SYNTHESIS OF ISOQUINOLINE AND PYROCATECHOL DERIVATIVES AS POTENTIAL DRUG MOLECULES ACTIVE AGAINST PROSTATE CANCER

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ABSTRACT

SYNTHESIS OF ISOQUINOLINE AND PYROCATECHOL DERIVATIVES AS POTENTIAL DRUG MOLECULES ACTIVE AGAINST PROSTATE CANCER

Prostate cancer is one of the most common cancer types among male worldwide. It has been found that about 80 percent of prostate cancer patients have high level of androgen levels. Prior researches have stated that reducing the androgen level in the body can be a good solution to prevent the growth of cancer cells. Androgens are synthesized in both testes and adrenal glands. Hence, inhibition of androgen biosynthesis in not only testes, but also adrenal glands is essential to suppress the androgen biosynthesis. The last step of androgen biosynthesis in both testes and adrenal glands is catalyzed by a single enzyme, 17α-hydroxylase-17,20-lyase (CYP17). On account of this information, selective inhibition of CYP17 enzyme results in reduction or complete annihilation of androgen production. Thus, CYP17 is the foremost target enzyme for the treatment of prostate malignancy. Accordingly, computational screening method was used to find out lead compounds active against CYP17 enzyme by Prof. Metin Türkay and Assoc. Prof. Halil Kavaklı from Koç University. As a result of the drawbacks of steroidal compounds, a non-steroidal lead compound with an IC_{50} value of 35 μM was found. In order to find compounds with better IC_{50} values, derivatives of the lead compound were examined by using Autodock program. Among the derivatives, the compounds with better docking and binding energies were synthesized. In this project, synthesis of isoquinoline and pyrocatechol derivatives was targeted. For this purpose, ten compounds were synthesized. Biological tests were performed by the research group of Assoc. Prof. İbrahim Halil Kavaklı from Koç University. As a result, a new compound with hundred percent inhibition at 5 μM concentration was discovered.

ÖZET

PROSTAT KANSERİNE KARŞI AKTİF, POTANSİYEL İLAÇ MOLEKÜLLERİ OLARAK İZOKİNOLİN VE PİROKATEKOL TÜREVLERİNİN SENTEZİ

Prostat kanseri tüm dünya çapında erkekler arasında en yaygın kanser türlerinden biridir. Prostat kanseri hastalarının yüzde 80'inde yüksek miktarda androjen biyosentezi tespit edilmiştir. Yapılan çalışmalar vücuttaki androjen seviyesini azaltmanın kanser hücrelerinin büyümesini engellemek için iyi bir çözüm olabileceğini gösteriyor. Androjenler testislerde ve böbrek üstü bezlerinde sentezlenir. Bu yüzden vücuttaki androjen biyosentezini tamamen durdurmak için hem testislerdeki hem de böbrek üstü bezlerindeki androjen biyosentezini inhibe etmek gereklidir. Testislerdeki ve böbrek üstü bezlerindeki androjen biyosentezinin son basamağında 17α-hidroksilaz-C17,20-liyaz (CYP17) enzimi katalizör olarak görev alır. Bu bilgiye göre CYP17 enziminin seçici bir şekilde inhibe edilmesi vücuttaki androjen üretiminin azalmasına ya da tamamen durmasına neden olur. Bu nedenle CYP17 enzimi prostat kanseri tedavisinde en önemli hedef enzimdir. Buna dayanarak, Koç Üniversitesi'nden Prof. Dr. Metin Türkay ve Doç. Dr. Halil Kavaklı CYP17 enzimini engelleyebilecek öncü moleküller bulabilmek için bilgisayar taraması yaptılar. Steroid bazlı moleküllerin dezavantajlarından dolayı steroid bazlı olmayan, IC_{50} değeri 35 μM olan bir öncü bileşik bulundu. IC_{50} değeri daha iyi bileşikler bulmak için Autodock programı kullanılarak öncü bileşiğin türevleri incelendi. Türevler arasından yaklaşma ve bağlanma enerjileri daha iyi olanlar sentezlendi. Bu projede izokinolin ve pirokatekol türevlerinin sentezi amaçlanmıştır. Bu amaçla on bileşik sentezlendi. Biyolojik testler Koç Üniversitesi'nde Doç. Dr. İbrahim Halil Kavaklı'nın çalışma grubu tarafından yapıldı. Sonuç olarak beş μM konsantrasyonda yüzde yüz inhibisyon gösteren yeni bir molekül bulundu.

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LIST OF ACRONYMS/ABBREVIATIONS

Ac_2O	Acetic Anhydride
ADMET	Adsorption, Distribution, Metabolism, Elimination and Toxicity
ADT	Androgen Deprivation Therapy
BE	Binding Energy
$CDCl_3$	Deuterated chloroform
CH_2Cl_2	Dichloromethane
CYP	Cytochrome P-450
CYP 17	17 alpha-hydroxylase cytochrome P-450 (P-$450_{17\alpha}$)
DCC	N,N'-dicyclohexylcarbodiimide
DHT	5α-dihydrotestosterone
DE	Docking Energy
DMAP	N,N'-dimethylaminopyridine
DRE	Digital Rectal Exam
FSH	Follicle stimulating Hormones
GnRH	Gonadotropin Releasing Hormone
IC_{50}	Inhibitory Concentration
LC-MS	Liquid Chromatography-Mass Spectroscopy
LH	Luteinizing Hormone
NMR	Nuclear Magnetic Resonance
PC	Prostate Cancer
PDB ID	Protein Data Bank Identification
PSA	Prostate-Specific Antigen
QSAR	Quantitative Structure Activity Relationship
RT	Room Temperature
SBDD	Structure Based Drug Design
T	Testosterone
TEA	Triethylamine
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography

1. INTRODUCTION

1.1. Cancer

Cancer is an extensive group of a variety of diseases. The common thing of these diseases is the unregulated cell growth. Cancerous cells divide and grow hysterically, form malignant tumors, and attack nearby parts of the body. The spread of cancer to other locations in the body is called metastasis. The cancer can also expand to more far-away parts of the body through the lymphatic system or bloodstream. On the other hand, not all tumors are malignant. Benign tumors do not have unregulated growth, do not attack adjacent tissues, and do not expand throughout the body. There are more than 200 different identified cancers that badly affect humans [1].

Even though cancer can affect people from all ages, and there are a small number of types of cancer that are more common in children, the risk of coming down with cancer generally increases with age. In 2007, about 13% of all human deaths worldwide, which means 7.9 million, resulted from cancer. Ratio is increasing at the same time as more people live to an old age and as lifestyle of people changes widely in the developing world [2].

Stating the causes of cancer is complicated. There is no absolute explanation concerning to having cancer. The main known factors that increase the risk of cancer are [3,4]:

- Tobacco use
- Certain infections
- Radiation
- Lack of physical activity
- Obesity
- Environmental pollutants

- Genetic failures

Symptoms of cancer differ in accordance with the type of the disease. However general symptoms are unintentional weight loss, fever, being excessively tired, and changes to the skin [5].

Type of treatment that is used depends upon the type, location and stage of the cancer. Nevertheless, cancer is generally treated with chemotherapy, radiation therapy and surgery. The likelihood of curing cancer varies significantly by the type and location of cancer and the grade of disease when treatment is started [2].

1.2. Prostate Cancer

Prostate cancer (PC) is the most common cancer type and age-related cause of death among male all around the world. In 2007, it was expected that 220,000 new cancer incidents would be diagnosed and about 30,000 men would die of cancer in the US [6]. Indeed aside from the lung cancer, it is the second leading cause of death from cancer in the US [7].

There are several factors that increase the development of prostate cancer. The main factors are [8]:

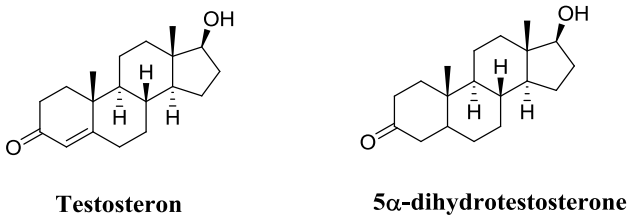
- Age
- Family history
- Race
- Physical inactivity
- Diet

Generally, no symptoms are observed at the early stages of prostate cancer. Nonetheless, prostate cancer sometimes causes symptoms. These symptoms consist of [9]:

- Frequent urination
- Increased urination at night
- Difficulty with starting and maintaining a steady stream of urine
- Blood in the urine
- Painful urination
- Erection problems
- Bone pain

1.3. Relationship between Androgens and Prostate Cancer

Prostate cancer is androgen dependent in more than 80% of the incidents [10]. Androgens are steroid based male sex hormones and have an significant role in the development, growth and progression of prostate cancer [11,12]. There are two important androgens in this respect. These androgens are testosterone (T) and dihydrotestosterone (DHT) (Figure 1.1).



Testosteron **5α-dihydrotestosterone**

Figure 1.1. Structures of testosterone (T) and dihydrotestosterone (DHT).

About 90% of testosterone (T) is synthesized in testes and while the rest 10% is synthesized in adrenal glands. Subsequently testosterone is converted to the more potent androgen, dihydrotestosterone (DHT), by the enzyme 5α-reductase that is localized primarily in the prostate [13].

Several studies point out a correlation between serum testosterone levels and high risk of prostate cancer [14]. Consequently, elimination of release, production or behavior of androgenic hormones is a main therapeutic option for the treatment of this malignancy [15].

1.4. Diagnosis of Prostate Cancer

The majority of cancers occurs in the periphery of the prostate gland, and triggers symptoms only when they have grown to compress the urethra, or spread over the sphincter or neurovascular bundle [16,17]. The stage of the cancer is useful in understanding the symptoms that it produces (if any). Development PC can be divided into 4 stages [18]:

- Stage 1: Tumors are by definition clinically silent
- Stage 2: Palpable rectally
- Stage 3: Tumors have invaded local structures, such as the bladder, seminal vesicles or the prostatic capsule
- Stage 4: Tumors have invaded more widely

Prostate specific antigen (PSA) is a physiologic product of normal epithelial cells. It is known that PC patients have relatively higher serum PSA in their blood with respect to healthy men. For that reason, PSA test is a screening method for the determination of PSA level in blood [19].

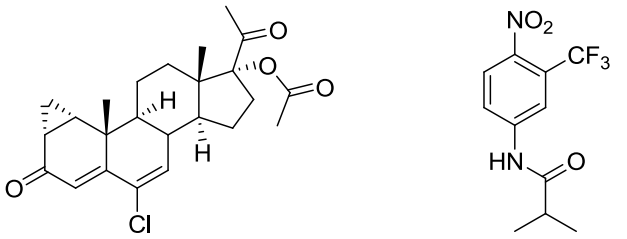
Digital rectal examination (DRE) is a different method to detect PC. In DRE, a rectal examination is carried out to control the size and texture of the prostate gland, and also to detect any local abnormalities in its periphery. An abnormal rectal examination is a strong forecaster of the cancer.

As a result, the typical management of a man with lower urinary tract symptoms includes a rectal examination and a PSA test [20,21].

1.5. Treatment of Prostate Cancer

Treatment for patients whose PC is in early stages can be local therapy such as prostatectomy (surgical removal of part or all of the prostate gland) or radiation therapy [22].

For patients in further stages, the widespread treatment of PC is orchidectomy or chemical castration by gonadotropin releasing hormone (GnRH) analogues which decrease the androgen synthesis in testes. Although these treatments suppress androgen synthesis in testes, there is a significant drawback, they do not affect the androgen biosynthesis in the adrenal glands. For that reason they are commonly combined with androgen receptor antagonists. Flutamide and cyproterone acetate are two androgen receptor antagonists which reduce the stimulatory effects of the remaining androgens (Figure 1.2).



Cyproterone acetate Flutamide

Figure 1.2. Structures of Cyproterone acetate and Flutamide.

However this combination is still not useful in all patients because of mutations in the androgen receptor. Due to the presence of mutations, anti-androgens may behave as agonists. That's why total inhibition of androgen synthesis in testes and adrenal glands is a potential alternative for the treatment [23,24].

1.6. Hormonal Therapy

It is found that elimination of androgens results in a decrease in the PSA level in the bloodstream [19]. In addition, about 90% of PC patients respond to androgen deficiency which means cancer cells need androgen for their growth [25]. Furthermore, it is observed that the growth of the tumor in metastatic prostate cancer is prevented by the hormonal

therapy. In view of that, hormonal therapy is frequently favored for the advanced PC in order to deactivate or slow down the spread of tumors [26]. There are several ways to control the androgen levels in PC:

- Antiandrogens interact with the androgen receptor and prevent androgens from their stimulating effect on tumor. Unfortunately they have many side effects like liver problems, anemia and nausea.

- The activity of estrogens is on hypothalamic level. They decrease the release of gonadorelin. Subsequently, there is a decrease in the formation of luteinizing hormones (LH) and follicle stimulating hormones (FSH) in pituitary gland which results in a decrease of the androgen production in testes. However, there are considerable cardiovascular side effects.

- The use of gonadorelin analogs is also a way to inhibit the testicular androgen production, but results in fewer side effects [27].

All of the current therapeutic approaches mentioned above are not able to inhibit androgen biosynthesis completely. In addition, there are many adverse effects of these treatments. Due to these disadvantages, it is required to develop a novel target which is capable of blocking androgen production completely and has minimum side effects.

1.7. New Therapeutic Approaches

Researchers have concentrated on developing novel targets for the treatment of PC due to the presence of adverse effects and the incompetence of the current treatment methods over the past 15 years. Considering all the studies done in this field, androgen deprivation therapy (ADT) is a prominent one which gives the most positive responses for the prostate patients [28].

As mentioned before, it is thought that diminishing the androgen biosynthesis in both testes and adrenal glands can be a good solution in the treatment of the prostate cancer.

Androgens induce the growth of prostate cancer. Therefore, annihilation of synthesis or release of androgenic hormones is the most important new therapeutic method in the treatment of the prostate cancer [29]. Since testosterone and dihydrotestosterone are two important androgens, it is essential to study the mechanism of synthesis of testosterone and dihydrotestosterone.

1.7.1. Biological Synthesis of Testosterone (T) and Dihydrotestosyerone (DHT)

Testicular and adrenal androgen biosynthesis begins from cholesterol. A number of enzymatic transformations of cholesterol which is frequently catalyzed by P450 enzymes end up with the formation of androgenic steroids. The last step of the androgen biosynthesis in both testes and adrenal glands is catalyzed by the enzyme P-450, 17α-hydroxylase-C17, C20 lyase (CYP17) which is a member of P-450 enzyme family. As it can seen from Figure 1.3 [31], CYP17 is the key enzyme which first hydroxylates pregnenolone and progesterone in 17α-position (hydroxylase). Afterward, the acetyl group is cleaved (lyase) and the 17-keto androgens dehydroandrostendione and androstendione are synthesized. Subsequently, these compounds are transformed enzymatically to testosterone and dihydrotestosterone steroids [27].

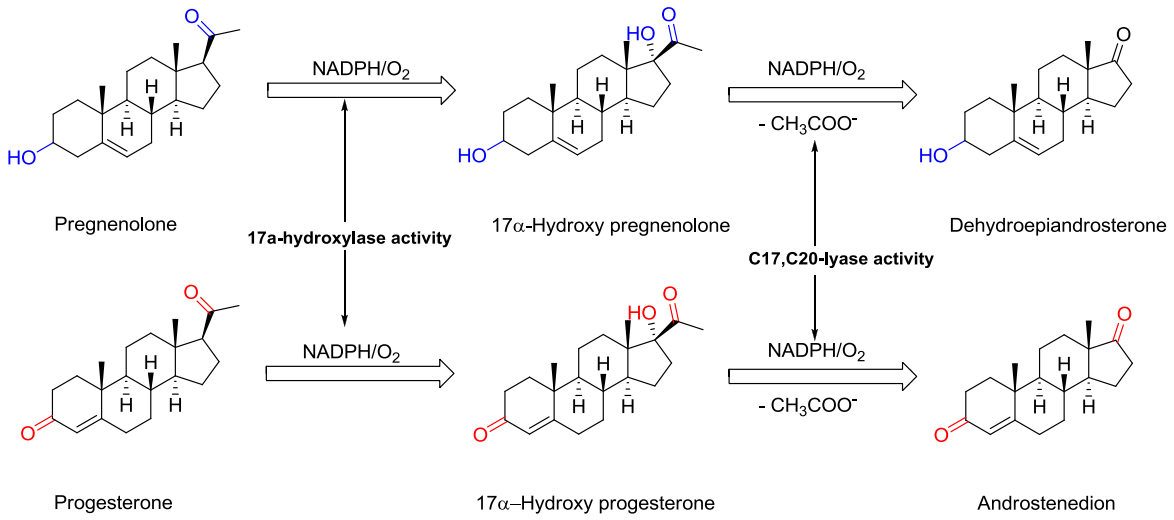


Figure 1.3. CYP17 catalyzed androgen biosynthesis.

1.7.2. Target Enzyme CYP17

As indicated before, androgen biosynthesis occurs in both testes and adrenal glands. Hence, total androgen production in the body can be blocked only by inhibiting the androgen biosynthesis in both testes and adrenal glands.

The enzymatic pathway of androgen biosynthesis is given in Figure 1.4. [12]. P-450, 17α-hydroxylase-C17, C20 lyase (CYP17) catalyzes the synthesis of both testosterone and DHT. In order to keep away from undesired side effects, androgen biosynthesis inhibitors should be specific and not affect corticoid biosynthesis. Therefore strong and selective inhibition of CYP17 gives rise to total and exclusive removal of androgen production [27]. For this reason, CYP17 is the new therapeutic target enzyme for the treatment of prostate cancer.

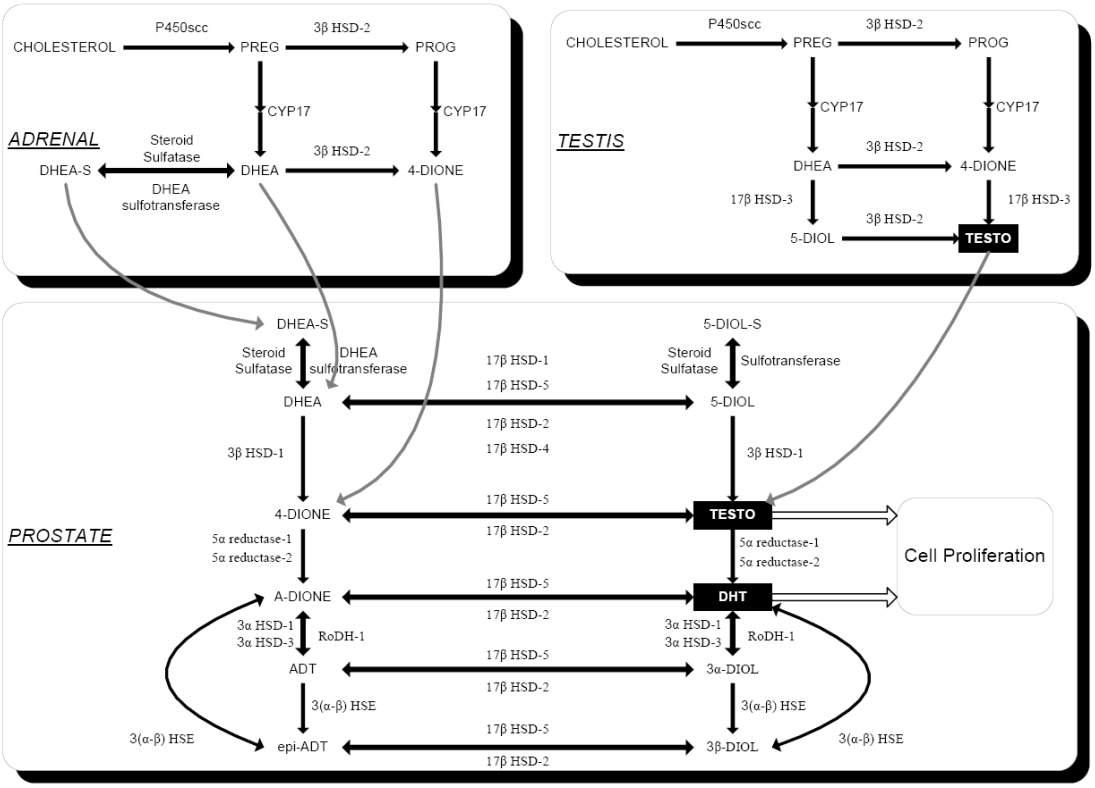


Figure 1.4. Enzymatic synthesis of androgens in testes and adrenals.

1.8. Drug Discovery, Design and Optimization

1.8.1. Drug Discovery

Primary stage of drug discovery is to choose a disease which is intended to be treated. Subsequently, identification of important biomacromolecules as target molecules which are related to the disease has to be done. In the determination of target molecules, selectivity is a key issue. Undesired side effects can be removed thanks to the selectivity. After determination of the target molecule, finding a lead compound is of great importance. Lead compounds as basis for drug design can be described as chemical substances which show pharmacological activity on the target molecules [31].

1.8.2. Drug Design

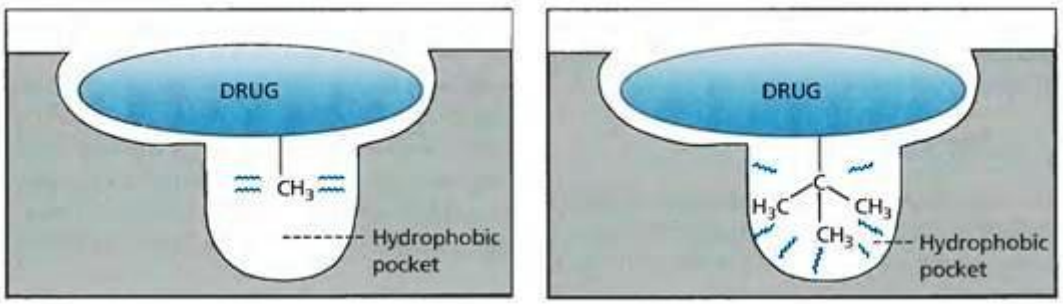
It is extremely essential for a drug molecule to be complementary to its bio-molecular target regarding the shape and charge. Otherwise, there is a danger of poor selectivity, low level of activity and undesired side effects [31]. For that reason, drug design has to be a repetitive process and new compounds have to be derivatized from the target molecules to find the most complementary target-drug couple [32].

1.8.3. Drug Optimization

Subsequent to discovering a lead compound, optimization studies on the structure of the lead compound have to be done in order to obtain higher efficiency. In fact, a lead compound should fit the target bio-macromolecule very tightly. At this point, investigating the functional groups and the type of intermolecular forces that target molecule and lead compound have gain importance to understand the drug-enzyme interactions [31].

Previous studies have stated that many functional groups on the structure such as amines, alcohols, ketones, aldehydes, amides, alkyl chains can either increase or decrease the interaction between the lead compound and target enzyme. As it is given in the Figure 1.5, changing the alkyl chain results in better filling of the hydrophobic pocket of the

enzyme because of van der Waals interactions and increases the enzyme-drug interaction [31].



.

Figure 1.5. Addition of alkyl chain to fill a hydrophobic pocket.

Furthermore, modification of lead compound can also increase the efficiency of drug molecule. For instance, statins are a class of drugs which have been discovered to reduce cholesterol levels. Statin derivatives are given in Figure 1.6 [31].

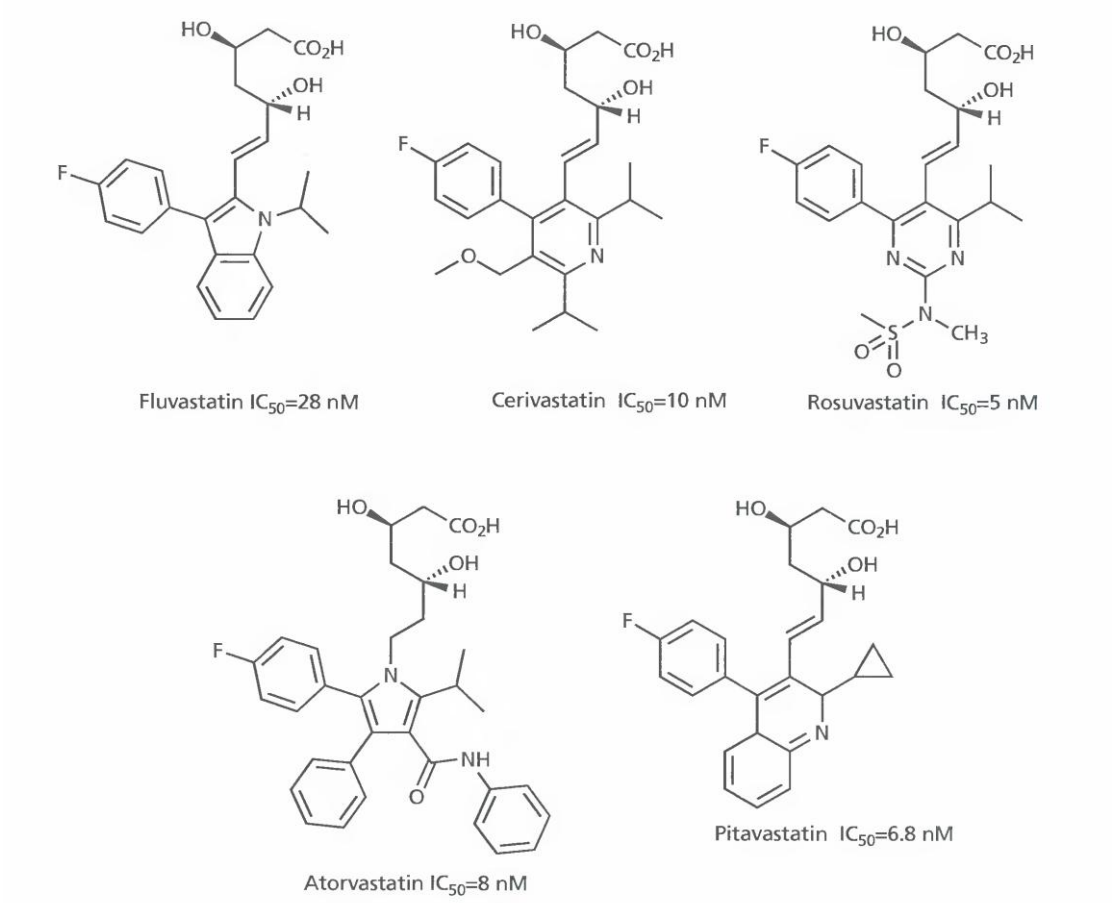


Figure 1.6. Development of enalaprilate.

Studies have indicated that statin derivatives which have a lower hydrophobic nature are more selective for the liver cells where the largest part of cholesterol synthesis takes place. Additionally, statins with lower hydrophobic character have fewer side effects. Among the statins the most efficient one is rosuvastatin. Sulfonamide group of rosuvastatin reduces the hydrophobic character of the compound and results in enhanced binding interactions [31].

Basically there are two drug design approaches. These approaches are ligand based drug design and structure based drug design [32]. In this project, structure based drug design is used.

1.8.4. Structure based drug design

Structure-based drug design (SBDD) is an effective procedure, specifically if it is used as a tool in an armamentarium for determining new drug leads against considerable targets [33].

The most important step in SBDD for a particular disease is the determination of lead compounds for a biomolecular target. The discovery procedure begins with the determination of the 3-D structure of the target enzyme by X-ray crystallography, NMR, or homology modeling. Next, by using computational methods, primary drug candidates are found. Virtual screening that is using docking analysis of the drug candidates on the active or regulatory site of the target protein is one of the most commonly used methods. Virtual screening supplies a result which is based on the steric and electrostatic interactions between the drug candidates and the target enzyme. Subsequently, drug candidates that give comparatively high binding affinity are tested with *in vitro* and *in vivo* analyses. Even if the compounds have low binding energies in accordance with virtual screening, these compounds can give significantly high IC_{50} values in experimental tests. Thus, it is crucial to examine the relationship between binding energies and experimentally tested activities of drug candidates [34].

Additionally, although a drug candidate with high binding affinity can be found, there are many more factors to be considered such as bioavailability; lack of toxicity,

carcinogenicity and teratogenicity of the compound and its metabolites; and overall on shelf and *in vivo* stabilities before clinical trials [35].

1.9. Computational Studies

1.9.1. Computational Modeling of CYP-7

In order to examine the catalytic activities, substrate and reaction selectivity of an enzyme, it is highly necessary to know its structure. If not, it is impossible to design a specific drug to inhibit the target enzyme. Accordingly, determination of the structure of CYP17 is required in this project. A computer model of CYP17 enzyme with PDB ID code 2c17 is shown in Figure 1.7. This model of CYP17 is based on class II P450 crystal structure, namely P450BMP. As a common characteristic of all P450 enzymes, CYP17 enzyme contains a heme group and a hydrophobic domain [36].

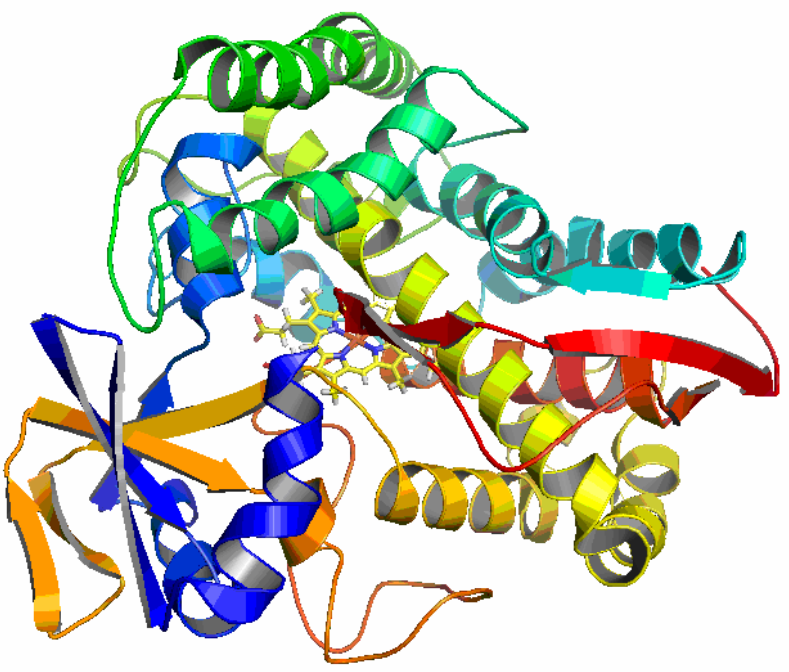


Figure 1.7. Computer generated model of CYP17.

Computational modeling of CYP17 is carried out at Koc University by Prof. Metin Türkay. The computer models of CYP17 propose good prediction of IC_{50} values and a favorable preliminary model for further studies [37].

1.9.2. CYP17 Inhibitors

In a reaction which is catalyzed by an enzyme, enzyme supplies a particular field which is the active site of the enzyme to bind to a substrate. At the same time an inhibitor binds to the active site of the enzyme reversibly, the natural substrate cannot move toward the active site of the enzyme and the catalytic reaction stops as long as the inhibitor stays there. This case is called as competitive inhibition. Substrate and inhibitor compete for binding to the active site of the enzyme.

CYP17, CYP19 and four other enzymes (CYP11A1, CYP11B1, CYP11B2, CYP21) that have duty on steroid hormone production are P450 enzymes. As stated earlier, they include a heme part as a form of a porphyrin ring with a central iron ion (Figure 1.8) [27]. The duty of the iron is to activate molecular oxygen which is necessary for the following reaction of the substrate. As a result, it is expected from a CYP17 inhibitor to make complex with heme iron [27].

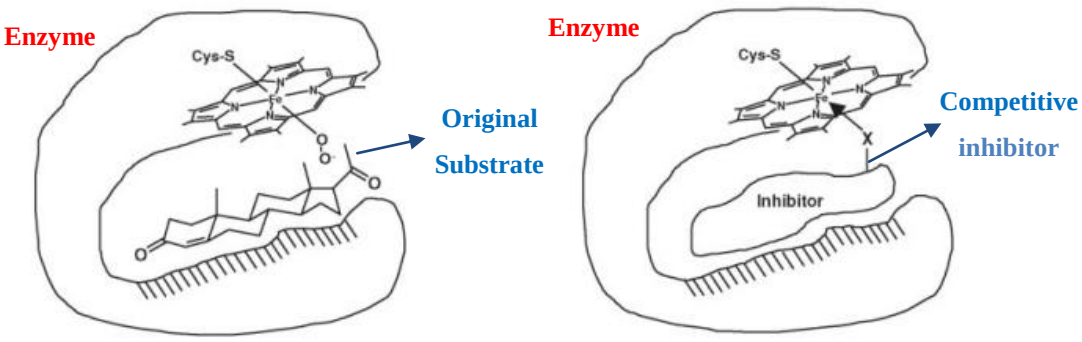


Figure 1.8. Progesterone vs Inhibitor binding to the active site of CYP17.

1.9.3. Alternatives of CYP17 Inhibitors

CYP17 inhibitors can be divided as steroidal and non-steroidal inhibitors. Figure 1.9 [38] shows some of the steroidal and non-steroidal inhibitors of CYP17.

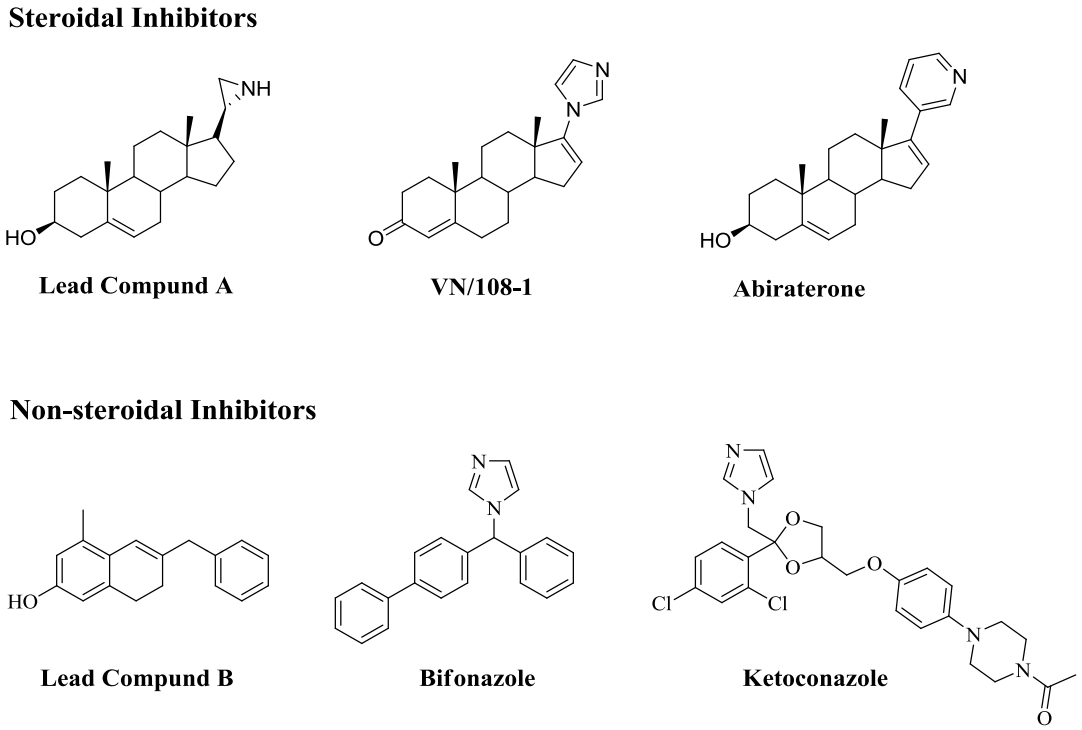


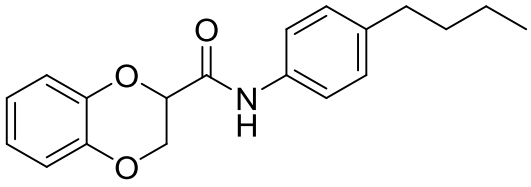
Figure 1.9. Literature examples of CYP17 inhibitors.

Even though there are steroidal compounds which have quite high activity, it is necessary to change steroidal drugs by non-steroidal drugs because of the fact that steroidal compounds cause side effects due to interactions with steroid receptors [39].

Although among the non-steroidal compounds ketoconazole which is actually discovered as an antimycotic drug is a potent inhibitor [40], it has dose-limiting toxicity [41]. Hence it is not used for the treatment of PC anymore [40].

Since non-steroidal compounds have advantages to steroidal compounds, synthesis of non-steroidal compounds is targeted in this project. Thus, researchers at Koç University screened about 50000 molecules and 23 non-steroidal drug candidates were found. Among those 23 candidates a lead molecule was determined (Figure 1.10) which gave the best

results in docking and binding energy, ADMET (adsorption, distribution, metabolism, elimination and toxicity), Quantitative Structure Activity Relationship (QSAR) studies and IC_{50} measurements.



Docking Energy: -9.38kcal/mol
Binding Energy: -7.45 kcal/mol
IC_{50} : 35μM

Figure 1.10. Structure of the Lead Molecule.

1.9.4. Substituents on the Lead Molecule and Their Functions

Modification of the lead compound is necessary in order to find the best molecule with the highest activity. For this reason, possible functions of the functional groups of the lead compound have to be examined carefully. Figure 1.11 shows substituents and their predicted functions for the inhibition of the CYP17.

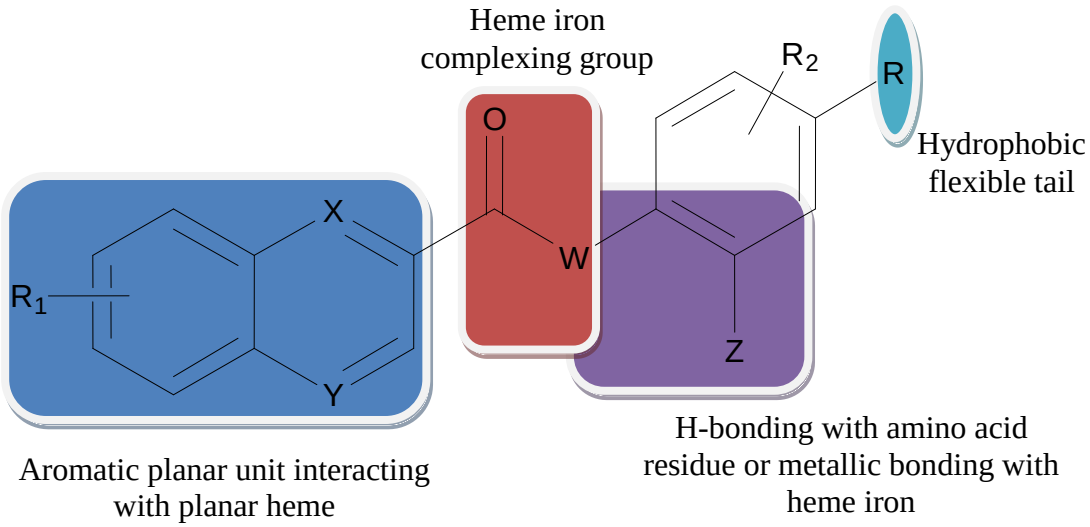


Figure 1.11. Functional groups on the lead compound and their possible functions.

Furthermore, Table 1.1 shows the possible modifications on the lead copound.

Table 1.1. Possible modifications on the lead compound.

X and Y atoms	C, O
W atom	N, O, C
Z group	-OR, -NR_2 (R= -H, Alkyl)
R	C_2 - C_6 alkyl or alkoxy, Branching alkyl
R_1	-OMe, -H, -F (at different positions)

1.9.5. Docking and Binding Energy Studies

Binding and docking energies are two important terms in energy studies. Binding energy is the strength of the interaction between enzyme and candidate drug molecule. Docking energy is the energy related to the approach of candidate drug molecule to enzyme. Docking and binding energy calculations were carried out at Koç University by using AutoDock program. Table 1.2 and Table 1.3 show the docking and binding energies of the lead compound and derivatives of the lead compound.

Table 1.2. Docking and Binding Energies of Lead Compound.

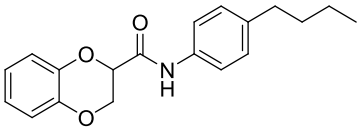
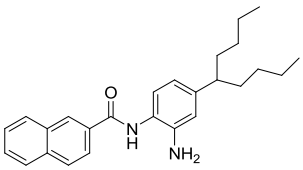
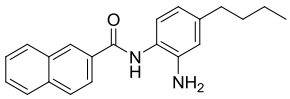
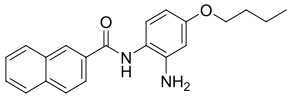
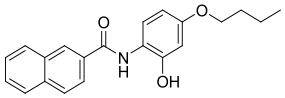
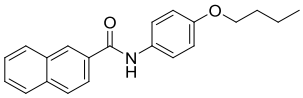
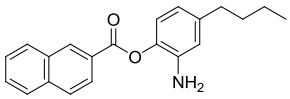
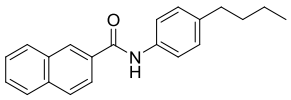
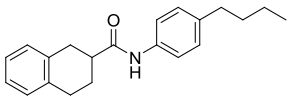
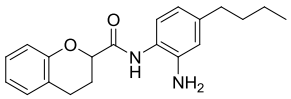
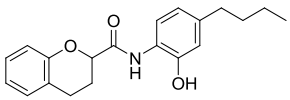
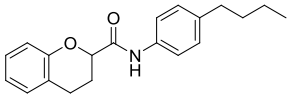
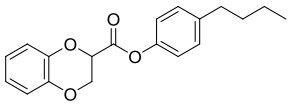
Lead Compound	**Docking Energy (D.E.) (kcal/mol)**	**Binding Energy (B.E.) (kcal/mol)**
	**-9.38**	**-7.45**

Table 1.3. Docking and Binding Energies of some Lead Compound Derivatives.

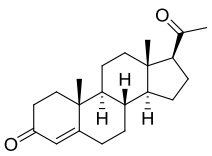
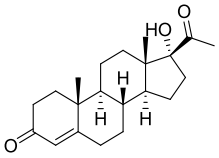
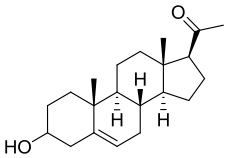
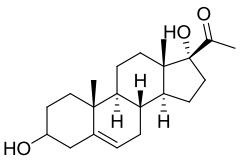
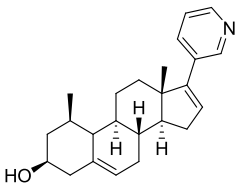
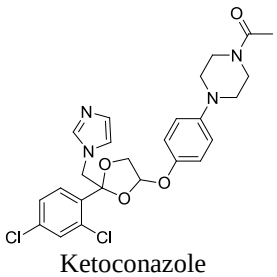
Compounds	D.E. (kcal/ mol)	B.E. (kcal/ mol)	Compounds	D.E. (kcal/ mol)	B.E. (kcal/ mol)
	-11.85	-9.44		-10.56	-8.47
	-10.66	-8.39		-10.38	-8.10
	-9.85	-7.63		-10.14	-8.26
	-9.89	-7.77		-9.50	-7.85
	-9.31	-7.33		-9.23	-7.30
	-9.43	-7.61		-8.54	-6.67

From Table 1.2 and Table 1.3, it can be concluded that some lead compound derivatives have better docking and binding energy values than the lead compound.

1.9.6. Docking and Binding Energy Calculations of Natural Substrates and Current Drug Molecules

With the purpose of evaluating the docking and binding energies of the lead compound derivatives, energy calculations of the natural substrates and current drug molecules for prostate cancer were done, as well (Table1.4).

Table 1.4. Docking and Binding Energies of Natural Substrates and Current Drugs.

Compounds	**Docking** **(kcal/mol)**	**Binding** **(kcal/mol)**	**Compounds**	**Docking** **(kcal/mol)**	**Binding** **(kcal/mol)**
 Progesterone	-10.04	-9.73	 17hydroxyprogesterone	-9.61	-9.68
 Pregnenolone	-9.71	-9.44	 17hydroxypregnenolone	-10.15	-10.10
 Abiraterone	-9.30	-9.33	 Ketoconazole	-11.13	-9.33

2. AIM OF THE STUDY

The lead compound can be examined in two parts, the right hand side and the left hand side. Figure 2.1 shows the parts of the lead compound.

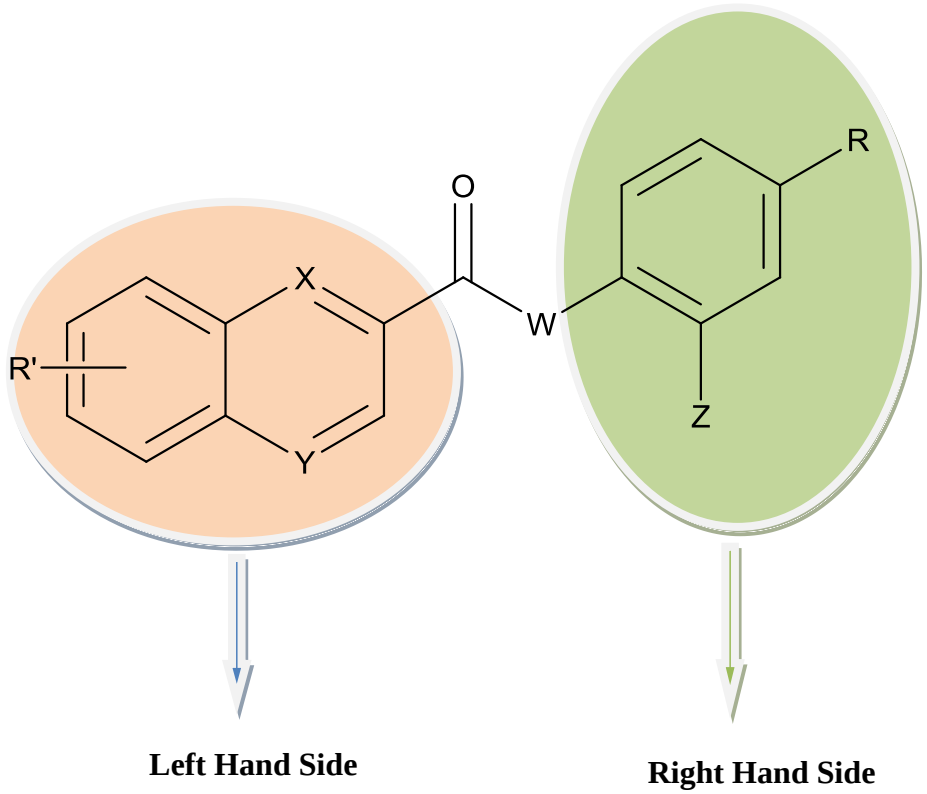
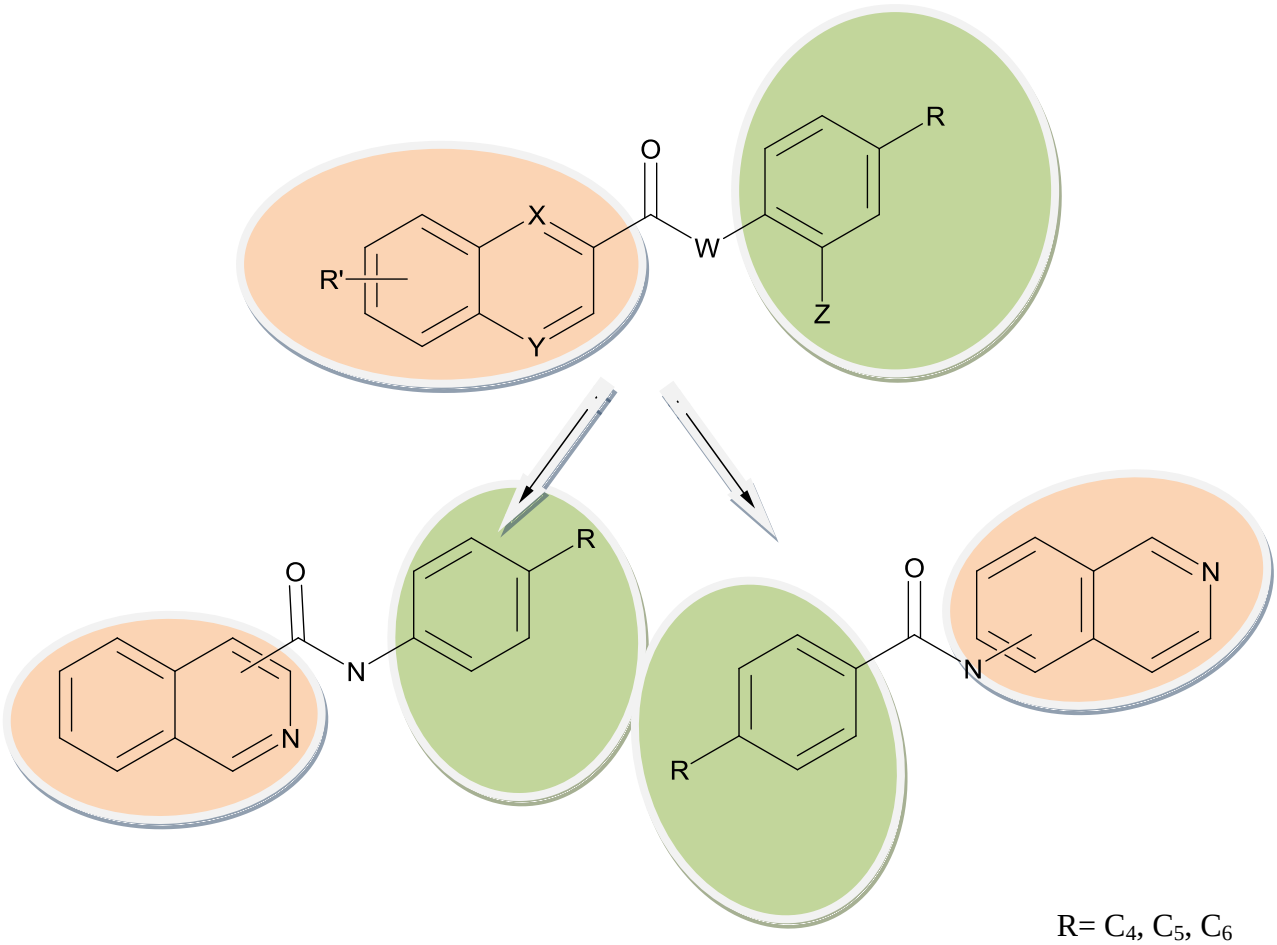


Figure 2.1. Parts of the Lead Compound.

In the first part of the project, X and Y atoms were chosen as C atoms and R` group was chosen as H atom. However, the lead compound was altered and instead of napthoic acid derivatives isoquinoline derivatives were used at the left hand side. It is thought that N atom of isoquinoline can generate a complex with heme group of CYP17. Heme iron complexing group located in the middle part of the compound was chosen as the amide group which is a coupling product of carboxylic acid and amine. Both aminoisoquinolines and isoquinolinecarboxylic acids were used at the left hand side. For the right hand side of the lead molecule Z was determined as H atom and R as C_4, C_5, C_6 alkyl groups. Figure 2.2 summarizes the lead molecule optimizations of the first part.



R= C_4, C_5, C_6

Figure 2.2. Lead Molecule Optimization of the First Part.

In the second part, optimization was done on another compound which is synthesized by our research group and has an IC_{50} value of 9.1 µM. This compound (Figure 2.3), namely AK74, has a better IC_{50} value than that of the lead compound. Consequently, derivatives of AK74 were targeted.

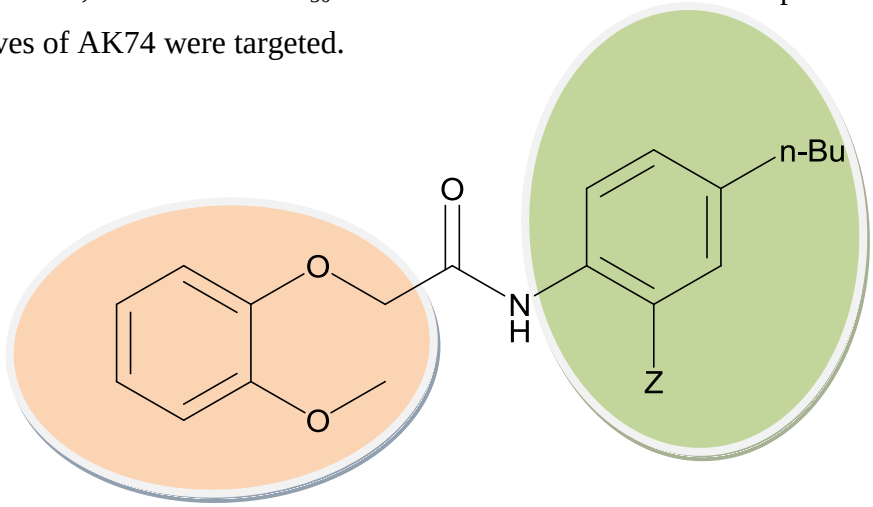
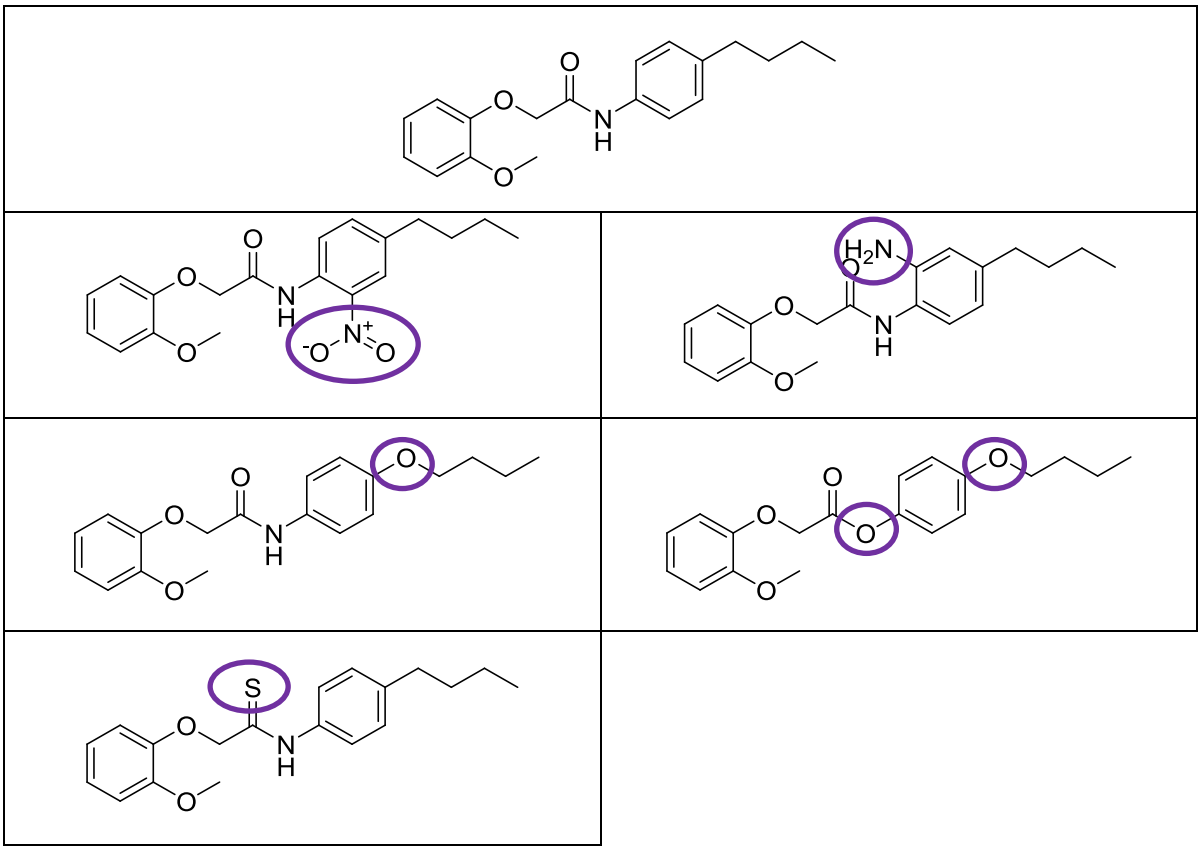


Figure 2.3. Structure of AK74.

Table 2.1 demonstrates all the modifications that have been done on AK74.

Table 2.1. Optimization of AK74.



In addition, before the synthesis of these targeted compounds, their docking and binding energies were calculated and it was found that almost all of them had better values compared to the lead molecule (Table 2.2, Table 2.3).

Table 2.2. Docking and Binding Energy of the Lead Molecule.

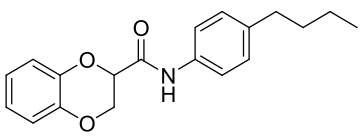
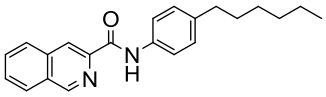
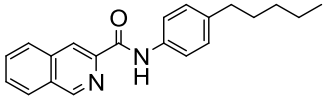
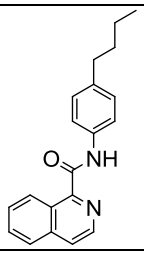
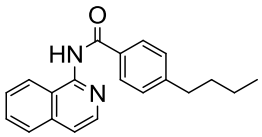
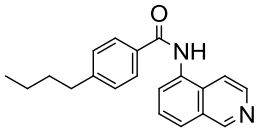
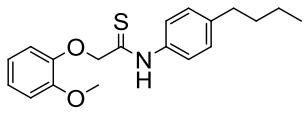
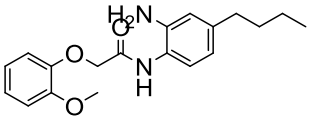
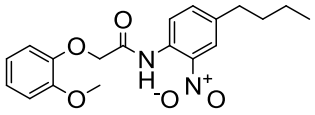
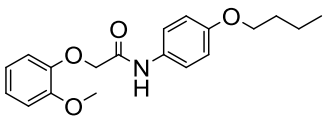
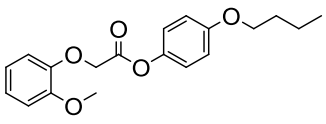
Lead Compound	**Docking Energy (D.E.) (kcal/mol)**	**Binding Energy (B.E.) (kcal/mol)**
	**-9.38**	**-7.45**

Table 2.3. Docking and Binding Energy Results of Targeted Molecules.

Final Product	D.E (kcal/ mol)	B.E (kcal/ mol)	Final Product	D.E (kcal/ mol)	B.E (kcal/ mol)
	-10.54	-7.89		-11.27	-8.94
	-9.34	-7.20		-10.55	-8.67
	-11.21	-9.26		-12.43	-9.09
	-11.30	-7.91		-10.22	-6.94
	-11.36	-8.02		-11.55	-7.87

3. RESULTS AND DISCUSSION

In this project, synthesis of isoquinoline and pyrocatechol derivatives were targeted in order to understand the effect of N bearing heterocycle and changing the functional groups of AK74.

In the first part, isoquinoline derivatives were obtained by coupling reactions of aminoisoquinolines with carboxylic acids and isoquinolinecarboylic acids with amines. All of the starting materials were commercially available.

The common approach followed in the second part was as follows; 2-(2-methoxyphenoxy)acetic acid which is a pyrocatechol derivative was used as the left hand side of the molecule, and it was coupled with different phenyl derivatives to obtain final products. Coupling reactions were done with commercially available phenyl derivatives. On the other hand, two of the phenyl derivatives were not commercially available. That's why these phenyl derivatives were synthesized through multistep reactions. Only in the synthesis of one final product in which the amide was converted to thioamide, there was one more step after the coupling reaction. The last step was converting the carbonyl group to the thionyl group by using Lawesson's reagent.

To confirm these reactions ^{1}H-NMR Spectroscopy, ^{13}C-NMR Spectroscopy were used.

3.1. Synthetic Approaches for Isoquinoline Derivatives

Synthesis of isoquinoline derivatives was carried out by *in-situ* coupling reactions. The first step of this reaction was bromination of carboxylic acid using PBr_3 in dry dichloromethane (DCM) and under nitrogen to convert carboxylic acids to the corresponding acid bromides. Three hours later, triethylamine (TEA) was added to the reation mixture to neutralize HBr which liberates during the reaction and finally amine was added. The reaction mixture was stirred at room temperature for 1 hour. Figure 3.1 shows the synthetic approach used to synthesize all isoquinoline derivatives.

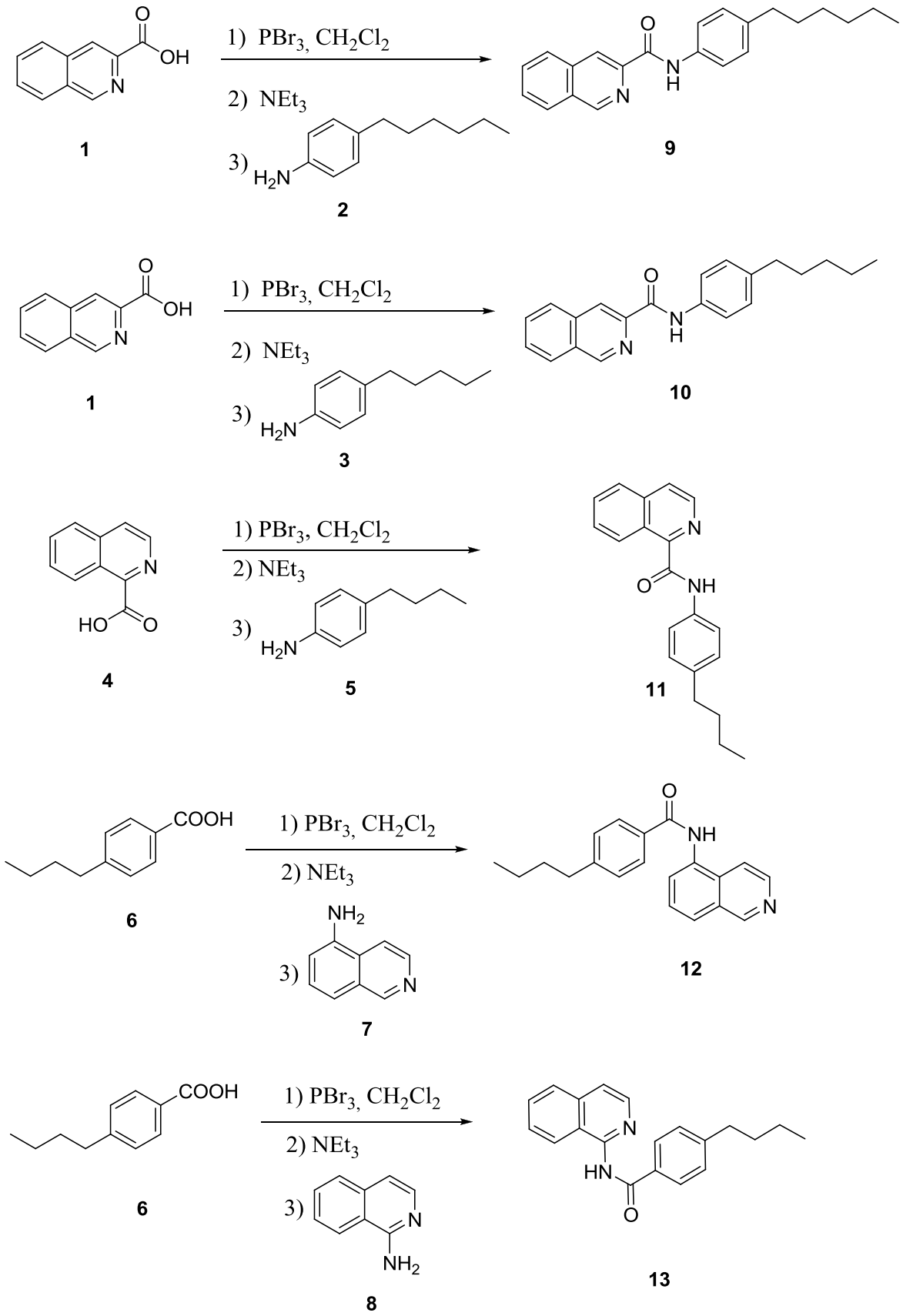


Figure 3.1. The synthetic approach to synthesize isoquinoline derivatives.

By synthesizing these compounds, it can be possible to see the effect of changing the coupling position of isoquinolines. The effect of alkyl chain length can also be observed by comparing the compounds 9 and 10.

3.2. Synthesis of the Lead Compound Derivatives from Alkyl, Nitro and Amino Substituted Phenyl Derivatives

For the synthesis of alkyl, nitro and amino substituted phenyl derivatives, 4-butyl aniline (compound 5) was used as the starting material. The first step is the amine protection by using acetic anhydride in water. After the protection reaction, the resulting compound (compound 14) was subjected to nitration reaction by using nitric acid and sulfuric acid. Once nitration reaction was completed, there was no more need for amine protection. Therefore, the next step was deprotection of amine group by using 20% sulfuric acid in order to obtain compound 16. Subsequently, coupling reaction of compound 16 with 2-(2-methoxyphenoxy)acetic acid (compound 17) was carried out by using PBr_3 which is the same approach with the one used in the synthesis of isoquinolines. Compound 18 which is the product of the coupling reaction was synthesized as a final product. However, another final product which is still worked on was also targeted from compound 18 with reduction of nitro group to amine group by using 57% hydroiodic acid (HI). Synthesis of compounds 18 and 19 are shown in Figure 3.2.

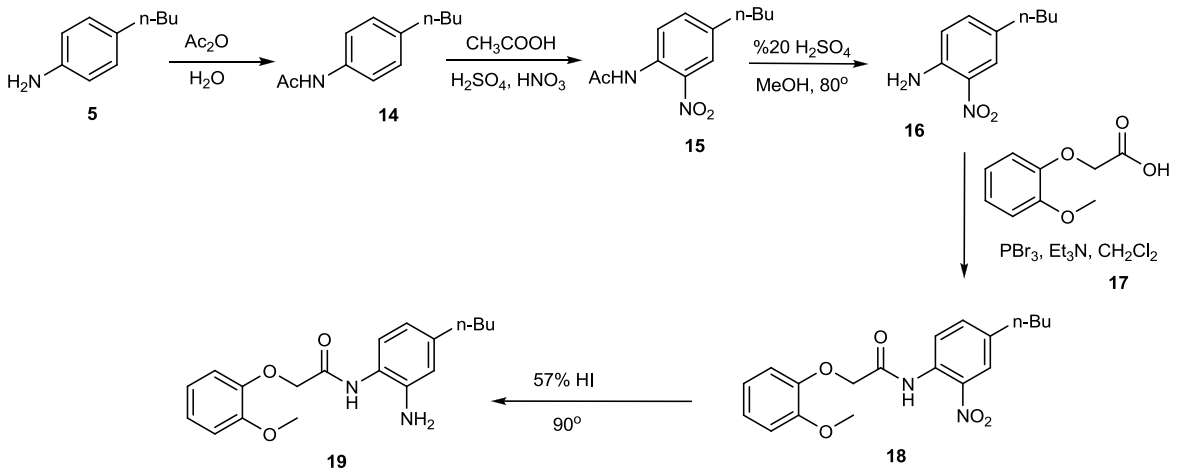


Figure 3.2. Synthesis of compounds 18 and 19.

Compound 18 and compound 19 are useful to examine the effect of an additional nitro or amine group on phenyl ring.

3.3. Synthesis of the Lead Compound Derivatives from Alkoxy and Hydroxy Substituted Phenyl Derivativesa

Coupling reaction of 2-(2-methoxyphenoxy)acetic acid (compound 17) and 4-butoxyphenol was done with a different approach from the one with PBr_3. This type of coupling was carried out by using N,N'-dicyclohexylcarbodiimide (DCC) and N,N'-dimethylaminopyridine (DMAP). As a result, compound 21 was synthesized. Figure 3.3 demonstrates the synthesis of 21.

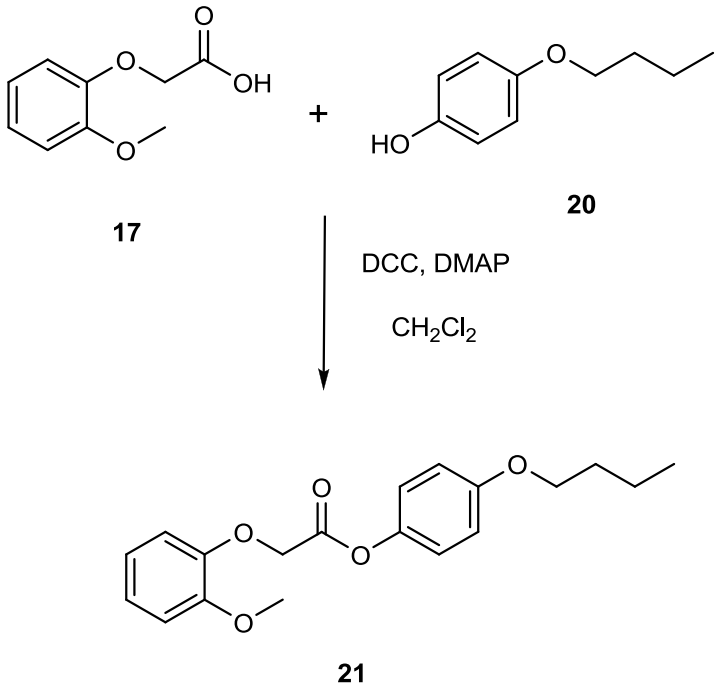


Figure 3.3. Coupling reaction of 2-(2-methoxyphenoxy)acetic acid (compound 17) and 4-butoxyphenol.

From the testing of the compound 21, the effects of both the alkoxy group instead of alkyl group, and ester moiety instead of amide can be understood.

3.4. Synthesis of the Lead Compound Derivatives by Converting Amides to Thioamides

Before converting amide to thioamide, coupling reaction of compounds 5 and 17 with PBr_3 was done in order to obtain the amide, 22. Afterward, by refluxing the mixture of compound 22 and Lawesson's reagent in toluene, compound 23 was obtained. Figure 3.4 illustrates the synthesis of 23.

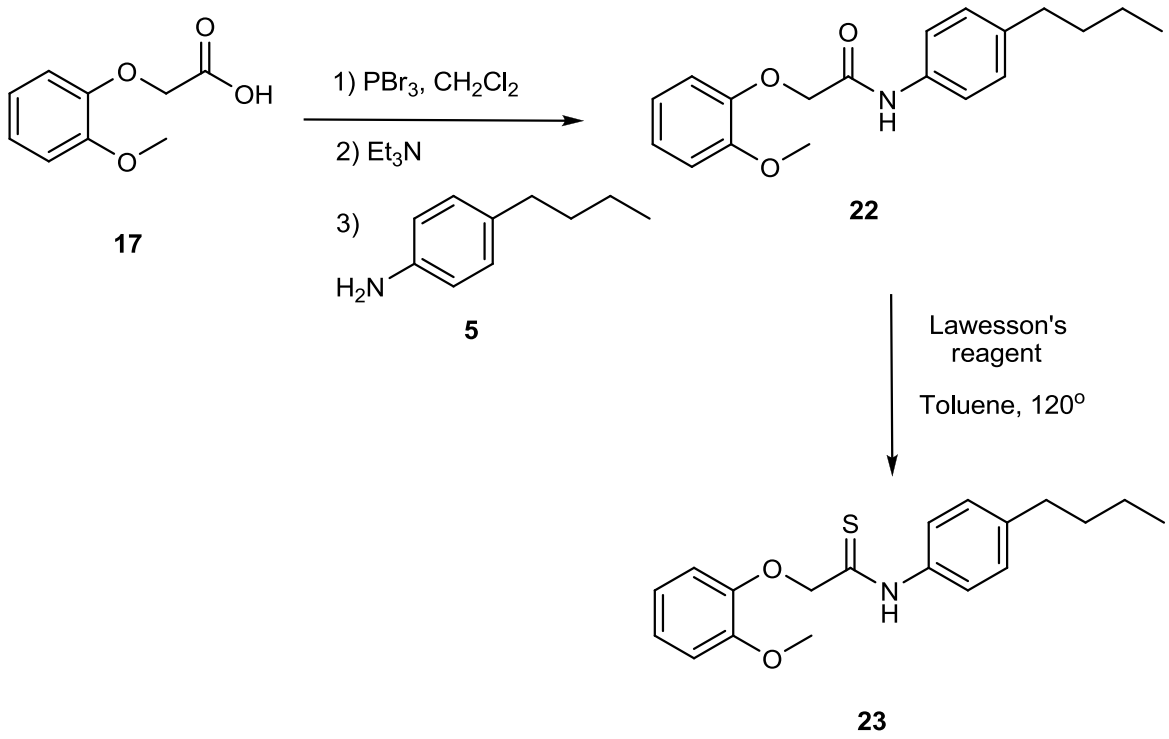


Figure 3.4. Synthesis of Thioamides.

This compound can give an idea about the effect of changing O atom of the carbonyl group to S atom.

3.5. Synthesis of the Lead Compound Derivatives from Alkoxy and Amino Substituted Phenyl Derivatives

Synthesis of the compound 25 was accomplished with the coupling procedure in which PBr_3 was used. Figure 3.5 shows the synthesis.

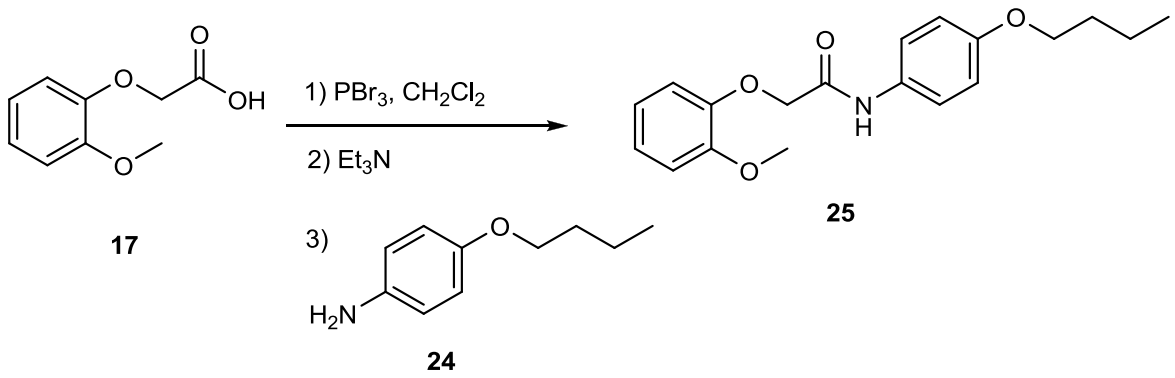


Figure 3.5. Coupling reaction of 2-(2-methoxyphenoxy)acetic acid and 4-butoxyaniline.

Compound 25 can be a good example to compare the effectiveness of compounds with an alkoxy chain and compounds with an alkyl chain.

4. EXPERIMENTAL

4.1. Methods and Materials

All chemicals were used as received from the manufacturer (Merck, Aldrich, Alfa Aesar, and Riedel de Haen). Dry solvents (CH_2Cl_2, THF and Toluene) were obtained from ScimatCo Purification System; other solvents were dried with molecular sieves. Column chromatography was performed using silicagel-60 (43-60 nm). Thin layer chromatography was performed using silica gel plates (Kiesel gel 60 F254, 0,2mm, Merck) and aluminum oxide plates.

4.2. Instrumentation

Thin layer chromatography (TLC) plates were viewed under 254 nm UV lamp. ^{1}H-NMR, ^{13}C-NMR spectra were recorded by using a Varian Gemini 400 MHz spectrometer Varian Associates, Palo Alto, CA) in $CDCl_3$ as solvent at the Advanced Technologies Research and Development Center at Bogazici University.

4.3. Synthesis of Lead Compound Derivatives

4.3.1. Synthesis of N-(4-hexylphenyl)isoquinoline-3-carboxamide (9)

The coupling reaction was done according to the literature procedure [42]. The experiment was done under nitrogen by using dry CH_2Cl_2. Isoquinoline-3-carboxylic acid (1) (48.84 mg, 0.28 mmol) was dissolved in 3 mL dry CH_2Cl_2 under N_2. To this solution, PBr_3 (0.05 mL, 0.56 mmol) was added at 0°C and the reaction mixture was mixed for 3 hours at room temperature. After 3 hours, triethyl amine (0.12 mL, 0.85 mmol) was added and at that time fuming was observed because of the neutralization of HBr. 4-hexylaniline (2) (50mg, 0.28 mmol) was added to the reaction mixture. Reaction mixture was stirred over-night. To this mixture, 10 mL CH_2Cl_2 was added and it was extracted with H_2O. When the water was added, fume occurred. The organic layer was dried over Na_2SO_4,

filtered and concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel and hexane/ CH_2Cl_2 (1:2) as the eluent phase (39% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.87 (t, 3H, CH_2CH_3), 1.24 (m, 4H, CH_2CH_2), 1.30 (m, 2H, CH_2CH_2), 1.58 (m, 2H, CH_2CH_2), 2.59 (t, 2H, $ArCH_2$), 7.20 (d, 2H, Ar*H*), 7.72 (m, 3H, Ar*H*), 7.79 (m, 1H, Ar*H*), 8.05 (m, 2H, Ar*H*), 8.71 (s, 1H, Ar*H*), 9.22 (s, 1H, Ar*H*), 10.19 (s, 1H, N*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 13.09 (CH_3), 21.34 (CH_2), 27.93 (CH_2), 28.68 (CH_2), 30.77 (CH_2), 34.38 (CH_2), 118.71 (2C, Ar*C*), 119.60 (Ar*C*), 126.24 (Ar*C*), 126.42 (Ar*C*), 126.74 (Ar*C*), 127.21 (Ar*C*), 127.93 (2C, Ar*C*), 128.73 (Ar*C*), 130.16 (Ar*C*), 134.59 (Ar*C*), 135.13 (Ar*C*), 138.02 (Ar*C*), 149.94 (Ar*C*), 168.87 (*C*=O) ppm.

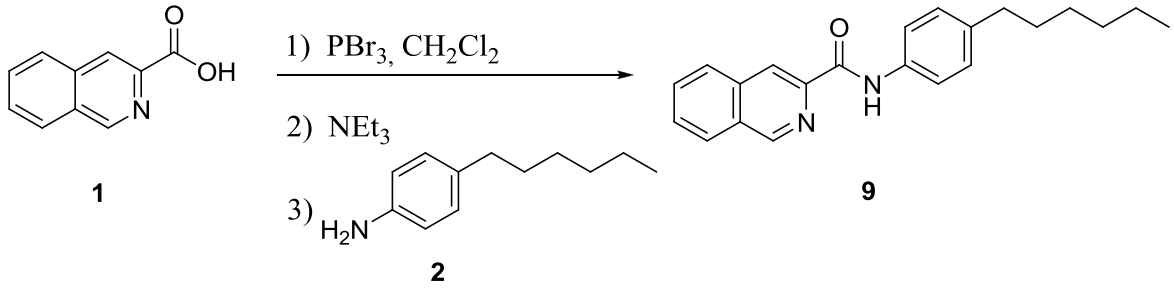


Figure 4.1. Synthesis of N-(4-hexylphenyl)isoquinoline-3-carboxamide (9).

4.3.2. Synthesis of N-(4-pentylphenyl)isoquinoline-3-carboxamide (10)

The coupling reaction was done according to the literature procedure [42]. The experiment was done under nitrogen by using dry CH_2Cl_2. Isoquinoline-3-carboxylic acid (1) (100 mg, 0.577 mmol) was dissolved in 3 mL dry CH_2Cl_2 under N_2. To this solution, PBr_3 (0.11 mL, 1.15 mmol) was added at 0°C and the reaction mixture was mixed for 3 hours at room temperature. After 3 hours, triethyl amine (0.24 mL, 1.73 mmol) was added and at that time fuming was observed because of the neutralization of HBr. 4-Pentylaniline (3) (94.27 mg, 0.577 mmol) was added to the reaction mixture. Reaction mixture was stirred over-night. To this mixture, 10 mL CH_2Cl_2 was added and it was extracted with H_2O. When the water was added, fume occurred. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel and hexane/ CH_2Cl_2 (1:2) as the eluent phase (46% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.90 (t, 3H, CH_2CH_3), 1.28 (m, 4H, CH_2CH_2), 1.63 (m, 2H, CH_2CH_2), 2.61 (t, 2H, $ArCH_2$), 7.22 (d, 2H, Ar*H*), 7.74 (m, 4H, Ar*H*), 8.05

(m, 2H, Ar*H*), 8.72 (s, 1H, Ar*H*), 9.22 (s, 1H, Ar*H*), 10.2 (s, 1H,Ar*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 13.82 (*C*H_3), 22.68 (*C*H_2), 29.21 (*C*H_2), 31.21 (*C*H_2), 34.80 (*C*H_2), 119.73 (2C, Ar*C*), 120.62 (Ar*C*), 127.38 (Ar*C*), 127.72 (2C, Ar*C*), 128.20 (Ar*C*), 128.98 (Ar*C*), 129.01 (Ar*C*), 129.74 (Ar*C*), 130.87 (Ar*C*), 131.22 (Ar*C*), 135.58 (Ar*C*), 138.88 (Ar*C*), 151.03 (Ar*C*), 170.45 (*C*=O) ppm.

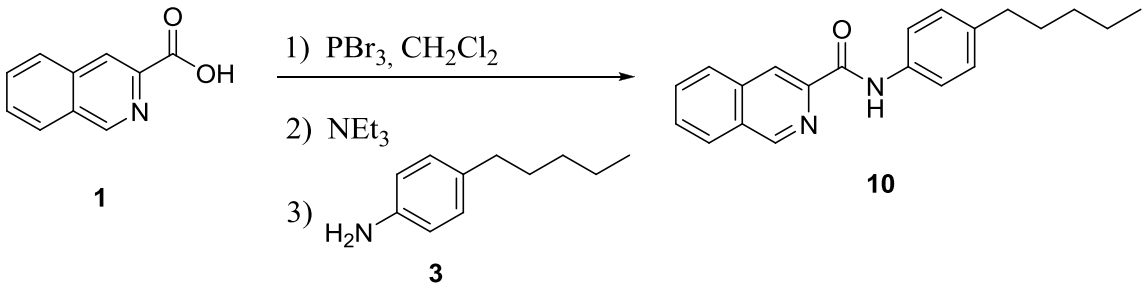


Figure 4.2. Synthesis of N-(4-pentylphenyl)isoquinoline-3-carboxamide (10).

4.3.3. Synthesis of N-(4-butylphenyl)isoquinoline-1-carboxamide (11)

The coupling reaction was done according to the literature procedure [42]. The experiment was done under nitrogen by using dry CH_2Cl_2. Isoquinoline-1-carboxylic acid (4) (100 mg, 0.577 mmol) was dissolved in 3 mL dry CH_2Cl_2 under N_2. To this solution, PBr_3 (0.11 mL, 1.15 mmol) was added at 0°C and the reaction mixture was mixed for 3 hours at room temperature. After 3 hours, triethyl amine (0.24 mL, 1.73 mmol) was added and at that time fuming was observed because of the neutralization of HBr. 4-butylaniline (5) (86.17 mg, 0.577 mmol) was added to the reaction mixture. Reaction mixture was stirred over-night. To this mixture, 10 mL CH_2Cl_2 was added and it was extracted with H_2O. When the water was added, fume occurred. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel and hexane/ CH_2Cl_2 (1:1) as the eluent phase (43% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.95 (t, 3H, CH_2CH_3), 1.38 (m, 2H, CH_2CH_2), 1.62 (m, 2H, CH_2CH_2), 2.63 (t, 2H, $ArCH_2$), 7.22 (d, 2H, Ar*H*), 7.73 (d, 4H, Ar*H*), 7.86 (m, 2H, Ar*H*), 8.52 (d, 1H, Ar*H*), 9.53 (d, 1H, Ar*H*), 10.29 (s, 1H, N*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 14.00 (*C*H_3), 22.34 (*C*H_2), 33.72 (*C*H_2), 35.16 (*C*H_2), 119.86 (Ar*C*), 124.80 (Ar*C*), 126.93 (Ar*C*), 127.28 (Ar*C*), 127.87 (Ar*C*), 128.87 (2C, Ar*C*), 128.95 (2C, Ar*C*),

130.62 (Ar*C*), 135.60 (Ar*C*), 137.62 (Ar*C*), 139.01 (Ar*C*), 139.92 (Ar*C*), 147.67 (Ar*C*), 163.56 (*C*=O) ppm.

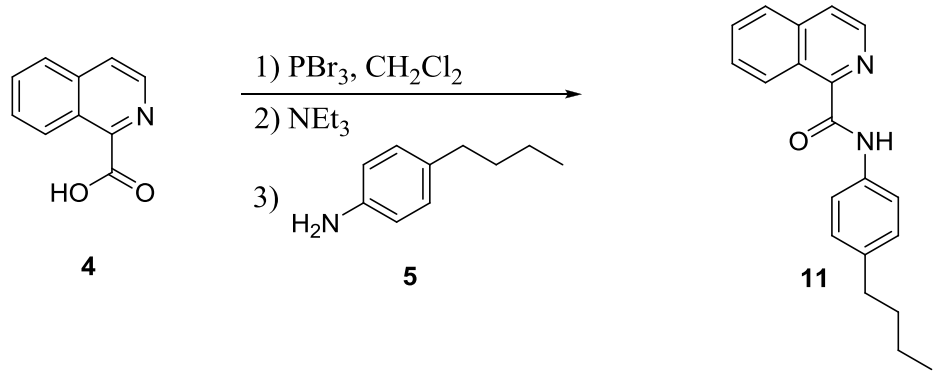


Figure 4.3. Synthesis of N-(4-butylphenyl)isoquinoline-1-carboxamide (11).

4.3.4. Synthesis of 4-butyl-N-(isoquinolin-5-yl)benzamide (12)

The coupling reaction was done according to the literature procedure [42]. The experiment was done under nitrogen by using dry CH_2Cl_2. 4-butylbenzoic acid (6) (98.90 mg, 0.555 mmol) was dissolved in 3 mL dry CH_2Cl_2 under N_2. To this solution, PBr_3 (0.10 mL, 1.11 mmol) was added at 0°C and the reaction mixture was mixed for 3 hours at room temperature. After 3 hours, triethyl amine (0.23 mL, 1.66 mmol) was added and at that time fuming was observed because of the neutralization of HBr. 5-aminoisoquinoline (7) (80 mg, 0.555 mmol) was dissolved in 1 mL dry CH_2Cl_2 and it was added to the reaction mixture. Reaction mixture was stirred over-night. To this mixture, 10 mL CH_2Cl_2 was added and it was extracted with H_2O. When the water was added, fume occurred. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel and ethyl acetate/ CH_2Cl_2 (1:3) as the eluent phase (47% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.89 (t, 3H, CH_2CH_3), 1.32 (m, 2H, CH_2CH_2), 1.58 (m, 2H, CH_2CH_2), 2.63 (t, 2H, ArCH_2), 7.21 (d, 2H, Ar*H*), 7.50 (t, 1H, Ar*H*), 7.57 (d, 1H, Ar*H*), 7.72 (d, 1H, Ar*H*), 7.82 (d, 2H, Ar*H*), 8.05 (d, 1H, Ar*H*), 8.40 (d, 1H, Ar*H*), 8.48 (s, 1H, Ar*H*), 9.14 (s, 1H, N*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 12.91 (CH_3), 21.29(CH_2), 32.32 (CH_2), 34.57 (CH_2), 113.34 (Ar*C*), 124.23 (2C, Ar*C*), 124.28 (Ar*C*), 126.25 (2C, Ar*C*), 126.34 (Ar*C*), 127.88 (Ar*C*), 128.03 (Ar*C*),

129.22 (Ar*C*), 130.60 (Ar*C*), 131.02 (Ar*C*), 142.09 (Ar*C*), 146.76 (Ar*C*), 151.94 (Ar*C*), 165.45 (*C*=O) ppm.

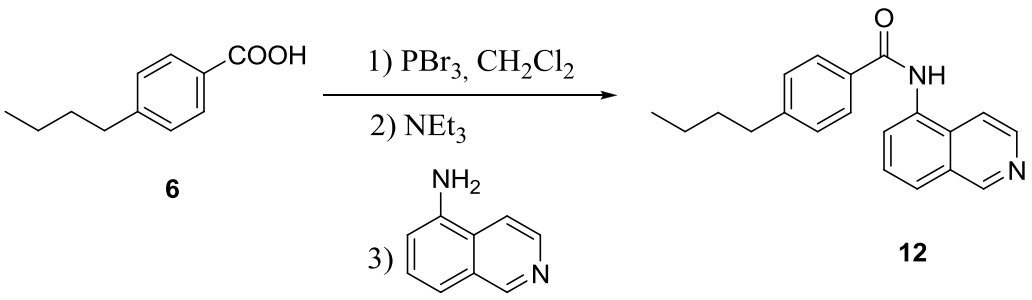


Figure 4.4. Synthesis of 4-butyl-N-(isoquinolin-5-yl)benzamide (12).

4.3.5. Synthesis of 4-butyl-N-(isoquinolin-1-yl)benzamide (13)

The coupling reaction was done according to the literature procedure [42]. The experiment was done under nitrogen by using dry CH_2Cl_2. 4-butylbenzoic acid (6) (111.26 mg, 0.624 mmol) was dissolved in 3 mL dry CH_2Cl_2 under N_2. To this solution, PBr_3 (0.12 mL, 1.25 mmol) was added at 0°C and the reaction mixture was mixed for 3 hours at room temperature. After 3 hours, triethyl amine (0.26 mL, 1.87 mmol) was added and at that time fuming was observed because of the neutralization of HBr. 1-aminoisoquinoline (8) (60 mg, 0.416 mmol) was dissolved in 1 mL dry CH_2Cl_2 and it was added to the reaction mixture. Reaction mixture was stirred over-night. To this mixture, 10 mL CH_2Cl_2 was added and it was extracted with H_2O. When the water was added, fume occurred. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel. **^{1}H NMR** ($CDCl_3$), δ: 0.85 (t, 3H, CH_2CH_3), 1.31 (m, 2H, CH_2CH_2), 1.54 (m, 2H, CH_2CH_2), 2.58 (t, 2H, $ArCH_2$), 7.16 (d, 2H, Ar*H*), 7.41 (m, 2H, Ar*H*), 8.08 (d, 2H, Ar*H*), 8.20 (d, 4H, Ar*H*), 8.93 (s, 1H, N*H*) ppm.

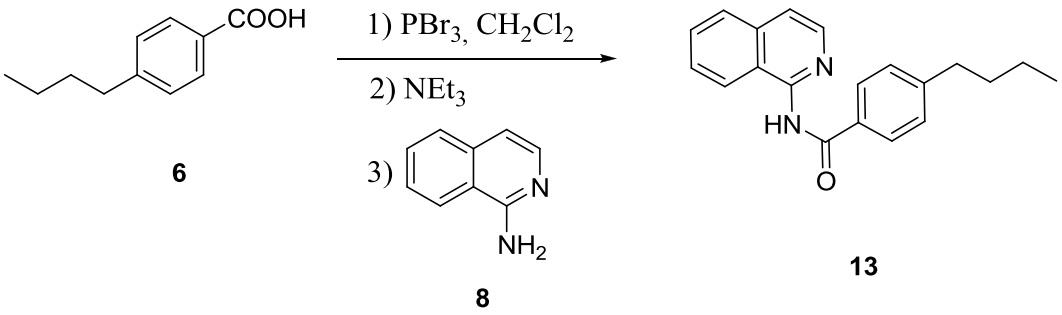


Figure 4.5. Synthesis of 4-butyl-N-(isoquinolin-1-yl)benzamide (13).

4.3.6. Synthesis of N-(4-butylphenyl)acetamide (14)

The reaction was done according to literature procedure [43]. 4-butylaniline (5) (1.5 g, 10.05 mmol) was added to 4 mL of water with vigorous stirring. Then, acetic anhydride (1.54 g, 15.07 mmol) was added to this heterogeneous solution. After stirring 2 hours at room temperature, white precipitation was observed. The reaction was monitored by using TLC silica plates and CH_2CL_2 as the eluent phase. One spot on silica gel plate was observed as the only product. The reaction was poured into stirring icy water beaker in order to precipitate all products that were not precipitated. Suction filtration was next step to remove the water and the remaining acetic anhydride (99.5% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.91 (t, 3H, CH_2CH_3), 1.33 (m, 2H, CH_2CH_2), 1.55 (m, 2H, CH_2CH_2), 2.09 (s, 3H, CH_3CO), 2.54 (t, 2H, $ArCH_2$), 7.06 (d, 2H, Ar*H*), 7.41 (d, 2H, Ar*H*), 8.67 (s, 1H, N*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 13.91 (CH_3), 22.27 (CH_2), 24.15 (CH_3CO), 33.63 (CH_2), 35.04 ($ArCH_2$), 120.44 (2C, Ar*C*), 128.65 (2C, Ar*C*), 135.82 (Ar*C*), 138.86 (Ar*C*), 169.23 (*C*=O) ppm.

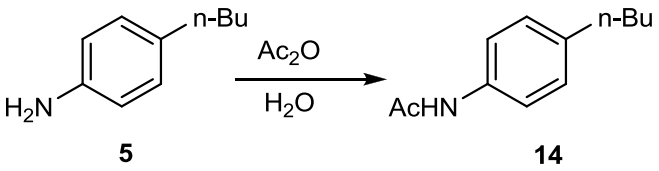
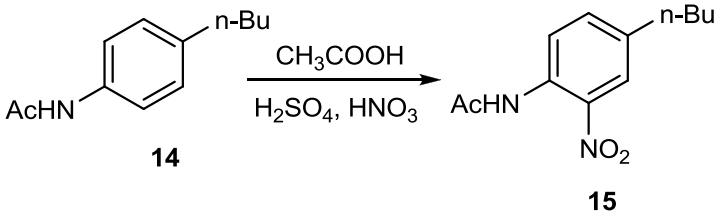


Figure 4.6. Synthesis of N-(4-butylphenyl)acetamide (14).

4.3.7. Synthesis of N-(4-butyl-2-nitrophenyl)acetamide (15)

The nitration of compound (14) was done according to the literature procedure [44]. N-(4-butylphenyl)acetamide (14) (1.73 g, 9.05 mmol) was dissolved in 2,5 mL glacial acetic acid. The solution was warmed gently in order to dissolve all the solid material, then the solution was cooled in an iced bath. 2,5 mL H_2SO_4 (96%) at 5^oC was slowly added to the solution. Then the mixture of 1 mL HNO_3 (65%) and 1 mL H_2SO_4 (96%) at 5^oC was added in small portions. After the addition was complete, the reaction mixture was stirred at room temperature for 50 minutes. Then, viscous reaction mixture was added to the mixture of 50 mL water and 10 mL ice. Solution was extracted with 3x50 mL CH_2Cl_2, dried over anhydrous Na_2SO_4, filtered and evaporated. The crude product was impure and a column was prepared by using silica gel and ethyl acetate/hexane (1:5) as the eluent phase (84% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.92 (t, 3H, CH_2CH_3), 1.35 (m, 2H, CH_2CH_2), 1.60 (m, 2H, CH_2CH_2), 2.27 (s, 3H, CH_3CO), 2.63 (t, 2H, $ArCH_2$), 7.45 (d, 1H, Ar*H*), 7.99 (d, 1H, Ar*H*), 8.62 (s, 1H, Ar*H*), 10.20 (s, 1H, N*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 13.82 (CH_3), 22.12 (CH_2), 25.52 (CH_3CO), 33.07 (CH_2), 34.53 ($ArCH_2$), 122.18 (Ar*C*), 124.83 (Ar*C*), 132.52 (Ar*C*), 136.18 (2C, Ar*C*), 138.51 (Ar*C*), 168.89 (*C*=O) ppm.



14 **15**

Figure 4.7. Synthesis of N-(4-butyl-2-nitrophenyl)acetamide (15).

4.3.8. Synthesis of 4-butyl-2-nitroaniline (16)

Deprotection was done by the hydrolysis of the amide group to amine by using 20% H_2SO_4. Compound 15 (1.08 g, 4.57 mmol) was dissolved in 24 mL of CH_3OH in a two-necked round bottom flask fitted with a reflux condenser and a magnetic stirrer. 24 mL of 20% H_2SO_4 was added drop by drop to the reaction flask and refluxed at 80^oC for 2 hours. The reaction was monitored by using TLC silica gel plates and CH_2CI_2 as the eluent phase. At the end of the 2 hours, the reaction mixture was cooled to RT and made weakly alkaline

by slowly adding 5% aqueous solution of $NaHCO_3$. The resultant solution was extracted with DCM; washed with water, dried with Na_2SO_4 by leaving overnight. The product was pure so no purification process was done (97.4% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.92 (t, 3H, CH_2CH_3), 1.34 (m, 2H, CH_2CH_2), 1.57 (m, 2H, CH_2CH_2), 2.53 (t, 2H, $ArCH_2$), 5.95 (s, 2H, NH_2), 6.83 (d, 1H, Ar*H*), 7.27 (d, 1H, Ar*H*), 7.95 (s, 1H, Ar*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 13.86 (CH_3), 22.12 (CH_2), 33.26 (CH_2), 34.13 ($ArCH_2$), 113.78 (Ar*C*), 118.70 (Ar*C*), 124.74 (Ar*C*), 125.84 (Ar*C*), 137.15 (Ar*C*), 141.70 (Ar*C*) ppm.

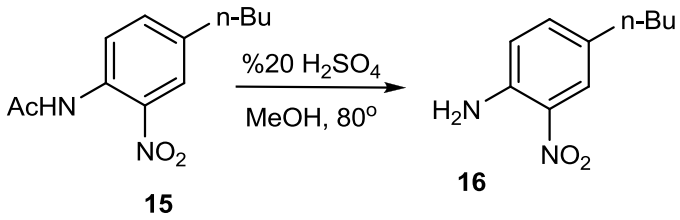


Figure 4.8. Synthesis of 4-butyl-2-nitroaniline (16).

4.3.9. Synthesis of N-(4-butyl-2-nitrophenyl)-2-(2-methoxyphenoxy)acetamide (18)

The coupling reaction was done according to the literature procedure [42]. The experiment was done under nitrogen by using dry CH_2Cl_2. 2-(2-methoxyphenoxy)acetic acid (17) (302.50 mg, 1.65 mmol) was dissolved in 3 mL dry CH_2Cl_2 under N_2. To this solution, PBr_3 (0.32 mL, 3.30 mmol) was added at 0°C and the reaction mixture was mixed for 3 hours at room temperature. After 3 hours, triethyl amine (0.70 mL, 4.95mmol) was added and at that time fuming was observed because of the neutralization of HBr. Compound 16 (215 mg, 1.10 mmol) was dissolved in 1 mL dry CH_2Cl_2 and it was added to the reaction mixture. Reaction mixture was stirred over-night. To this mixture, 10 mL CH_2Cl_2 was added and it was extracted with H_2O. When the water was added, fume occurred. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel and hexane/ CH_2Cl_2 (1:1) as the eluent phase (18% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.94 (t, 3H, CH_2CH_3), 1.36 (m, 2H, CH_2CH_2), 1.62 (m, 2H, CH_2CH_2), 2.66 (t, 2H, $ArCH_2$), 3.94 (s, 3H, OCH_3), 4.69 (s, 2H, OCH_2CO), 6.97 (m, 4H, Ar*H*), 7.48 (d, 1H, Ar*H*), 8.03 (s, 1H, Ar*H*), 8.72 (d, 1H, Ar*H*), 11.37 (s, 1H, N*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 13.83 (CH_3),

22.14 (*C*H_2), 33.08 (*C*H_2), 34.60 (*C*H_2), 55.83 (O*C*H_3), 69.12 (O*C*H_2CO), 112.19 (Ar*C*), 115.05 (Ar*C*), 120.75 (Ar*C*), 122.31 (Ar*C*), 123.28 (Ar*C*), 125.00 (Ar*C*), 131.39 (Ar*C*), 135.83 (2C, Ar*C*), 139.13 (Ar*C*), 146.69 (Ar*C*), 150.00 (Ar*C*), 167.91 (*C*=O) ppm.

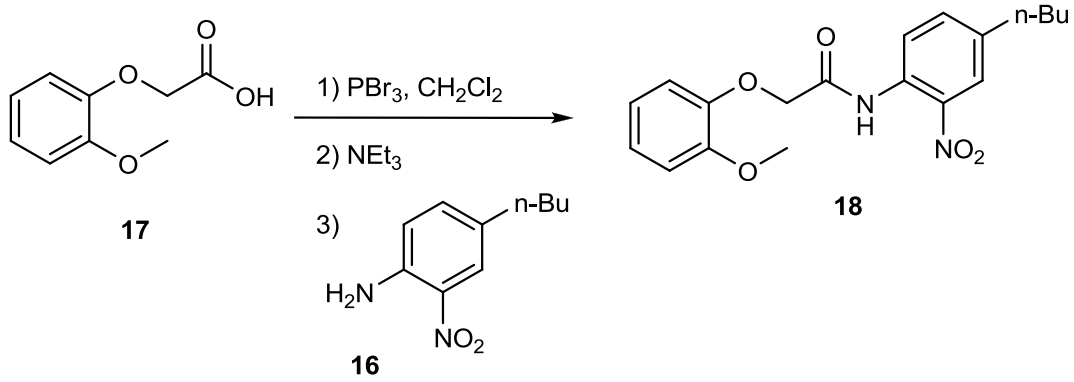


Figure 4.9. Synthesis of N-(4-butyl-2-nitrophenyl)-2-(2-methoxyphenoxy)acetamide (18).

4.3.10. Synthesis of N-(2-amino-4-butylphenyl)-2-(2-methoxyphenoxy)acetamide (19)

The reaction was done according to the literature procedure [45]. A suspension of compound 18 (73 mg, 0.22 mmol) in unstabilized 57% HI (0.67 mL) was heated at 90^{o}C for 3 hours. After cooling to RT, the mixture was diluted with ethyl acetate, and washed successively with saturated aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel and methanol/CH_2Cl_2 (1:20) as the eluent phase.

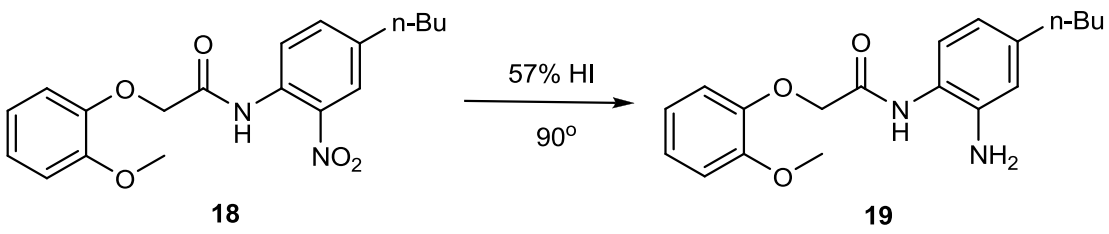


Figure 4.10. Synthesis of N-(2-amino-4-butylphenyl)-2-(2-methoxyphenoxy)acetamide (19).

4.3.11. Synthesis of 4-butoxyphenyl 2-(2-methoxyphenoxy)acetate (21)

The coupling reaction was done according to the literature procedure [46]. First 4-butoxyphenol (20) (45.62 mg, 0.27 mmol) and DMAP (40.24 mg, 0.33 mmol) were dissolved in 2 mL of dry CH_2Cl_2. To this solution, 2-(2-methoxyphenoxy)acetic acid (17) (50 mg, 0.27 mmol) and DCC (67.96 mg, 0.33 mmol) in 2 mL of dry CH_2Cl_2 were added at RT under nitrogen over a period of 2 hours. The reaction mixture was stirred at RT for 24 hours. At the end of this period, resulting precipitate was filtered and the solution was concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel and hexane/CH_2Cl_2 (1:2) as the eluent phase (51% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.97 (t, 3H, CH_2CH_3), 1.48 (m, 2H, CH_2CH_2), 1.75 (m, 2H, CH_2CH_2), 3.90 (s, 3H, OCH_3), 3.93 (t, 2H, OCH_2CH_2), 4.91 (s, 2H, OCH_2CO), 6.87 (d, 2H, Ar*H*), 6.94 (d, 2H, Ar*H*), 7.00 (m, 4H, Ar*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 13.85 (CH_3), 19.21 (CH_2), 31.26 (CH_2), 55.89 (OCH_3), 66.70 (OCH_2CO), 68.04 (OCH_2CH_2), 112.19 (2C, Ar*C*), 114.91 (Ar*C*), 115.01 (2C, Ar*C*), 120.77 (Ar*C*), 122.01 (Ar*C*), 122.88 (Ar*C*), 143.39 (Ar*C*), 147.14 (Ar*C*), 149.77 (Ar*C*), 157.01 (Ar*C*), 168.08 (*C*=O) ppm.

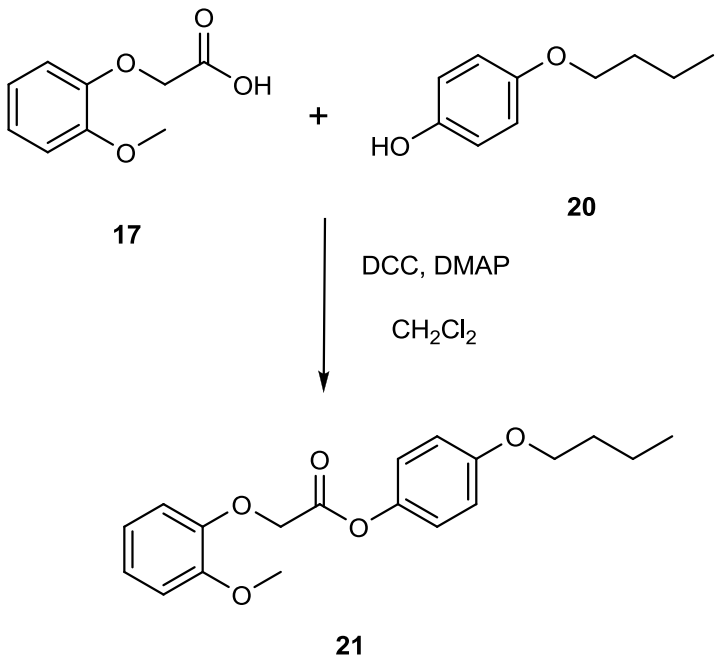


Figure 4.11. Synthesis of 4-butoxyphenyl 2-(2-methoxyphenoxy)acetate (21).

4.3.12. Synthesis of N-(4-butylphenyl)-2-(2-methoxyphenoxy)acetamide (22)

The coupling reaction was done according to the literature procedure [42]. The experiment was done under nitrogen by using dry CH_2Cl_2. 2-(2-methoxyphenoxy)acetic acid (17) (200 mg, 1.10 mmol) was dissolved in 3 mL dry CH_2Cl_2 under N_2. To this solution, PBr_3 (0.21 mL, 2.20 mmol) was added at 0°C and the reaction mixture was mixed for 3 hours at room temperature. After 3 hours, triethyl amine (0.46 mL, 3.30 mmol) was added and at that time fuming was observed because of the neutralization of HBr. 4-butylaniline (5) (163.84 mg, 1.10 mmol) was added to the reaction mixture. Reaction mixture was stirred over-night. To this mixture, 10 mL CH_2Cl_2 was added and it was extracted with H_2O. When the water was added, fume occurred. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel and hexane/ethyl acetate (3:1) as the eluent phase (36% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.94 (t, 3H, CH_2CH_3), 1.36 (m, 2H, CH_2CH_2), 1.59 (m, 2H, CH_2CH_2), 2.59 (t, 2H, $ArCH_2$), 3.91 (s, 3H, OCH_3), 4.63 (s, 2H, OCH_2CO), 6.97 (m, 4H, Ar*H*), 7.16 (d, 2H, Ar*H*), 7.52 (d, 2H, Ar*H*), 8.97 (s, 1H, N*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 13.94 (CH_3), 22.26 (CH_2), 33.64 (CH_2), 35.07 (CH_2), 55.89 (OCH_3), 70.50 (OCH_2CO), 112.16 (2C, Ar*C*), 116.60 (Ar*C*), 119.92 (Ar*C*), 121.35 (2C, Ar*C*), 123.59 (Ar*C*), 128.91 (Ar*C*), 134.89 (Ar*C*), 139.29 (Ar*C*), 147.36 (Ar*C*), 149.88 (Ar*C*), 166.78 (*C*=O) ppm.

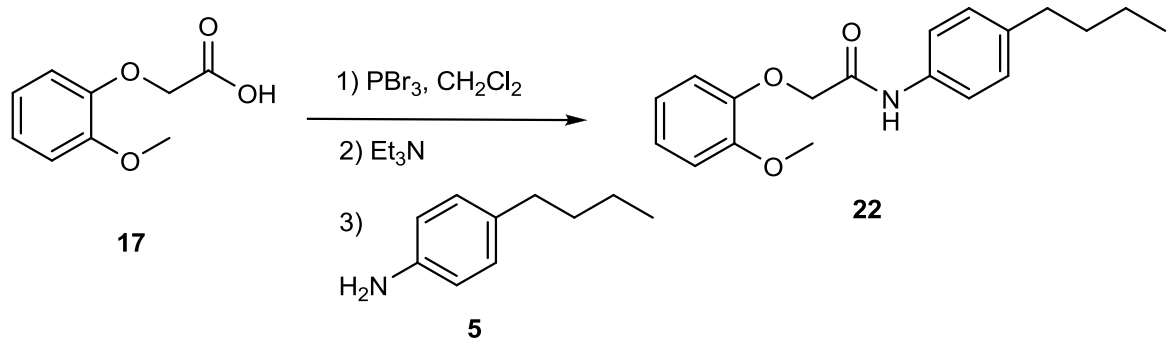


Figure 4.12. Synthesis of N-(4-butylphenyl)-2-(2-methoxyphenoxy)acetamide (22).

4.3.13. Synthesis of N-(4-butylphenyl)-2-(2-methoxyphenoxy)ethanethioamide (23)

The reaction was done according to the literature procedure [47]. Compound 22 and Lawesson's reagent were dissolved in toluene and the reaction mixture was refluxed at 120°C for 24 hours. At the end of 24 hours, the solution was concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel and hexane/ethyl acetate (15:1) as the eluent phase (38% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.94 (t, 3H, CH_2CH_3), 1.37 (m, 2H, CH_2CH_2), 1.61 (m, 2H, CH_2CH_2), 2.63 (t, 2H, $ArCH_2$), 3.90 (s, 3H, OCH_3), 5.02 (s, 2H, OCH_2CO), 6.99 (m, 4H, Ar*H*), 7.24 (d, 2H, Ar*H*), 7.78 (d, 2H, Ar*H*), 10.53 (s, 1H, N*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 13.93 (CH_3), 22.26 (CH_2), 33.64 (CH_2), 35.07 (CH_2), 55.90 (OCH_3), 70.49 (OCH_2CO), 112.29 (2C, Ar*C*), 116.60 (Ar*C*), 119.83 (2C, Ar*C*), 121.39 (2C, Ar*C*), 123.60 (Ar*C*), 126.64 (Ar*C*), 128.92 (Ar*C*), 134.79 (Ar*C*), 139.39 (Ar*C*), 214.37 (*C*=S) ppm.

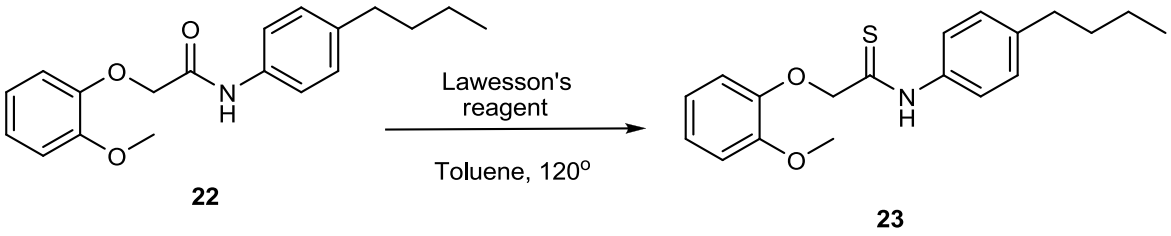


Figure 4.13. Synthesis of N-(4-butylphenyl)-2-(2-methoxyphenoxy)ethanethioamide (23).

4.3.14. Synthesis of N-(4-butoxyphenyl)-2-(2-methoxyphenoxy)acetamide (25)

The coupling reaction was done according to the literature procedure [42]. The experiment was done under nitrogen by using dry CH_2Cl_2. 2-(2-methoxyphenoxy)acetic acid (17) (100 mg, 0.55 mmol) was dissolved in 3 mL dry CH_2Cl_2 under N_2. To this solution, PBr_3 (0.11 mL, 1.1 mmol) was added at 0°C and the reaction mixture was mixed for 3 hours at room temperature. After 3 hours, triethyl amine (0.23 mL, 1.65 mmol) was added and at that time fuming was observed because of the neutralization of HBr. 4-butoxyaniline (24) (90.71 mg, 0.55 mmol) was added to the reaction mixture. Reaction mixture was stirred over-night. To this mixture, 10 mL CH_2Cl_2 was added and it was extracted with H_2O. When the water was added, fume occurred. The organic layer was

dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was impure and a column was prepared by using silica gel and hexane/ethyl acetate (2:1) as the eluent phase (41% yield). **^{1}H NMR** ($CDCl_3$), δ: 0.90 (t, 3H, CH_2CH_3), 1.42 (m, 2H, CH_2CH_2), 1.69 (m, 2H, CH_2CH_2), 3.87 (s, 3H, OCH_3), 3.88 (t, 2H, OCH_2CH_2), 4.59 (s, 2H, OCH_2CO), 6.81 (d, 2H, Ar*H*), 6.92 (m, 4H, Ar*H*), 7.41 (d, 2H, Ar*H*), 8.74 (s, 1H, N*H*) ppm. **^{13}C-NMR** ($CDCl_3$), δ: 12.84 (CH_3), 18.20 (CH_2), 30.28 (CH_2), 54.89 (OCH_3), 66.92 (OCH_2CO), 69.39 (OCH_2CH_2), 111.06 (Ar*C*), 113.78 (2C, Ar*C*), 115.46 (Ar*C*), 120.32 (2C, Ar*C*), 122.53 (Ar*C*), 129.12 (Ar*C*), 146.27 (Ar*C*), 148.78 (Ar*C*), 155.13 (Ar*C*), 165.60 (*C*=O) ppm.

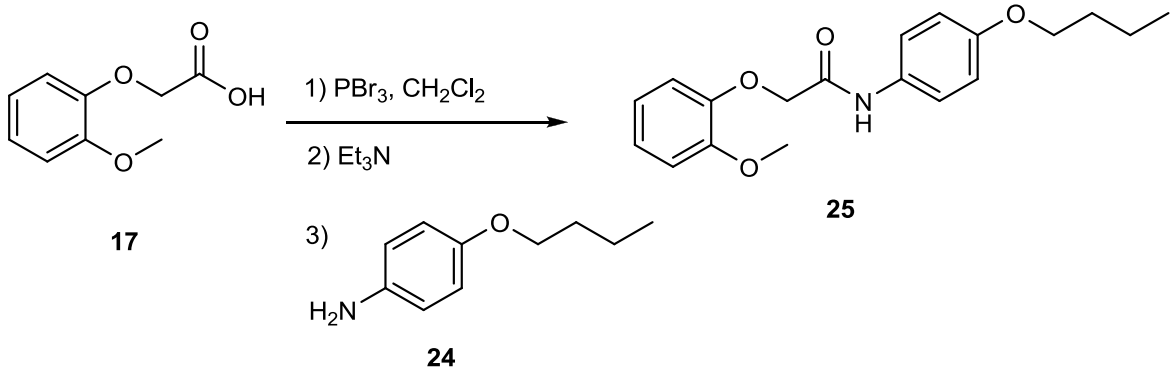


Figure 4.14. Synthesis of N-(4-butoxyphenyl)-2-(2-methoxyphenoxy)acetamide (25).

5. FUTURE WORK

As future work, *in vitro* tests of the synthesized drug candidates will be performed on mammalian cells (percent inhibition test at 5μM) to find whether they are biologically active or not. Among them, potential drug molecules will be tested to find their IC_{50} values. Based on the results new drug candidates will be derivatized.

In addition, elemental and LC-MS analyses of the synthesized drug candidates will be performed for publication.

6. CONCLUSION

The aim of the study was to synthesize isoquinole derivatives and derivatives of the compound AK74 which have an IC_{50} value of 9.1 μM. The modifications included using aminoisoquinolines or isoquinolinecarboxylic acids; variation of phenylene ring with alkyl, alkoxy, nitro, amino or hydroxyl group and converting carbonyl group to thionyl group. By synthesizing these derivatives, decreasing IC_{50} value of the lead compound was targeted. To this end, compounds in the Table 2.3 were synthesized successfully in order to send for biological testing. As a result, a new compound with 100% inhibition at 5 μM concentration was discovered.

APPENDIX A: SPECTROSCOPY DATA

^{1}H and ^{13}C NMR and spectroscopy of the synthesized products are included. Necessary expansions were made on the NMR data.

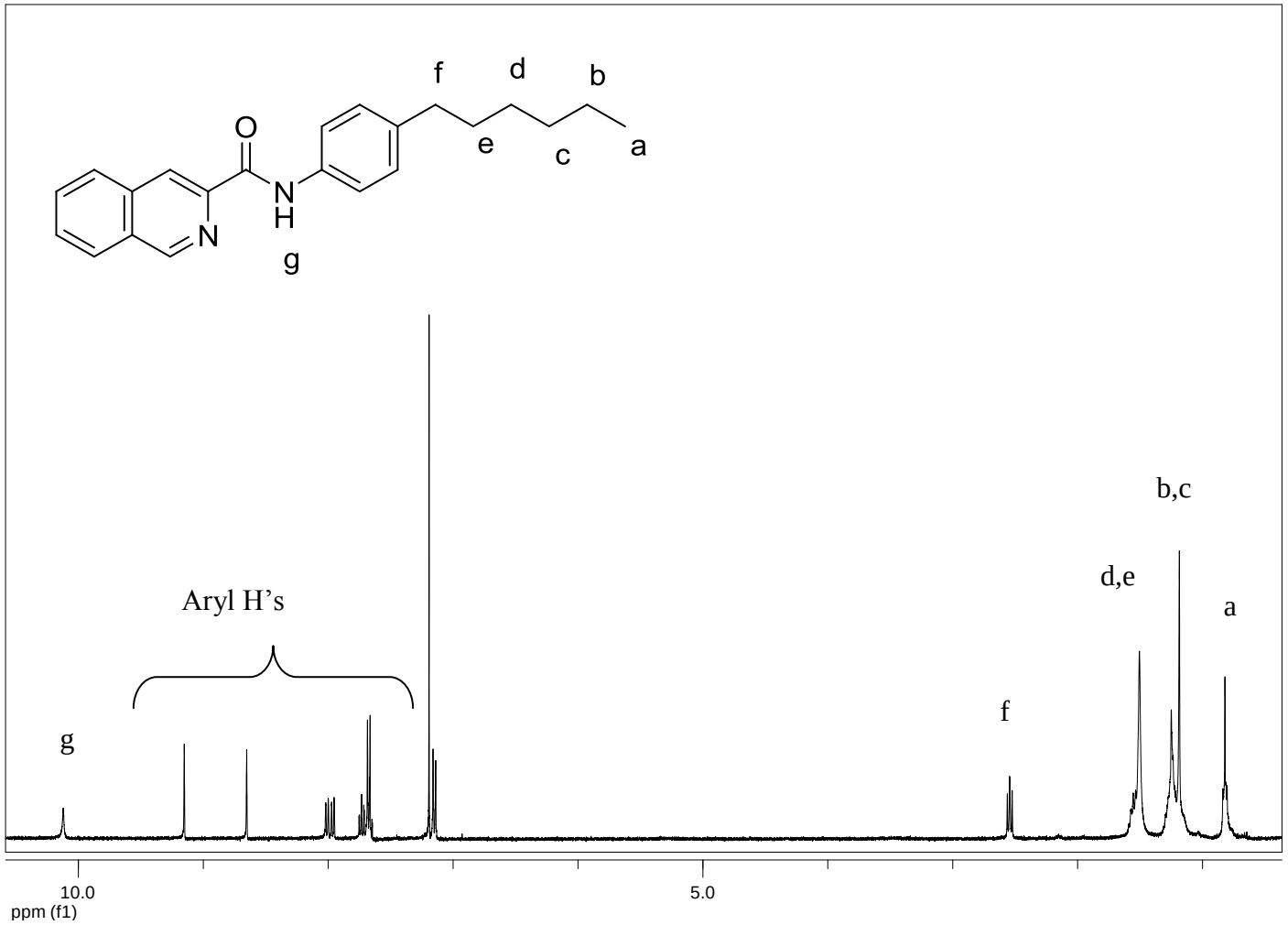


Figure A.1. ^{1}H-NMR Spectrum of N-(4-hexylphenyl)isoquinoline-3-carboxamide.

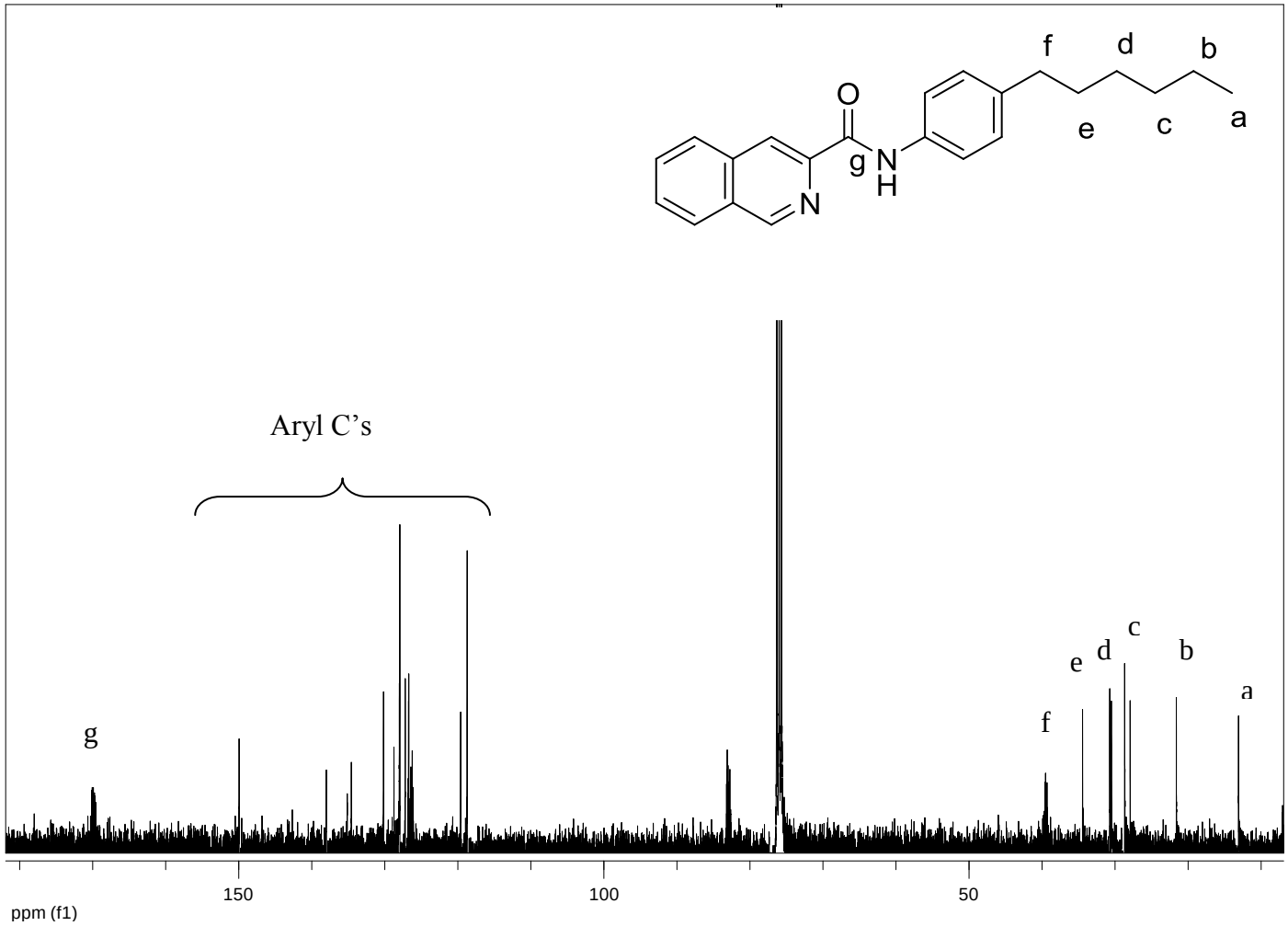


Figure A.2. ^{13}C-NMR Spectrum of N-(4-hexylphenyl)isoquinoline-3-carboxamide.

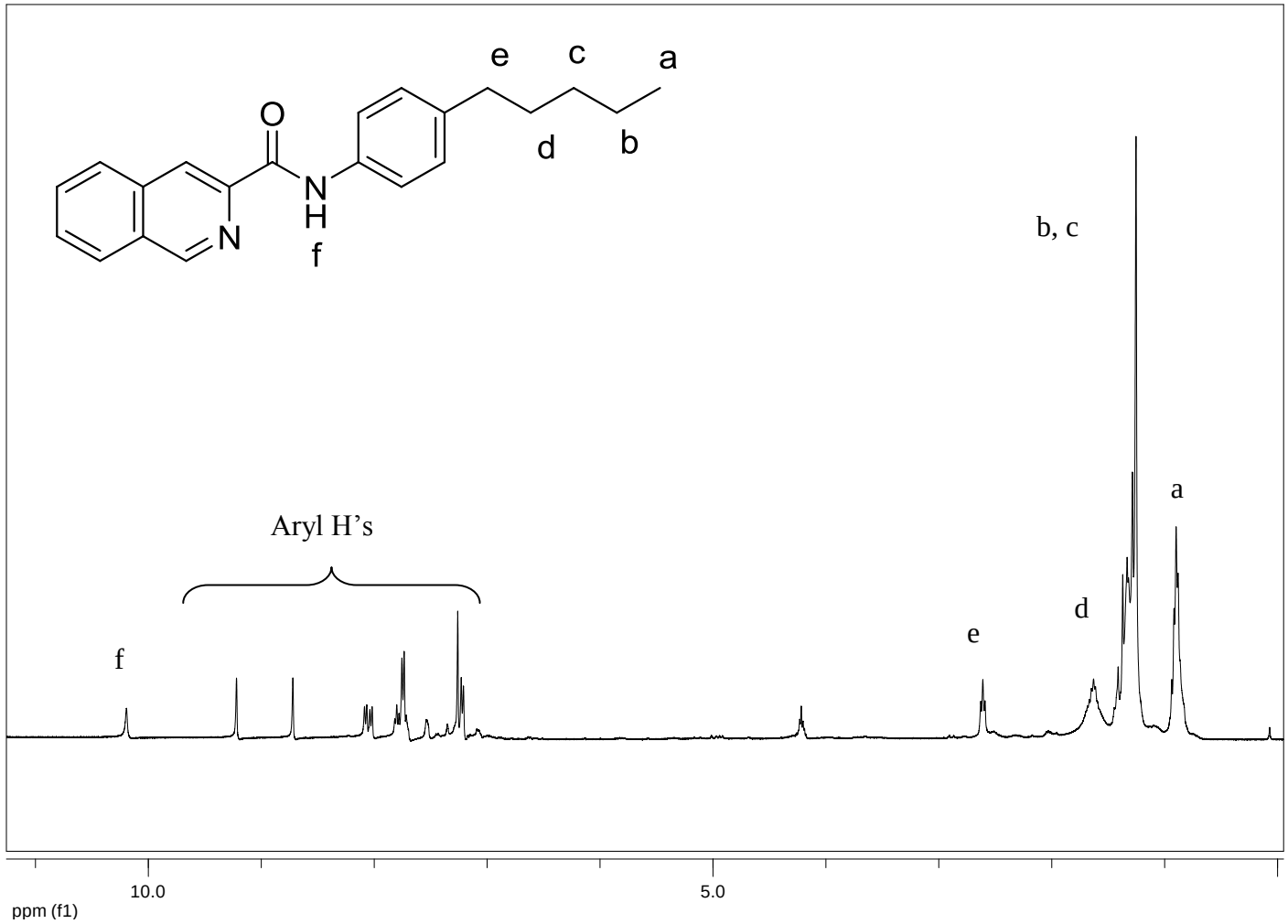


Figure A.3. ^{1}H-NMR Spectrum of N-(4-pentylphenyl)isoquinoline-3-carboxamide.

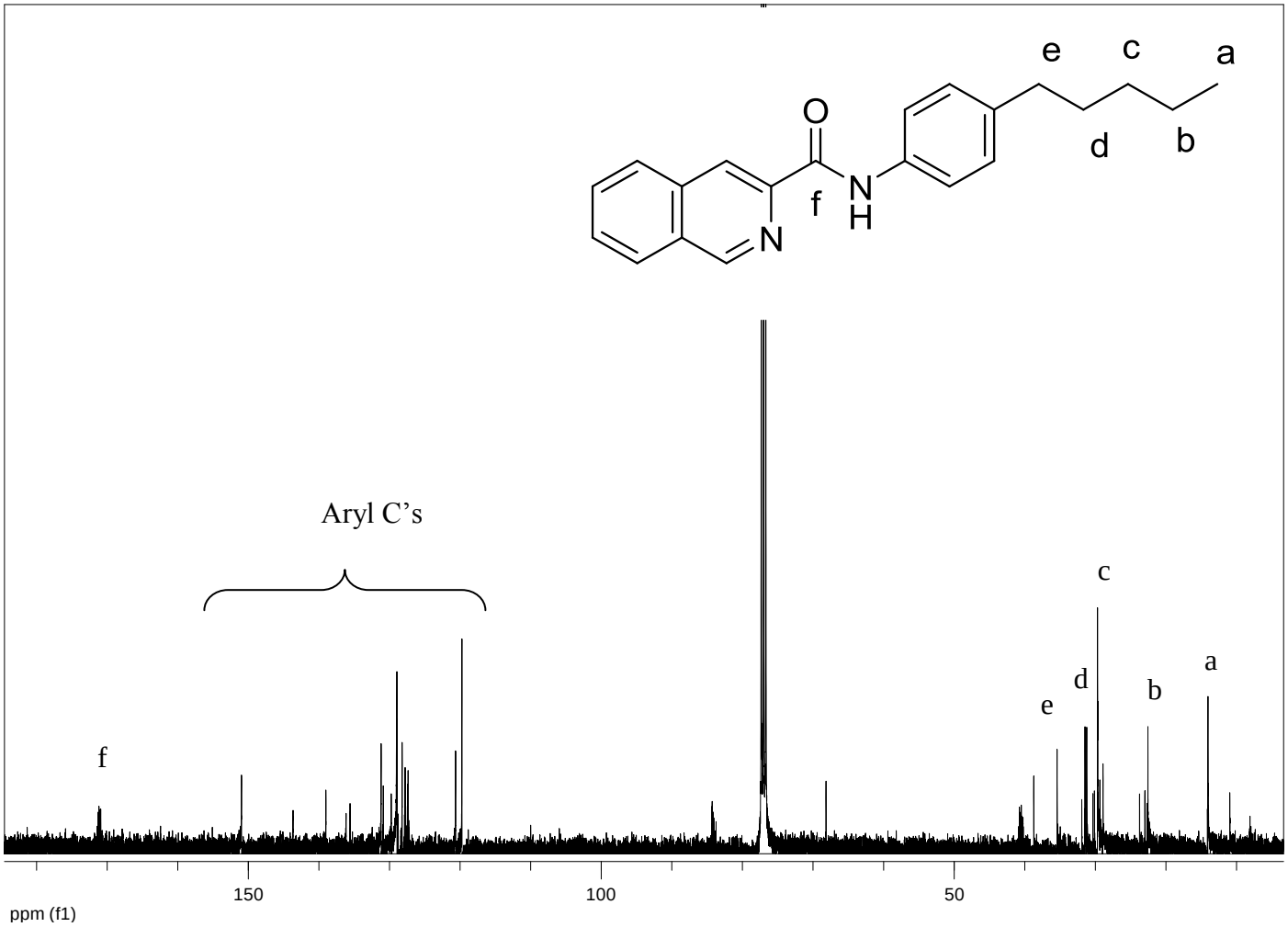


Figure A.4. ^{13}C-NMR Spectrum of N-(4-pentylphenyl)isoquinoline-3-carboxamide.

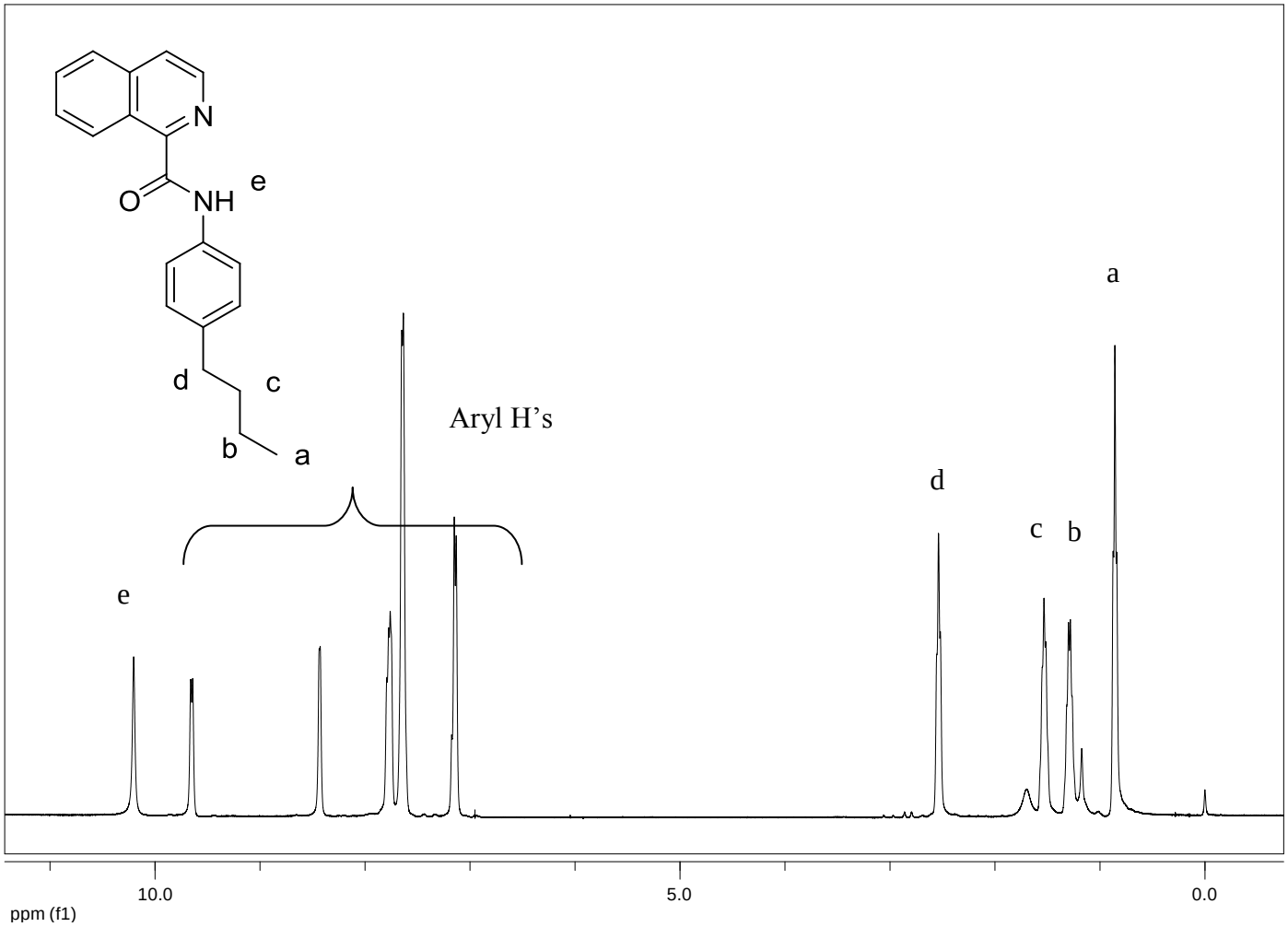


Figure A.5. ^{1}H-NMR Spectrum of N-(4-butylphenyl)isoquinoline-1-carboxamide.

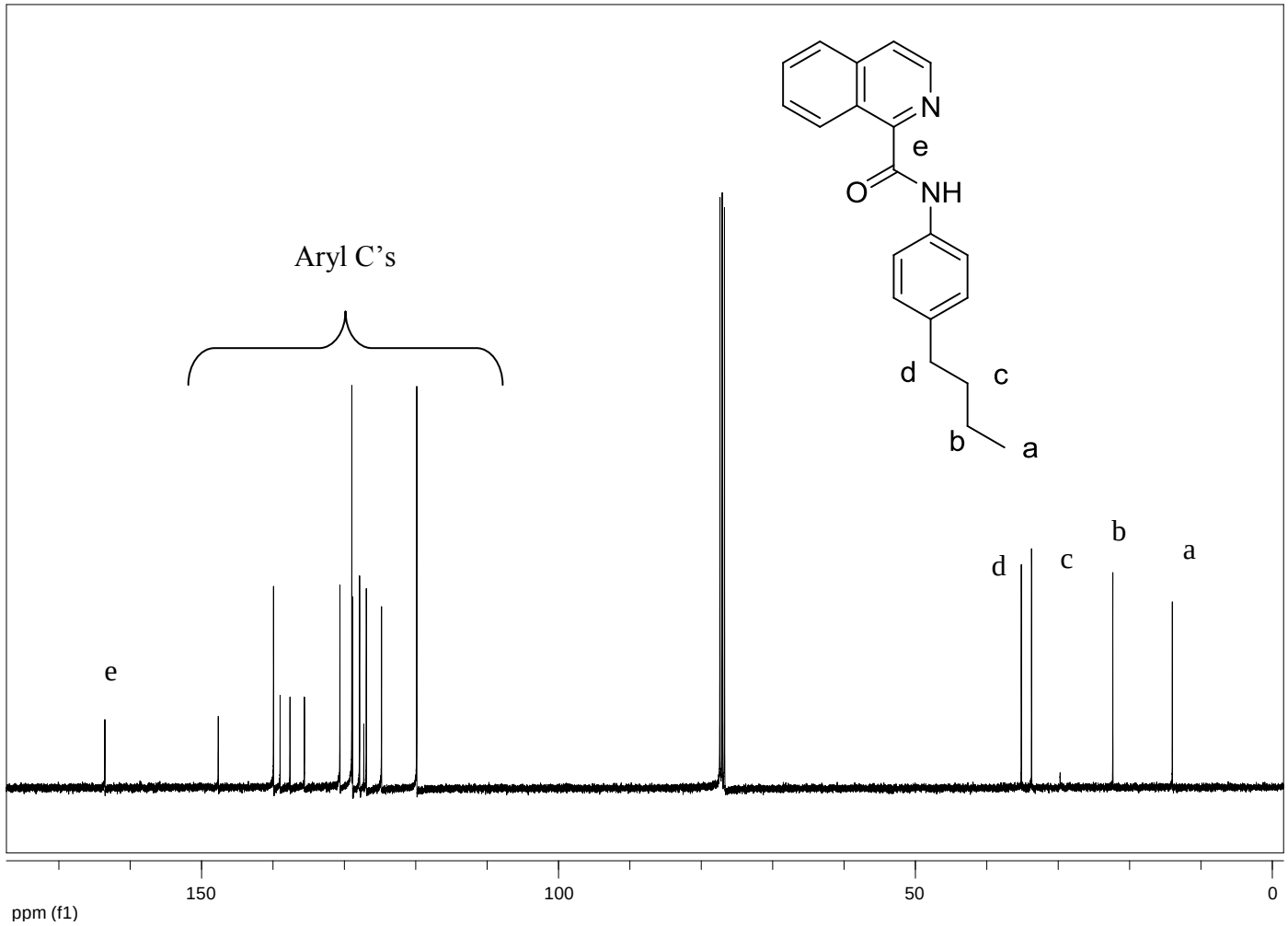


Figure A.6. ^{13}C-NMR Spectrum of N-(4-butylphenyl)isoquinoline-1-carboxamide.

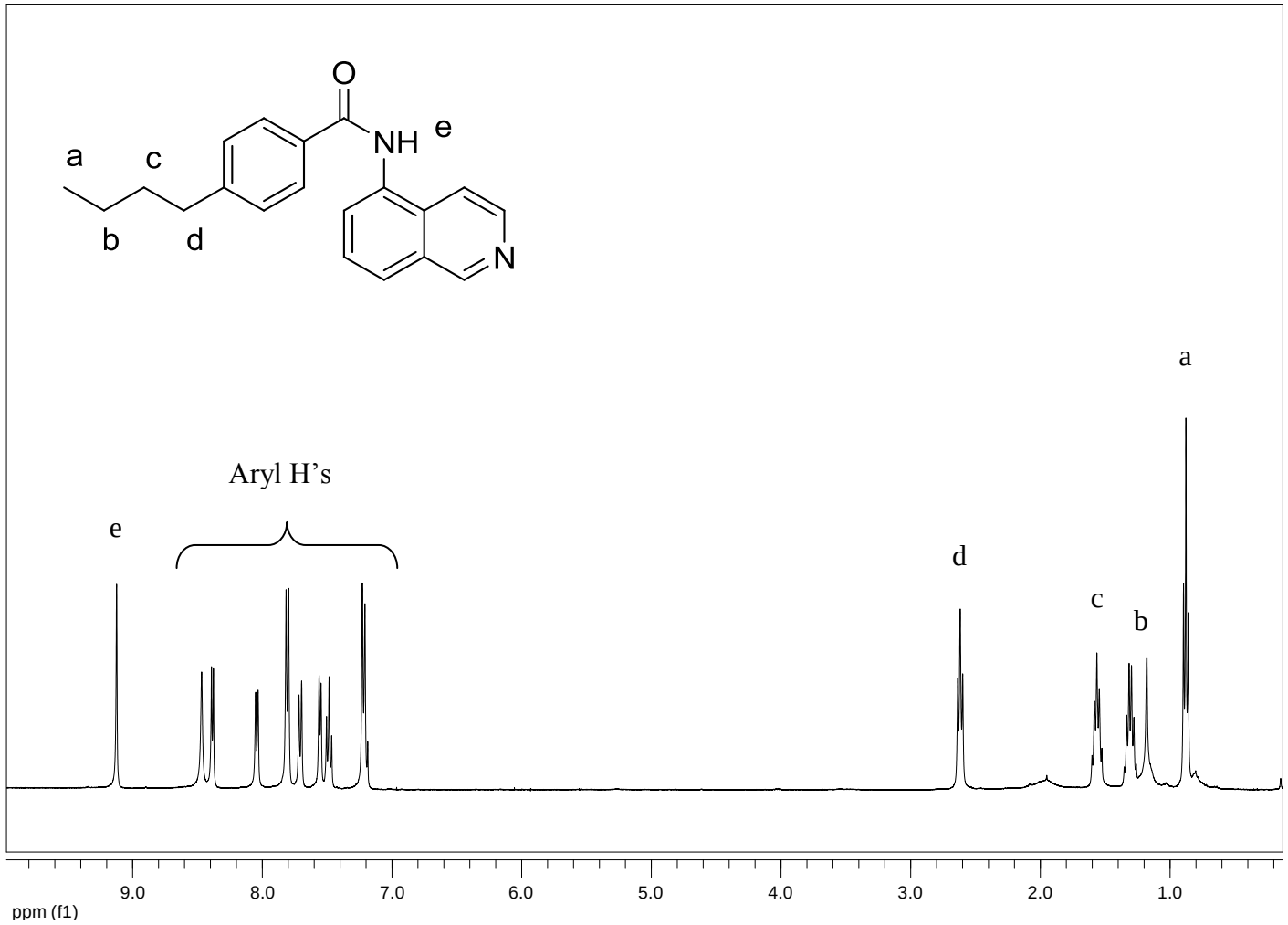


Figure A.7. ^{1}H-NMR Spectrum of 4-butyl-N-(isoquinolin-5-yl)benzamide.

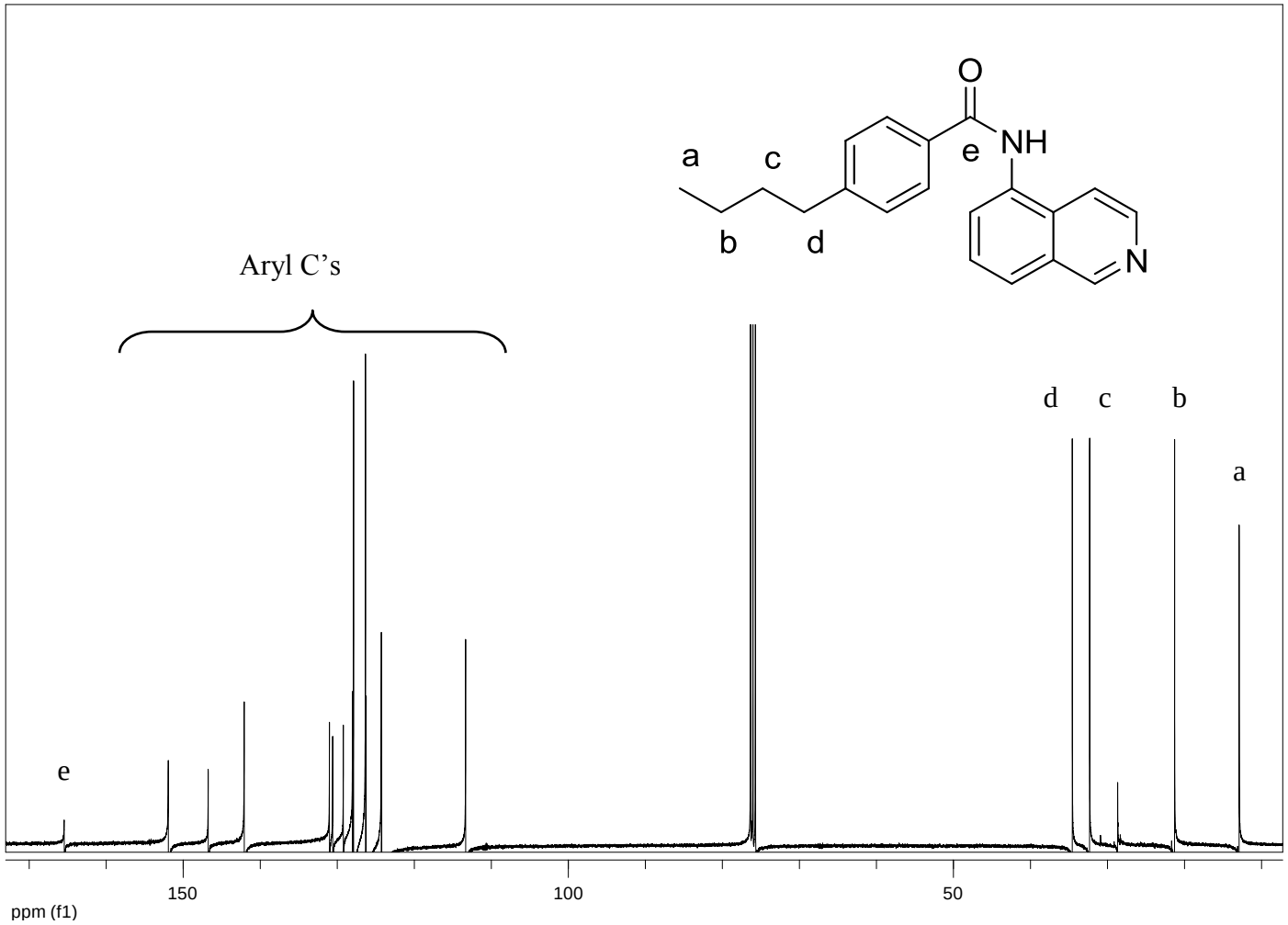


Figure A.8. ^{13}C-NMR Spectrum of 4-butyl-N-(isoquinolin-5-yl)benzamide.

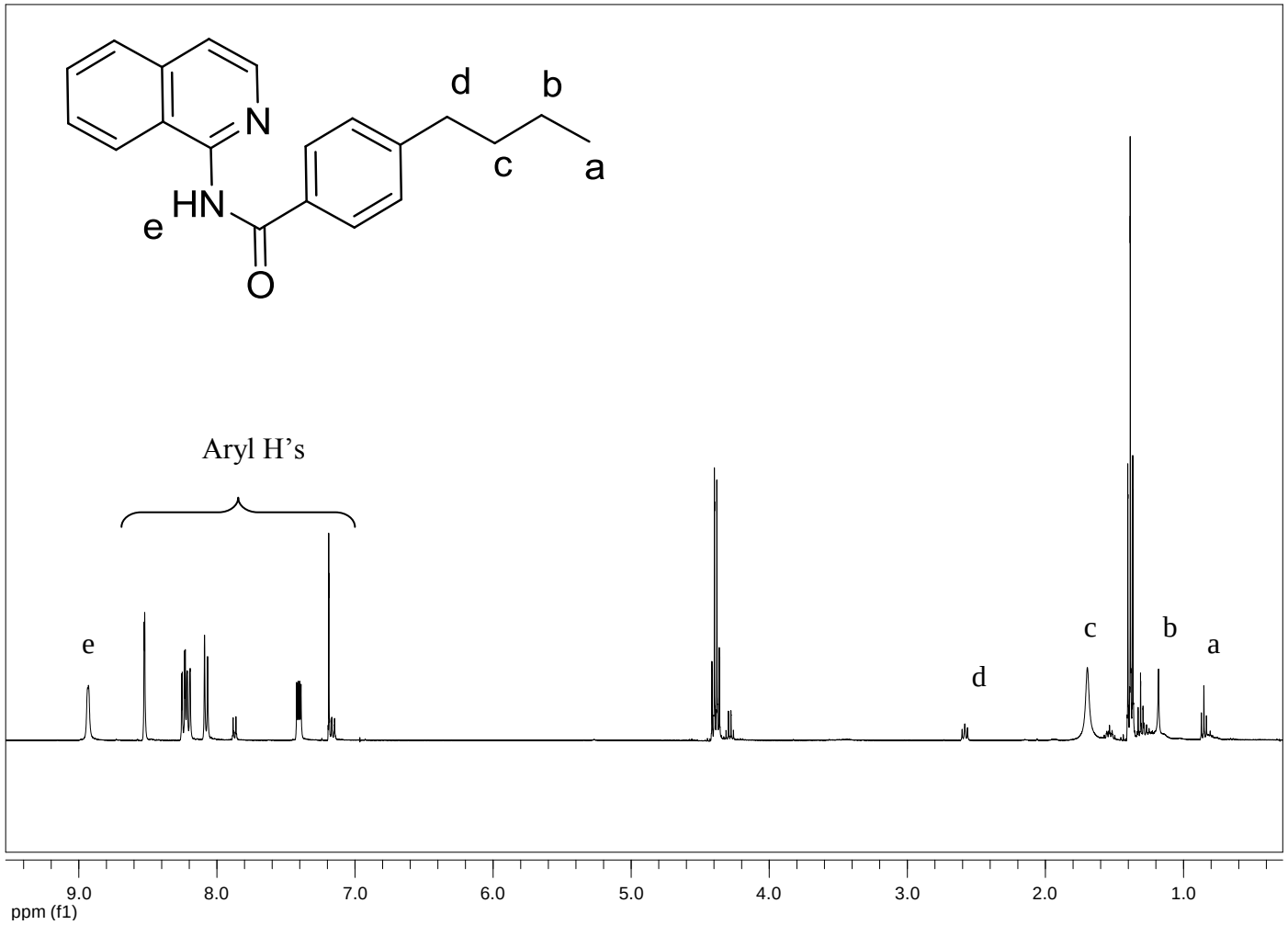


Figure A.9. ^{1}H-NMR Spectrum of 4-butyl-N-(isoquinolin-1-yl)benzamide.

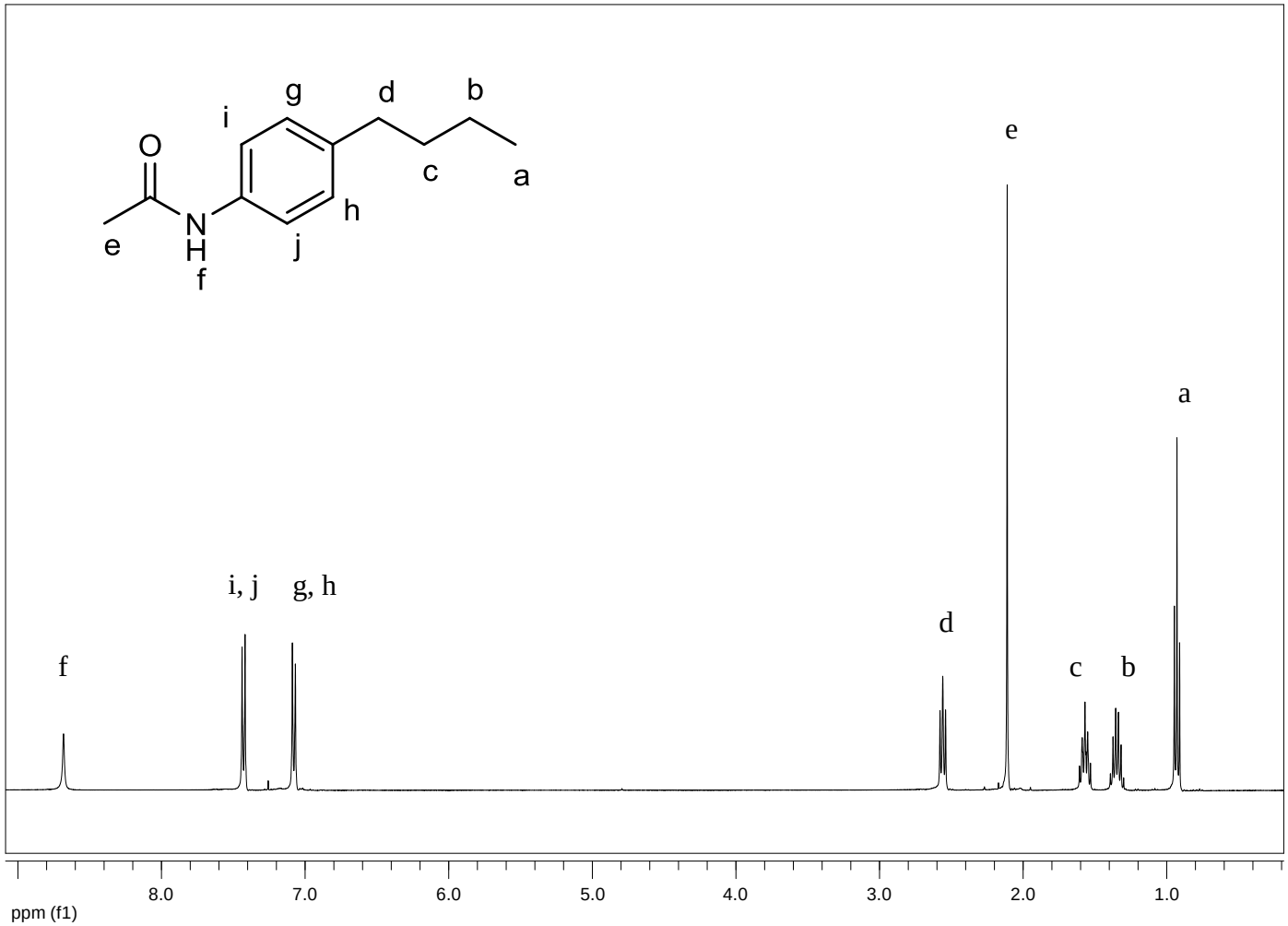


Figure A.10. ^{1}H-NMR Spectrum of N-(4-butylphenyl)acetamide.

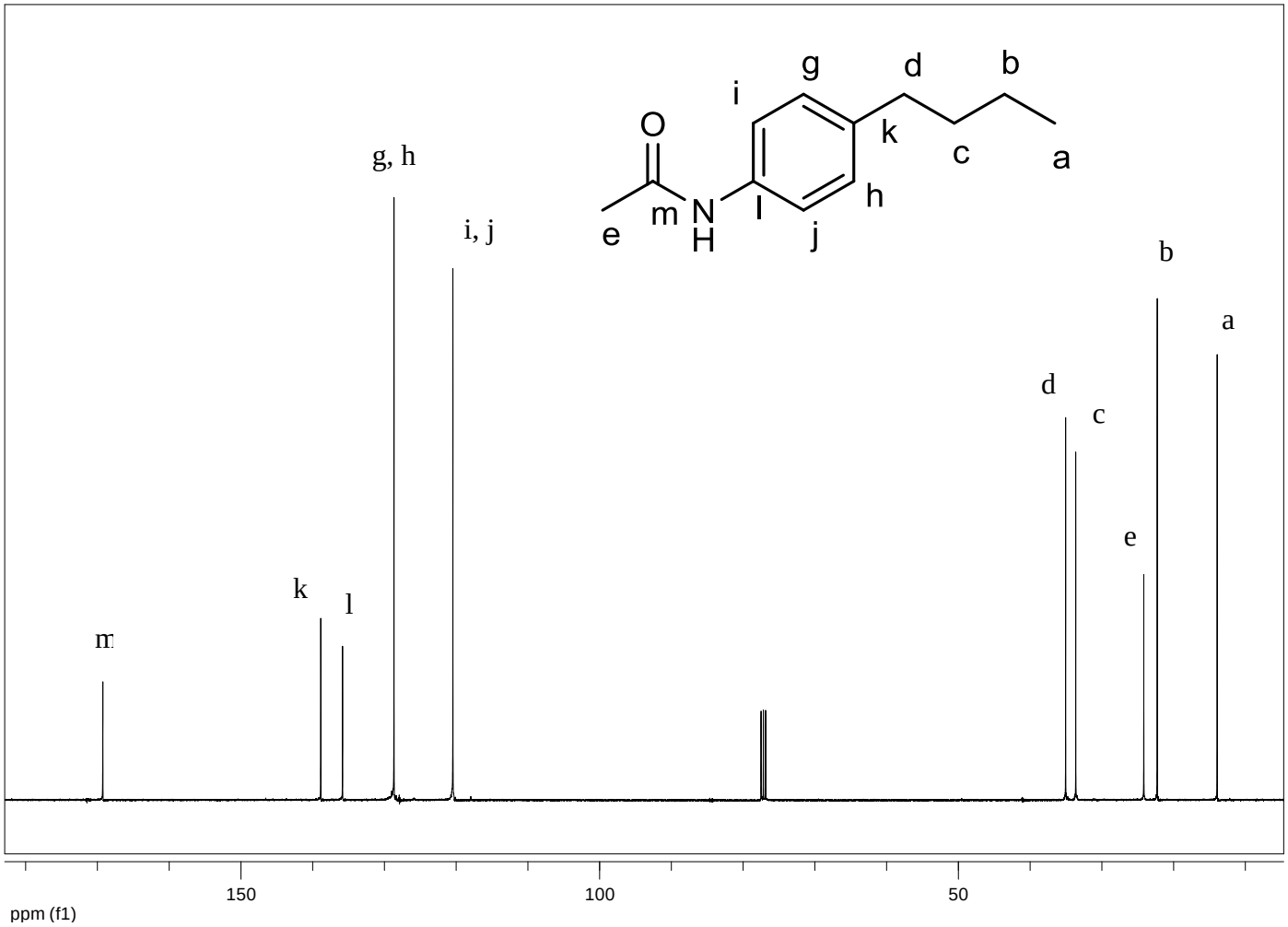


Figure A.11. ^{13}C-NMR Spectrum of N-(4-butylphenyl)acetamide.

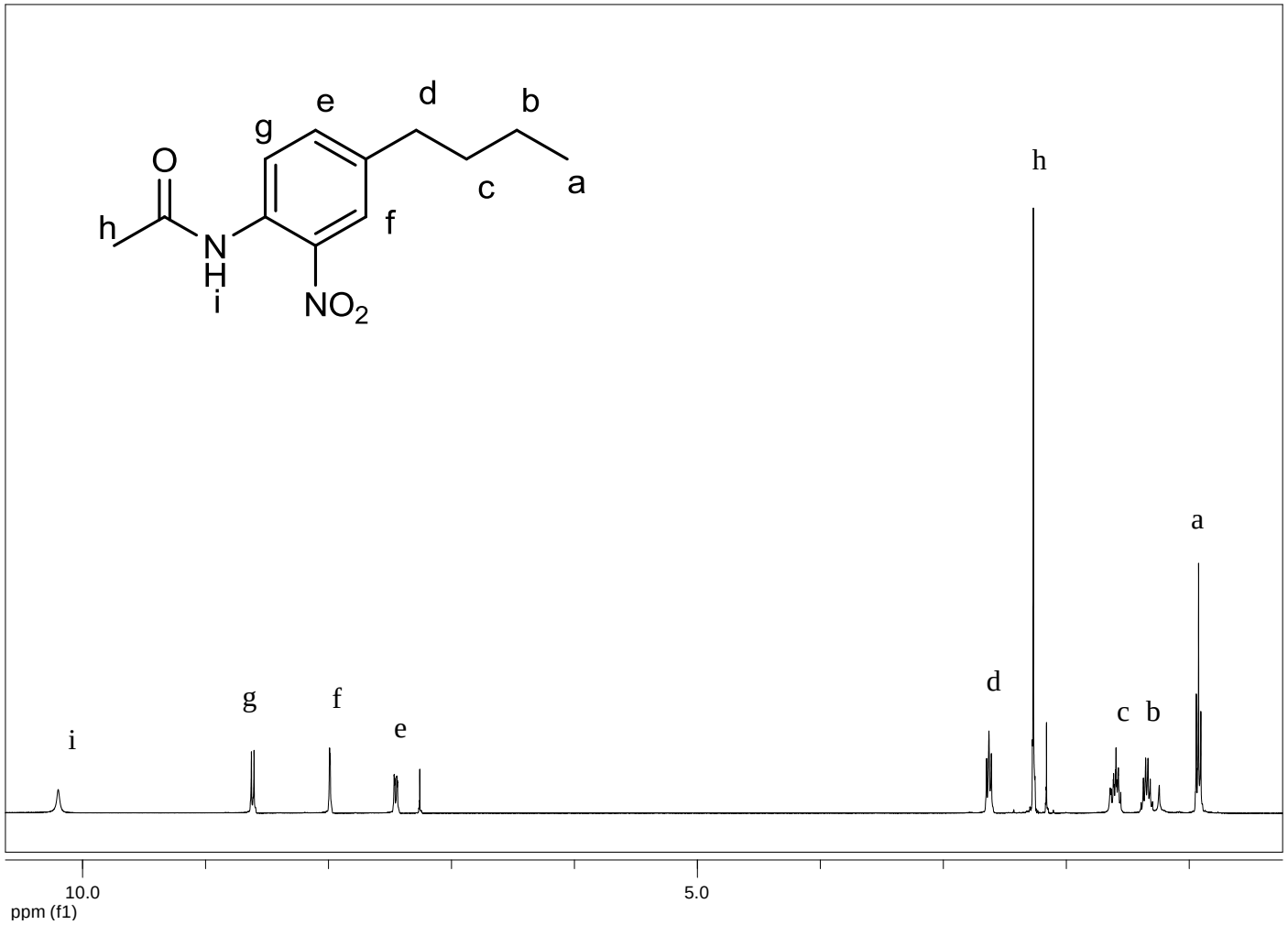


Figure A.12. ^{1}H-NMR Spectrum of N-(4-butyl-2-nitrophenyl)acetamide.

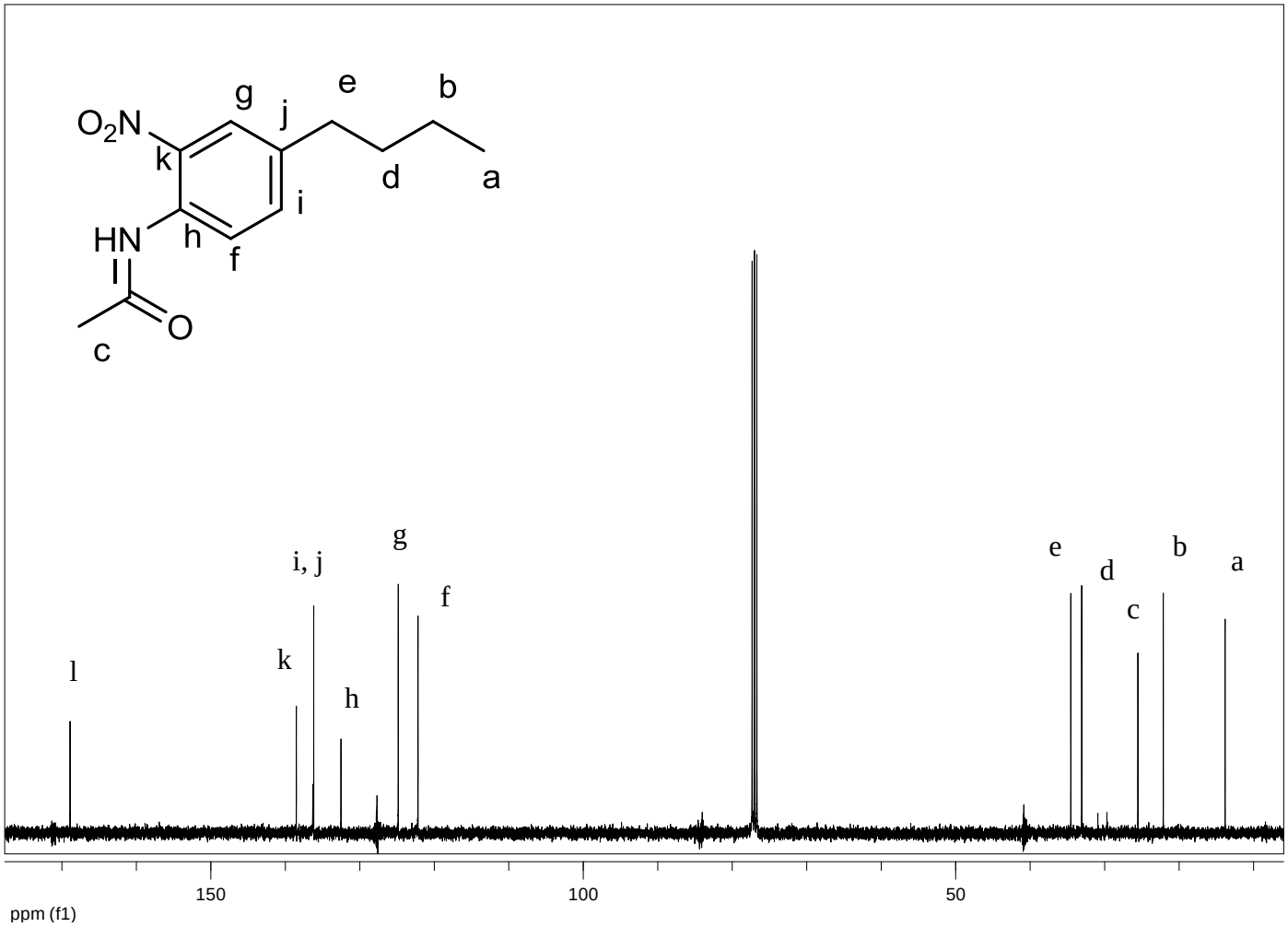


Figure A.13. ^{13}C-NMR Spectrum of N-(4-butyl-2-nitrophenyl)acetamide.

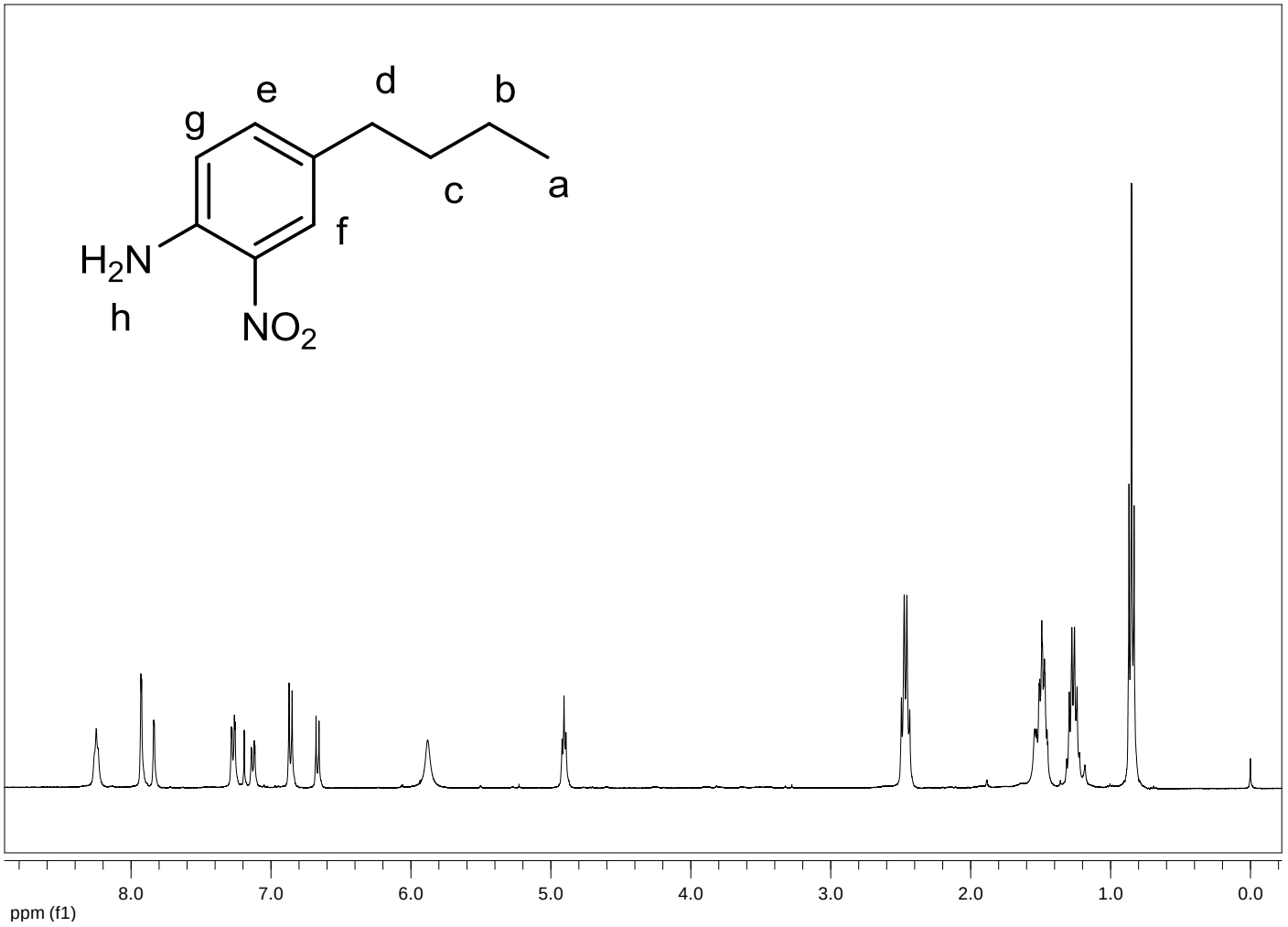


Figure A.14. ^{1}H-NMR Spectrum of 4-butyl-2-nitroaniline.

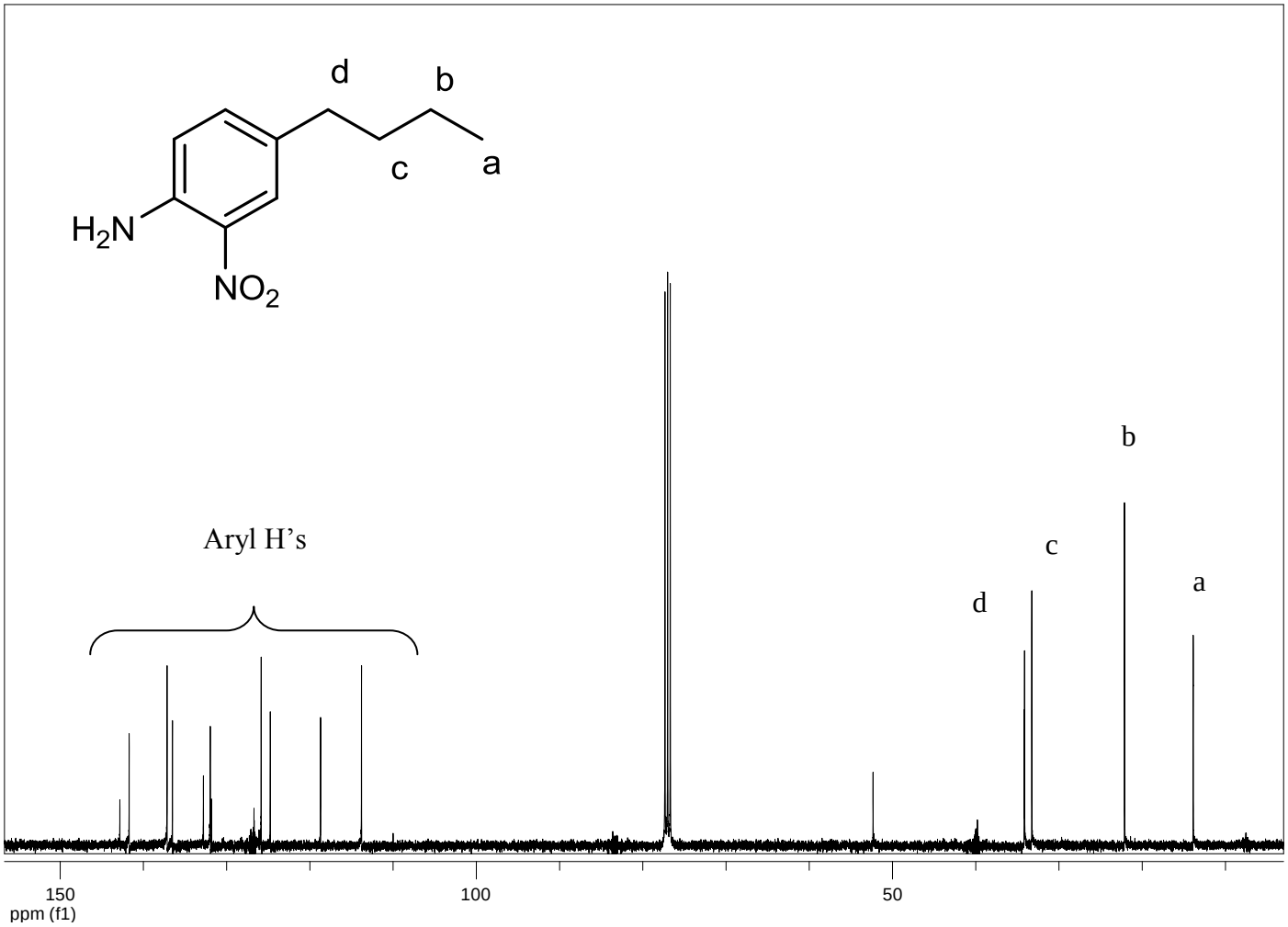


Figure A.15. ^{13}C-NMR Spectrum of 4-butyl-2-nitroaniline.

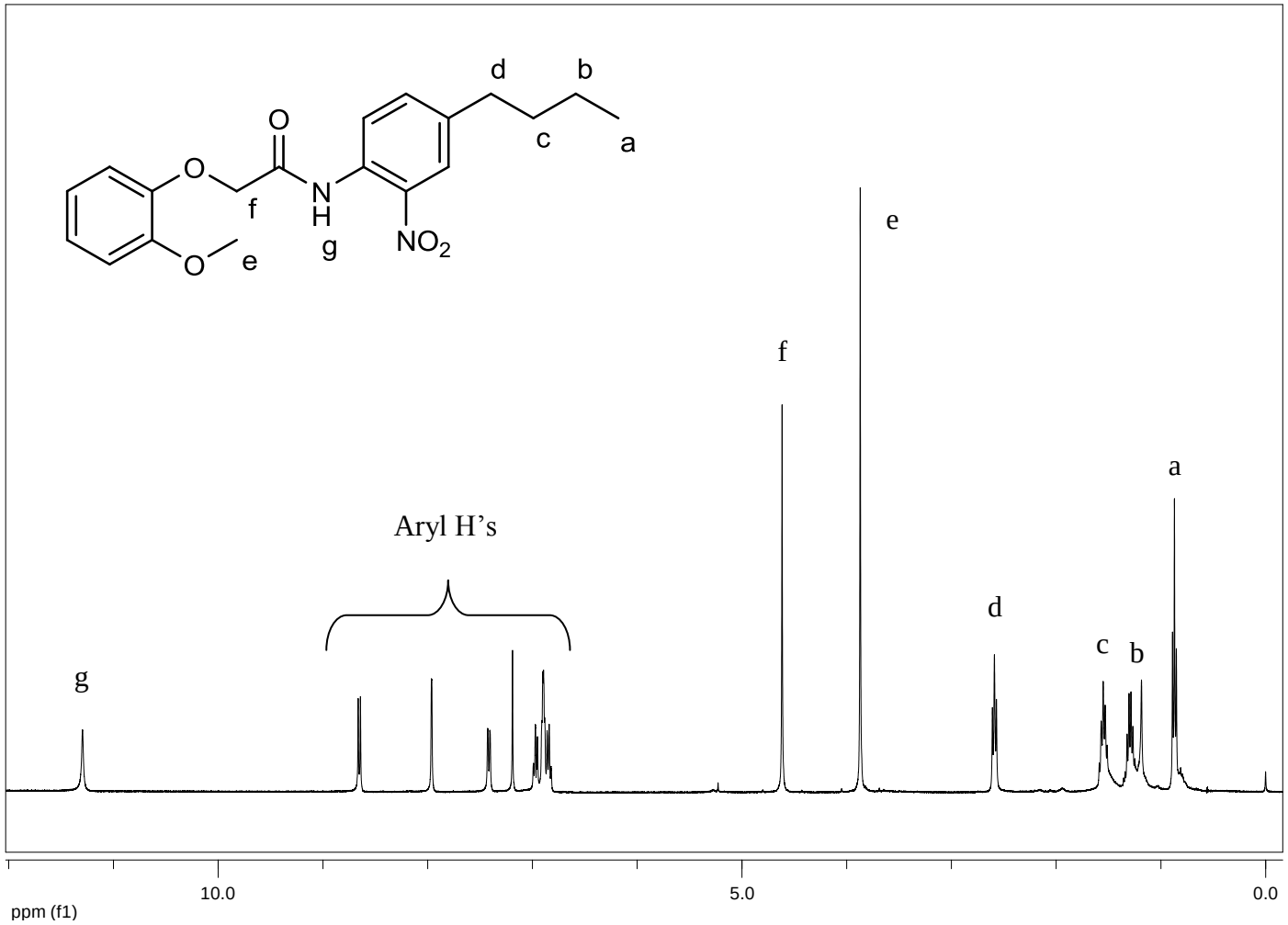


Figure A.16. ^{1}H-NMR Spectrum of N-(4-butyl-2-nitrophenyl)-2-(2-methoxyphenoxy)acetamide.

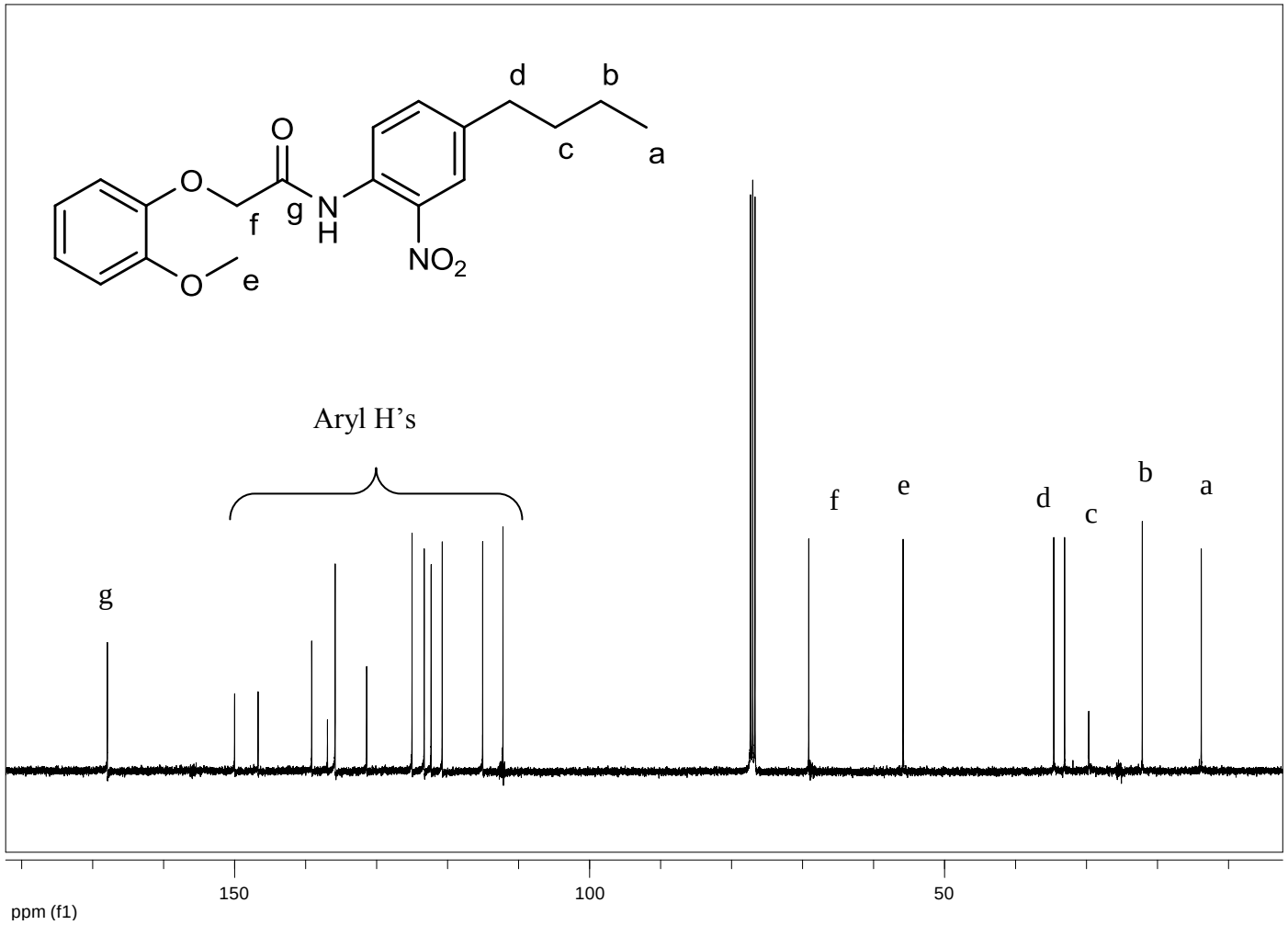


Figure A.17. ^{13}C-NMR Spectroscopy of N-(4-butyl-2-nitrophenyl)-2-(2-methoxyphenoxy)acetamide.

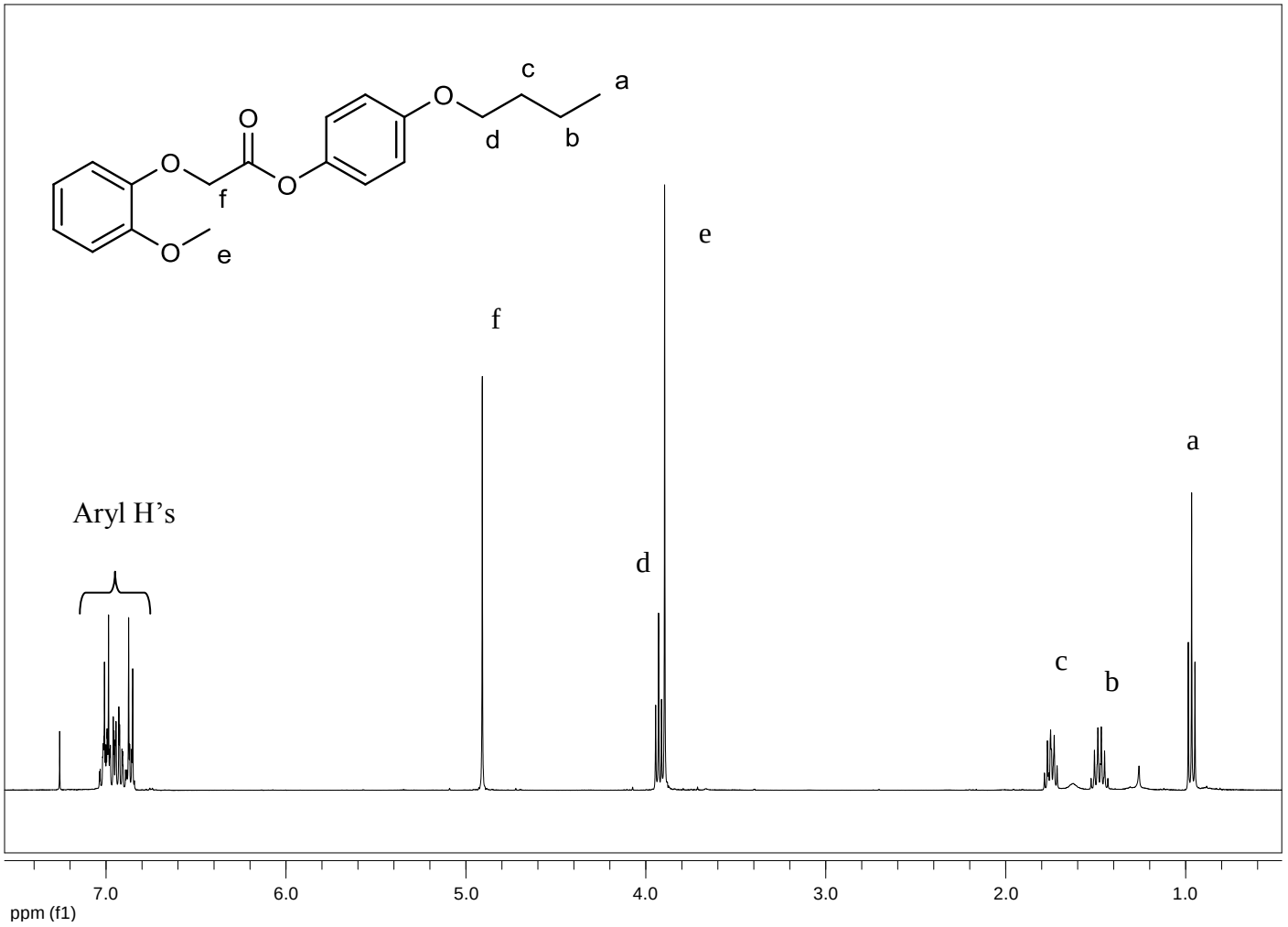


Figure A.18. ^{1}H-NMR Spectroscopy of 4-butoxyphenyl 2-(2-methoxyphenoxy)acetate.

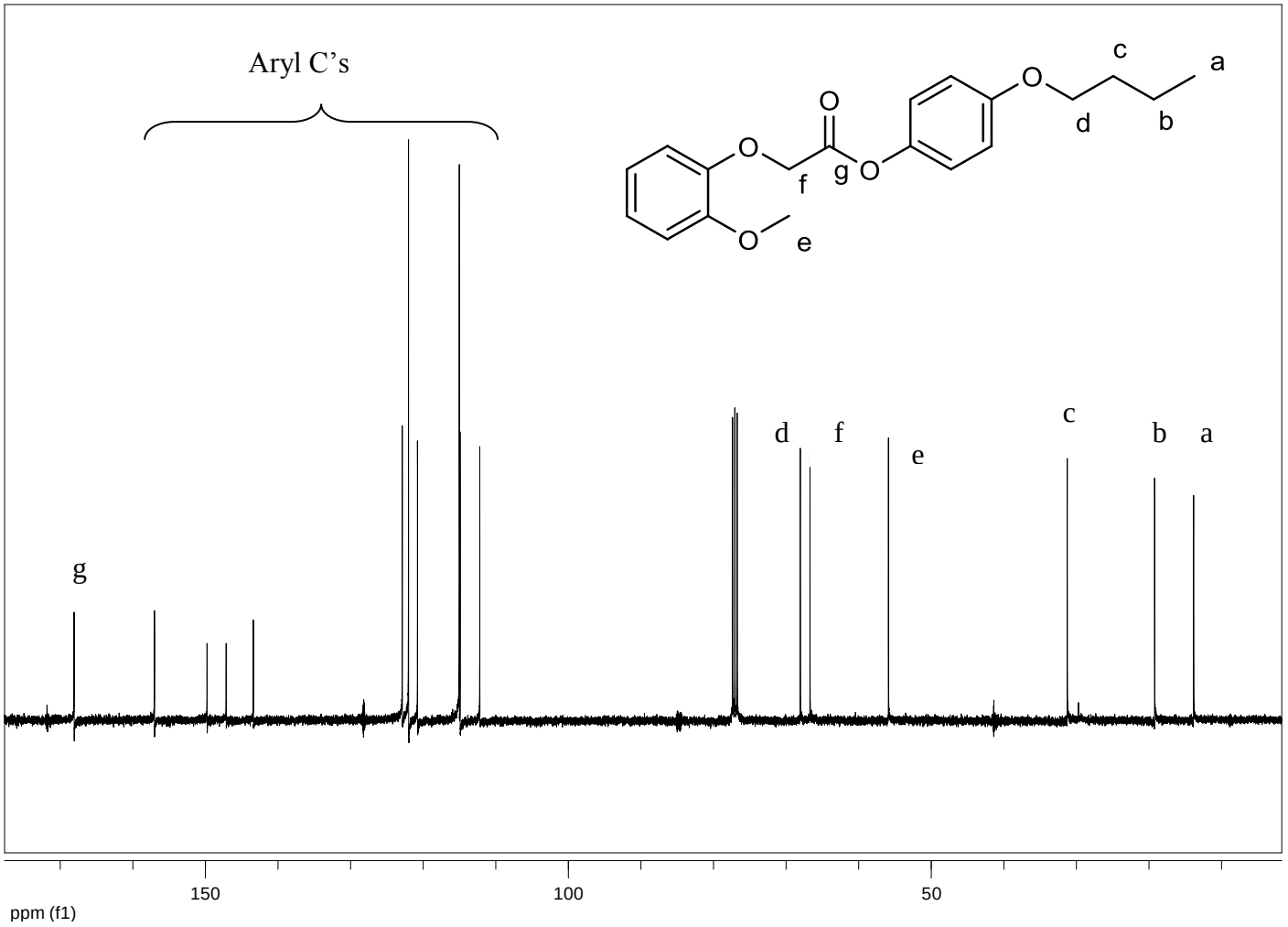


Figure A.19. ^{13}C-NMR Spectroscopy of 4-butoxyphenyl 2-(2-methoxyphenoxy)acetate.

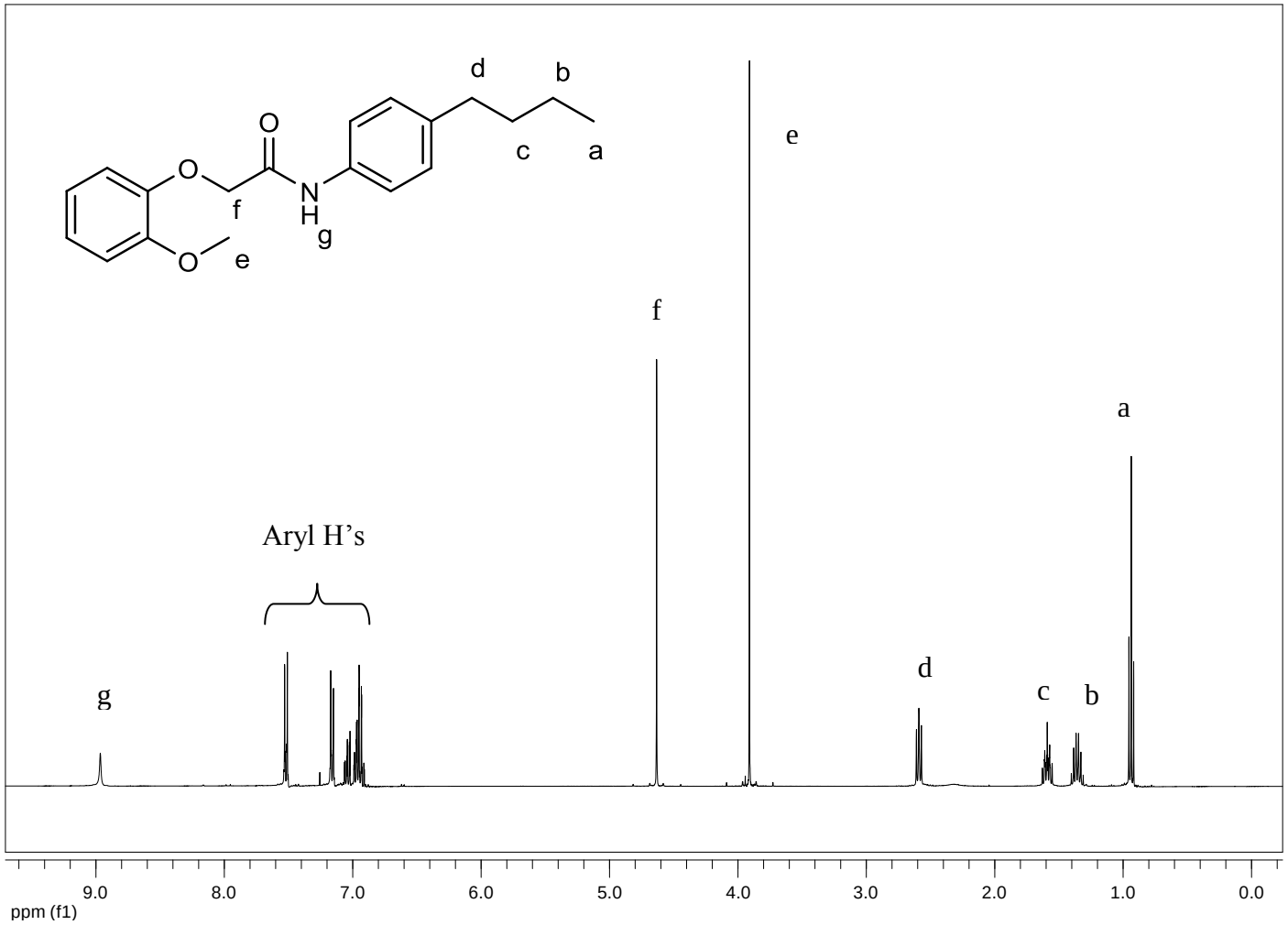


Figure A.20. ^{1}H-NMR Spectroscopy of N-(4-butylphenyl)-2-(2-methoxyphenoxy)acetamide.

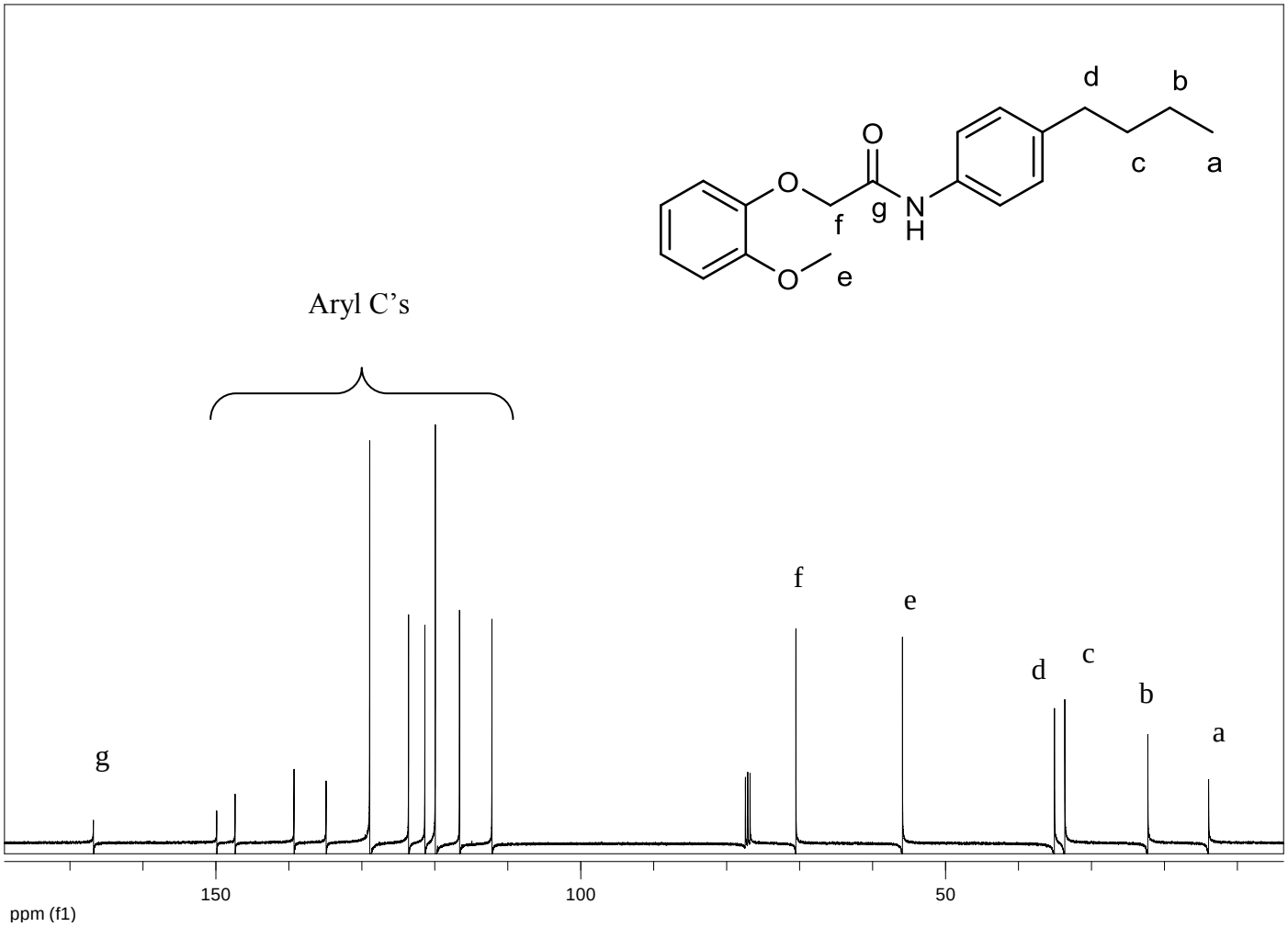


Figure A.21. ^{13}C-NMR Spectroscopy of N-(4-butylphenyl)-2-(2-methoxyphenoxy)acetamide.

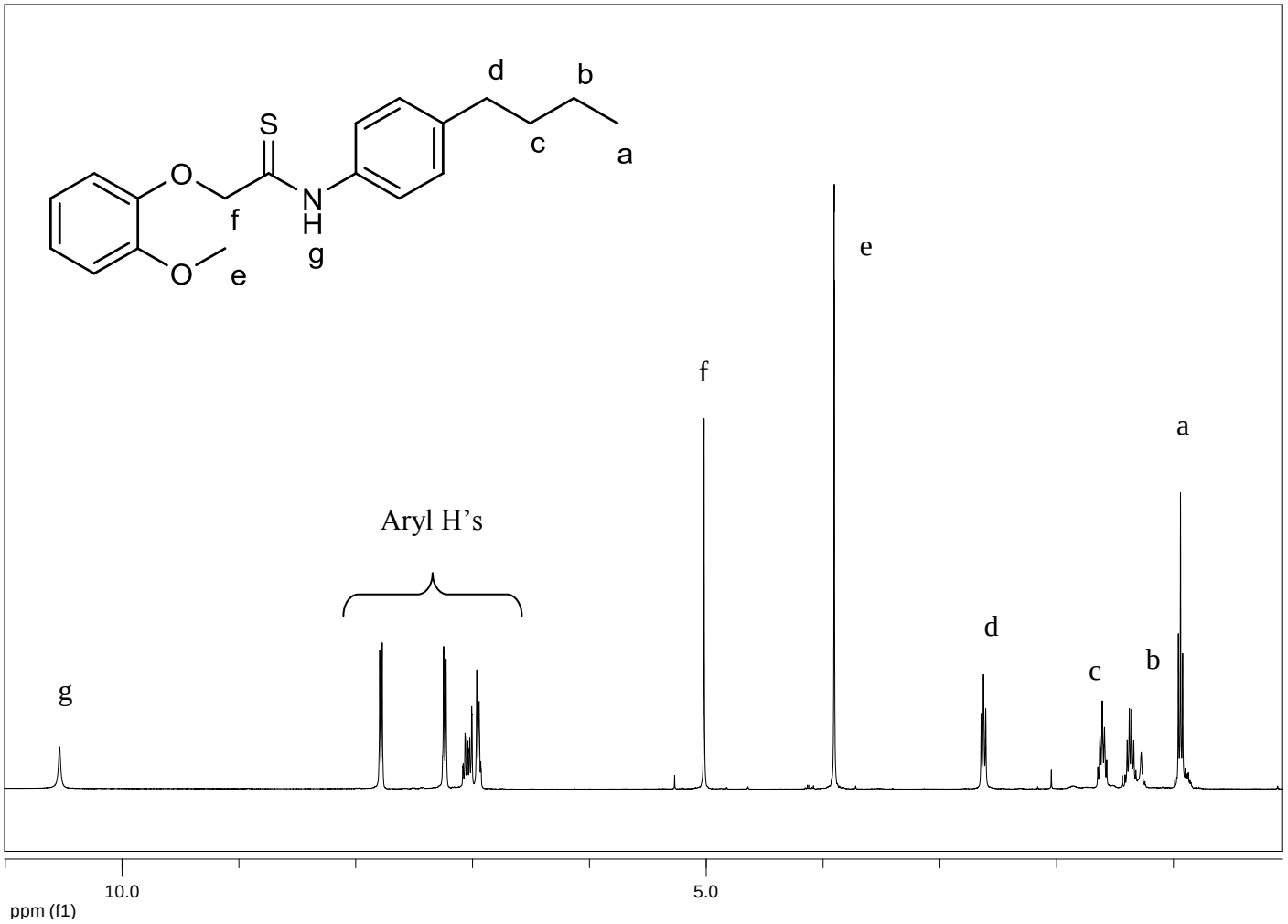


Figure A.22. ^{1}H-NMR Spectroscopy of N-(4-butylphenyl)-2-(2-methoxyphenoxy)ethanethioamide.

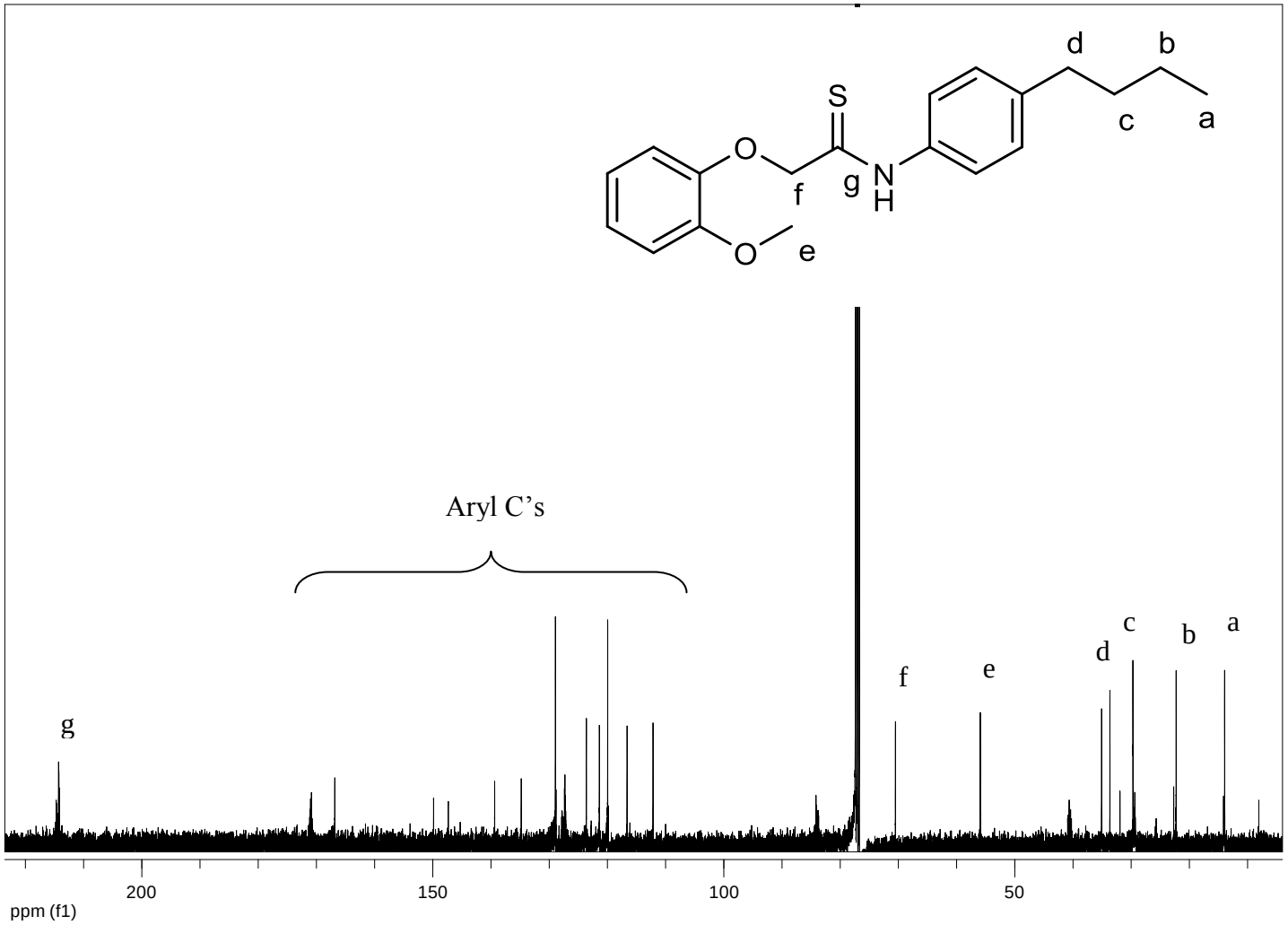


Figure A.23. ^{13}C-NMR Spectroscopy of N-(4-butylphenyl)-2-(2-methoxyphenoxy)ethanethioamide.

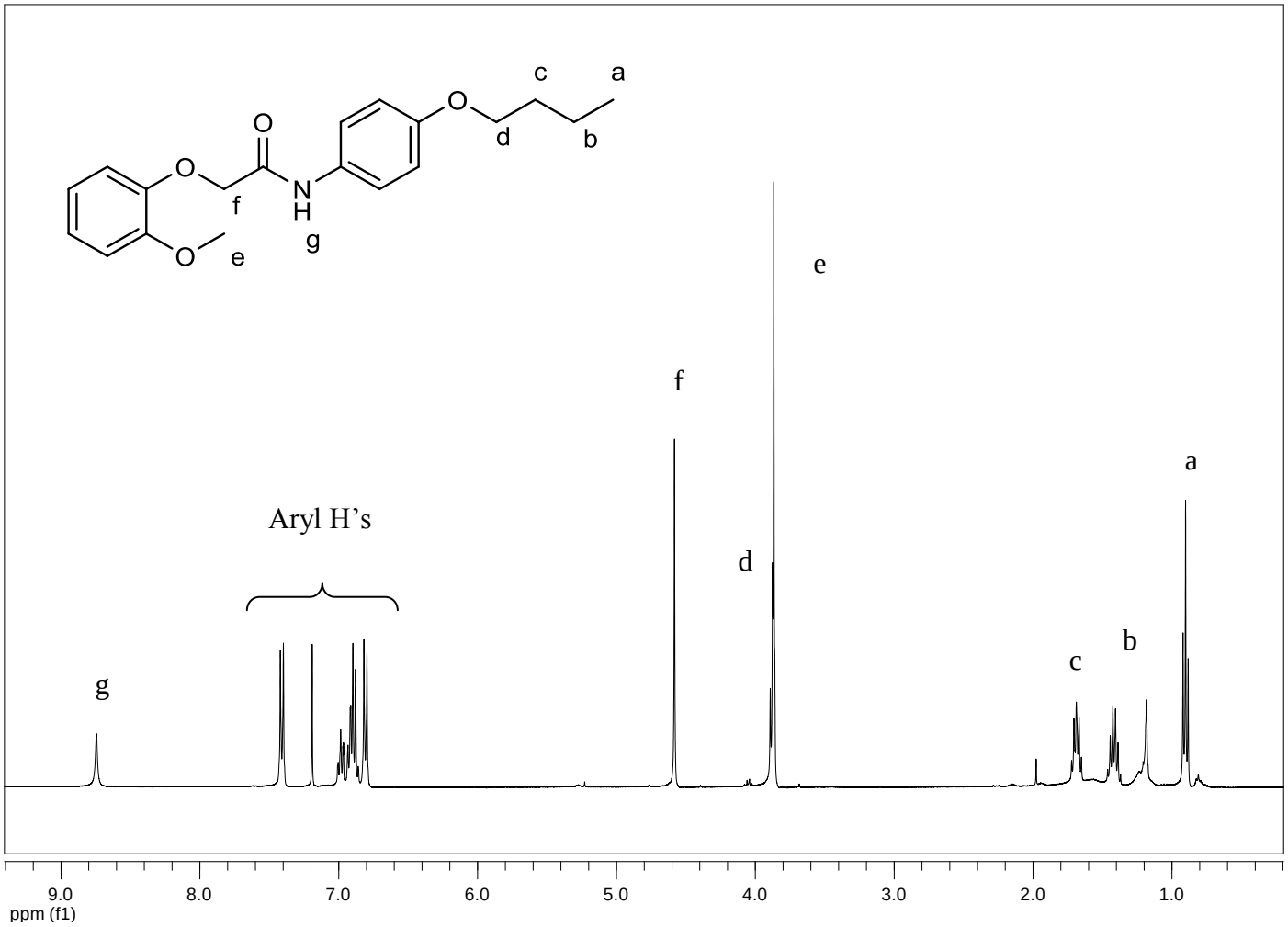


Figure A.24. ^{1}H-NMR Spectroscopy of N-(4-butoxyphenyl)-2-(2-methoxyphenoxy)acetamide.

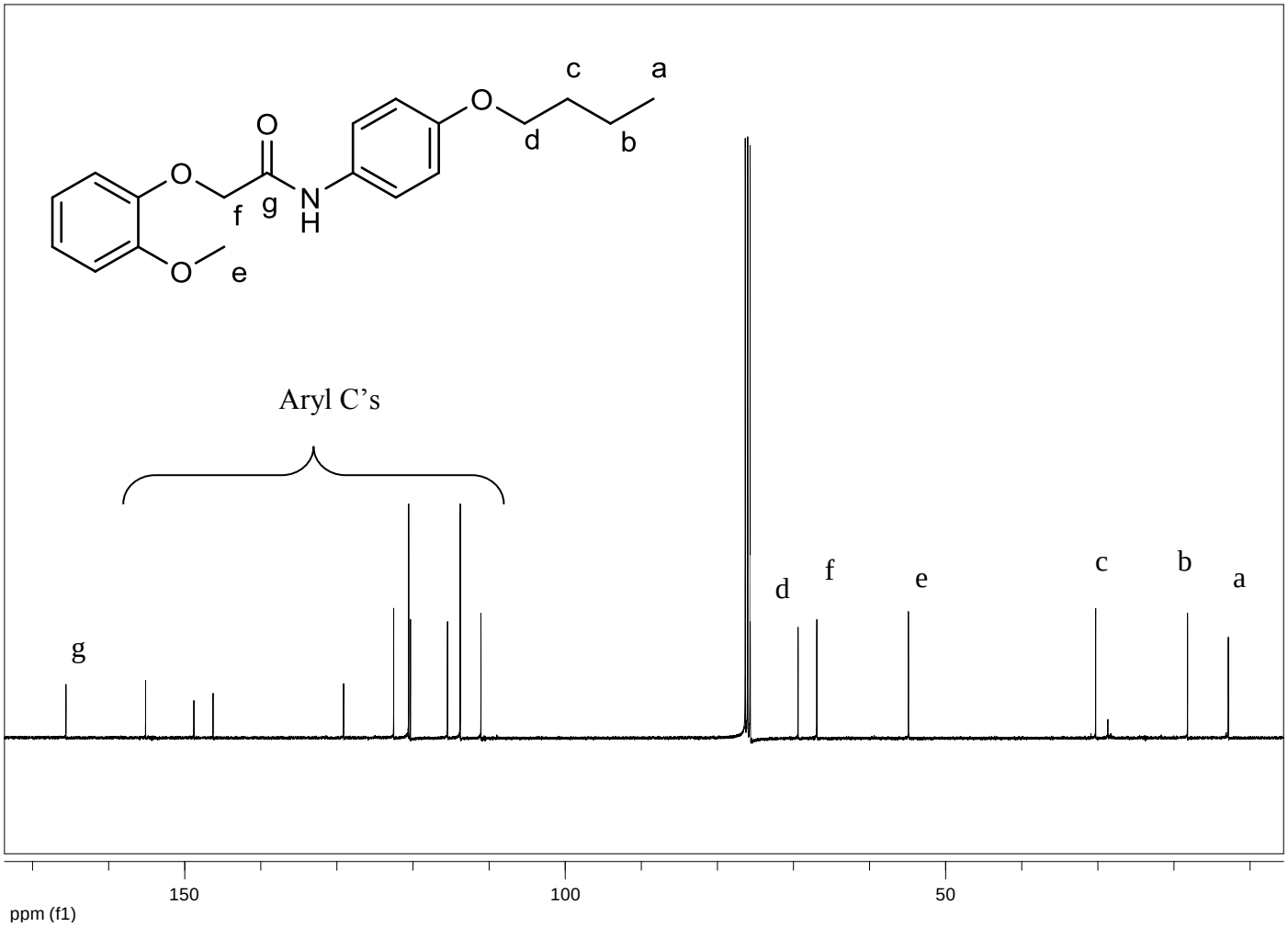


Figure A.25. ^{13}C-NMR Spectroscopy of N-(4-butoxyphenyl)-2-(2-methoxyphenoxy)acetamide.

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