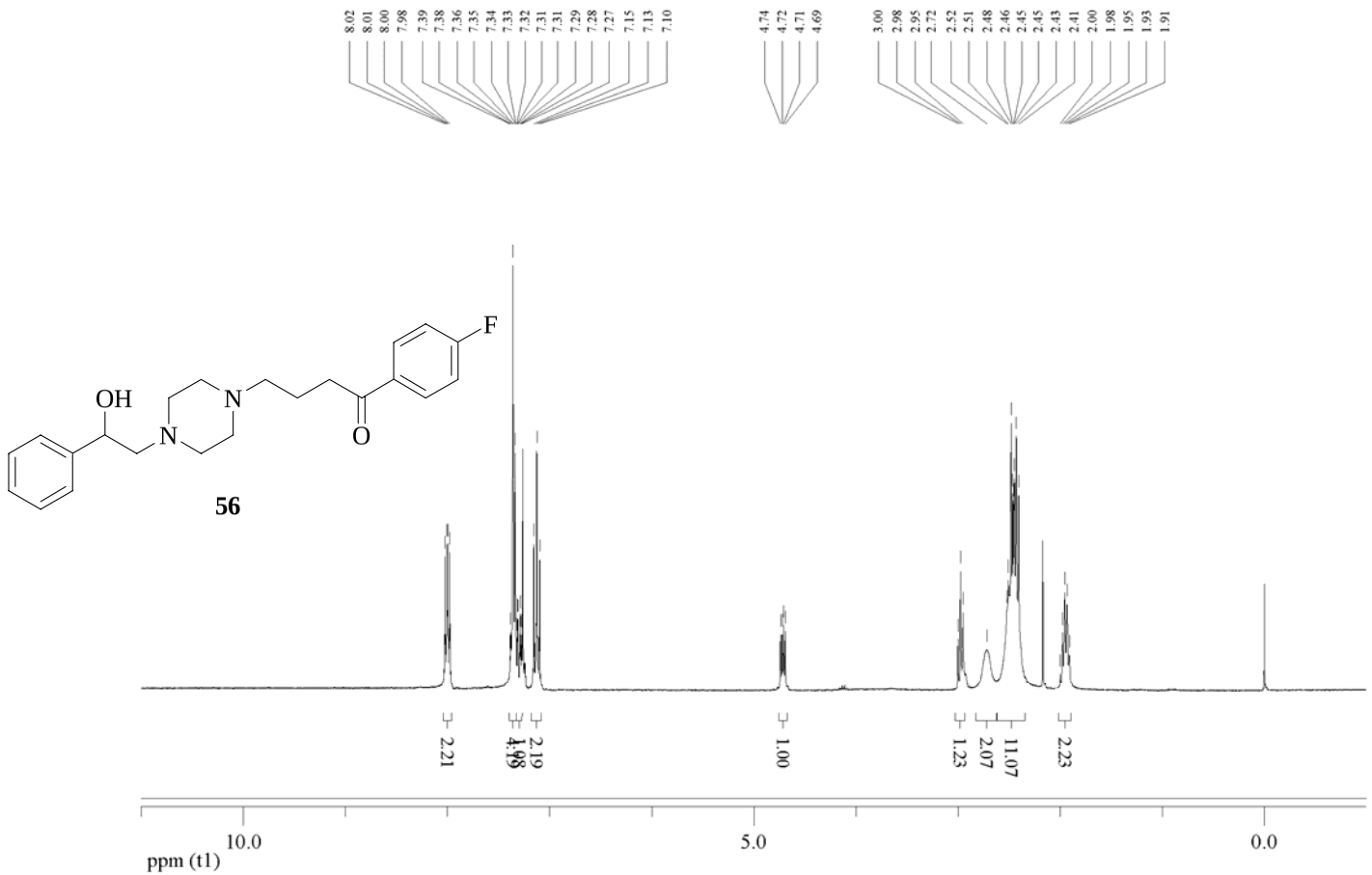
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **IX**



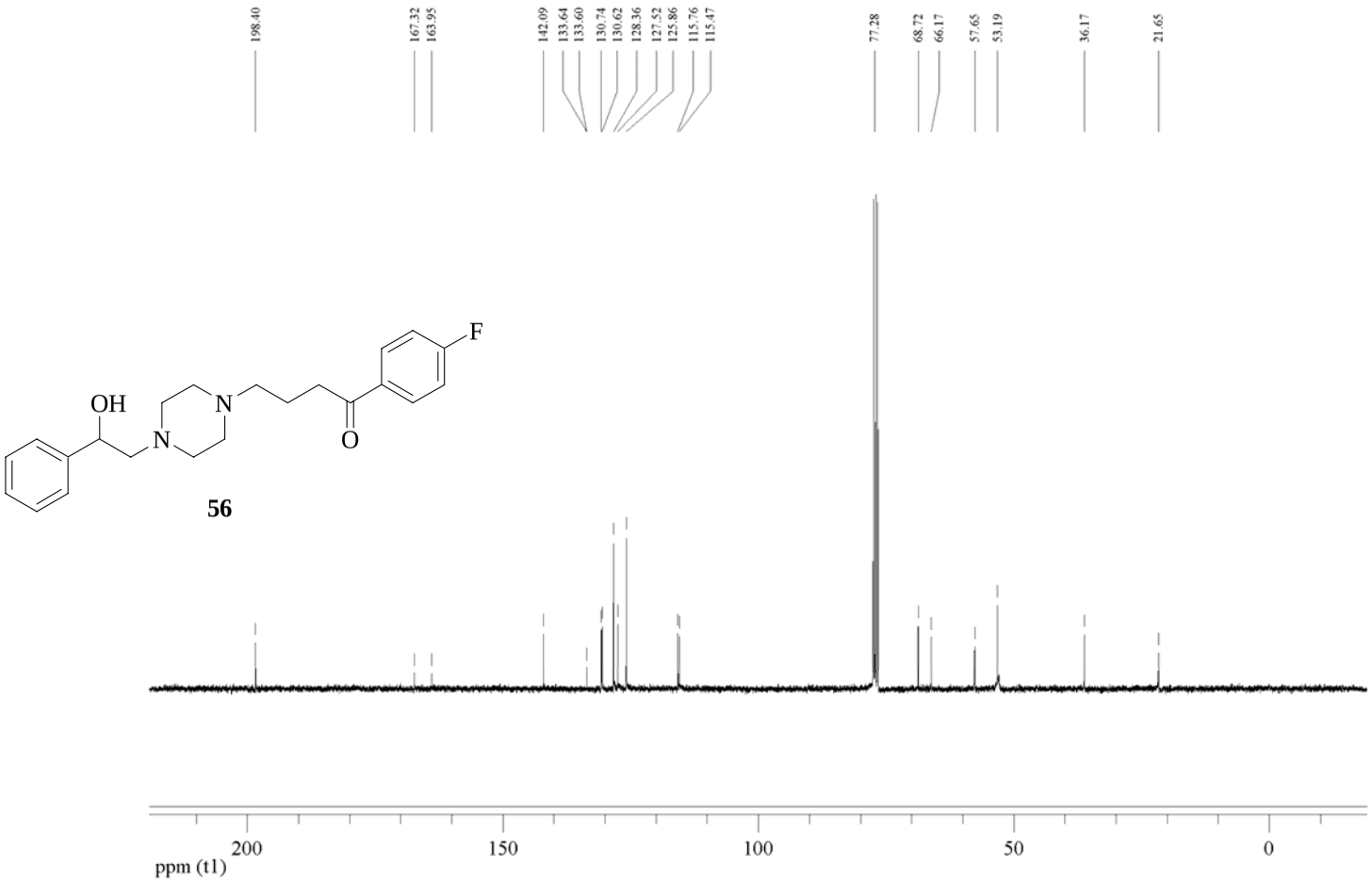
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **IX**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **56**

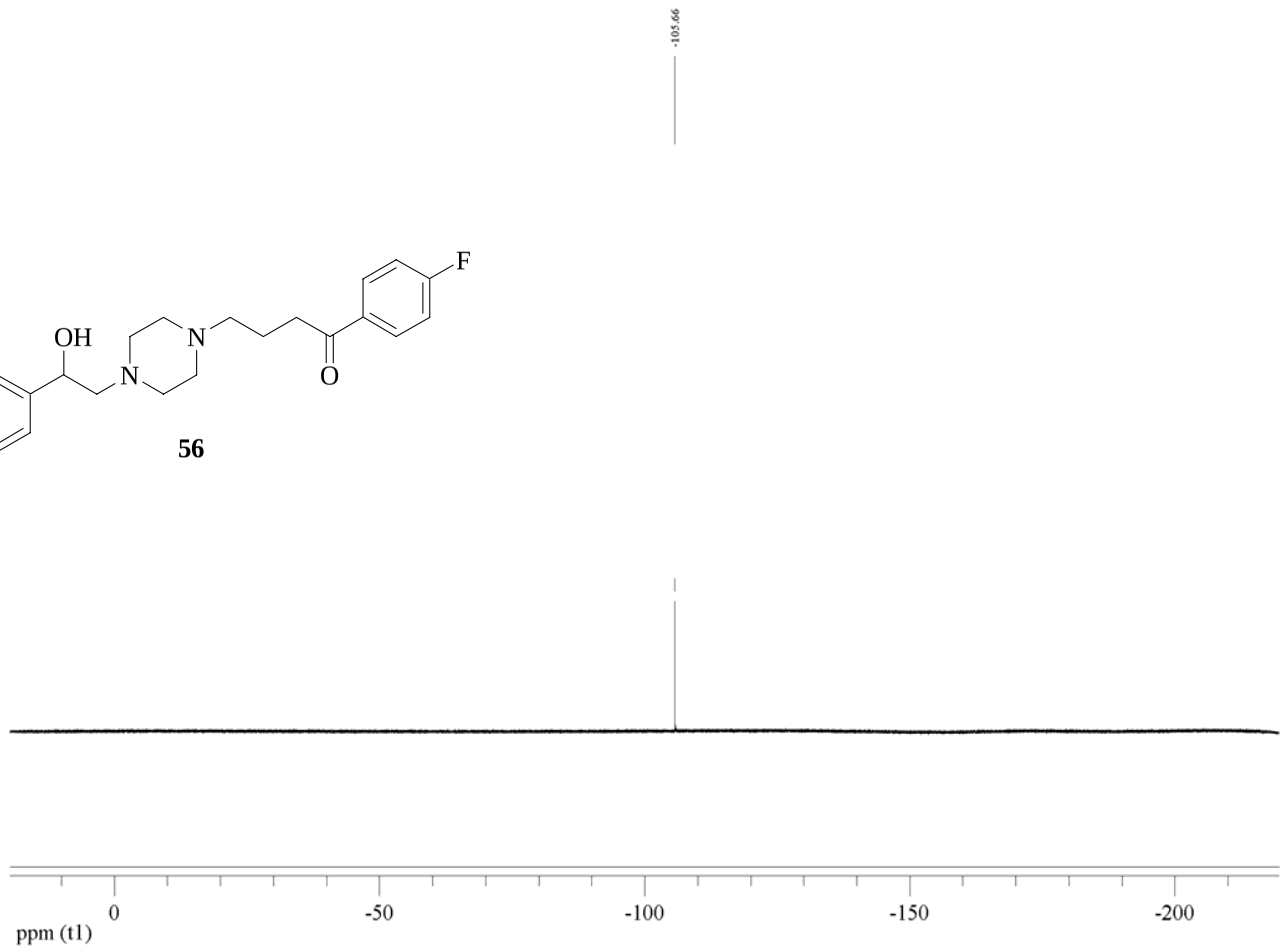


^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **56**

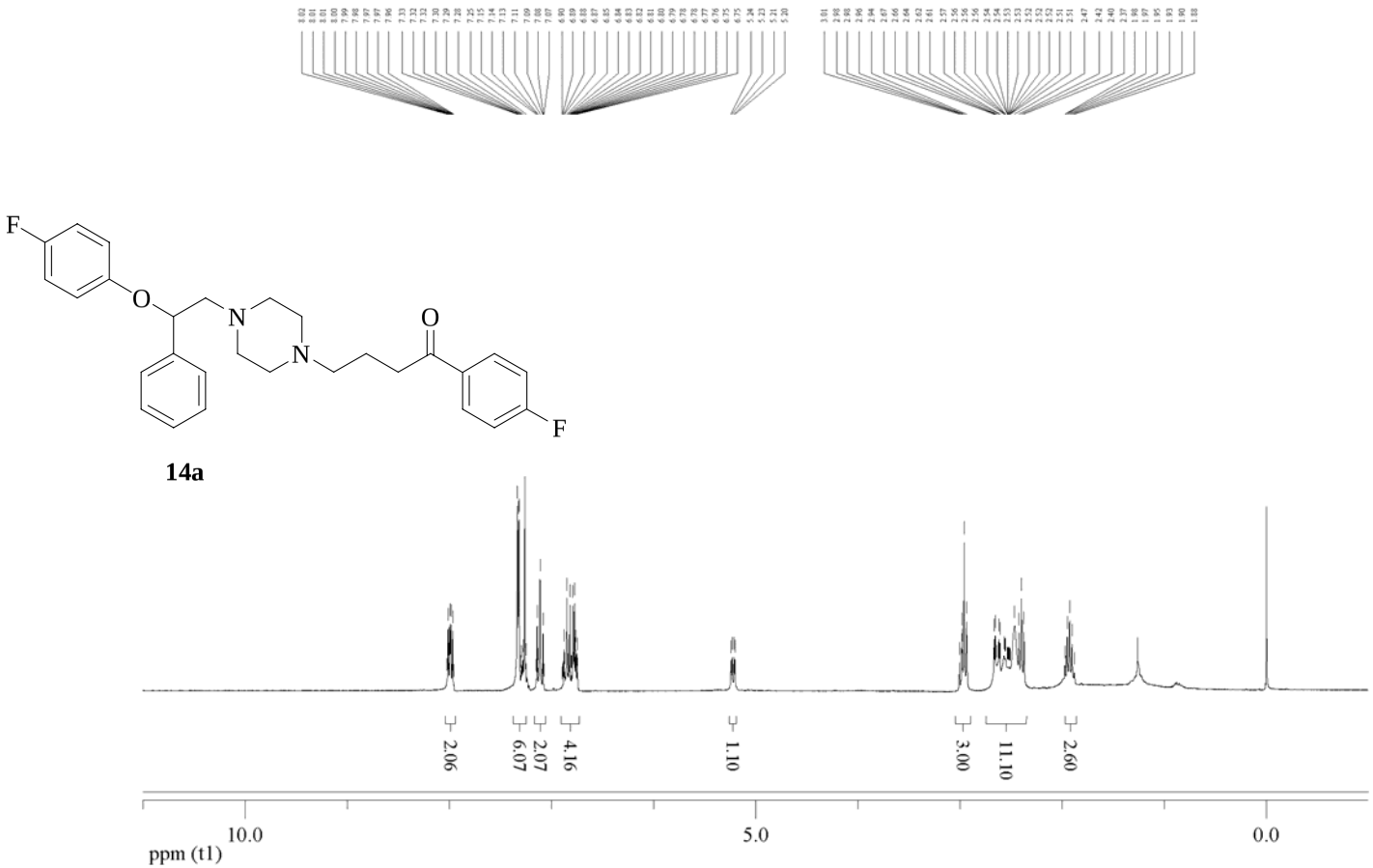


^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **56**

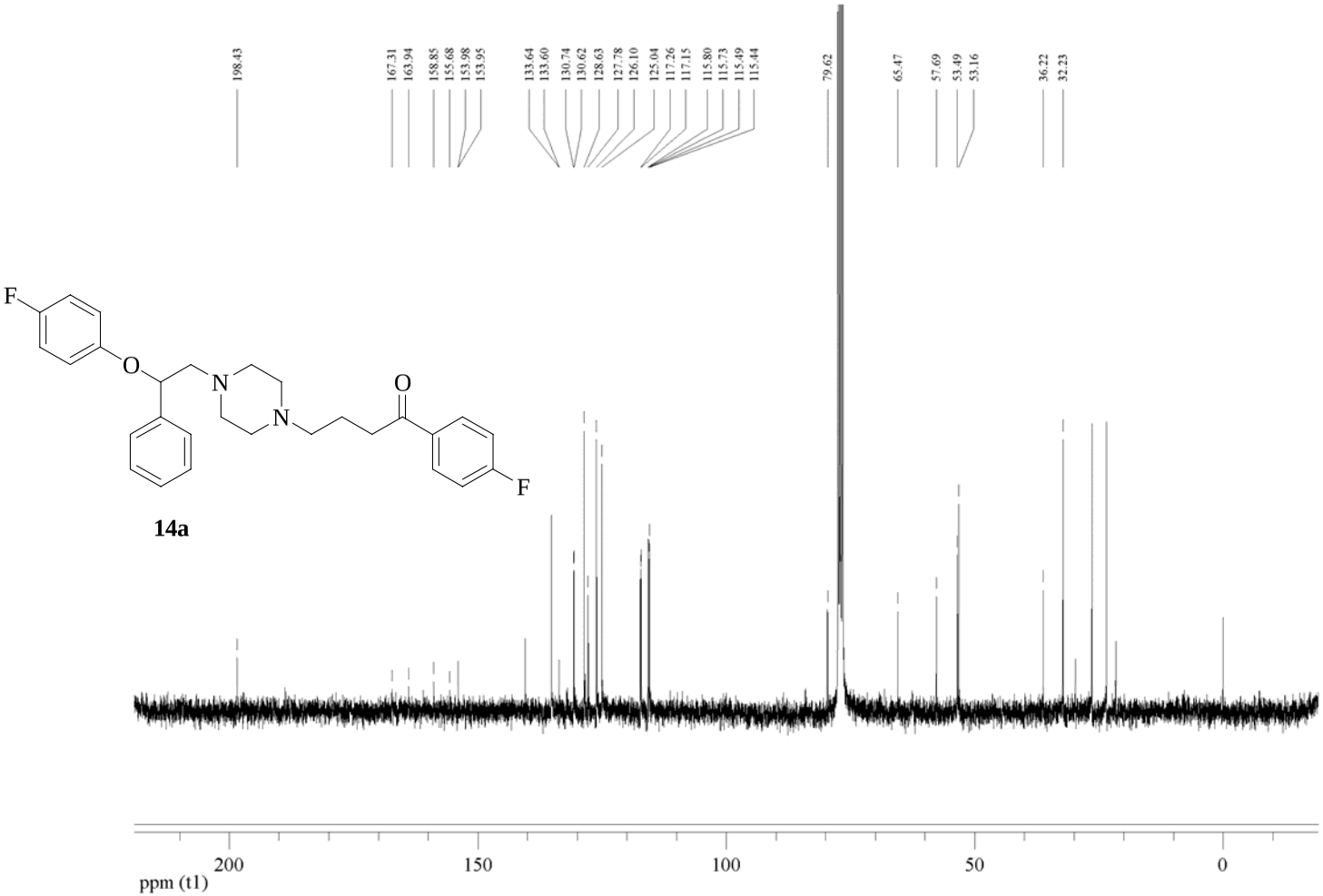
56



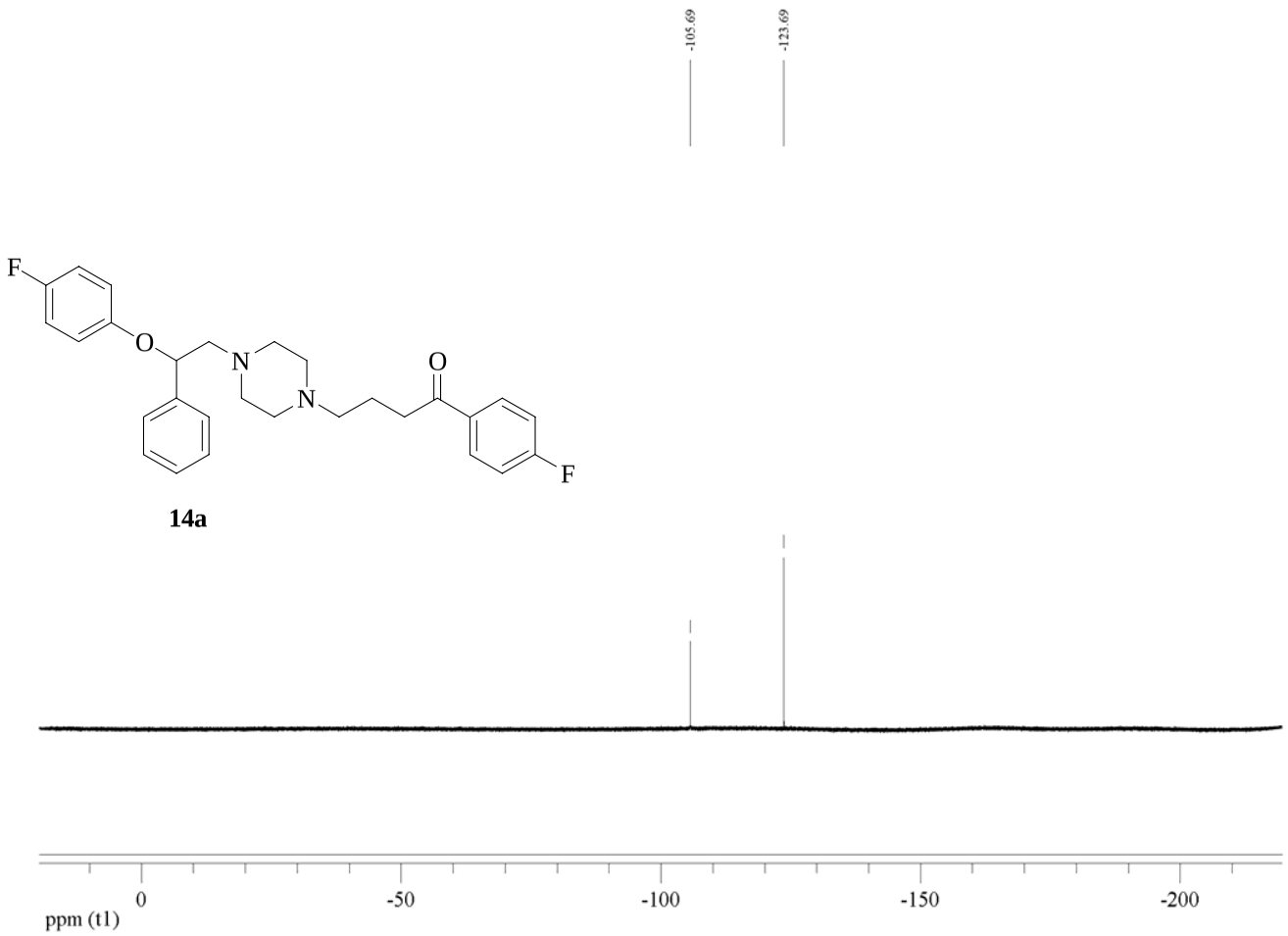
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **14a**



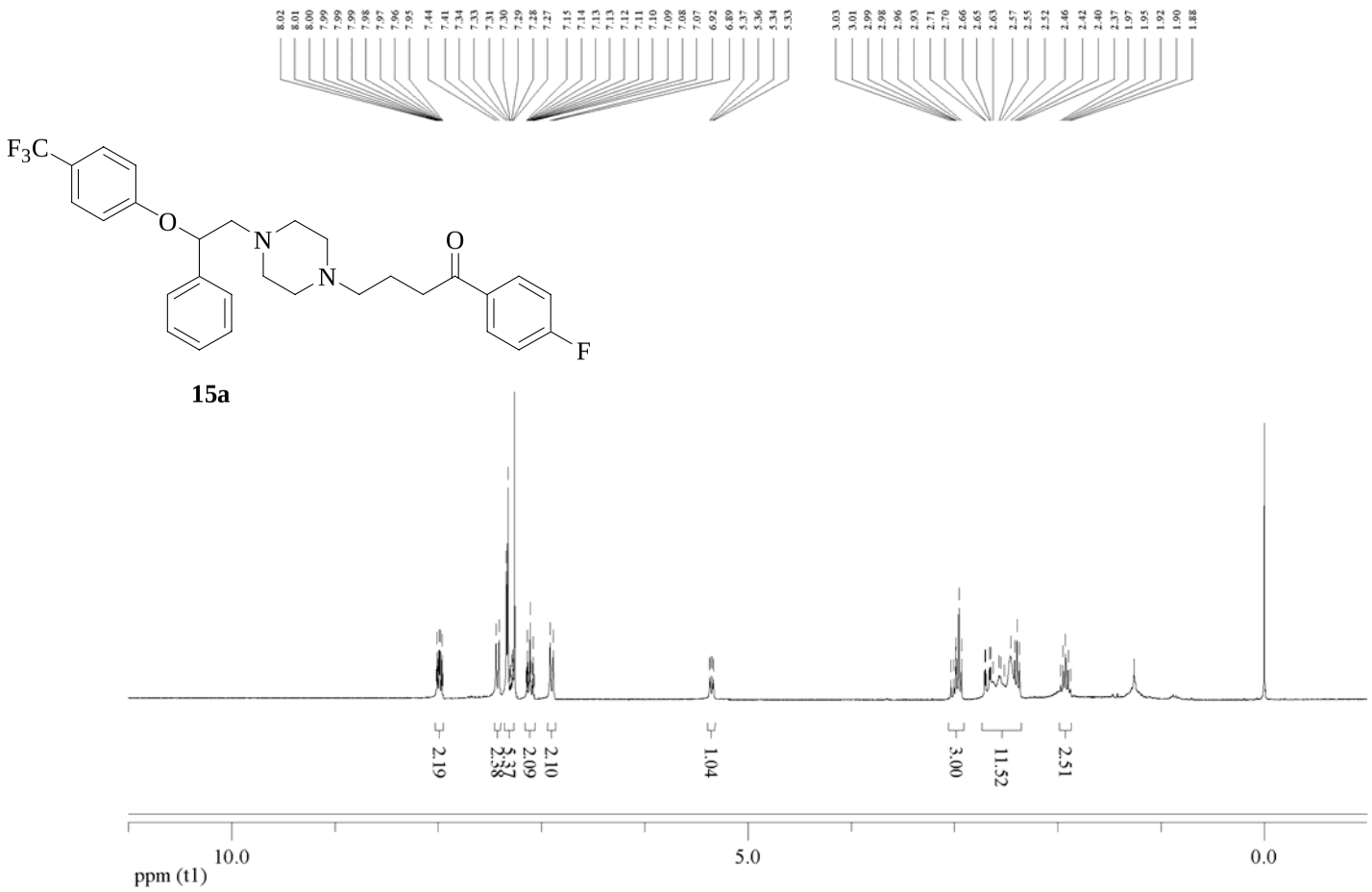
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **14a**



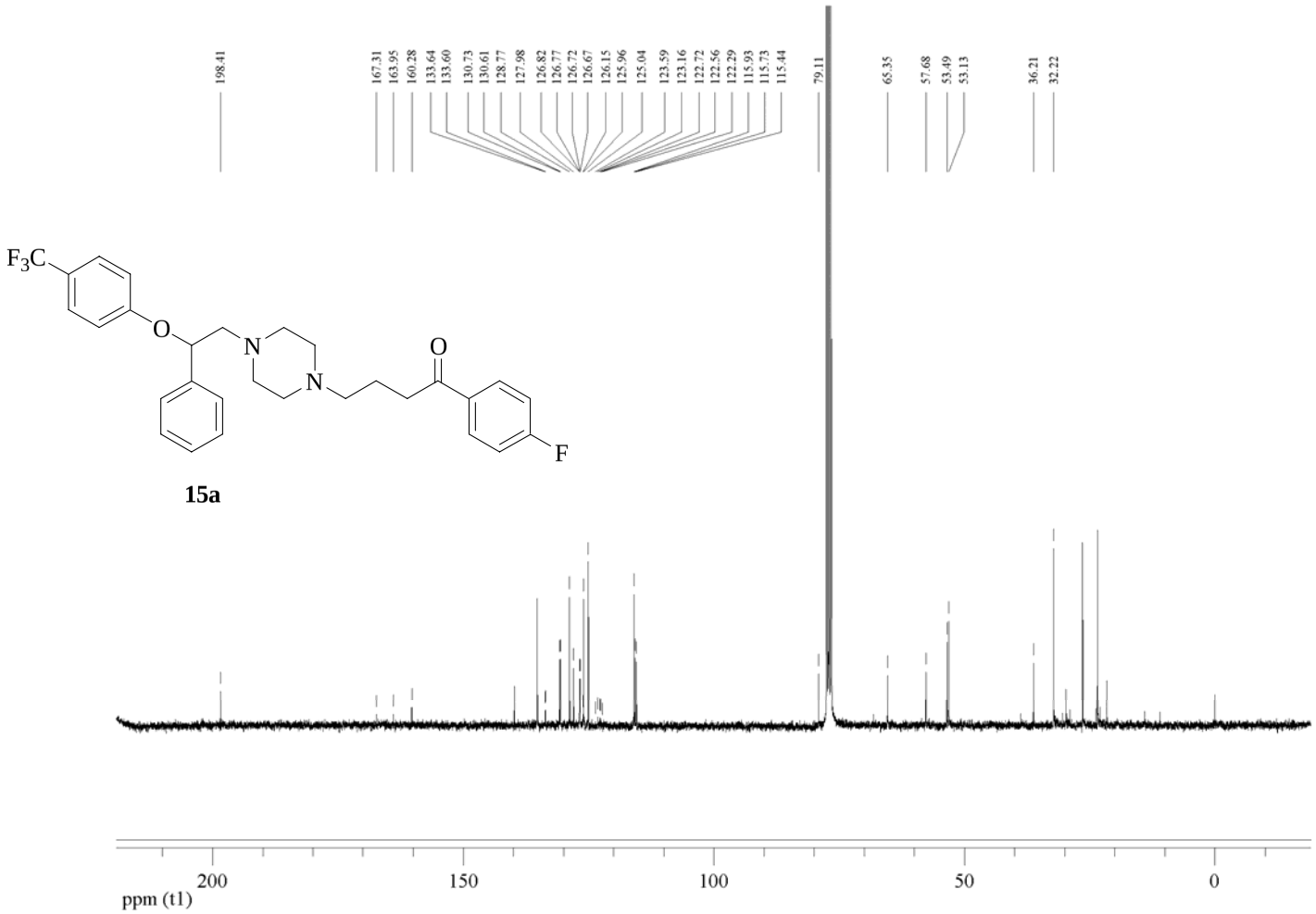
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **14a**



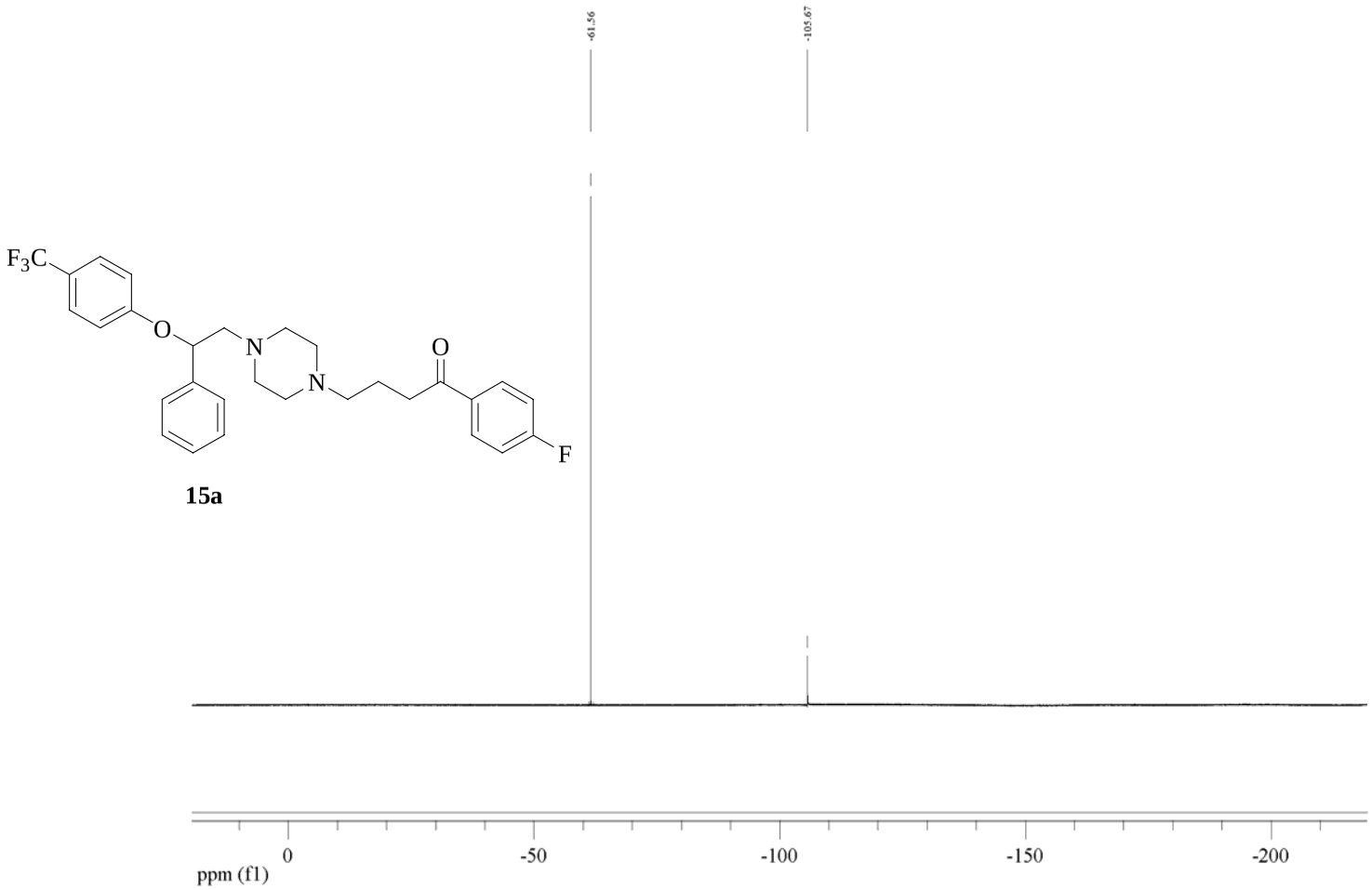
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **15a**



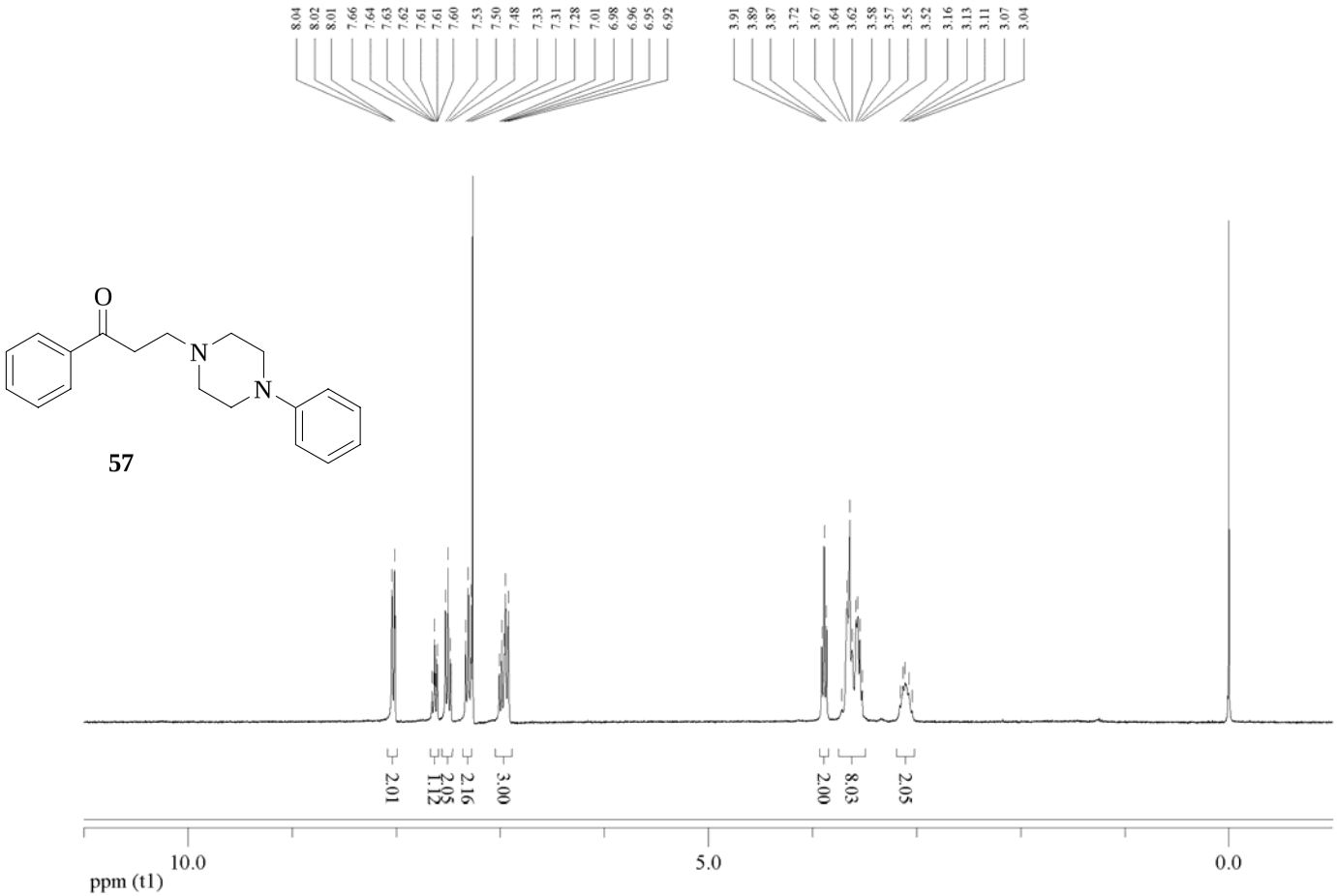
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **15a**



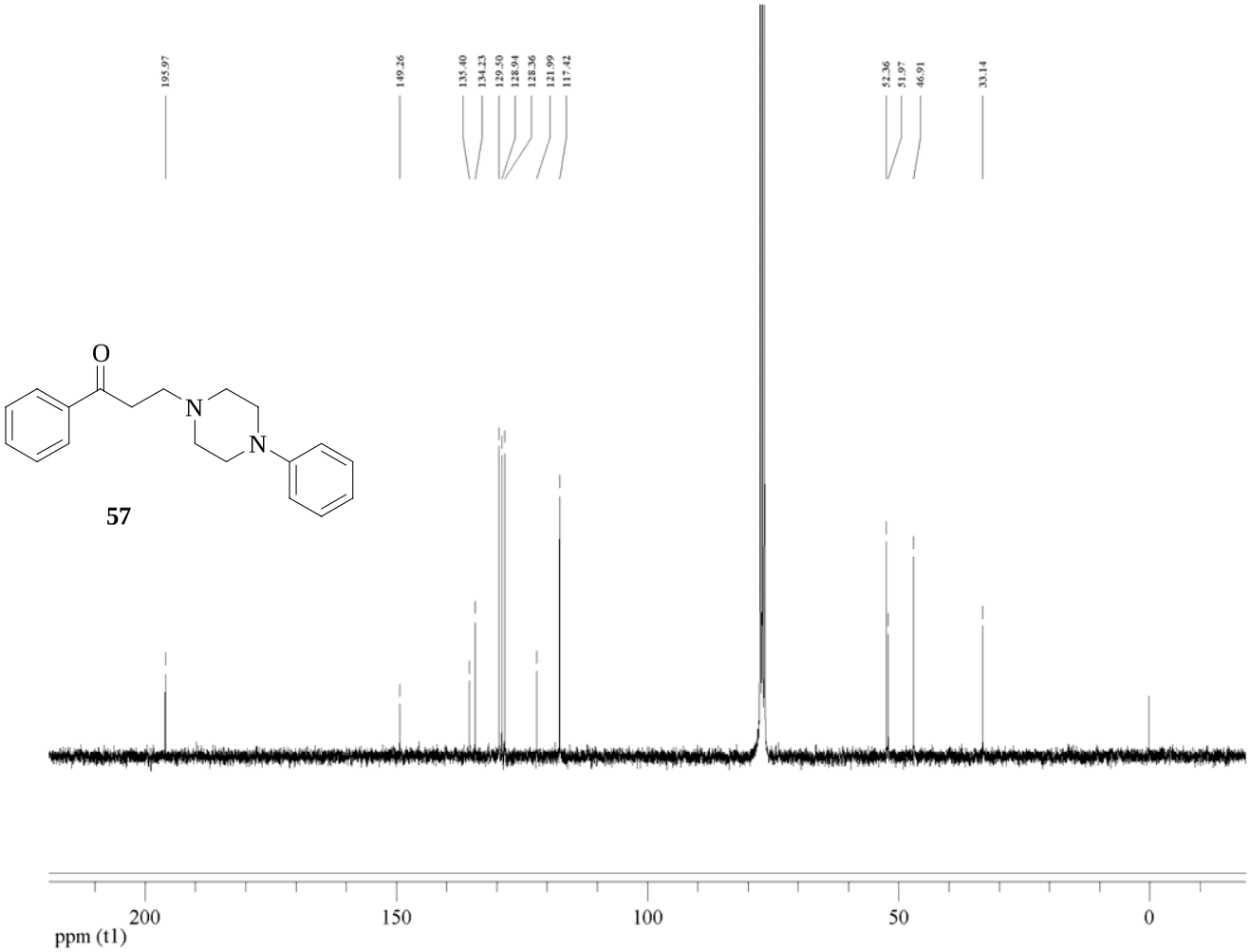
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **15a**



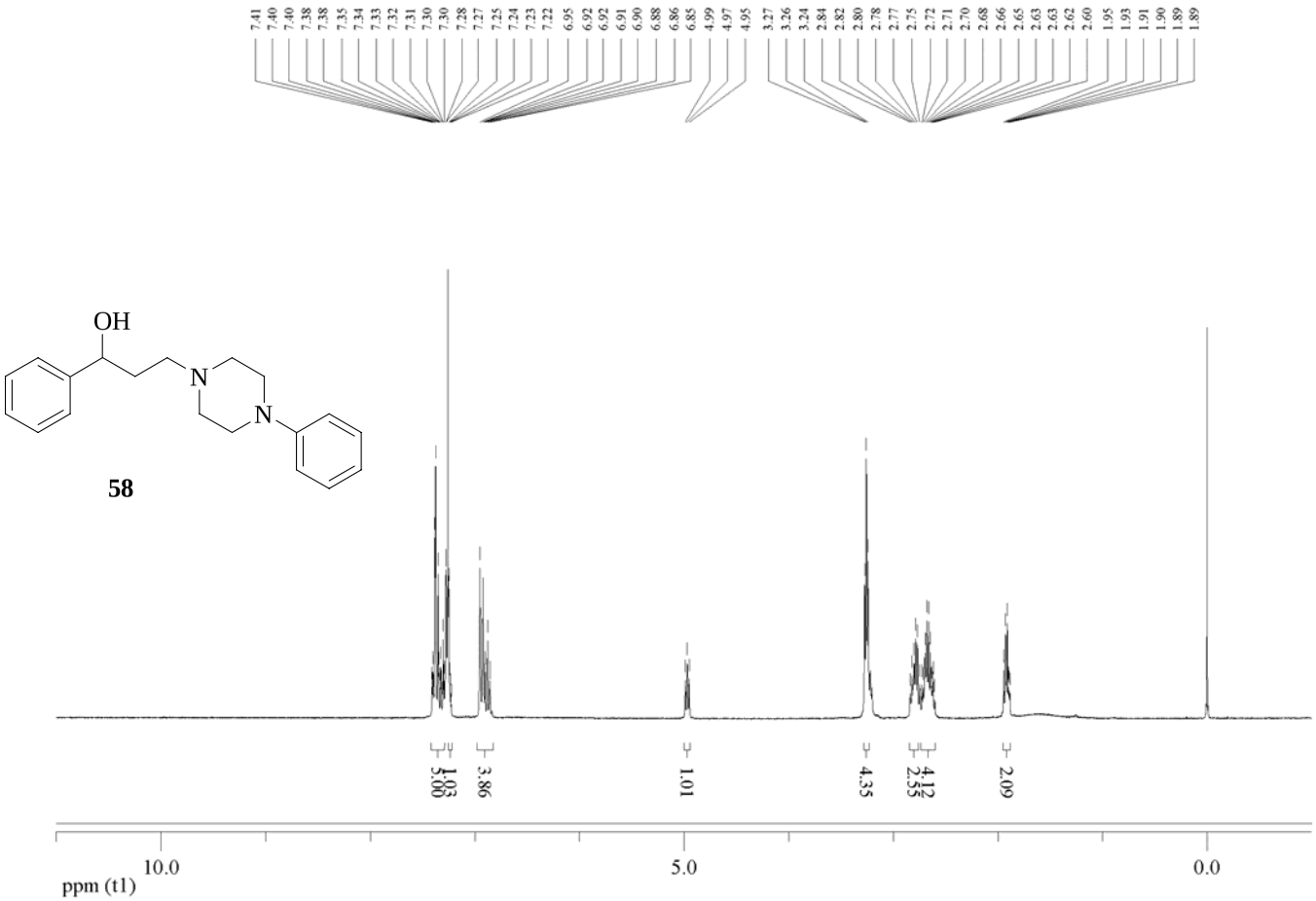
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **57**



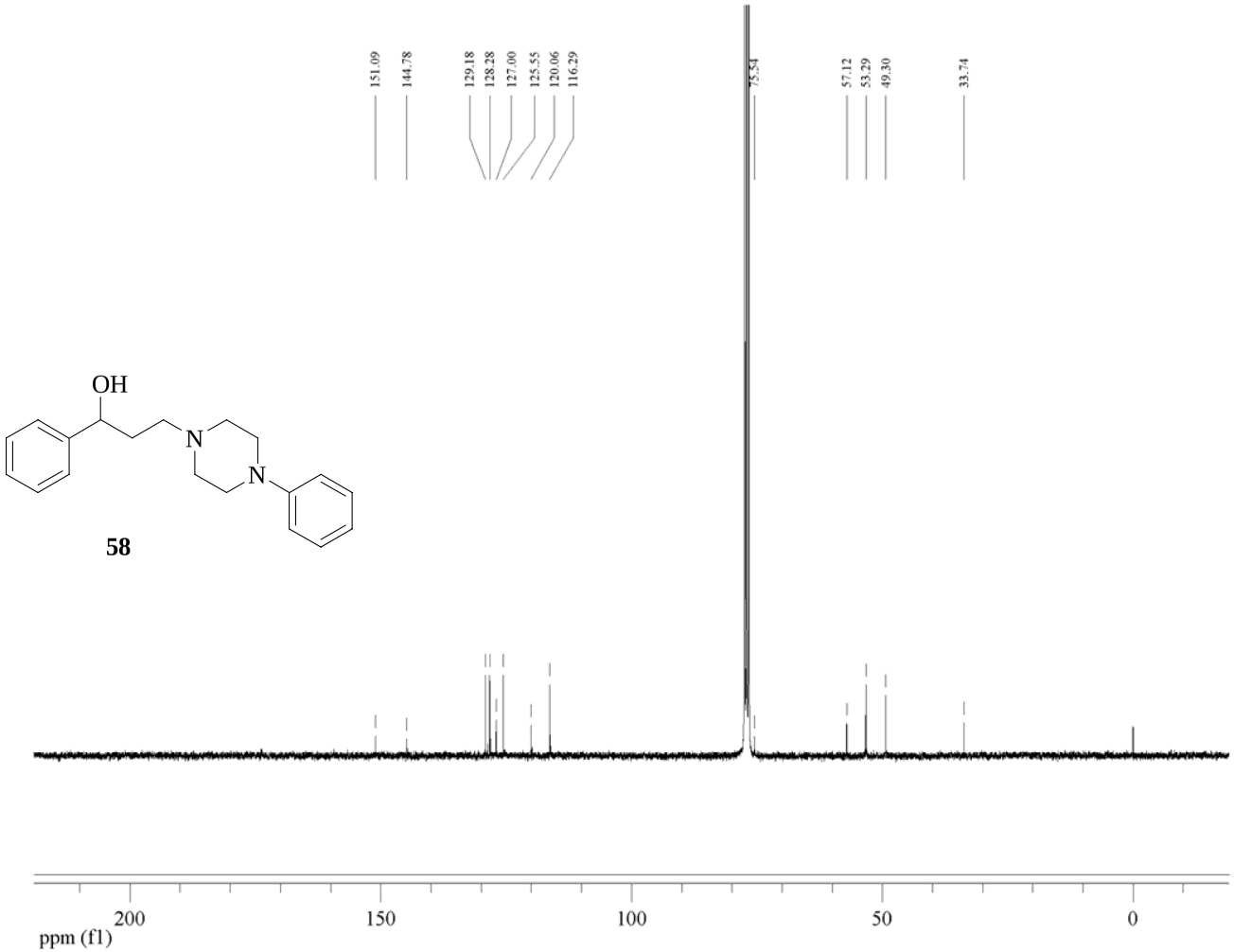
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **57**



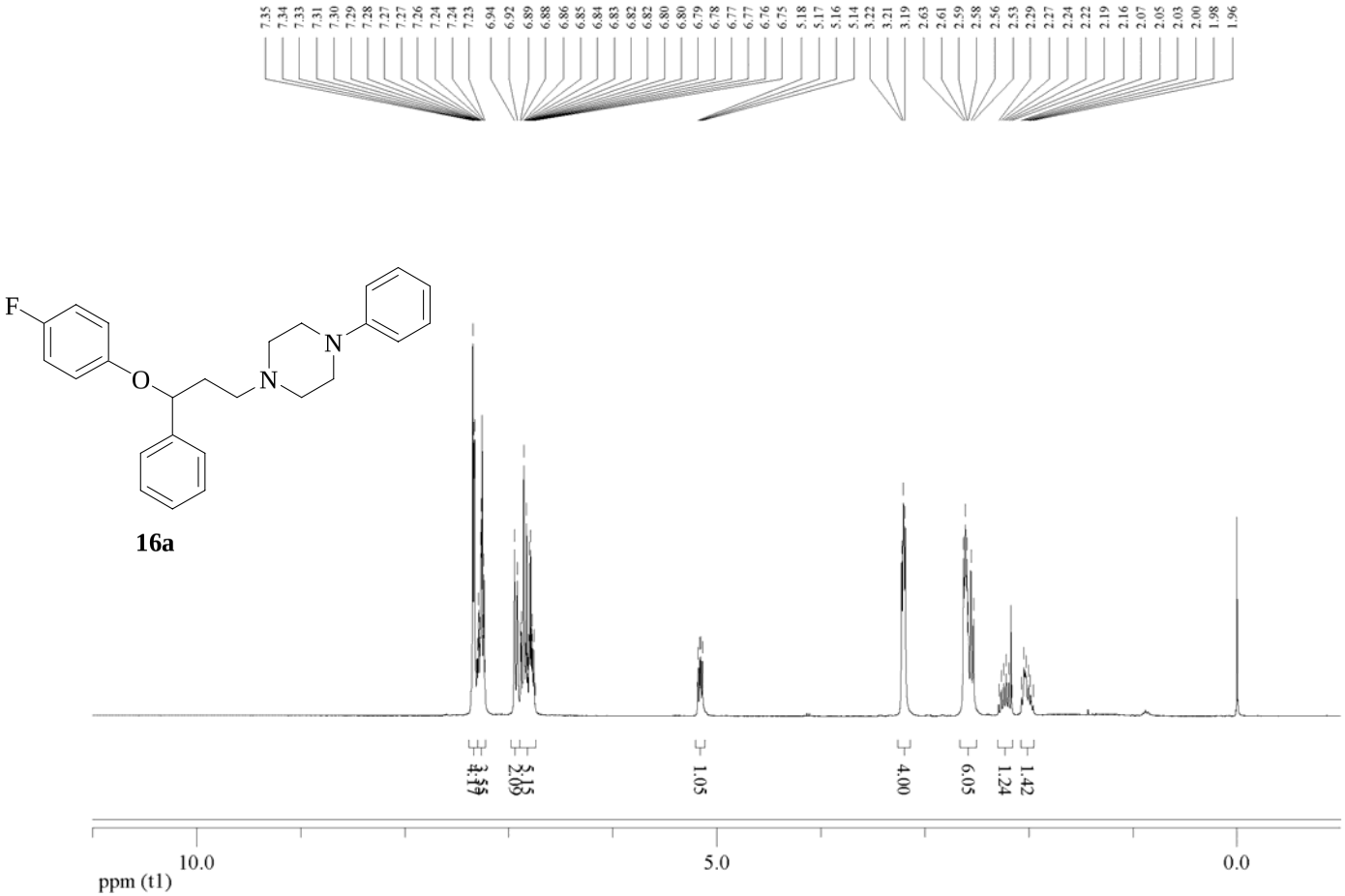
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **58**



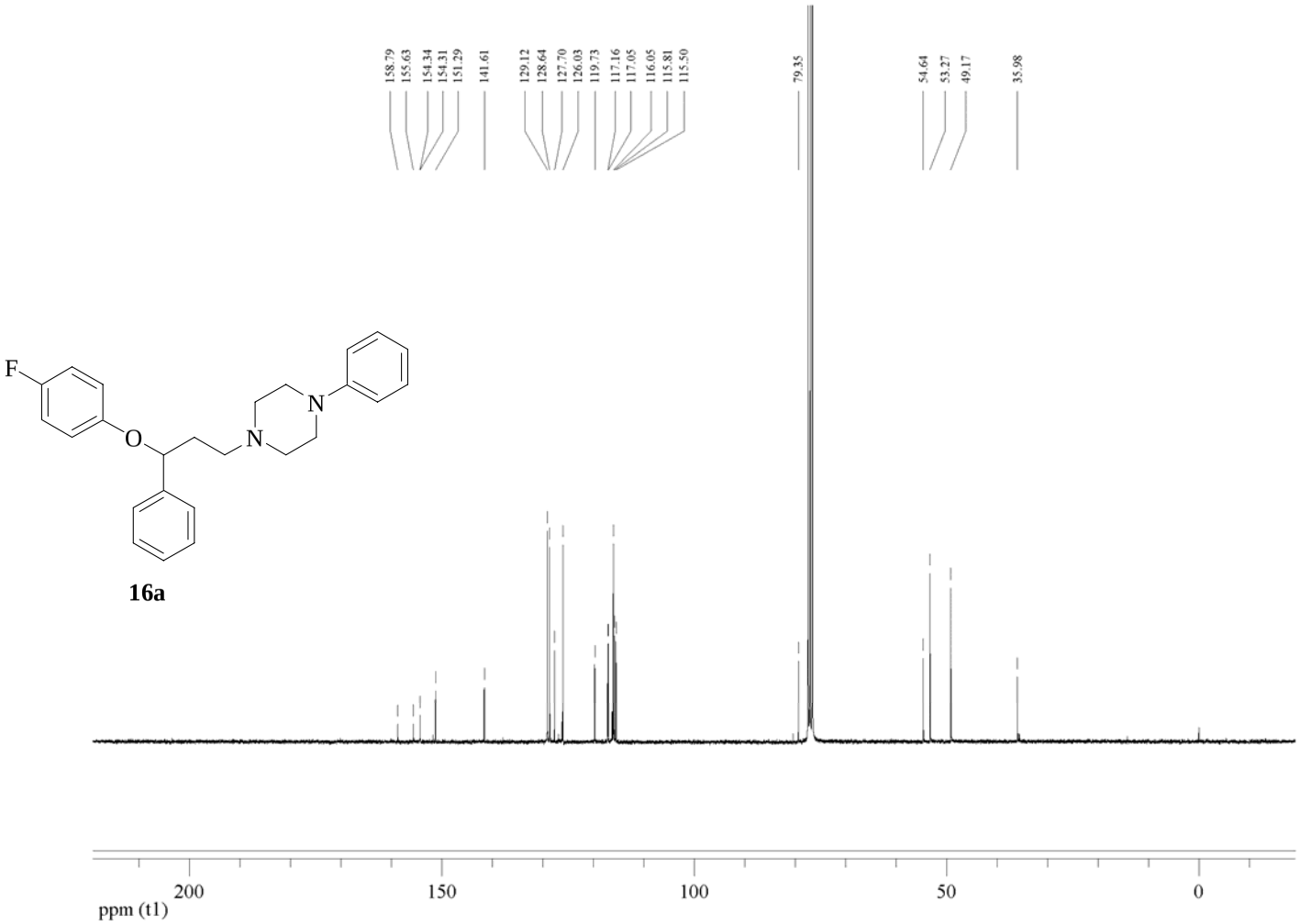
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **58**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **16a**

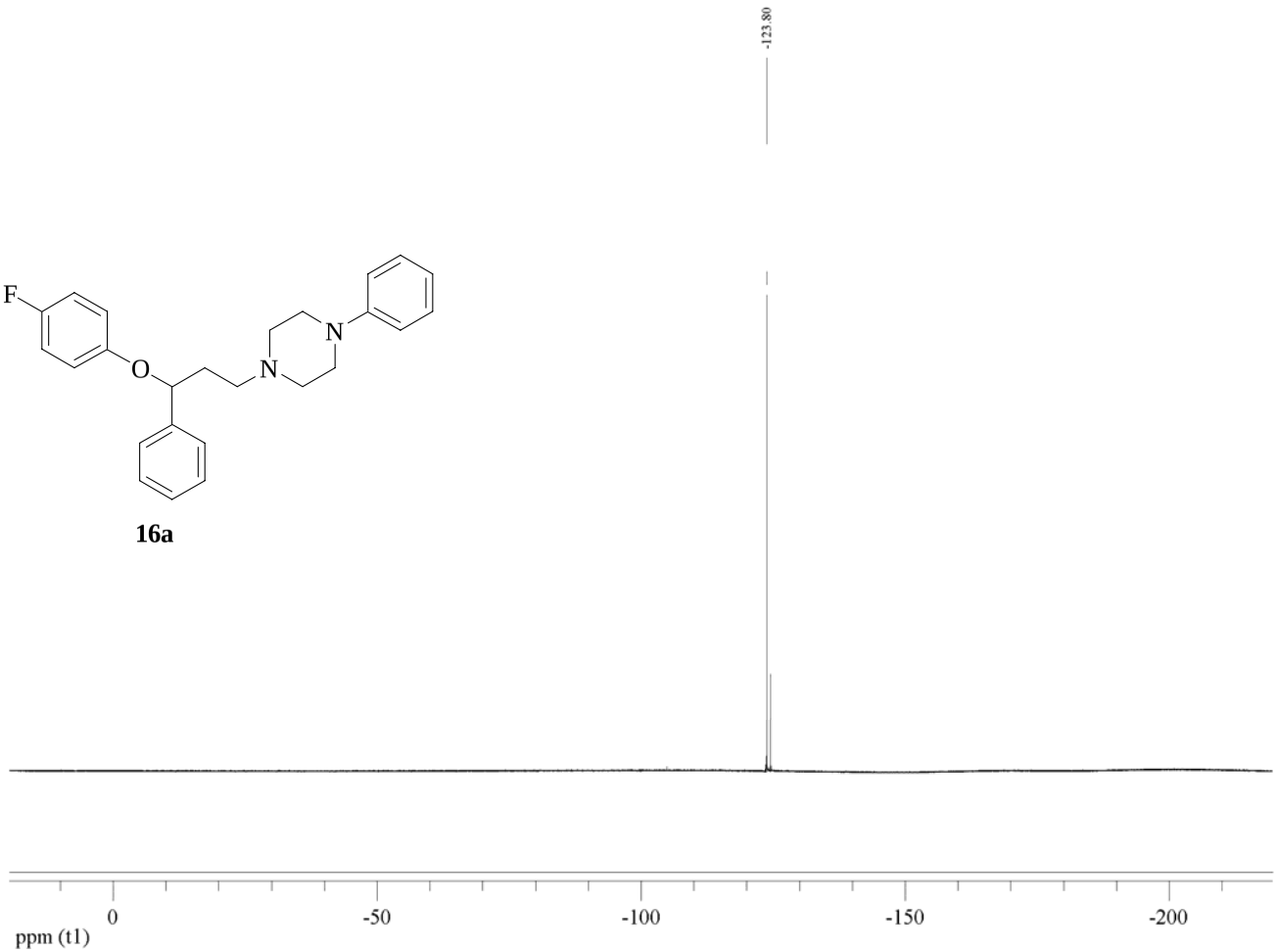


^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **16a**

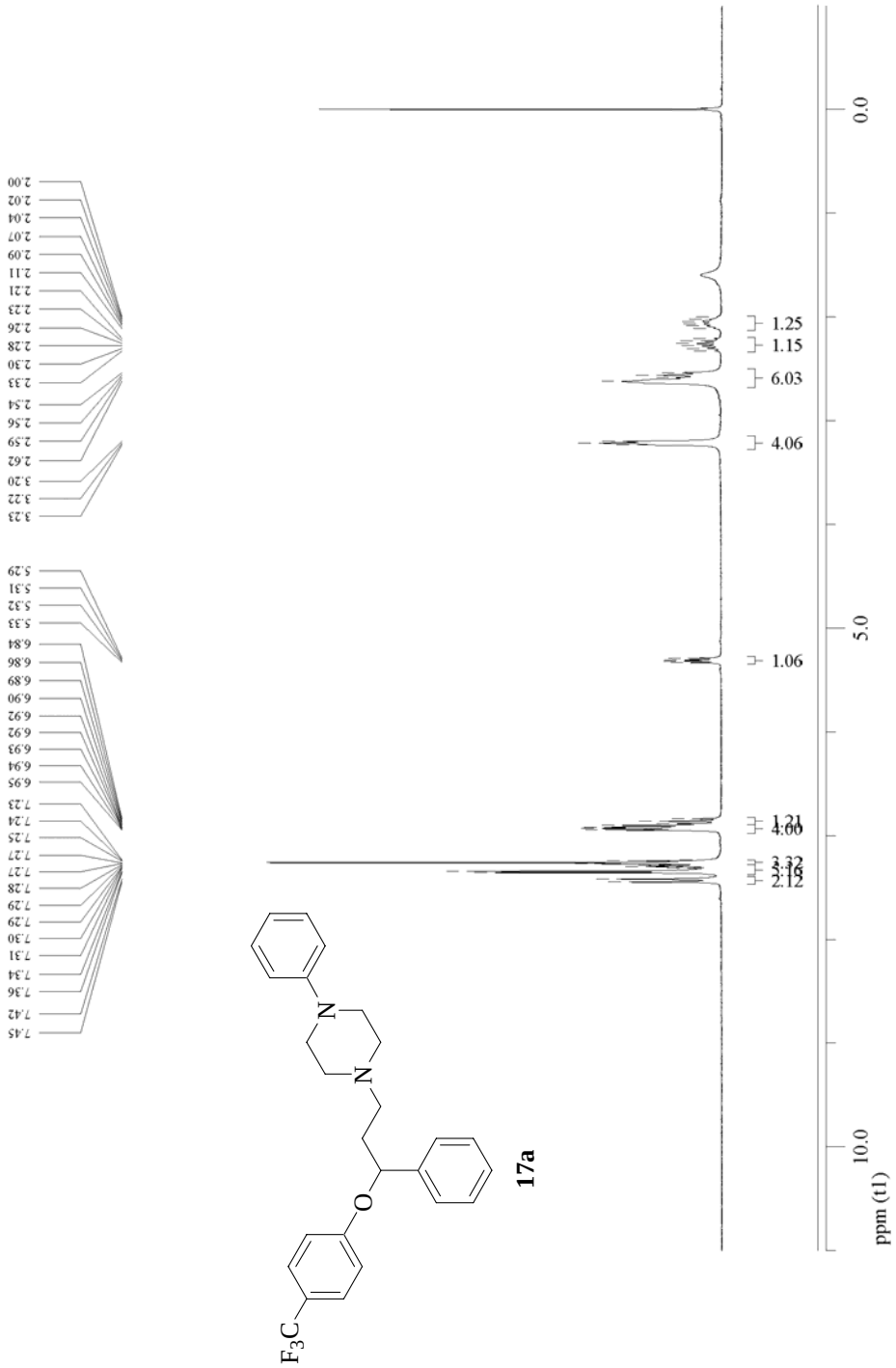


^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **16a**

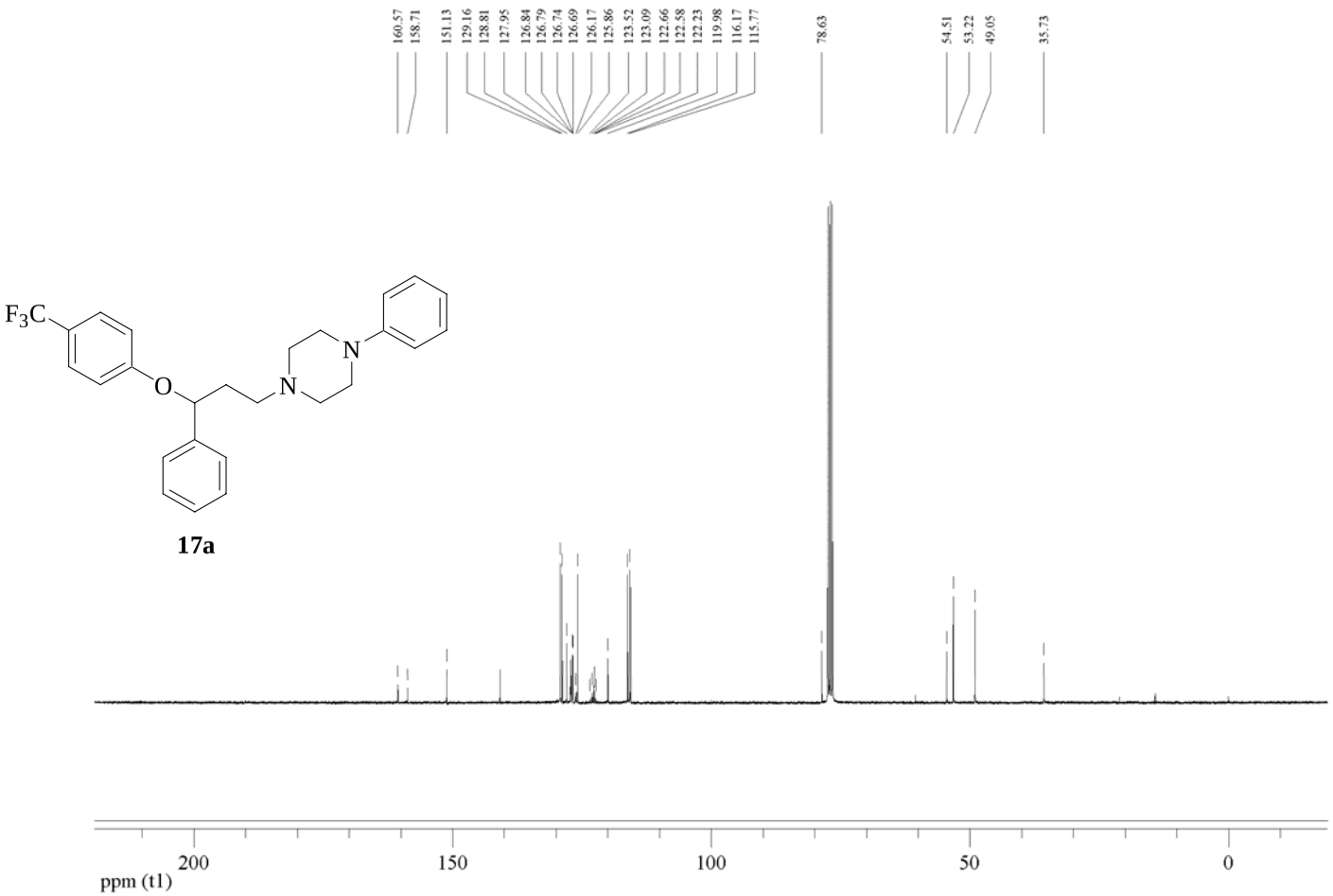
16a



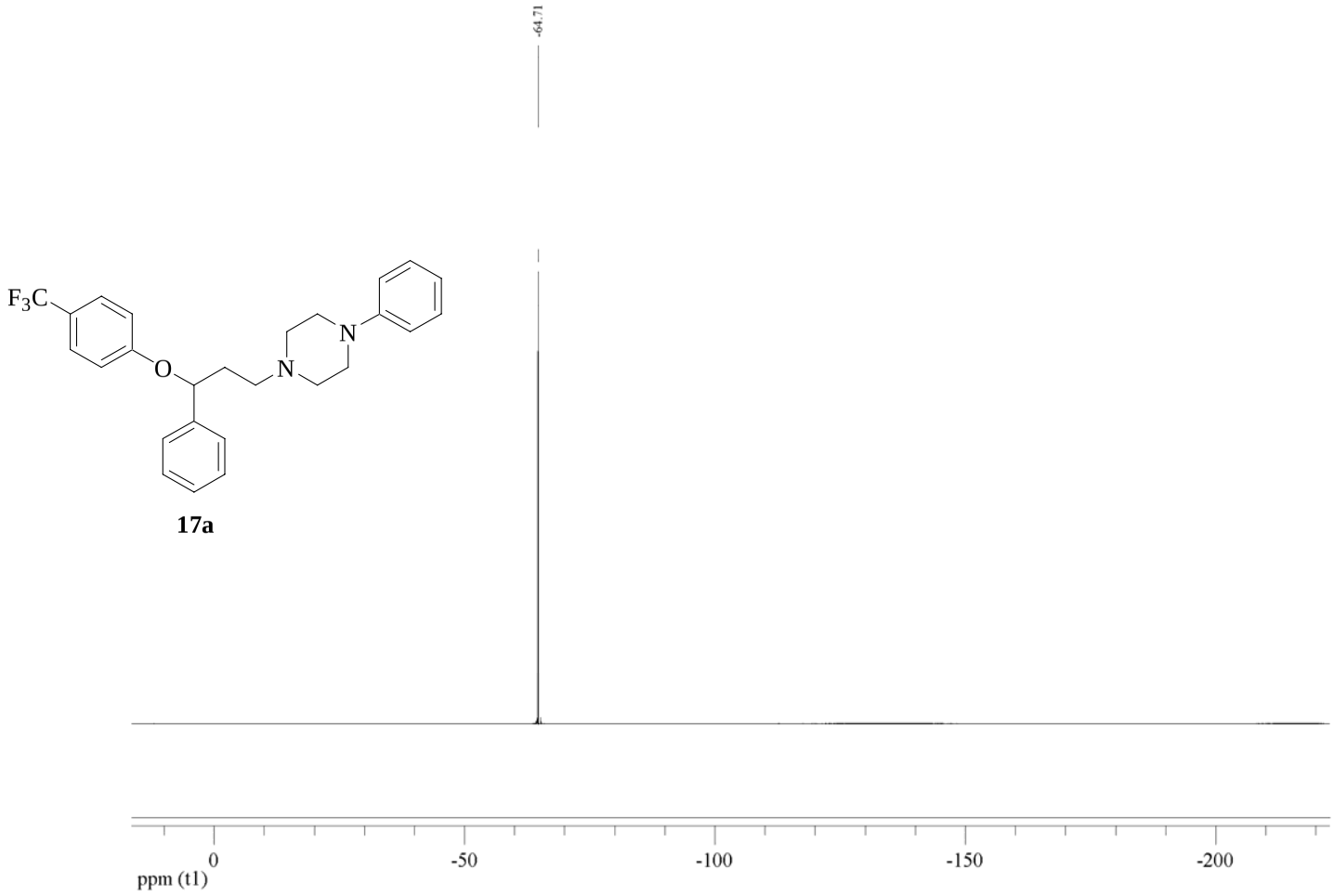
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **17a**



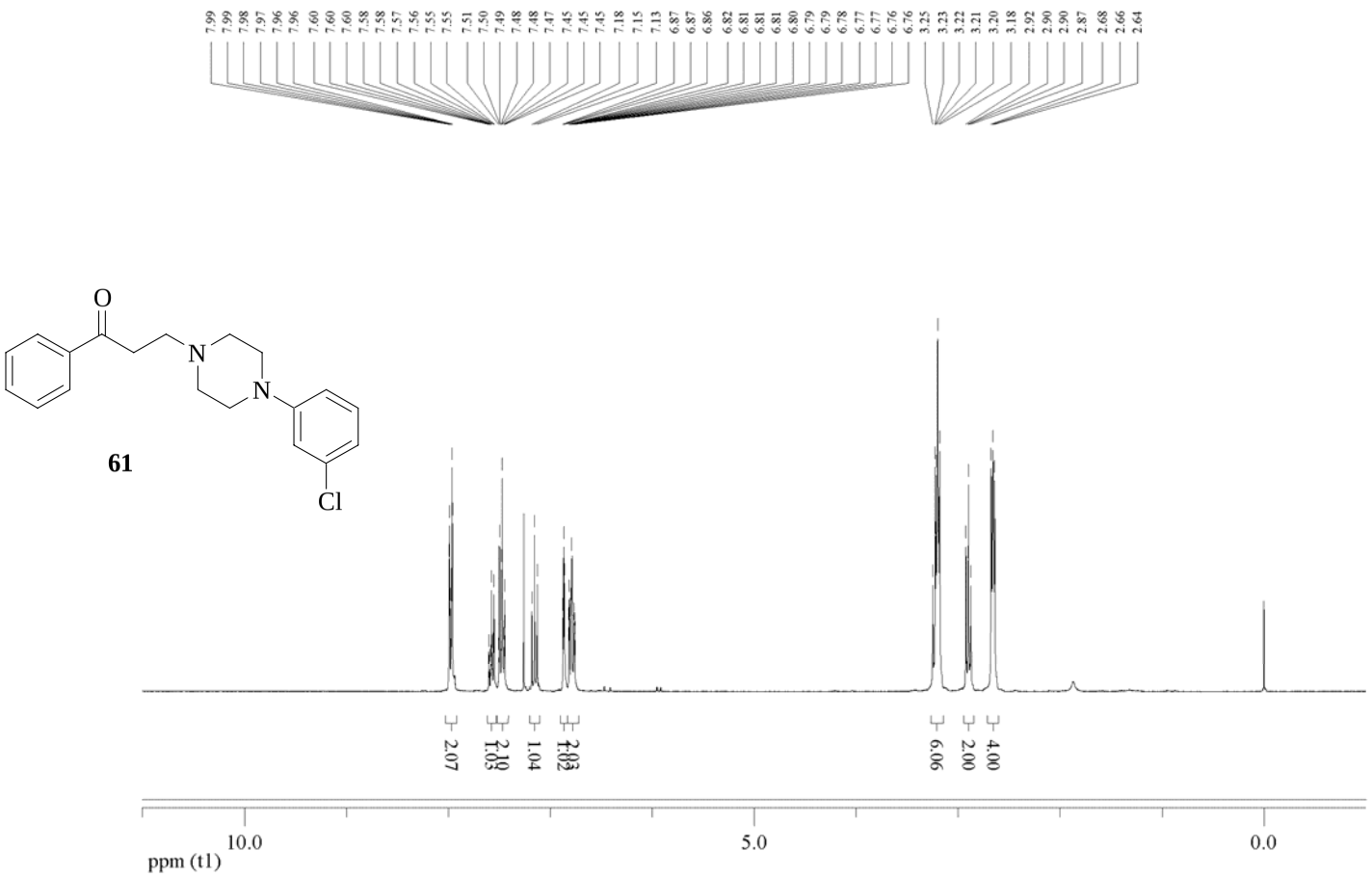
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **17a**



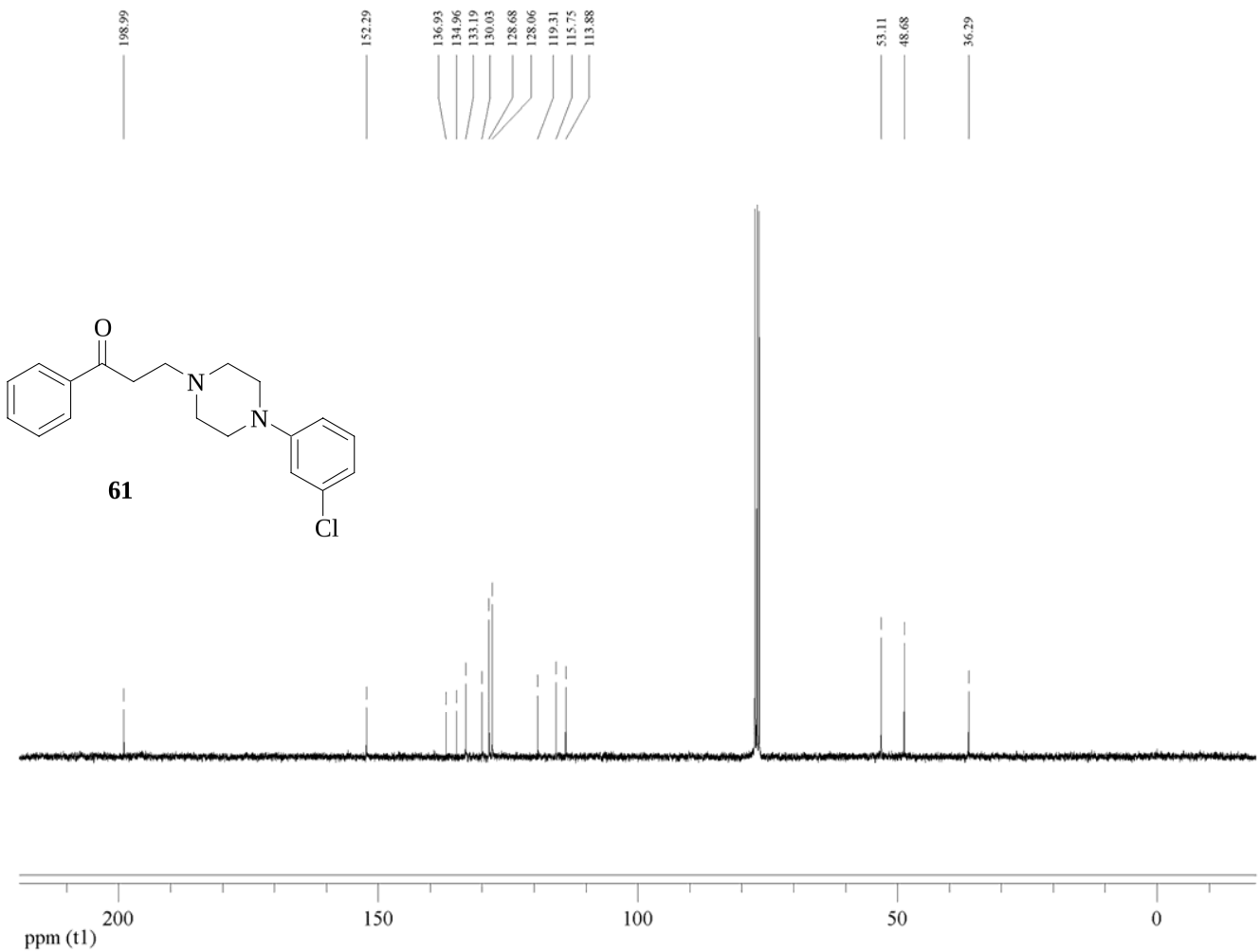
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **17a**



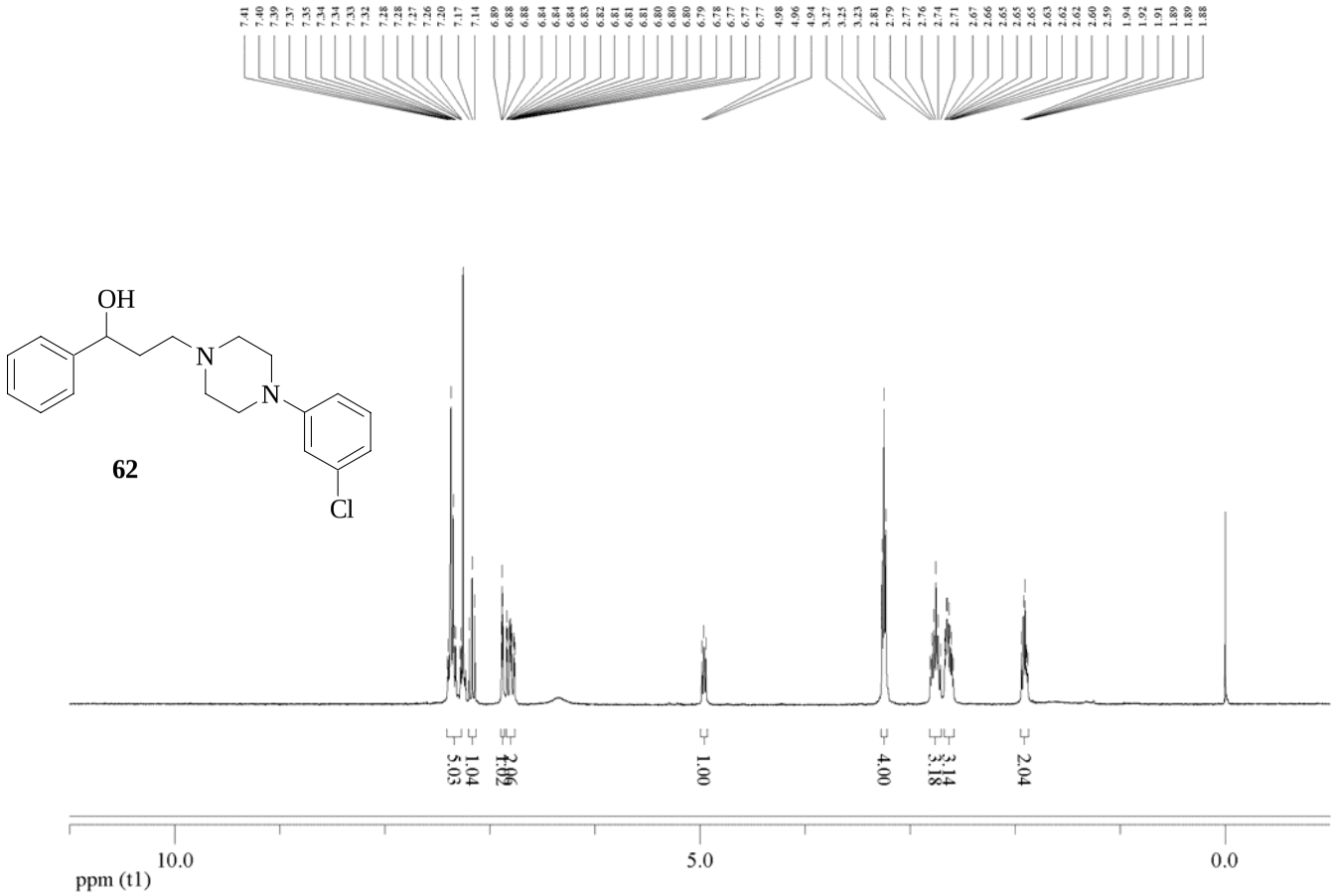
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **61**



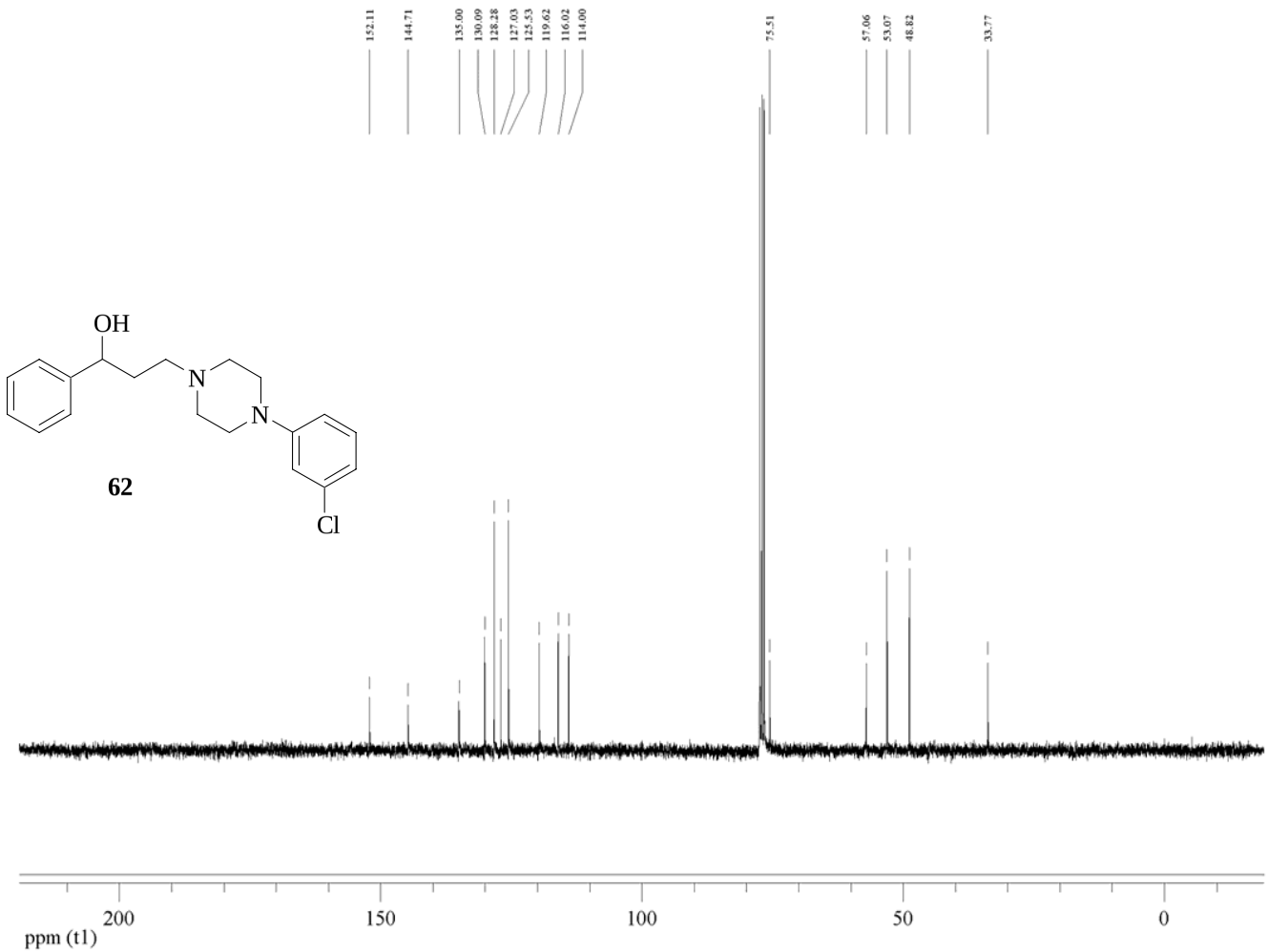
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **61**



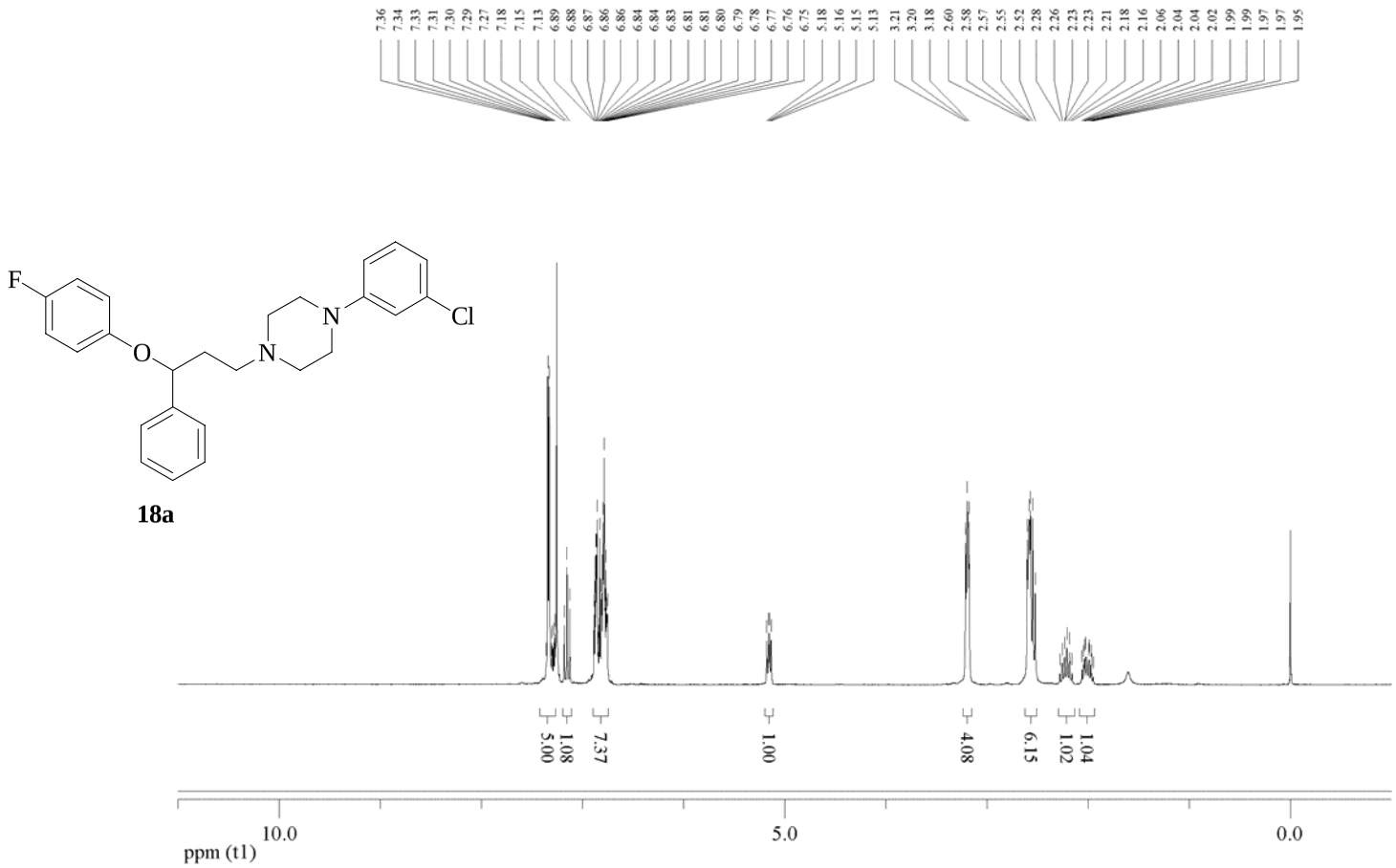
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **62**



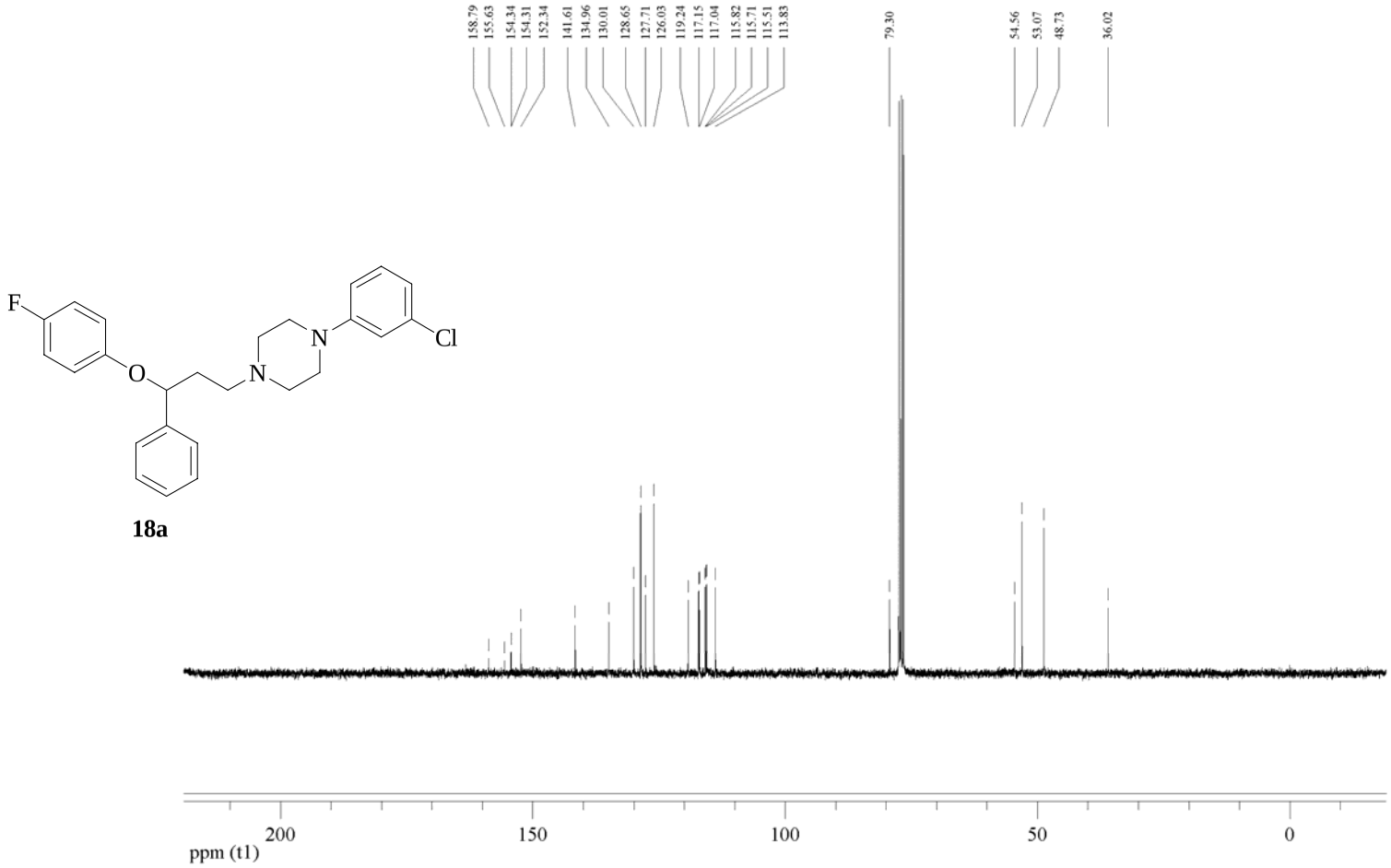
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **62**



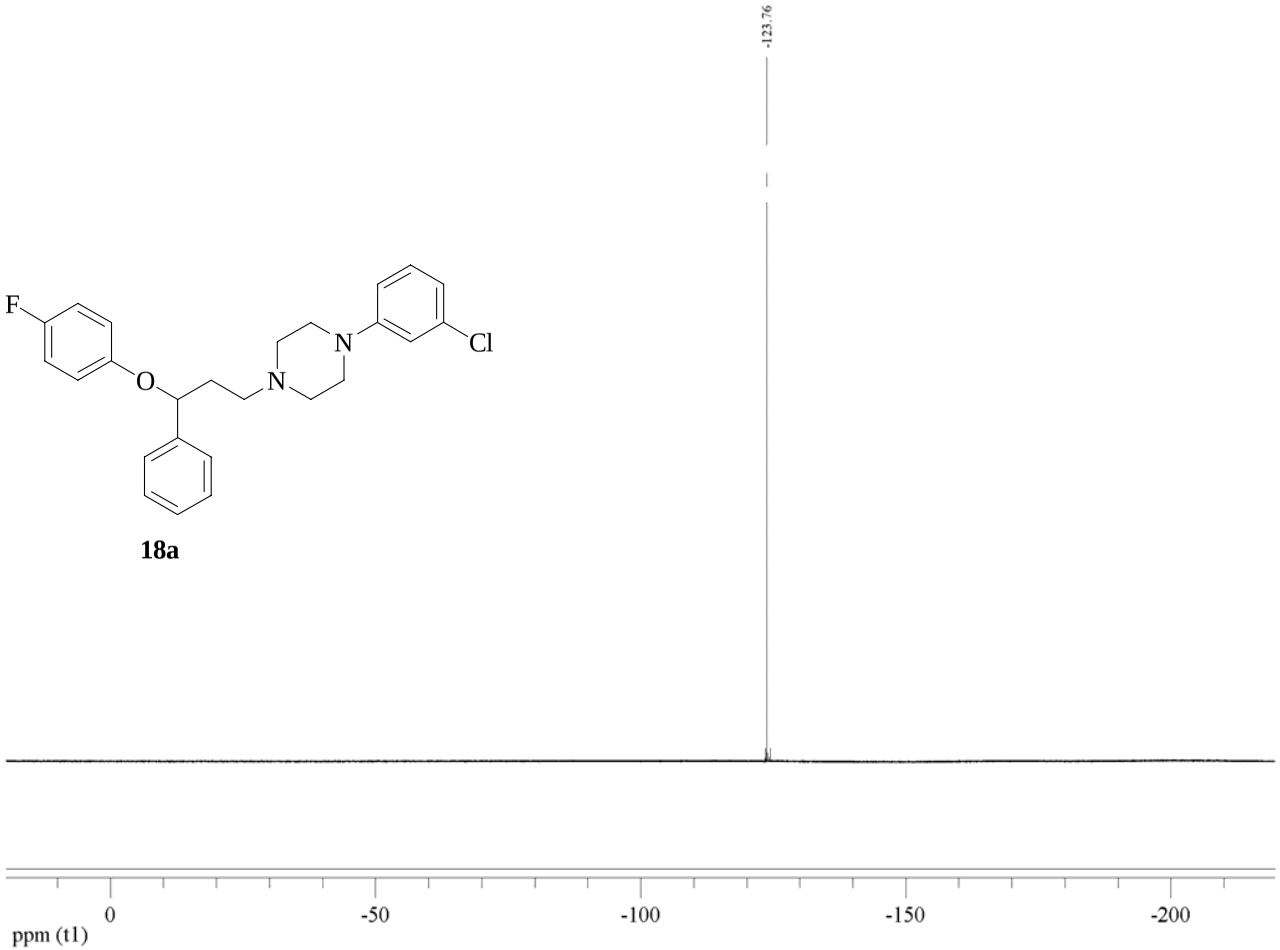
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **18a**



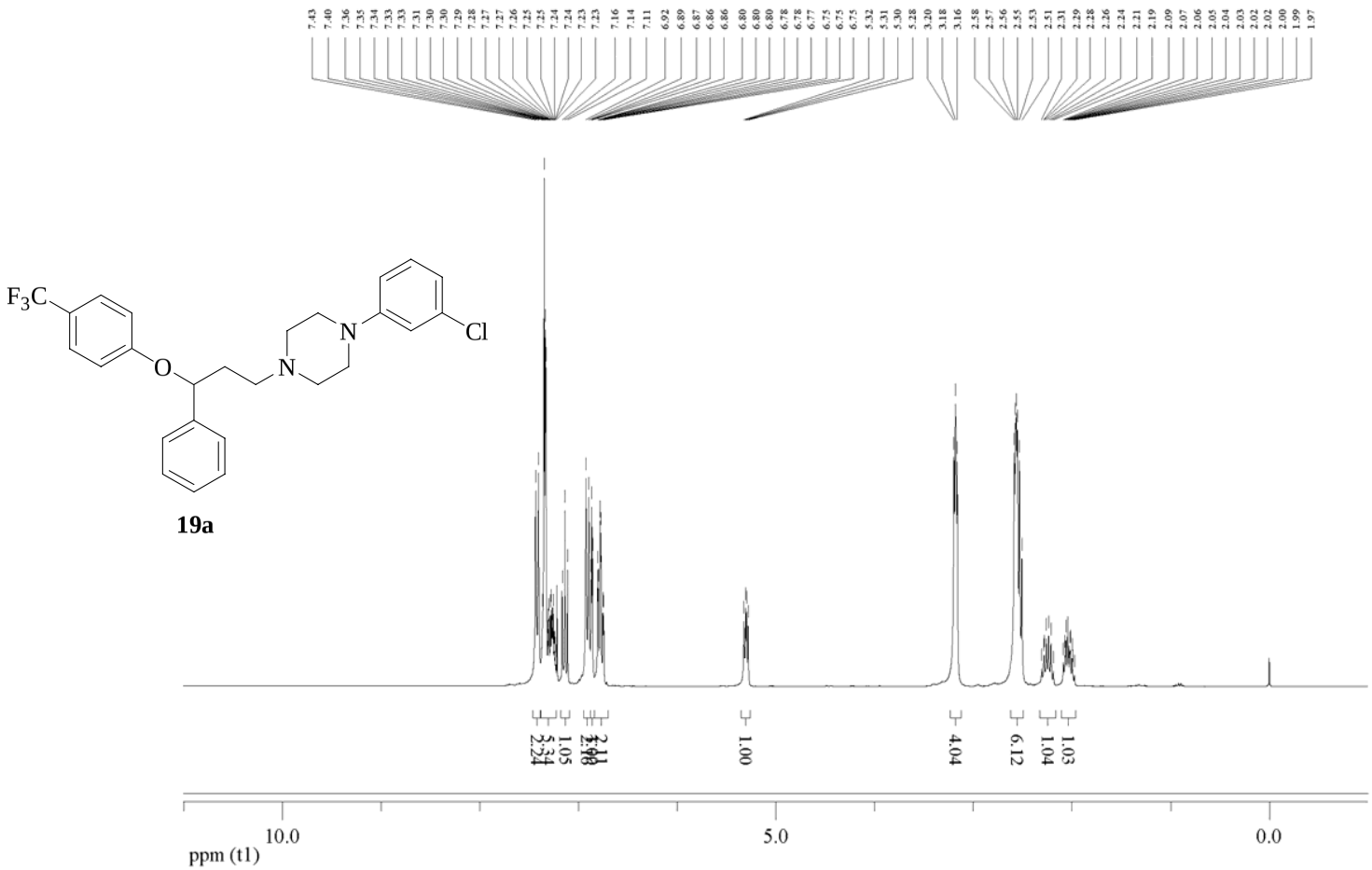
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **18a**



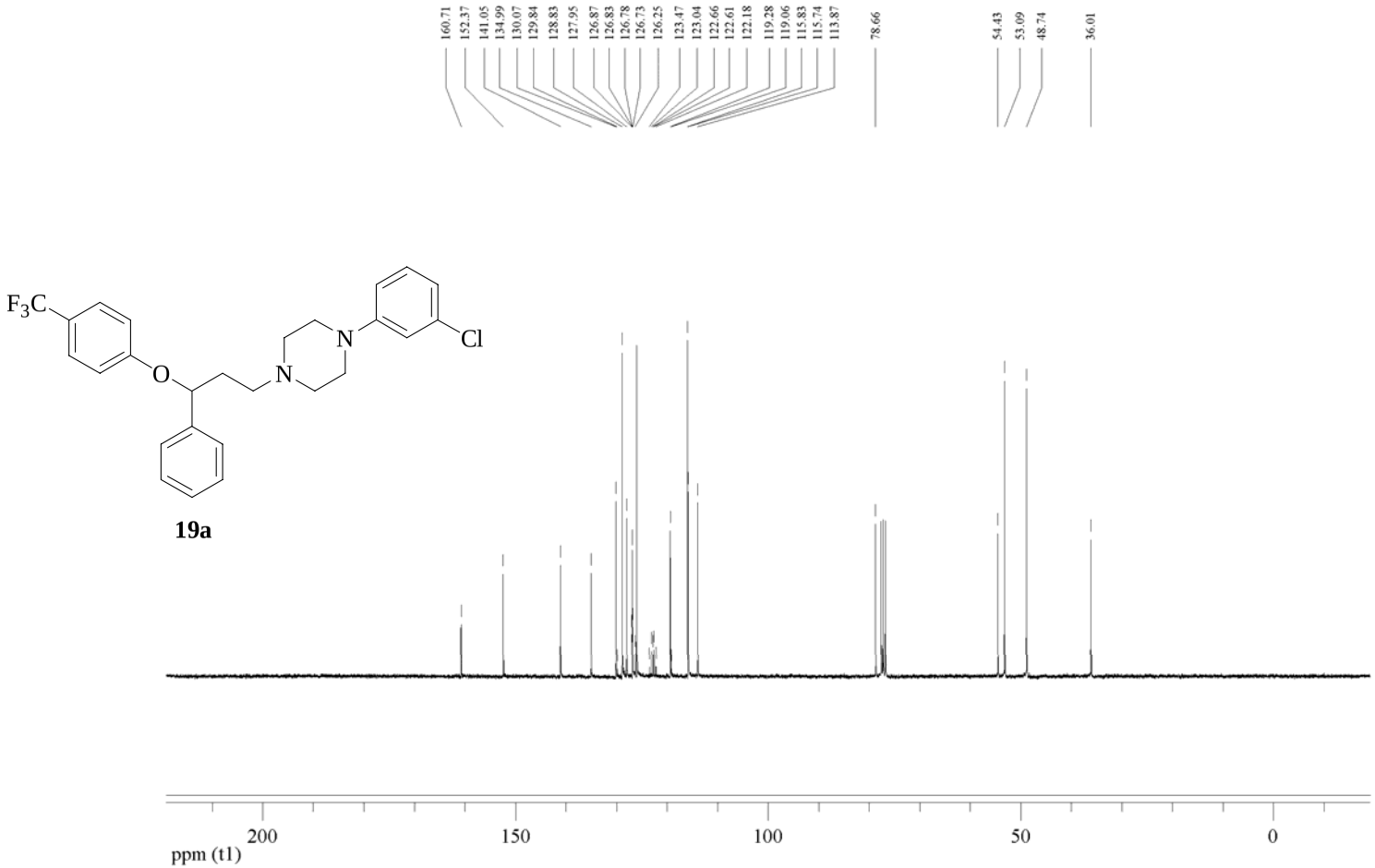
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **18a**



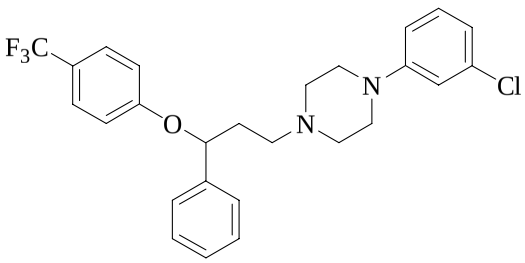
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **19a**



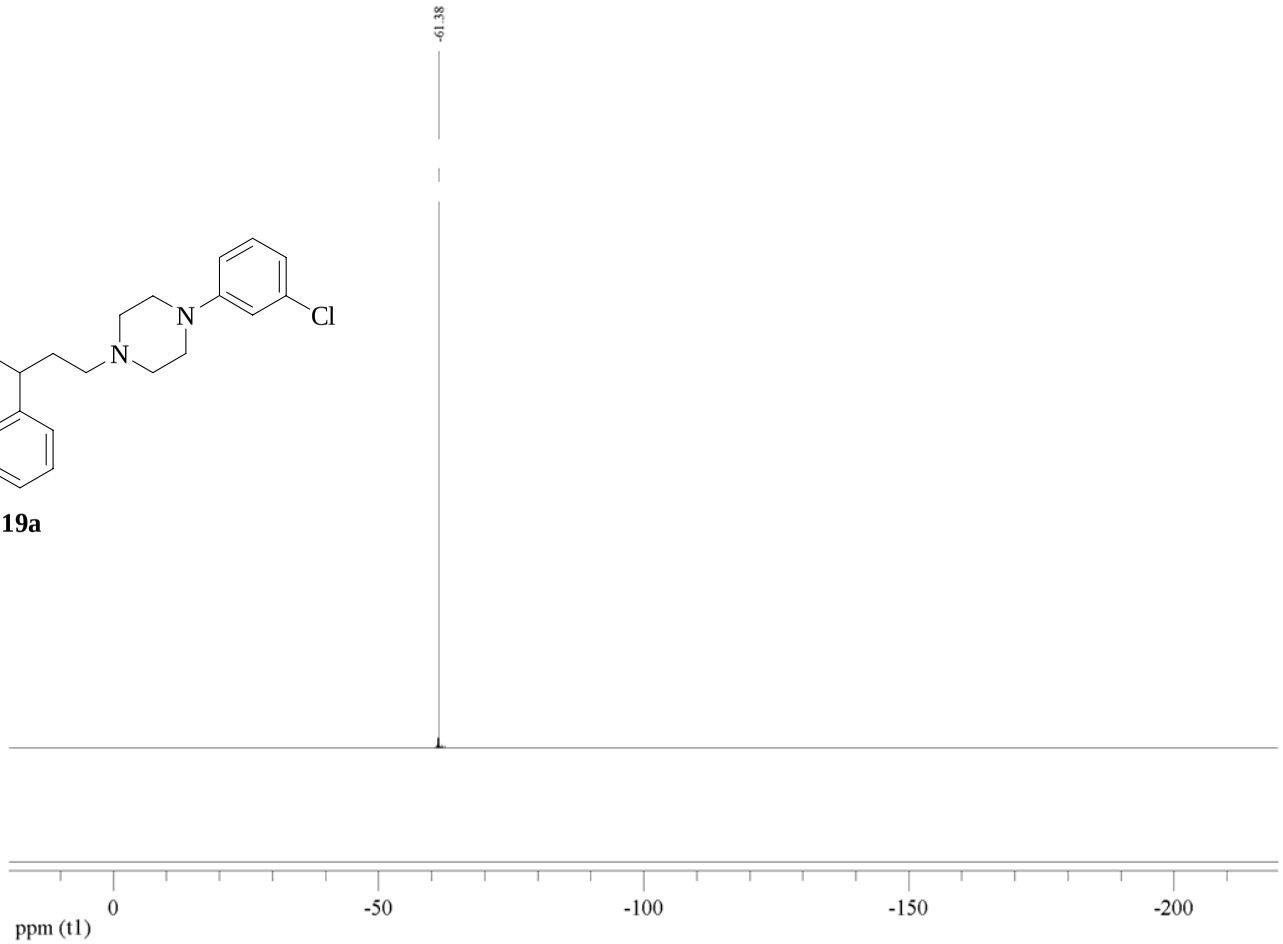
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **19a**



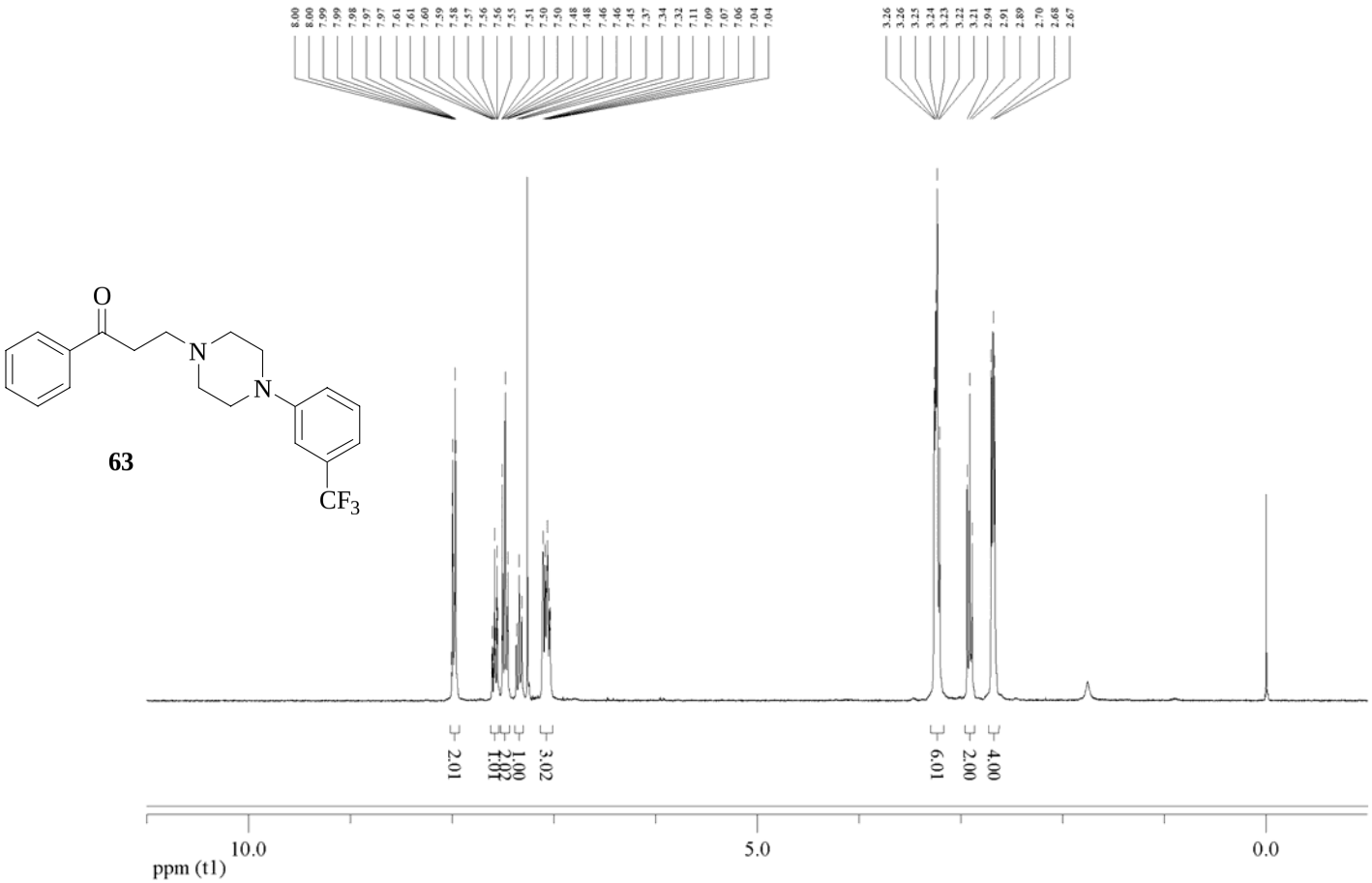
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **19a**



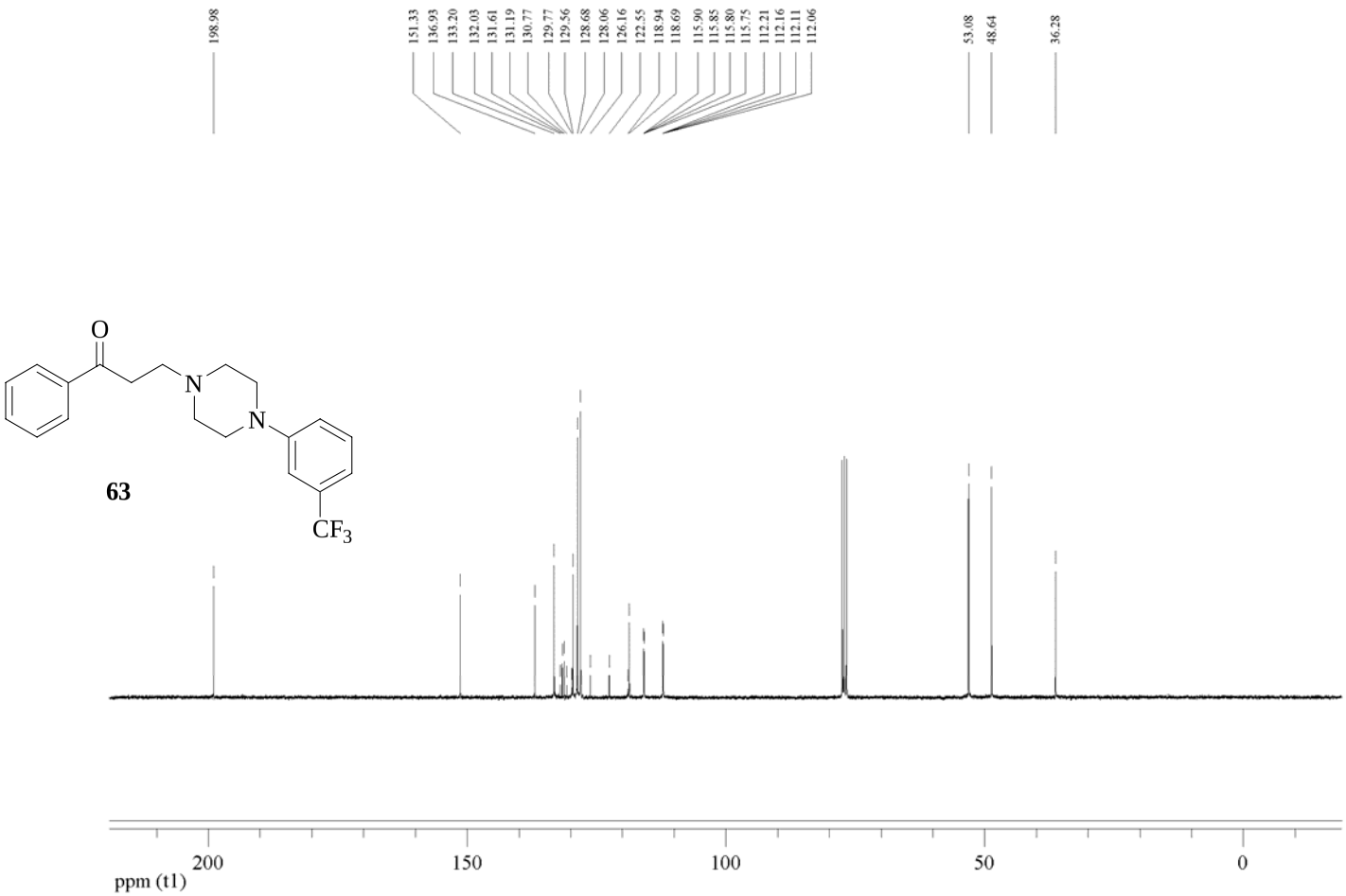
19a



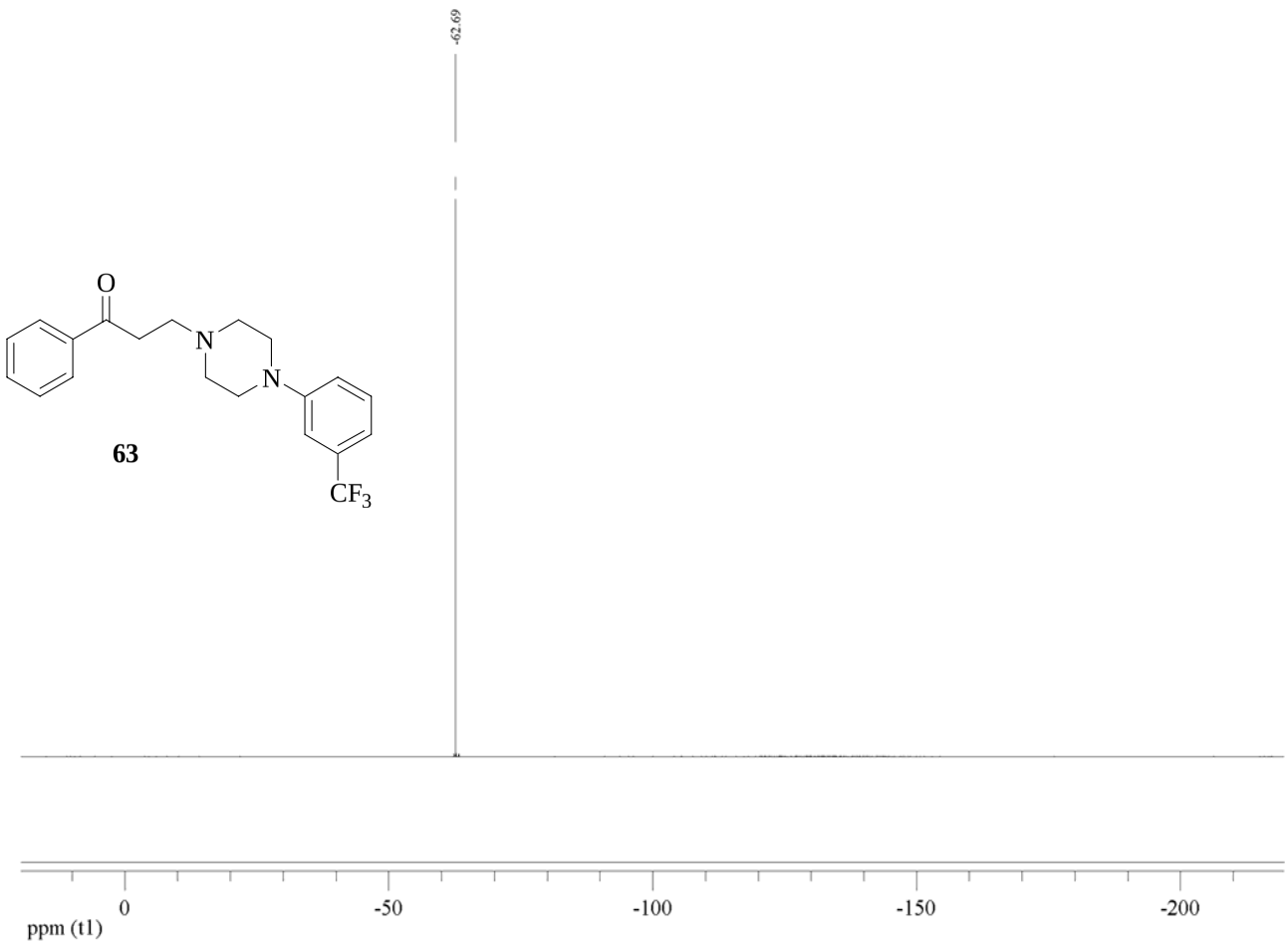
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **63**



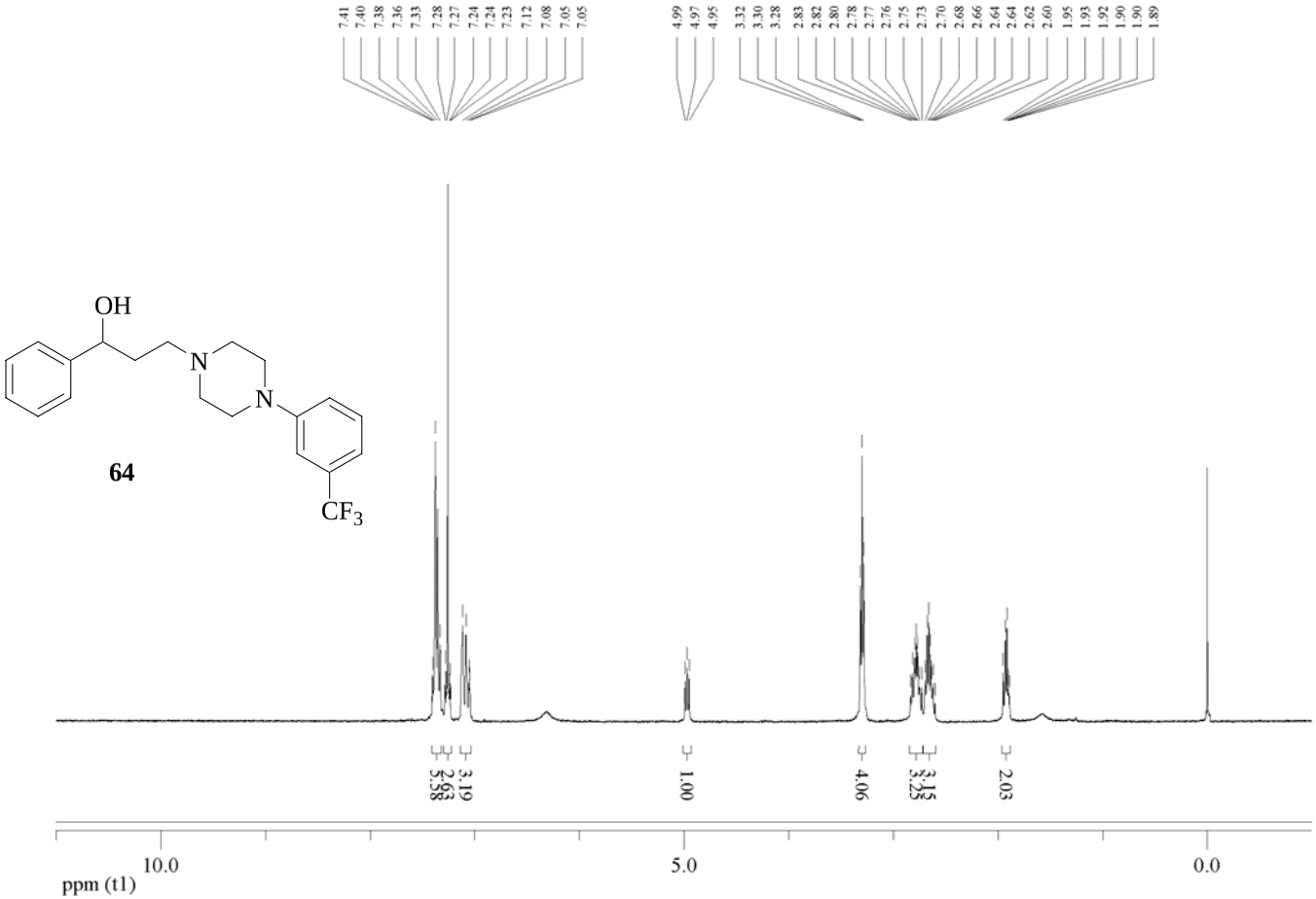
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **63**



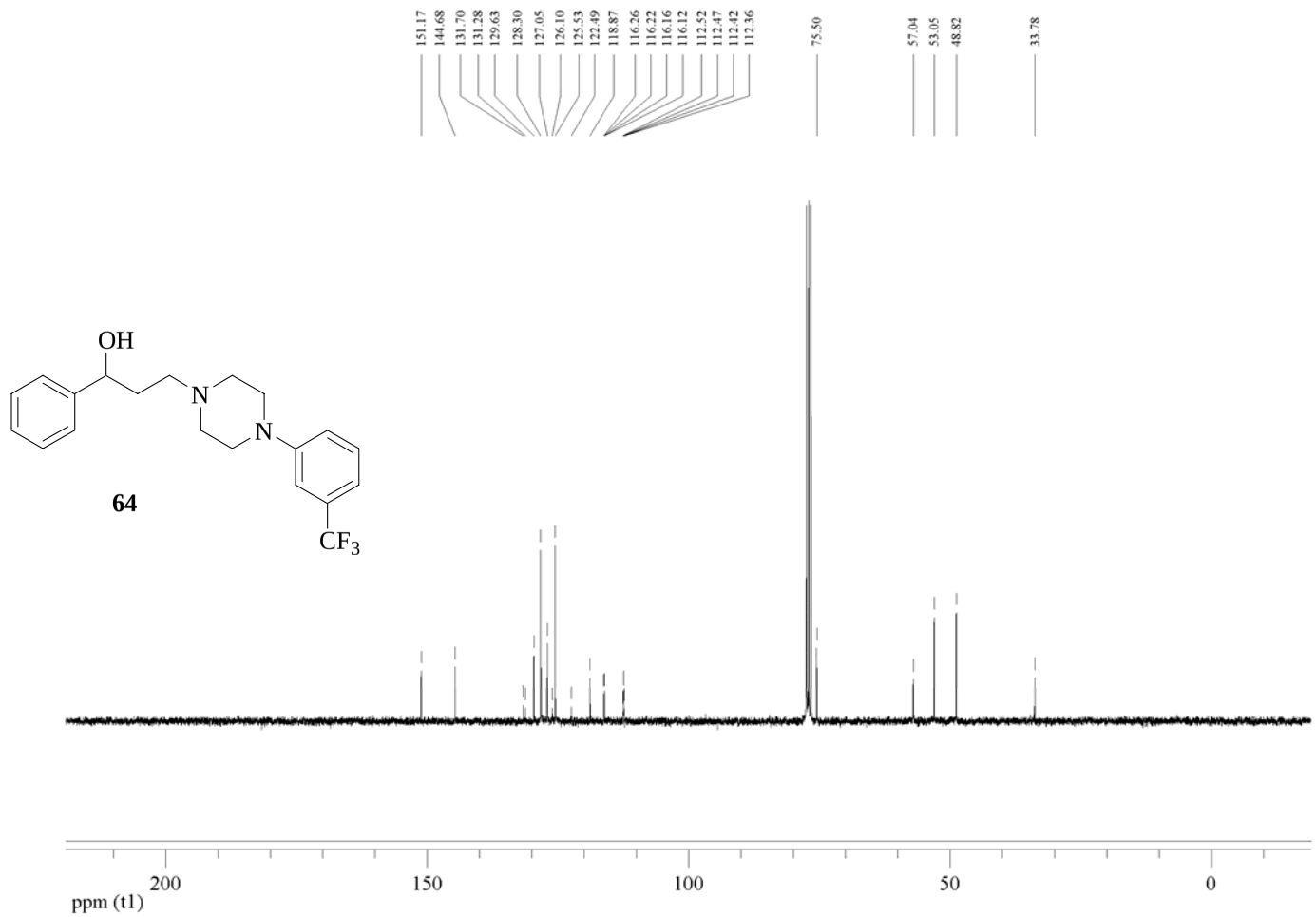
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **63**



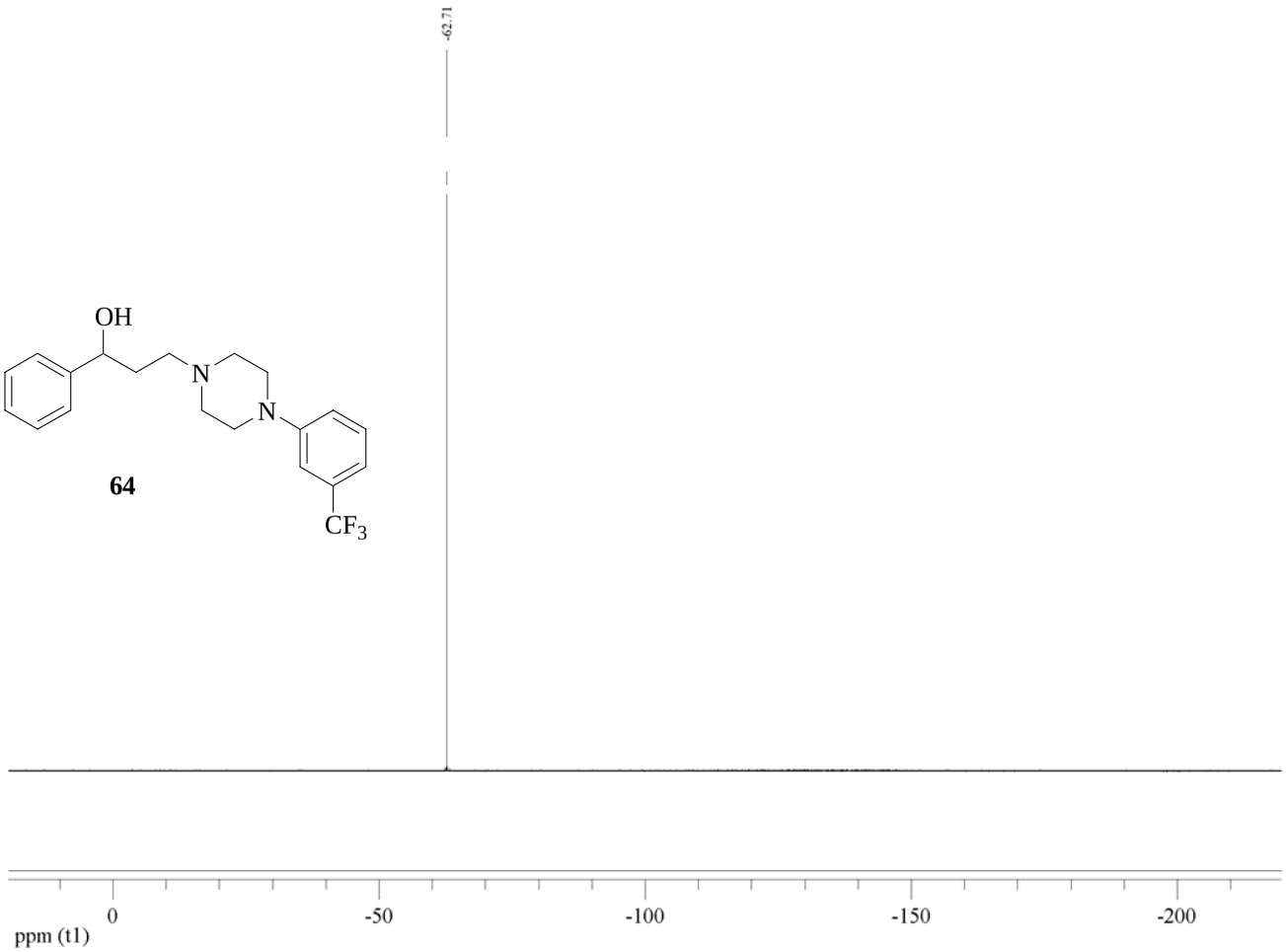
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **64**



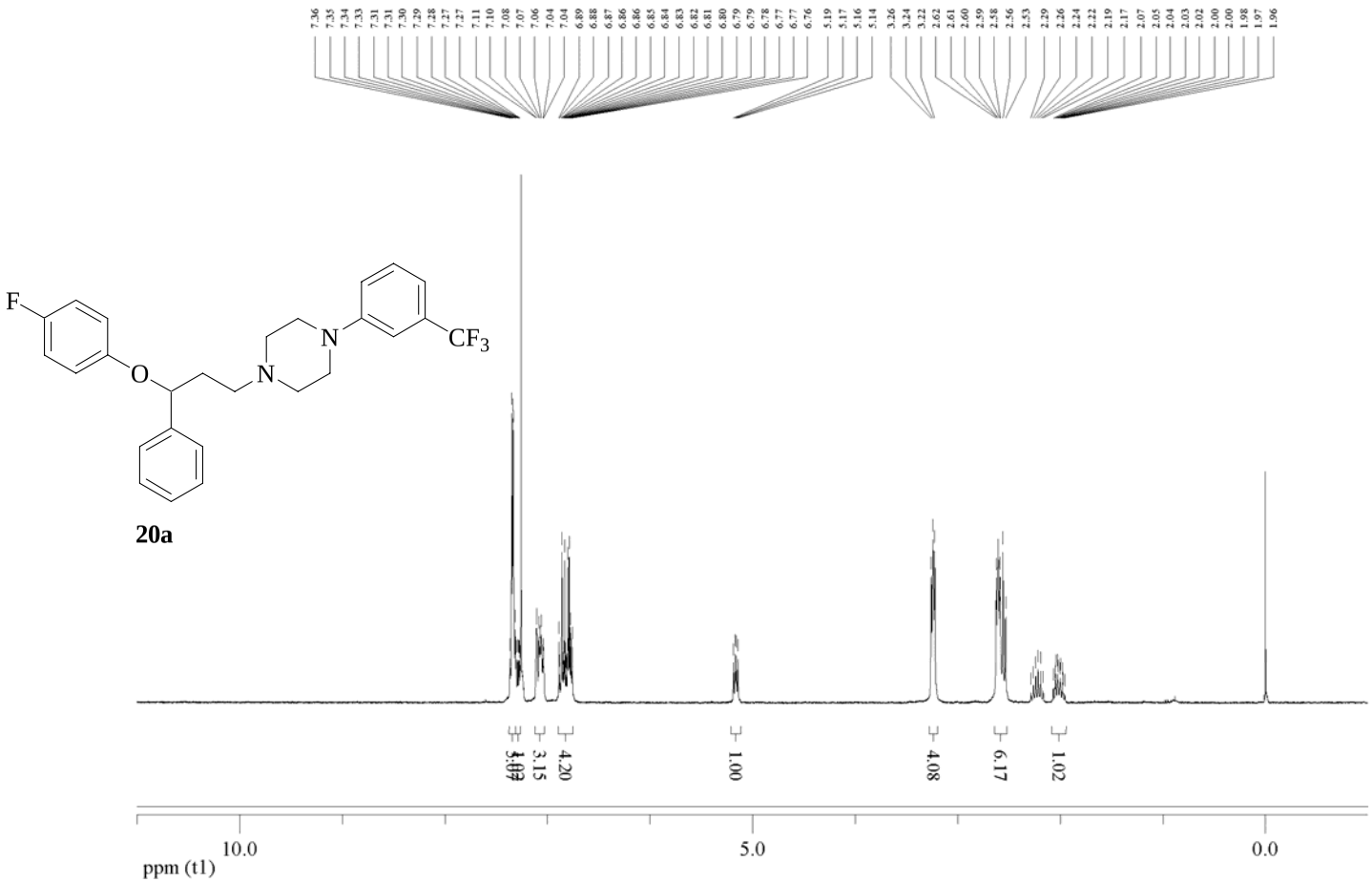
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **64**



^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **64**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **20a**



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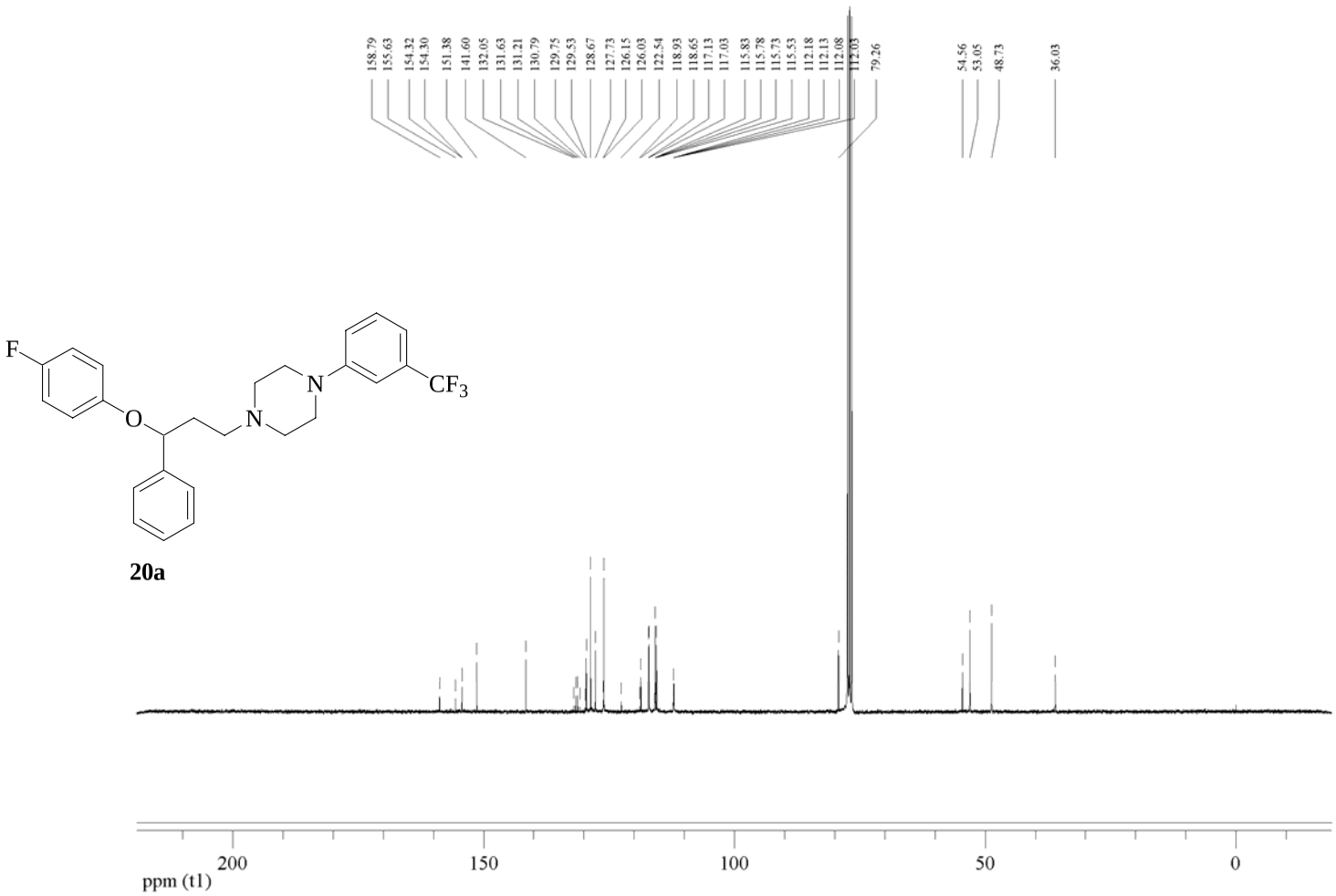

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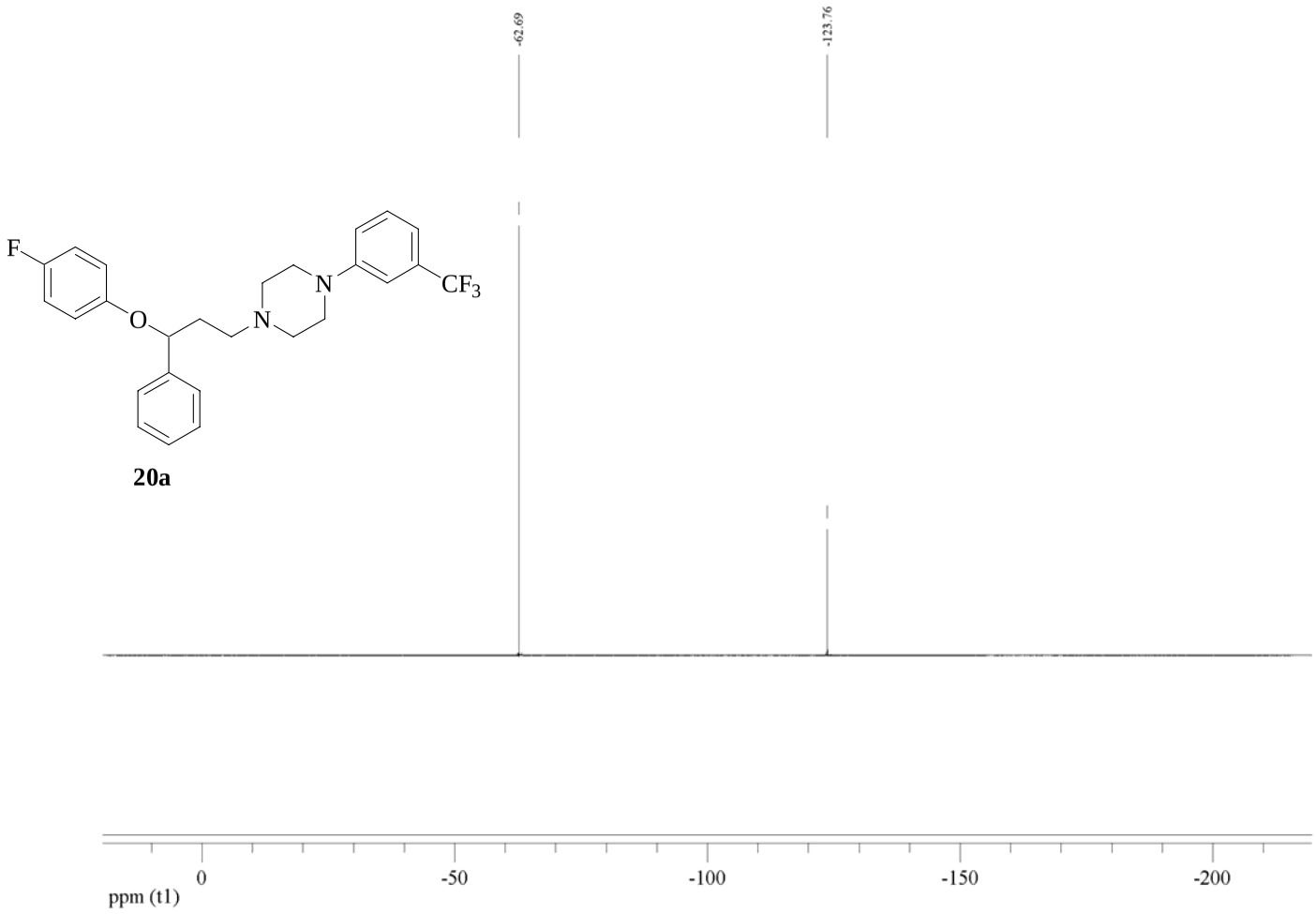

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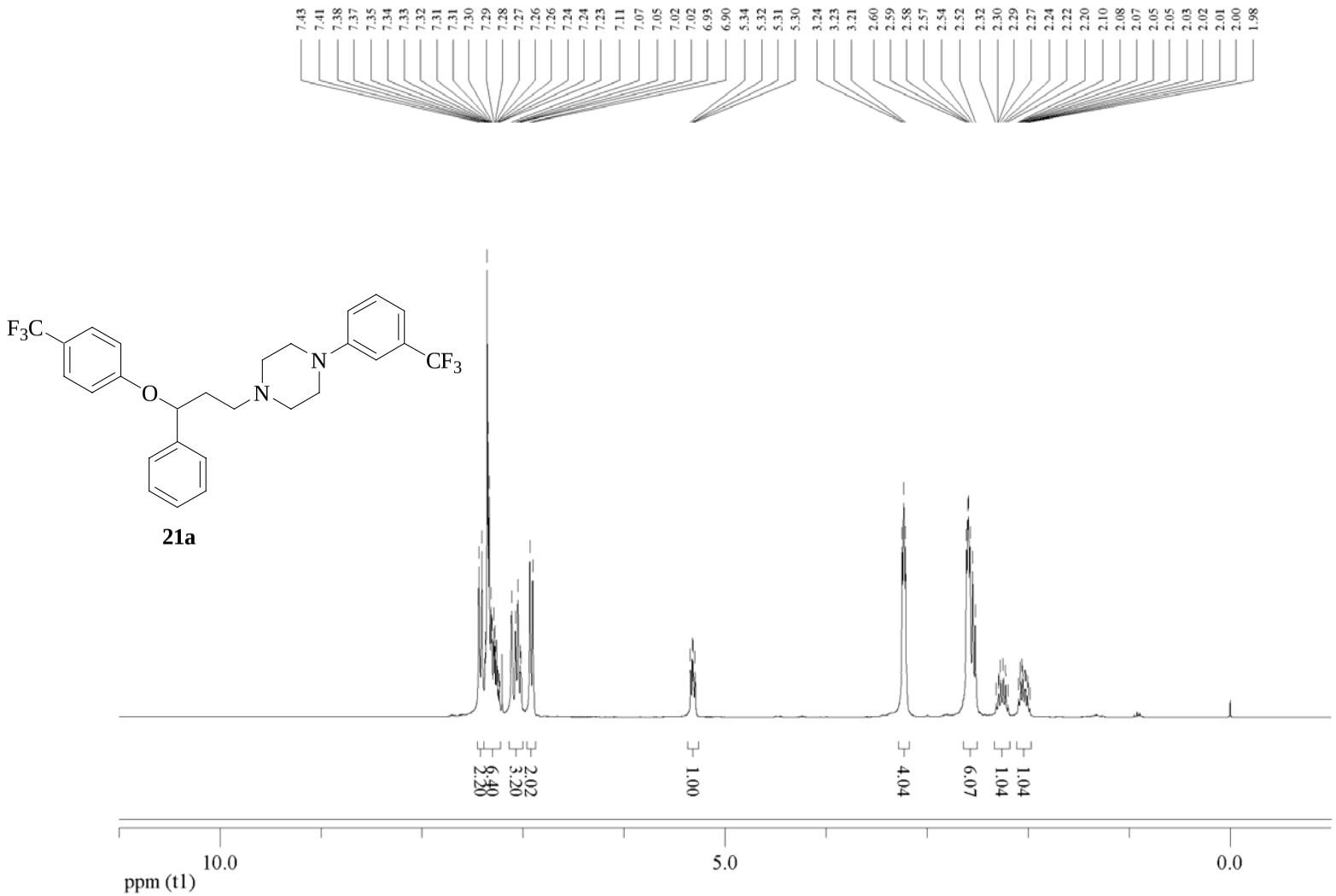

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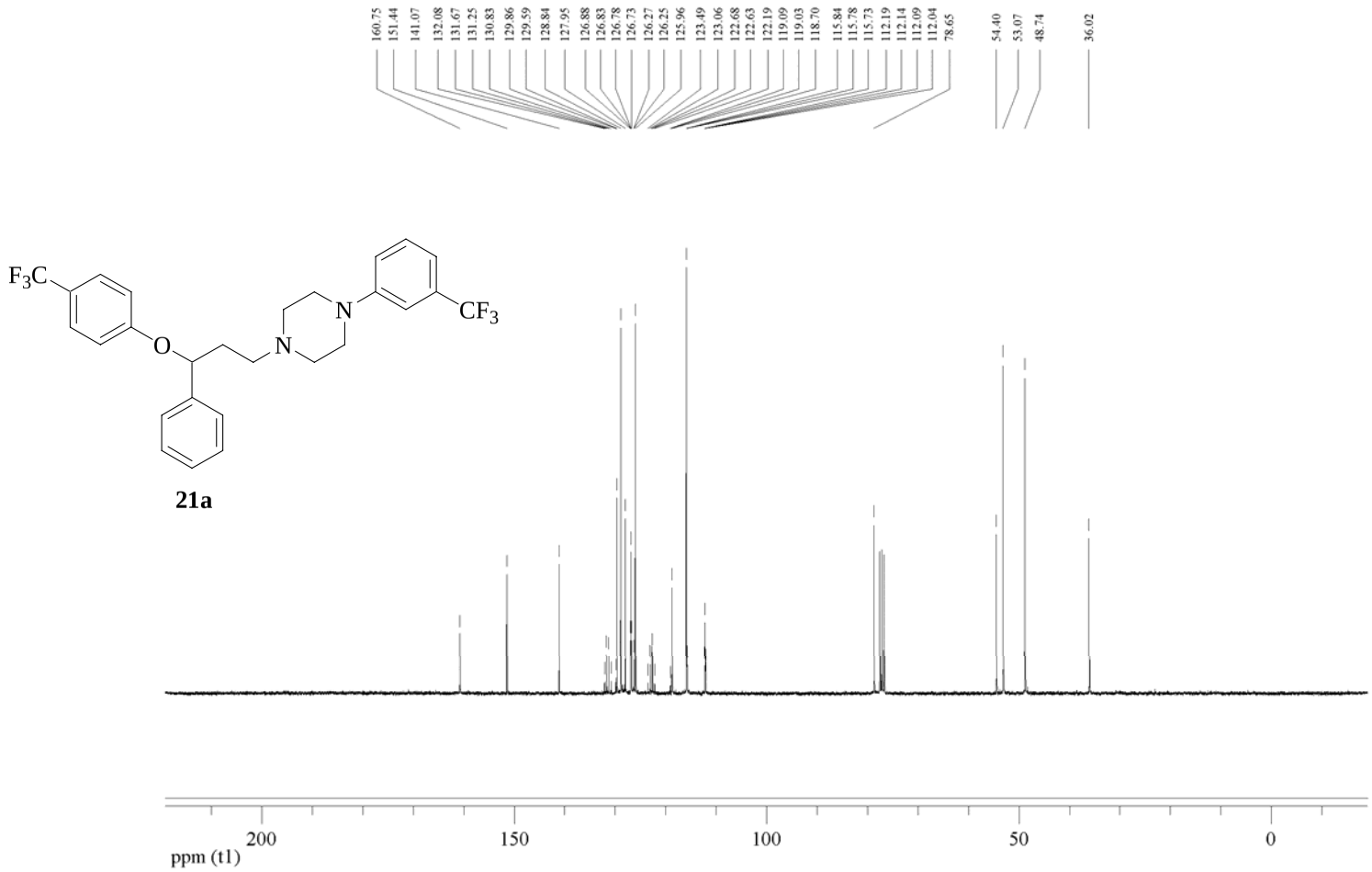
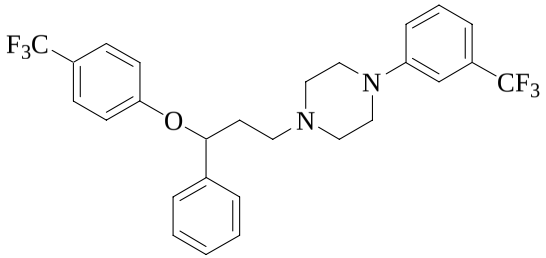
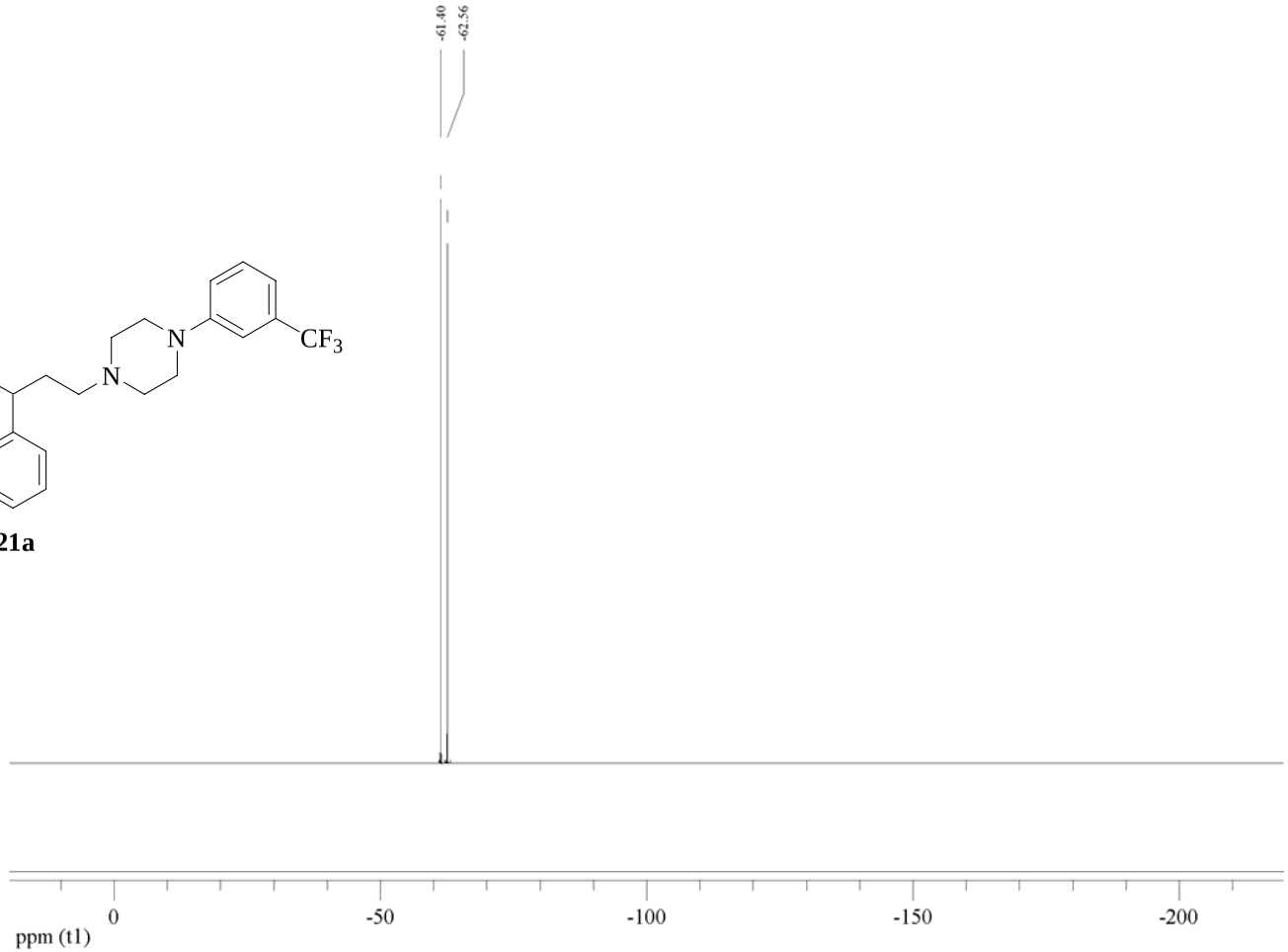


^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **21a**



21a



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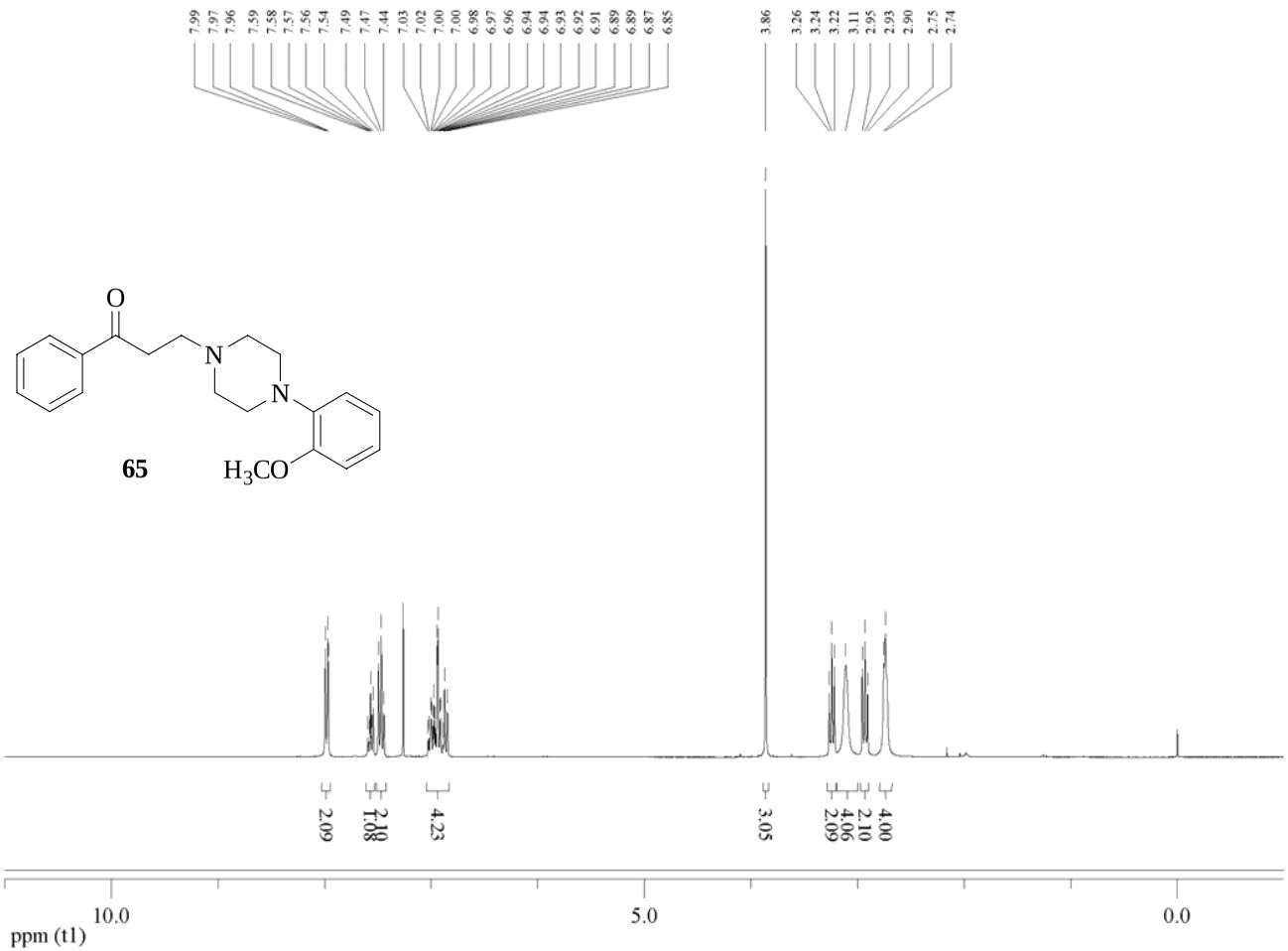
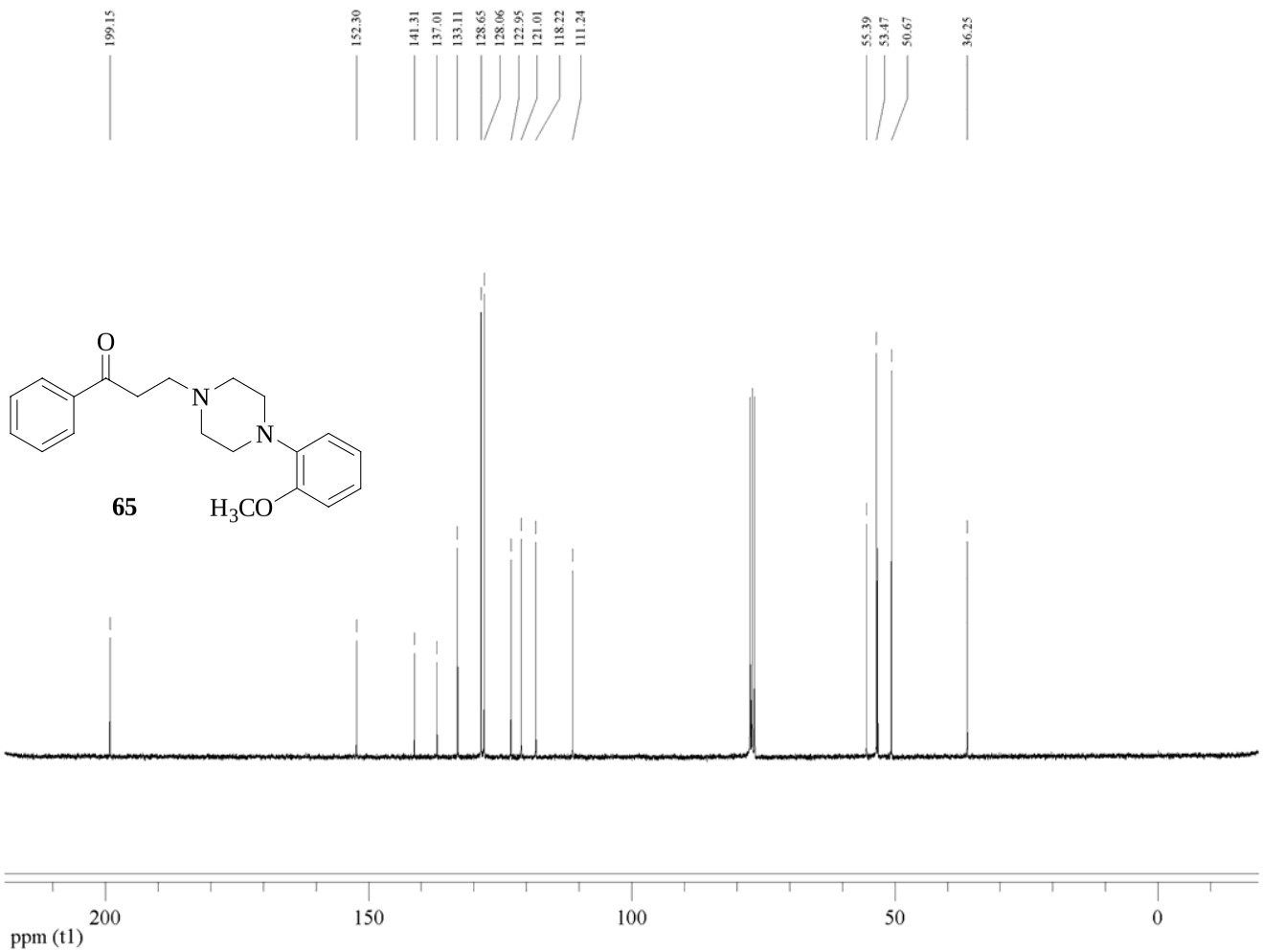


^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **65**



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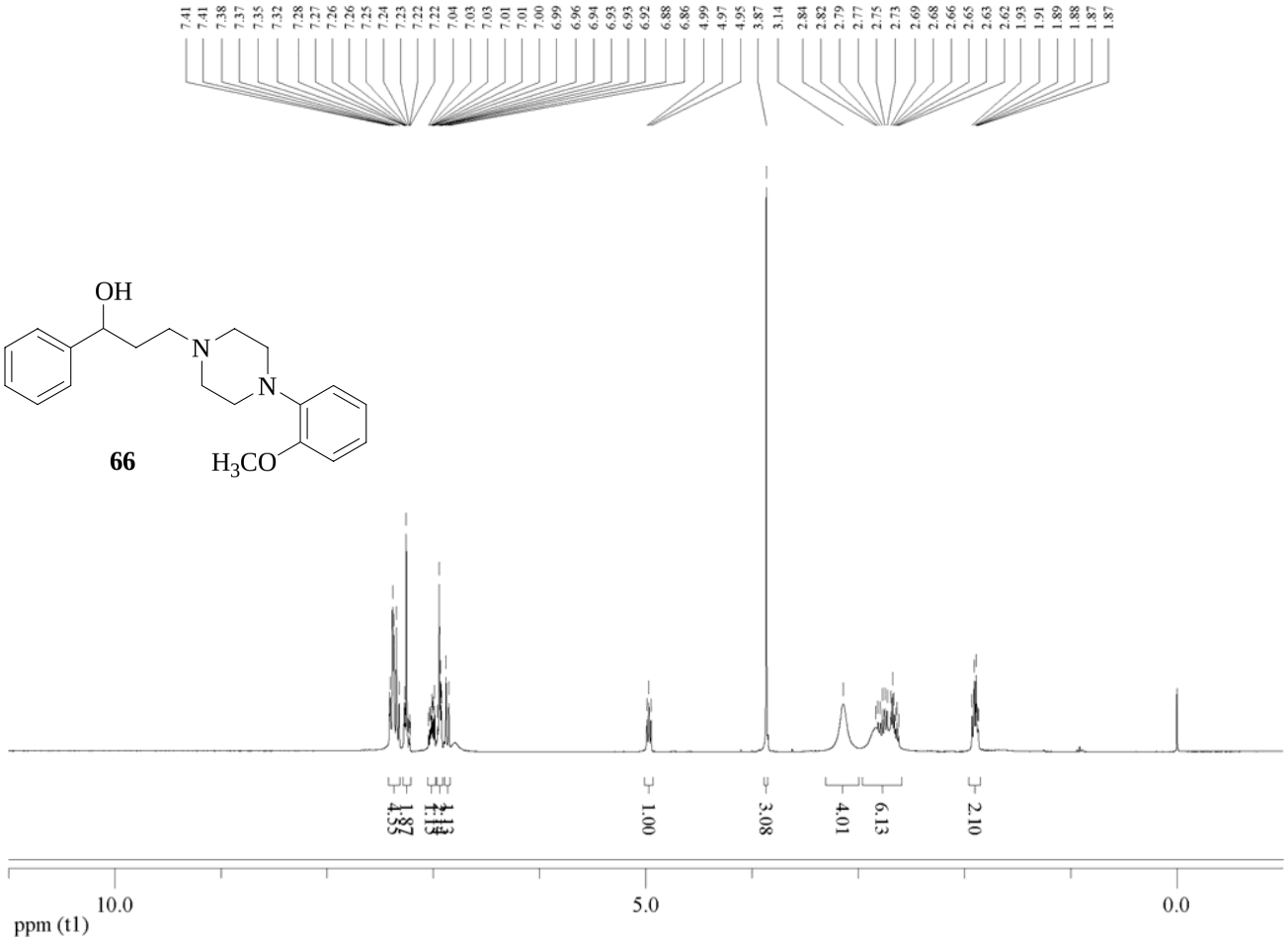

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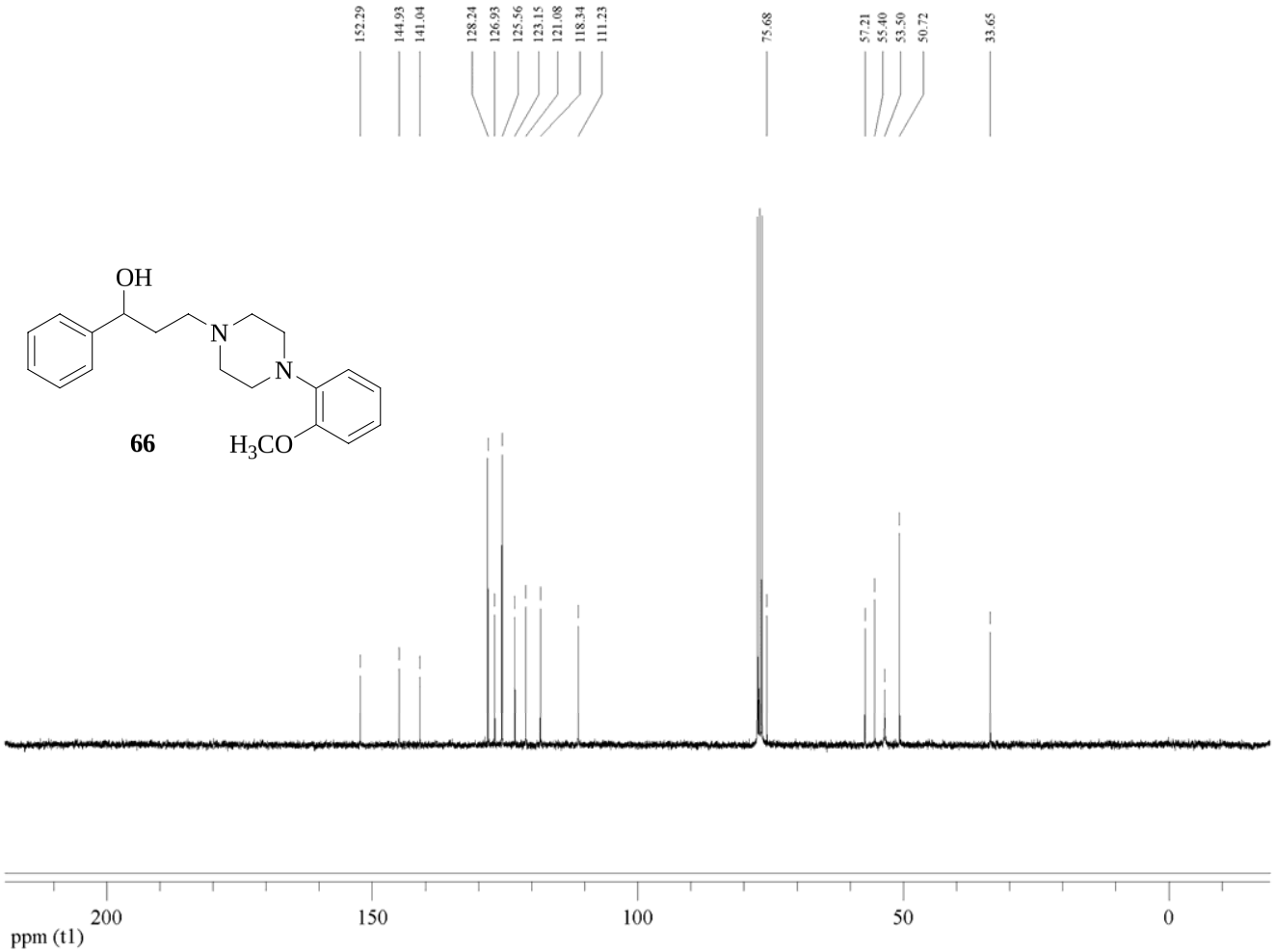

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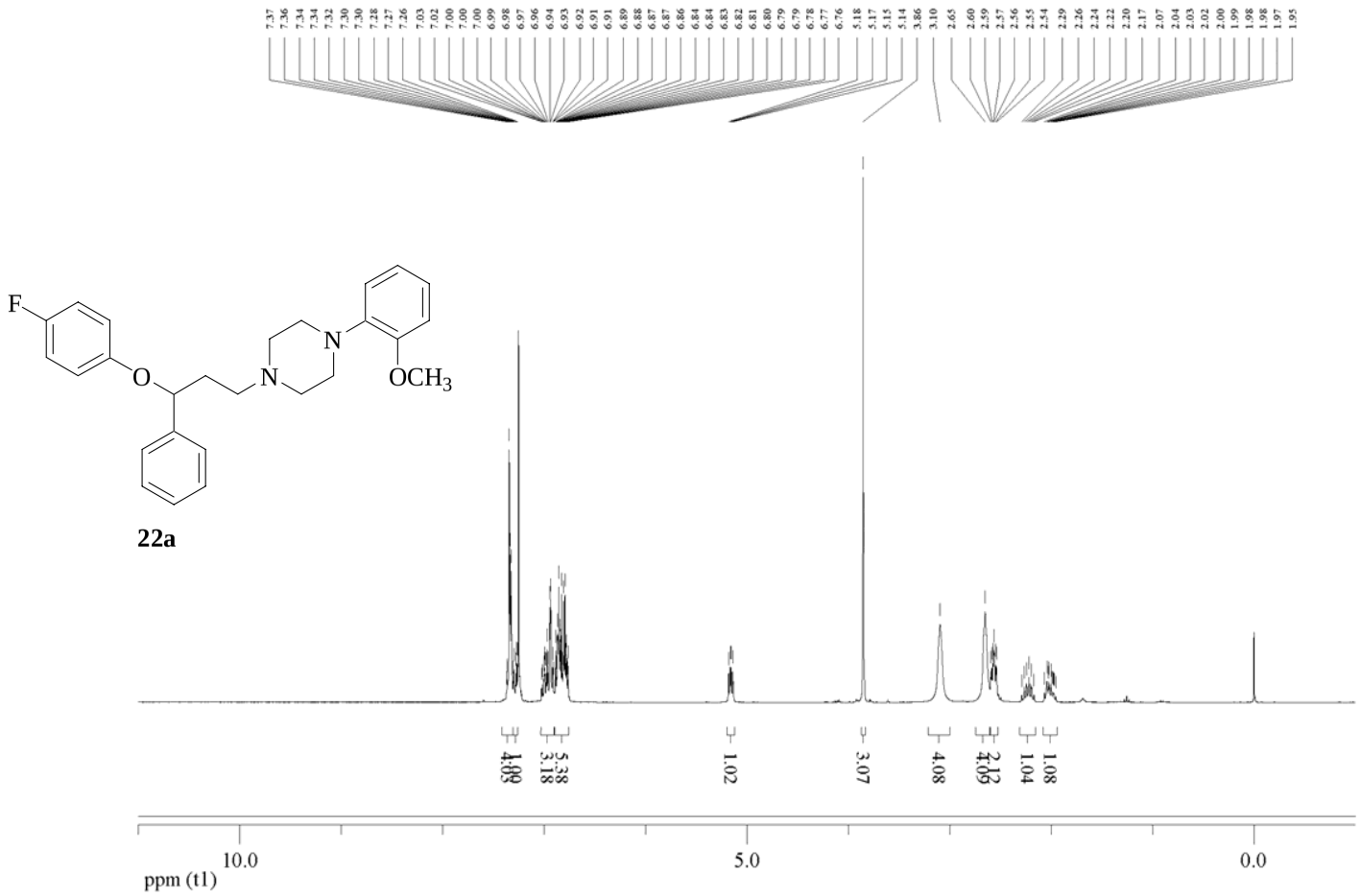
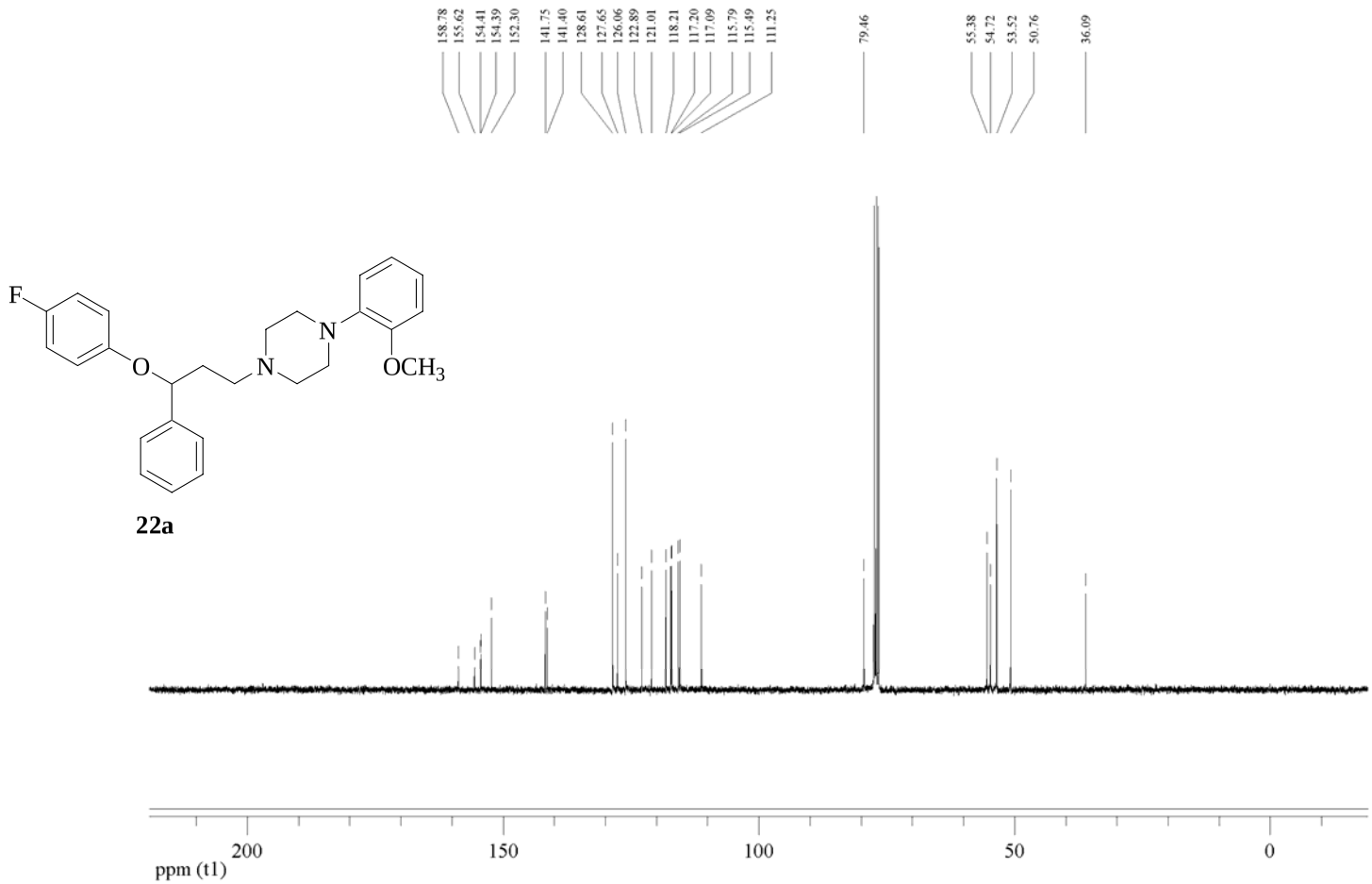
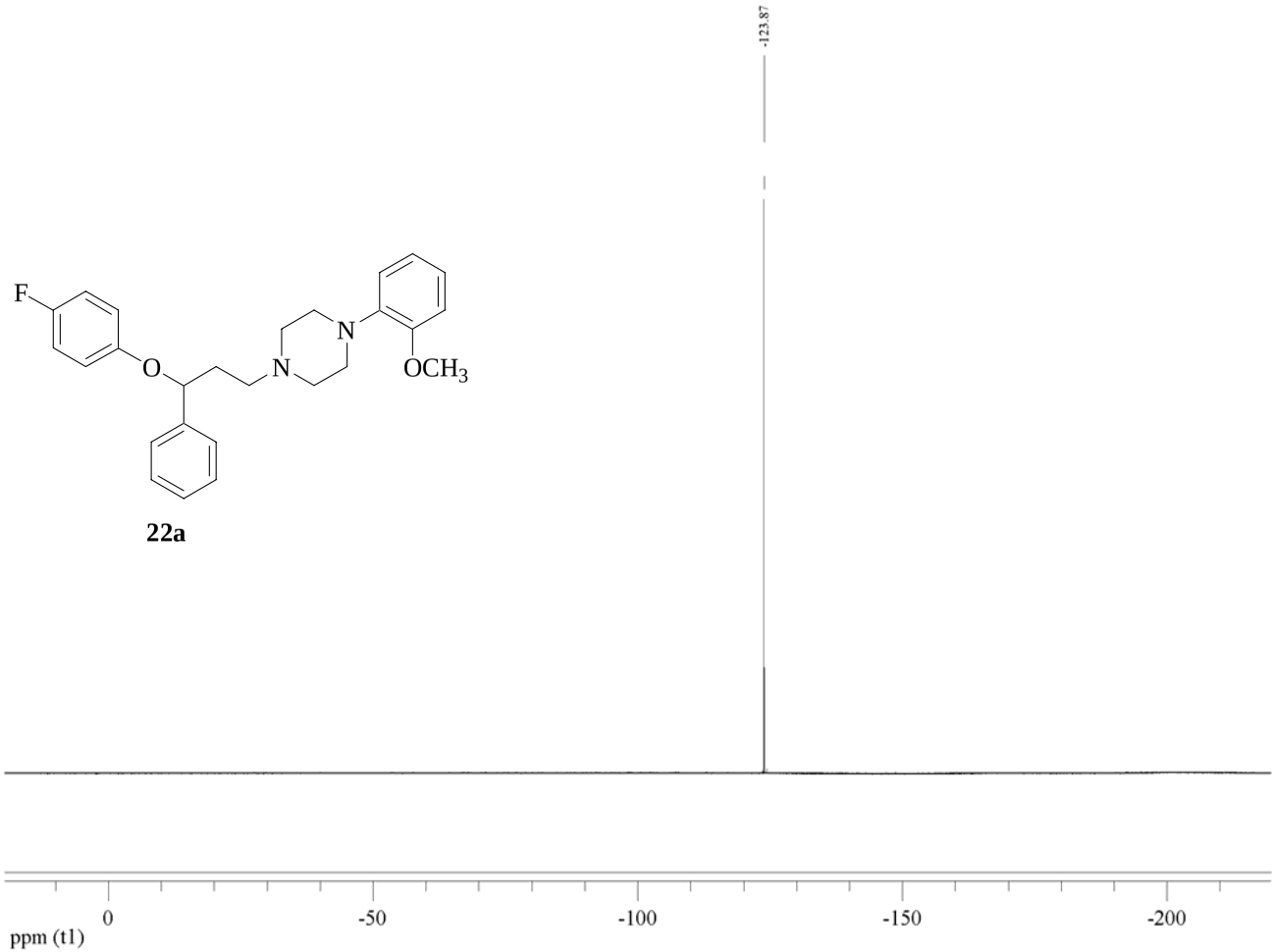


^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **22a**



^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **22a**



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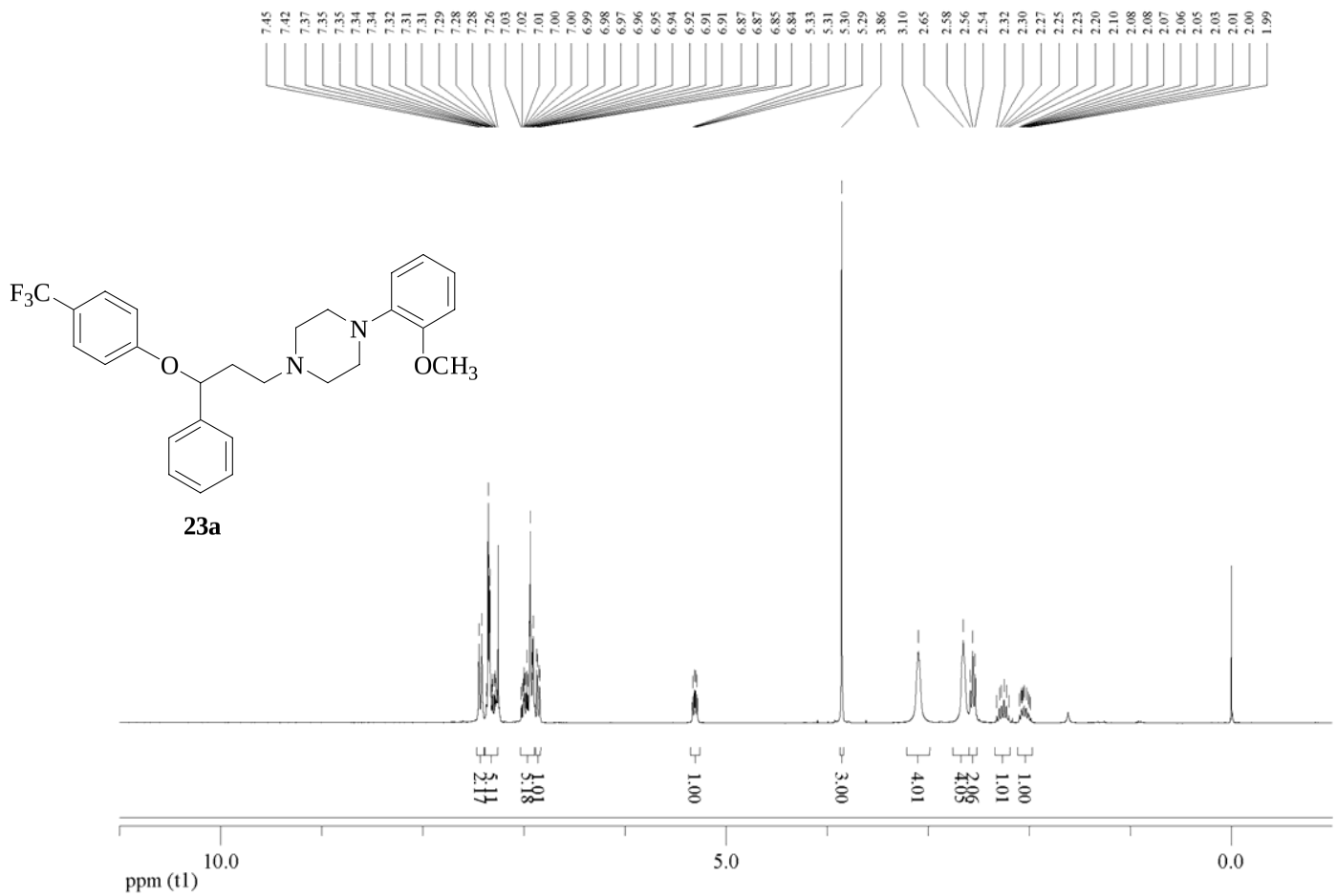

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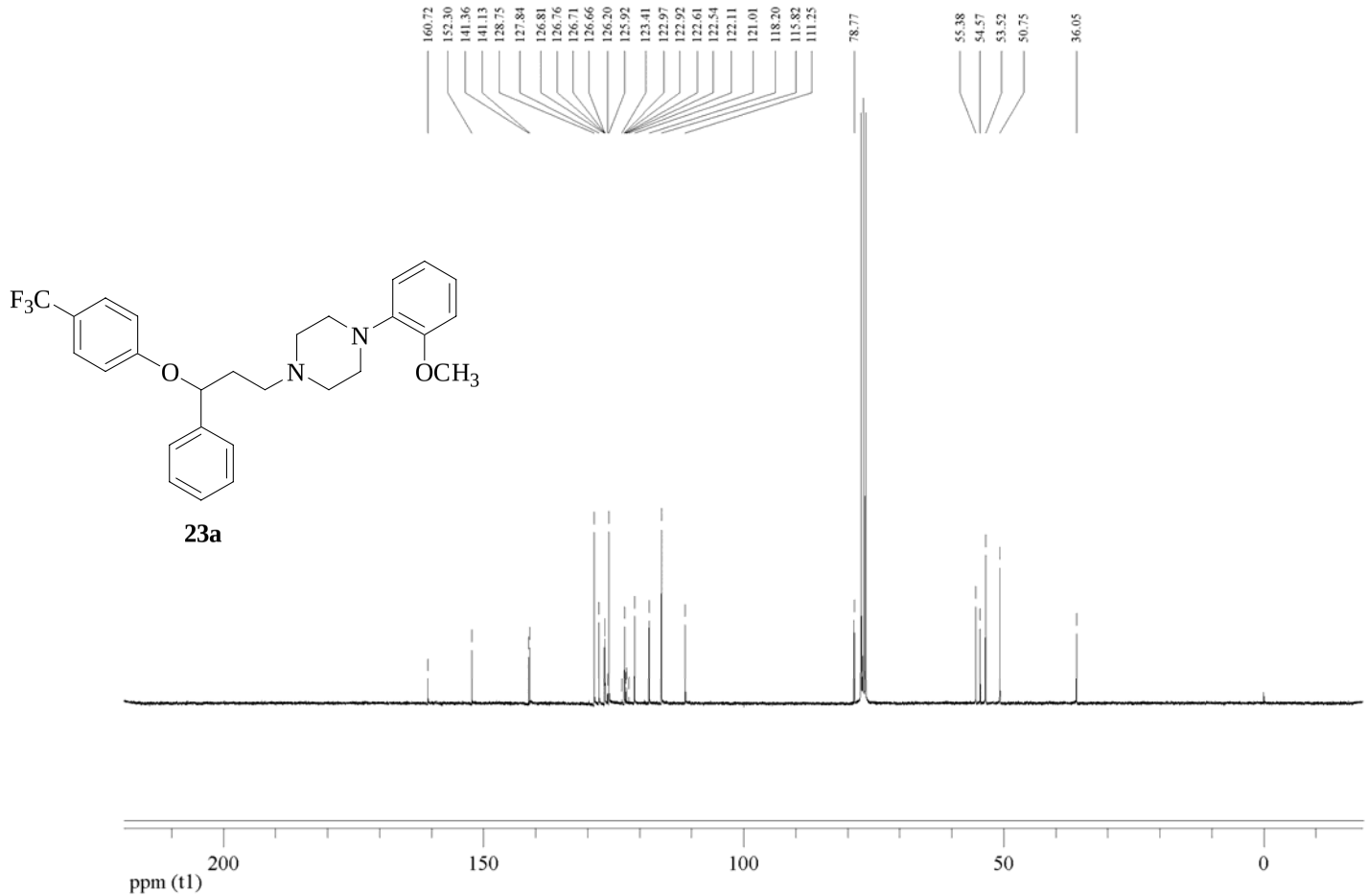
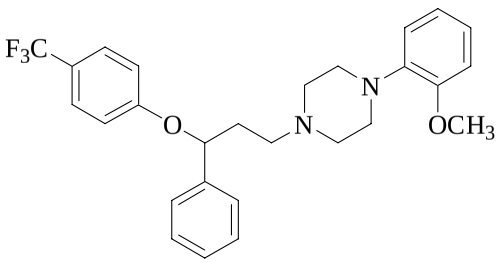
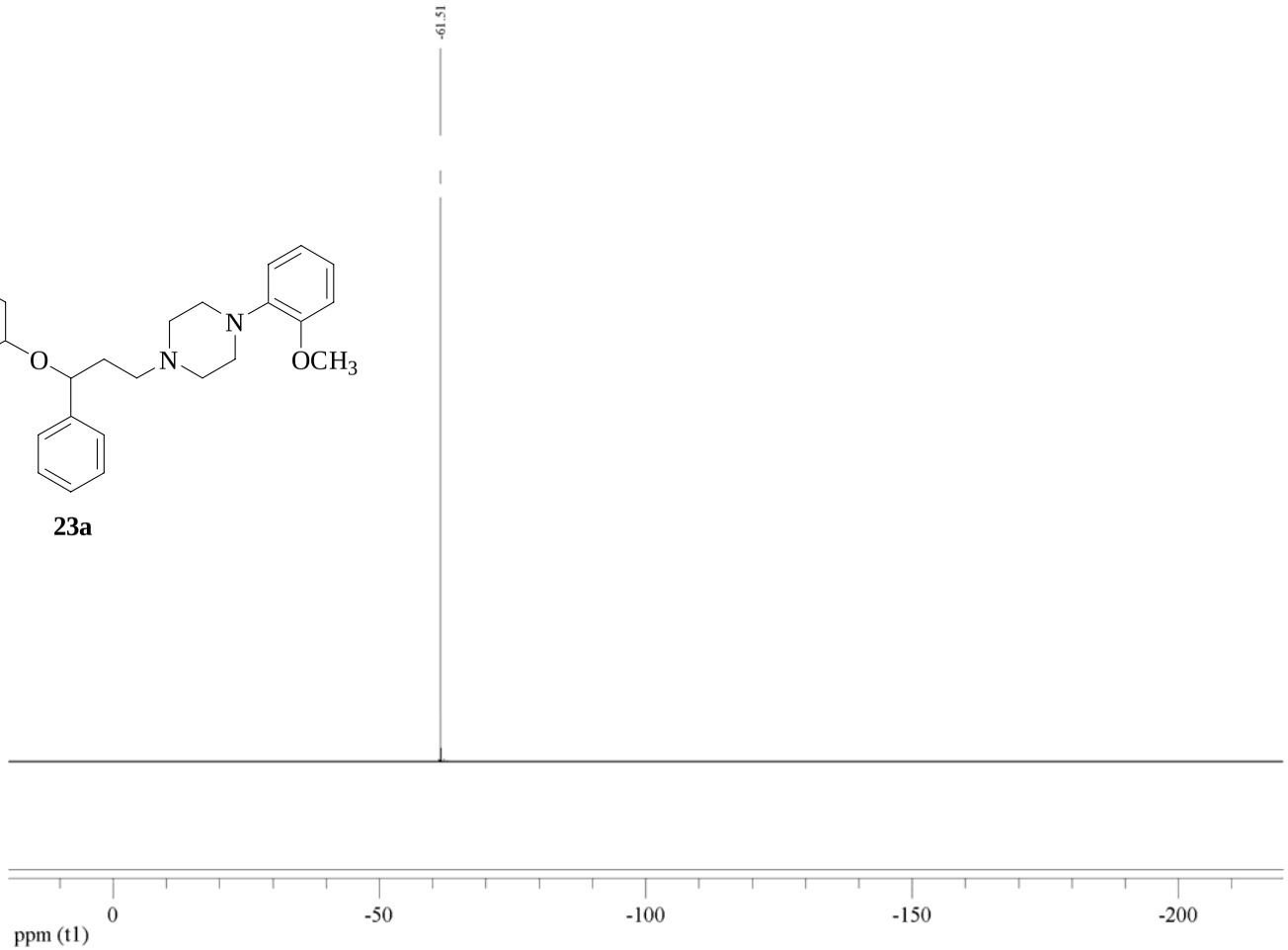


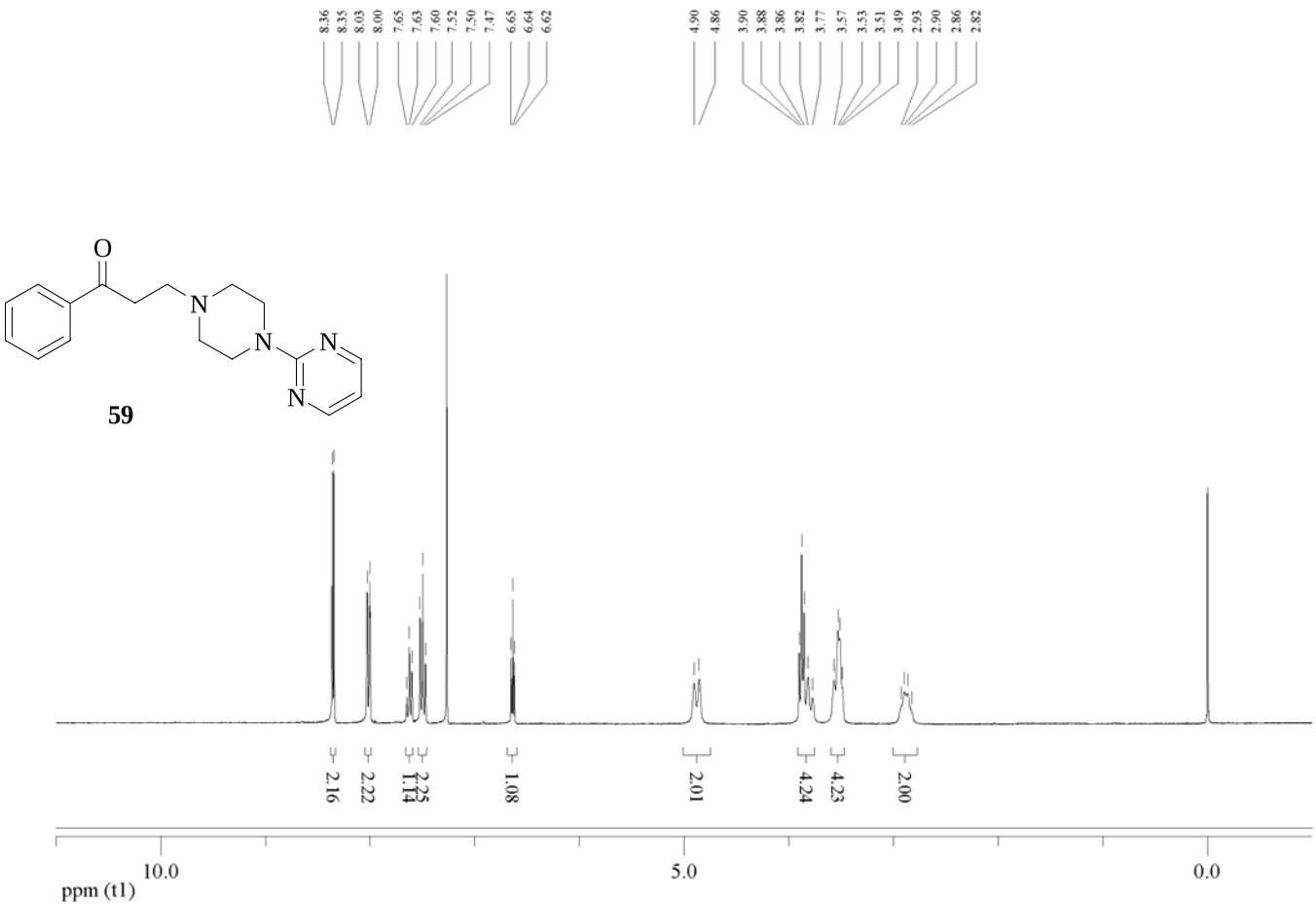
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **23a**



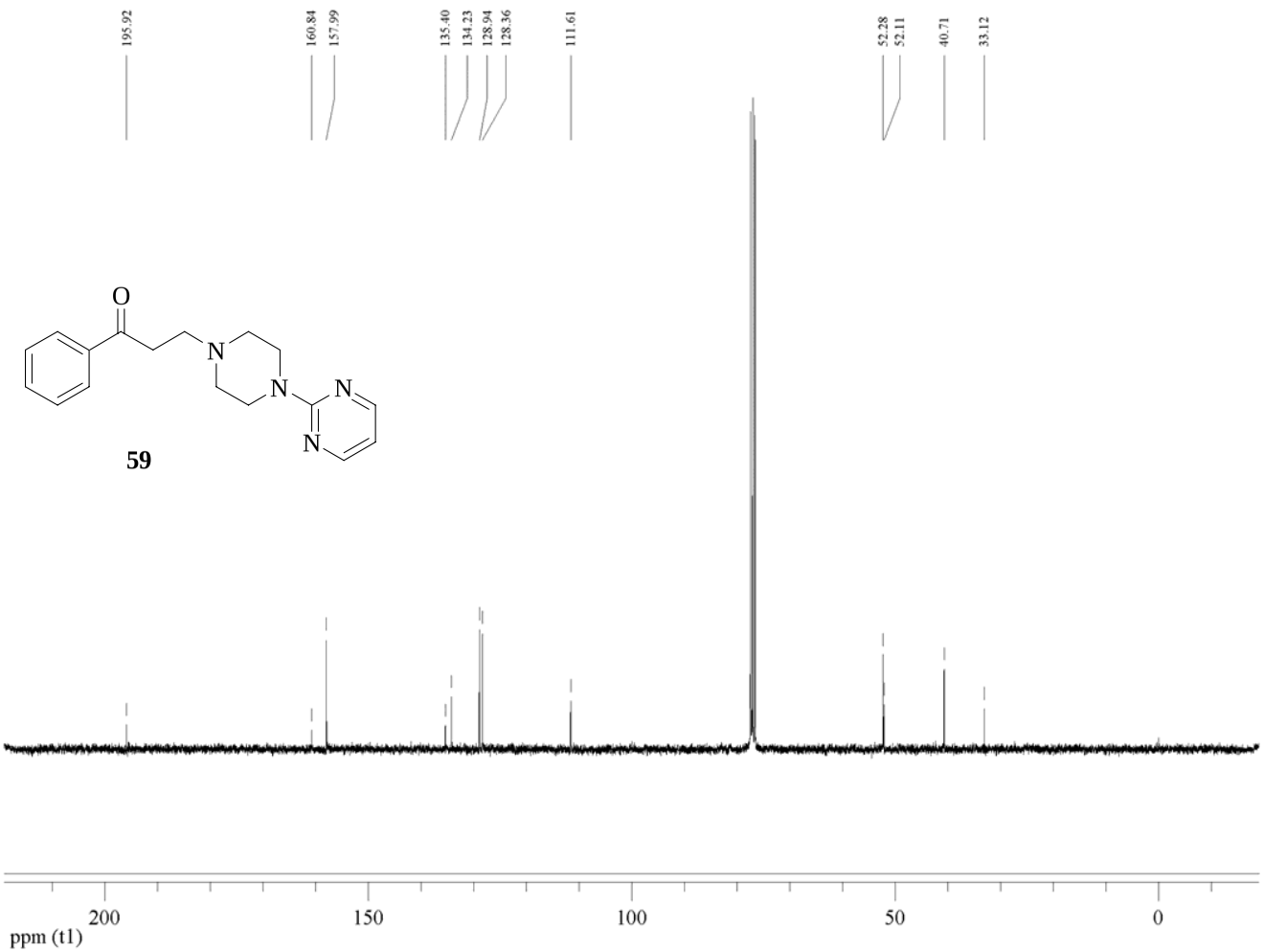
23a



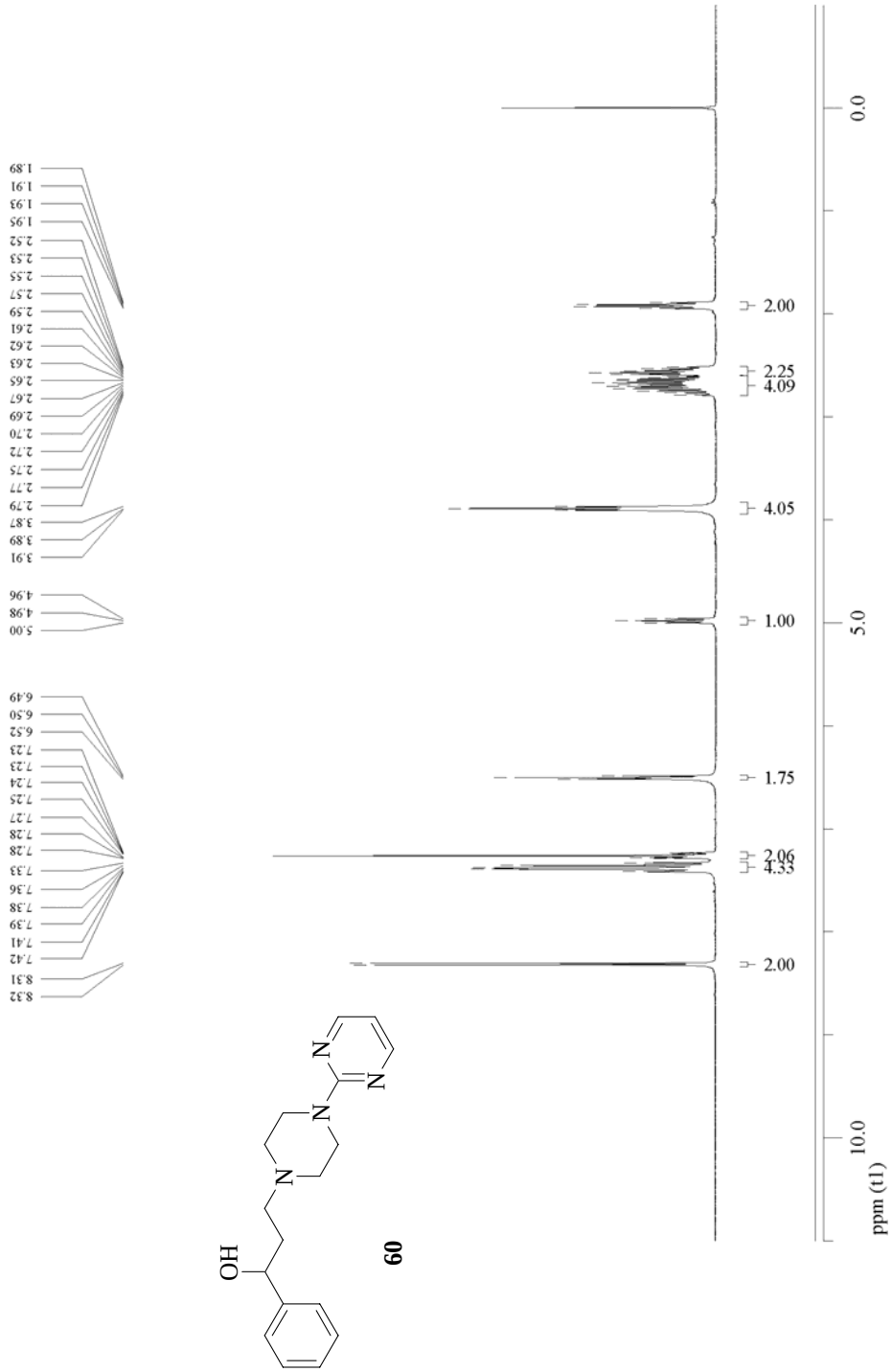
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **59**



^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **59**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **60**



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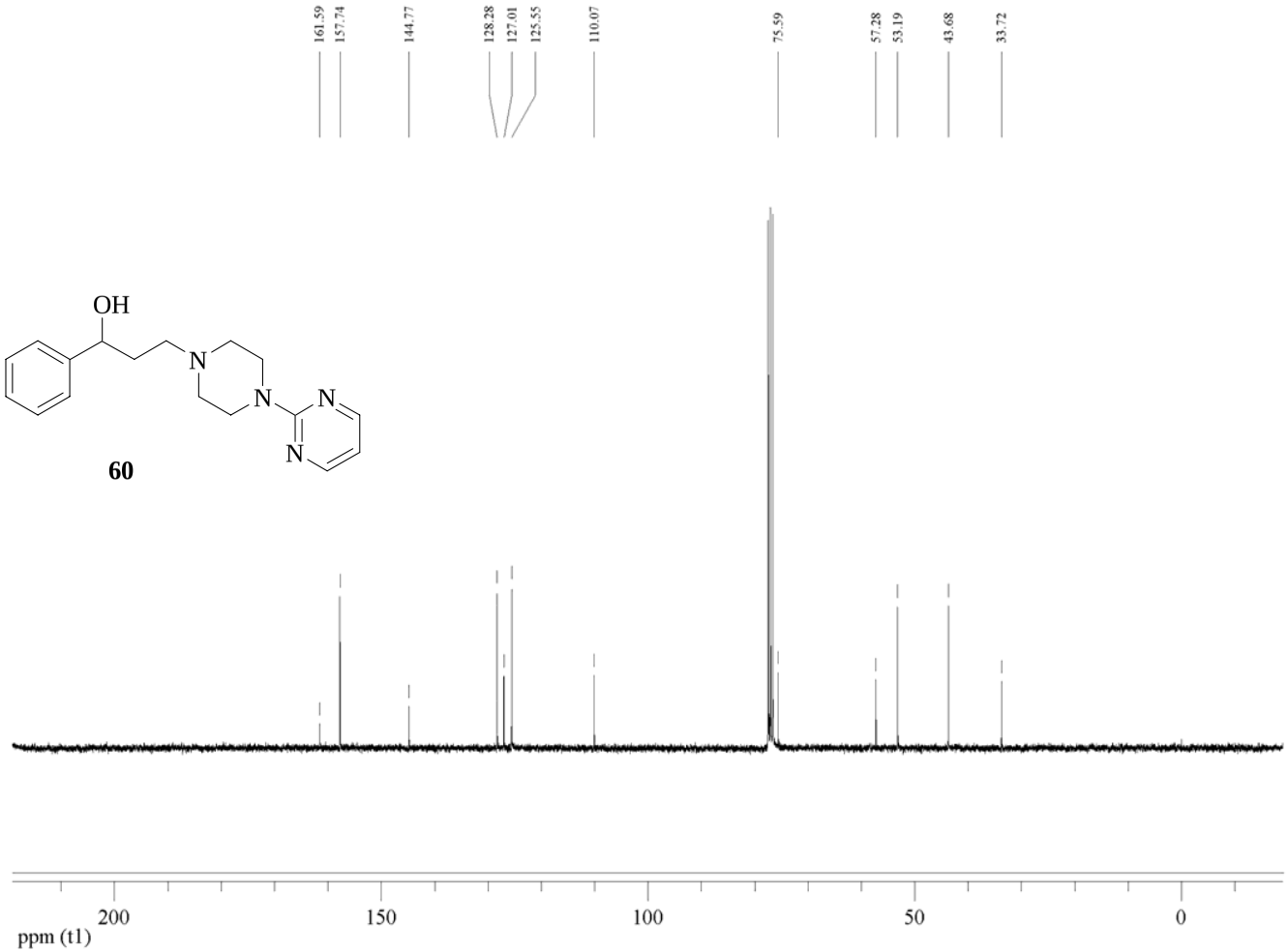
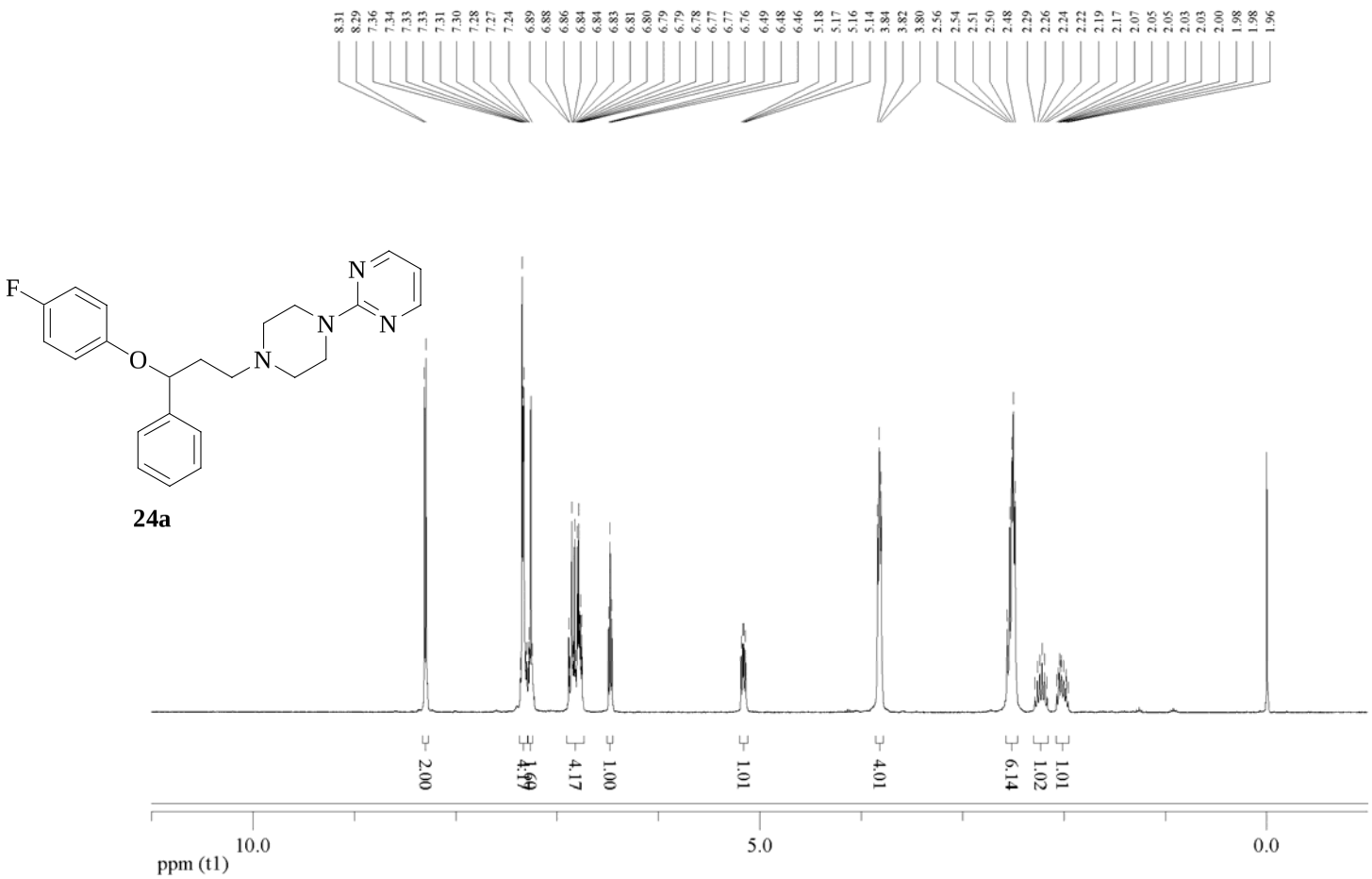
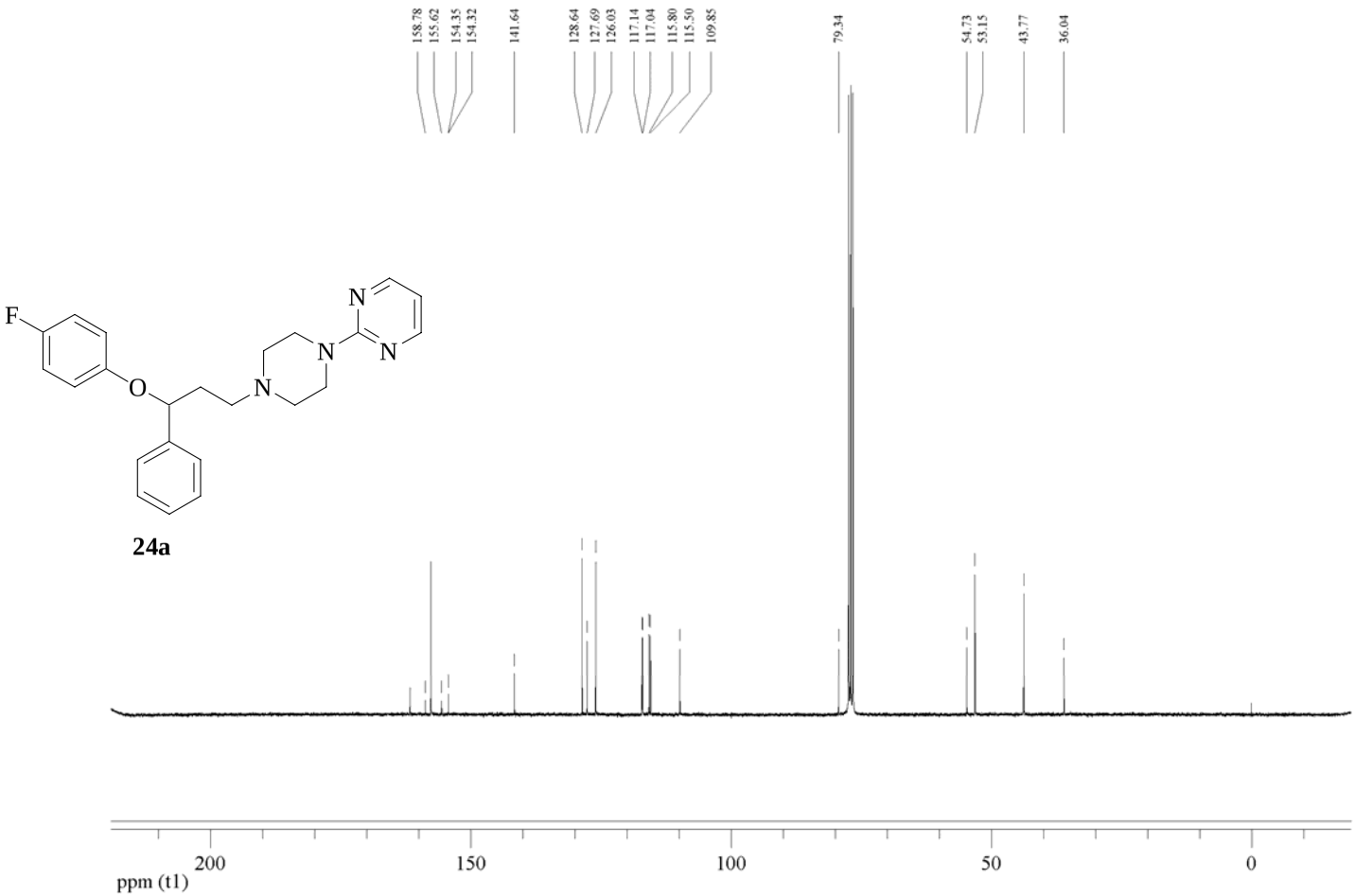


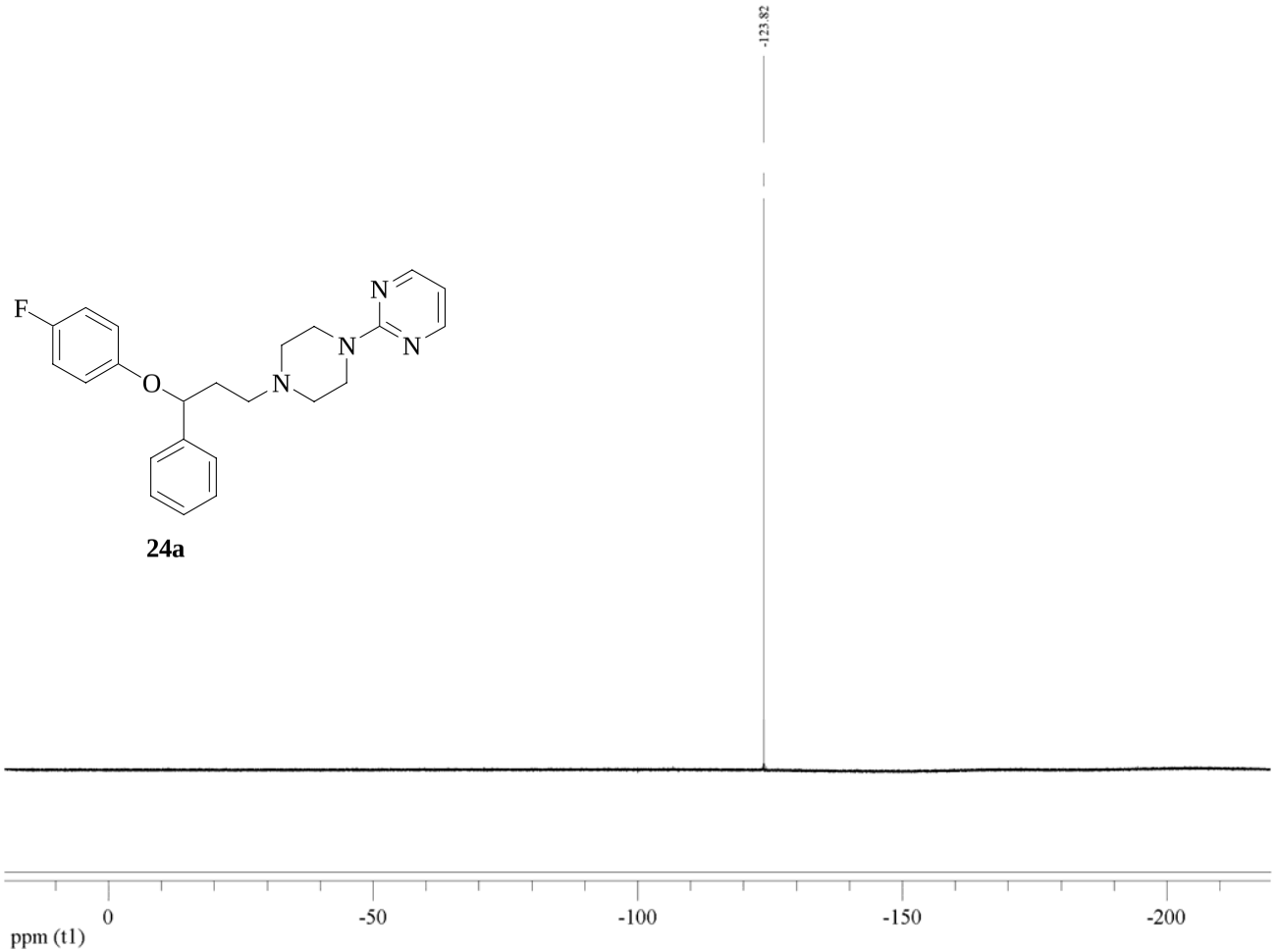
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **24a**



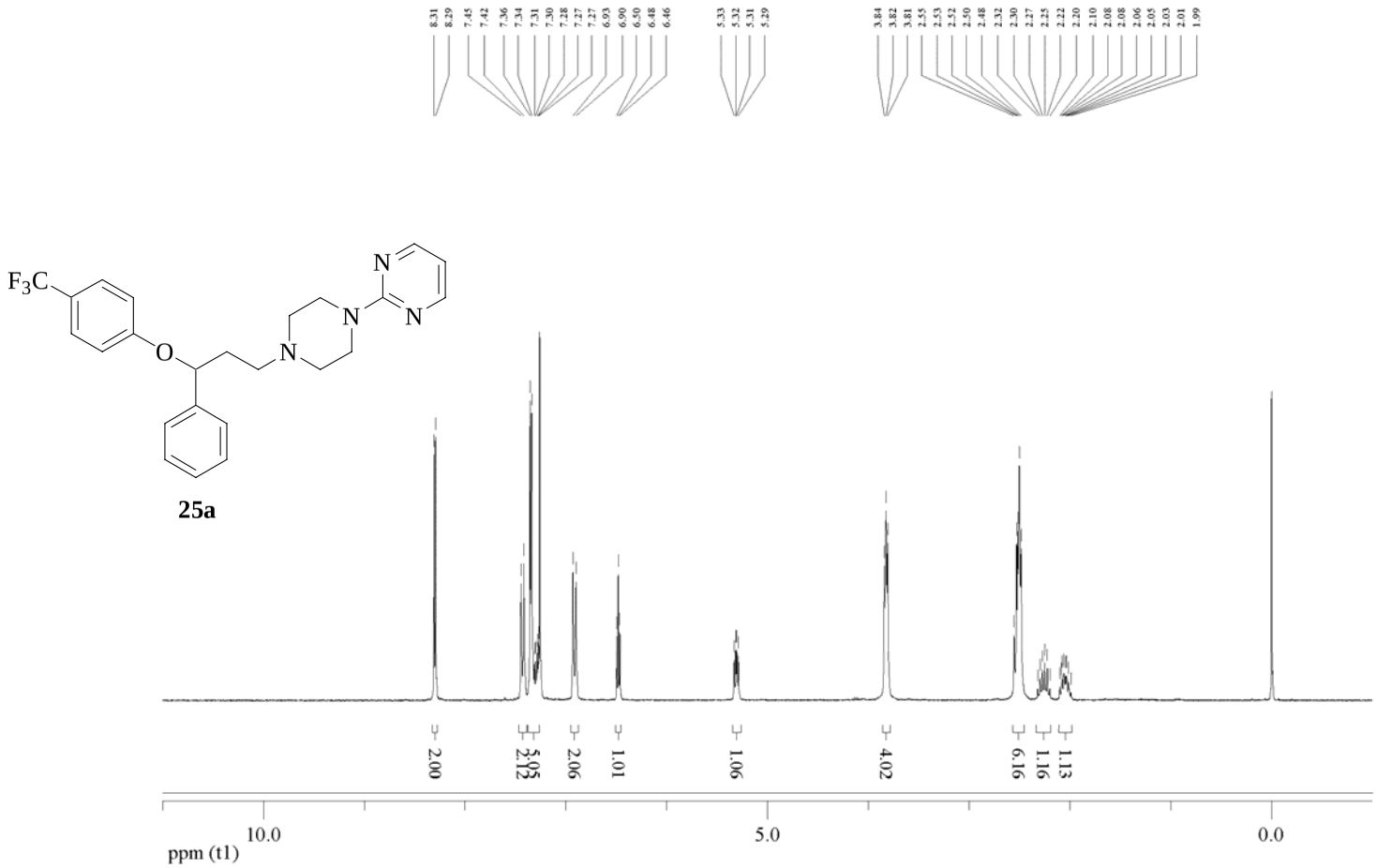
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **24a**



^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **24a**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **25a**



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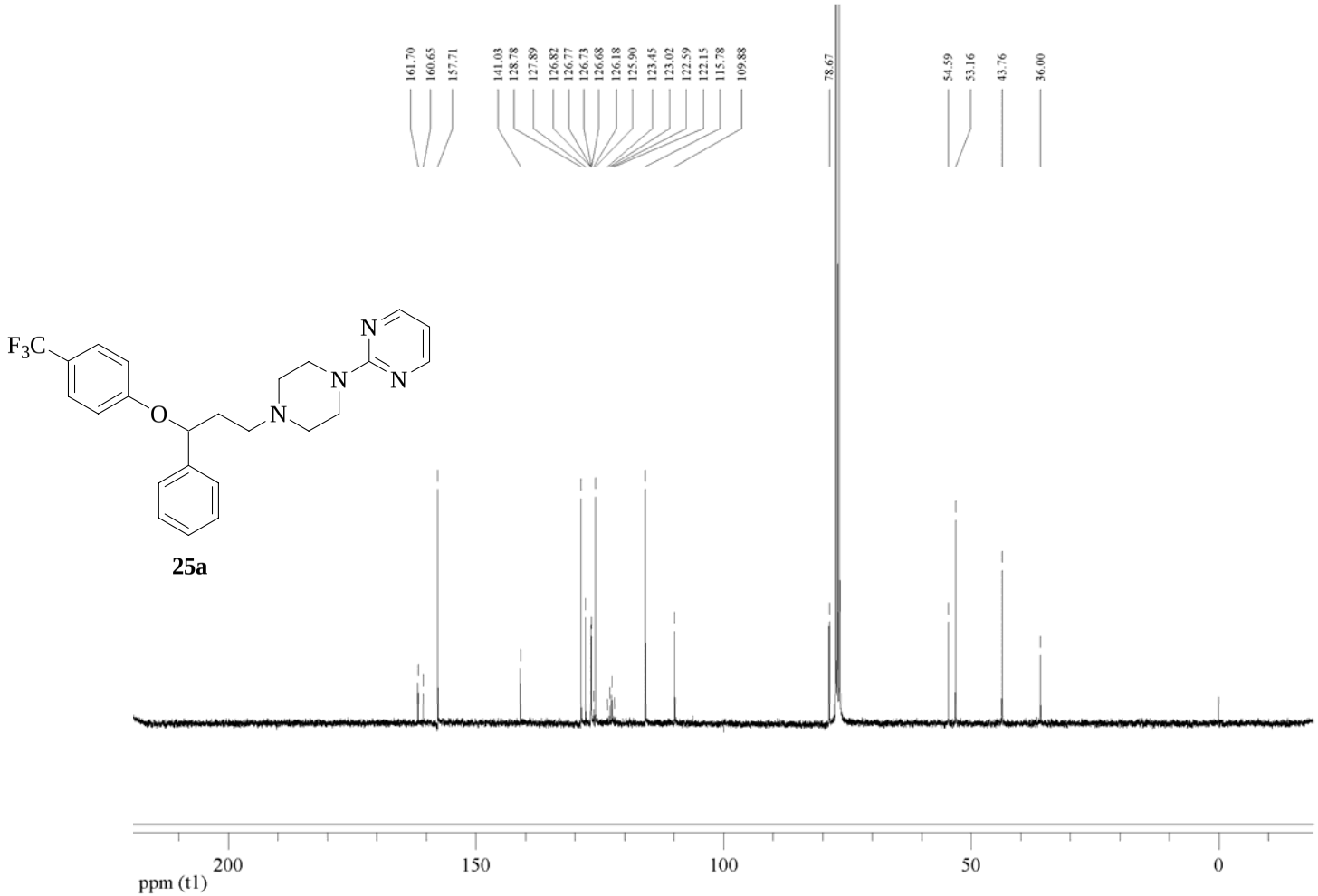

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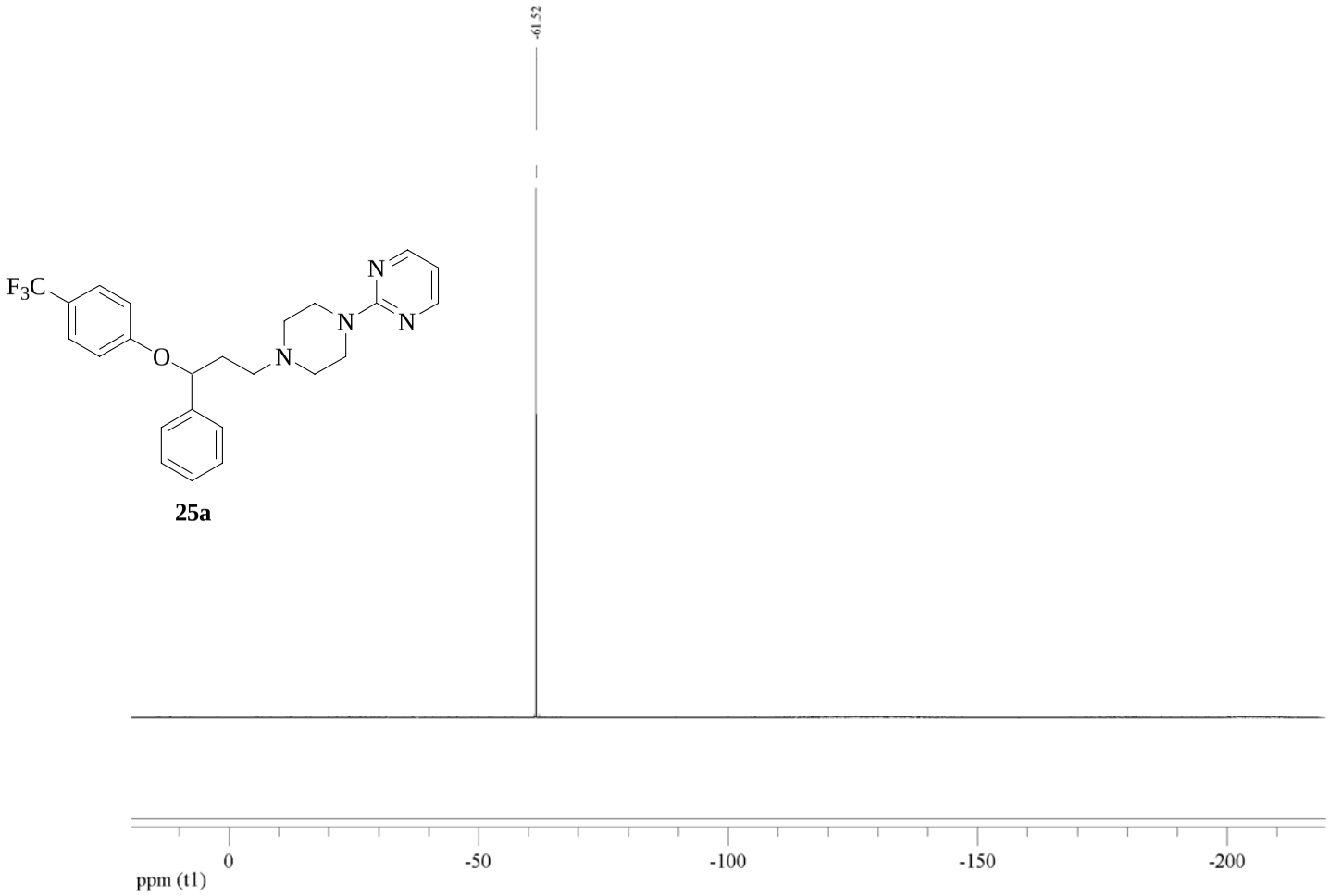
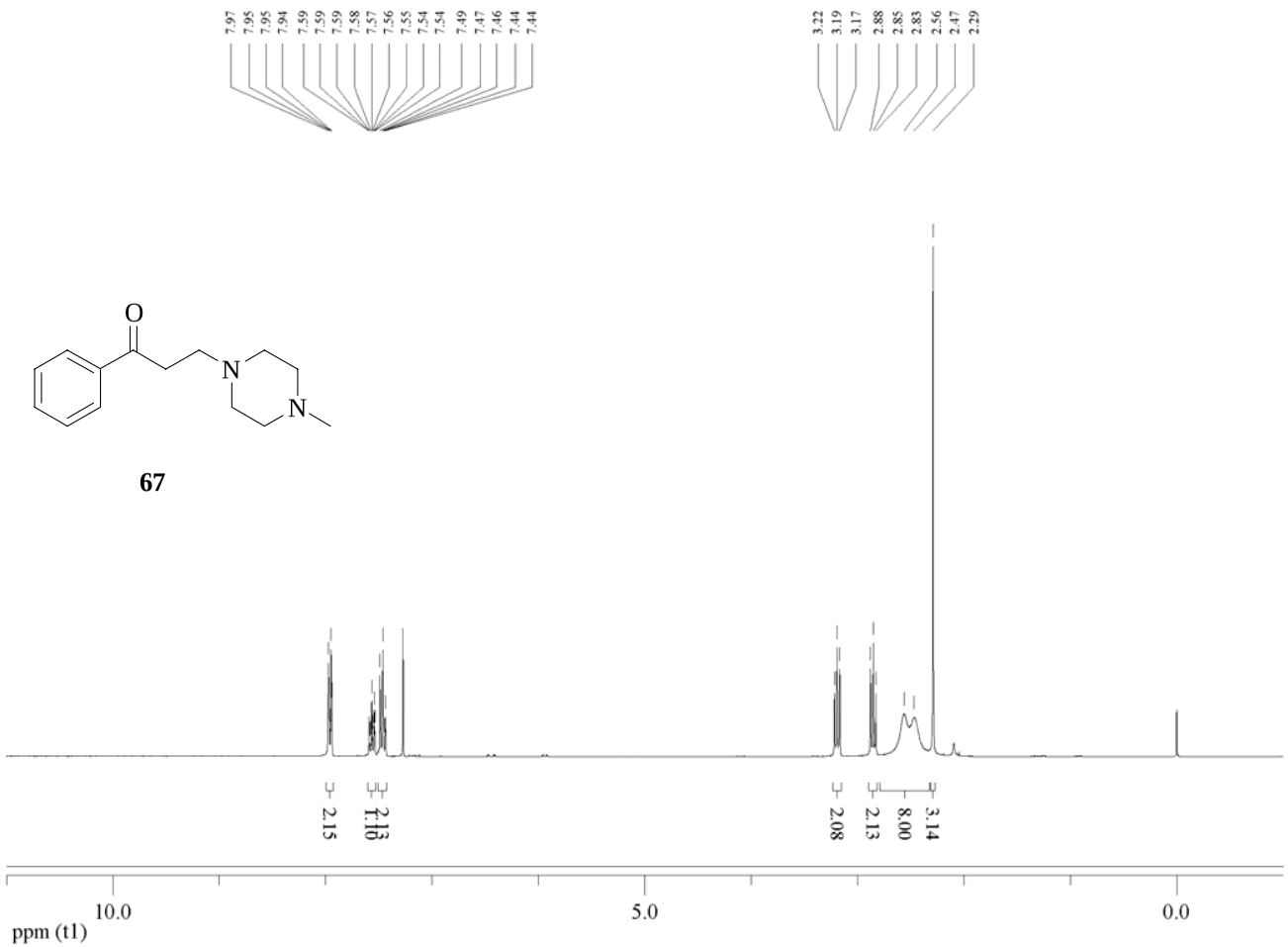
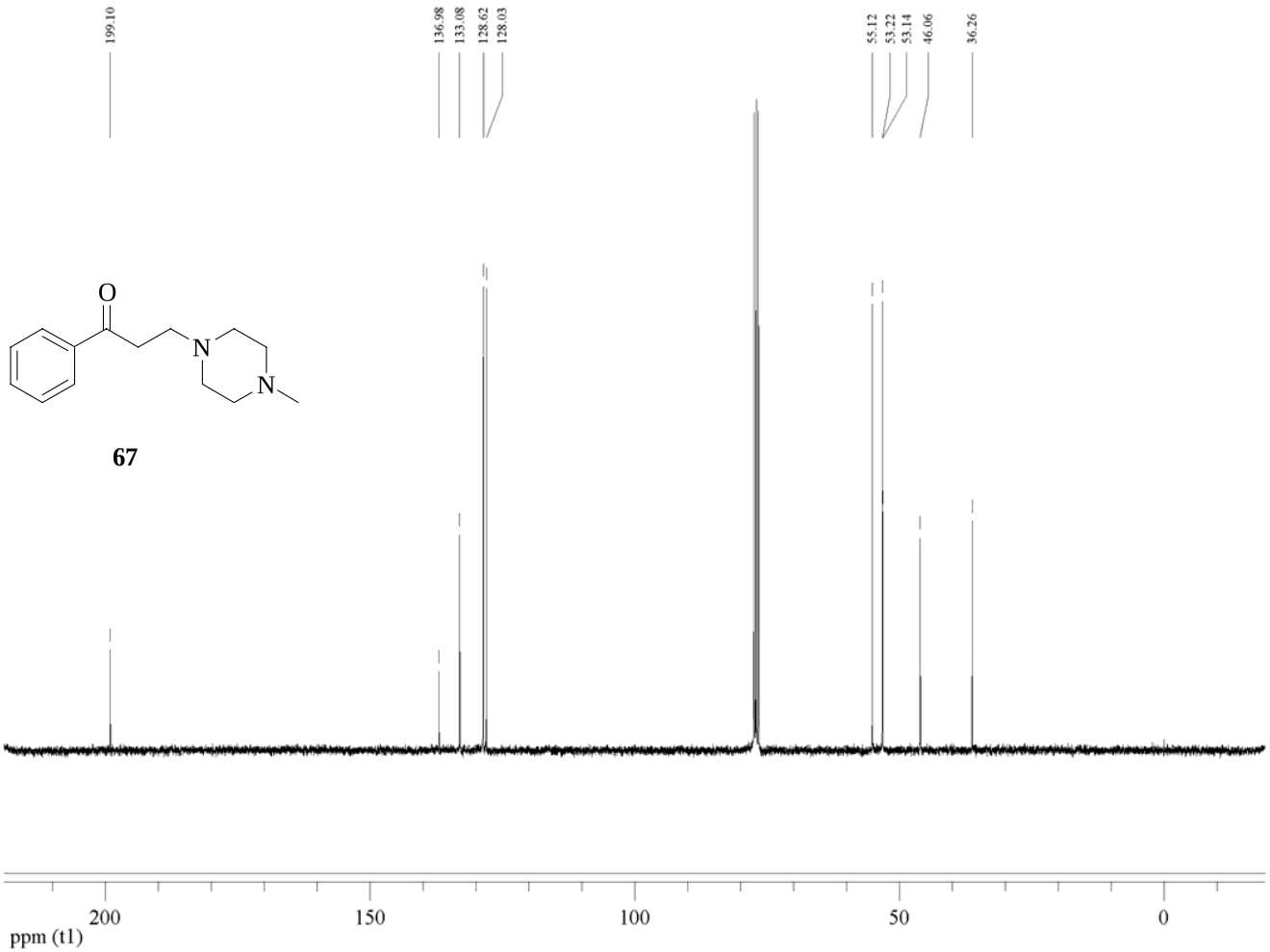


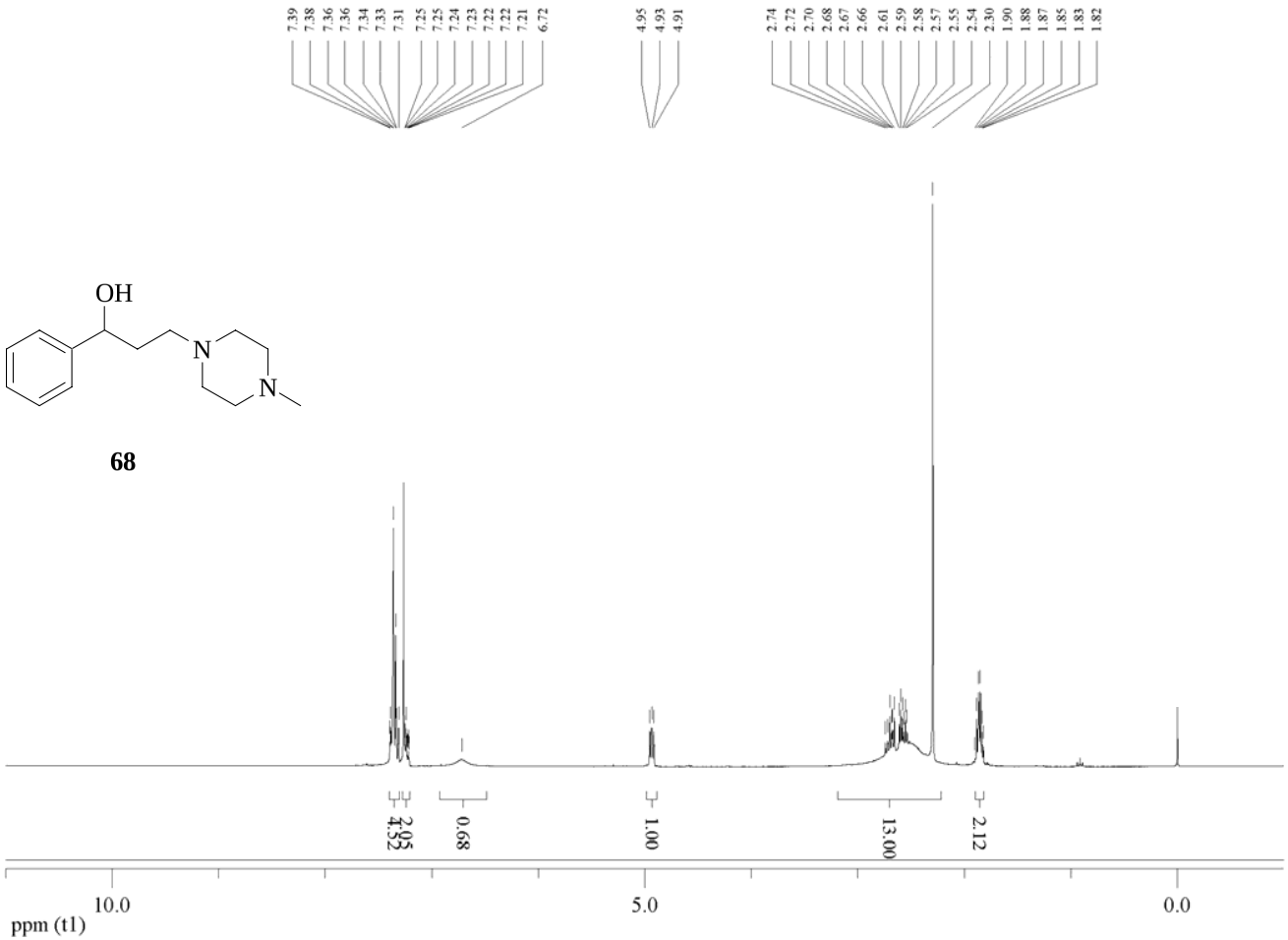
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **67**



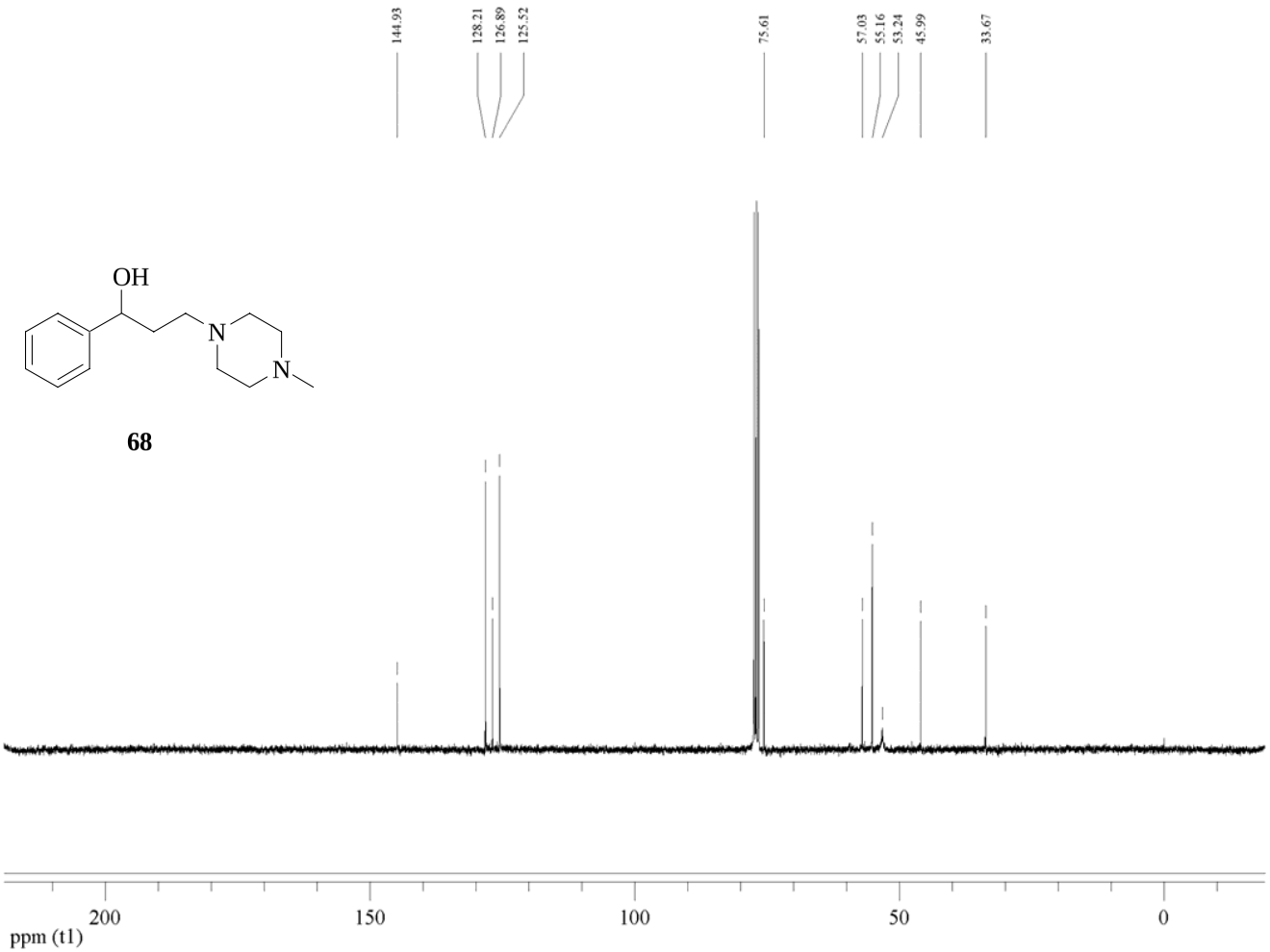
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **67**



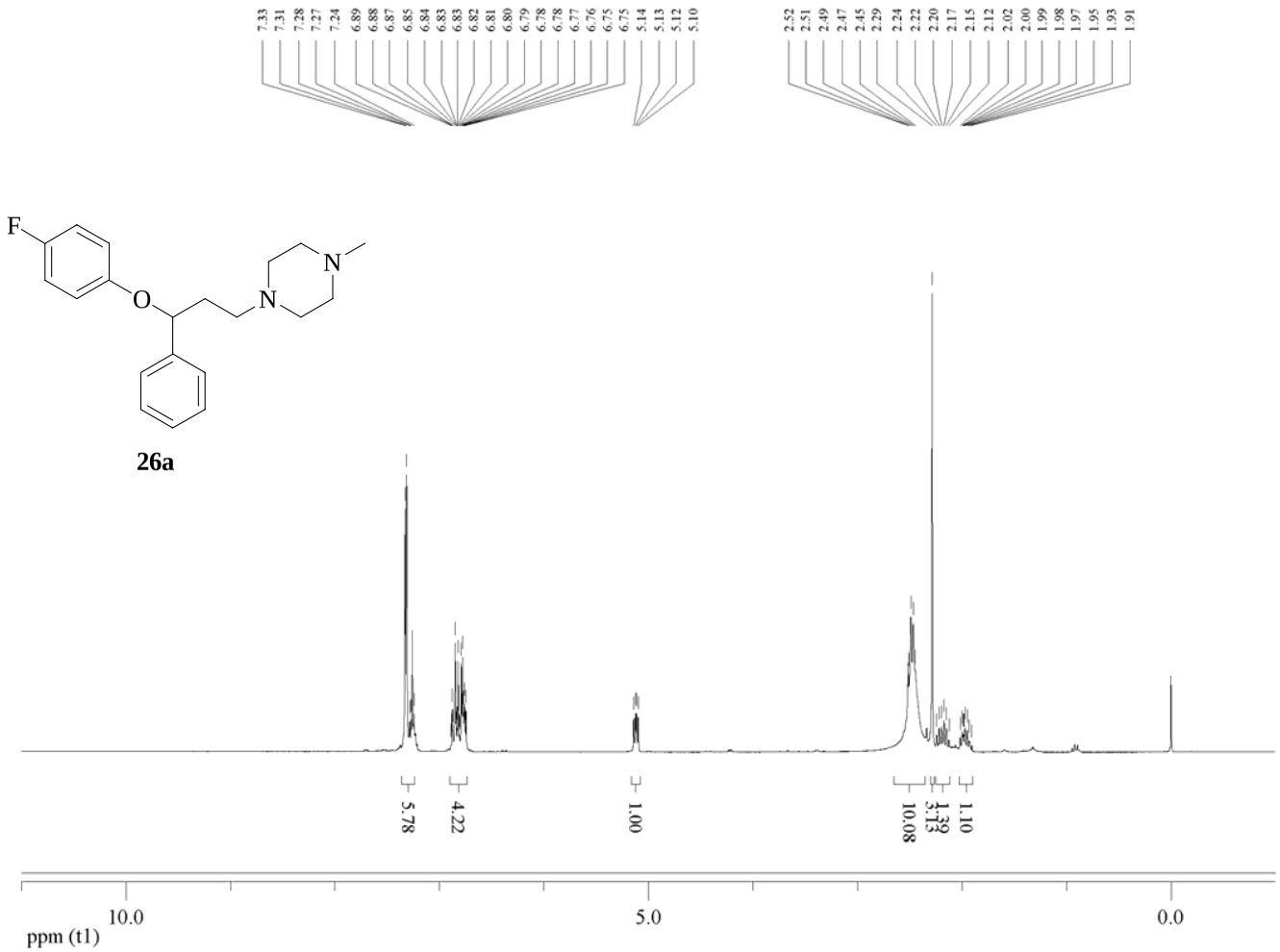
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **68**



^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **68**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **26a**



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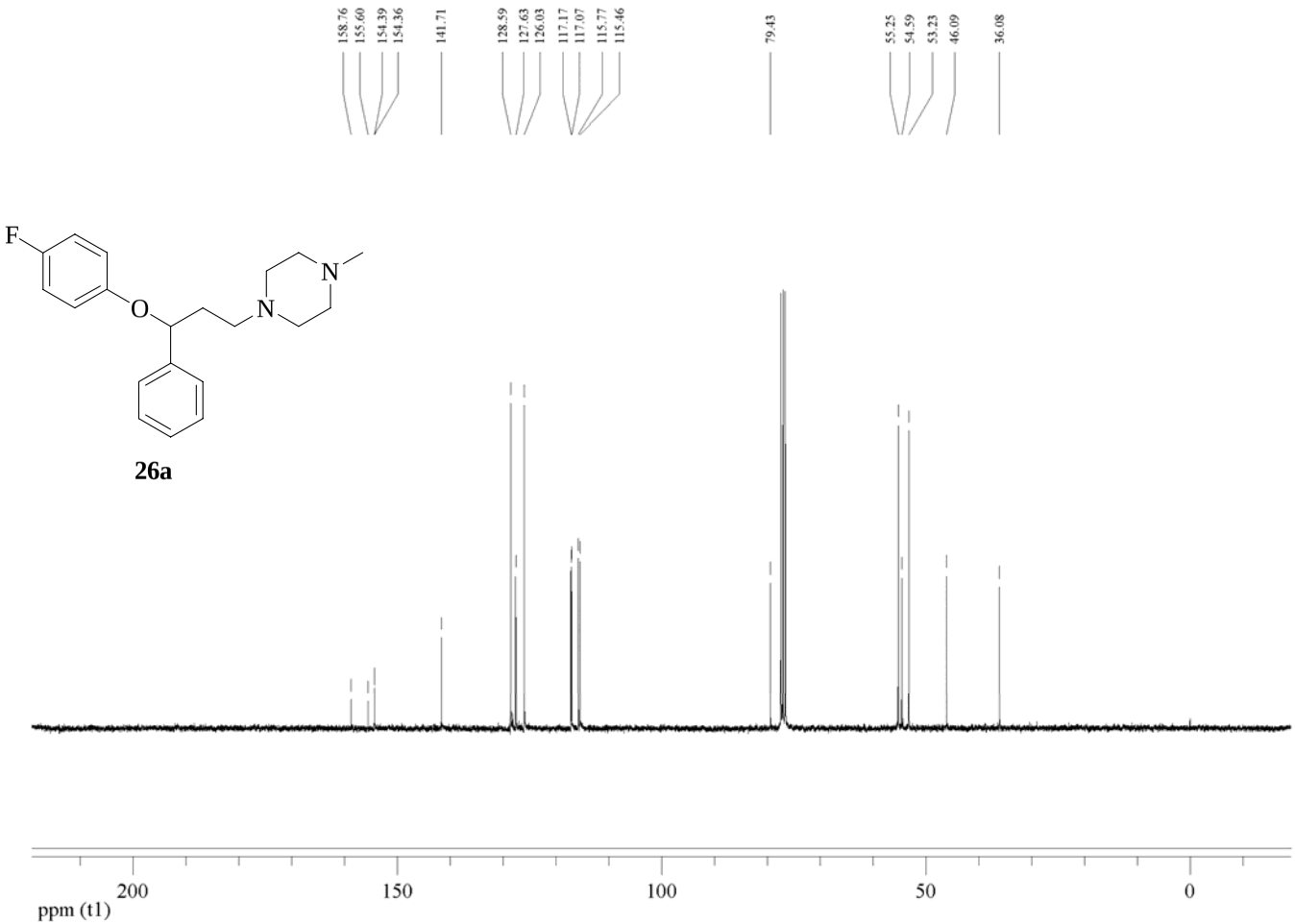
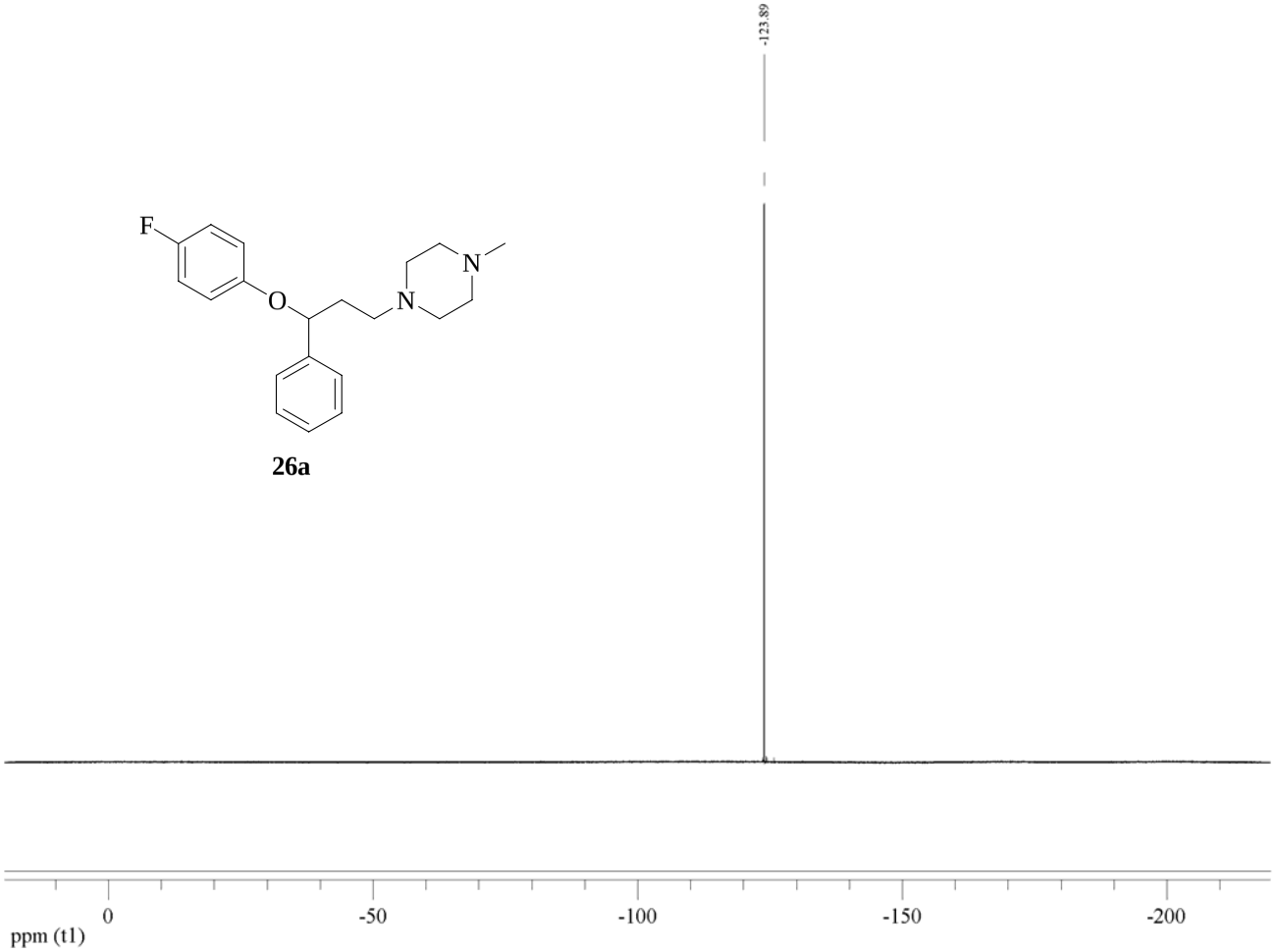
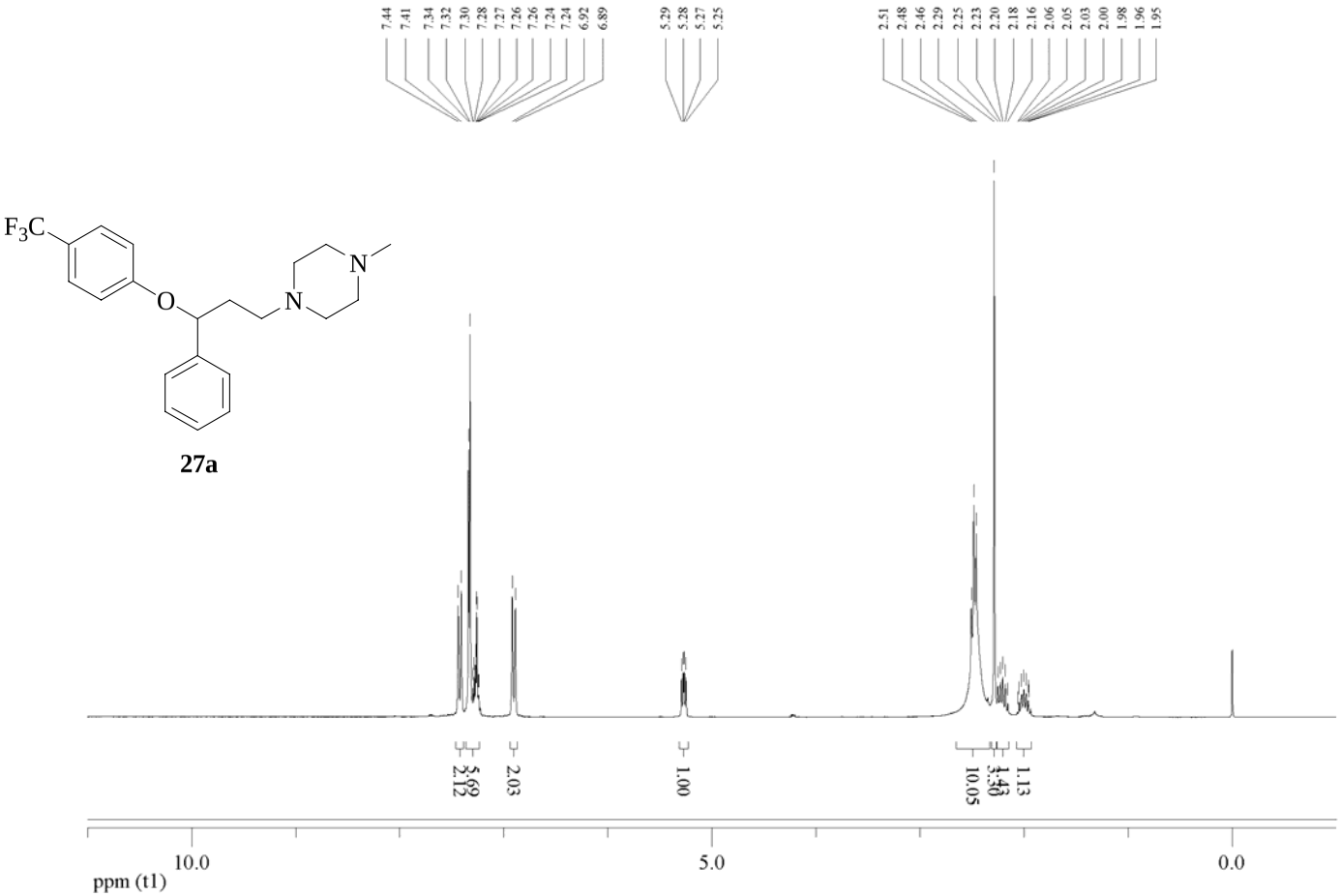


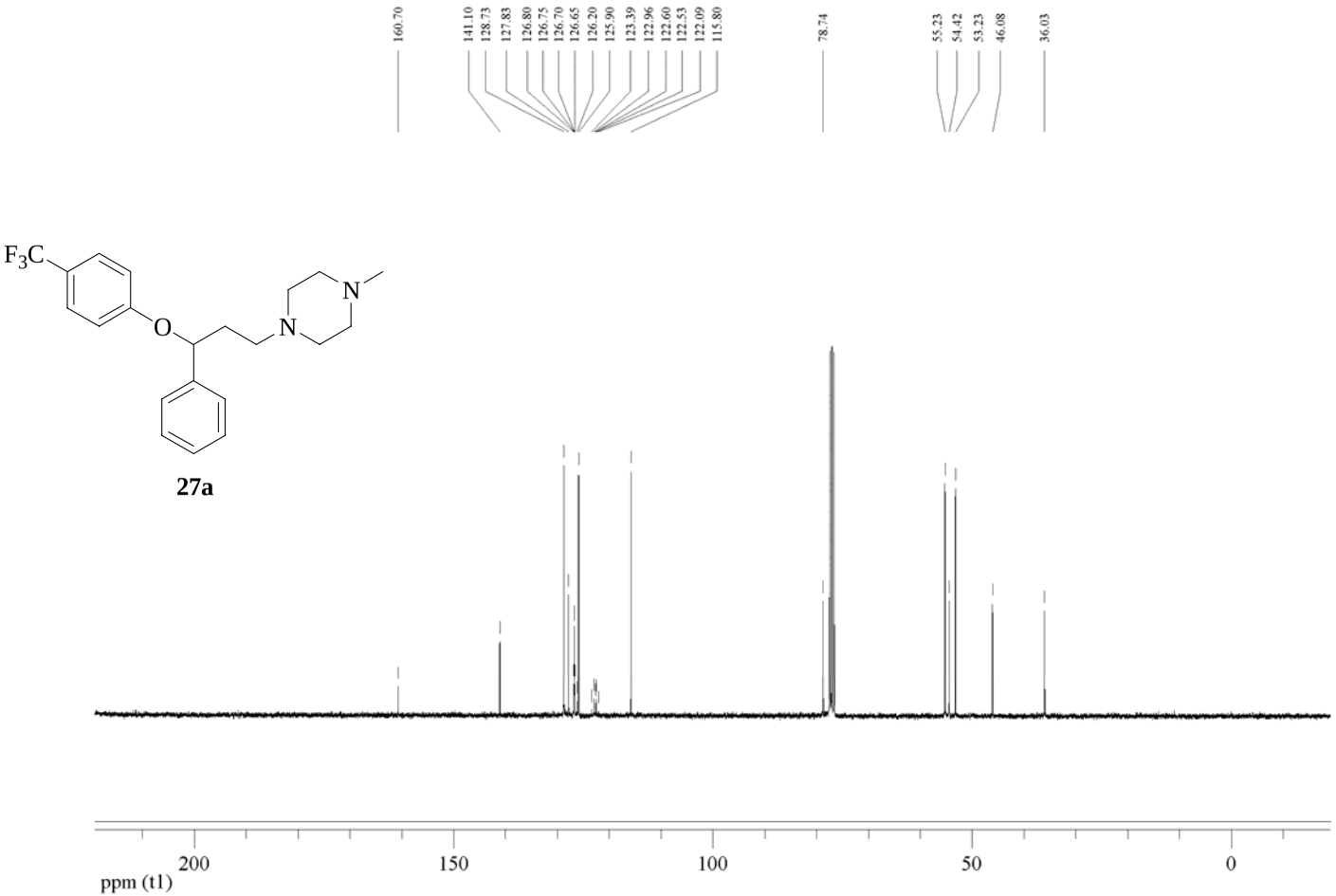
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **26a**



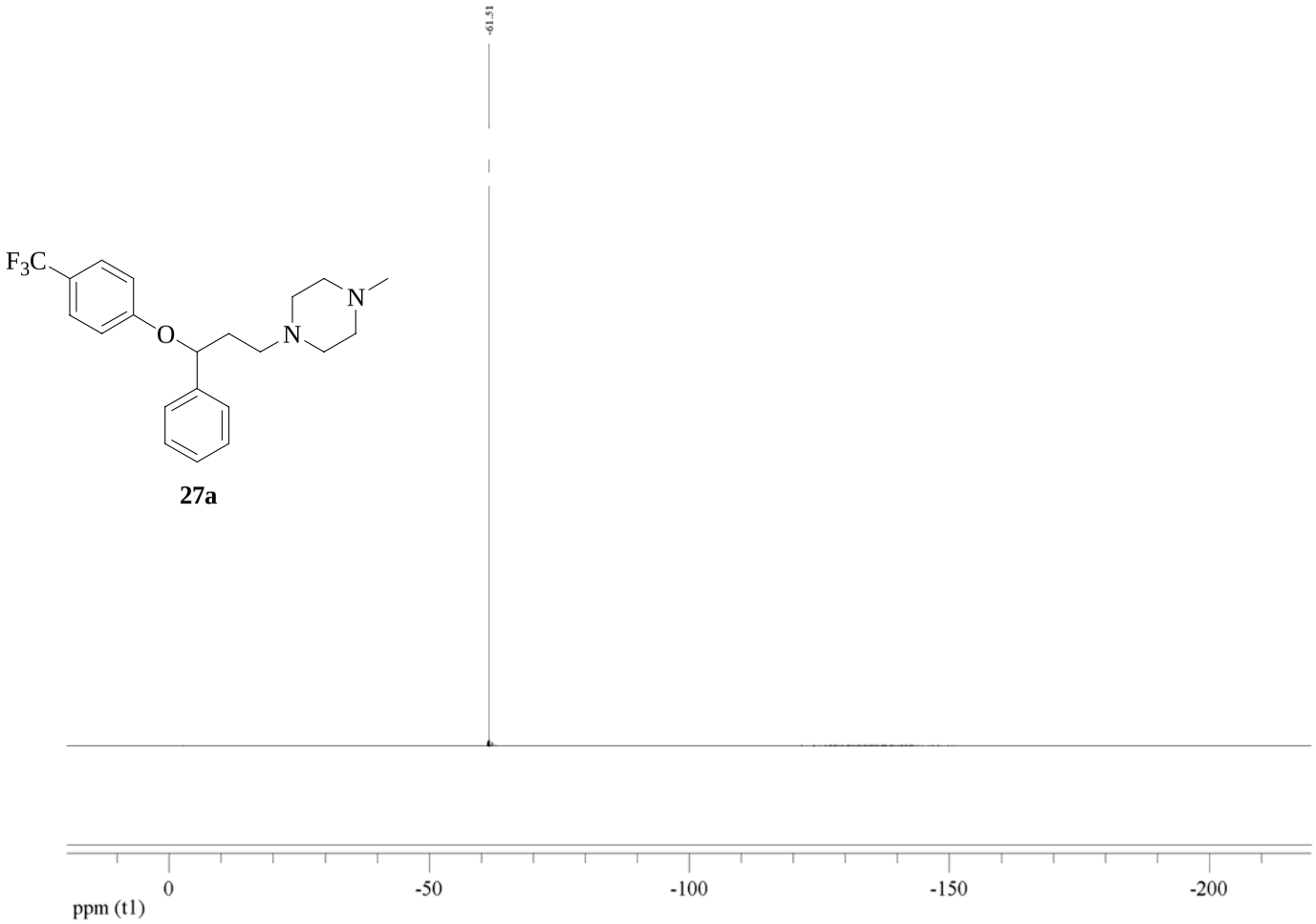
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **27a**



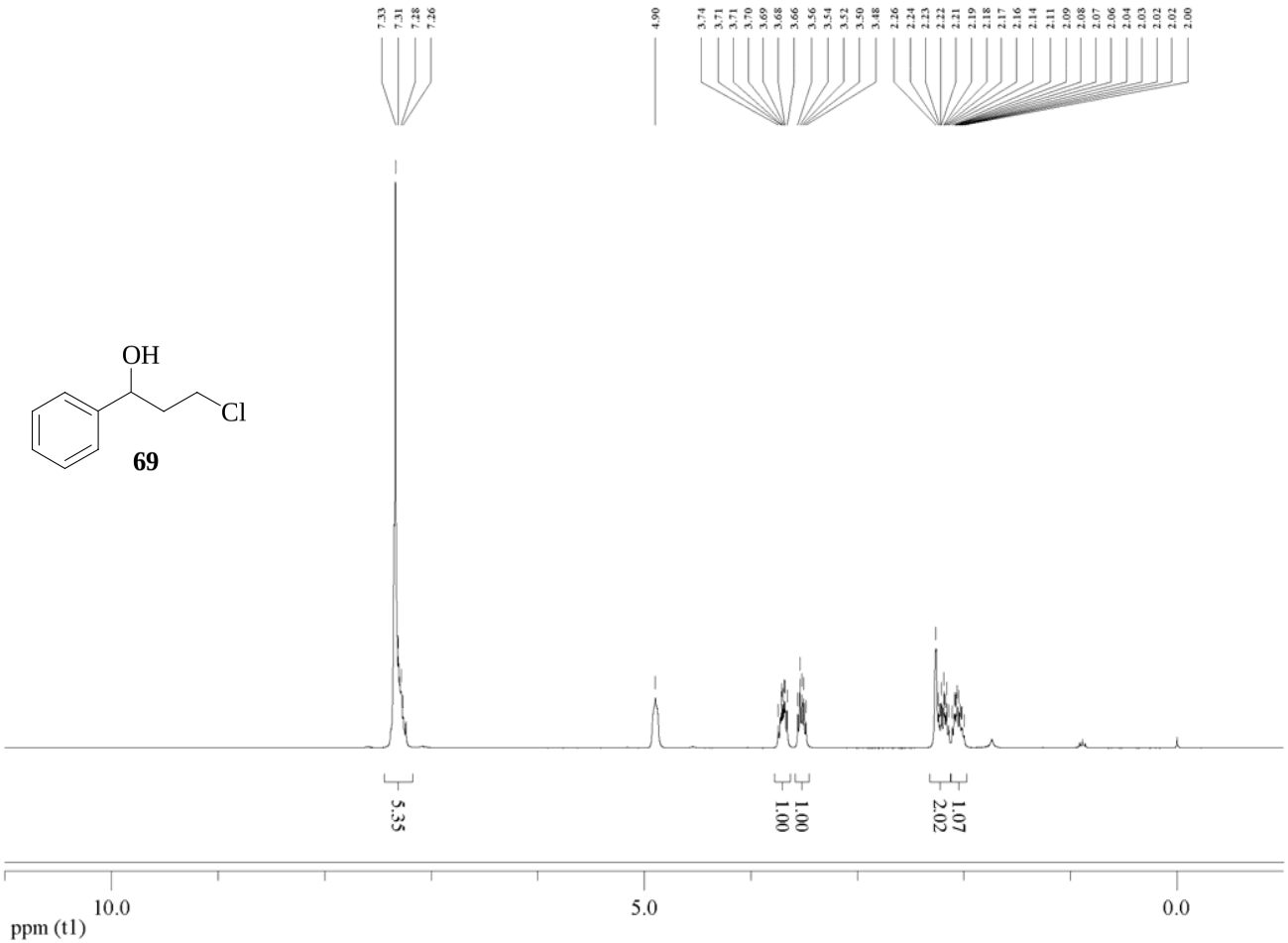
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **27a**



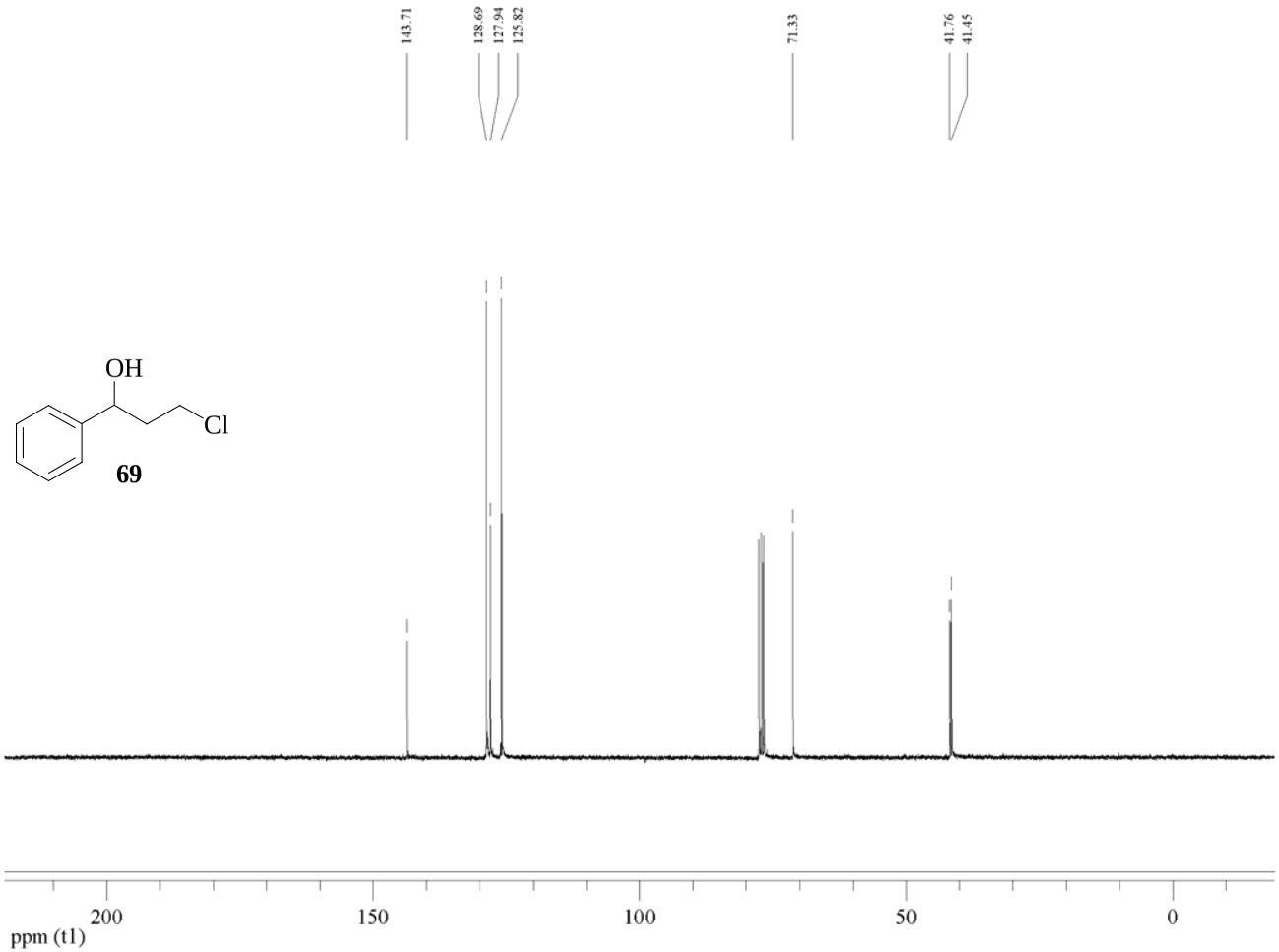
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **27a**



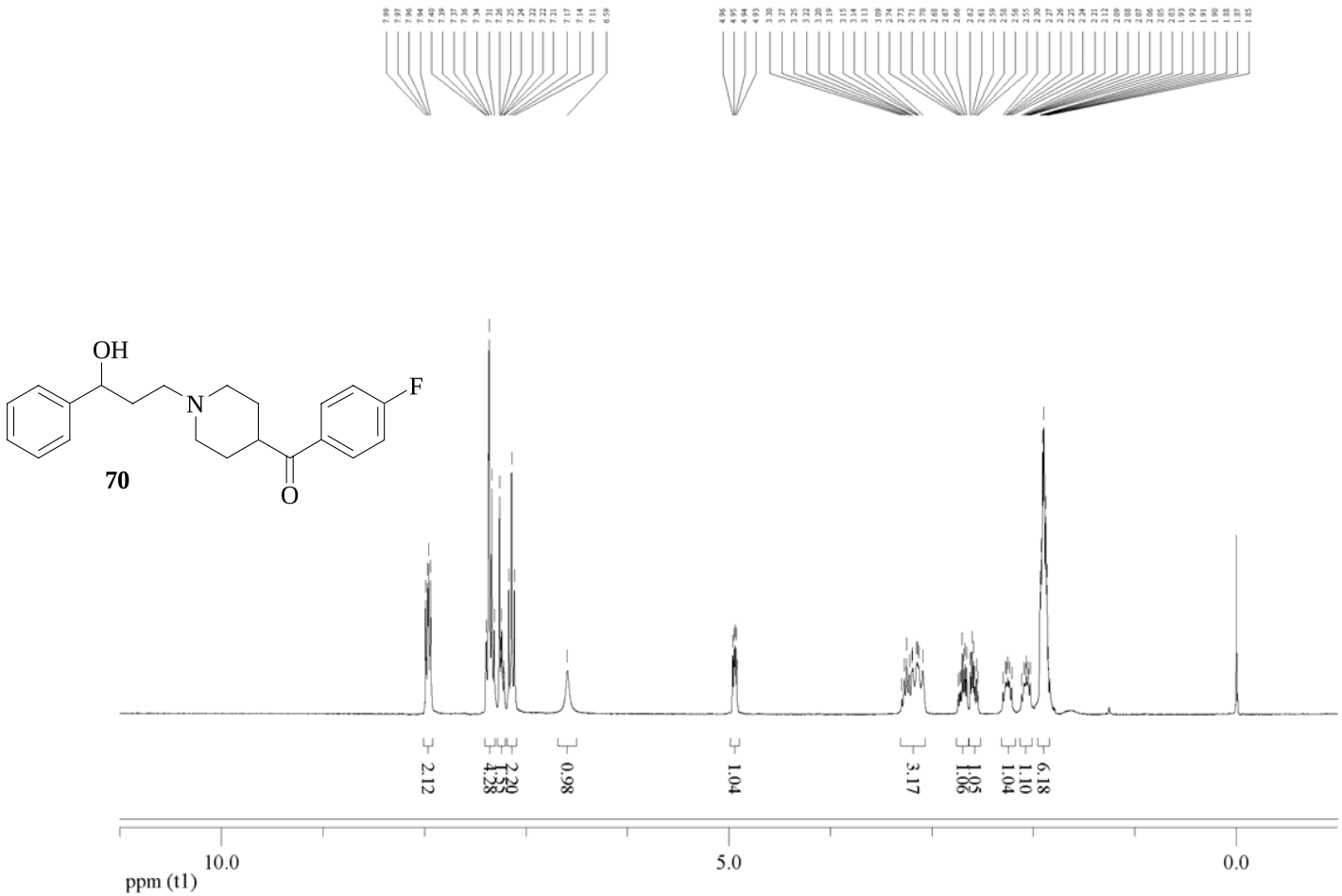
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **69**



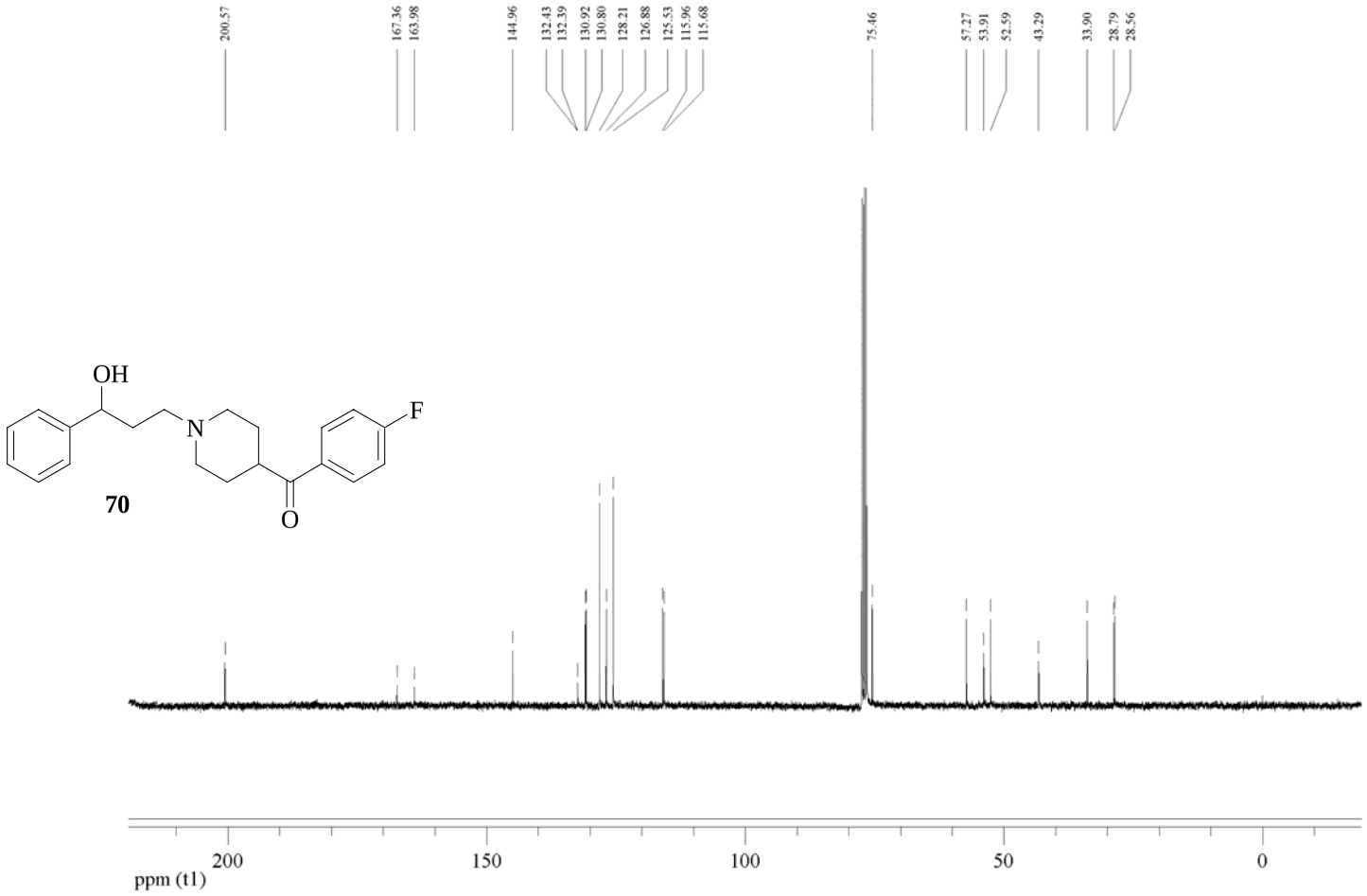
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **69**



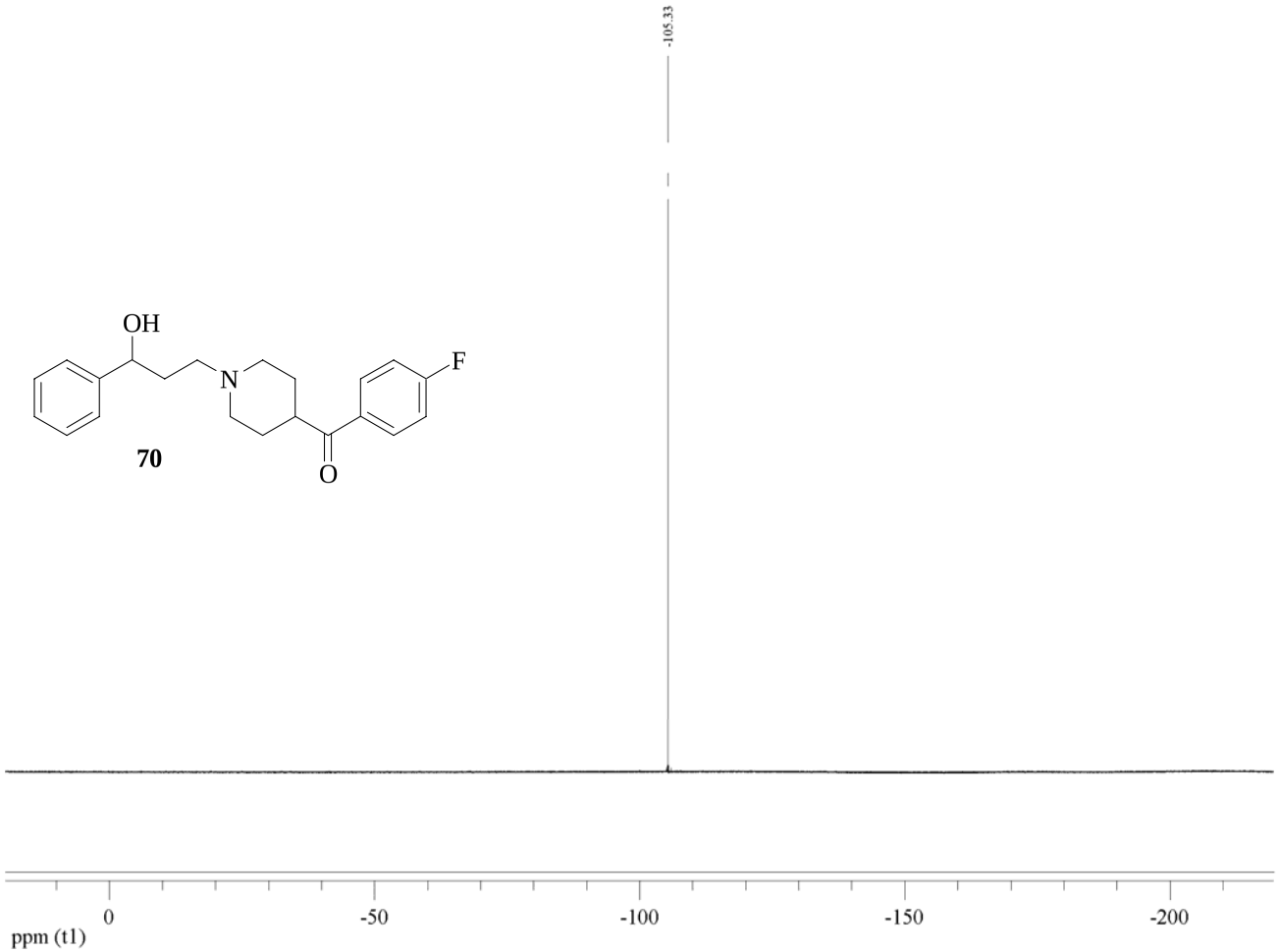
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **70**



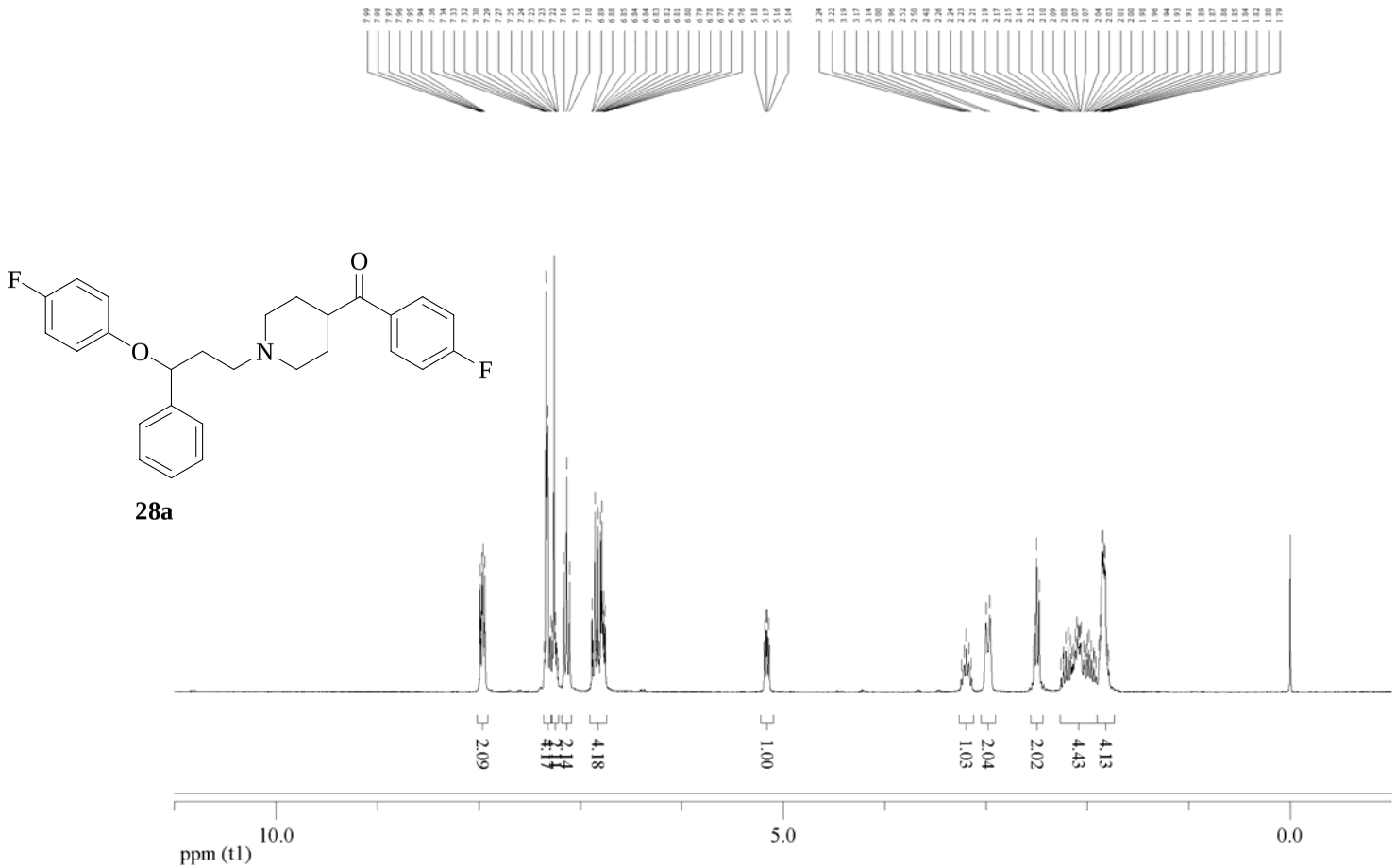
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **70**



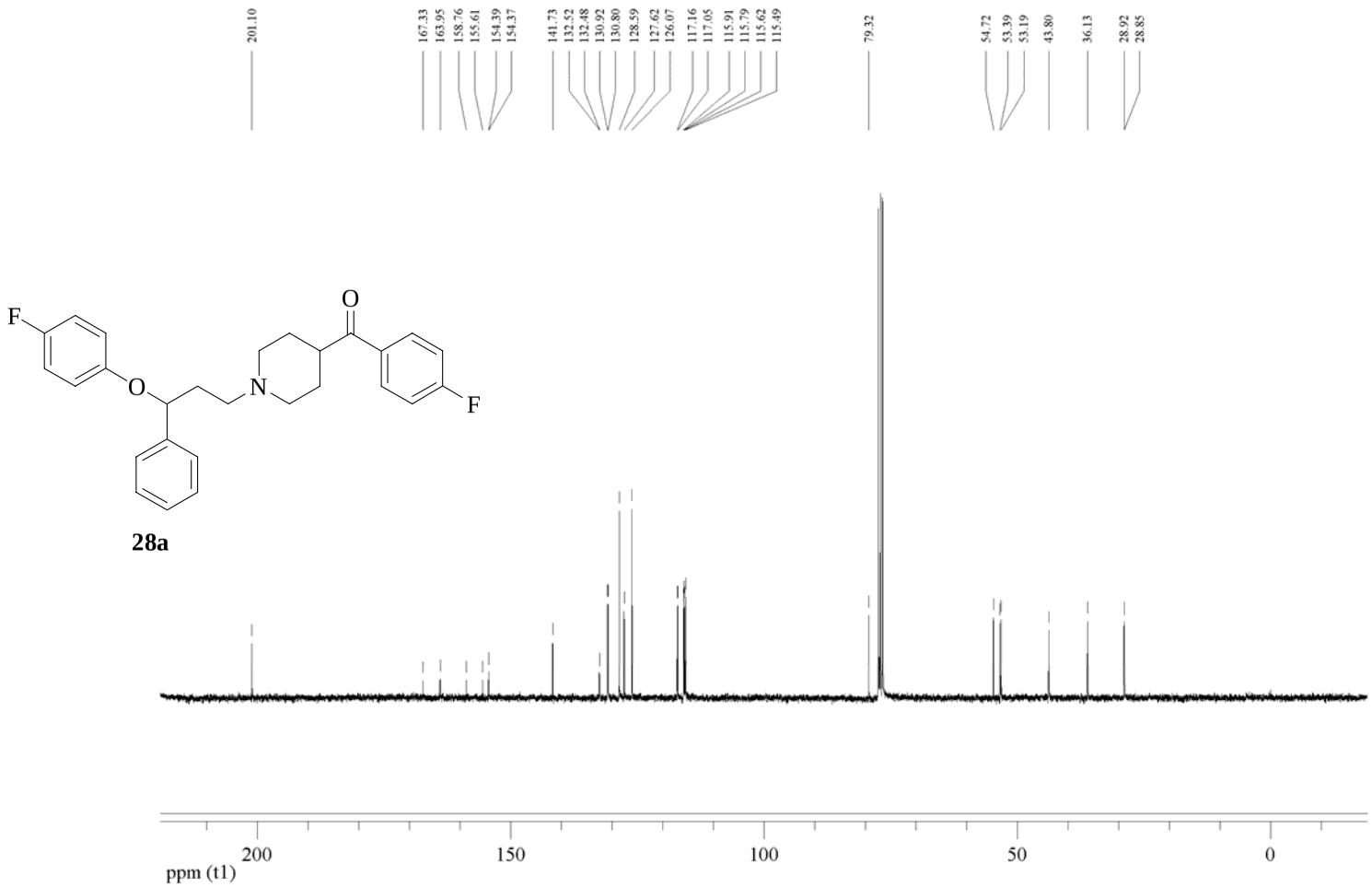
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **70**



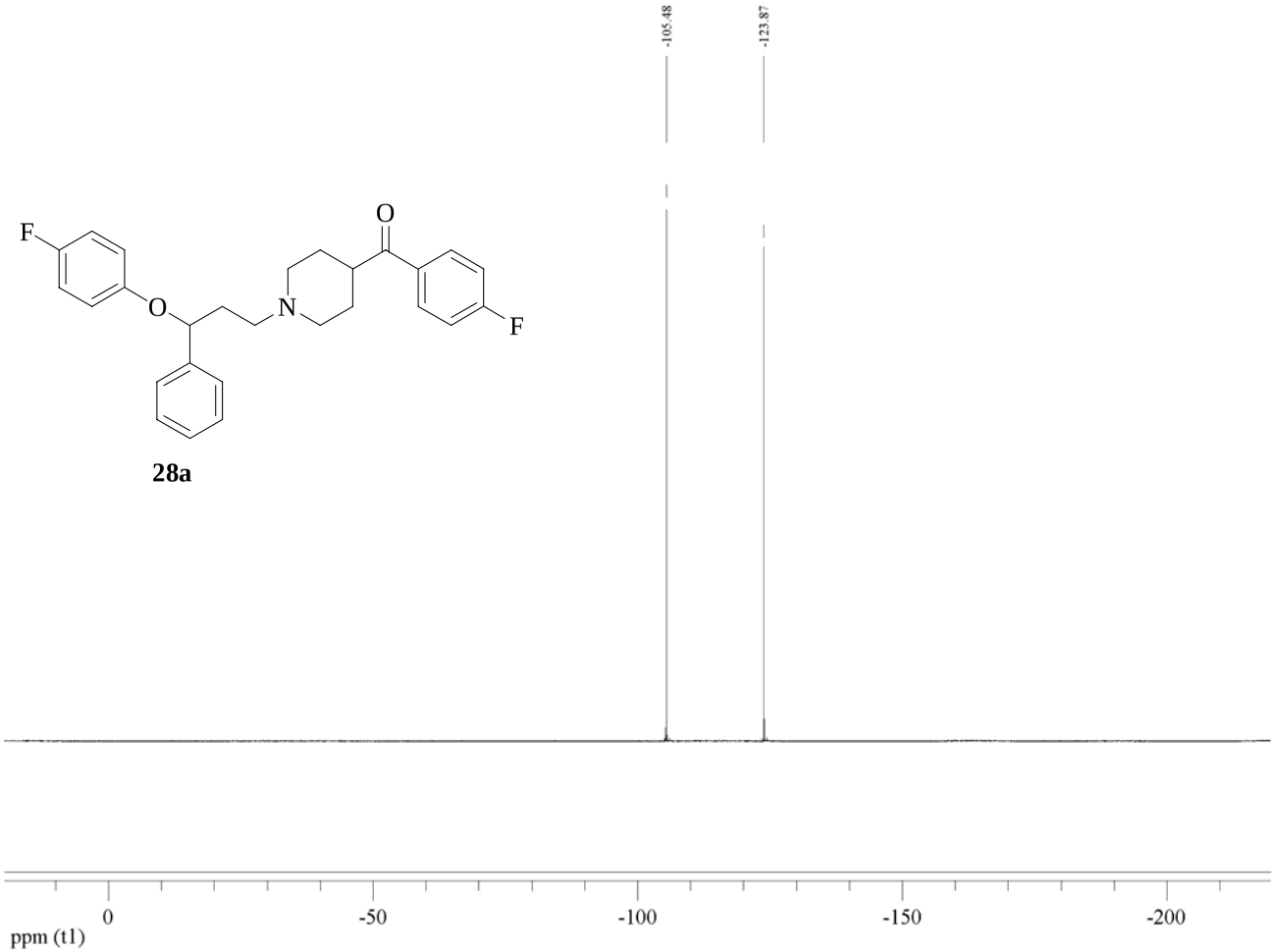
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **28a**



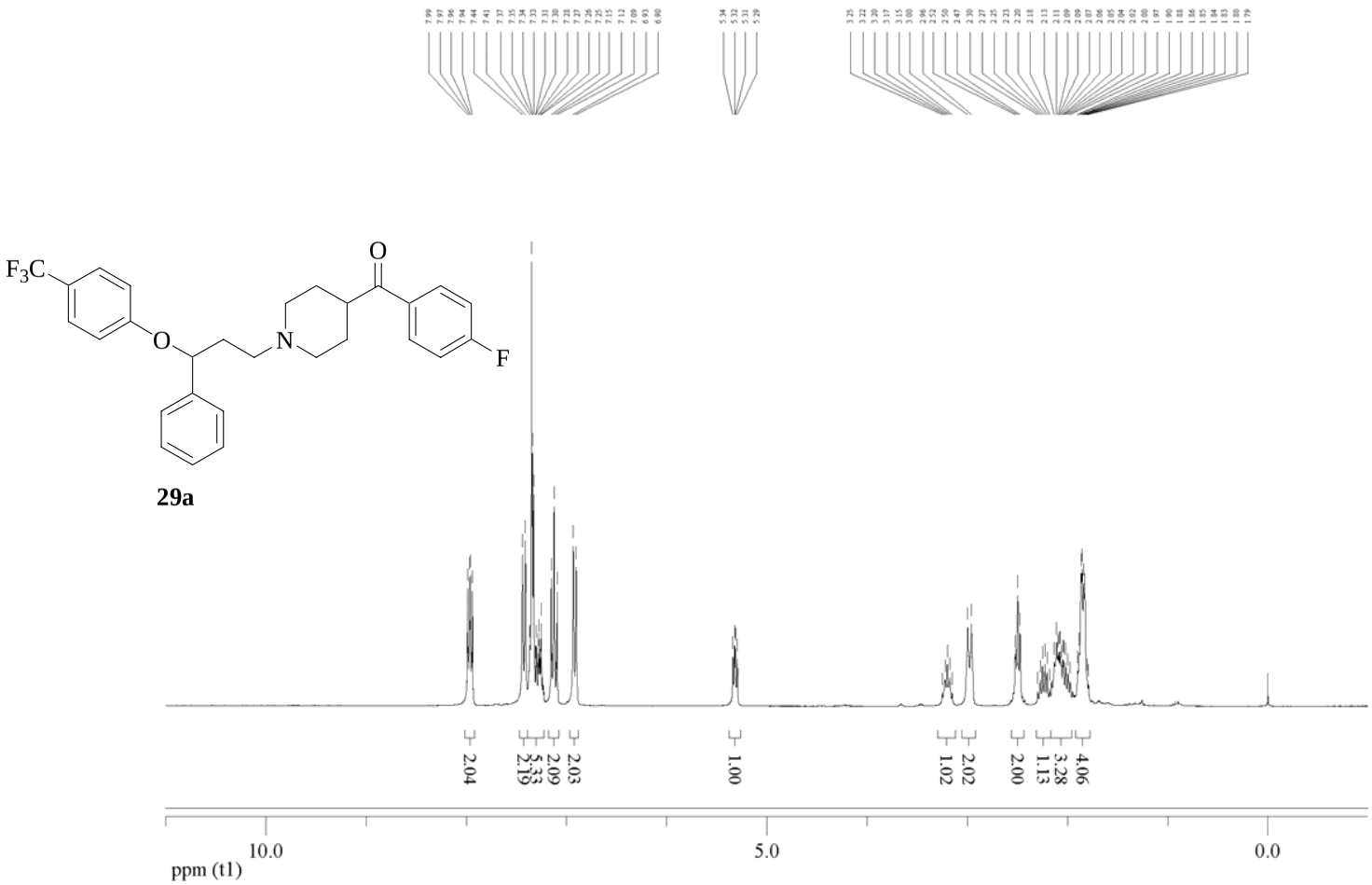
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **28a**



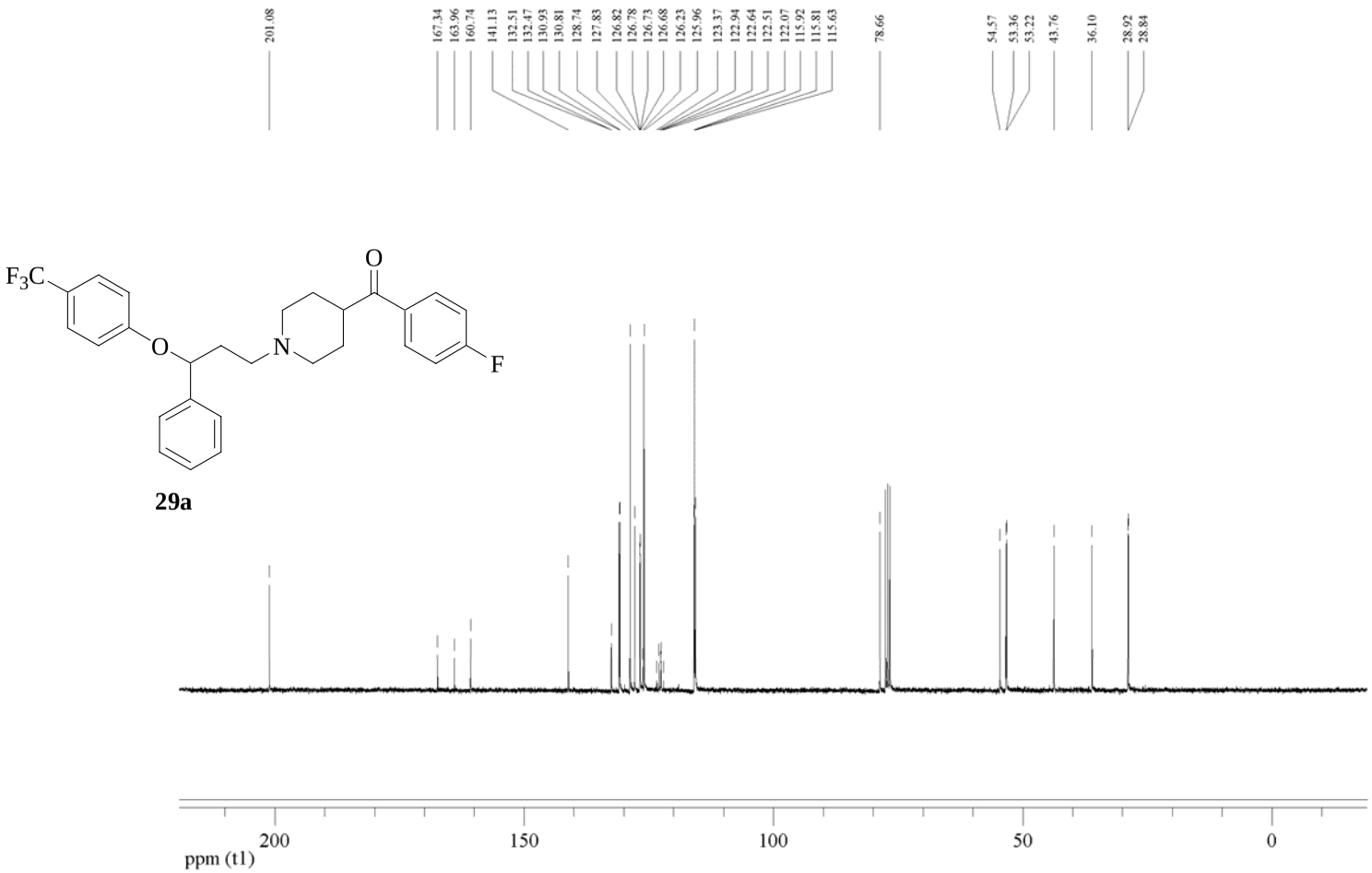
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **28a**



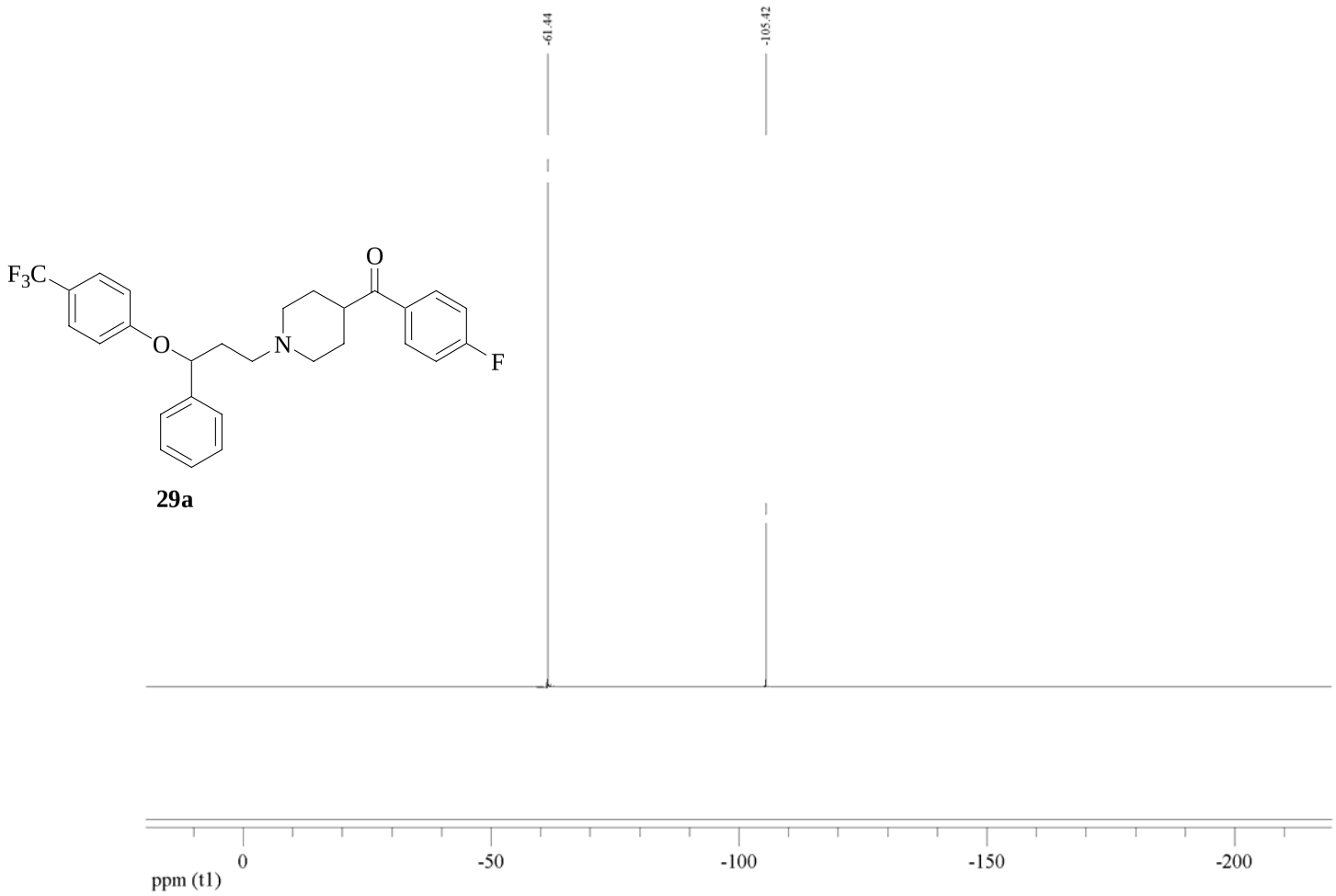
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **29a**



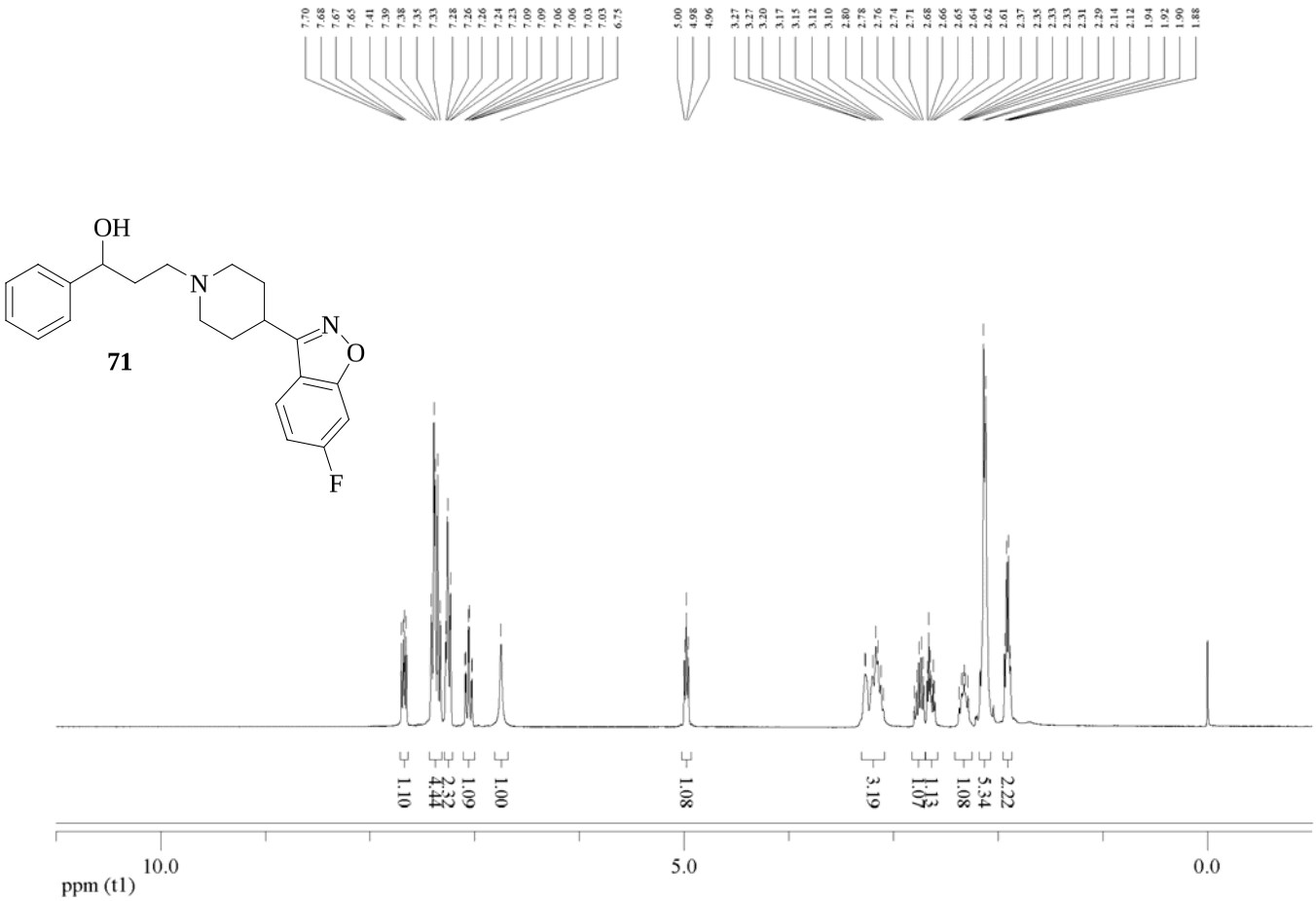
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **29a**



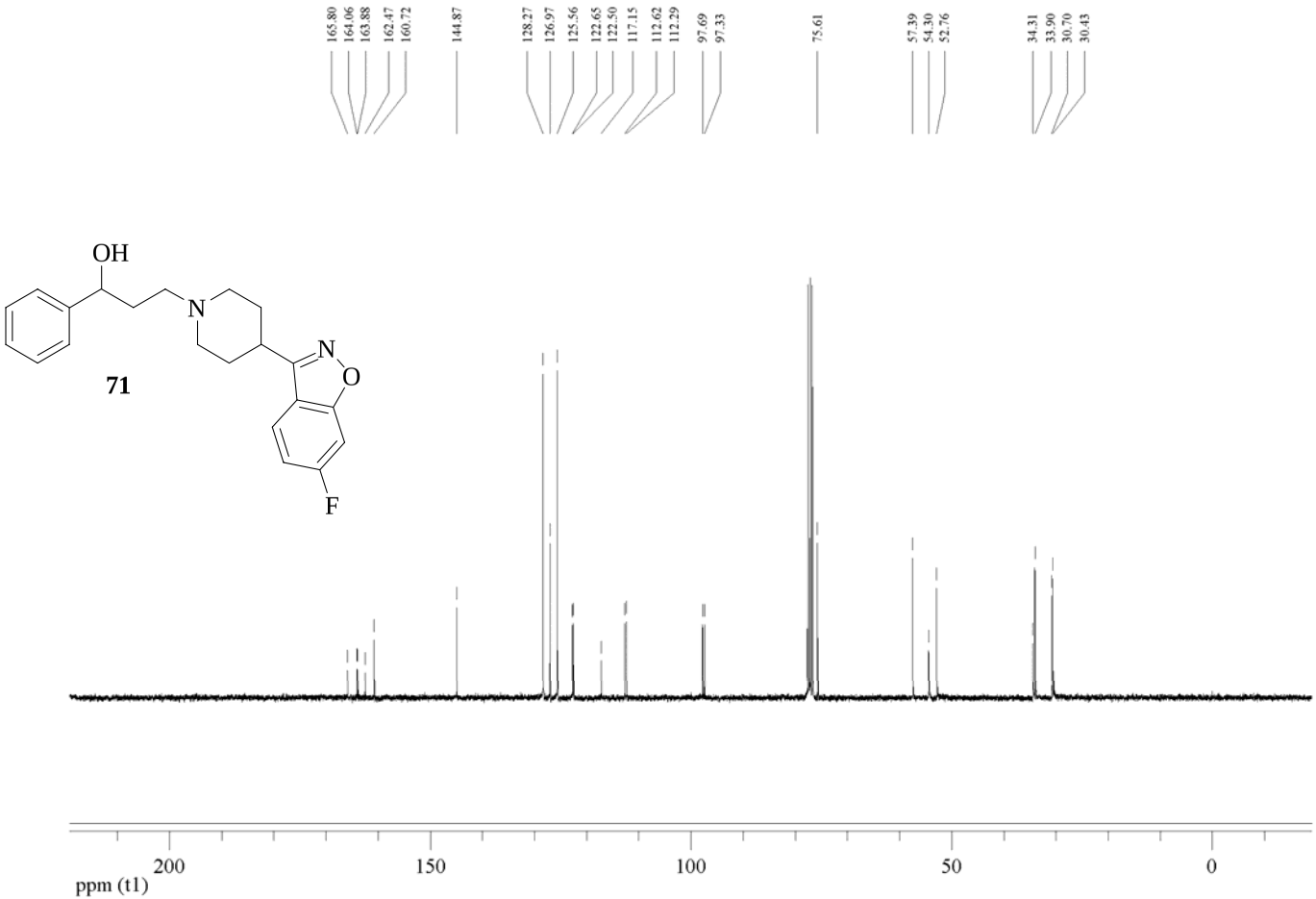
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **29a**



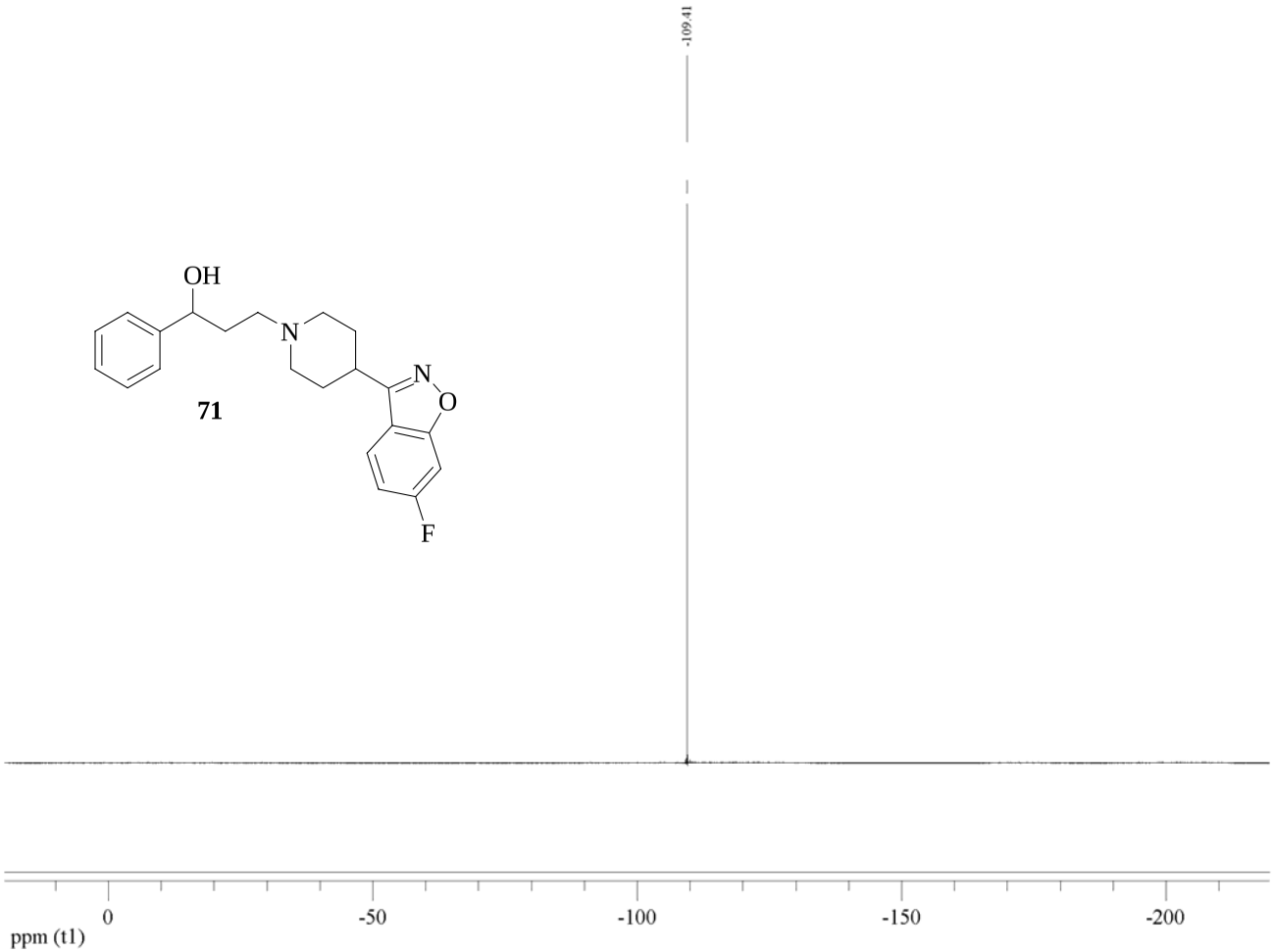
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **71**



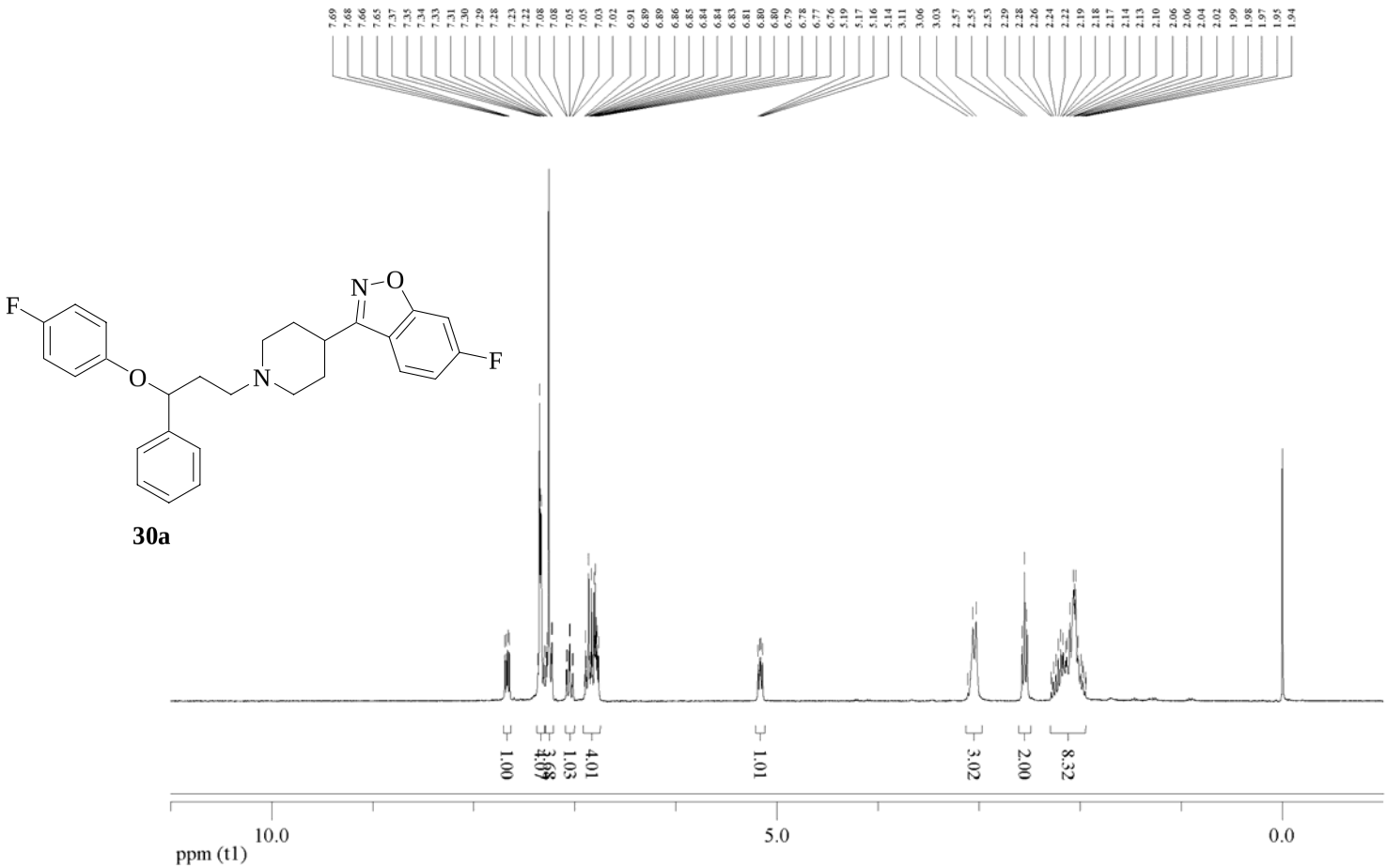
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **71**



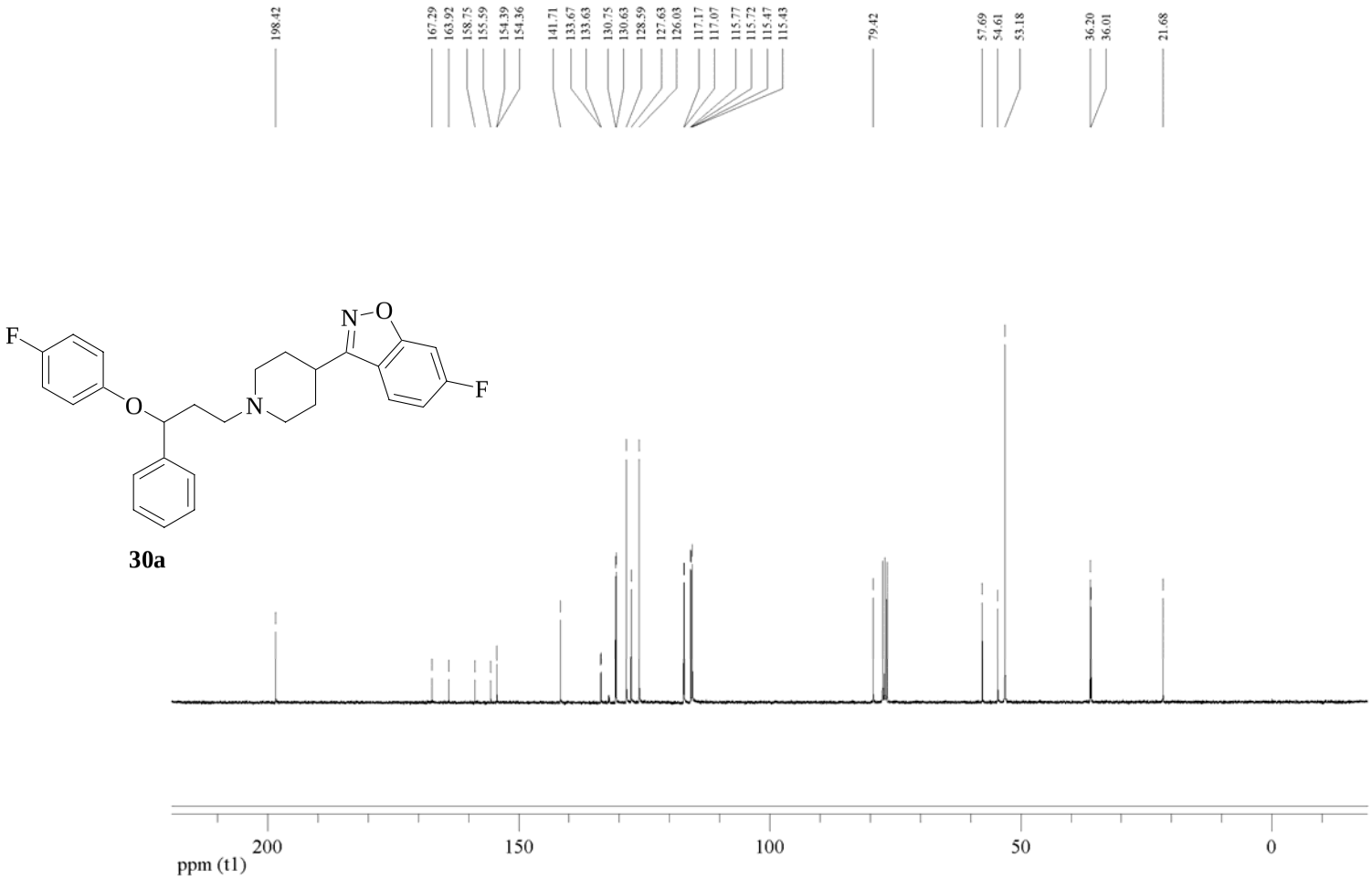
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **71**



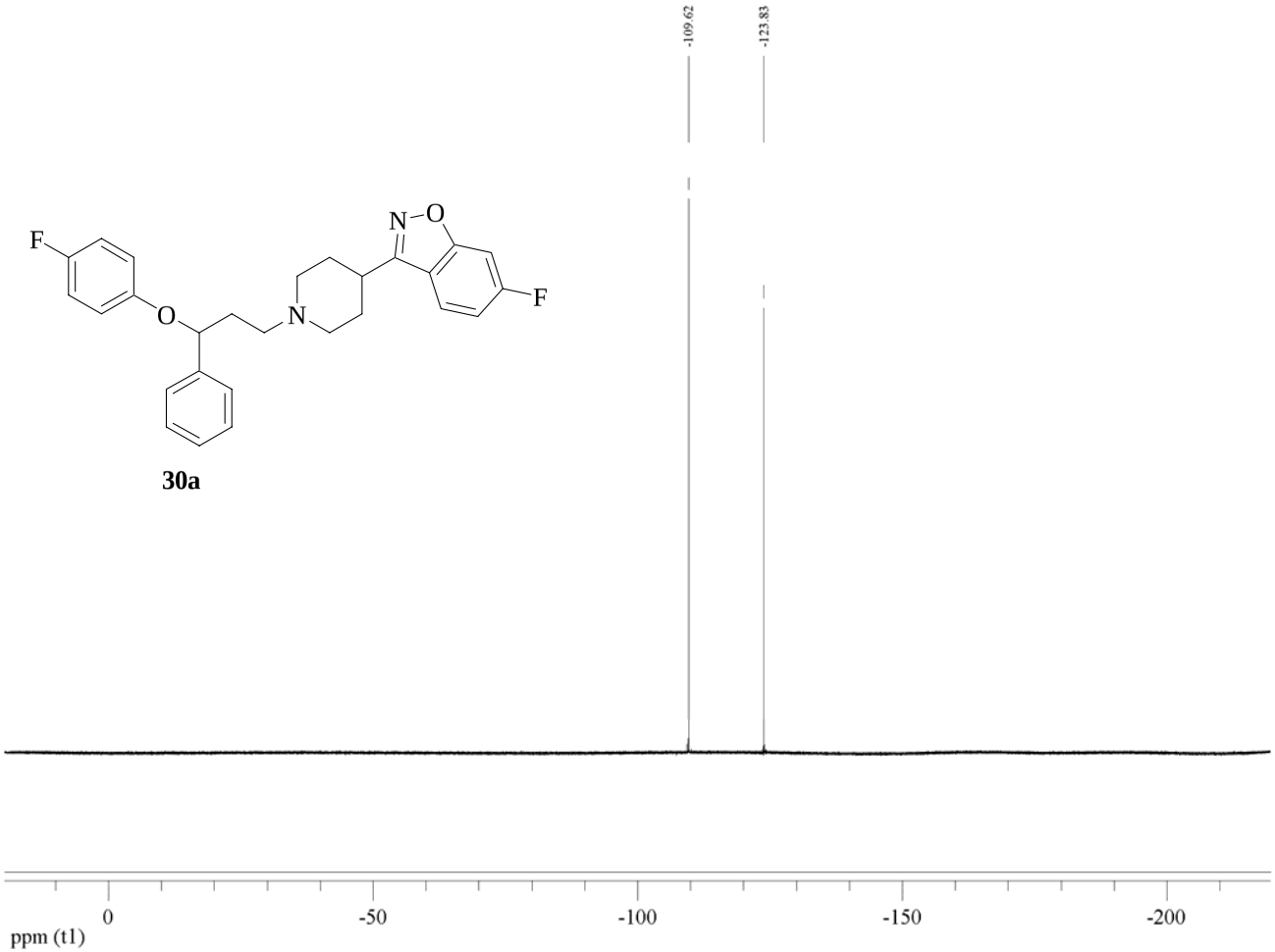
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **30a**



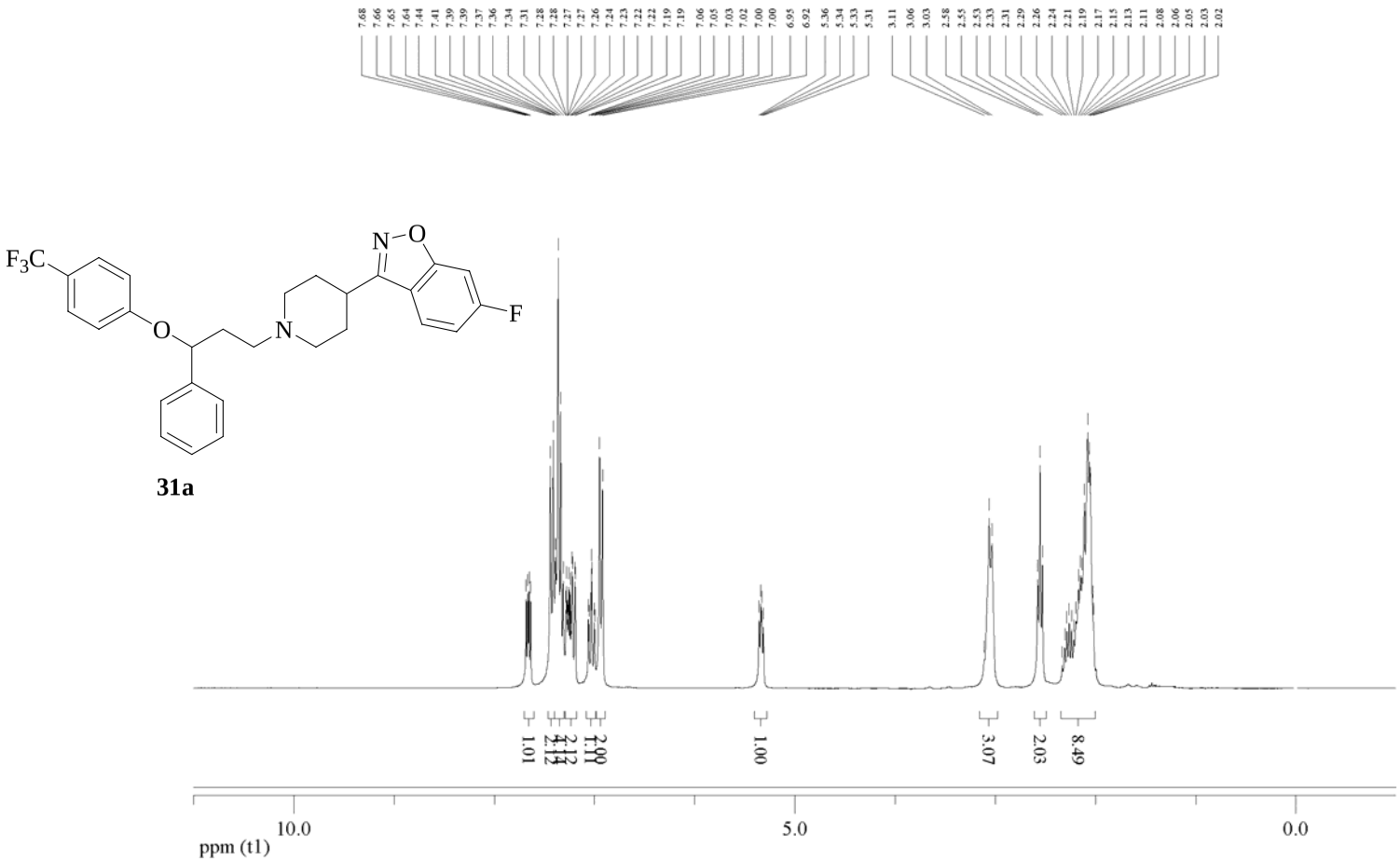
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **30a**



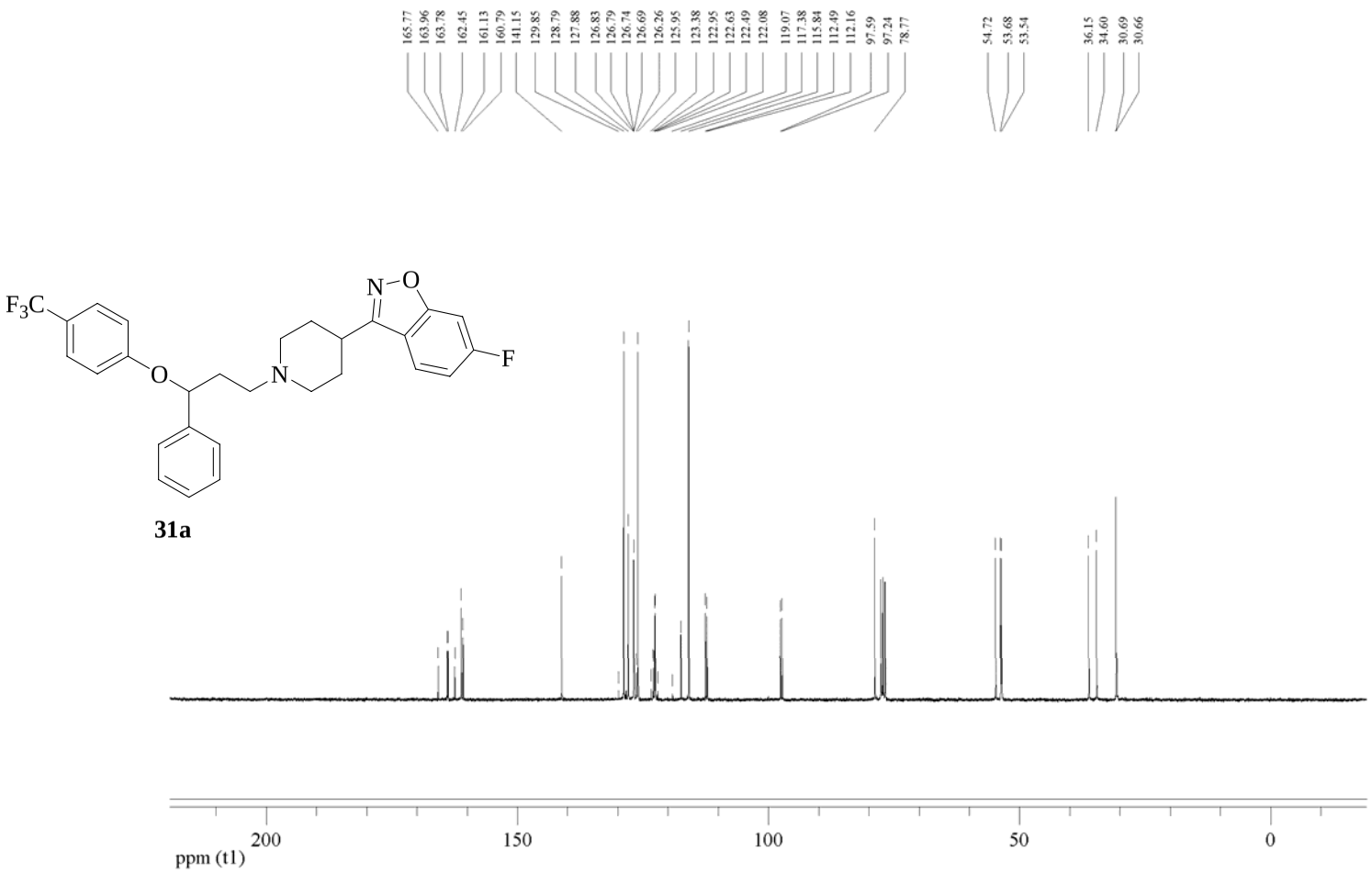
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **30a**



^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **31a**



^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **31a**



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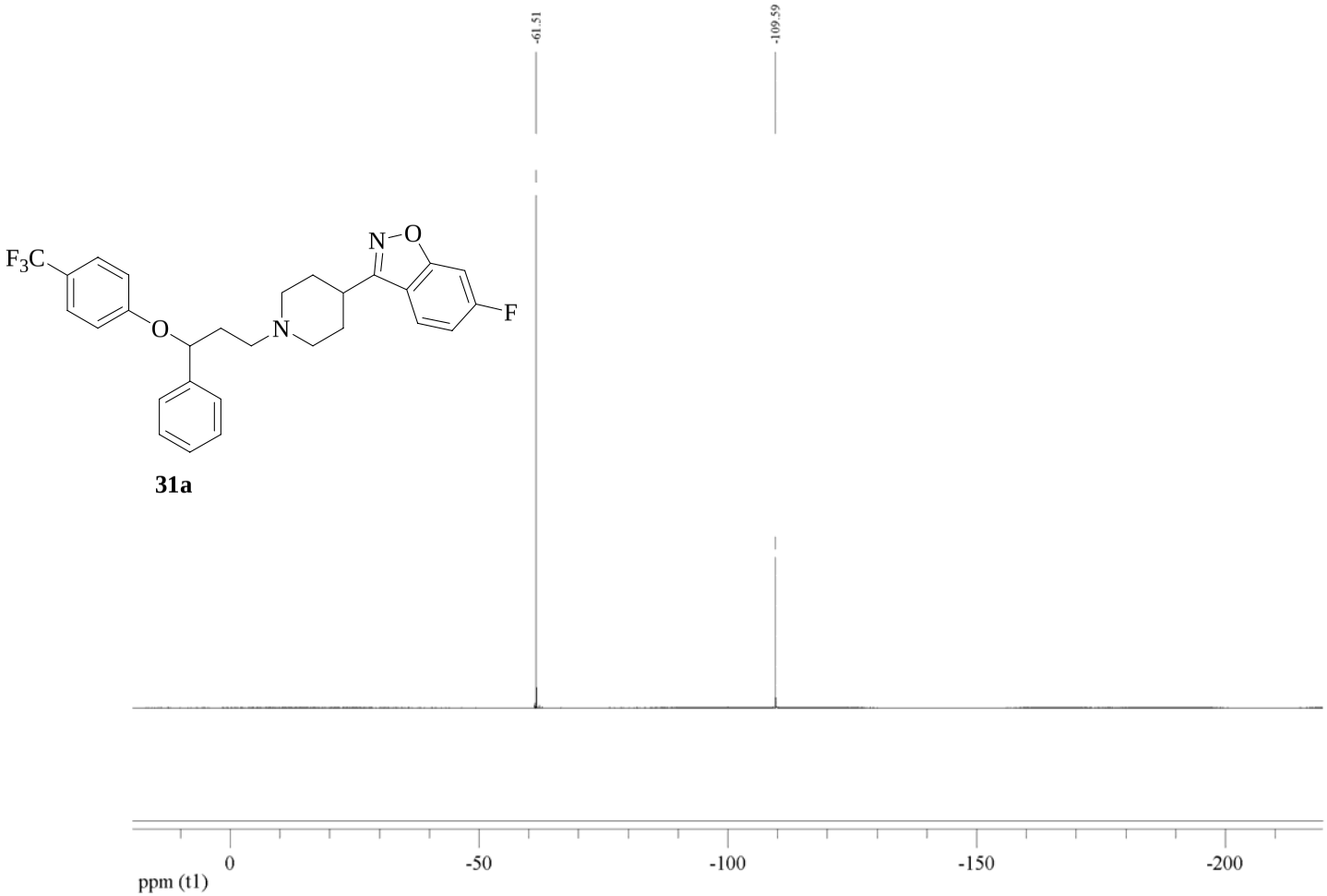
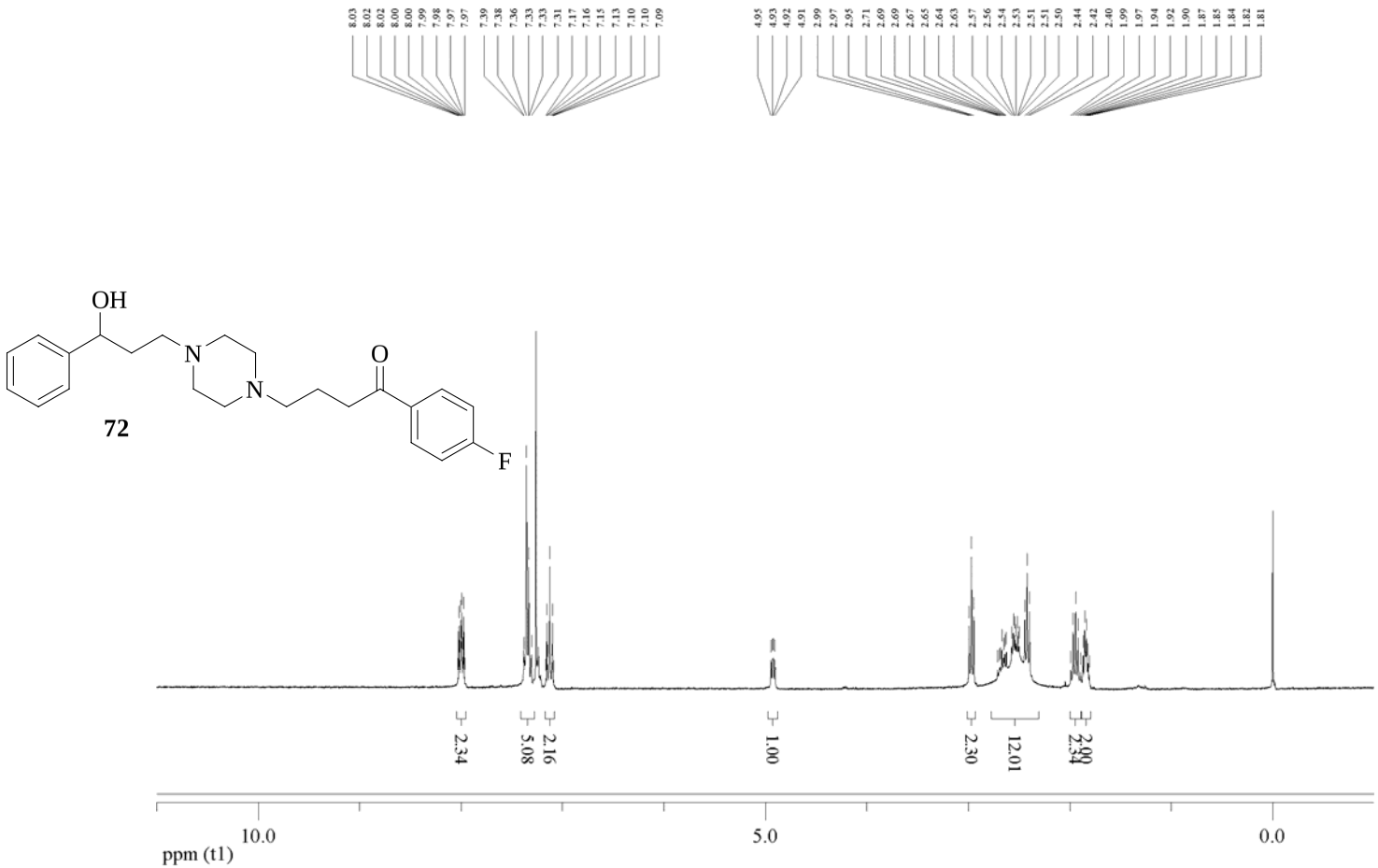
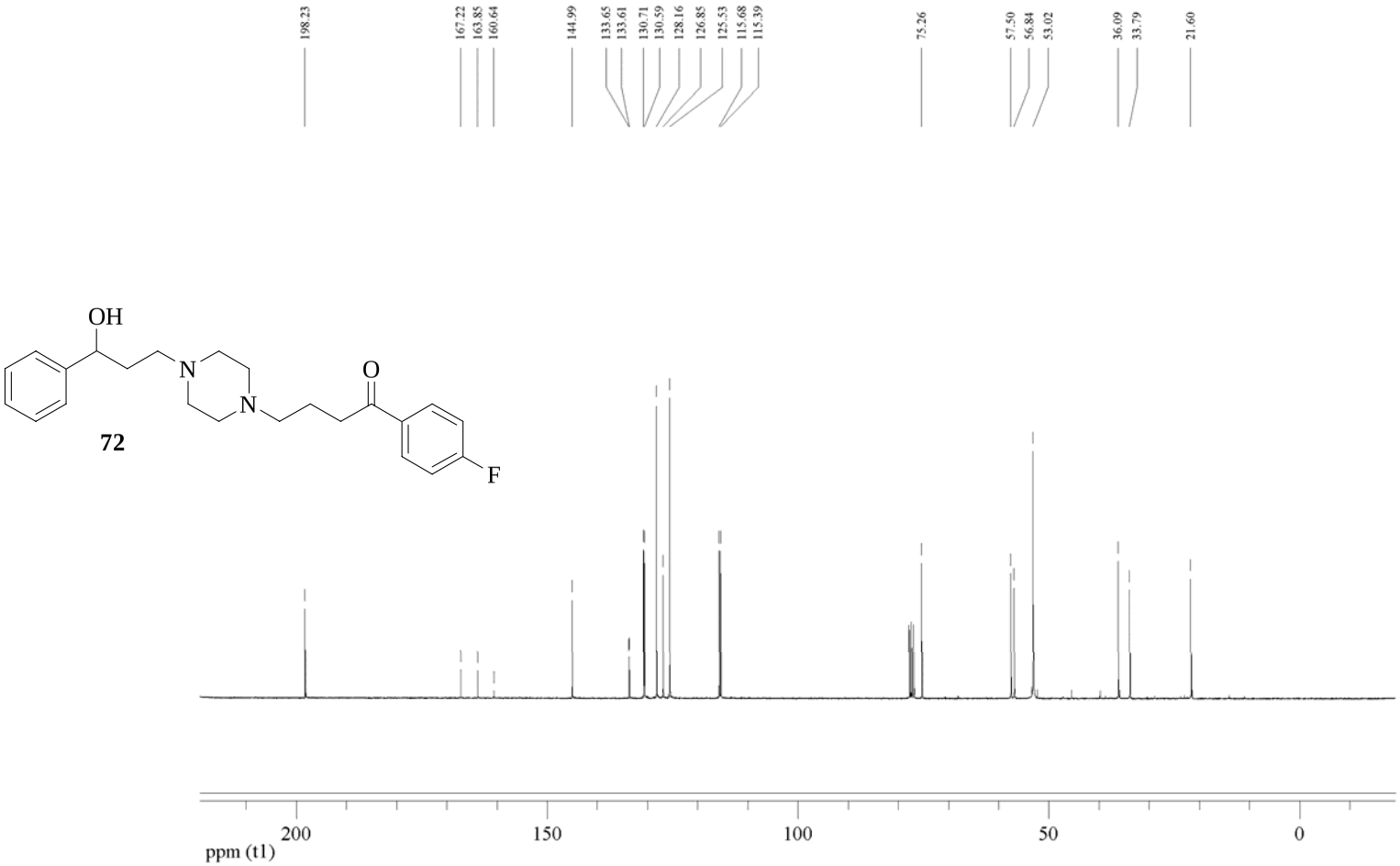


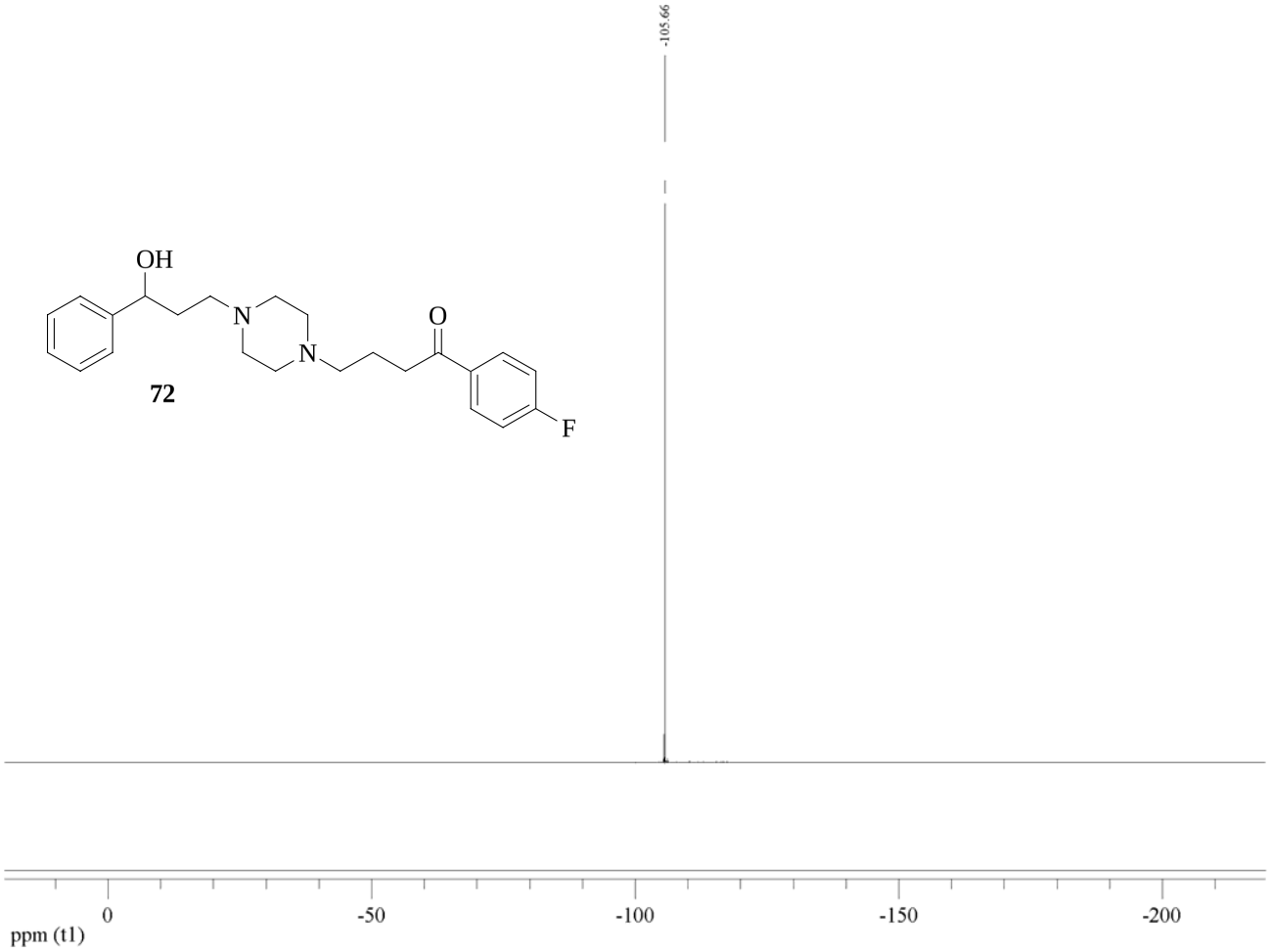
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **72**



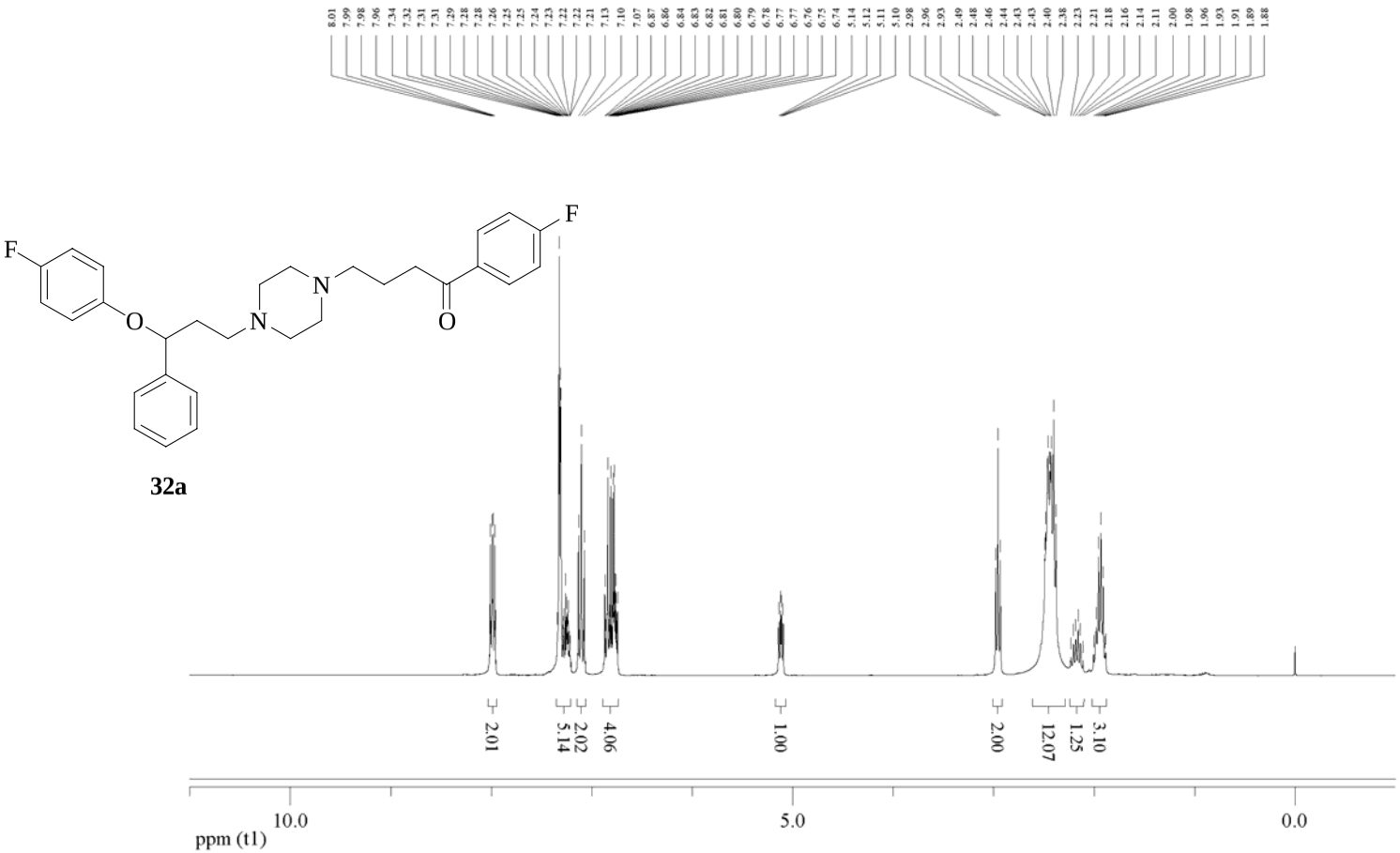
^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **72**



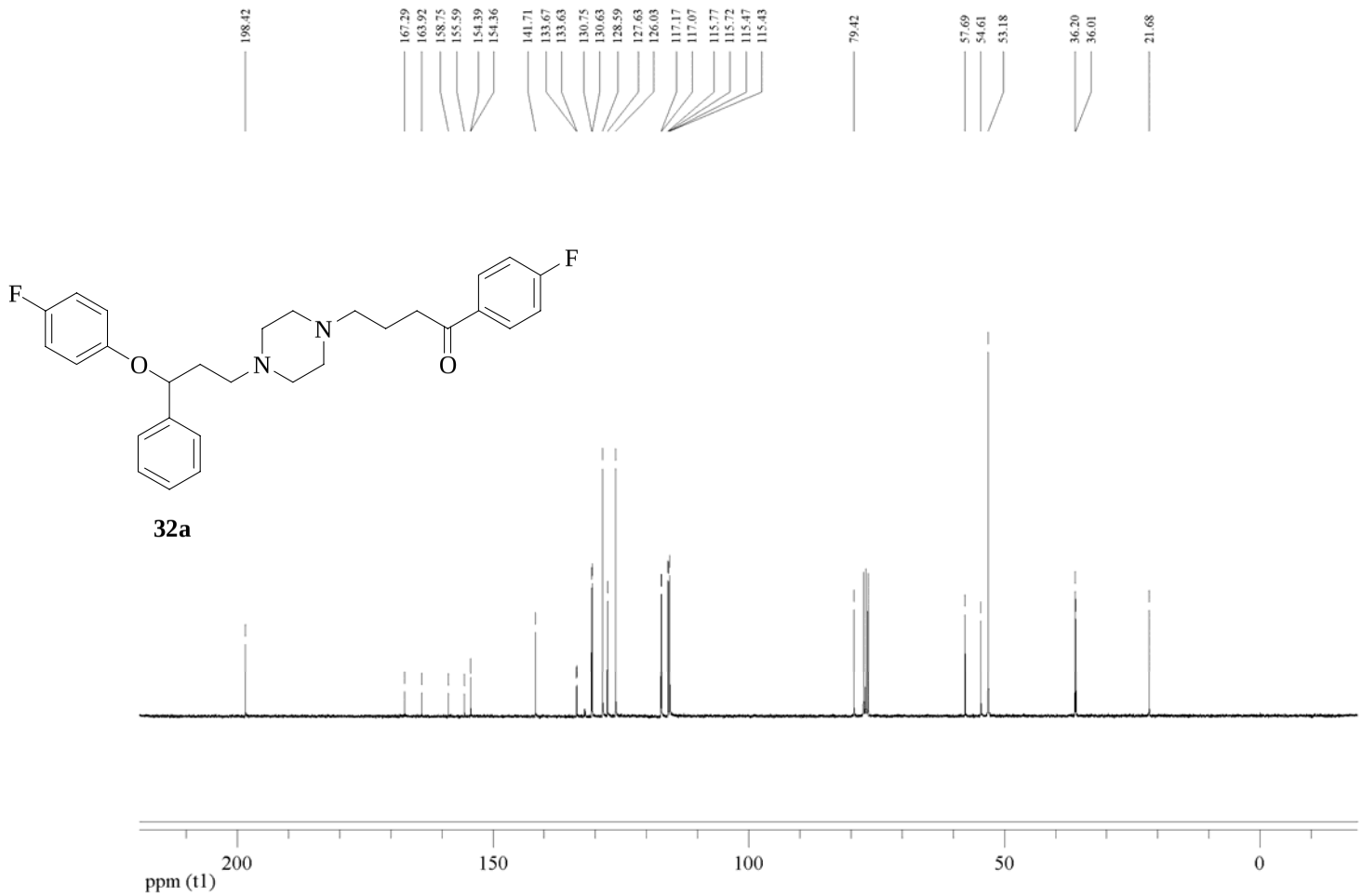
^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **72**



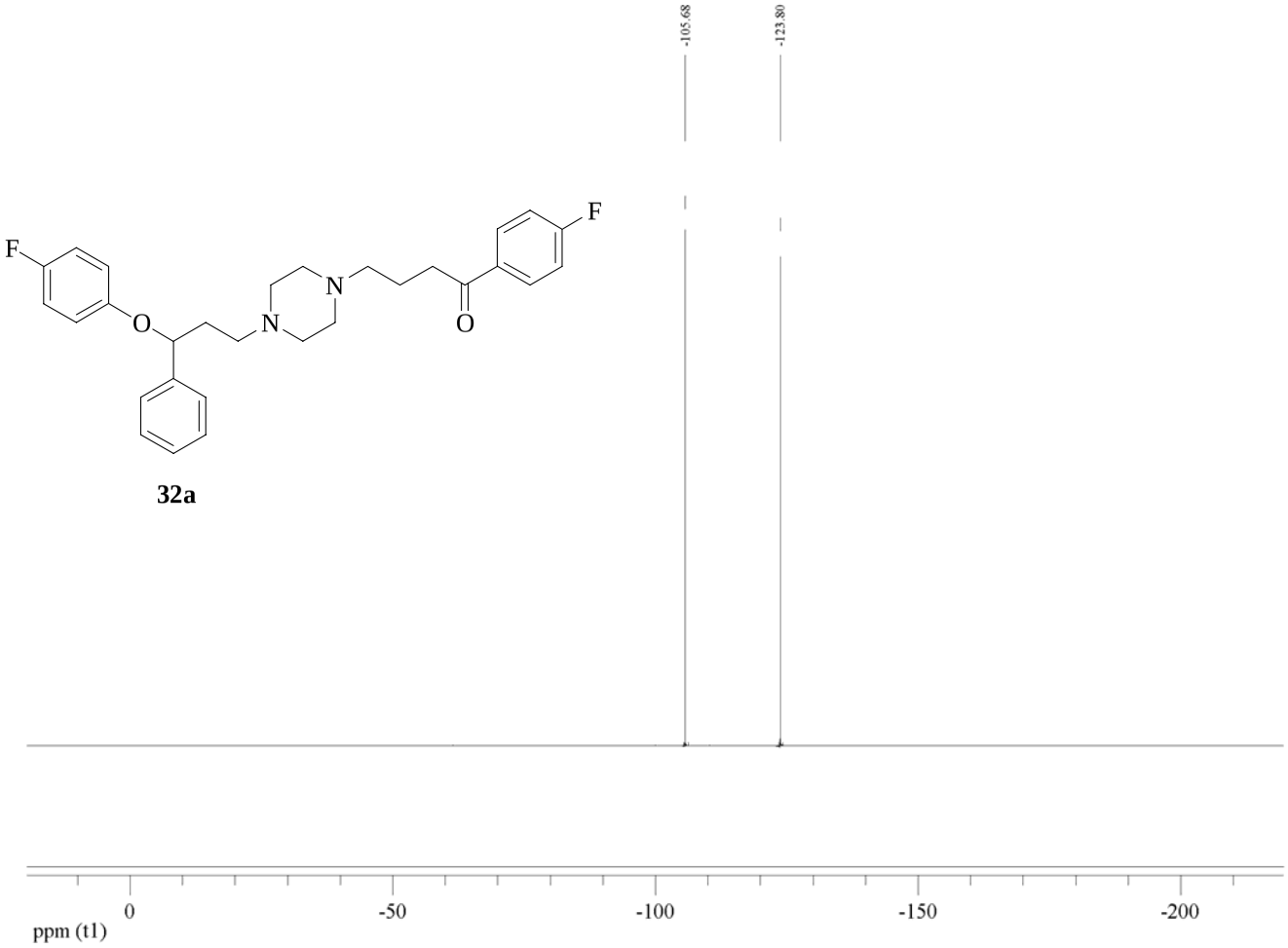
^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **32a**



^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **32a**



^{19}F-NMR ($CDCl_3$, 282 Mhz) of Compound **32a**



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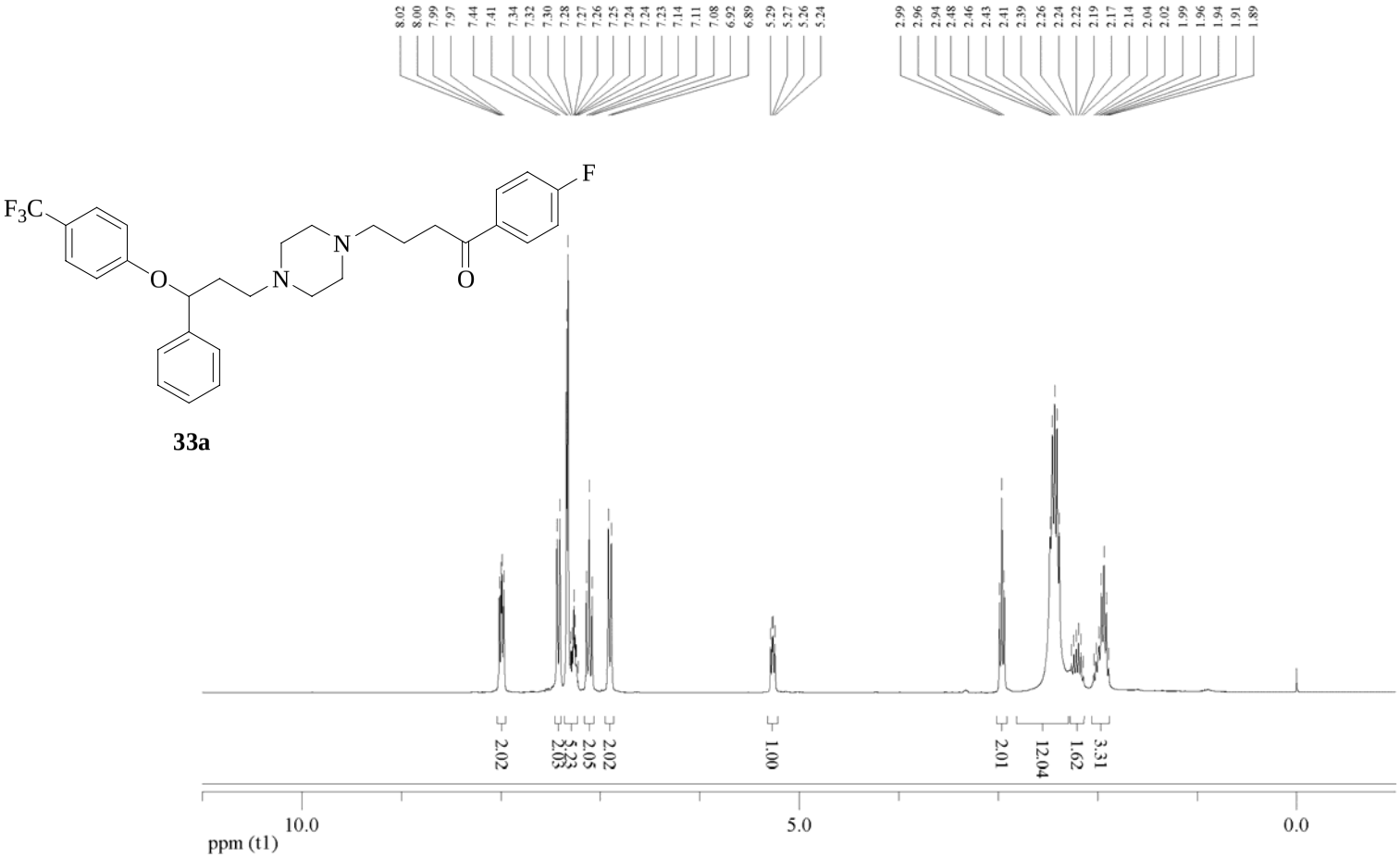

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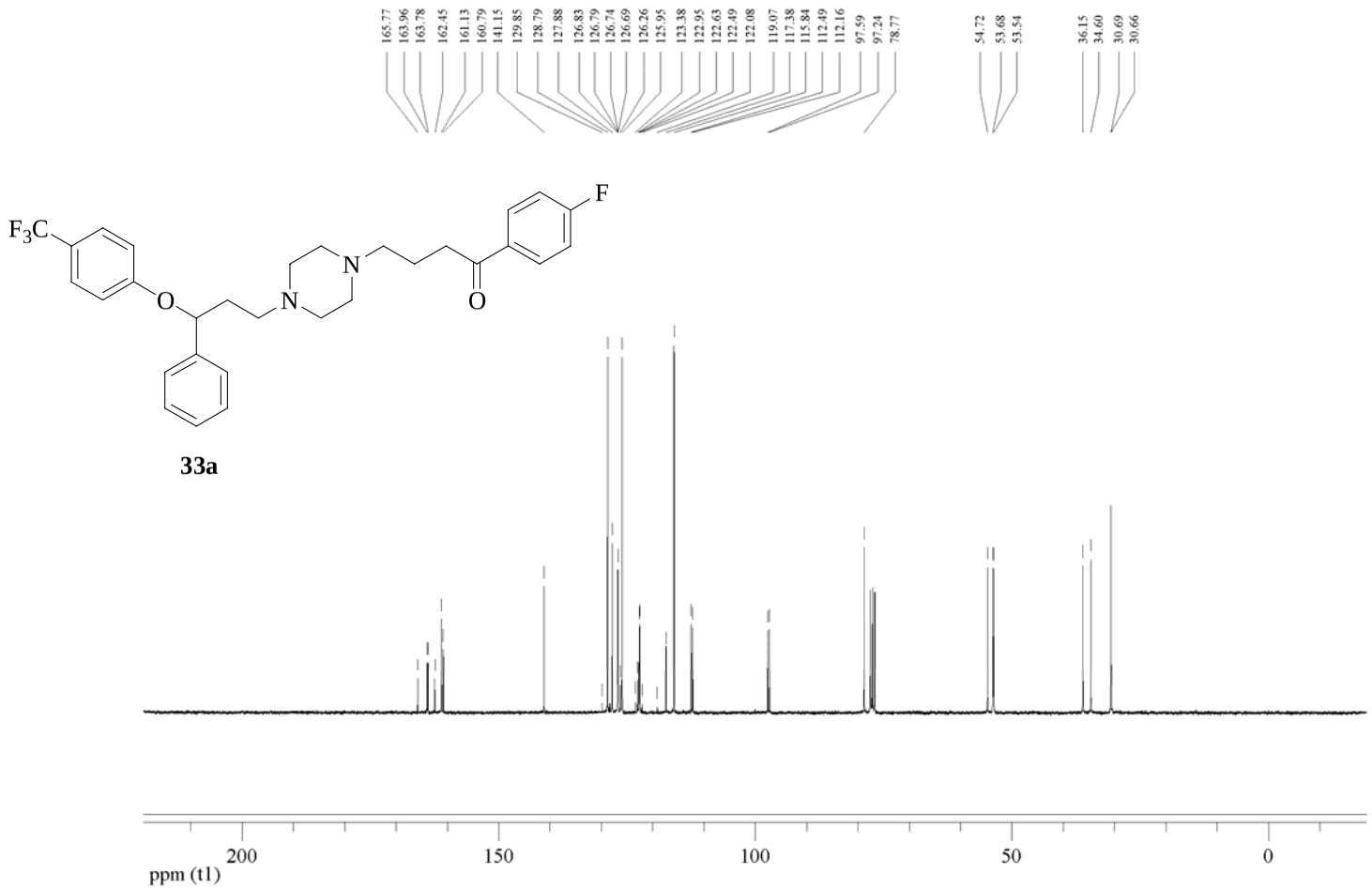

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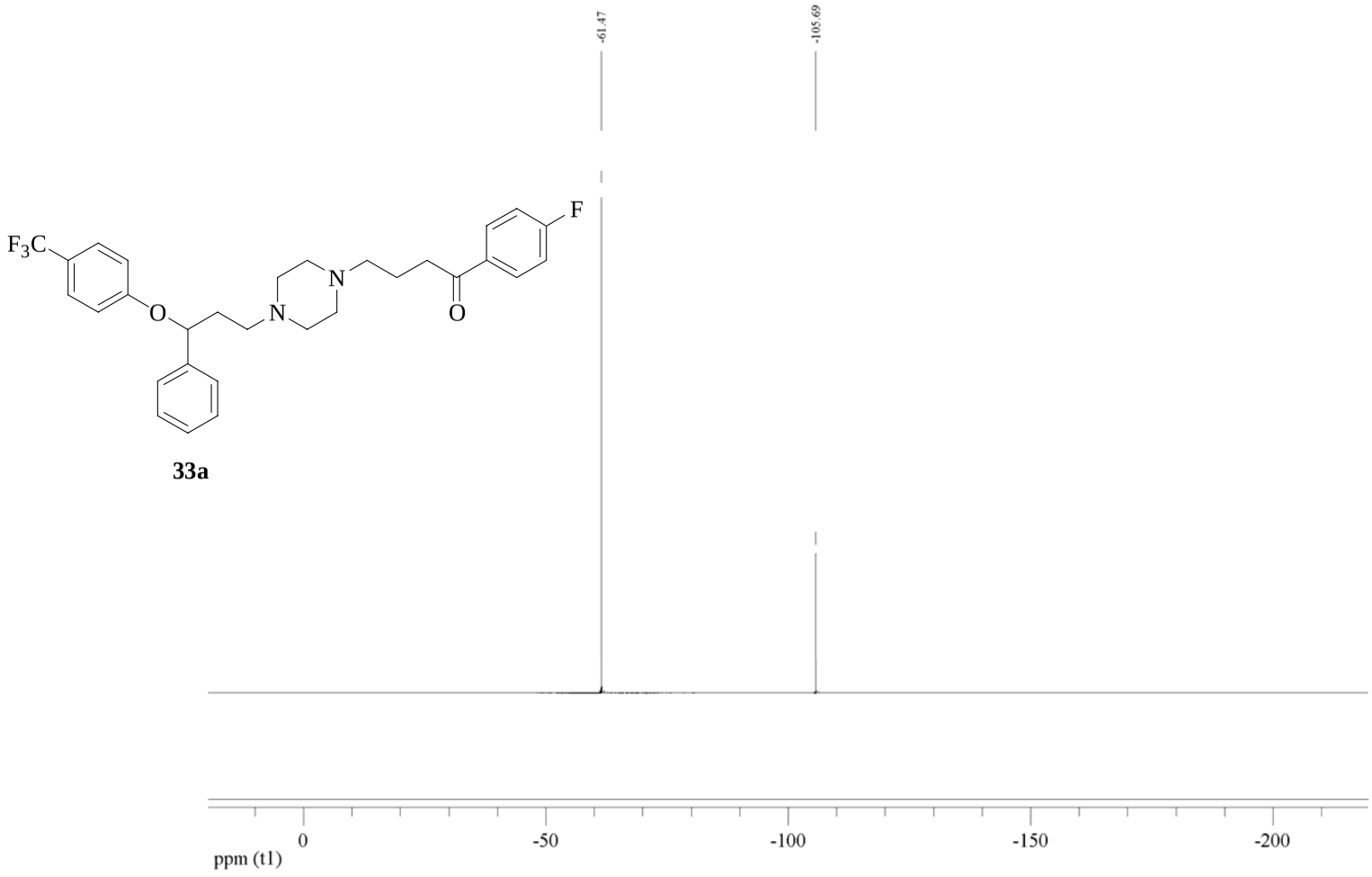
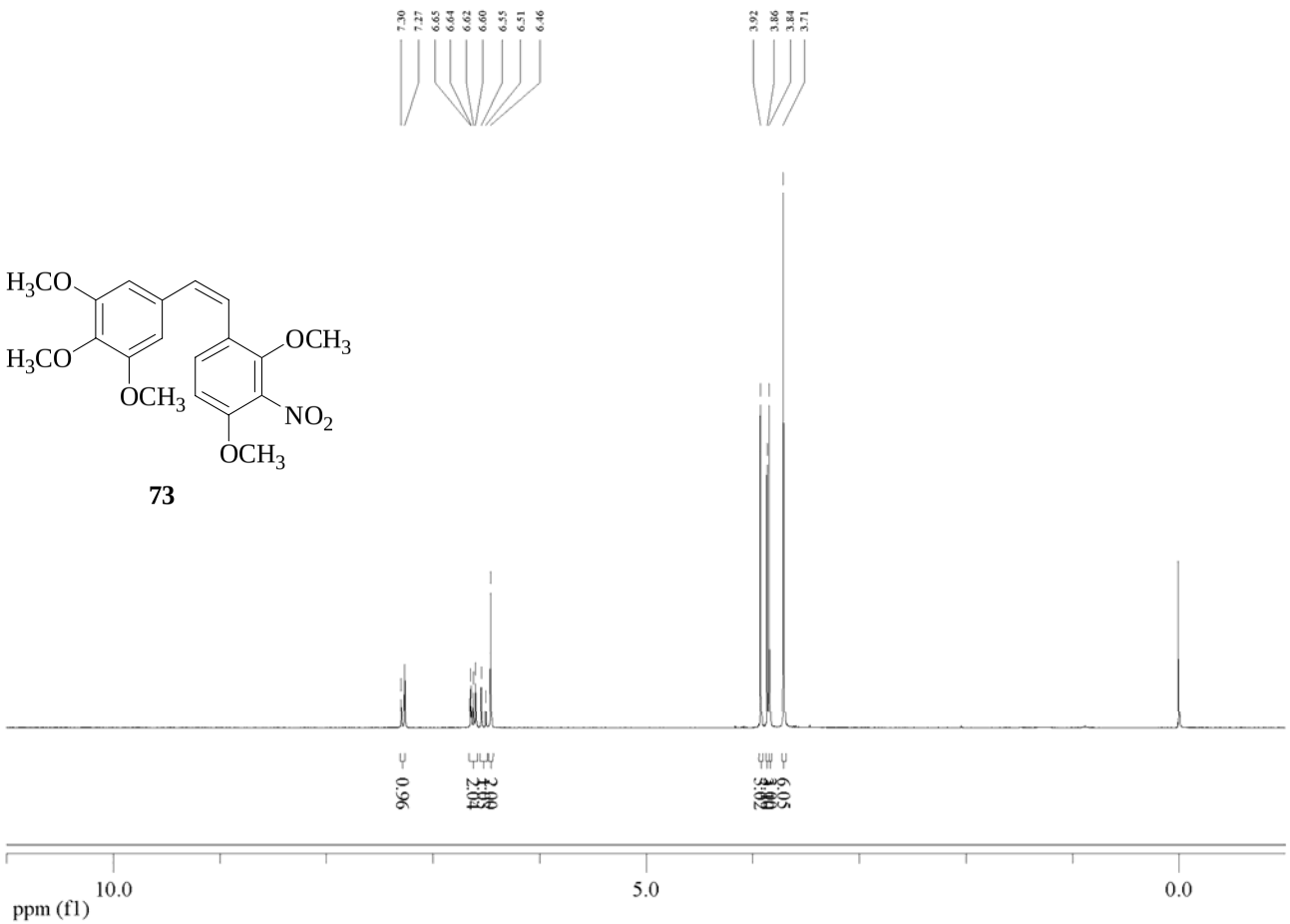
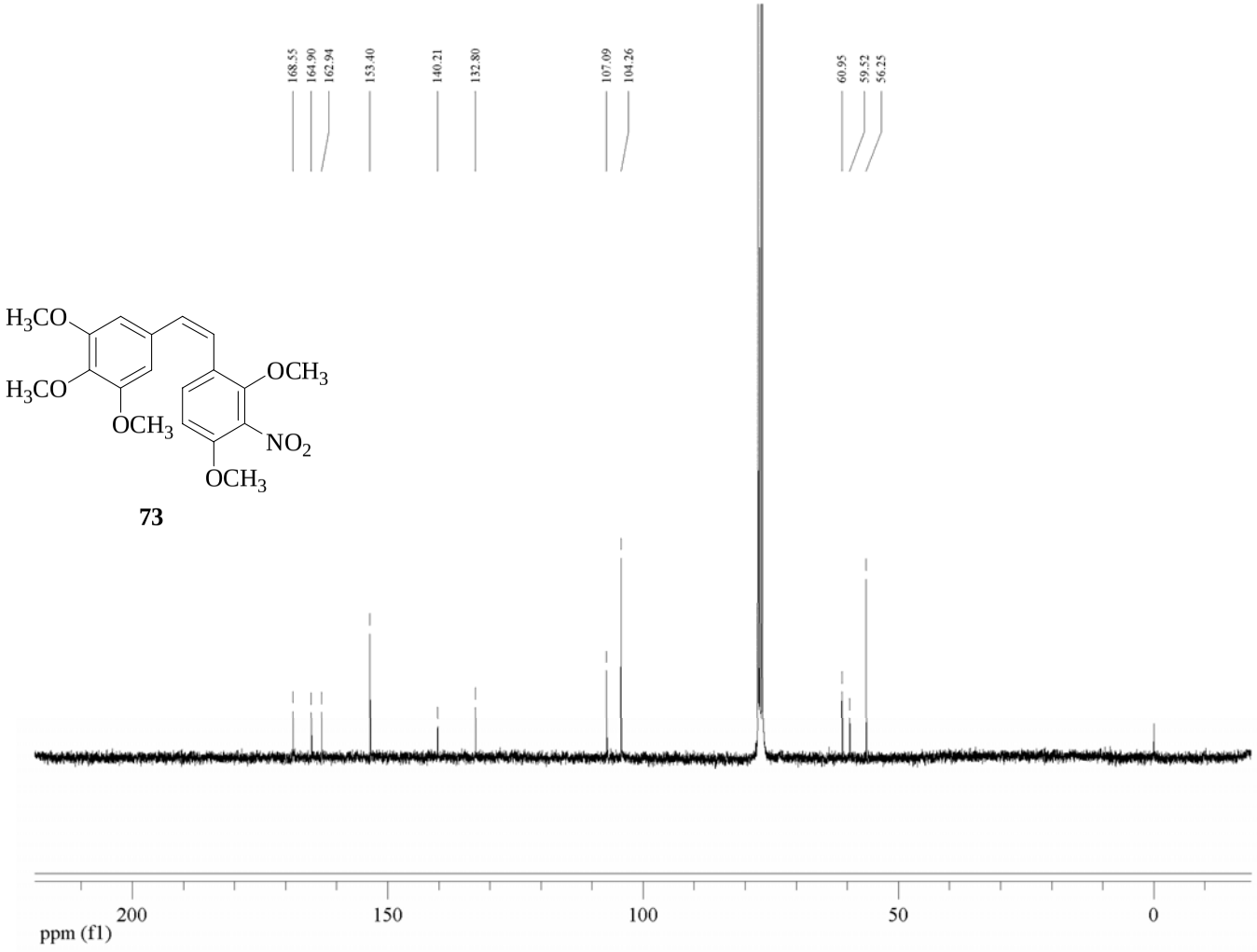


^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **73**

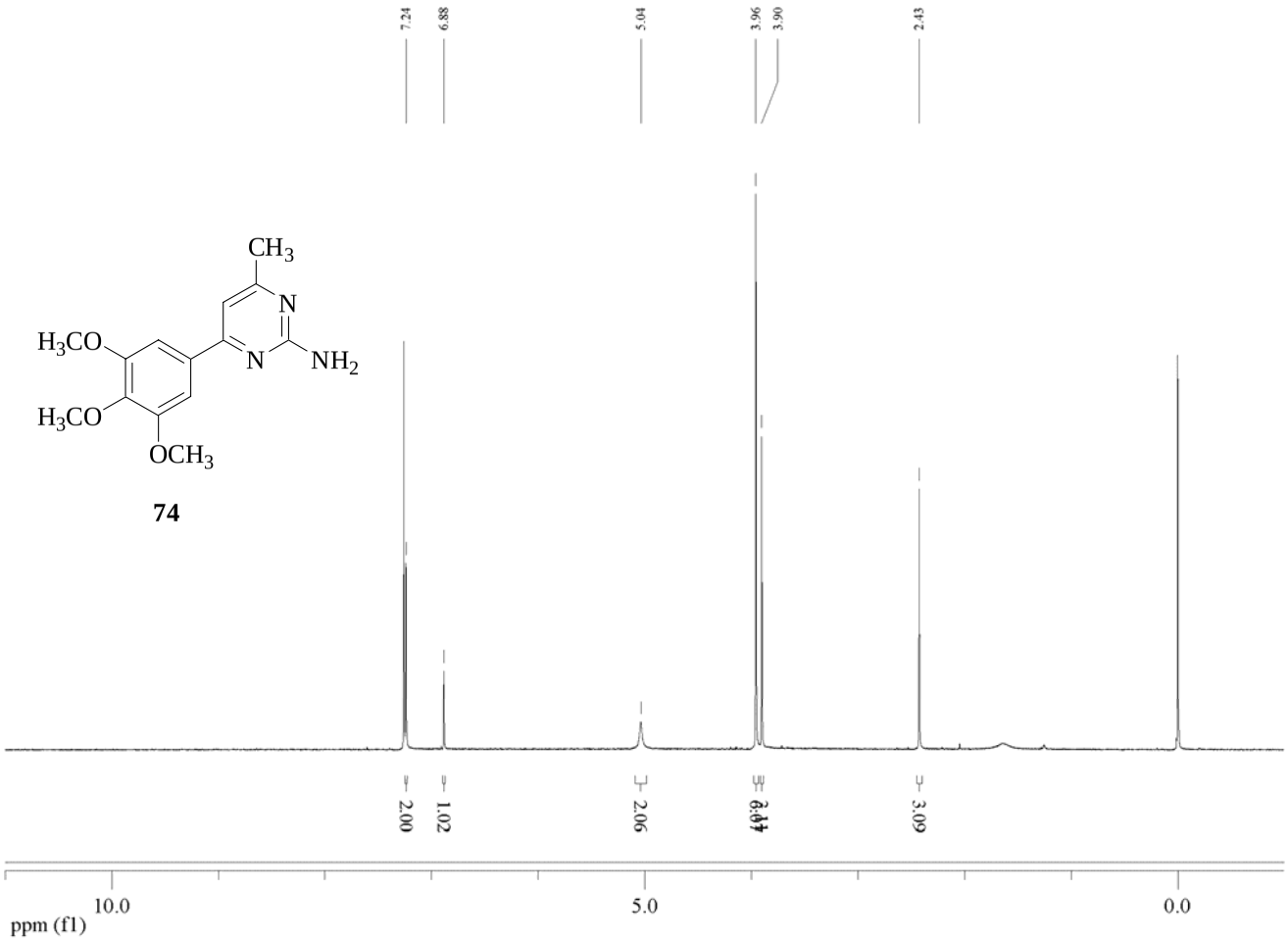


73

^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **73**

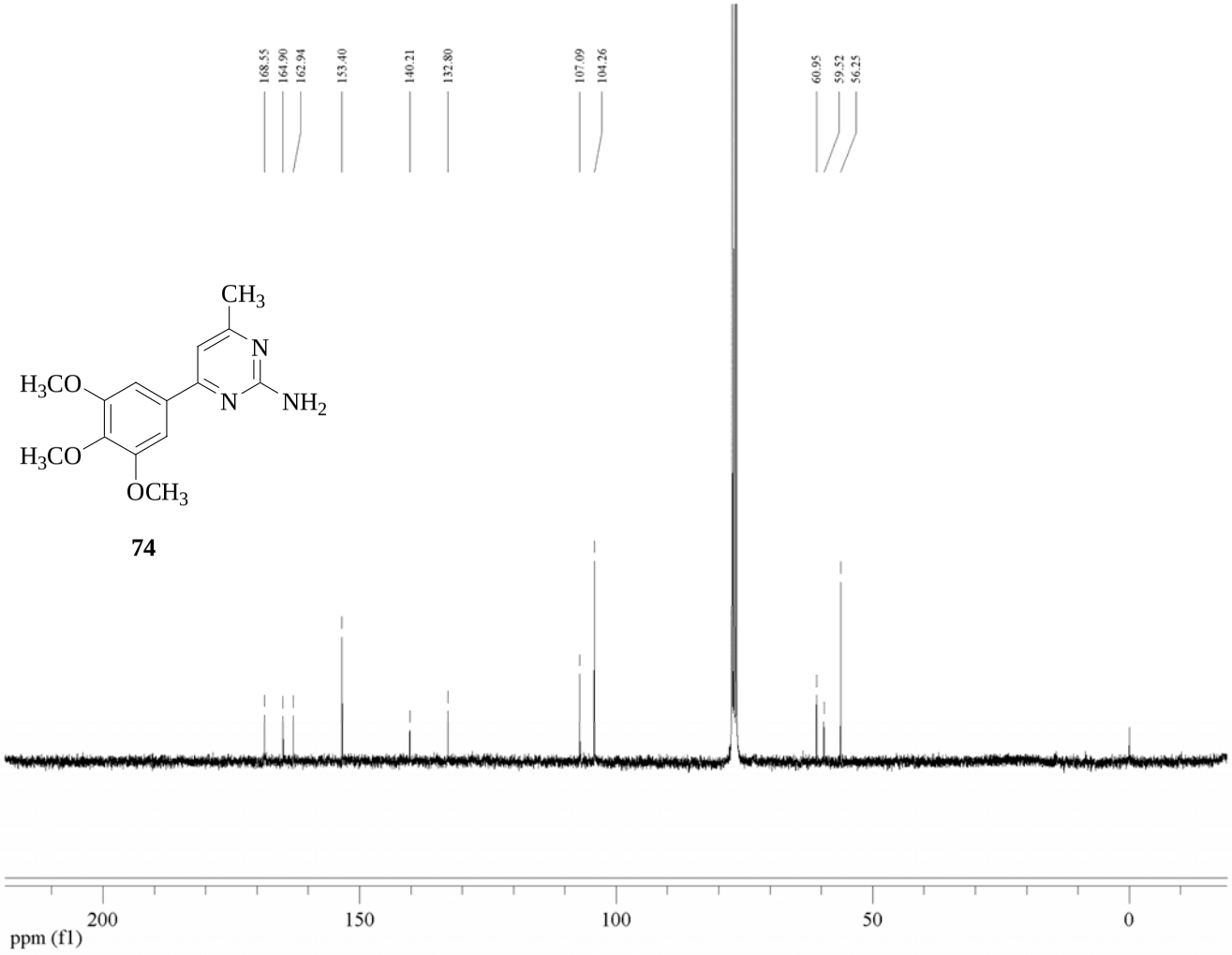


^{1}H-NMR ($CDCl_3$, 300 Mhz) of Compound **74**



74

^{13}C-NMR ($CDCl_3$, 75 Mhz) of Compound **74**



APPENDIX B

Selected HPLC Spectra

Spectrum		Page
1.	HPLC spectrum of compound 1b	369
2.	HPLC spectrum of compound 2b	370
3.	HPLC spectrum of compound 3b	371
4.	HPLC spectrum of compound 4b	372
5.	HPLC spectrum of compound 5b	373
6.	HPLC spectrum of compound 6b	374
7.	HPLC spectrum of compound 7b	375
8.	HPLC spectrum of compound 8b	376
9.	HPLC spectrum of compound 9b	377
10.	HPLC spectrum of compound 10b	378
11.	HPLC spectrum of compound 11b	379
12.	HPLC spectrum of compound 12b	380
13.	HPLC spectrum of compound 13b	381
14.	HPLC spectrum of compound 14b	382
15.	HPLC spectrum of compound 15b	383
16.	HPLC spectrum of compound 16b	384
17.	HPLC spectrum of compound 17b	385
18.	HPLC spectrum of compound 18b	286

19. HPLC spectrum of compound 19b 387

20. HPLC spectrum of compound 20b 388

21. HPLC spectrum of compound 21b 389

22. HPLC spectrum of compound 22b 390

23. HPLC spectrum of compound 23b 391

24. HPLC spectrum of compound 24b 392

25. HPLC spectrum of compound 25b 393

26. HPLC spectrum of compound 26b 394

27. HPLC spectrum of compound 27b 395

28. HPLC spectrum of compound 28b 396

29. HPLC spectrum of compound 29b 397

30. HPLC spectrum of compound 30b 398

31. HPLC spectrum of compound 31b 399

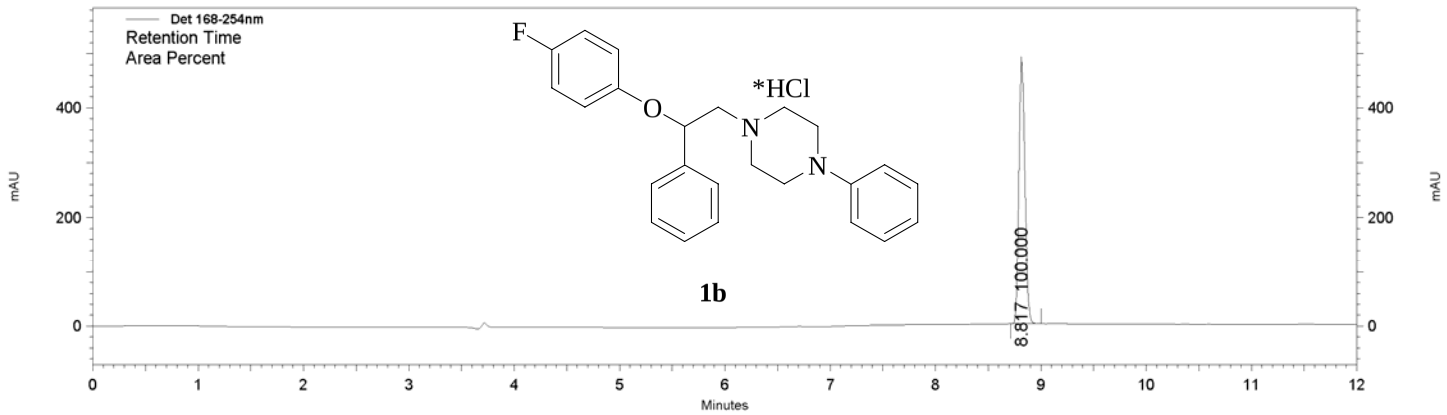
32. HPLC spectrum of compound 32b 400

33. HPLC spectrum of compound 33b 401

HPLC spectrum of Compound **1b**

Det 168-254nm Results

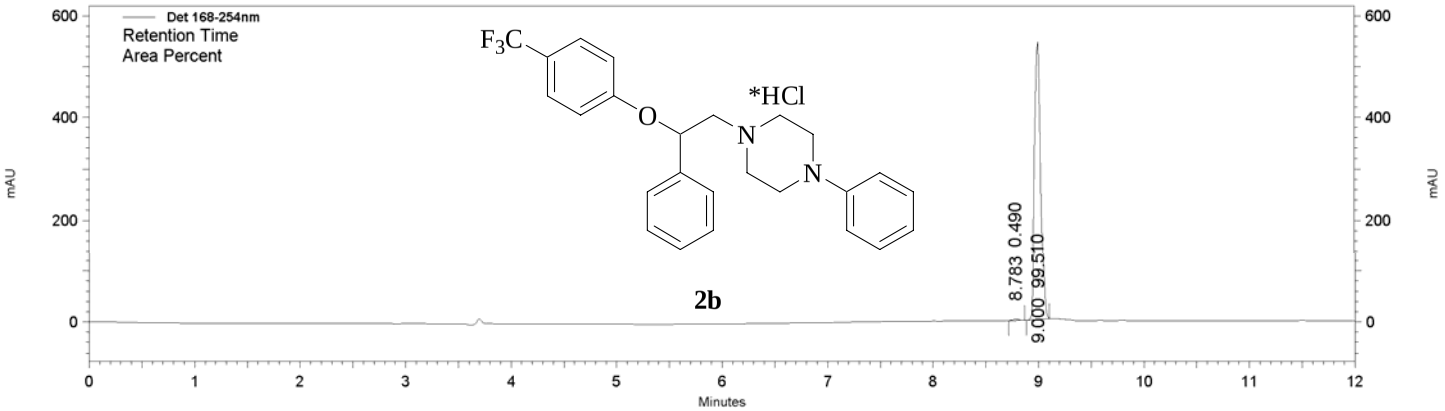
Pk #	Name	Retention Time	Area	Area Percent
1		8.817	1933647	100.000
Totals			1933647	100.000



HPLC spectrum of Compound **2b**

Det 168-254nm Results

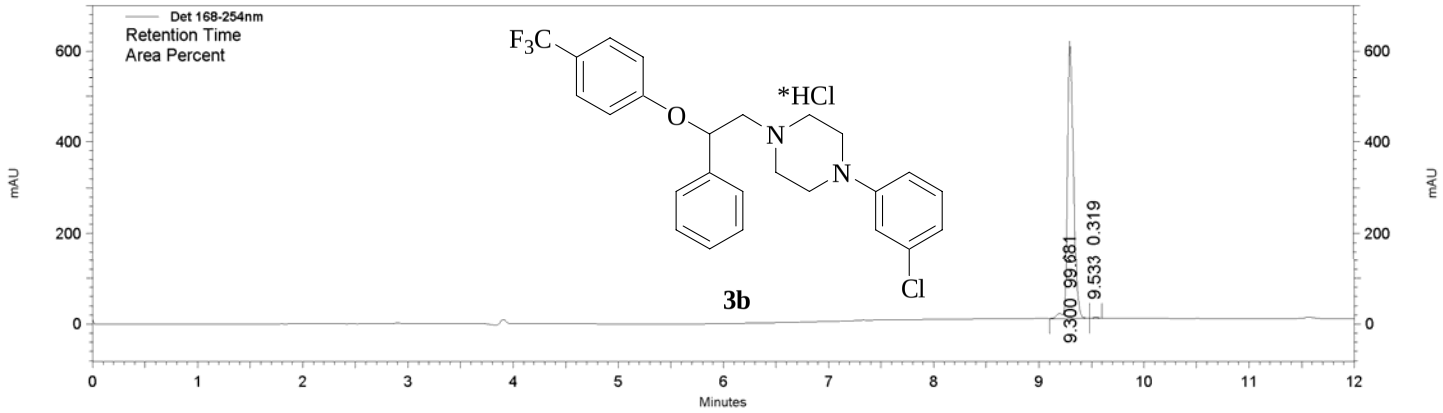
Pk #	Name	Retention Time	Area	Area Percent
1		8.783	10912	0.490
2		9.000	2216615	99.510
Totals			2227527	100.000



HPLC spectrum of Compound **3b**

Det 168-254nm Results

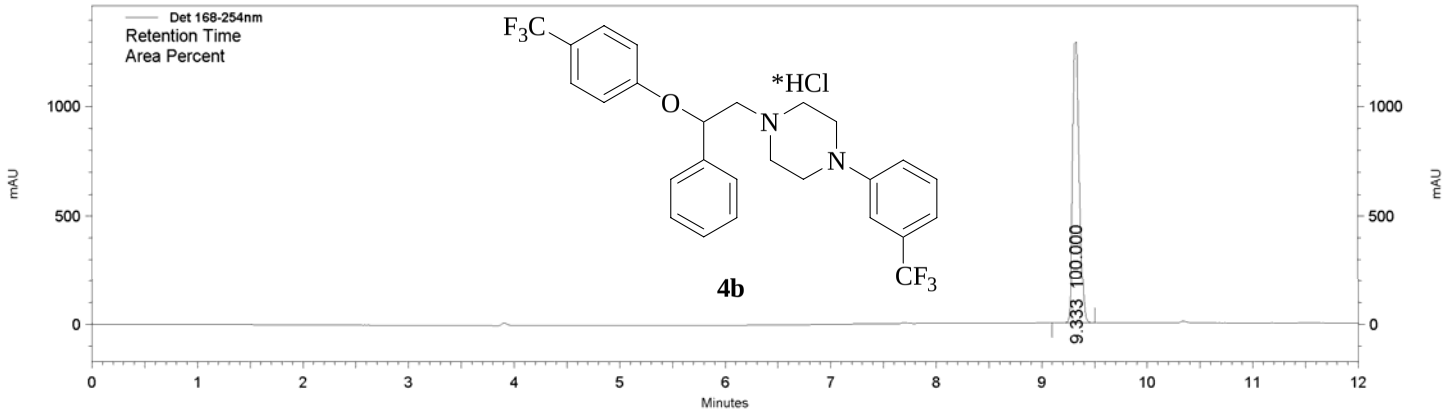
Pk #	Name	Retention Time	Area	Area Percent
1		9.300	2461520	99.681
2		9.533	7887	0.319
Totals			2469407	100.000



HPLC spectrum of Compound **4b**

Det 168-254nm Results

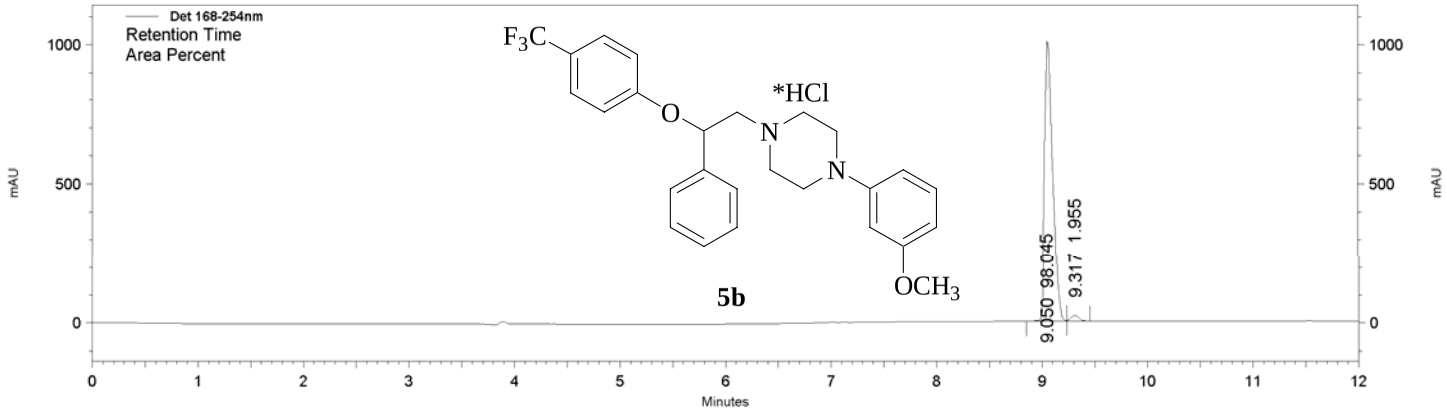
Pk #	Name	Retention Time	Area	Area Percent
1		9.333	5717945	100.000
Totals			5717945	100.000



HPLC spectrum of Compound **5b**

Det 168-254nm Results

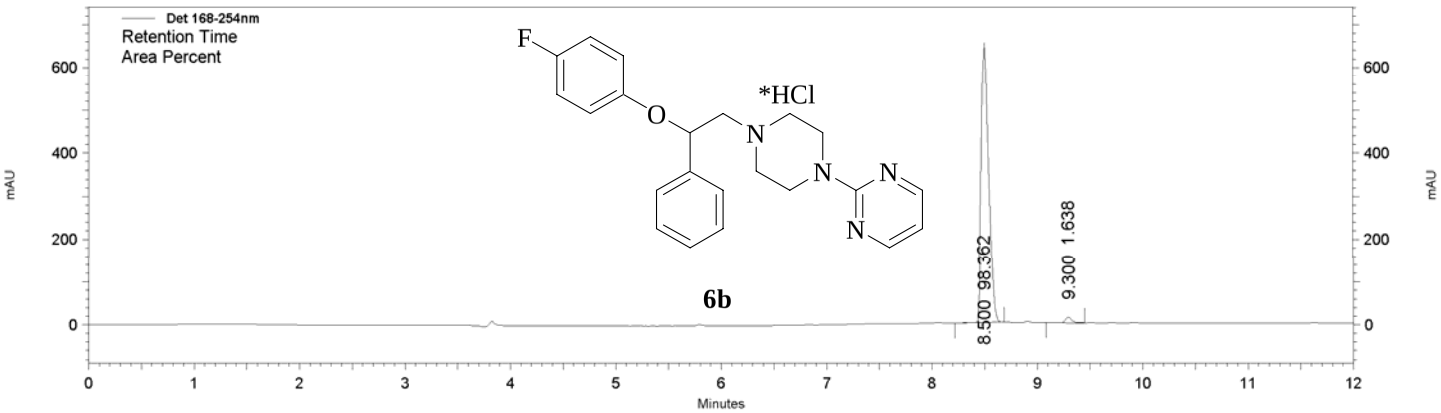
Pk #	Name	Retention Time	Area	Area Percent
1		9.050	5382435	98.045
2		9.317	107330	1.955
Totals			5489765	100.000



HPLC spectrum of Compound **6b**

Det 168-254nm Results

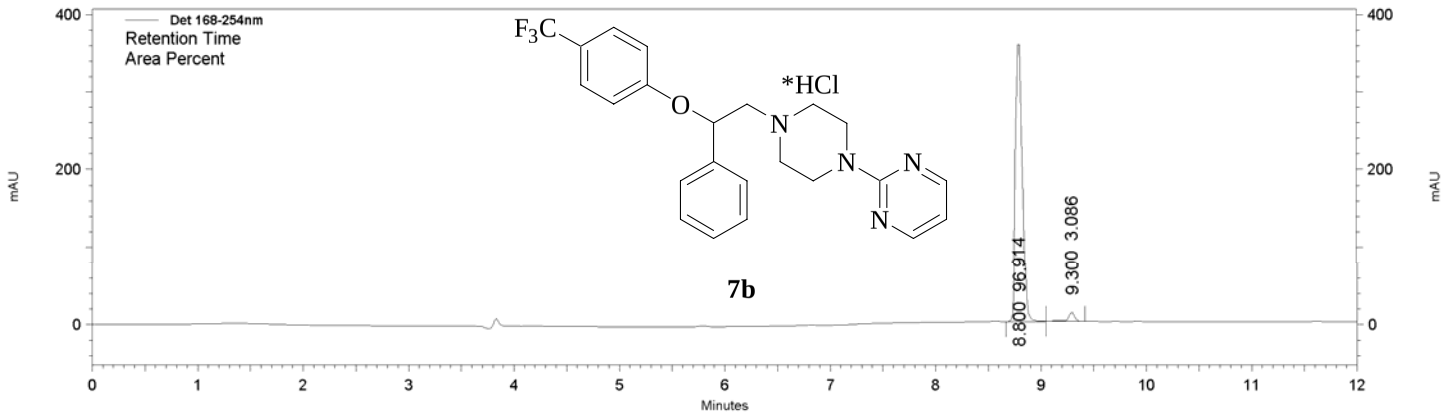
Pk #	Name	Retention Time	Area	Area Percent
1		8.500	3212512	98.362
2		9.300	53489	1.638
Totals			3266001	100.000



HPLC spectrum of Compound **7b**

Det 168-254nm Results

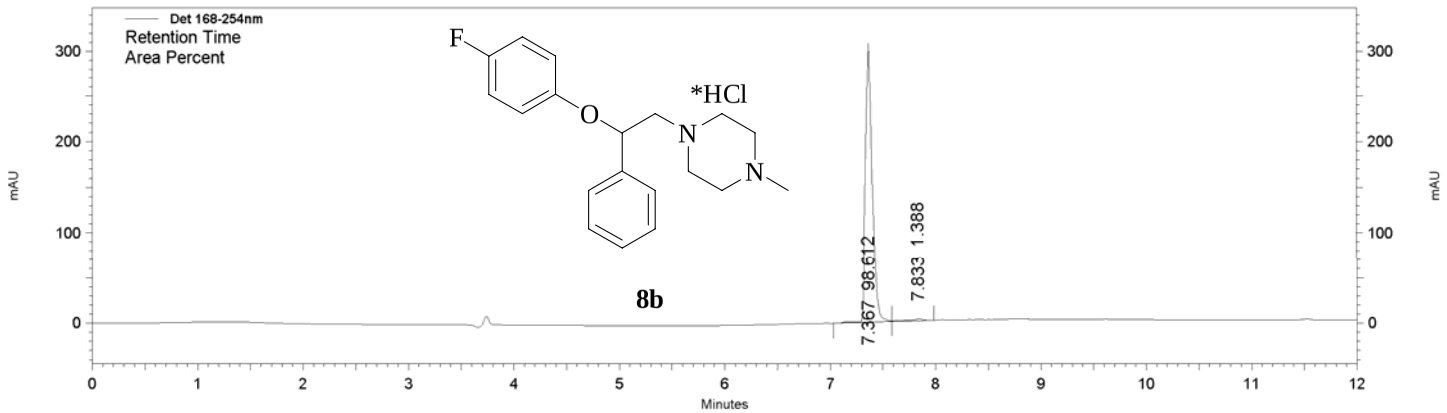
Pk #	Name	Retention Time	Area	Area Percent
1		8.800	1625651	96.914
2		9.300	51770	3.086
Totals			1677421	100.000



HPLC spectrum of Compound **8b**

Det 168-254nm Results

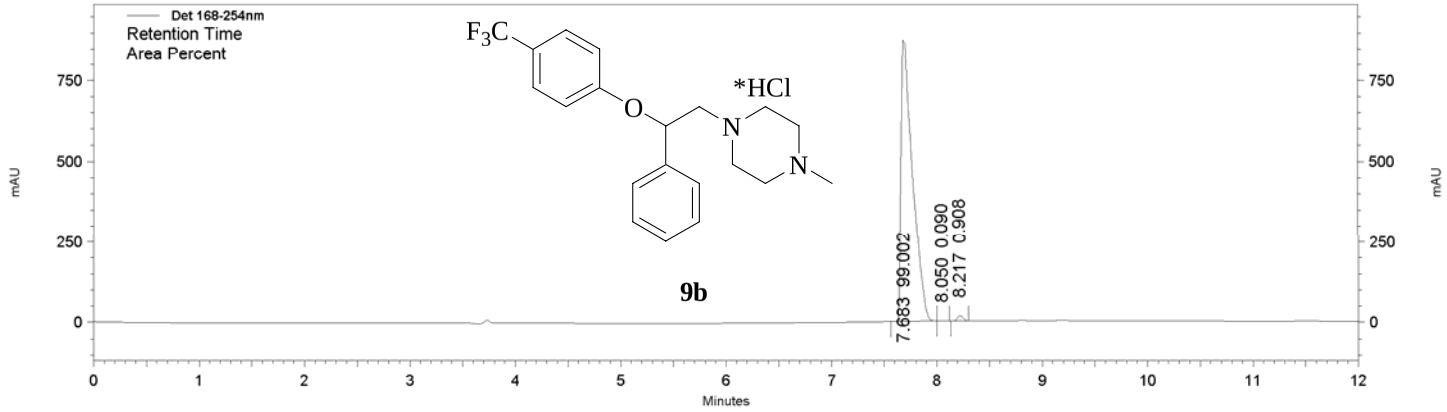
Pk #	Name	Retention Time	Area	Area Percent
1		7.367	1390805	98.612
2		7.833	19582	1.388
Totals			1410387	100.000



HPLC spectrum of Compound **9b**

Det 168-254nm Results

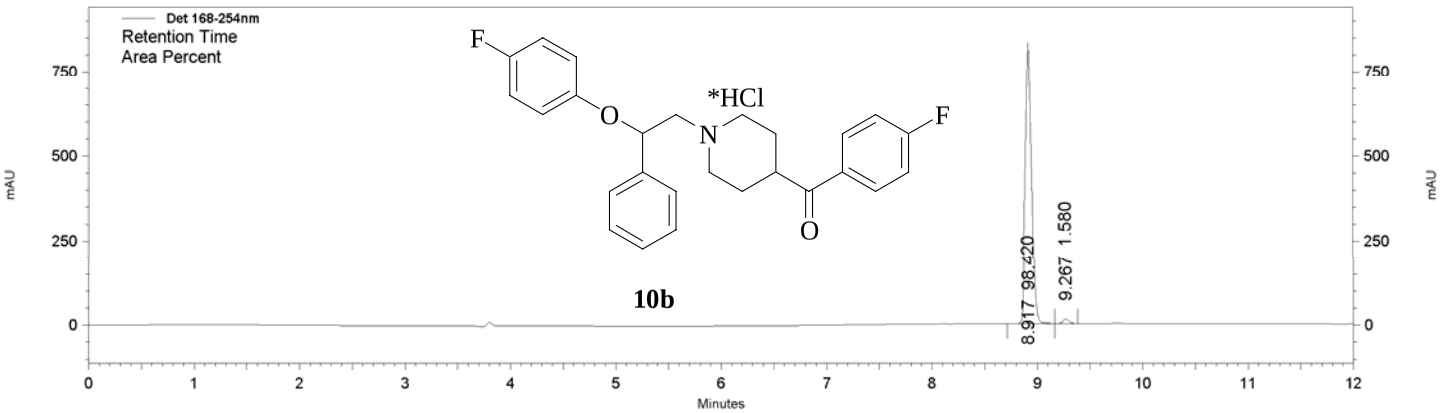
Pk #	Name	Retention Time	Area	Area Percent
1		7.683	6647499	99.002
2		8.050	6072	0.090
3		8.217	60941	0.908
Totals			6714512	100.000



HPLC spectrum of Compound **10b**

Det 168-254nm Results

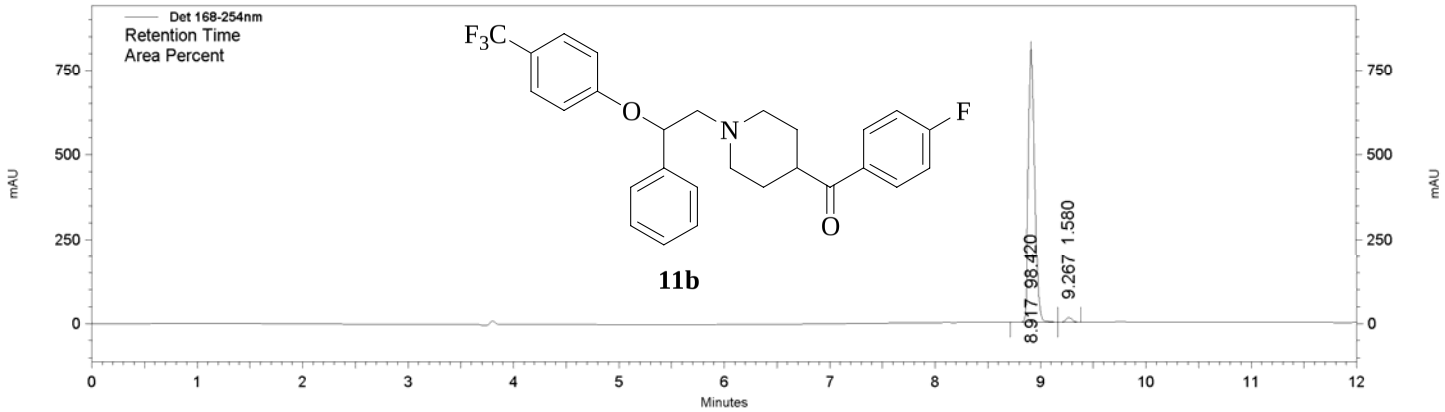
Pk #	Name	Retention Time	Area	Area Percent
1		8.917	3363483	98.420
2		9.267	54002	1.580
Totals			3417485	100.000



HPLC spectrum of Compound **11b**

Det 168-254nm Results

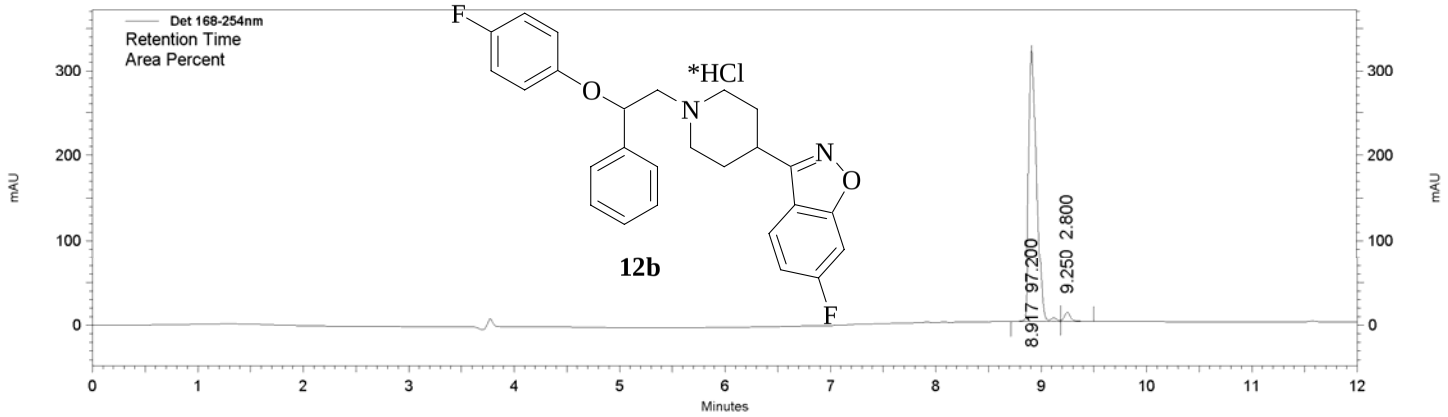
Pk #	Name	Retention Time	Area	Area Percent
1		8.917	3363483	98.420
2		9.267	54002	1.580
Totals			3417485	100.000



HPLC spectrum of Compound **12b**

Det 168-254nm Results

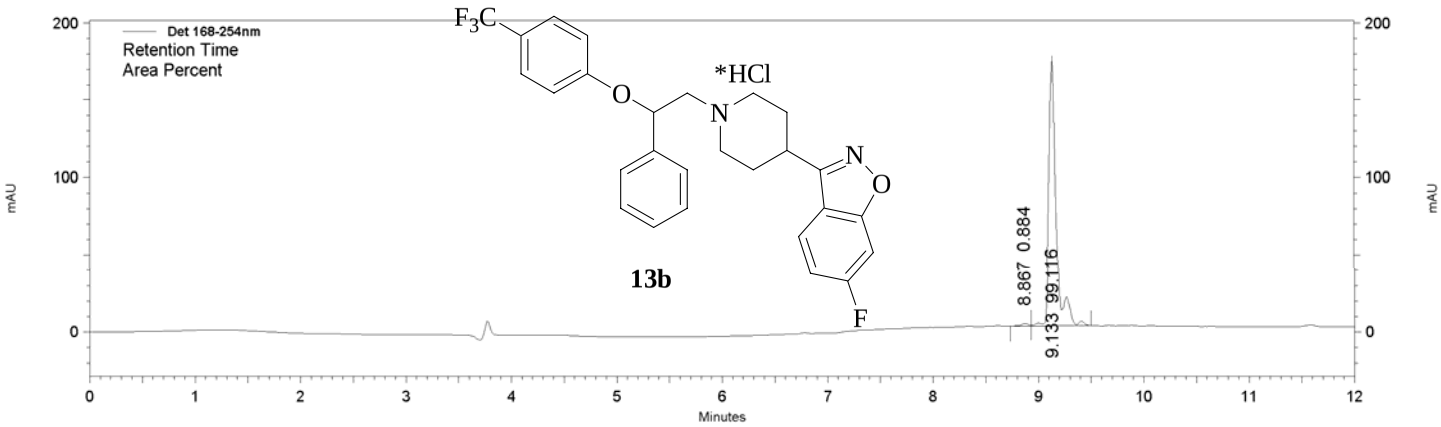
Pk #	Name	Retention Time	Area	Area Percent
1		8.917	1669985	97.200
2		9.250	48113	2.800
Totals			1718098	100.000



HPLC spectrum of Compound **13b**

Det 168-254nm Results

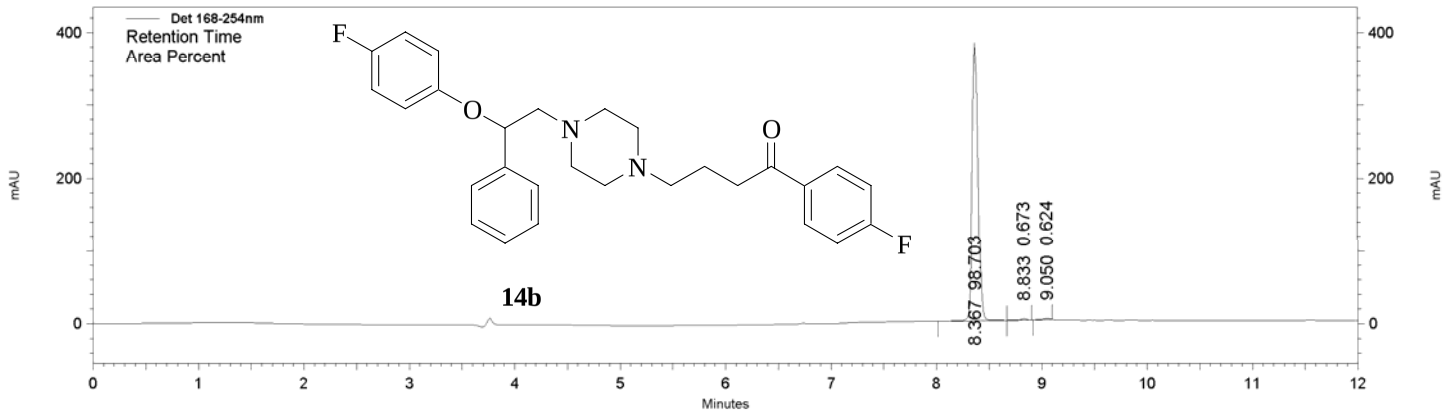
Pk #	Name	Retention Time	Area	Area Percent
1		8.867	7531	0.884
2		9.133	844271	99.116
Totals			851802	100.000



HPLC spectrum of Compound **14b**

Det 168-254nm Results

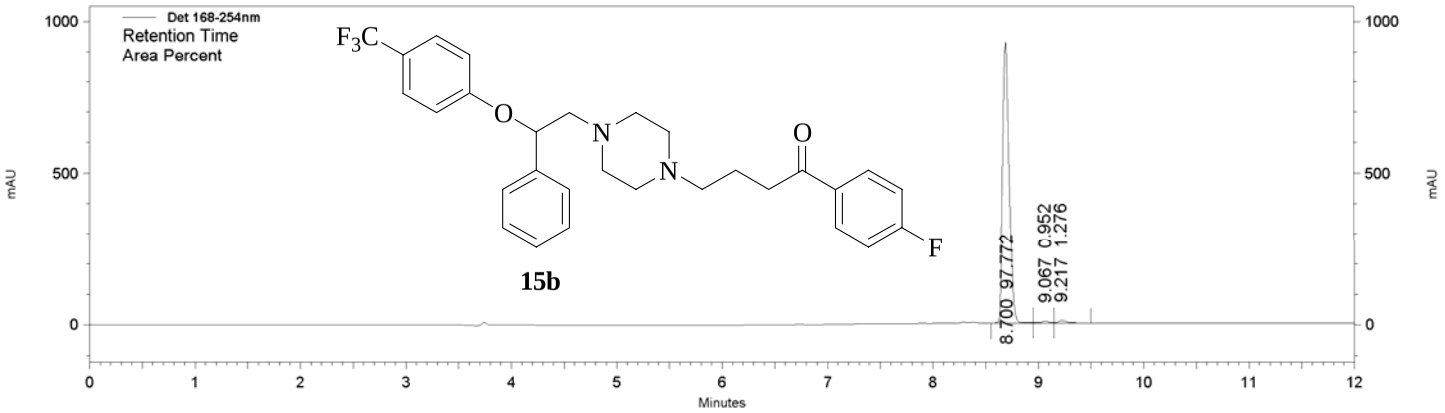
Pk #	Name	Retention Time	Area	Area Percent
1		8.367	1496808	98.703
2		8.833	10207	0.673
3		9.050	9463	0.624
Totals			1516478	100.000



HPLC spectrum of Compound **15b**

Det 168-254nm Results

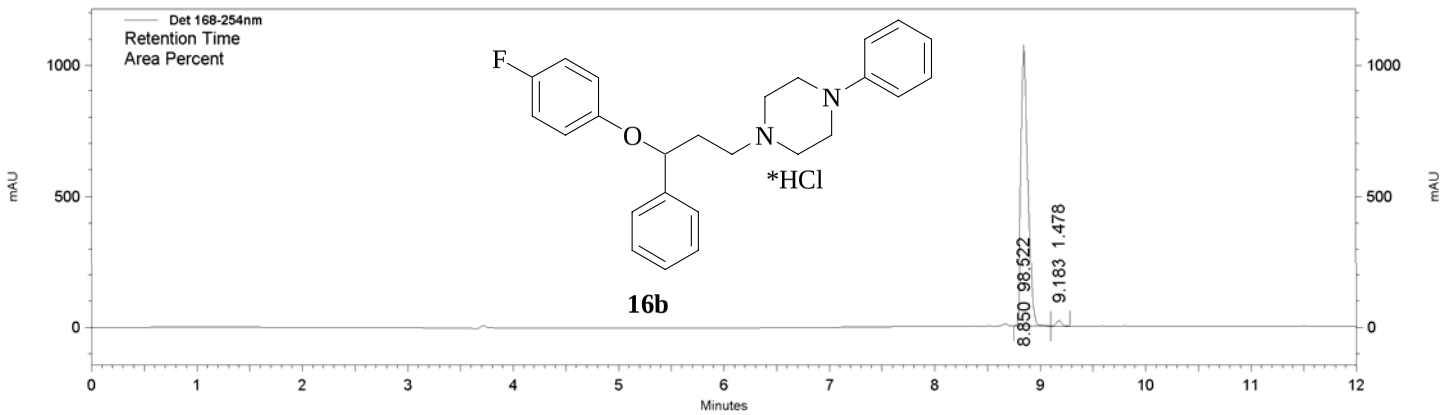
Pk #	Name	Retention Time	Area	Area Percent
1		8.700	4055283	97.772
2		9.067	39495	0.952
3		9.217	52922	1.276
Totals			4147700	100.000



HPLC spectrum of Compound **16b**

Det 168-254nm Results

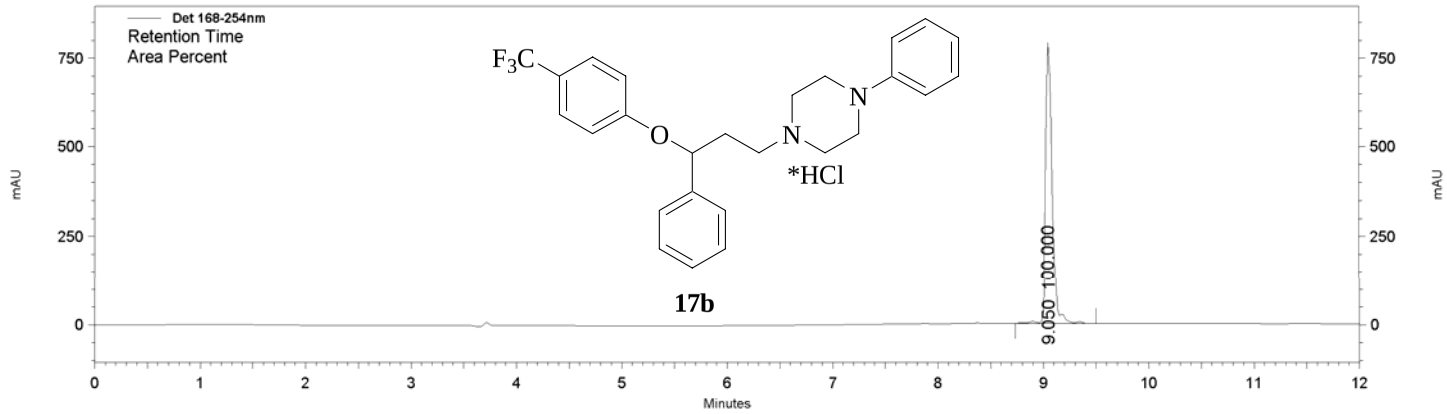
Pk #	Name	Retention Time	Area	Area Percent
1		8.850	4889325	98.522
2		9.183	73331	1.478
Totals			4962656	100.000



HPLC spectrum of Compound **17b**

Det 168-254nm Results

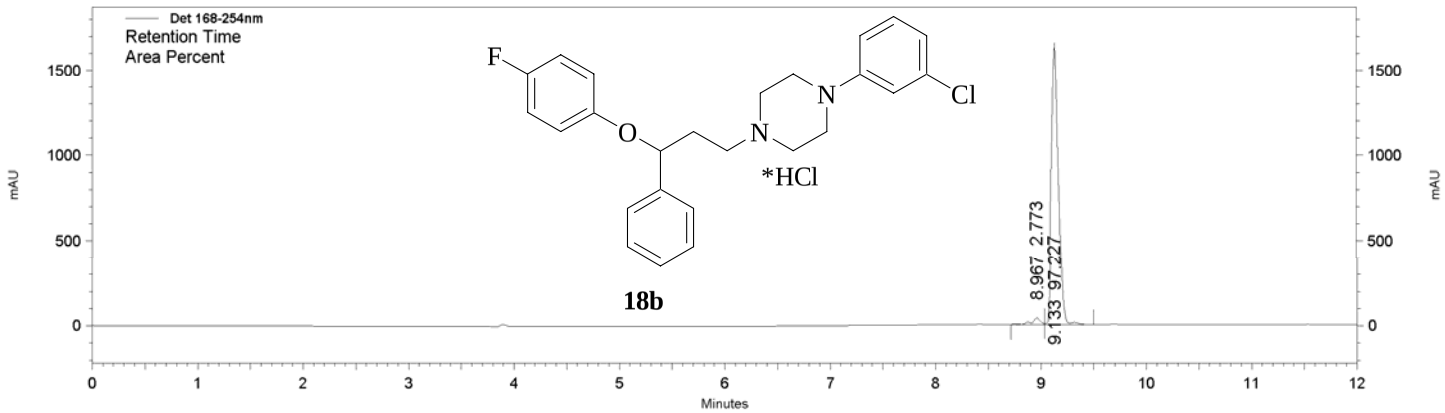
Pk #	Name	Retention Time	Area	Area Percent
1		9.050	3428741	100.000
Totals			3428741	100.000



HPLC spectrum of Compound **18b**

Det 168-254nm Results

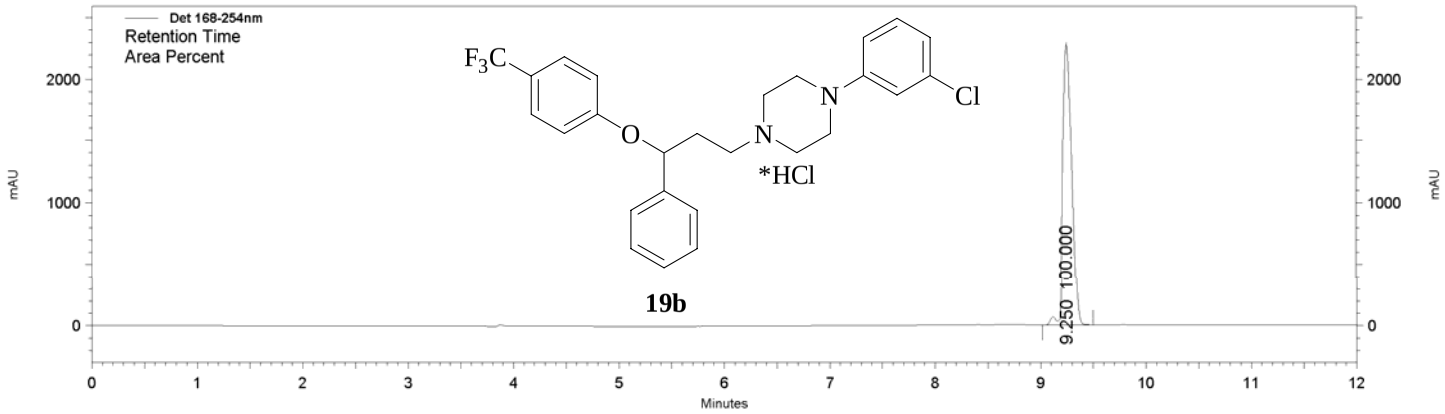
Pk #	Name	Retention Time	Area	Area Percent
1		8.967	217706	2.773
2		9.133	7633268	97.227
Totals			7850974	100.000



HPLC spectrum of Compound **19b**

Det 168-254nm Results

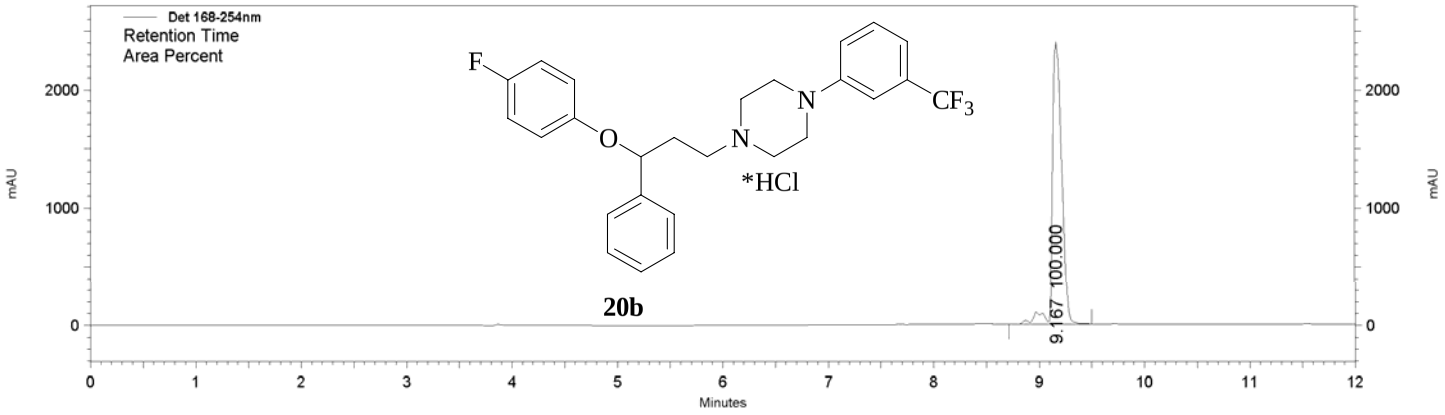
Pk #	Name	Retention Time	Area	Area Percent
1		9.250	13487651	100.000
Totals			13487651	100.000



HPLC spectrum of Compound **20b**

Det 168-254nm Results

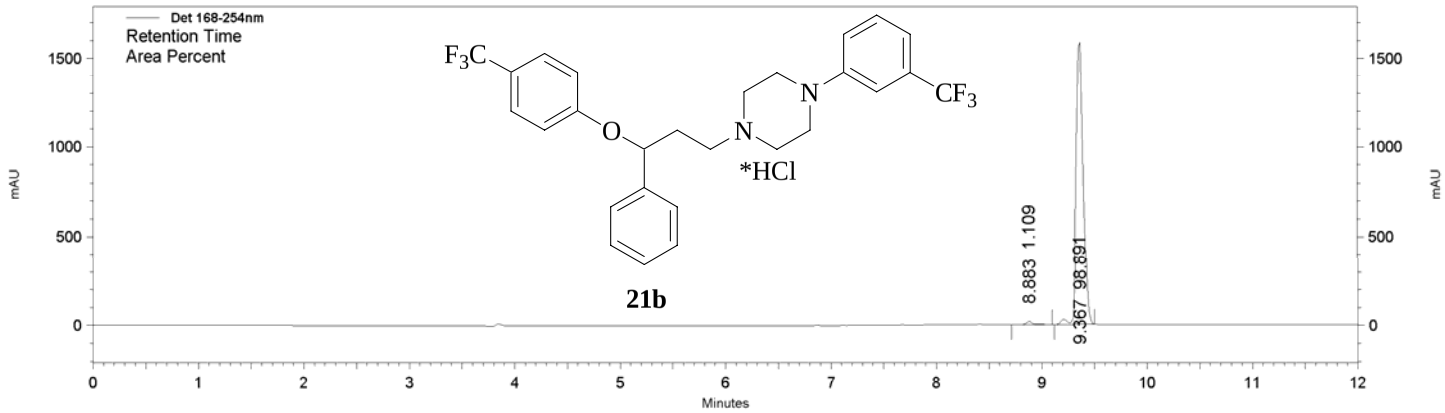
Pk #	Name	Retention Time	Area	Area Percent
1		9.167	14995027	100.000
Totals			14995027	100.000



HPLC spectrum of Compound **21b**

Det 168-254nm Results

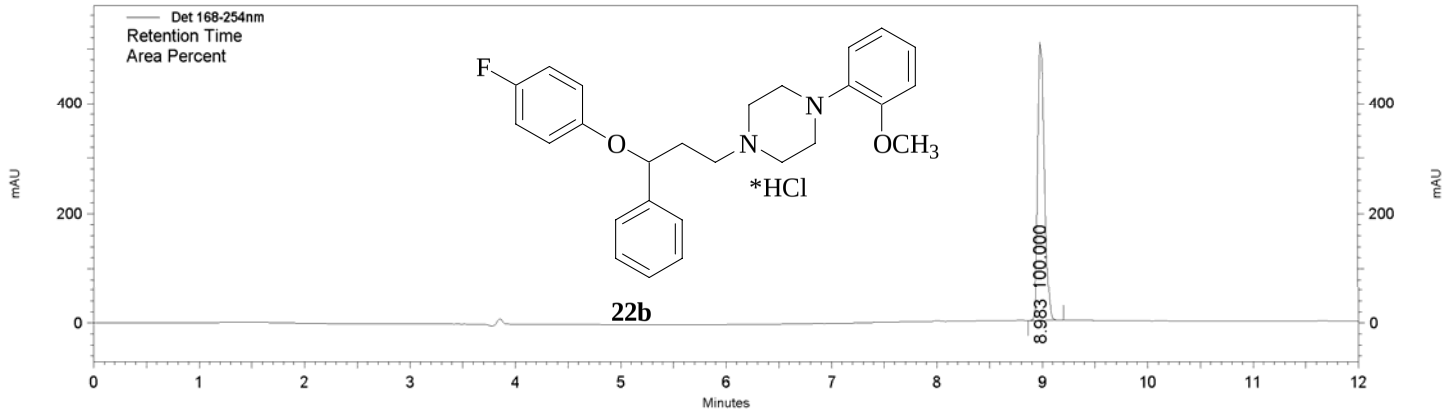
Pk #	Name	Retention Time	Area	Area Percent
1		8.883	83677	1.109
2		9.367	7462514	98.891
Totals			7546191	100.000



HPLC spectrum of Compound **22b**

Det 168-254nm Results

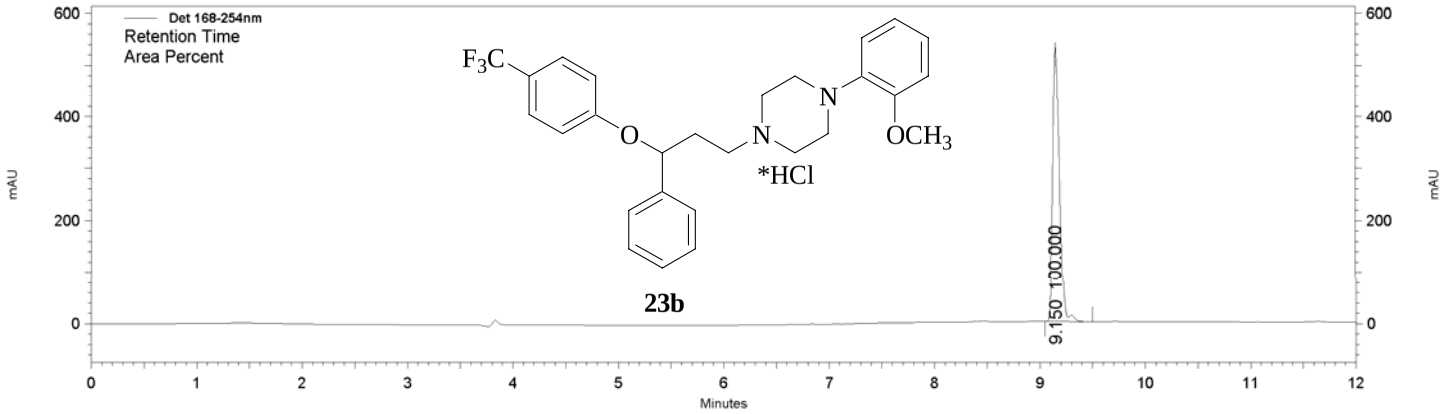
Pk #	Name	Retention Time	Area	Area Percent
1		8.983	2260172	100.000
Totals			2260172	100.000



HPLC spectrum of Compound **23b**

Det 168-254nm Results

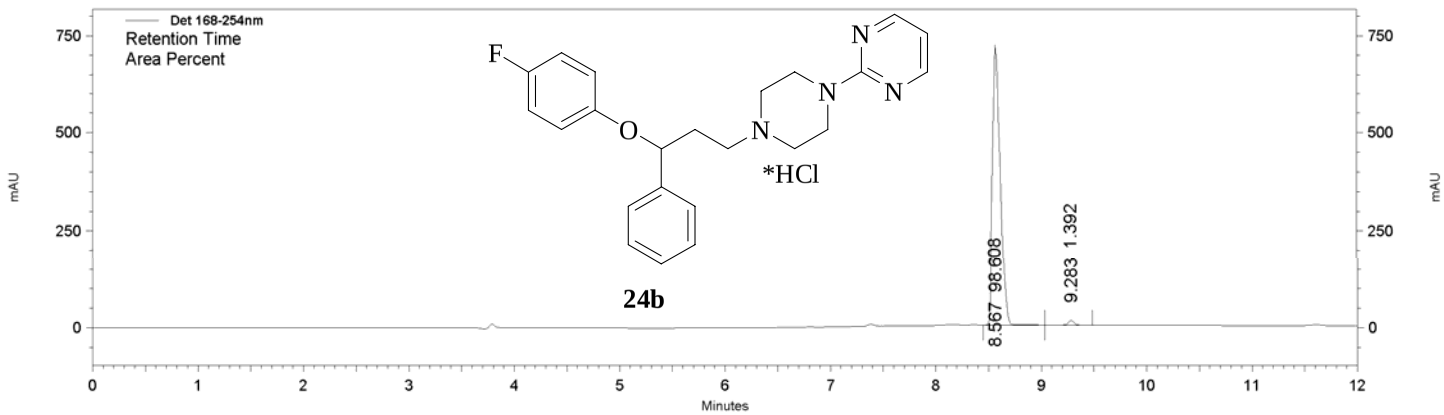
Pk #	Name	Retention Time	Area	Area Percent
1		9.150	2407414	100.000
Totals			2407414	100.000



HPLC spectrum of Compound **24b**

Det 168-254nm Results

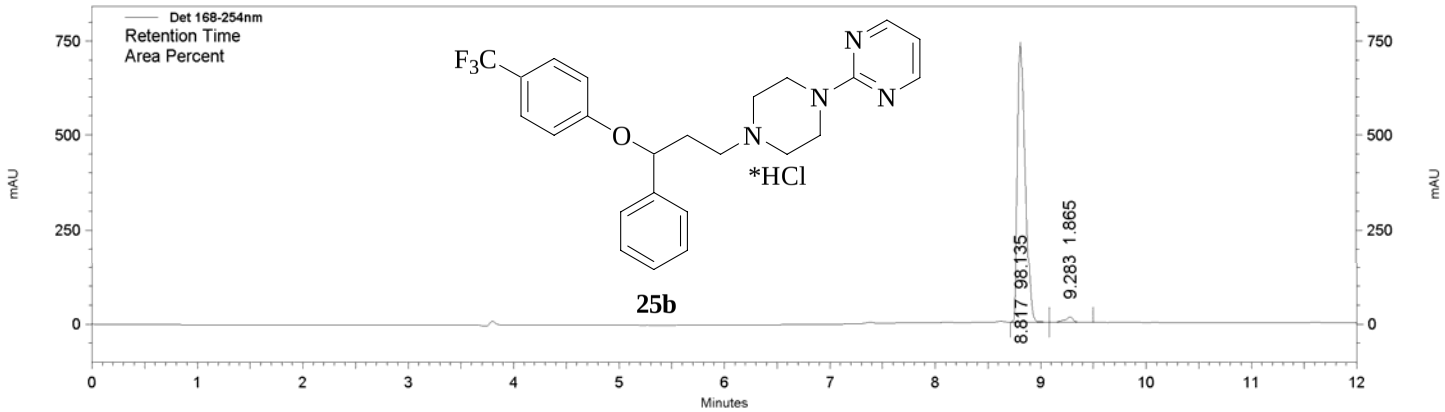
Pk #	Name	Retention Time	Area	Area Percent
1		8.567	3722821	98.608
2		9.283	52545	1.392
Totals			3775366	100.000



HPLC spectrum of Compound **25b**

Det 168-254nm Results

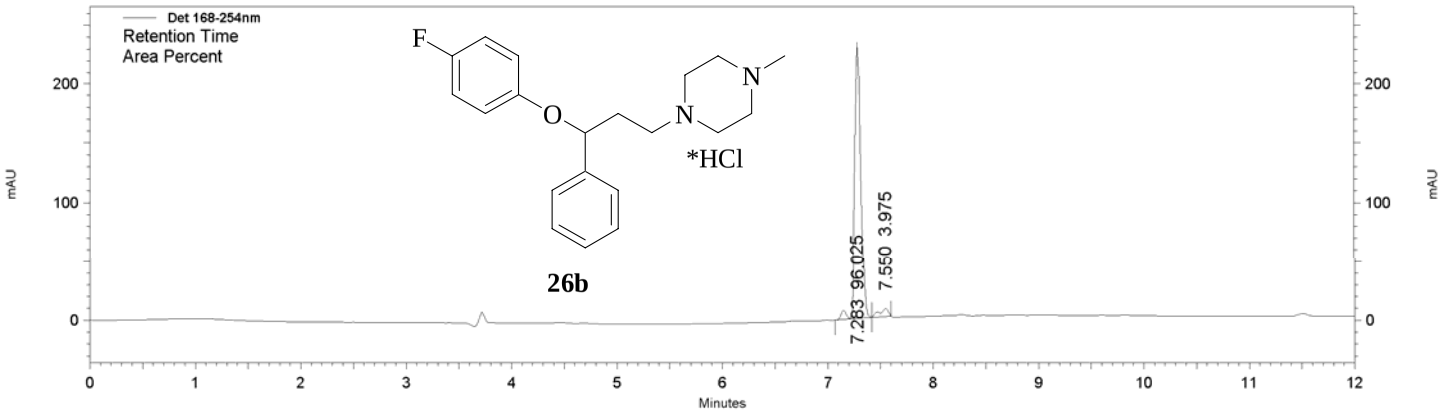
Pk #	Name	Retention Time	Area	Area Percent
1		8.817	3826203	98.135
2		9.283	72700	1.865
Totals			3898903	100.000



HPLC spectrum of Compound **26b**

Det 168-254nm Results

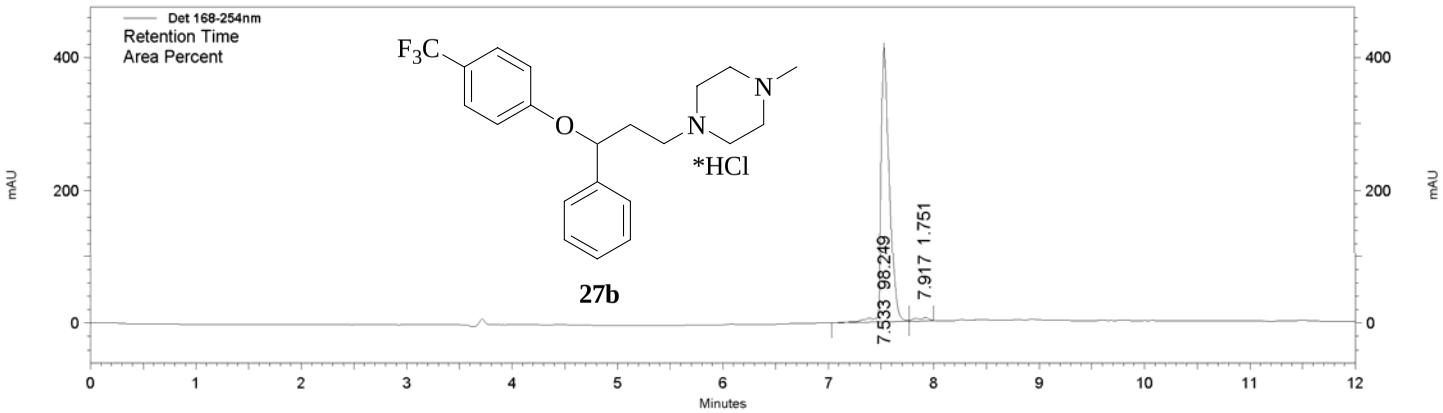
Pk #	Name	Retention Time	Area	Area Percent
1		7.283	947986	96.025
2		7.550	39240	3.975
Totals			987226	100.000



HPLC spectrum of Compound **27b**

Det 168-254nm Results

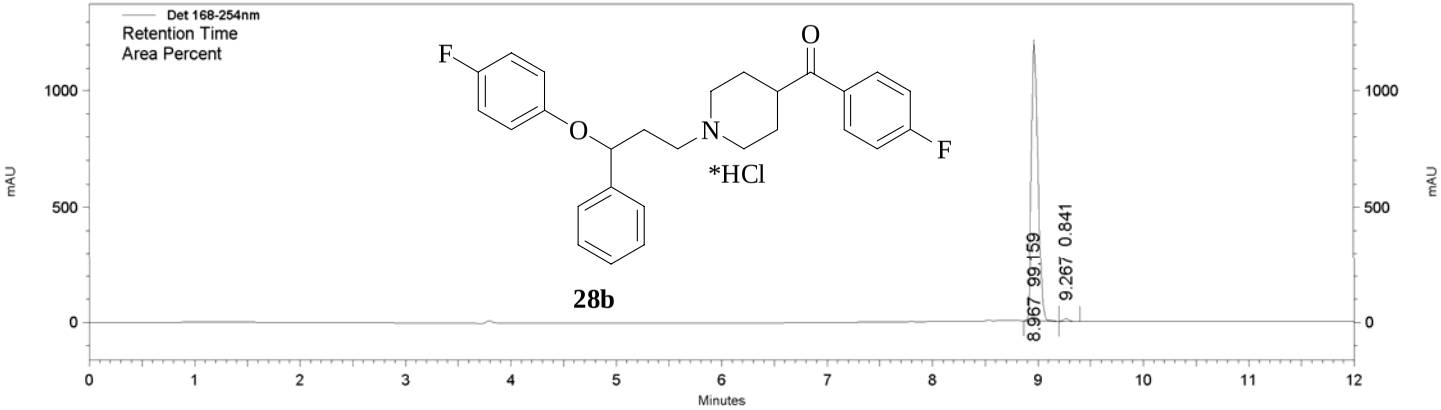
Pk #	Name	Retention Time	Area	Area Percent
1		7.533	2231265	98.249
2		7.917	39769	1.751
Totals			2271034	100.000



HPLC spectrum of Compound **28b**

Det 168-254nm Results

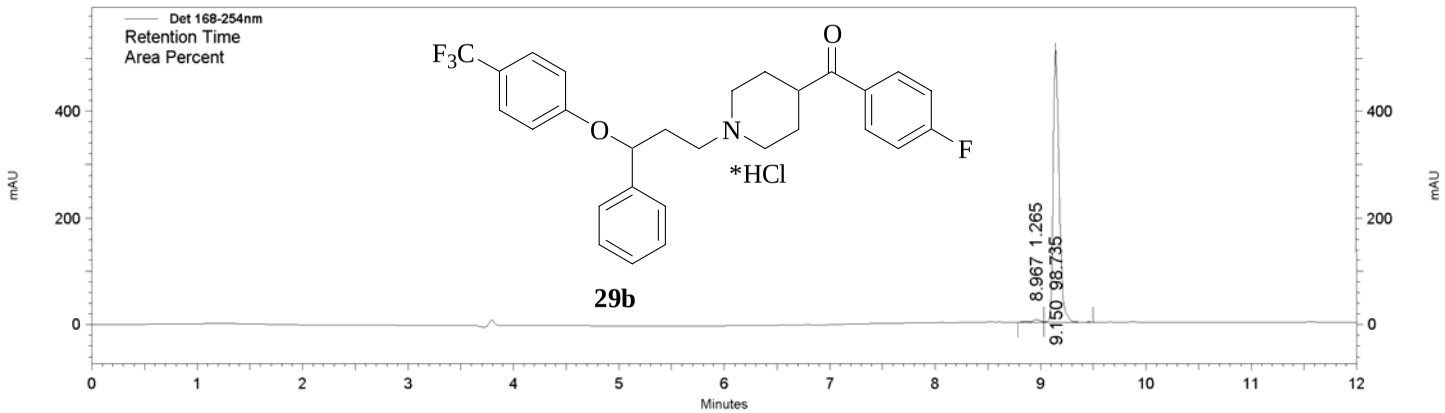
Pk #	Name	Retention Time	Area	Area Percent
1		8.967	5223244	99.159
2		9.267	44298	0.841
Totals			5267542	100.000



HPLC spectrum of Compound **29b**

Det 168-254nm Results

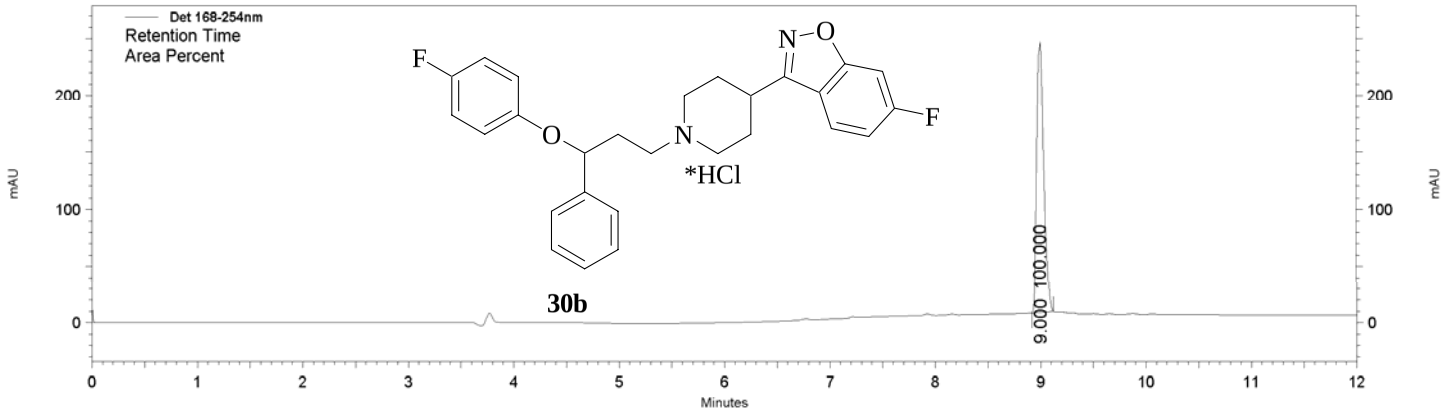
Pk #	Name	Retention Time	Area	Area Percent
1		8.967	26014	1.265
2		9.150	2030983	98.735
Totals			2056997	100.000



HPLC spectrum of Compound **30b**

Det 168-254nm Results

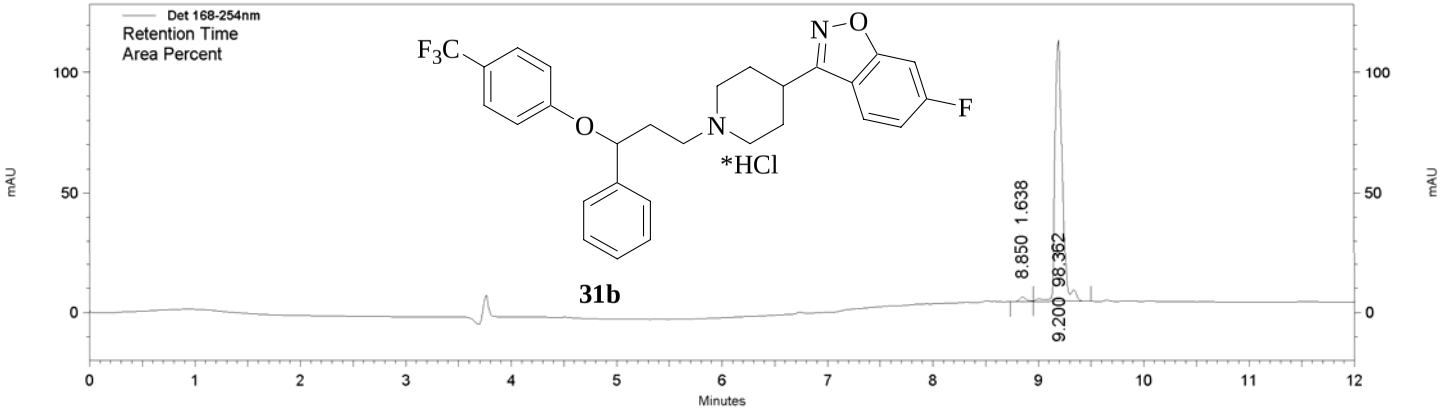
Pk #	Name	Retention Time	Area	Area Percent
1		9.000	1110239	100.000
Totals			1110239	100.000



HPLC spectrum of Compound **31b**

Det 168-254nm Results

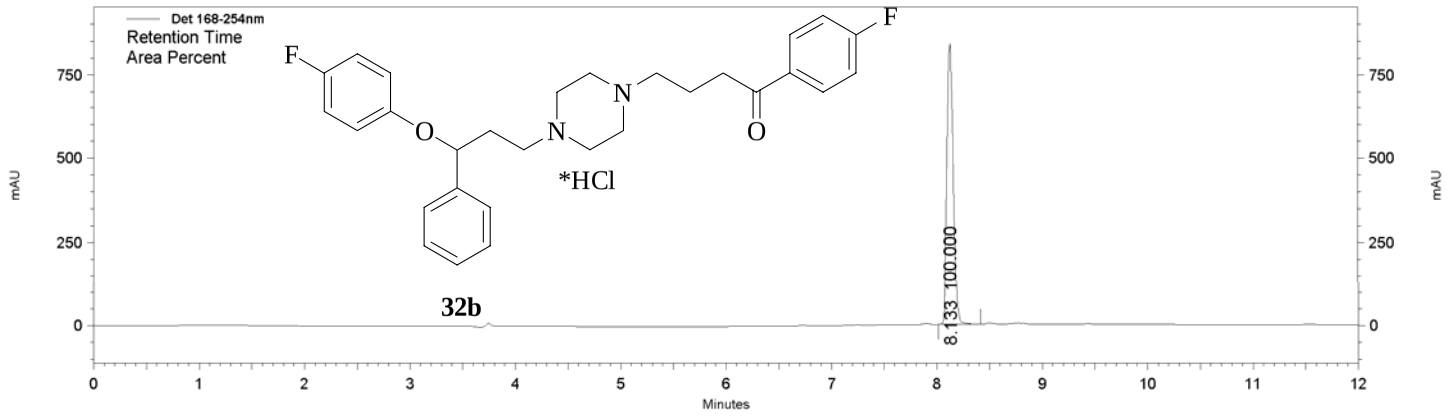
Pk #	Name	Retention Time	Area	Area Percent
1		8.850	8631	1.638
2		9.200	518313	98.362
Totals			526944	100.000



HPLC spectrum of Compound **32b**

Det 168-254nm Results

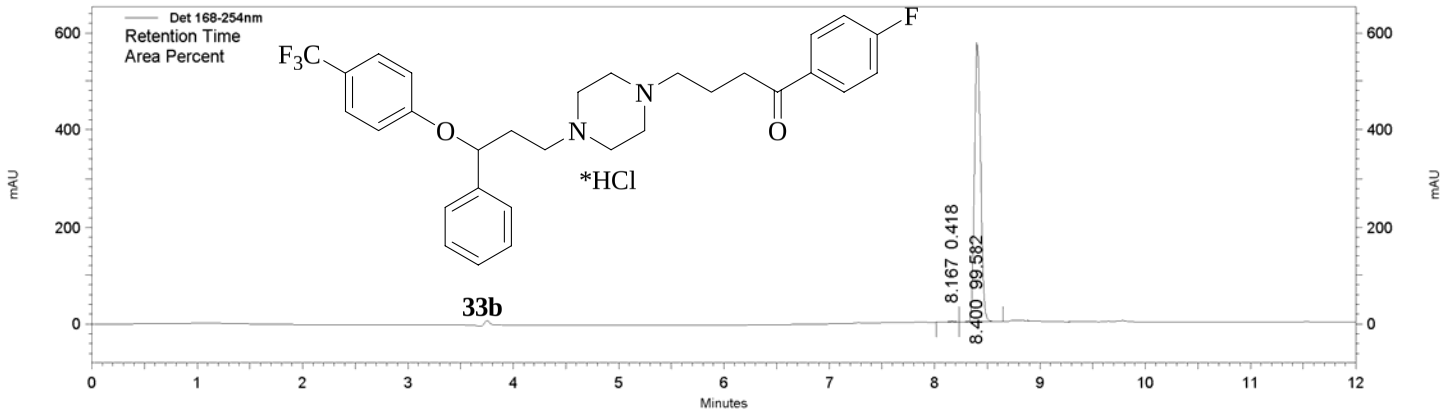
Pk #	Name	Retention Time	Area	Area Percent
1		8.133	3525951	100.000
Totals			3525951	100.000



HPLC spectrum of Compound **33b**

Det 168-254nm Results

Pk #	Name	Retention Time	Area	Area Percent
1		8.167	9794	0.418
2		8.400	2332258	99.582
Totals			2342052	100.000



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