DESIGN AND SYNTHESIS OF DRUG MOLECULES AGAINST PROSTATE CANCER

by

Tuba Şahin

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DESIGN AND SYNTHESIS OF DRUG MOLECULES AGAINST PROSTATE CANCER

APPROVED BY:

Prof. A. Ersin Acar
(Thesis Supervisor) .....

Prof. Metin H. Acar .....

Prof. İlknur Doğan .....

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To My Beloved Father

And My Family

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ABSTRACT

DESIGN AND SYNTHESIS OF DRUG MOLECULES FOR PROSTATE CANCER

Prostate cancer is a common disease in today's world; especially in the western world it is the most prevalent cancer among men. It is believed that there is a correlation between prostate cancer and increased levels of certain hormones. It has been also observed that lowering androgen levels often makes prostate cancers shrink or grow more slowly, and therefore, the target of the treatment becomes the androgen removal.

It is believed that effective inhibitors of the enzymes involved in testosterone and dihydrotestosterone (DHT) biosynthesis could be useful in the treatment of diseases associated with androgen excess in women and in the treatment of androgen-sensitive prostatic hyperplasia and cancer in men.

In the biosynthesis of testosterone and DHT there is a common enzyme, 17-alpha hydroxylase C17,20-lyase (CYP17), utilized both in testicular and adrenal androgen synthesis. Therefore inhibition of this enzyme is a valuable technique to lower or, if possible, to eliminate the availability of androgens to the prostate.

This project started with Structure Based Drug Design (SBDD) studies at Koc University using a computer generated model of the CYP17 enzyme where several thousands of molecules were subject to screening. In this screening study, the docking and binding energies were calculated and a lead compound was found. The *in-vivo* testing of the lead compound showed that the computer model's prediction for the potential activity was right. To increase the observed activity (IC_{50}= 35 micromolar) and specificity against the enzyme, the lead compound needed to be derivatized. The lead compound is constituted of two parts; the naphtyl and the phenyl subunits. In this study the aim was to synthesize lead compound derivatives which have branched alkyl susbstituents on the phenyl ring.

Thus benzoic acids substituted with amino and/or methoxy groups were subjected to functionalization using different techniques to obtain branched alkyl groups on the phenyl unit. Among these, the reaction of the benzoic acid ester derivative with a Grignard reagent proved to be the best approach in the synthesis of the branched alkyl derivatives.

ÖZET

PROSTAT KANSERİNE KARŞI İLAÇ MOLEKÜLLERİNİN DİZAYN VE SENTEZİ

Prostat kanseri günümüzde çok görülen bir hastalıktır; özellikle bati dünyasında erkekler arasında en yaygın olan kanser turudur. Bazı hormonların seviyelerindeki artış ile prostat kanseri arasında bir ilişki olduğuna inanılmaktadır. Ayrıca androjen seviyelerinin düşürülmesinin genellikle prostat kanserinin küçülmesine veya daha yavaş ilerlemesine sebep olduğu görülmüştür; bu yüzden prostat kanserinde tedavi hedefi androjen yoksunluğu haline gelmiştir.

Testosteron ve dihidrotestosteron (DHT) biyosentezinde görev yapan enzimlerin etkili inhibe edilmesinin kadınlarda androjen fazlılığı ve erkeklerde androjene duyarlı prostat büyümesi ve kanseri tedavilerinde yararlı olabileceğine inanılmaktadır.

Testosteron ve DHT biyosentezinde kullanılan ortak bir enzim vardır, 17-alfa hidroksilaz C17,20-laz (CYP17); bu enzim hem adrenal bezler hem de testislerdeki androjen sentezinde kullanılmaktadır. Bu sebeple bu enzimin inhibe edilmesi prostatta androjen varlığının azaltılması ya da mümkünse ortadan kaldırılması hususunda önemli bir tekniktir.

Bu proje Yapıya Dayalı İlaç Dizaynı çalışmasında CYP17 enziminin bilgisayar modeli kullanılarak birkaç bin molekülün taranmasıyla Koç Üniversitesi'nde başlamıştır. Bu taramada moleküllerin bağlanma ve kenetlenme enerjileri hesaplanmış ve bir oncu molekül bulunmuştur. Oncu molekülün *in-vivo* testi bilgisayar modelinin potansiyel aktivite tahminini doğrulamıştır. Enzime karşı gözlemlenen aktiviteyi (IC_{50}= 35 mikromolar) ve spesifikasyonunu arttırmak için oncu molekülün türevlendirilmesi gerekmektedir. Oncu molekül iki kısımdan oluşmaktadır, bunlar naftil ve fenil alt birimleridir. Bu çalışmadaki amaç fenil halkası üzerinde dallanmış alkil grupları bulunan oncu molekül türevleri sentezlemektir.

Bu sebeple amin ve/veya metoksi gruplarına sahip benzoik asit türevleri farklı teknikler kullanılarak fonksiyonelleştirilmiş ve fenil halkasinda dallanmış alkil gruplarına sahip moleküller elde edilmiştir. Bu teknikler arasında benzoik asit ester türevinin Grignard reaktifiyle reaksiyonunun dallanmış alkilli fenil türevi eldesinde en iyi yaklaşım olduğu görülmüştür.

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LIST OF ABBREVIATIONS

$CDCl_3$	Deutorated chloroform
CH_2Cl_2	Dichloromethane
CYP	Cytochrome P-450
CYP 17	17 alpha-hydroxylase cytochrome P-450 (P-450(17) alpha)
DMSO	Dimethyl sulfoxide
EtOAc	Ethylacetate
LHRH	Luteinizing Hormone-Releasing Hormone
PSA	Prostate Specific Antigen
SBDD	Structure-Based Drug Design
SEER	Surveillance Epidemiology and End Results
TLC	Thin Layer Chromatography
THF	Tetrahydrofuran
WHO	World Health Organization

1. INTRODUCTION

1.1 What is Cancer?

Cancer is a leading cause of death worldwide, according to World Health Organization (WHO). Every year more than 11 million people are diagnosed with cancer and seven million people die from the disease around the globe [1]. It is estimated that deaths from cancer worldwide will continue rising, with an estimated 12 million deaths in 2030 [2]. Surveillance Epidemiology and End Results (SEER) statistics show that from 2003 to 2007, the median age at diagnosis for cancer of all sites was 66 years of age. Approximately 1.1 % were diagnosed under age 20; 2.7 % between 20 and 34; 5.7 % between 35 and 44; 14. % between 45 and 54; 22.3 % between 55 and 64; 24.7% between 65 and 74; 21.8% between 75 and 84; and 7.7% 85+ years of age [3].

Cancer is a generic term for a group of diseases which are caused by the division of abnormal cells without control. In this disease cancerous cells can invade other tissues through the blood and lymph systems known as metastasis [4].

Actually the doctors cannot explain why some people develop cancer but others don't. However it has been proved scientifically that some factors increase the chances of developing cancer [5]. The most common risk factors for cancer are:

- Growing older
- Tobacco
- Sunlight
- Ionizing radiation
- Certain chemicals and other substances
- Some viruses and bacteria
- Certain hormones
- Family history of cancer
- Alcohol

1.2. Prostate Cancer

A major cause of death among men prostate cancer [6] is a common disease in today's world; especially in the western world it is the most prevalent cancer among men [7] It is estimated that approximately one in six men will be diagnosed with prostate cancer in their life [8]. In fact American Cancer [9] Society states that prostate cancer is the most common cancer in American men other than skin cancer. It is believed that there is a correlation between prostate cancer and increased levels of certain hormones [10].

It is estimated that three-quarters of prostatic cancer is sensitive to androgens [11], furthermore some scientists suggest that the percentage of tumors which are androgen-dependant is approximately 90 [12]. Androgen can be defined as a type of hormone that promotes the development and maintenance of male sex characteristics [13]. It was found that high levels of androgens promote prostate cell growth, and may contribute to prostate cancer risk in some men [14].

Androgens are important for the normal development and maintenance of the prostate gland [15]. These male sex hormones, in particular 5α-dihydrotestosterone (DHT), which is the reduced metabolite of testosterone, mediate growth and development of the cancerous tissue [11]. Also it has been suggested that prostate cancer risk may be increased in association with high serum concentrations of free testosterone [16].

1.3. Diagnosis and Treatment of Prostate Cancer

It has been found that prostate specific antigen (PSA) production is dependent on androgens, and PSA are associated with individuals having an increased prostate cancer risk, so this information can be used for the early detection of prostate cancer [17]. Actually PSA is not a tumor marker in itself; it is just a physiologic product of normal prostatic epithelial cells. Also it has been discovered that serum PSA concentration is relatively higher in men with prostate cancer compared to those without [18]. Other than PSA testing digital rectal examination (DRE) can be utilized for screening of prostate cancer [19].

The treatment options for localized prostate cancer are radical prostatectomy (The surgical removal of part or all of the prostate gland), external beam radiotherapy or brachytherapy (the insertion of radioactive seeds into the prostate gland), active surveillance and hormone therapy [19].

1.3.1. The Target of the Treatment of Prostate Cancer

As stated earlier high levels of androgens are believed to contribute to prostate cancer risk in some men; especially the main androgens: testosterone and dihydrotestosterone (DHT). The greatest source of androgens in males is the testes, where testosterone is produced and is subsequently converted in peripheral tissue to DHT, the active metabolite [18, 20]. Therefore the target of the disease becomes androgen removal and it is clearly recognized as one of the cornerstones of management in patients with advanced prostate cancer disease [18]. It has been also observed that lowering androgen levels often makes prostate cancers shrink or grow more slowly [21]. However, the testes are not the only source of testosterone; adrenal glands have also been shown to contribute to androgen production [18]; therefore for androgen removal this portion should be taken into account as well.

1.3.2. Androgen Removal

For many years androgen deprivation therapy has been the standard care in advanced prostate cancer [22]; to achieve this, surgical removal of the testicles (orchiectomy) was used as the only way to reduce testosterone [20]. Majority of the patients respond to androgen removal as demonstrated by a decline in PSA levels, slowed progression of metastatic disease, and improved survival [18]. However surgical intervention is not enough for the androgen removal since some amount of androgens are synthesized in the adrenal glands.

1.3.3. Hormone Therapy

The aim of hormonal therapy, androgen deprivation therapy (ADT), is the same: to reduce the amount of testosterone and androgens in the bloodstream, depriving prostate cancer cells of the fuel they need to grow. In this approach three ways of interfering with testosterone have been used [20]:

- Using drugs to interrupt testosterone production (luteinizing hormone-releasing hormone [LHRH] agonists ketoconazole and aminoglutethimide)
- Using drugs that block androgen action (antiandrogens: androgen receptor antagonist)
- Taking estrogen

However these drugs have serious side effects as many other drugs do as well. For example ketoconazole causes serious hepatic dysfunction and gastrointestinal disturbances. Ketoconazole, an imidazole antifungal agent, inhibits testicular and adrenal androgen synthesis by inhibiting the cytochrome P-450-dependent 14-demethylation step in the steroid synthesis pathway [23]. However, clinical use of this agent was limited due to its significant side effects caused by non-selective inhibition of several other cytochrome P-450 enzymes [18, 24]. Antiandrogens may cause anemia, nausea and liver problems to list some of the many side effects. Estrogens may cause water retention, breast growth and tenderness.

As these treatment options have many undesirable side effects, new therapeutic approaches, which are more efficient and effective with less adverse effects, are needed for the management of prostate cancer.

1.4. New Horizons

Over the past 20 years, most efforts have focused on maximizing the degree of androgen suppression therapy by combining agents that inhibit or block both testicular and adrenal androgens [17]. For this reason scientists tried to understand the mechanism of testosterone and DHT biosynthesis which are important androgens stimulating the prostate growth together with cancerous cells.

As mentioned earlier androgens play an important role in the development of prostate cancer; therefore suppression of release, synthesis, or actions of androgenic hormones has become a major therapeutic option in the management of this malignancy [11]. It is believed that effective inhibitors of the enzymes involved in testosterone and DHT biosynthesis could be useful in the treatment of diseases associated with androgen excess in women and in the treatment of androgen-sensitive prostatic hyperplasia and cancer in men [25]. Therefore clear understanding of the mechanism of the formation of testosterone and DHT is crucial to be able to choose a new target for a better therapeutic approach.

1.4.1. Biosynthesis of Testicular and Adrenal Androgens

In the biosynthesis of androgens many enzymes are necessary some of which belong to the family of cytochrome P-450 (CYP) enzyme system. CYP enzyme system consists of a large and ubiquitous family of medium-size proteins that contain a single iron protoporphyrin IX prosthetic group [26]. There are more than 50 enzymes in the CYP enzyme system and these enzymes are involved in the metabolism of xenobiotics, pharmaceuticals, and a variety of endogenous and exogenous steroids including testosterone [27].

The synthesis of testicular androgens involves conversion of pregnenolone to testosterone by a series of microsomal enzymes [28] CYP17, which is the key enzyme in androgen biosynthesis, catalyses both the 17α-hydroxylation of pregnenolone and progesterone and the subsequent 17,20-lyase reaction cleaving the C17–C20 bond to yield the 17-keto androgens androstendione and dehydroandrostendione (DHEA), the precursors of testosterone [29] as seen in Figure 1.1.

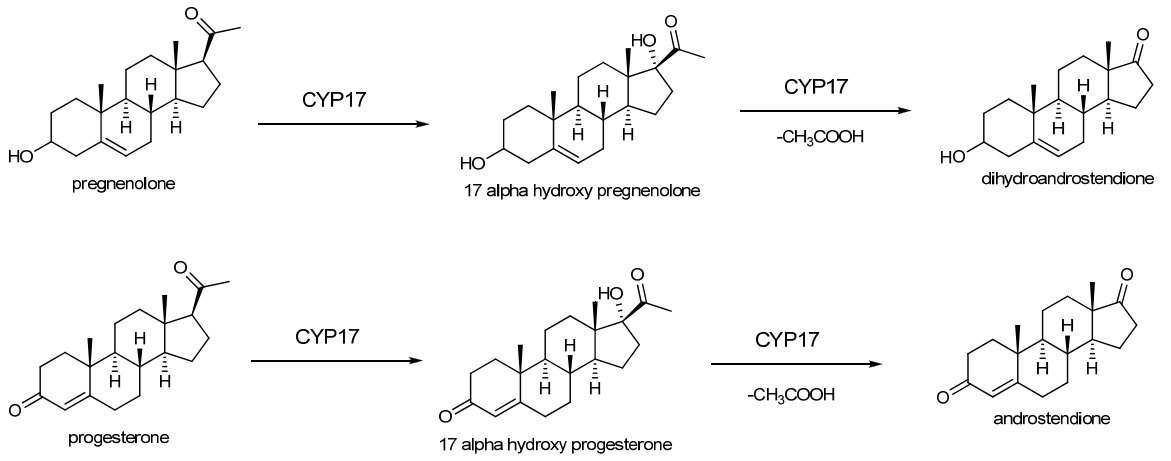


Figure 1.1. Reactions catalyzed by CYP17

1.4.2. Target Selection

To be able to eliminate androgen synthesis totally, a target should be chosen in such a way that by stopping the target both the testicular and adrenal androgen syntheses should be stopped. As mentioned before for the treatment of prostate cancer, testosterone and DHT concentrations should be lowered. For this reason, biosynthetic pathways leading to the production of these androgens should be analyzed and an appropriate target should be chosen.

Since the problem with existing therapeutics is the deficiency in coping with adrenal androgen synthesis the androgen production in adrenal glands should also be examined. It can be understood from the schematic representation of the synthesis of androgens in Figure.1.2 [30] that there is a common enzyme, 17-alpha hydroxylase C17,20 lyase (CYP17), utilized both in testicular and adrenal androgen synthesis. The biosynthesis of testicular and adrenal androgens depends on the action of CYP17 and inhibition of this enzyme is a valuable technique to lower or, if possible, to eliminate the availability of androgens to the prostate [11].

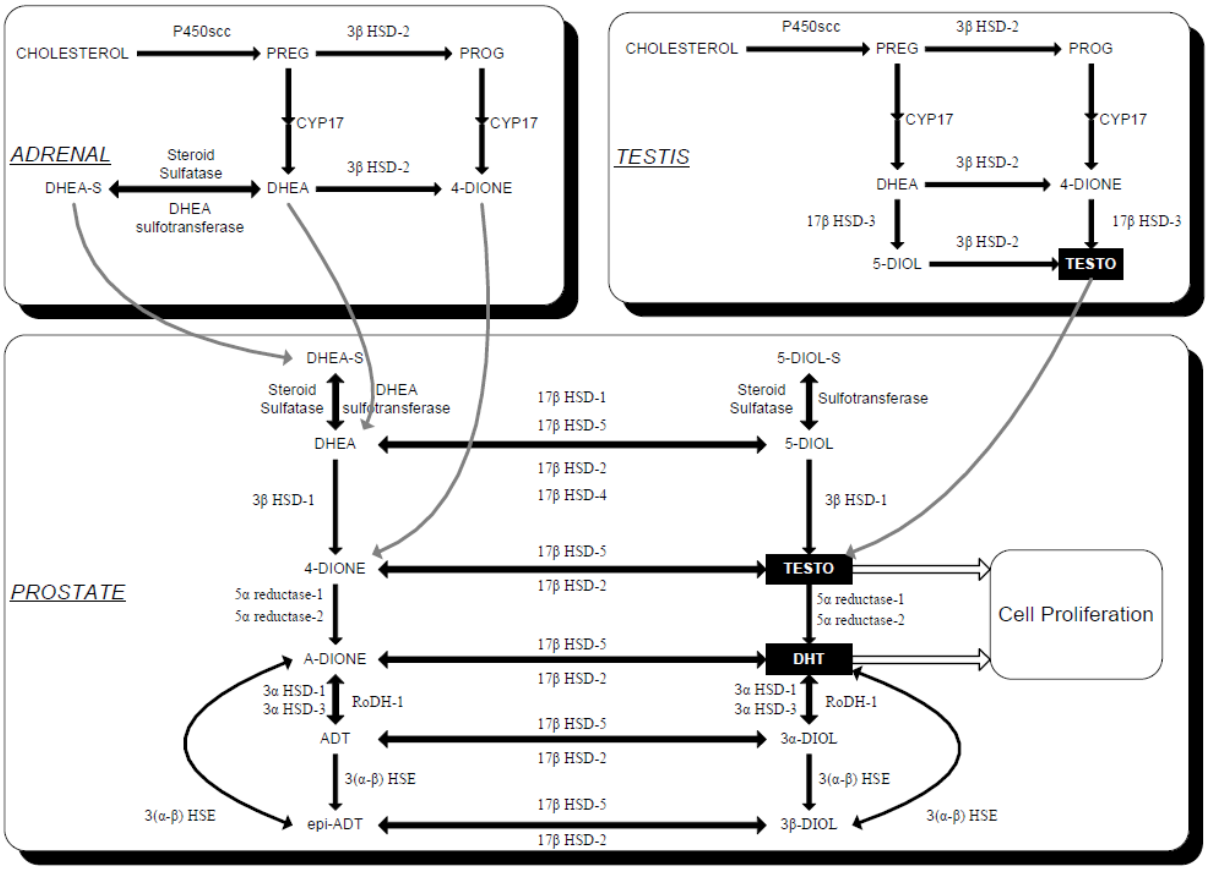


Figure 1.2. Synthetic pathways leading to the formation of androgens

1.5. Drug Design Problem

The drug design problem has been evaluated by both experimental and computational approaches. The experimental methods depend on high throughput screening methods; however this approach has some disadvantages: the number of available chemical substances for testing is small, experimentation costs are high and it is possible that the chemical substance may be interacting with another active site of the target protein. Computational simulation methods test chemical structures stored in databases for binding to the active site of the protein. In these methods chemical substances are tested for activity on the target protein. However this approach has some disadvantages as well: only a very small number of chemical structures in databases are available, databases often do not contain suitable molecules for the target protein [31].

1.6. Prior Art

1.6.1. Structure-Based Drug Design

Steps in drug discovery, design and development can be summarized as in Figure1.3

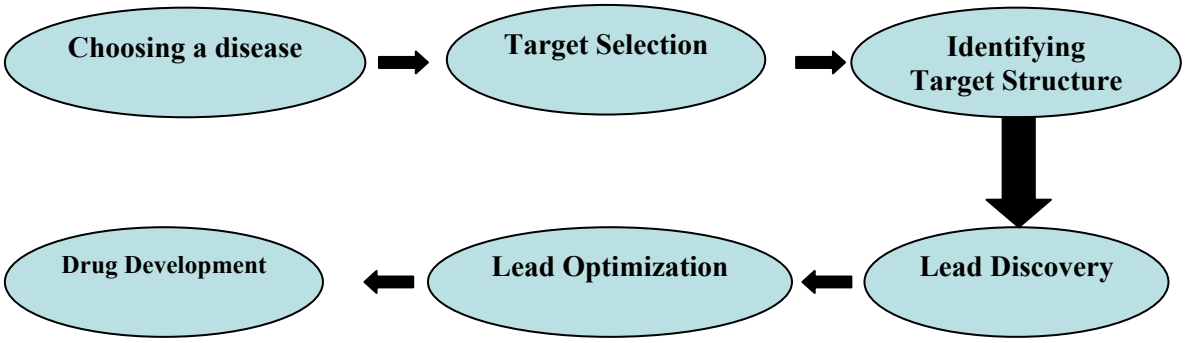


Figure 1.3. Steps in drug discovery, design and development

Structure-based drug design (SBDD) has been accepted as an established approach in pharmaceutical industry and academia [32] and this approach is used in this current project.

In this approach enzyme structure is crucial for understanding the catalytic activities together with substrate and reaction selectivity; therefore computational modeling of the target enzyme constitutes the first step of the design. The next step in the drug discovery process is to identify drug candidates that will interact with the binding site of the target protein. In general, a large number of chemical substances that may be suitable as drugs are tested on the target protein to observe the effect of the interaction. The chemical substances that show any level of interaction are categorized as a hit. The drug candidates interact with the active site to have therapeutic effect by blocking, accelerating, decelerating, reversing or initiating reactions depending on the way to cure the disease.

The active site of the protein is a space to be filled with a molecule that complements it in terms of shape, charge, and other interacting components [33]. To evaluate the relationship of a candidate molecule with the enzyme some parameters are examined. The two most important parameters are docking and binding energies. In docking, the interaction energy between the enzyme and the substrate (the drug molecule) is computed

by summing the energy contributions between all atoms of the two molecules forming the enzyme-substrate complex. The docking energy is considered as the sum of Van der Waals' and electrostatic energies among all the atoms of the complex. The comparison between the two docking energy values can be a useful tool to verify the stability of the complexes. This parameter represents the gain of potential energy due to interactions between the molecules forming the complex with respect to the sum of the energy of each molecule in their free state [34].

This phase ends up with a list of drug candidates which have good docking and binding energies. After that the compounds at the list are synthesized and tested for activity against the enzyme CYP17. The activities of drug molecules are compared by using the half maximal inhibitory concentration (IC_{50}). This quantitative measure indicates how much of a particular drug is needed to inhibit a given biological process (or component of a process, in this case an enzyme) by 50 %.

1.6.2. Computational Modeling of the Enzyme CYP17

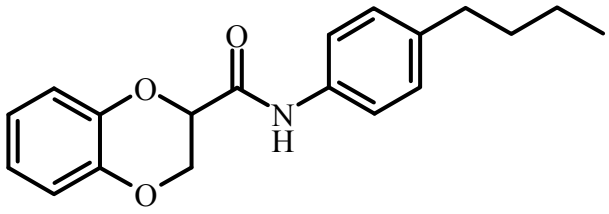
Enzyme structure is crucial for understanding the catalytic activities, substrate and reaction selectivity. Therefore, knowing the structure of CYP17 is mandatory for designing specific drugs to inhibit the catalytic activities of the enzyme. Although a crystal structure of CYP17 has not been reported in the literature or databases, a computer generated model exists as shown in Figure 1.4 with PDB ID code 2c17(cited therein).The binding site of the cytochrome P450 is defined with heme group, as the floor of the pocket; and surrounding its residues [31].



Figure 1.4. Model of CYP 17

At this point of the project computational chemists at Koc University worked on the model of the enzyme and they determined types of interactions between the enzyme and potential substrate. Based on the simulations they were able to reveal that the enzyme has a smaller substrate binding pocket than the previous models, letting only the planar substrates like steroids to accommodate. The comparison of docking and binding energies for two distinct groups of compounds was studied: steroidal and steroidomimetic compounds. It is argued in the literature that steroidomimetics may offer better results compared to steroidal drugs due to interaction of steroidal drugs with some other steroid eceptors including estrogen and gestagen receptor resulting in side effects [35].

Then the screening started: database search for candidate compounds was done and the lead compound in Figure 1.5 was identified with docking and binding values of -9.60 and -7.70 respectively having an IC_{50} value of 35μM *in vivo* testing.



1

Figure 1.5. Structure of the lead compound

1.6.3. Substituents on the Lead Copmpound and Their Possible Functions

To optimize the lead compound some changes are made on the structure. In Figure 1.6 a summary of the changes, substituents and their possible functions in the inhibition of CYP17 can be seen.

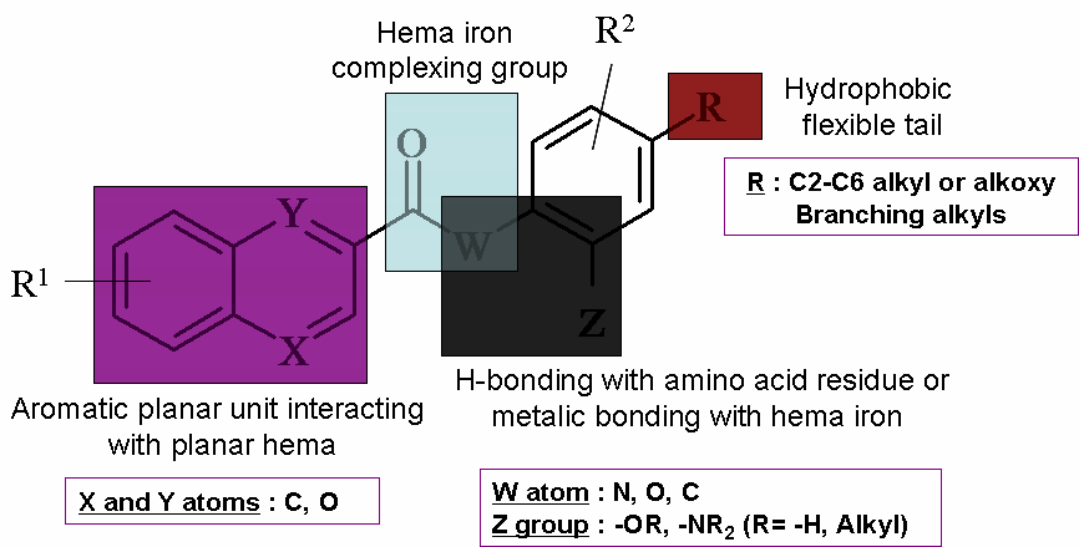
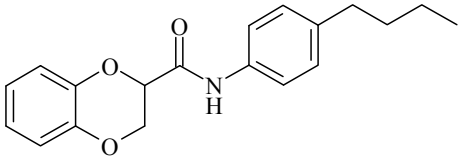


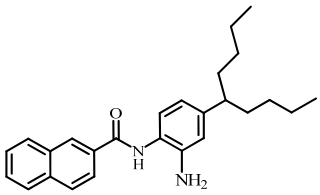
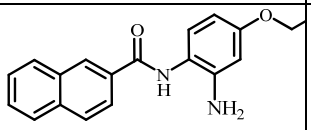
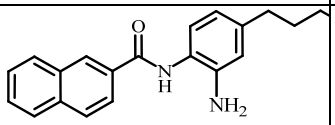
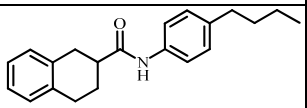
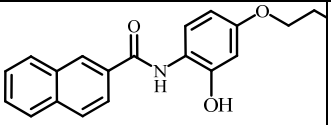
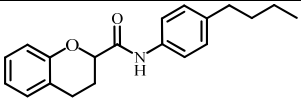
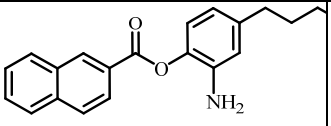
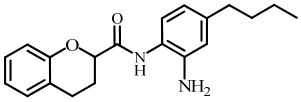
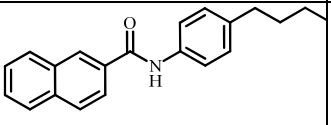
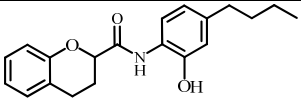
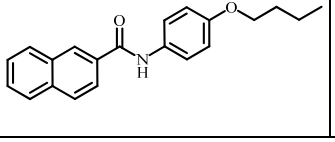
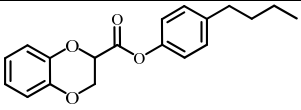
Figure 1.6. Substituents on the lead copmpound and their possible functions

1.6.4. Some Results of Docking and Binding Studies

Some of the docking and binding energies can be seen in Table 1.1.

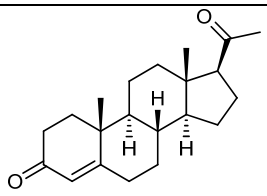
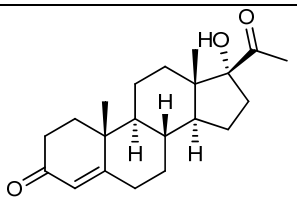
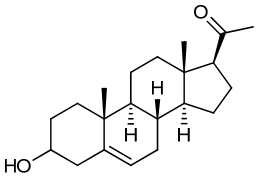
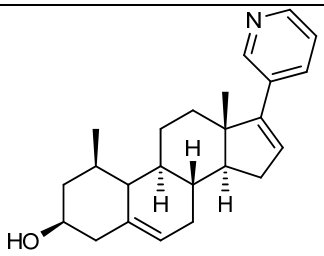
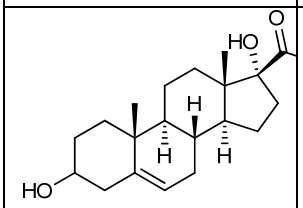
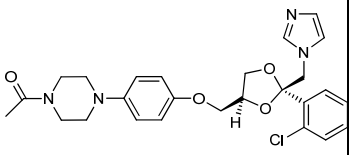
Table 1.1 Some of the docking and binding energies

Lead Compound	Docking	Binding
	-9.60	-7.70

Lead compound Derivatives	Docking	Binding	Lead compound Derivatives	Docking	Binding
	-11.85	-9.44		-10.66	-8.39
	-10.56	-8.47		-9.50	-7.85
	-10.38	-8.10		-9.43	-7.61
	-10.14	-8.26		-9.31	-7.33
	-9.89	-7.77		-9.23	-7.30
	-9.85	-7.63		-8.54	-6.671

1.6.5. Docking and Binding Studies of Natural Substrates and Current Drug Molecules

Table 1.2. Docking and binding studies of natural substrates and current drug molecules

Compounds	Docking	Binding	Compounds	Docking	Binding
 Progesterone	-10.04	-9.73	 17hydroxyprogesterone	-9.61	-9.68
 Pregnenolone	-9.71	-9.44	 Abiraterone	-10.15	-10.10
 17hydroxy pregnenolone	-9.30	-9.33	 Ketoconazole	-11.13	-9.33

1.7. To Find the Tightest Fit for the Enzyme

It is possible to optimize a lead compound into the tightest fit for an enzyme by making small structural changes on it. The effect will be observed by a lower IC_{50} value and it will be a more potent drug. Also by adding structural units to the lead compound its structure becomes more complicated but yet more specific to its target. For example by making changes on two lead compounds against kinase enzyme (developed by Astex and Vernalis/Novartis respectively) it was possible to increase the activities of the drugs by 1600-2500 times as can be seen in Figue 1.7.

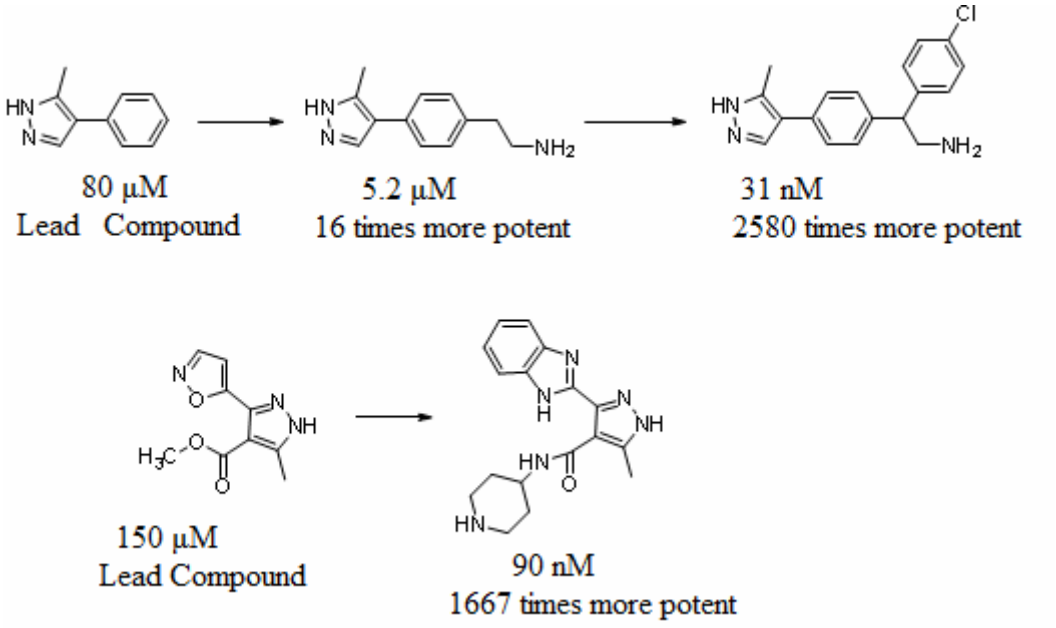


Figure 1.7. The effect of changes on lead compound to ic_{50} values

1.8. Selectivity Against Other Enzymes

As mentioned earlier in CYP enzyme system there are more than 50 enzymes and their common feature is that they contain a single iron protoporphyrin IX prosthetic group. Since the selectivity of a drug molecule with respect to its target is very important to eliminate the side effects, the drug candidates the interaction between the drug molecule and the other non-target enzymes should be minimal in an ideal drug. Therefore drug candidates should also be tested against some other enzymes like CYP11B1, CYP11B2, CYP3A4, and CYP19 [36-38] to evaluate the selectivity and possible side effects.

2. AIM OF THE STUDY

The lead compound in this research can be assessed in two parts: left-hand side and right hand side as can seen in Figure 1.8

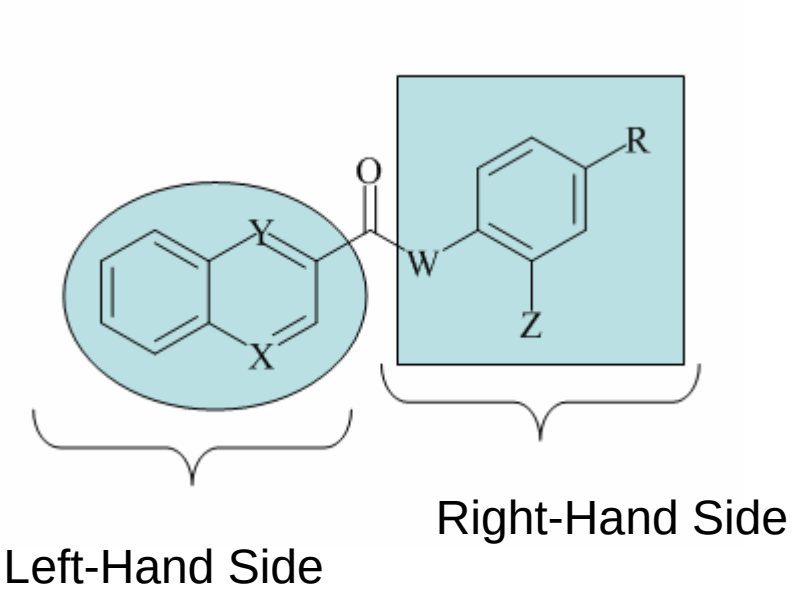


Figure 2.1. Parts of the lead compound

At this point of the project X and Y atoms were chosen as C atoms and therefore some available naphthoic acid derivatives was used as the left-hand side. For the right-hand side phenyl derivatives with W as N atom and Z as N or O atoms were used. From the first entry in Table1.1 it can be seen that the drug candidate with branched R group has higher binding and docking values compared to drug candidates with linear R groups. Also if the docking and binding values of the first entry in Table 1.1 are compared to the values of the natural substrates and current drug molecules in Table 1.2 it is understood that the drug candidate with branched R group has comparable energies to the natural substrates and current drugs. Therefore phenyl derivatives with branched alkyl groups (-R) were targeted in this thesis. Then these synthesized phenyl derivatives will be coupled with various available naphthoyl chloride derivatives to reach final products.

3. RESULTS AND DISCUSSION

In this study the aim was to synthesize the derivatives of the lead compound 1 active against prostate cancer. Since the derivative which has the branched alkyl group (Table 1.1, entry 1) had relatively higher binding and docking values, the derivatives with branched alkyl substituents were targeted.

The general synthetic approach used was to start from a benzoic acid derivative which had the desired substituents at the desired positions on the aromatic ring. To synthesize the branched alkyl group, the acid was first converted to methyl ester, and then a Grignard reaction was done on the ester with the desired alkyl magnesium bromide reagent. The product of this reaction should in principle have identical branches on the benzylic position since the Grignard reagent first attacks the ester and forms a ketone then the Grignard reagent attacks the resulting ketone *in-situ* to give an alcohol after the addition of water. Because of this double attack of the Grignard reagent on the ester, the reagent was used twice as much the ester. Therefore to obtain different type of branches, the structure of the alkyl magnesium bromide can be changed as desired. After Grignard reaction the produced alcohol should be reduced to alkyl group and for this transformation some possible routes have been identified and some of them have been tried (Figure 3.2).

For the verification of these transformations ^{1}H-NMR Spectroscopy, ^{13}C-NMR Spectroscopy, Liquid Chromatography-Mass Spectroscopy and Elemental Analysis methods have been used.

For the right-hand side of the compounds diamino substituted phenyl, amine-hydroxy substituted phenyl and dihydroxy substituted phenyl rings with branched alkyl substituents on the benzylic position have been synthesized.

3.1. Synthetic Strategies Used in Branched Alkyl Diamino Phenyl Derivative

Synthetic strategies undertaken for the synthesis of branched alkyl diamino phenyl derivative in this study can be seen in Figure 3.1

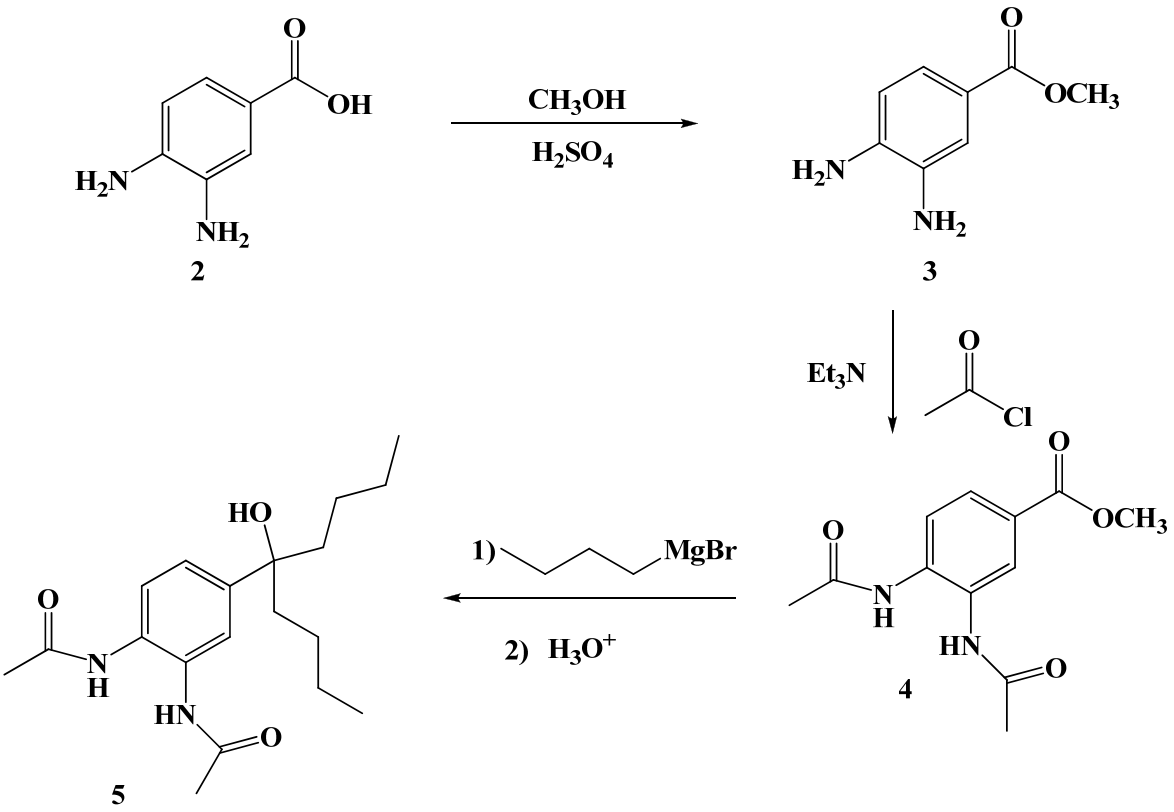


Figure 3.1. Synthetic strategies used in branched alkyl diamino phenyl derivative

For the synthesis of diamino phenyl derivative, compound 2 was used as the starting benzoic acid derivative. The acid was converted to methyl ester via a Fischer esterification reaction in the presence of concentrated sulphuric acid as a catalyst in CH_3OH (74 % yield). The isolated yield of this reaction is lower than it should be because of the high solubility of the product due to two amine groups in aqueous phase.

To achieve the branched alkyl substitution on the benzylic position a Grignard reaction was going to be used; however first the amine substituents on the benzene ring had to be protected since a Grignard reaction cannot be done on a compound with free amine groups. For protection, the amines were acetylated with acetyl chloride in the presence of triethyl amine with 85 % yield.

The Grignard reaction was done on compound 4 by using *n*BuBr and magnesium metal in dry THF. Up to this point the yields of the reactions were usually high; however in the Grignard reaction too many side products were formed which both lowered the yield and made the purification process more complicated. For the purification first column chromatography using various ratios of various solvents has been tried. Then the purification was done first by dissolving the crude product in 3 mL of CH_3OH then precipitating in water. After precipitation the number of impurities was decreased and the product was further purified with column chromatography using silica gel as the packing material and CH_2Cl_2 as the eluent phase. The isolated yield was found to be only 35 % due to the formation of side-products.

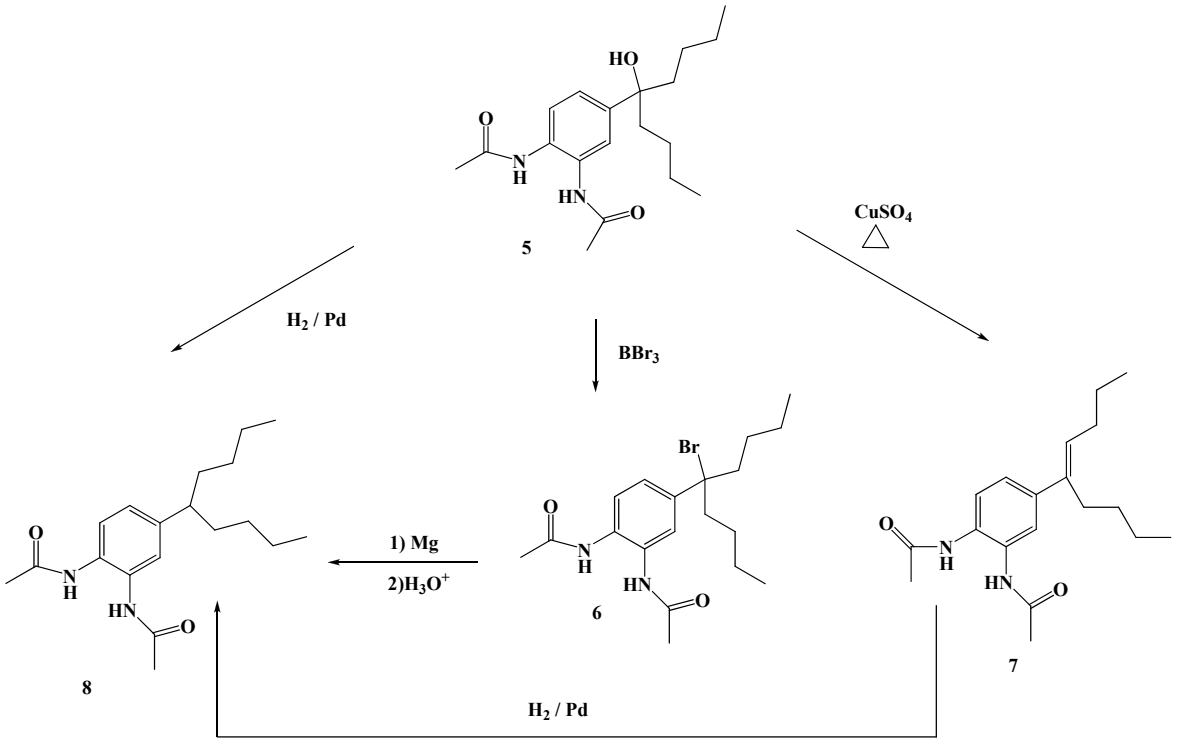
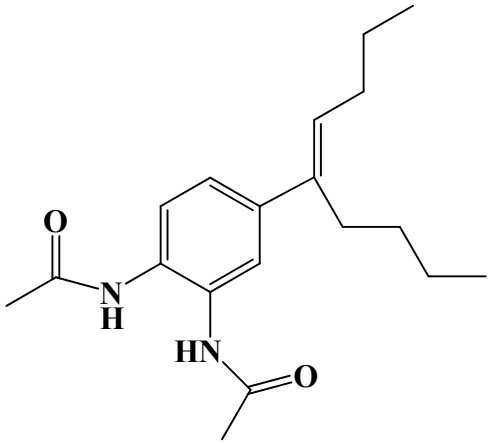


Figure 3.2. Reduction of alcohol to alkyl in branched alkyl diamino phenyl derivative

To remove the benzylic alcohol formed in Grignard reaction (compound 5) three different possible routes as can be seen in Figure 3.2 have been investigated. First the bromination pathway was done and it was found that this reaction did not result in bromination at the benzylic position. In the NMR analysis there were two triplets at 5.54

and 5.33 (J= 6.8) which indicated the formation of compound 7 instead of compound 6 with its E and Z isomers (Figure 3.3). Since this route proved to be unsuccessful in our synthesis the other possible reactions needs to be explored in future.



7

Figure 3.3. (E)-N,N'-(4-(non-4-en-5-yl)-1,2-phenylene)diacetamide

As a future work, after the formation of compound 8 as summarized in Figure 3.4 it is planned to be deprotected by using 20 % sulphuric acid, and then coupled with naphthoyl chloride. After the coupling reaction the final target molecule, compound 12, should be separated from other possible products, compounds 10 and 11, by using appropriate purification methods.

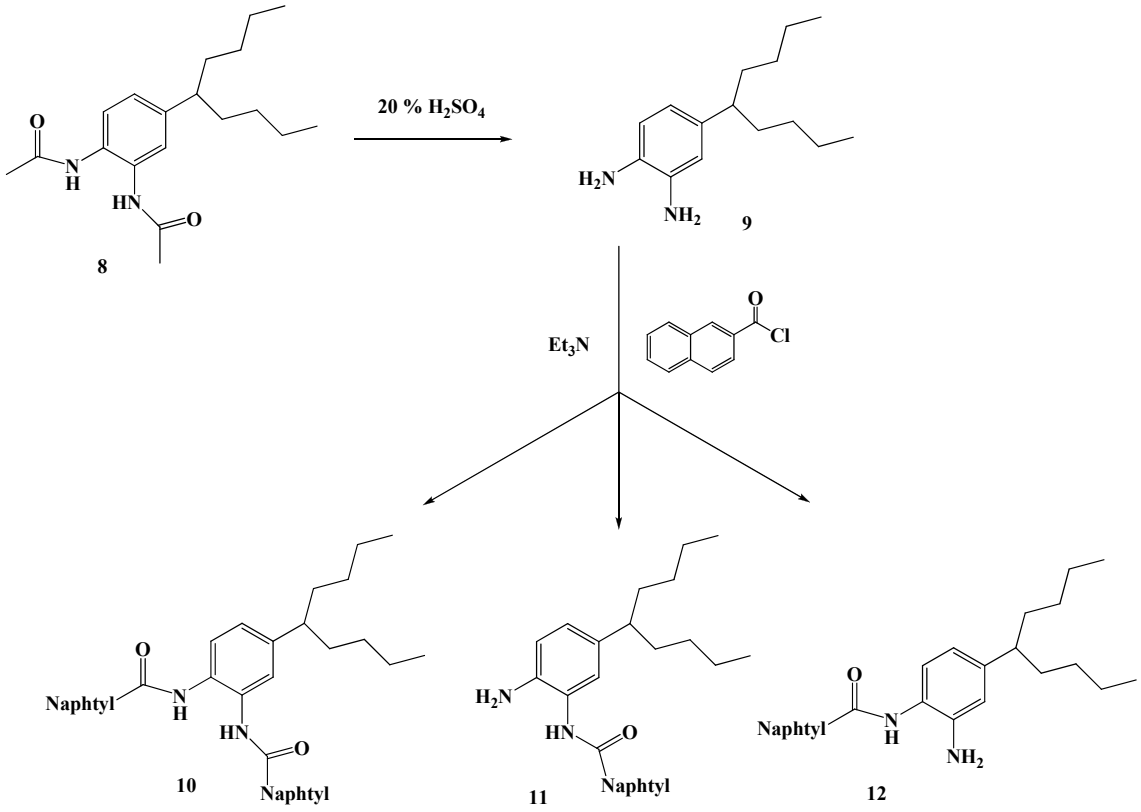


Figure3.4. Deprotection and coupling with naphthoyl chloride in branched alkyl diamino phenyl derivative

3.2. Synthetic Strategies Used in Branched Alky Amine-Hydroxy Phenyl Derivative

Synthetic strategies undertaken for the synthesis of branched alkyl amine-hydroxy phenyl derivative in this study can be seen in Figure 3.5

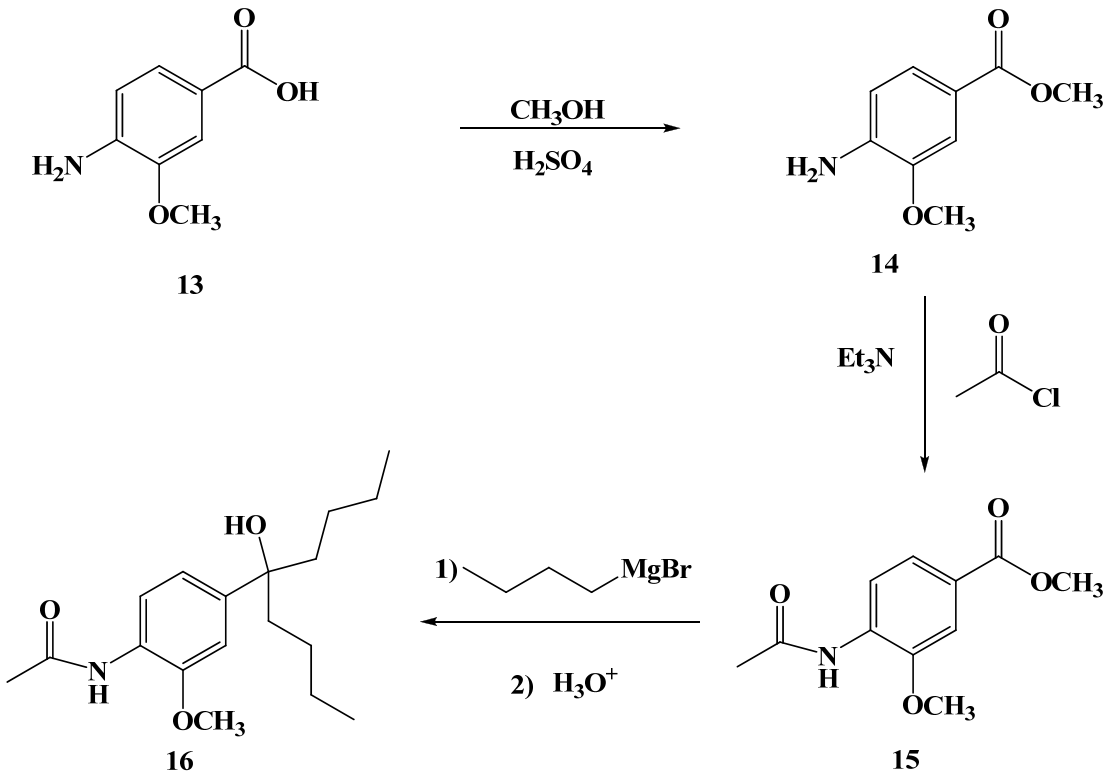


Figure 3.5. Synthetic strategies used in branched alkyl amine-hydroxy phenyl derivative

For the synthesis of amine-methoxy phenyl derivative, compound 12 was used as the starting benzoic acid derivative. The acid was converted to methyl ester via a Fischer esterification reaction in the presence of concentrated sulphuric acid as a catalyst in CH_3OH (96.5 % yield).

To achieve the branched alkyl substitution on the benzylic position a Grignard reaction was going to be used; however first the amine substituent on the benzene ring had to be protected since a Grignard reaction cannot be done on a compound with a free amine group. For the protection, the amines were acetylated with acetyl chloride in the presence of triethyl amine with 87 % yield.

The Grignard reaction was done on compound 15 by using *n*BuBr and magnesium metal in dry THF. Up to this point the yields of the reactions were usually high; however in the Grignard reaction too many side products were formed which both lowered the yield and made the purification process more complicated. For the purification first column chromatography using various ratios of various solvents has been tried. Then the purification was done first by dissolving the crude product in 3 mL of CH_3OH then precipitating in water. After precipitation the number of impurities was decreased and the product was further purified with column chromatography using silica gel as the packing material and CH_2Cl_2 as the eluent phase. The isolated yield was found to be 67 % which is higher than the yield of Grignard reaction of compound 4.

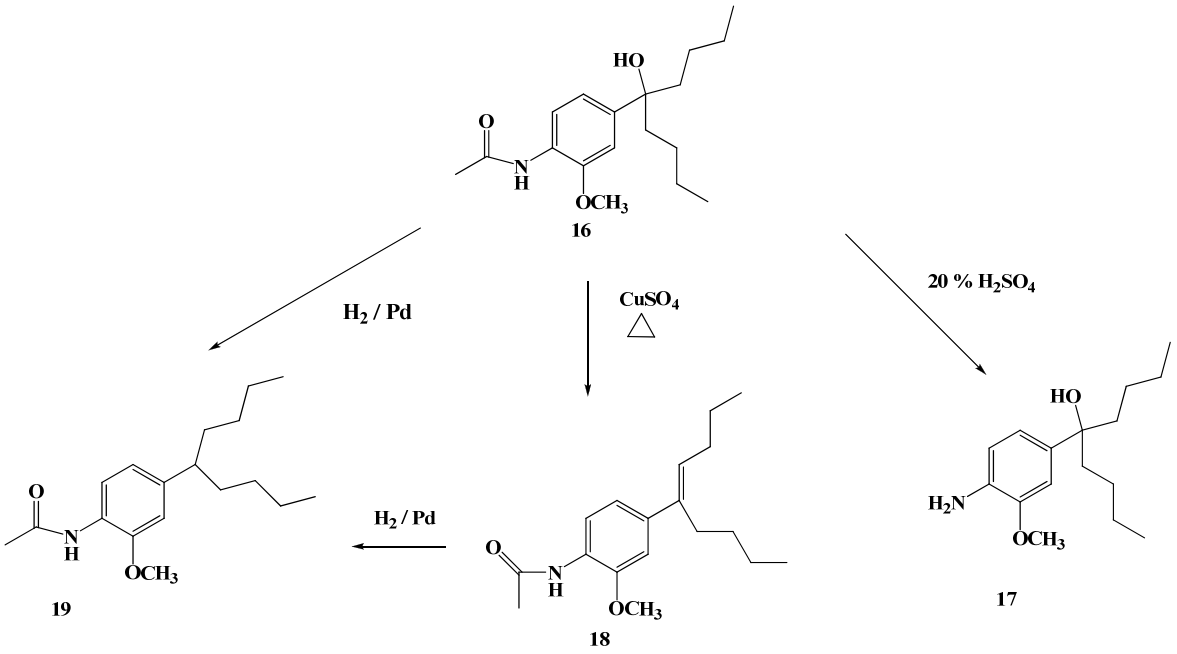
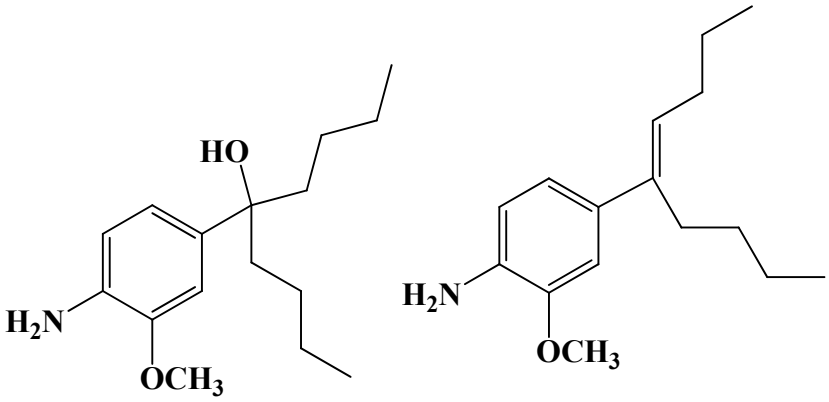


Figure 3.6. Reduction of alcohol to alkyl in branched alkyl amine-hydroxy phenyl derivative

To remove the benzylic alcohol formed in Grignard reaction (compound 16) two different possible routes as can be seen in Figure 3.6 have been identified. One method was to employ hydrogenolysis using palladium and hydrogen gas, the second was to obtain a double bond upon dehydration of the benzylic alcohol followed by the reduction of the double bond by a hydrogenation reaction. In both methods, whether the amine and the phenol were protected or not did not matter.

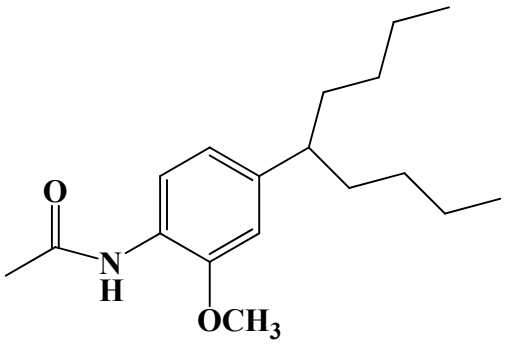
Thus, when first the deprotection was targeted and carried out using 20 % sulphuric acid and it was found that this reaction not only deprotected the amine group; but also resulted in elimination of water at the benzylic position. In the NMR analysis, there were two triplets at 5.48 and 5.30 (J= 7.8) which indicated the formation of compound 20 instead of compound 17 with its E and Z isomers (Figure 3.7).



17 20

Figure 3.7. Deprotection of compound 16

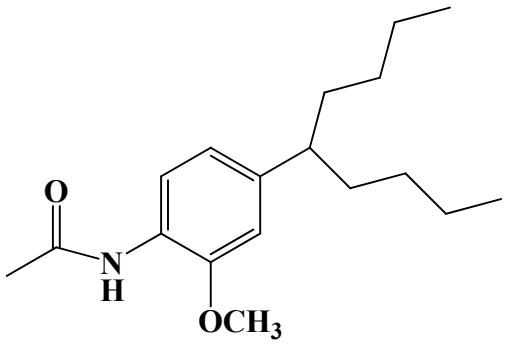
The yield of the deprotection-dehydration reaction is not available because it was run in small scale. Also, the dehydration of the alcohol at the benzylic position has been tried on the protected amino derivative 16 with anhydrous Cu_2SO_4 as catalyst, and compound 18 (Figure 3.8) was synthesized successfully again in small scale. The hydrogenation of the double bond, in principle, should then lead to the target intermediate 19.



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Figure 3.8. (E)-N-(2-methoxy-4-(non-4-en-5-yl)phenyl)acetamide

Finally the direct hydrogenolysis of compound 16 in the presence of palladium as catalyst to obtain the desired branched alkyl derivative 19 was investigated. This reaction was carried out in small scale but due to the formation of large number of impurities and problems in the purification it is not known if the desired compound is synthesized; so as a future work this reaction is planned to be run again in a larger scale to be able to isolate the products.



19

Figure 3.9. N-(2-methoxy-4-(nonan-5-yl)phenyl)acetamide

As a future work (Figure 3.10), after the synthesis of compound 19, a deprotection will be done by using 20 % sulphuric acid to obtain compound 21. Then, compound 21 will be coupled with naphthoyl chloride to form compound 22. Then the methoxy group on compound 22 will be converted to hydroxyl group by using BBr_3. If this reaction is done before coupling with naphthoyl chloride there will be a competition between nitrogen and oxygen atoms about the attack to the naphthoyl chloride. Therefore this reaction is planned to be the last step to of the synthesis to avoid the competition.

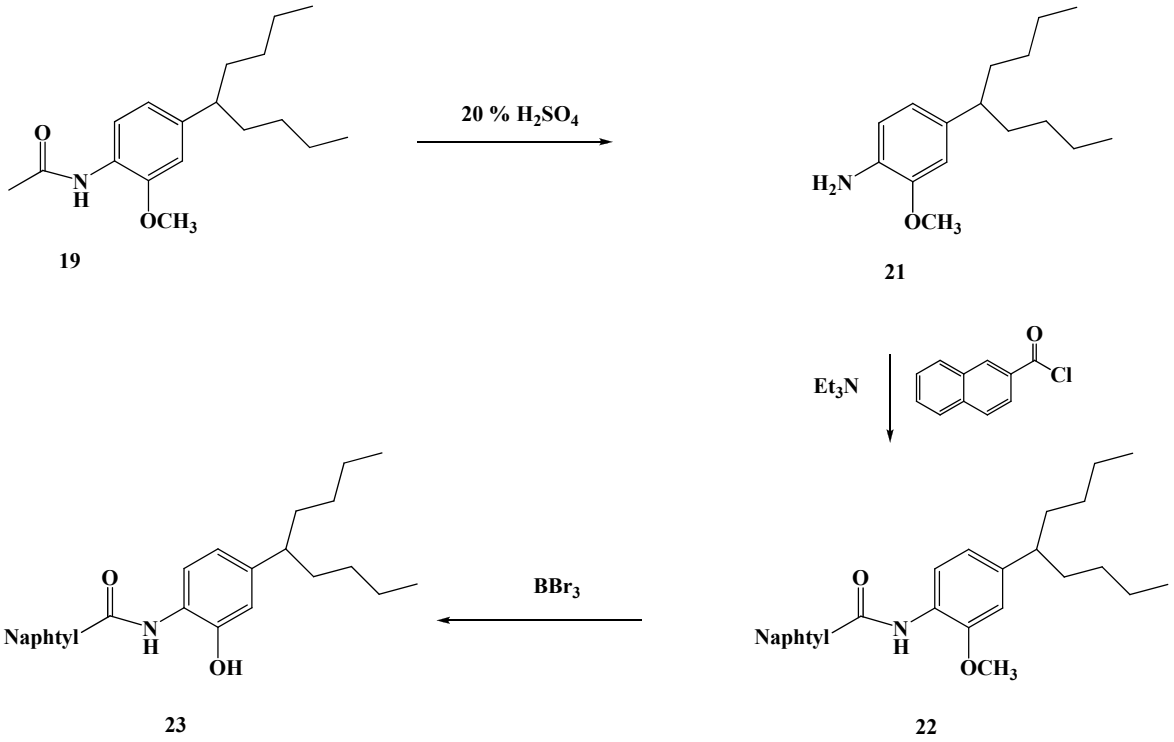


Figure 3.10. Deprotection and coupling with naphthoyl chloride in branched alkyl amine-methoxy phenyl derivative

3.3. Synthetic Strategies Used in Unsymmetrical Branched Alkyl Dihydroxy Phenyl Derivative

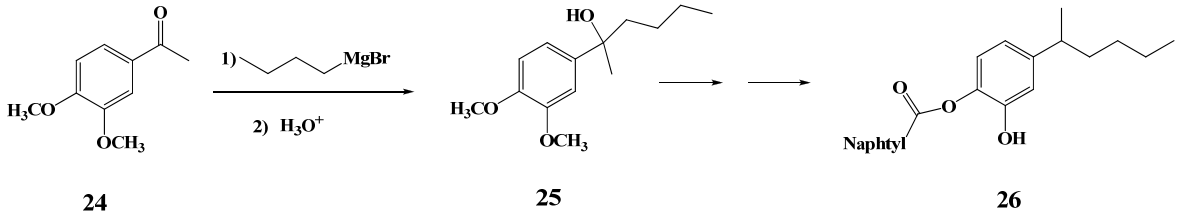


Figure 3.11. Synthetic strategies used in unsymmetrical branched alkyl dihydroxy phenyl derivative

In this synthesis Grignard reaction was done successfully; however it was decided that symmetrically branched alkyls should be synthesized first. Therefore this synthesis was suspended for the time being.

4. EXPERIMENTAL

4.1. Methods and Materials

All chemicals were used as received from manufacturer (Merck, Aldrich, Alfa Aesar, Riedel de Haen). Dry solvents (CH_2Cl_2, THF, toluene) was obtained from ScimatCo Purification System, other dry solvents were dried of 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

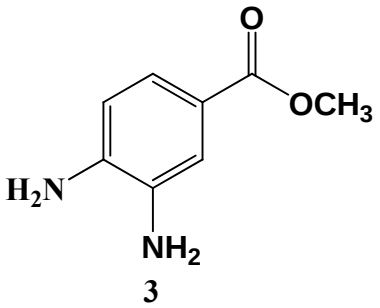
Thin layer chromatograhy plates were viewed under 254 nm UV lamp. Infrared spectroscopy was carried out on Thermo Scientific Nicolet 380 FT-IR spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Varian Gemini 400 MHz spectrometer Varian Associates, Palo Alto, CA) in $CDCl_3$, DMSO and CD_3OD as solvent at the Advanced Technologies Research and Development Center at Boğaziçi University.

4.3. Synthesis of the Derivatives

4.3.1. Methyl 3,4-diaminobenzoate (3)

The synthesis was done using Fischer esterification method. The experiment was carried out under inert atmosphere using nitrogen. Compound 2 (2.00 g, 0.013 mol) and methanol (20 mL) were added to a 50 mL three necked round bottom flask fitted with a condenser and magnetic stirrer, the solution was refluxed at 65°C. Concentrated sulphuric acid (2 mL) was added in a dropwise manner via an injector to the reaction flask. The mixture was stirred for 3 hours. The reaction progress was monitored by thin layer chromatography (TLC) using silica gel plates and 2 CH_3OH / CH_2Cl_2 as the eluent phase. At the end of 3 hours the mixture was diluted with 10 mL of water and 5 mL of 5 was added to the mixture to basify the medium. Then the water phase was extracted 3 times

with 20 mL of CH_2Cl_2. The organic layer was dried over $CaCl_2$ and the product was concentrated and light brown solid compound 3 (Figure 4.1) in was obtained. ^{1}H NMR ($CDCl_3$) δ 3.40 (s, 2H, NH_2), 3.78 (s, 2H, NH_2), 3.84 (s, 3H, CH_3), 6.66 (d, J= 8, 1H, ArH), 7.40 (d, J= 2, 1H, ArH), 7.47 (d of d, J= 8, 1H, ArH) ppm.

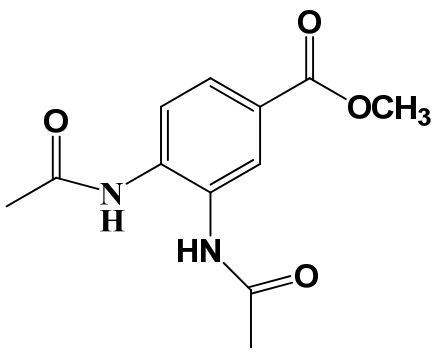


3

Figure 4.1. Methyl 3,4-diaminobenzoate (3)

4.3.2. Methyl 3,4-diacetamidobenzoate (4)

The synthesis was done according to literature procedure [39]. The experiment was carried out under inert atmosphere using nitrogen at 0 °C. Methyl 3,4-diaminobenzoate (0. 89 g, 0. 003 mol) and anhydrous CH_2Cl_2 (10 mL) were added to a 25 mL three necked round bottom flask fitted rubber septa and a magnetic stirrer. Triethyl amine (0.75 mL, 0.0052 mol) was added dropwise to the mixture. Then acetyl chloride (07 mL, 0.01mol) was added to the reaction drop by drop. After these additions the reaction mixture was stirred at room temperature for one hour. The reaction progress was monitored by TLC using silica gel plates and 2% CH_3OH / CH_2Cl_2 as the eluent phase. When the starting material was consumed at the end of one hour the mixture was poured into water (15 mL) and extracted 3 times with 15 mL of CH_2Cl_2. The extracts were collected and washed with saturated salt solution. The organic layer was dried over $CaCl_2$ and the product was concentrated. Then the product was purified by recrystallization in methanol and after filtration light brown-white solid compound 4 (Figure 4.2) was obtained. ^{1}H NMR (DMSO) δ 2.06 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 7.68 (d of d, J= 8.8 1H, Af, 7.83 (d, J=8.4, 1H, Ar, 8.10 (s, J= 1.6, 1H, Ar), 9.44 (s, 1H, NH), 9.47 (s, 1H, NH) ppm.



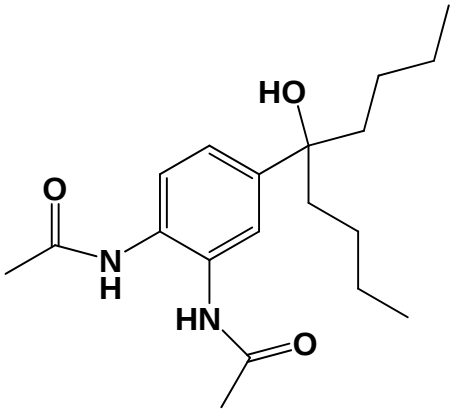
4

Figure 4.2. Methyl 3,4-diacetamidobenzoate (4)

4.3.3. N,N'-(4-(5-hydroxynonan-5-yl)-1,2-phenylene)diacetamide (5)

The synthesis was done according to literature procedures [40, 41] utilizing a Grignard reagent. The experiment was carried out under inert atmosphere using nitrogen. First Grignard reagent was prepared. Mg metal (0.2 g, 0.008 mol kept in oven for two days at 120°C) was placed in a 25 mL three necked round bottom flask fitted with a condenser and a magnetic stirrer. The flask was heated with a $CaCl_2$ tube to remove any humidity remaining on the metal. Then the $CaCl_2$ tube was removed and the flask was purged with nitrogen gas to deoxygenate. 5 mL of dry THF was transferred flask via a deoxygenated syringe and the temperature was set to 40°C. *n*BuBr (0.8 mL, 0.008 mol) was added to the flask slowly drop by drop , bubbling started and the color of the solution turned to grey. After stirring the mixture for half an hour the bubbling ceased and the formation of the Grignard reagent was complete. In a one necked round bottom flask compound 4 (0.6 g, 0.0024 mol) was dissolved in 10 mL of dry THF and the flask was cooled to 0°C. Then the Grignard reagent was added to this solution drop by drop with a syringe. The reaction progress was monitored by TLC using aluminum oxide gel plates and 2% CH_3OH / CH_2Cl_2 as the eluent phase. The reaction was left overnight and the next day the mixture was poured into %5 NH_4Cl solution (20 mL). This mixture was extracted 3 times with 15 mL of CH_2Cl_2. The extracts were collected and washed with saturated salt solution and the organic layer was dried over $CaCl_2$ and the product was concentrated. The crude product

was impure and it was purified by dissolving in 2 ml of CH_3OH and precipitating in water. After filtration light white solid compound 5 (Figure 4.3) in was obtained. The molecular weight of the compound is 334 g/mol and in LCMS analysis the parent peak was observed as 357. ^{1}H NMR (DMSO) δ 9.34 (s, 1H, NH), 9.15 (s, 1H, NH), 7.43 (m, 2H, ArH), 7.07 (d, J=8.8, 1H ArH), 4.51(s, 1H, OH), 2.03 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.62 (m, 4H, CH_2), 1.15 (m, 6H, CH_2), 0.86 (m, 2H, CH_2), 0.75 (t, J= 7.2, 6H, CH_3).

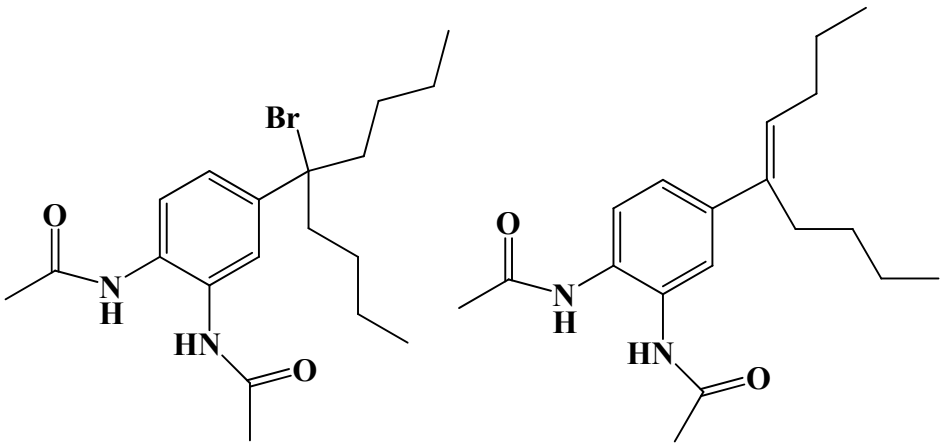


5

Figure 4.3. N,N'-(4-(5-hydroxynonan-5-yl)-1,2-phenylene)diacetamide (5)

4.3.4 N,N'-(4-(5-bromononan-5-yl)-1,2-phenylene)diacetamide (6)

The synthesis was done according to literature procedure [42] by using BBr_3. The experiment was carried out under inert atmosphere using nitrogen. 0.0072 g compound 4 (0.214 mmol) was dissolved in 5mL of dry CH_2Cl_2 in a one necked round bottom flask and the solution temperature was kept at 0°C. 0.5 mL of BBr_3 (0.5 mmol) was added dropwise and the reaction was left overnight. The reaction was quenched with 10 mL of water. The aqueous phase was extracted 3 times with 10 mL of CH_2Cl_2. The organic phase was dried over $CaCl_2$ and upon evaporation of the solvent 0.0681 g (0.171 mmol, Figure 4.4) product was obtained with 80 % yield. ^{1}H NMR (DMSO) δ 9.26 (s, 1H, NH), 9.21 (s, 1H, NH), 7.44 (s, 1H, ArH), 7.39 (d, J= 8.4 1H, ArH), 7.04 (d, J= 7.2 1H, ArH), 5.54 (t, J= 6.8, CH), 2.36 (m, 3H, CH2), 1.35 (m, 4 H, CH_2), 1.18 (m,6 H, CH_2), 0.84 (m, 3H, CH_3), 0.75 (m, 3H, CH_3).

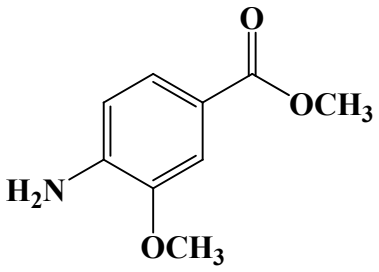


6 **7**

Figure 4.4. N,N'-(4-(5-bromononan-5-yl)-1,2-phenylene)diacetamide (6)

4.3.5 Methyl 4-amino-3-methoxybenzoate (14)

The synthesis was done according to literature procedure of Fischer esterification. The experiment was carried out under inert atmosphere using nitrogen. Compound 15 (1.00 g, 0.006 mol) and methanol (20 mL) were added to a 50 mL three necked round bottom flask fitted with a condenser and magnetic stirrer. The reaction bath temperature was adjusted to 65°C such that the solvent refluxed smoothly. Concentrated sulphuric acid (1.5 mL) was added in a dropwise manner via a syringe to the reaction flask. The mixture was stirred for 3 hours. The reaction progress was monitored by thin layer chromatography (TLC) using aluminum gel plates and CH_2Cl_2 as the eluent phase. At the end of 3 hours the mixture was diluted with 10 mL of water and 5 mL of 5% was added to the mixture to basify the medium. Then the water phase was extracted 3 times with 20 mL of CH_2Cl_2. The organic layer was dried over $CaCl_2$ and the product was concentrated and white solid material, compound 14 (1.047 g, Figure 4.5) was obtained in 96.5 % yield. The molecular weight of the compound is 181 g/mol and in LCMS analysis the parent peak was observed as 182. ^{1}H NMR (DMSO) δ 7.36 (d of d, J= 8.4, 1H, ArH), 7.26 (d, 1H, J= 1.6, ArH), 6.60 (d, J= 8.4, 1H, ArH), 5.59 (s, 2H, NH_2), 3.77 (s, 3H, CH_3), 3.72 (s, 3H, CH_3) ppm.

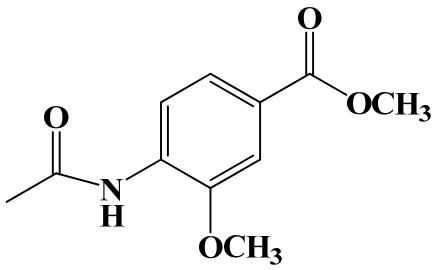


14

Figure 4.5. Methyl 4-amino-3-methoxybenzoate (14)

4.3.6. Methyl 4-acetamido-3-methoxybenzoate (15)

The synthesis was done according to literature procedure [39]. The experiment was carried out under inert atmosphere using nitrogen. Compound 15 (1.047 g, 0. 006 mol) and anhydrous CH_2Cl_2 (15 mL) were added to a 25 mL three necked round bottom flask fitted rubber septa and a magnetic stirrer. The reaction was cooled to 0 °C and triethyl amine (1.3 mL, 0.009 mol) was added dropwise to the mixture. Then acetyl chloride (0.7 mL, 0.009 mol) was added to the reaction drop by drop. After these additions the reaction mixture was stirred at room temperature for one hour. The reaction progress was monitored by TLC using aluminum oxide gel plates and CH_2Cl_2 as the eluent phase. When the starting material was consumed at the end of two hours the mixture was poured into water (15 mL) and extracted 3 times with 15 mL of CH_2Cl_2. The extracts were collected and washed with saturated salt solution and. The organic layer was dried over $CaCl_2$ and the product was concentrated. The crude product was not pure and it was purified with a silica gel column chromatography using CH_2Cl_2 as the eluent phase. After purification white solid material, compound 15 (1.122 g, Figure 4.6) was obtained in 87% yield. ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 3.94(s, 3H, CH_3), 7.55 (d, 1H, J= 1.6, ArH), 7.68 (d of d, 1H, J= 8.4, ArH), 7.92 (s, 1H, NH), 8.47 (d, 1H, J= 8, CH).ppm.



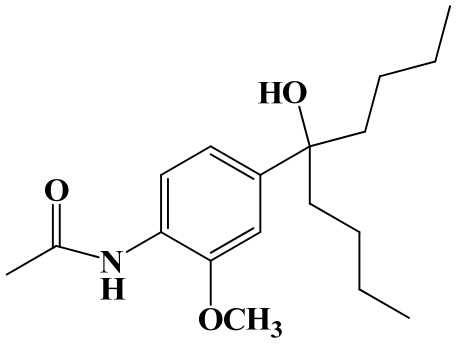
15

Figure 4.6. Methyl 4-acetamido-3-methoxybenzoate (15)

4.3.7. N-(4-(5-hydroxynonan-5-yl)-2-methoxyphenyl)acetamide (16)

The synthesis was done according to literature procedures [40,41] utilizing a Grignard reagent. The experiment was carried out under inert atmosphere using nitrogen. First Grignard reagent was prepared. Mg metal (0.45 g, 0.018 mol kept in oven for two days at 120°C) was placed in a 25 mL three necked round bottom flask fitted with a condenser and a magnetic stirrer. The flask was heated with a $CaCl_2$ tube to remove any humidity remaining on the metal. Then the $CaCl_2$ tube was removed and the flask was purged with nitrogen gas to deoxygenate. 5 mL of dry THF was transferred flask via a deoxygenated syringe and the temperature was set to 40°C. *n*BuBr (1.9 mL, 0.018 mol) was added to the flask slowly drop by drop , bubbling started and the color of the solution turned to grey. After stirring the mixture for half an hour the bubbling ceased and the formation of the Grignard reagent was complete. In a one necked round bottom flask. Compound 16 (1.043g, 0.0047 mol) was dissolved in 10 mL of dry THF and the flask was cooled to 0°C using an ice bath. Then the Grignard reagent was added to this solution drop by drop with a syringe. The reaction progress was monitored by TLC using aluminum oxide gel plates and CH_2Cl_2 as the eluent phase. The mixture was stirred two hours and then it was poured into %5 NH_4Cl solution (15 mL). This mixture was extracted 3 times with 15 mL of CH_2Cl_2. The extracts were collected and washed with saturated salt solution and the organic layer was dried over $CaCl_2$ and the product was concentrated. The crude product was impure and it was purified by dissolving in 2 mL of CH_3OH and precipitating in 20 mL water. After filtration light white solid material, compound 16(Figure 4.7) was obtained. Theoretical elemental analysis values are as follows: C, 70.32; H, 9.51; N, 4.56;

O, 15.61 and experimental values are: C, 70.03; H, 9.86; N, 4.60; O, 15.50. ^{1}H NMR (DMSO) δ 8.27 (d, J= 8.4, 1H, ArH), 7.71 (s, 1H, NH), 7.00 (d, J= 2, 1H, ArH) 6.85 (d of d, J= 8.4, 1H, ArH), 3.89 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 1.77 (m, 4H, CH_2), 1.23 (m, 6H, CH_2), 1.04 (m, 2H, CH_2), 0.83(m, 6H, CH_3) ppm.

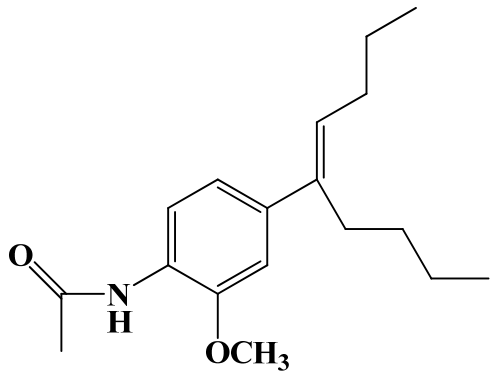


16

Figure 4.7. N-(4-(5-hydroxynonan-5-yl)-2-methoxyphenyl)acetamide (16)

4.3.8. Dehydration of N-(4-(5-hydroxynonan-5-yl)-2-methoxyphenyl)acetamide (18)

The dehydration was done according to the literature procedure [43] with anhydrous copper (II) sulphate as the catalyst. For the preparation of the catalyst copper(II) sulphate was dried by heating at 240°C for two days. 0.1 g compound 19, (0.325 mmol) and 80 mg of anhydrous copper(II) sulphate (0.29 mmol) was placed in a 25 mL three-necked round bottom flask fitted with a condenser and a magnetic stirrer. 10 mL of dry toluene was added to the reaction mixture and the temperature was set to 110°C. The reaction continued overnight and at the end copper (II) sulphate was filtered and the solvent evaporated. The crude product was purified twice by using silica gel columns with CH_2Cl_2 and 10% EtOAc / CH_2Cl_2 respectively. The product compound 18 (Figure 4.8) obtained was used for NMR analysis. Both E and Z isomers are obtained. ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J= 8, 1H, ArH), 7.64 (s, 1H, NH), 6.86 (d of d, J= 8, ArH), 6.77 (d, J= 1.6, ArH), 5.54 (t, J= 6.8, 1H, CH), 3.81 (s, 3H, CH_3), 2.38-0.79 (m, 16H)ppm.

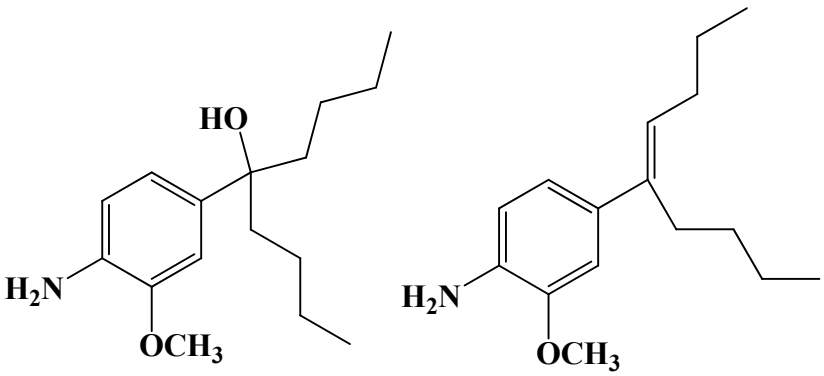


18

Figure 4.8. (E)-N-(2-methoxy-4-(non-4-en-5-yl)phenyl)acetamide

4.3.9. Deprotection of N-(4-(5-hydroxynonan-5-yl)-2-methoxyphenyl)acetamide (16)

Deprotection was done via hydrolysis of amide group to amine by using 20% H_2SO_4. 50 mg alcohol (compound 17, 0.16 mmol) was dissolved in 5 mL of CH_3OH in a three-necked round bottom flask fitted with a reflux condenser and a magnetic stirrer. 5 mL of 20% H_2SO_4 was added to the reaction flask and refluxed at 80°C for three hours. The reaction was monitored by using TLC using aluminum oxide gel plates and CH_2Cl_2 as the eluent phase. At the end of three hours the medium was basified with 10mL of 5% NaOH then extracted three times with 15 mL of CH_2Cl_2. The organic layer was extracted once with 20 mL of saturated NaCl solution. Then the organic phase was dried over $CaCl_2$. ^1H NMR ($CDCl_3$) δ 7.39 (m, 1H, ArH), 6.70 (m, 1H, ArH), 6.59 (m, 1H, ArH), 5.48 (t, J= 7. 8, 1H CH), 4.2 (s, 1H, NH), 3.8 (s, 3H, CH_3), 2.80-0.78 (m, 16H) ppm.

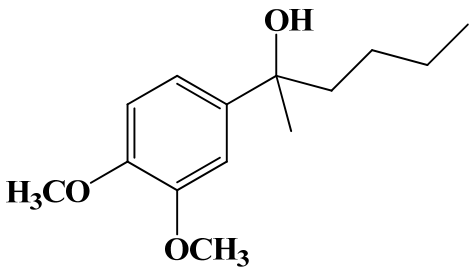


16 **20**

Figure 4.9. 5-(4-amino-3-methoxyphenyl)nonan-5-ol (16)

4.3.10. 2-(3,4-dimethoxyphenyl)hexan-2-ol (26)

The synthesis was done according to literature procedures [40, 41] utilizing a Grignard reagent. The experiment was carried out under inert atmosphere using nitrogen. First Grignard reagent was prepared. Mg metal (0.2 g, 0.008 mol kept in oven for two days at 120°C) was placed in a 25 mL three necked round bottom flask fitted with a condenser and a magnetic stirrer. The flask was heated with a $CaCl_2$ tube to remove any humidity remaining on the metal. Then the $CaCl_2$ tube was removed and the flask was purged with nitrogen gas to deoxygenate. 5 ml of dry THF was transferred flask via a deoxygenated syringe and the temperature was set to 40°C. *n*BuBr (1 mL, 0.009 mol) was added to the flask slowly drop by drop , bubbling started and the color of the solution turned to grey. After stirring the mixture for half an hour the bubbling ceased and the formation of the Grignard reagent was complete. In a one necked round bottom flask compound 24 (1 g, 0.006 mol) was dissolved in 10 mL of dry THF and the flask was cooled to 0°C using an ice bath. Then the Grignard reagent was added to this solution drop by drop with a syringe. The reaction progress was monitored by TLC using aluminum oxide gel plates and 1 % CH_3OH / CH_2Cl_2 as the eluent phase. The mixture was stirred two hours and then it was poured into %5 NH_4Cl solution (15 mL). This mixture was extracted 3 times with 15 mL of CH_2Cl_2. The extracts were collected and washed with saturated salt solution and the organic layer was dried over $CaCl_2$ and the product was concentrated on a rotarory evaporator. 0.0638 g oily crude product was obtained. Impure crude product was purified by flash chromatography with 15 % EtOAc / Hexane as eluent phase on a silica gel column. Then the product was further purified with another silica gel column with hexane. After the evaporation of the solvent a little amount (just enough for NMR analysis) of compound 26 in Figure 4.10 was obtained. ^{1}H NMR ($CDCl_3$) δ 7.00 (d, J= 2, 1H, ArH), 6.88 (d of d, J= 8 H ArH), 6.78 (d, J= 8, 1H, ArH), 3.86 (s, 3H, CH_3), 3.84(s, 3H, CH_3), 1.83 (s, 1H, OH), 1.75 (p, J= 4.8 2H, CH_2), 1.51 (s, 3H, CH_3), 1.20 (m, 4H, CH2), 0.83 (t, J= 7.2, CH_3) ppm. ^{13}C NMR ($CDCl_3$) δ 148.48 (ArC), 147.43 (ArC), 140.86 (ArC), 116.78 (ArC), 110.57 (ArC), 108.56 (ArC), 74.38 (C), 55.75 (2C, CH_3), 43.89 (C), 29.93 (CH_2), 26.14 (CH_3), 22.91 (CH_2), 13.91 (CH_2), 13.91 (CH_3)



26

Figure 4.10. (3,4-dimethoxyphenyl)hexan-2-ol (26)

5. CONCLUSION

In this study the aim was to synthesize the derivatives of the lead compound 1 active against prostate cancer. The left-hand side of the product was derivatized starting from an amine and/or methoxy substituted benzoic acid derivative to phenyl derivative having a symmetrical or unsymmetrical branched alkyl group at the benzylic position. Specifically methyl 3,4-diaminobenzoate (compound 3), methyl 3,4-diacetamidobenzoate (compound 4), N,N'-(4-(5-hydroxynonan-5-yl)-1,2-phenylene)diacetamide (compound 5), (E)-N,N'-(4-(non-4-en-5-yl)-1,2-phenylene)diacetamide (compound 7), methyl 4-amino-3-methoxybenzoate (compound 13), methyl 4-acetamido-3-methoxybenzoate (compound 14), N-(4-(5-hydroxynonan-5-yl)-2-methoxyphenyl)acetamide, (E)-N-(2-methoxy-4-(non-4-en-5-yl)phenyl)acetamide (compound 17), N-(4-(5-hydroxynonan-5-yl)-2-methoxyphenyl)acetamide (compound 15), (E)-2-methoxy-4-(non-4-en-5-yl)aniline (compound 20), (E)-N-(2-methoxy-4-(non-4-en-5-yl)phenyl)acetamide (compound10) and (3,4-dimethoxyphenyl)hexan-2-ol (compound 25) have been synthesized successfully.

APPENDIX A: SPECTROSCOPY DATA

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

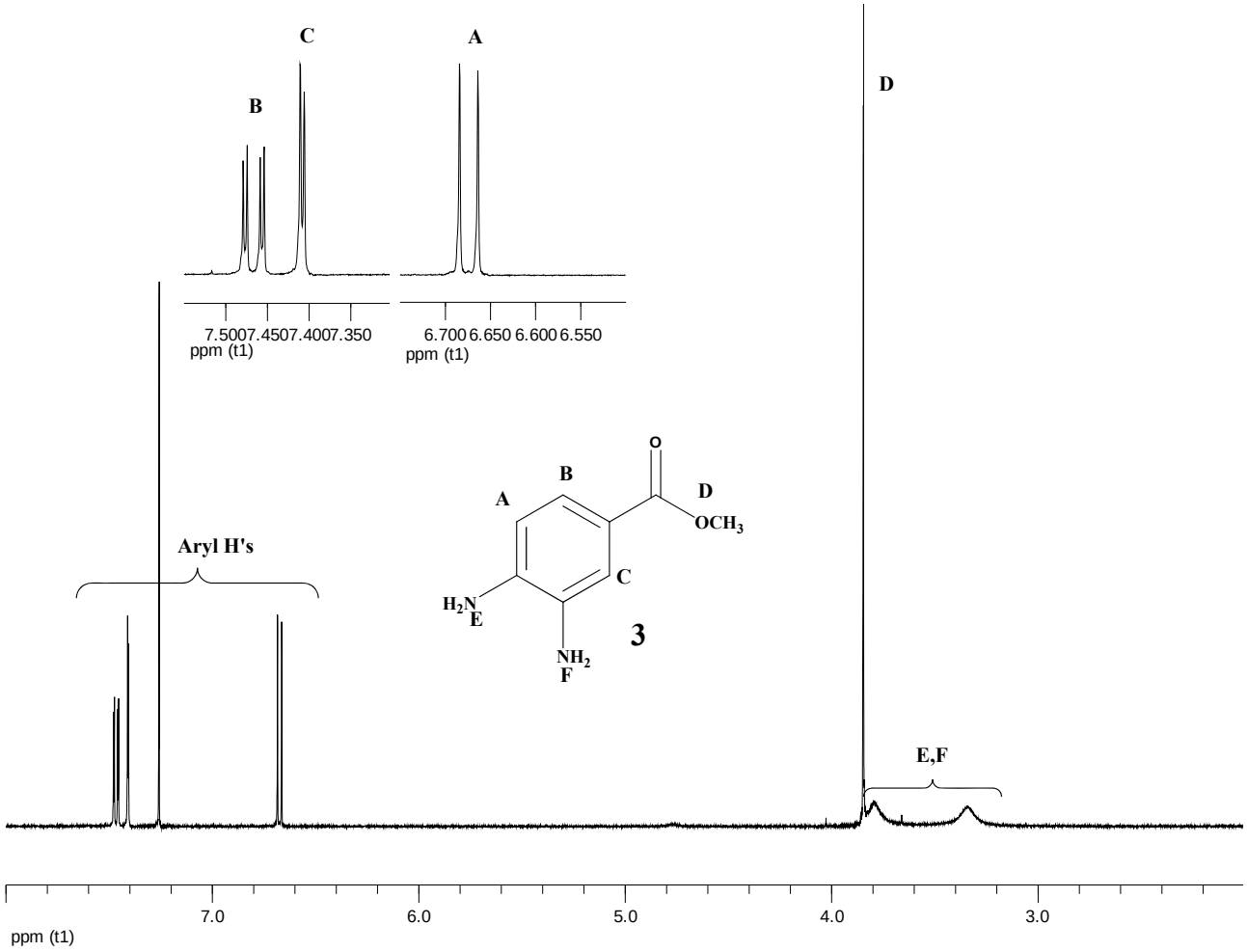


Figure A.1. ^{1}H-NMR of Methyl 3,4-diaminobenzoate (3)

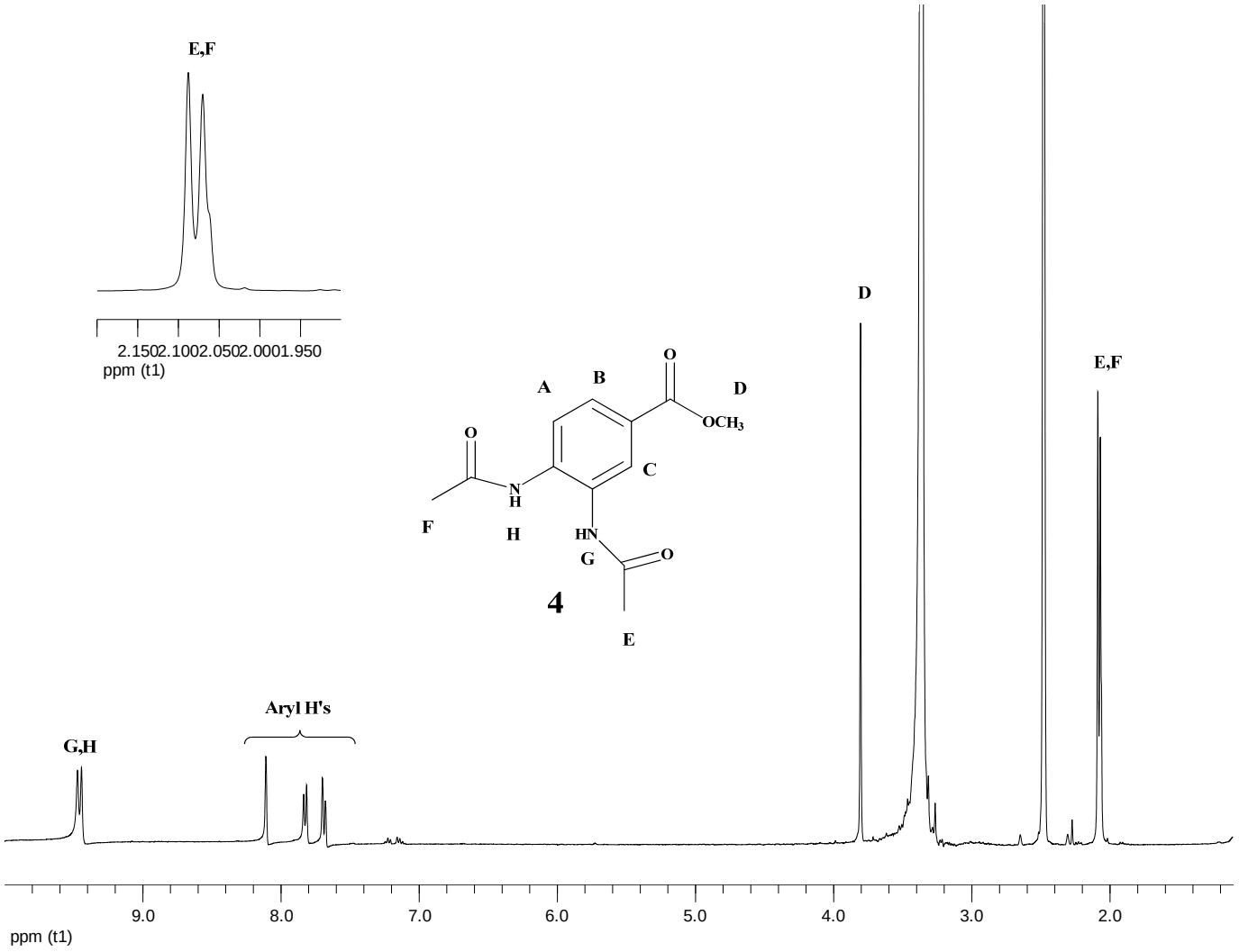


Figure A.2. ^{1}H-NMR of Methyl 3,4-diacetamidobenzoate (4)

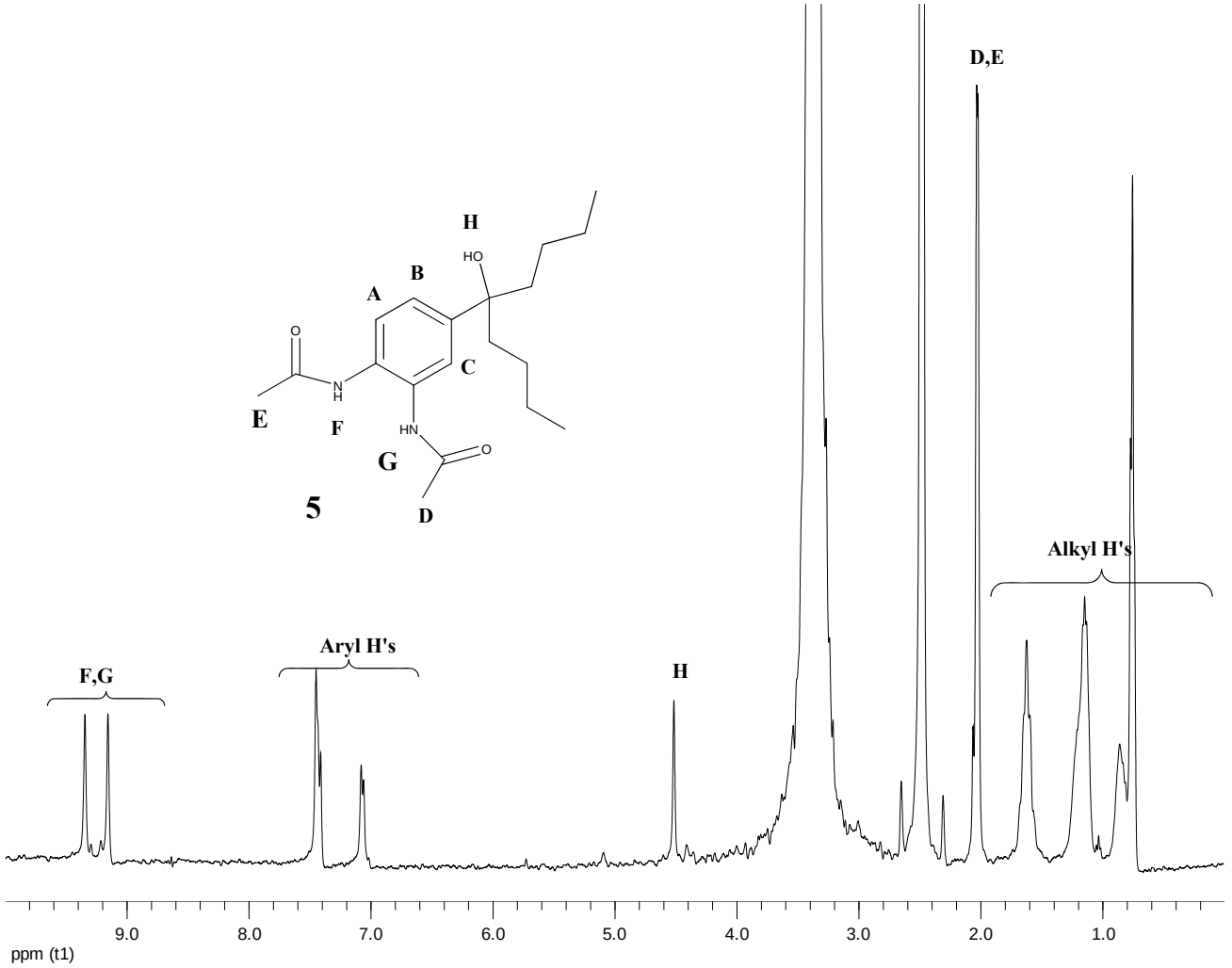


Figure A.3. ^{1}H-NMR of N,N'-(4-(5-hydroxynonan-5-yl)-1,2-phenylene)diacetamide (5)

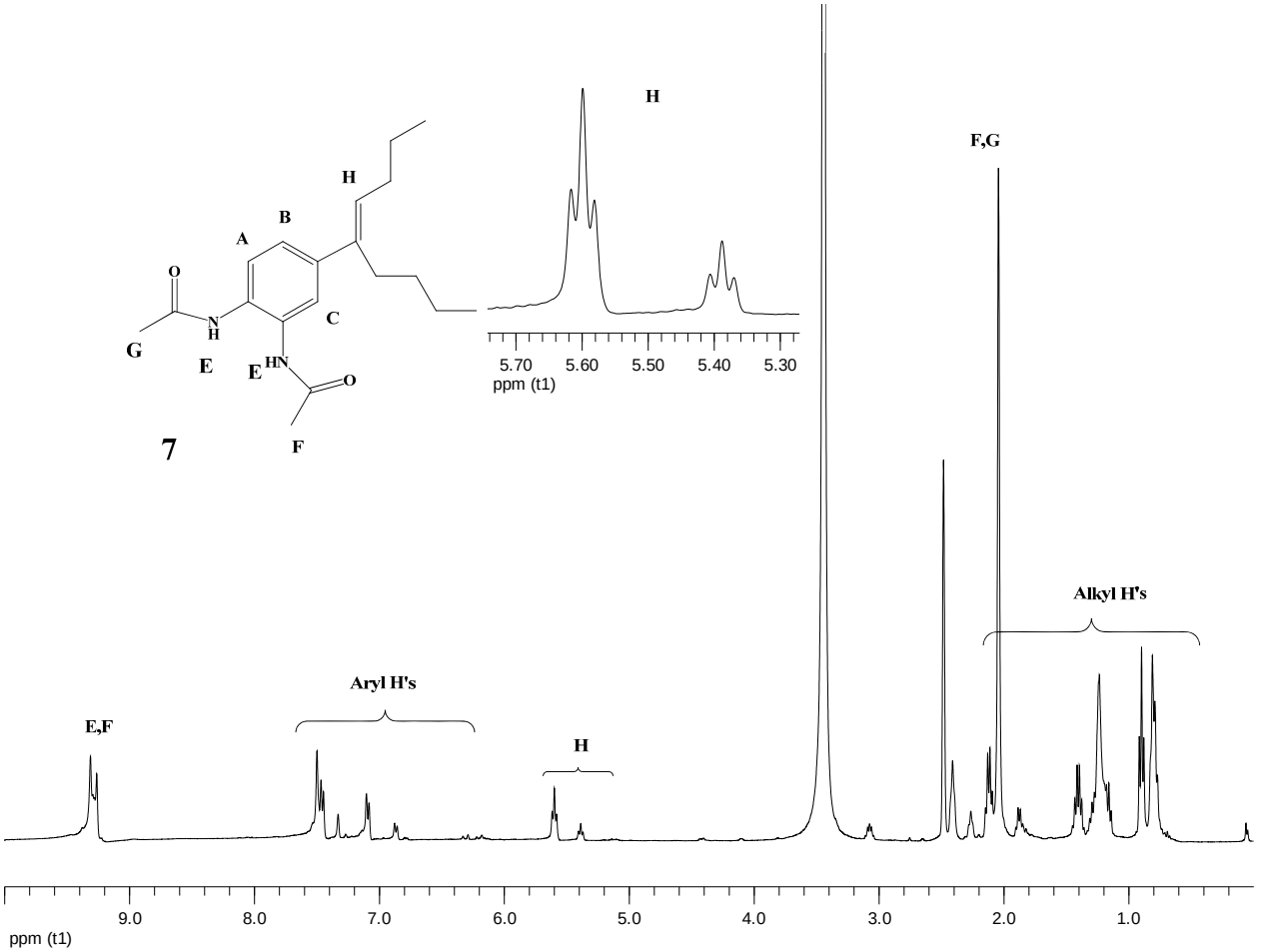


Figure A.4. ^{1}H-NMR of (E)-N,N'-(4-(non-4-en-5-yl)-1,2-phenylene)diacetamide (7)

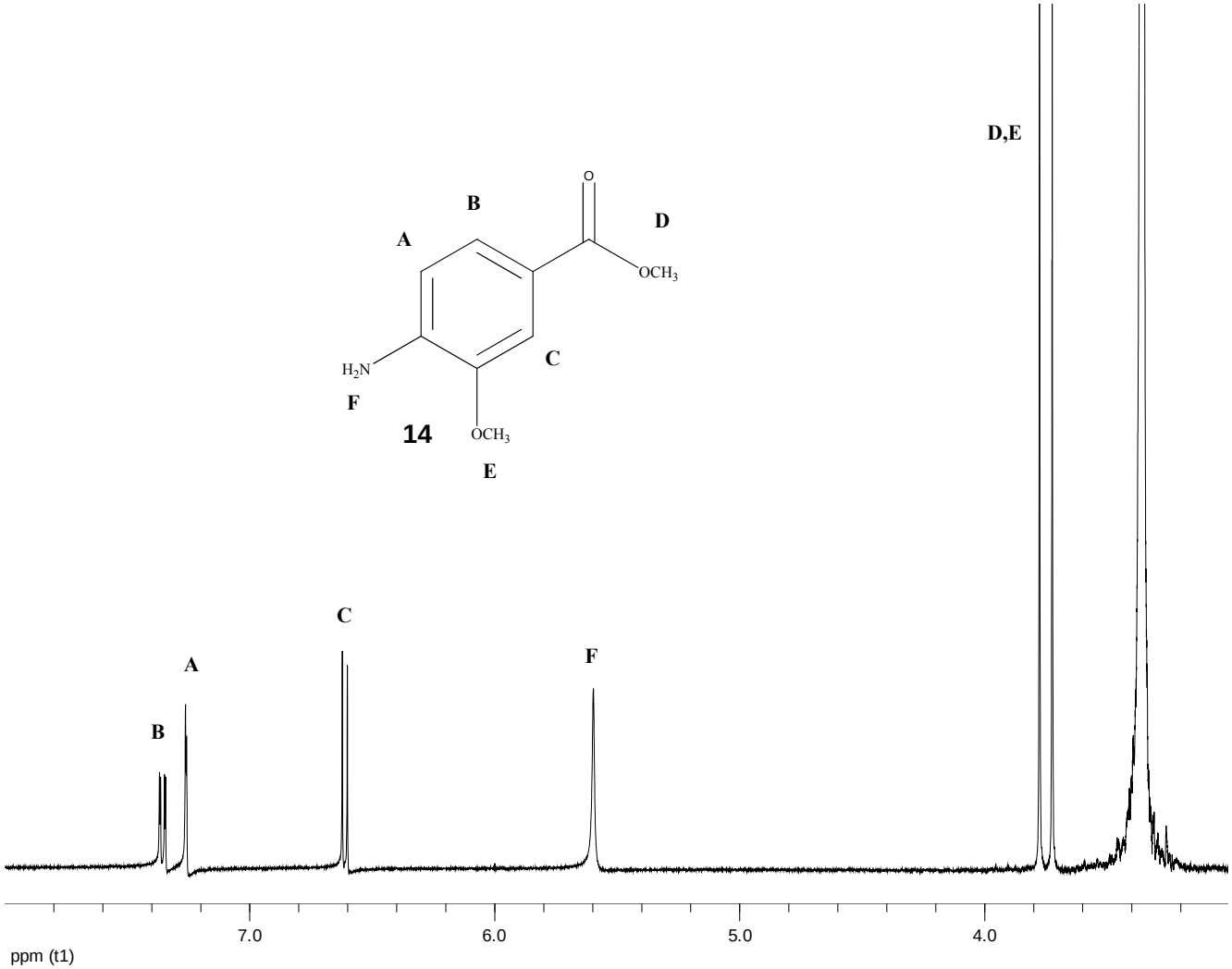


Figure A.5. ^{1}H-NMR of Methyl 4-acetamido-3-methoxybenzoate (14) [44]

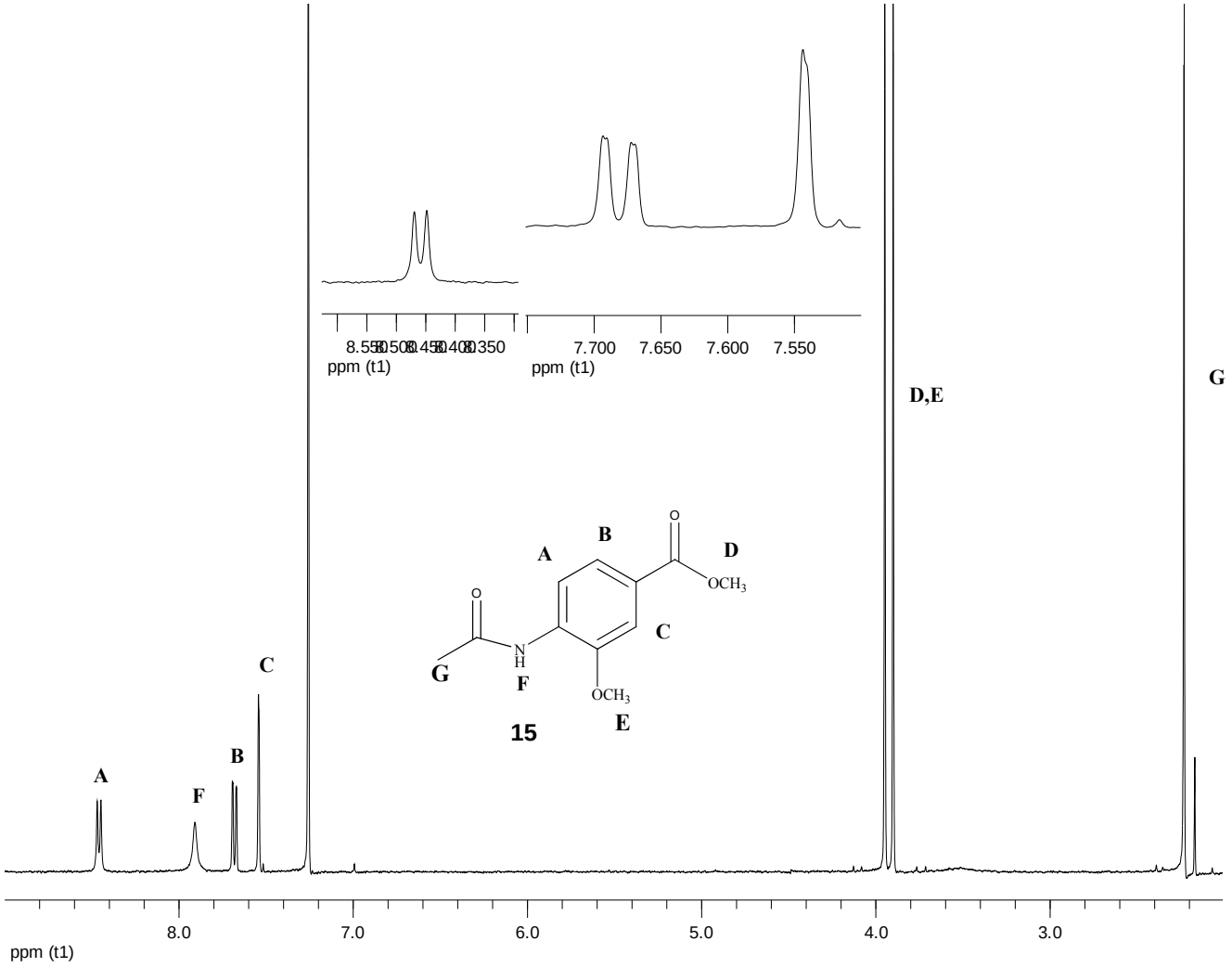


Figure A.6. [1]H-NMR of Methyl 4-acetamido-3-methoxybenzoate (15) [44]

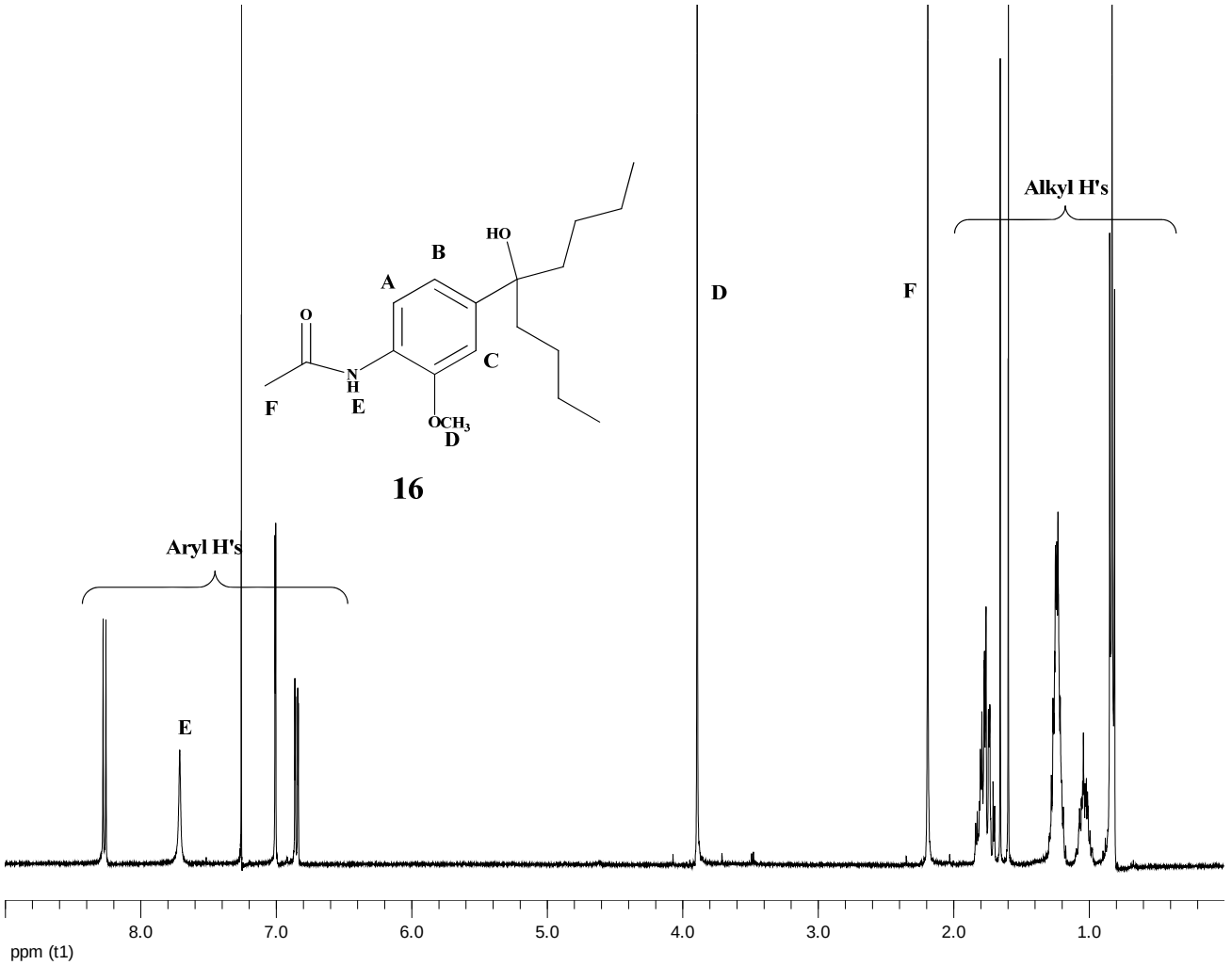


Figure A.7. ^{1}H-NMR of N-(4-(5-hydroxynonan-5-yl)-2-methoxyphenyl)acetamide (16)

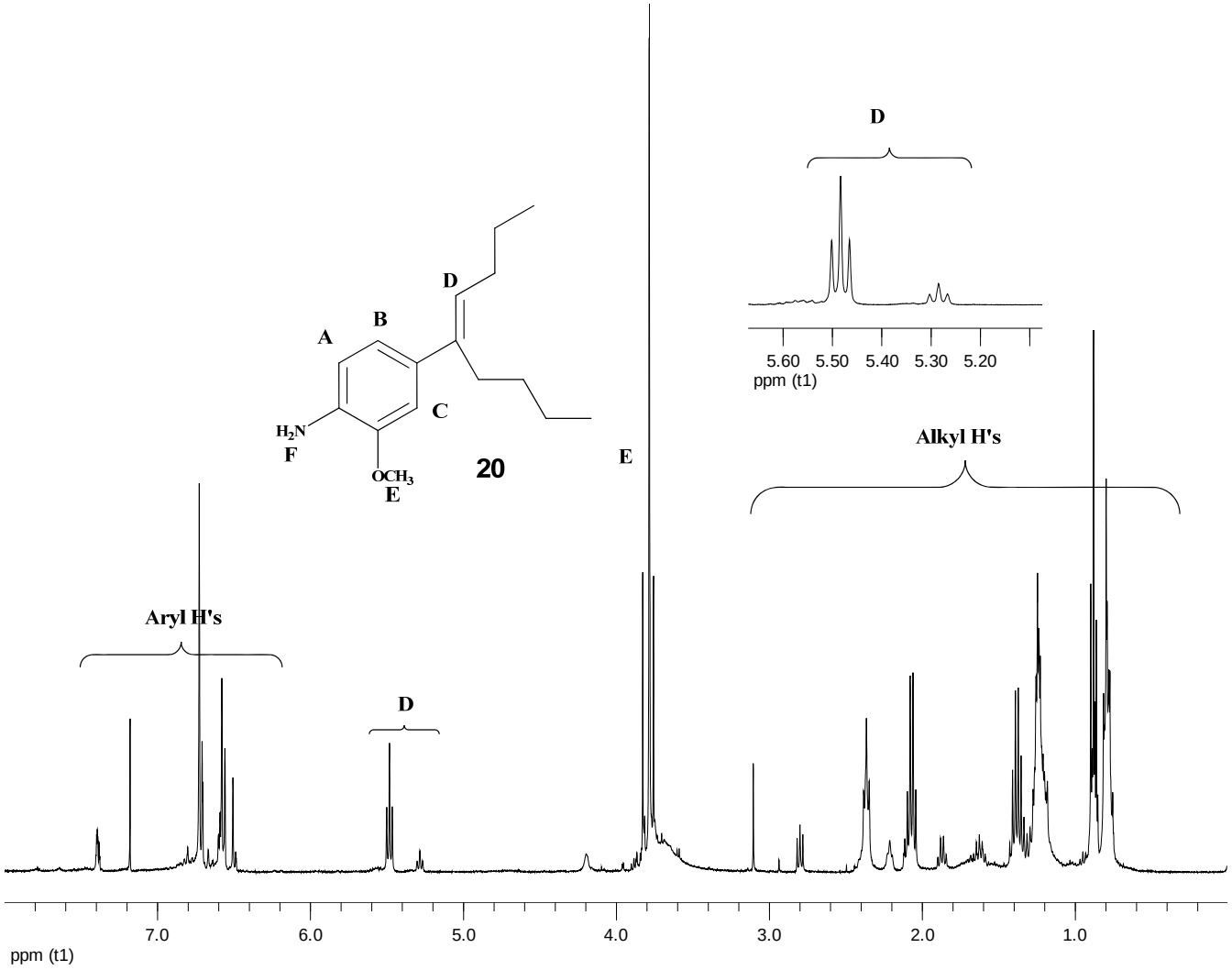


Figure A.8. ^{1}H-NMR of (E)-2-methoxy-4-(non-4-en-5-yl)aniline (20)

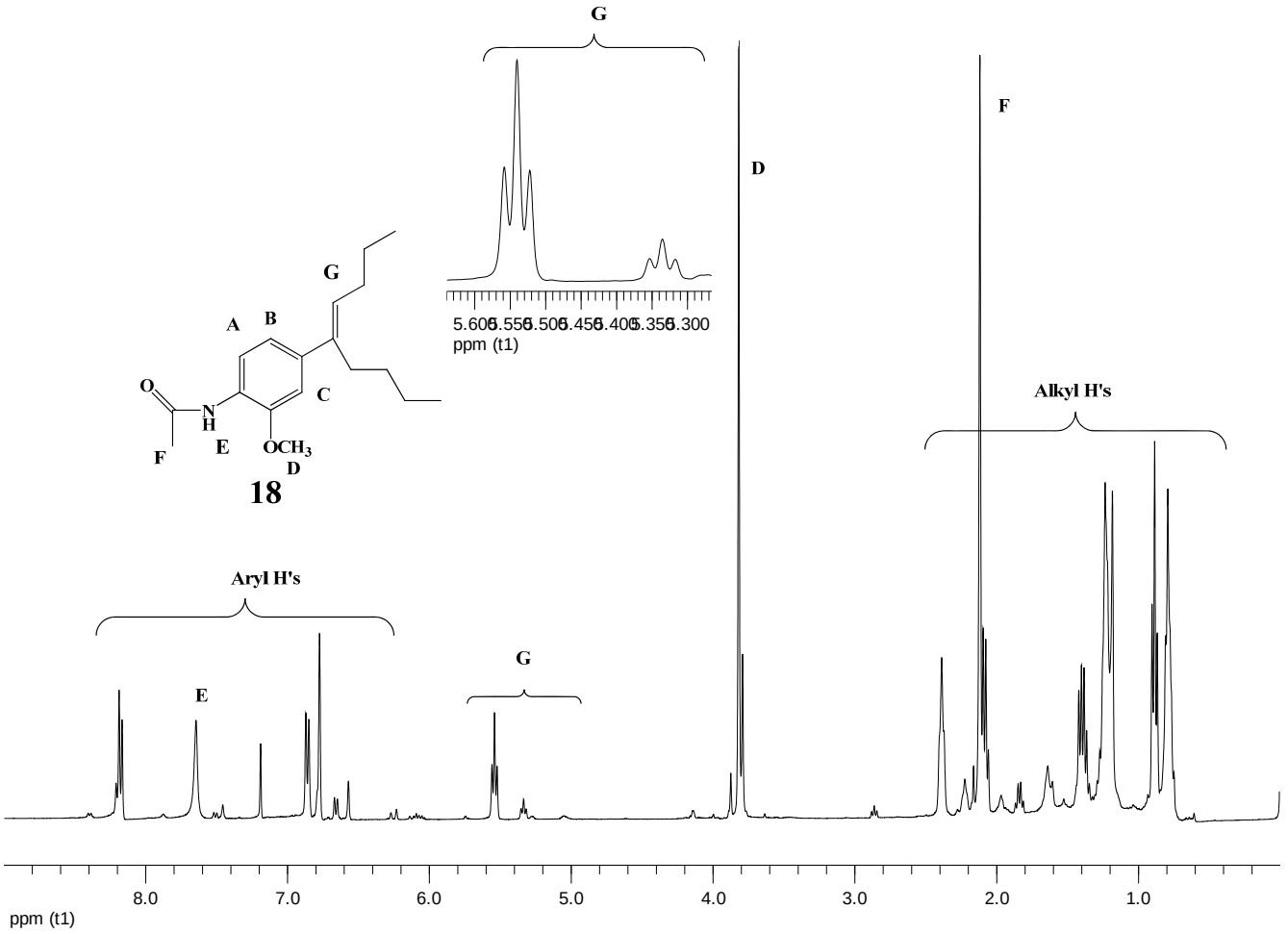


Figure A.9. ^{1}H-NMR of (E)-N-(2-methoxy-4-(non-4-en-5-yl)phenyl)acetamide (18)

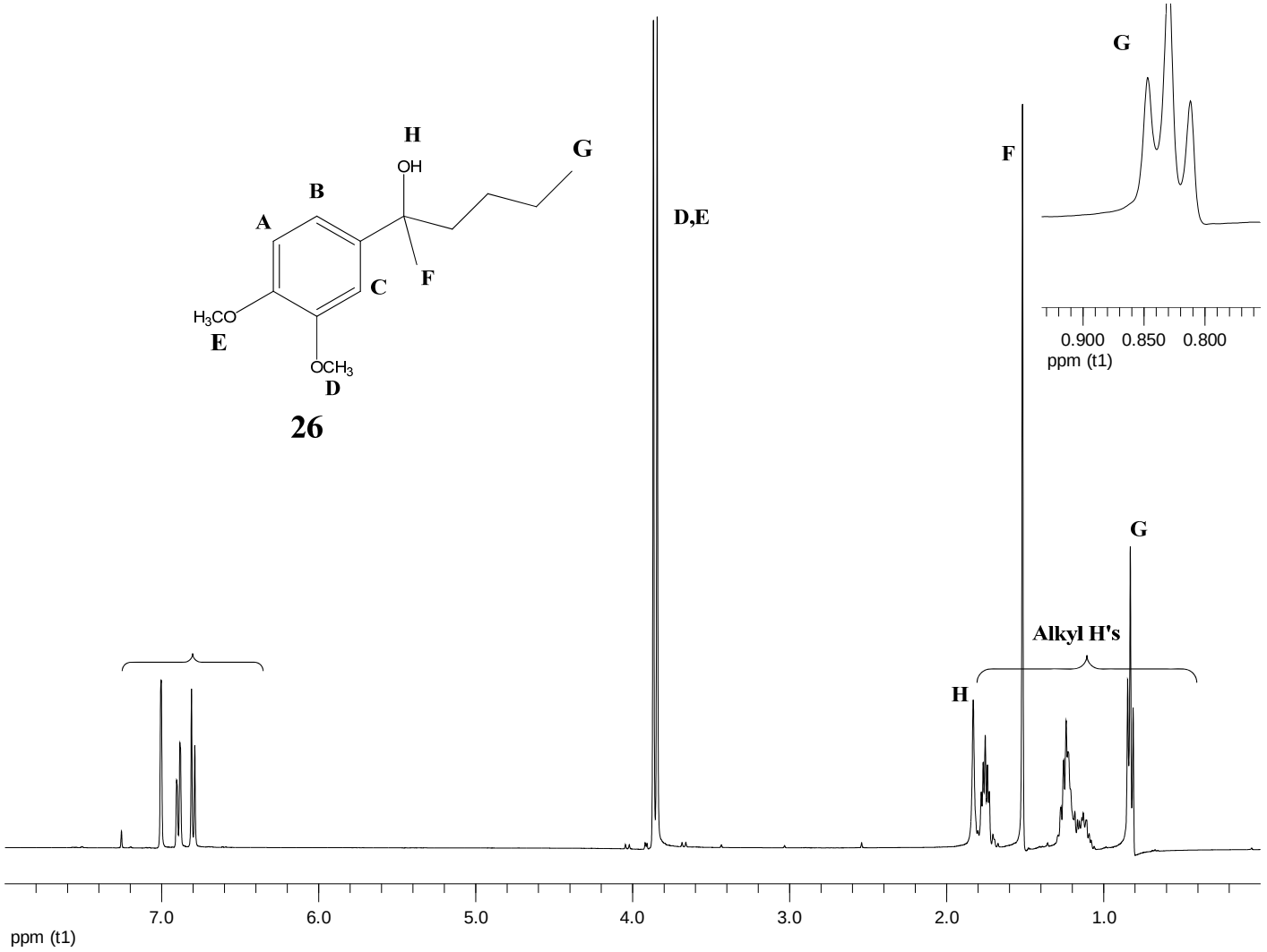


Figure A.10. ^{1}H-NMR of (3,4-dimethoxyphenyl)hexan-2-ol (26)

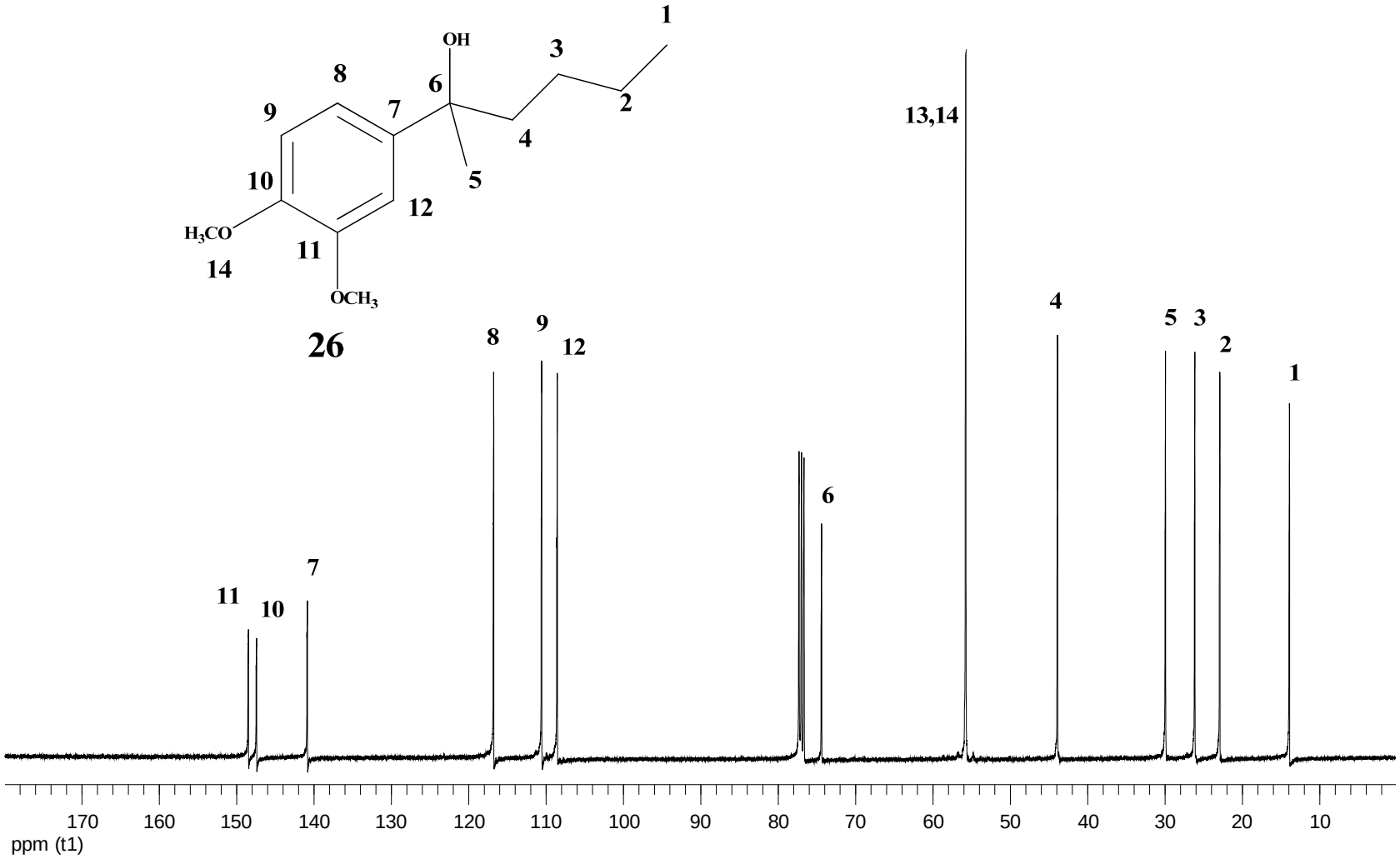


Figure A.11. ^{13}C-NMR of (3,4-dimethoxyphenyl)hexan-2-ol (25)

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