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Synthesis of Resveratrol Ester Derivatives

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Synthesis of Resveratrol Ester Derivatives

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by

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ABSTRACT

Synthesis of Resveratrol Ester Derivatives

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The goal of this research project was to synthesize derivatives of transresveratrol. In order for resveratrol to be activated and used by the body it needs to bind to Human Serum Albumin (HSA), a protein in blood plasma. The derivatives were synthesized to improve the ability of resveratrol to enter cells as well as improve their ability to bind to HSA. The three derivatives that were synthesized have converted one of the hydroxyl groups on resveratrol to an ether with a methylene chain terminated by a carboxylic acid. By varying the lengths of the methylene chain we varied the water solubility of the resveratrol derivative. This brought the research closer to the goal of determining how this would affect the binding ability to HSA. Currently three derivatives have been synthesized and purified once by column chromatography.

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CHAPTER 1

INTRODUCTION

When people seek inspiration, be it for art, philosophy, or math, nature is often where they turn, and it is no different for chemistry and medicine. The earliest records of natural products were depicted on clay tablets in cuneiform from Mesopotamia (2600 BC.) which documented oils from *Cupressus sempervirens* (Cypress) and Commiphora species (myrrh), both of which are still used to treat coughs, colds, and inflammation.[1] According to the World Health Organization (WHO), 80% of people still rely on plant-based traditional medicines for primary health care.[2] Today there remains a gap between modern medical approaches and traditional folk medicine, specifically the legitimacy and effectiveness of certain treatments. This gap is being closed as the field of medicinal chemistry evolves and as both chemists and medical professionals investigate various natural products. The most common example of a natural product is acetylsalicyclic acid, aspirin, which was derived from the natural product, salicin, that is isolated from the bark of the willow tree.[3] The structures for both salicine and acetylsalicyclic acid can be seen below in Figure 1.

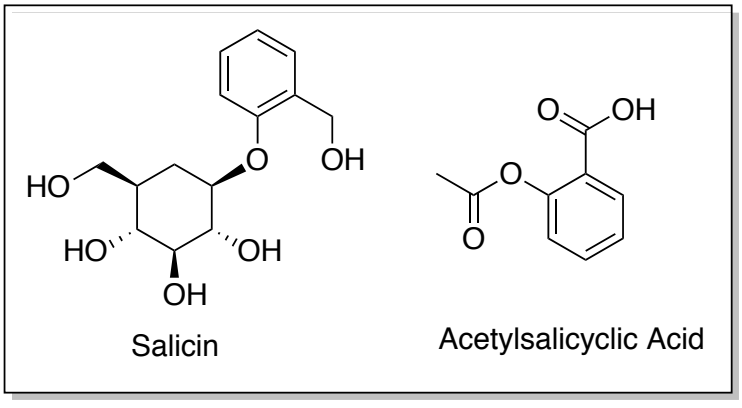


Figure 1. Chemical structures of various natural products

Another example of a natural product came from the investigation of *Papaver somniferum L*. (opium poppy) which resulted in the isolation of morphine, seen in Figure 2, for use as a commercially important drug, first reported in 1803.[4]

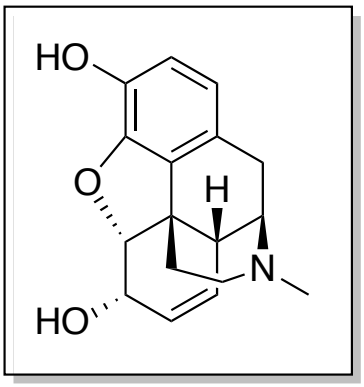


Figure 2. Chemical structure of morphine

Crucial investigations such as these are often be hindered by the accessibility of the molecule in question. For instance, in the Netherlands in 1974, C.J. Persons isolated 200 micrograms of periplanone B, a sex excitation pheromone, from the droppings of 75,000 virgin female cockroaches. [5] The chemical structure of periplanone B is shown in Figure 3. The process of isolating this molecule was extremely time consuming and clearly illustrated the problems encountered when working with natural products. It was not until 1979 that chemical synthesis was used as a guide to determine and produce periplanone B on a large scale in the laboratory.

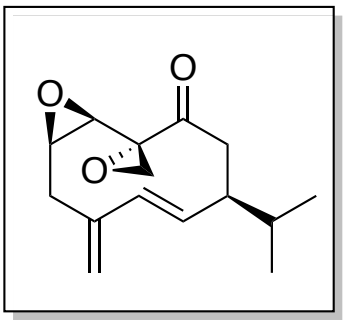


Figure 3. Chemical structure of periplanone B

Other challenges when working with natural products could include the size and complexity of the desired products. Though many natural products contain large carbon skeletons and diverse functional groups, their total chemical synthesis is important for investigating and characterizing these molecules.

Polyphenols

To understand the reactivity of polyphenols it is important to understand their origin. The term polyphenol first appeared in 1894 and was used to describe molecules derived mainly from plants.[6] Phenol, itself, is a compound originally extracted from coal tar.[7] Today, the polyphenol group ranges from natural products to synthetic products. From the name, it is clear that polyphenols are characterized chemically by the presence of multiple phenol groups; however, a more rigid definition was created by a group of chemists, Edwin Haslam, Edgar Charles Bate-Smith, Anthony Swain, and Theodore White.[8] This definition is known as the White-Bate-Smith-Swain-Haslam (WBSSH) definition of polyphenols and lists four properties that polyphenols must posses:

1) Polyphenols are moderately water soluble compounds

2) Polyphenols have a molecular weight of 500-4,000 Daltons

3) Polyphenols have greater than 12 phenolic hydroxyl groups

4) Polyphenols have 5-7 aromatic rings per 1,000 Daltons

This fairly precise definition was created when polyphenols strictly described a natural product; since then the definition has changed.

In 2011, Stéphane Quideau, a chemist, introduced a modified definition stating that polyphenols should be defined as: compounds exclusively derived from 1) shikimate/ phenylpropanoid and/or 2) the polyketide pathway featuring more than one phenolic unit and deprived of nitrogen based functionality.[9] As the interest in polyphenols grew, this branch of molecules was further divided into two main groups, hydrolyzable tannins and phenylpropanoids. Hydrolyzable tannins include gallic acid esters of glucose and other sugars or cyclitols.[10] These tannins continue to attract attention due to their importance in the process used to tan, or preserve, leather throughout the world. Phenylpropanoids include lignins, flavonoids, nonflavonoids, and condensed tannins. This group developed because lignins are important in soil chemistry in addition to plant structure. Furthermore, flavonoids in this phenylpropanoid group exist as secondary metabolites used for plant defense and flower color. Flavonoid compounds include (+) catechin and (-) epicatechin, seen in Figure 4, which have high free radical scavenging activities.

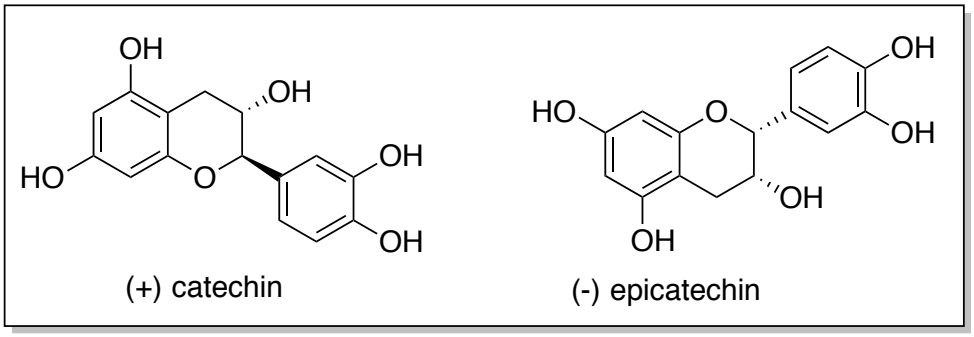


Figure 4. Chemical structures of (+) catechin and (-) epicatechin

Several nonflavonoids can be seen in Figure 5 including transpiceid, astringin, and astringinin; they are very effective free radical scavengers and structural derivatives of resveratrol.[11]

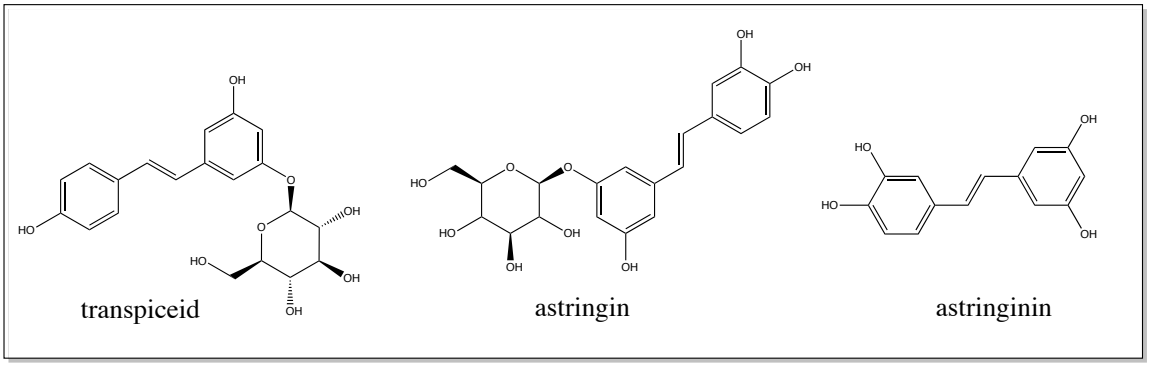


Figure 5. Chemical structures of transpiceid, astringin, and astringinin

As a group, polyphenols undergo several standard reactions that significantly contribute to their medicinal properties. These reactions include 1) ionization and oxidations to ortho and para quinones; 2) nucleophilic additions; and 3) oxidative and hydrolytic bond cleavages. Due to these characteristics, polyphenols tend to have oxidative activity, although it is not a defining characteristic of the group.

The chemical nature of polyphenols leads to commercial uses. The production of creosote, with its antiseptic and preservative properties, is widely known for the treatment and maintenance of wood. Some polyphenols are used as dyes; for example, pomegranate peels produce colorful fabrics in India, the high level of tannins and other polyphenols contributing to the color steadfastness. Additionally, polyphenol compounds are used as hepatotoxins, drugs, also endocrine disruptors and may play a role in the prevention of degenerative diseases including cardiovascular diseases, cancers, and tooth decay.

Polyphenols are the most abundant antioxidants in our diet. The total dietary intake may be as high as 1 g/d, 10 times higher than the intake of vitamin C and 100 times higher than the intake of vitamin E.[12] Chemists, initially intrigued by the

antioxidant behavior of polyphenols, focused on antioxidant vitamins, carotenoids, and minerals. It was not until 1995 that these antioxidant properties began to be more thoroughly investigated.[13] This delay was somewhat because polyphenols are plant derivatives and, therefore, possess complex molecular structures that were difficult to reproduce.

The most abundant polyphenols in the environment are the condensed tannins that are found in almost all families of plants and responsible for up to 50% of the dry weight of leaves.[14] Easily encountered in daily dietary items, polyphenols include many fruits, vegetables, cereals, chocolate, dry legumes, and plant-based drinks such as fruit juices, tea, coffee, and red wine. Current research into polyphenols has surged with much focus on medicinal properties of polyphenols as a group. One of the initial studies that generated interest around the medicinal properties of polyphenols, including resveratrol, was published in the 1990s and is known as the French paradox. The French paradox refers to the inconsistency that while the French traditionally have fatty diets, and many of them smoke, only a small percentage suffer from coronary heart disease. It was hypothesized that this phenomenon was directly linked to the French consumption of wine, specifically red wine. Further investigation revealed the presence of multiple polyphenolic compounds in red wines, which was potentially overriding the harmful dietary and lifestyle habits.

Given the vast benefits associated with these polyphenol family, scientists in 1995 began focusing on the medicinal properties of molecules similar to resveratrol, hoping to

enhance these benefits through chemical derivatives. Many different compounds make up the polyphenol family. This paper is concentrated on stilbenoids.

Stilbenoids

Stilbenoids are a small group of plant based nutrients and include pterostilbene and resveratrol. Overall, stilbenoids have anti-inflammatory affects; they prevent cancers and protect against diabetes. Pterostilbene is a potent antioxidant found mainly in blueberries, also in grapes. Similarly, resveratrol can be found in grapes and red wines, with red grapes containing up to 0.78 mg/L of resveratrol.[15]

In 2008, worldwide it was estimated that there were 12 million new cancer diagnoses and more than 7 million deaths from cancer.[16] A significant amount of research is in progress exploring methods for prevention of cancer and treatment options. When looking for new chemo-preventatives, it is not unusual to turn to the natural world for insight. One molecule of particular interest is resveratrol, first isolated from the roots of white hellebore plant in 1940.[17] Two natural geometric isomers of resveratrol, cis and trans, exist and can be seen in Figure 6.

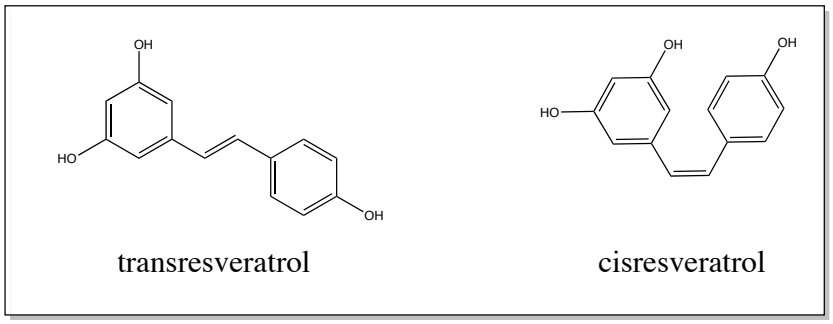


Figure 6. Geometric isomers of resveratrol

The transisomer is the main focus for most research because it mimics calorie restriction (CR)-type effects on a diverse group of organisms.[18] Resveratrol has been isolated in at least 72 plant species as well as from common foods such as grapes, mulberries, peanuts, and legumes. Studies indicate resveratrol acts as a phytoalexin, a compound produced by plants in response to stress from various sources including fungal infections, UV radiation, and temperature changes.[17] Since its discovery in 1940 resveratrol has been found to have numerous health benefits including antioxidant, an antimutagen, antifungal, antimicrobial, antitumor, and anti-inflammatory properties.[19] Due to these many benefits resveratrol has become a molecule of interest in multiple applications and the number of times it has been referenced has greatly increased since the 1990s. This growth can be seen in Figure 7.

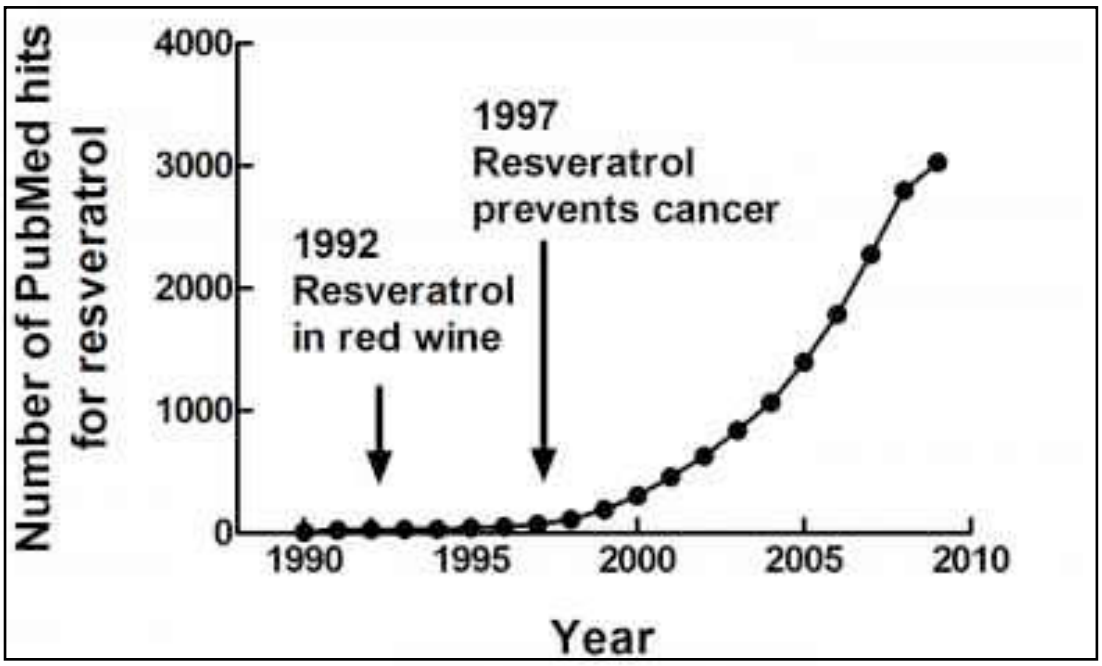


Figure 7. Citations of resveratrol[20]

Antiaging Possibilities

Resveratrol has also been found to have antiaging properties.[21] One of the ways this was discovered was due to resveratrol's antimicrobial characteristics. Resveratrol activates sirtuin which has functions including the caloric restriction-longevity effect.[21] Another antiaging effect of resveratrol stems from the fact that plants produce resveratrol when they are under stressful conditions, including UV stress. For that reason, it has also been demonstrated to play a role in prevention of photoaging.

Photoaging is defined as a characteristic change following chronic UVA and UVB exposure. Resveratrol has been demonstrated to act on cellular signaling mechanisms related to UV-mediated photoaging; more specifically, resveratrol lowers the levels of reactive oxygen species in UVA-exposed HaCat keratinocytes.[21]

Resveratrol falls into a category known as phytoestrogens. These are being evaluated as possible selective cytoplasmic and membrane surface estrogen receptor agonists, which are known to slow skin aging without the potential risk of estrogen. Further research in this area needs to be conducted; however, estrogen has been determined to enhance dermal water-holding capacity, to increase glycosaminoglycan content, to maintain skin elasticity and collagen content, and to diminish wrinkling.[21]

Antioxidant Properties

A relatively new health trend is concentrated around antioxidants. The ability of stilbenoids to serve as efficient antioxidants is directly linked to the molecular structure of the stilbenoid, specifically the location of various hydroxyl groups. Antioxidants are important because they protect the cells from damage caused by reactive oxygen species

(ROS). ROS such as superoxide, peroxyl radicals, and hydroxyl radicals form as a natural byproduct during the metabolism of oxygen and they have important roles in cell signaling and homeostasis.[22] ROS can also be generated from other sources including ionizing radiation and environmental stress such as UV or heat exposure. An imbalance between ROS and antioxidants can lead to oxidative stress, which in turn leads to cellular damage. This type of wide ranging damage has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation, and neurodegenerative diseases such as Alzheimer's Disease.[23] Oxidative stress in the brain, caused by the buildup of beta-amyloid plaques, is strongly correlated with the progression of Alzheimer's disease.[24] New research into the relationship between flavonoids and Alzheimer's disease has found that epicatchin, a molecule from the family of catechin flavonoids, is able to protect brain cells, however, not through the originally proposed antioxidant mechanism.[25] This study by McShea et al. showed that epicatchin was present in the bloodstream for a number of hours after ingestion.[25] This suggests that epicatchin is able to last long enough to cross the blood-brain barrier and could assist in protecting against Alzheimer's disease.

Effects on Heart Health

When researching resveratrol a reoccurring theme, "the French Paradox", is often cited. Epidemiological studies reveal an inverse relationship between heart disease and flavonoid consumption. A common example of the relationship between flavonoids and heart disease is often referred to as the French Paradox. Described earlier, this phenomenon focuses on the trend that French people suffer less from coronary heart diseases than other Europeans and consume significantly more cholesterol rich foods.

Many studies investigated this paradox and confirmed that one or two glasses daily of red wine, which contains high levels of flavonoids mainly quercetin and ruitin, can protect against heart disease.[26] Current work asserts that the flavonoids in red wine play a significant role in the heart health of many French people.

Some researchers believe that this can be explained by the high quantity of resveratrol present in red wine, (4-20mg/L).[27] It was also found that a wine supplemented diet was more than twice as effective at reducing serum markers of oxidative stress than a vegetable-added diet.[8]

Acknowledged earlier, research has found that resveratrol decreases the risk of coronary heart attacks by inhibiting platelet aggregation, reducing cholesterol levels, and displaying anti-inflammatory activity.[28] It has also been noted that resveratrol has protective effects against oxidation of lipoproteins.[29] Several projects have investigated the effects of resveratrol and similar molecules on individuals' diets. The overall conclusion is that polyphenols, rather than antioxidant vitamins alone, are responsible for the antiaging benefits of the Mediterranean diet.

Resveratrol may play a crucial role for the prevention of heart disease because it has been reported to inhibit platelet aggregation and coagulation.[30] Additionally, the resveratrol molecule displays neurological benefits, allowing it to play a role in prevention of neurological disorders including strokes, ischemia, Huntington's disease, and Alzheimer's disease.[28] Its most exciting attributes, however, are its anticancer properties. Resveratrol has been shown to inhibit the development of preneoplastic lesions in carcinogen-treated mouse mammary glands, suggesting it could potentially be a

cancer chemo-preventative agent in humans. This paper contains a review of current research regarding resveratrol and its beneficial properties previously mentioned.

Cancer

Cancer comes from the word karcinos, the term Hippocrates used in 400 B.C to describe tumors.[31] A devastating disease, cancer is defined as abnormal cells dividing uncontrollably as well as invading other tissues. It can spread throughout the body using either the vascular or the lymphatic systems.

The American Cancer Society has identified more than 100 types of cancer. The prevalence and magnitude can be illustrated by the most common of which is prostate cancer with more than 240,000 new cases expected in the United States in 2012.[20] Other predominant cancers include: bladder, breast, colorectal, endometrial, kidney, lung, pancreatic, and thyroid cancer. Cancers are grouped into five main categories: carcinoma, sarcoma, leukemia, lymphoma, and myeloma, or central nervous system cancers:

-Carcinoma encompasses cancers that begin in the skin or within tissues that line internal organs.

-Sarcoma includes cancer that initially starts in connective or supportive tissues such as bone, cartilage, fat, muscle, or blood vessels.

-Leukemia is a type of cancer that originates in blood-forming tissues, for example, bone marrow; producing large numbers of abnormal blood cells that enter the blood stream.

-Cancers that originally form in cells of the immune system fall into the category of lymphoma and myeloma. Myelomas, central nervous system cancers, include those that begin in the tissues of the brain and spinal cord.

While differences in origin dictate the specific type of cancer, all cancers involve the uncontrolled growth of cells.

Cancer Causes and Prevention

There is a large body of research regarding cancer, with goals to learn more about its causes, preventions, and treatments. Causes of cancers include genetic predispositions, tobacco use, diet and physical activity, sun and UV exposure, as well as pollution and chemical carcinogens. Exploring causality of cancers provides valuable insight into early prevention. For example, smoking results in approximately 30% of all deaths in the United States from cancer;[32] therefore, by avoid smoking, secondhand smoke, and use of tobacco products one can prevent some types of cancers.

Another key component of overall good health includes proper diet. Studies have shown that poor nutritional diet, physical inactivity, and obesity are strongly linked to cancer; thus, it follows that maintaining good physical health is a means to prevention. Because the overuse of alcohol increases the risk factors for cancers of the oral cavity, pharynx, larynx, oesophagus, liver, colo-rectum, and breast, limiting consumption also falls in the category of proper diet.[33]

Given the cancer causing damage resulting from sun and UV overexposure, sunburn and UV protection are crucial for cancer prevention. Though healthy lifestyle

choices can lower risk factors for cancer formation, it is important to remember there are other important contributing factors, such as the ever-present role that genetics plays in cancer initiation.

Cancer Treatments

In 1846, anesthesia became commonly available to surgeons, resulting in an increase in the use of surgery as a cancer treatment option.[34] As expected, surgery entails removal of the tumor and often some tissue surrounding or adjacent to the tumor area. Today treatment selection depends upon the specific form of cancer and its progression. Current treatment options are sophisticated and widely varied; including targeted therapy, immunotherapy, hormonal therapy, stem cell or bone marrow transplantation, hyperthermia, photodynamic therapy, surgery, radiation, and chemotherapy, with each treatment pathway having inherent drawbacks as well as unique benefits.

Typically physicians and cancer professionals describe a cancer based on when the cancer was detected and how far it has progressed, referred to as staging. Surgery also provides a means to verify the presence and stage of cancer. One major drawback to surgery is the fact that sometimes the cancer is widespread and not all of it can be removed. For this reason and because of the natural cancer cell growth, surgery is usually used in tandem with an additional form of treatment.

In 1895 cancer treatment was drastically changed when Wilhelm Conrad Roentgen invented the x-ray, the resulting technology that would give rise to the option of radiation therapy for cancer treatment. Radiation therapy uses x-rays to target and kill

cancer cells. There are several types of radiation therapy, including internal radiation and proton therapy; the most common is external-beam radiation therapy in which a machine positioned outside the patient's body produces and projects the radiation beam.[35] One benefit of radiation therapy is that it is a local treatment, affecting the specific part of the body receiving the therapy. When used in conjunction with surgery, radiation often proves effective at initially shrinking a tumor to improve the success of subsequent surgery. As revolutionary as radiation therapy has been it has many side effects such as fatigue, mild skin reactions, upset stomach, and loose bowel movements.[36] Internal radiation therapy has been cited as causing bleeding, infections, and irritation after the implant is removed.[36] These are the short-term side effects, with long-term effects including the risk of a second cancer, infertility, heart problems, gastrointestinal problems, lung fibrosis, neurologic problems, thyroid problems, or osteoporosis.[37]

Another cancer treatment, chemotherapy, became available in 1919 when it was found that a component of mustard gas could reduce white blood cells.[38] These chemotherapeutic drugs halt a cancer cell's unchecked ability to grow and divide, causing cell death and reduction of the cancer. Chemotherapy drugs have resulted in more varied treatment plans using combinations of surgery, radiation, and chemotherapy drugs.

Side effects of chemotherapy depend upon the dosage level and how the patient reacts to the drugs but typically involve fatigue, risk of infection, nausea, vomiting, loss of appetite, and diarrhea. While the side effects of these chemotherapy drugs are usually temporary, disappearing when the treatment is over, all of the current treatments are

physically and psychologically taxing for the patient; thus, current research focuses on developing more effective therapeutics with fewer and less severe side effects.

Effects on Cancer

One of the most effective ways to reduce the risk of carcinogenesis once cancer has been detected is chemo-prevention, i.e., prevention of cancer by ingesting chemical agents that reduce the risk of carcinogenesis.[30] Chemotherapy drugs currently used include nonsteroidal anti-inflammatory drugs (NSAIDS); these drugs inhibit cyclooxygenase (COX). COX catalyzes the conversion of arachidonic acid to proinflammatory chemicals such as prostaglandis which, if present, can stimulate tumor cell growth and suppress immune surveillance.[30] The enzyme can also activate carcinogens into forms that are able to damage genetic material.

Resveratrol behaves in a similar fashion to the NSAIDS used at this time in that it inhibits the hydroperoxidase activity of COX-1. The inhibition activity of resveratrol extends to several human enzymes including F1 ATPase and Tyrosinase.[19] Furthermore, resveratrol can induce quinone reductase activity, significant because quinone reductase is capable of metabolically detoxifying carcinogens.[30]

It has been found that resveratrol exerts proapoptotic effect in a discriminatory fashion. In other words, resveratrol is able to target tumor cells and cause them to die, the affect is on tumor cells, normal cells remain unharmed. This is especially critical because many current methods of cancer treatment damage both cancerous cells and normal cells.

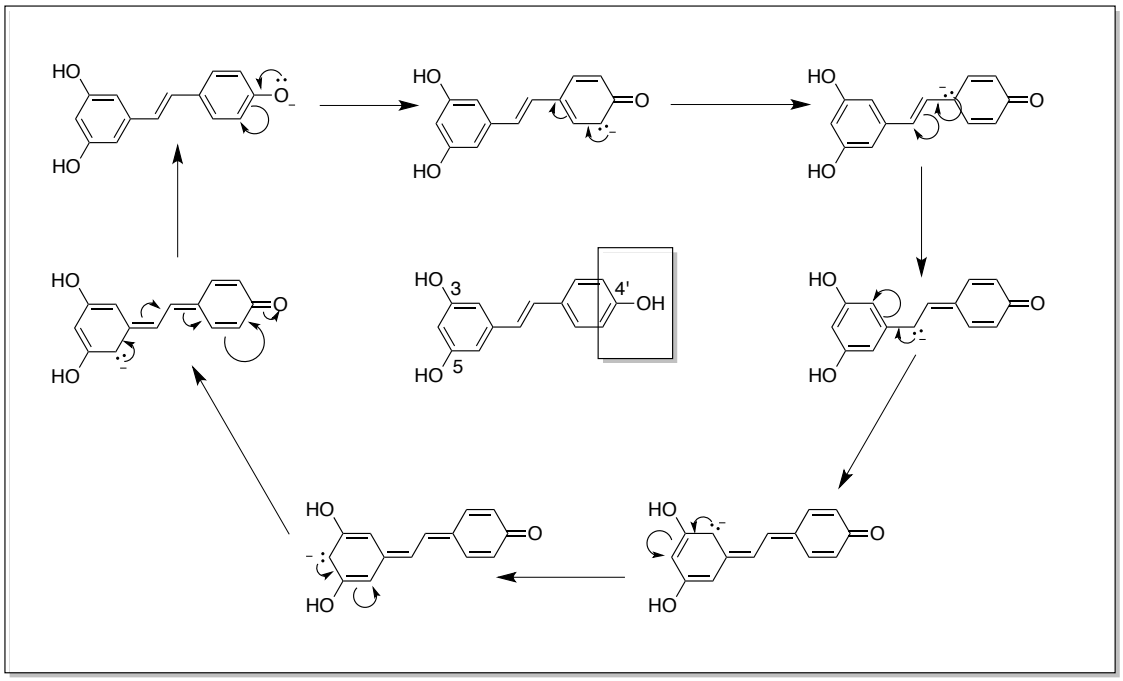
Resveratrol and other wine polyphenols have been shown to act as chemosensitizers and radiosensitizers, affecting tumor cells with an increased sensitivity to chemotherapy and radiation. These are exciting and important advances because a common problem with current cancer treatments is the ability of cancer cells to adapt and build up resistance to the treatment.

The majority of resveratrol's benefits stem from the fact that it is an extremely efficient free radical scavenger. Tests showed that resveratrol was 95% efficient at preventing lipid peroxidation, compared to 65% efficiency of vitamin E or vitamin C, which was only 37% efficient.[21] Furthermore, other studies indicate that resveratrol is the most active peroxyl radical scavenging compound when compared to previously known compounds including catechin, epicatechin, gallocatechin, gallic acid, and ellagic acid.[21]

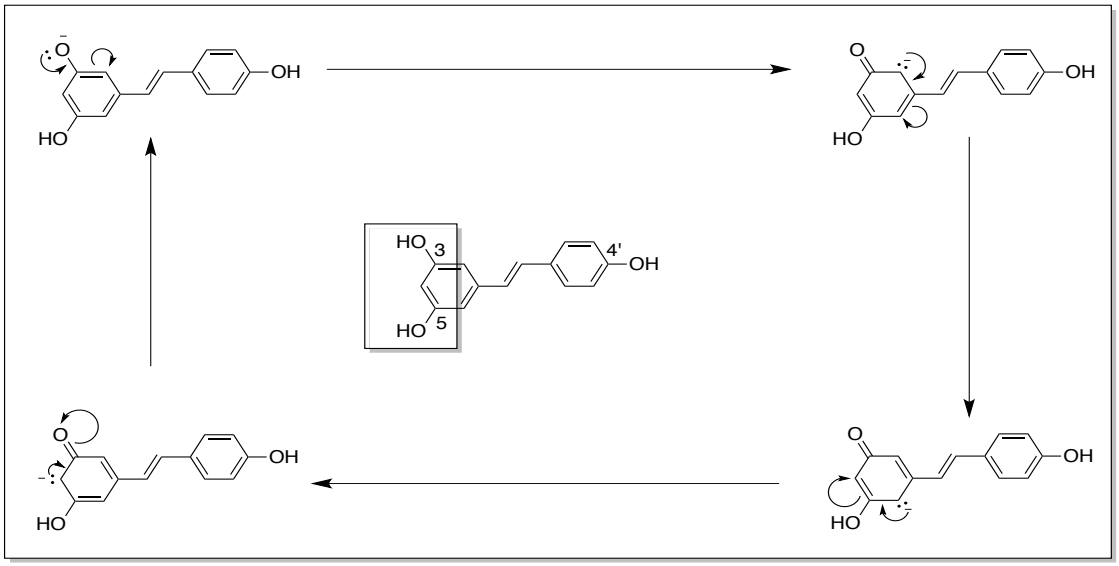
Though resveratrol shows potential as a therapeutic in many areas, especially as an anticancer drug, it not very water-soluble rendering it less effect as a therapeutic drug. In order for resveratrol to be active, it needs to be bound to Human Serum Albumin (HSA), protein found in human blood plasma. HSA binds fatty acids, salicylic acids, and many other commonly used drugs.[19] Experiments indicate that the 4' OH of resveratrol is the best group for derivatization. It has been shown that HSA has high affinities for carboxylic acids and that introducing a long chain carboxylate ester group will increase the binding to HSA.[30] There are several projects with the objective of synthesizing derivatives of resveratrol that have increased binding activity to HSA, which make it a better anticancer drug.

Resveratrol

Resveratrol's health benefits have been known for thousands of years. Both in ancient India and in ancient China medicines containing resveratrol have been used to treat a range of conditions from piles and indigestion to intestinal worms. Initially the molecule, resveratrol was discovered in 1939 by Michio Takaoka, a Japanese scientist but it was not until the 1990s that research into "the French paradox" caused to resveratrol studies to really take off. Since then there have been a wide variety of studies focusing on resveratrol with hopes of understanding why it has such a variety of medicinal properties as well as how to improve those medicinal properties. It is widely agreed that resveratrol's ability to act as an antioxidant gives it many of its medicinal benefits. Resveratrol's antioxidant activity can be attributed to its ability as a free radical scavenger. The hydroxyl groups on resveratrol are what make it such and effective free radical scavenger. These hydroxyl groups have been studied, and it has been found that the hydroxyl group in the 4' position has a pKa of ~9.3 while the other two hydroxyl groups at 3 and 5 have pKa values of 10.0 and 10.6.[39] This difference can be attributed to the resonance stability that the hydroxyl at 4' benefits from, which can be viewed below in schemes 1 and 2.



Scheme 1. Resonance scheme of resveratrol when 4' hydrogen is removed



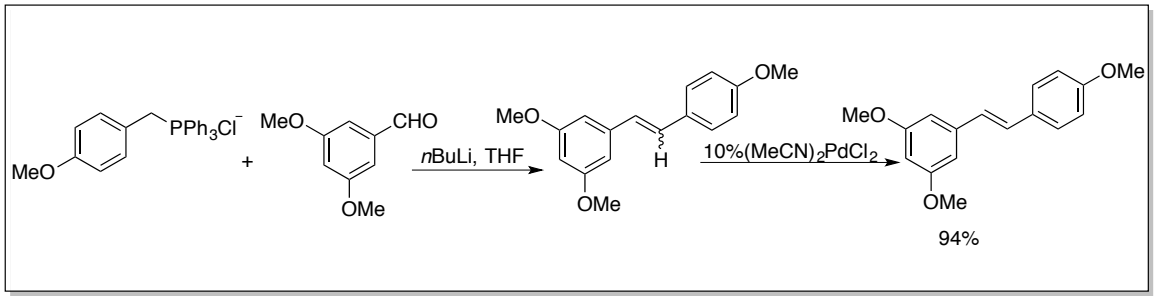
Scheme 2. Resonance scheme of resveratrol when 3 or 5 hydrogen is removed

As described earlier, resveratrol is an efficient radical scavenger and antioxidant that is able to reduce ROS, which can lead to a number of problems including developing Alzheimer's disease. Oxidative stress is caused by ROS that damage intracellular

macromolecules in neurons, including proteins, lipids, and DNA, and promote the dysfunction of various metabolic and signaling pathways in vulnerable brain tissues.[40] What makes resveratrol an especially affective antioxidant is that it has three hydroxyl groups that can donate their proton to a radical species that terminates the activity of the radicals, coupled with the fact that the now deprotonated resveratrol is a relatively stable nonreactive species that will not cause intercellular damage. The antioxidant properties of resveratrol have also been shown to contribute to the preservation of the mitochondria, which are very susceptible targets of free radical mediated damage.[40]

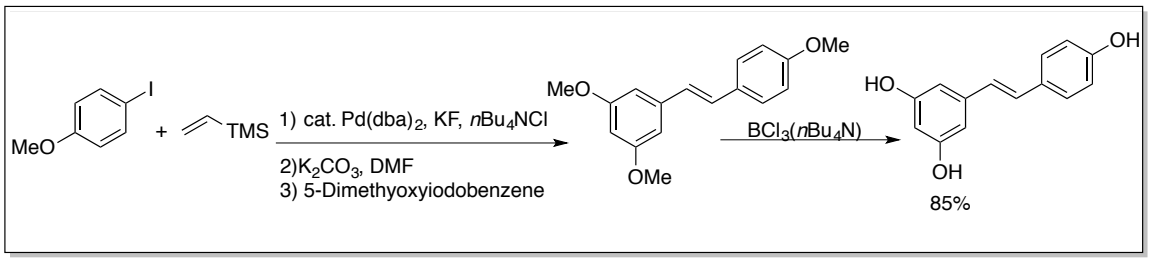
Synthesis of Resveratrol

Many synthetic syntheses of resveratrol exist, three of which are represented in the following schemes. Scheme 3 shows the synthesis of resveratrol using a Wittig reaction, a common procedure for the synthesis of alkenes.



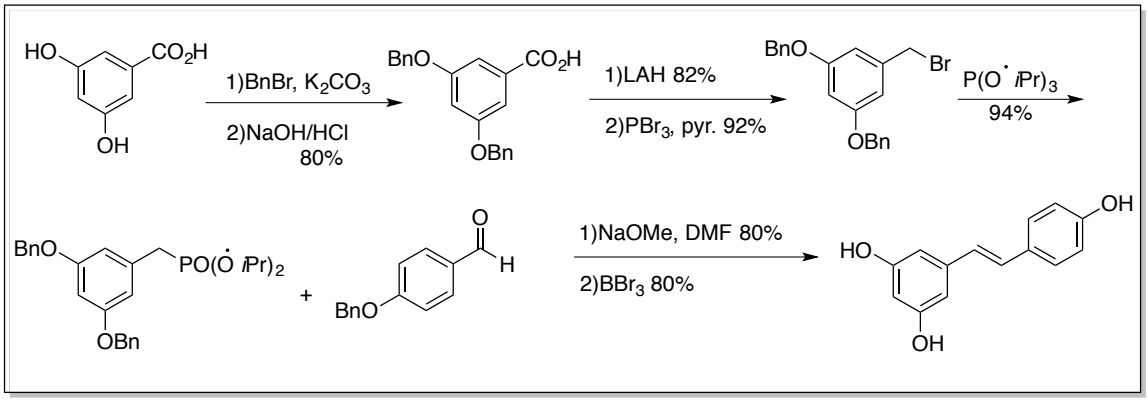
Scheme 3. Wittig synthesis of resveratrol

Scheme 4, below, shows the synthesis of resveratrol using a vinylsilane Heck reaction.



Scheme 4. Vinylsilane Heck synthesis of resveratrol

Scheme 5 shows the synthesis of resveratrol using an optimized Horner-Emmons reaction.



Scheme 5. Optimized Horner-Emmons synthesis of resveratrol

These are just a few ways that resveratrol has been synthetically synthesized, each has its own advantages and disadvantages and there is always a push to develop more efficient and cost-effective synthesis methods.

Synthesis of Resveratrol Derivatives

Once resveratrol was successfully synthesized the next step was to synthesize derivatives of resveratrol with the goal of improving its medicinal properties. A few derivatives can be viewed below in Figure 8. They are separated into two main groups, methyoxylated derivatives of resveratrol and hydroxylated derivatives of resveratrol.

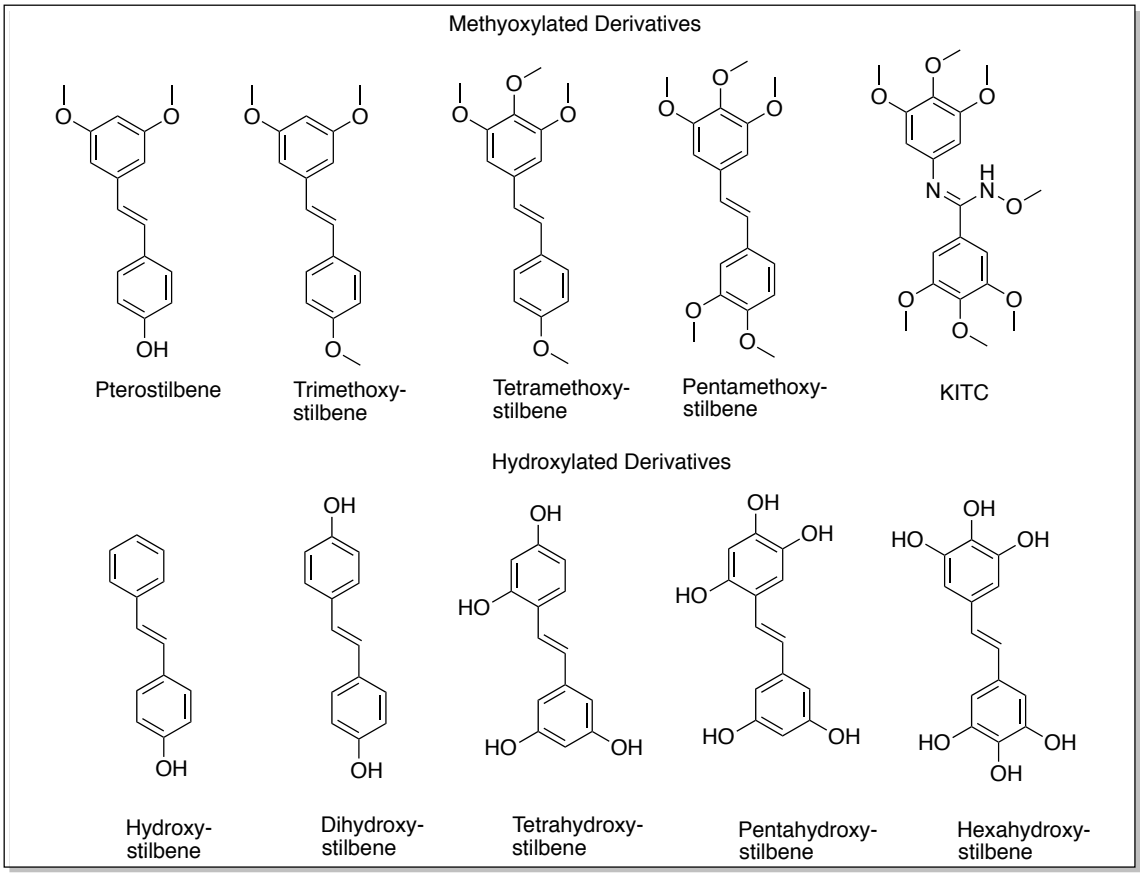


Figure 8. Methyoxylated and hydroxylated resveratrol derivatives

Methoxylated derivatives were synthesized with the aim of increasing the antitumor activity of resveratrol.[41] Research has shown that substituting methoxy groups in place of the hydroxyl groups on resveratrol substantially improved its cytotoxic activity.[42] The hydroxylated derivatives were synthesized based on research that indicated these hydroxyl groups improved the antiproliferative effects of resveratrol.[43,44]

Dr. Jiang's research is focused on improving resveratrol's anticancer properties, because of this the derivatives that I synthesized were ethoxylated and methoxylated derivatives (structures below in Figure 9).

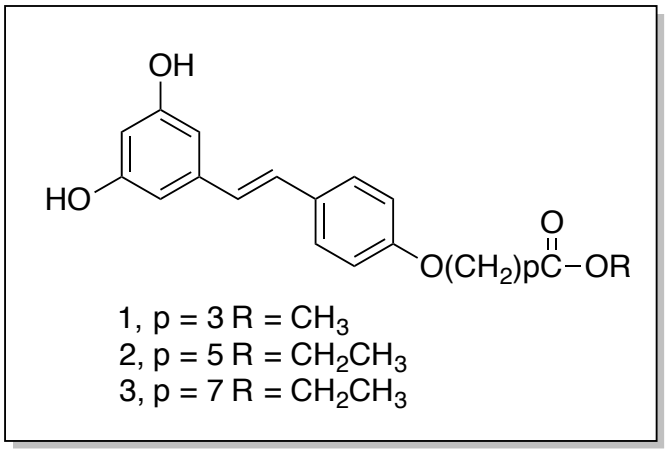


Figure 9. Structure of derivatives

Research has indicated that the 4'-OH group plays an important role in resveratrol's ability to bind to Human Serum Albumin (HSA) and this is what transports resveratrol into cells. Improving this binding ability would make resveratrol more bioavailable and therefore make it a more effective drug to help prevent cancer. The reason these three derivatives have a long chain is to help improve resveratrol's water solubility without changing too much of resveratrol's chemistry. The goal of this research, therefore, is to improve resveratrol's water solubility and its ability to bind to HSA.

CHAPTER 2

EXPERIMENTAL

General Methods

All commercial reagents were purchased from Sigma (St.Louis, MO, USA) and used without further purification. Solvents were used as purchased without further distillation and are mentioned in the experimental procedures. All proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance spectra were recorded with a JEOL-NMR Eclipse spectrometer operating at 400 MHz and a Varian 400 MHz multinuclear NMR. Chemical shifts were recorded as delta values in parts per million (ppm) relative to a TMS standard. The multiplicity for the signal peaks is reported as follows: s, singlet; d, doublet; m, multiplet. All NMR spectra are available in the appendices. Reported masses were obtained by using an Ohaus Adventurer Pro balance. Column chromatography was performed using silica gel as the stationary phase and different mixtures of solvents as eluents. The composition of these eluent systems are further detailed in the experimental procedures. Thin layer chromatography (TLC) was performed on silica gel plates using necessary solvents and visualized under a UVGL-58 UV lamp at 254nm wavelength. Melting points were determined using MEL-TEMP and recorded without correction.

Experimental Procedures

Synthesis of (E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)butanoate, **1**

Figure 10, below, shows the molecular structure of derivative 1.

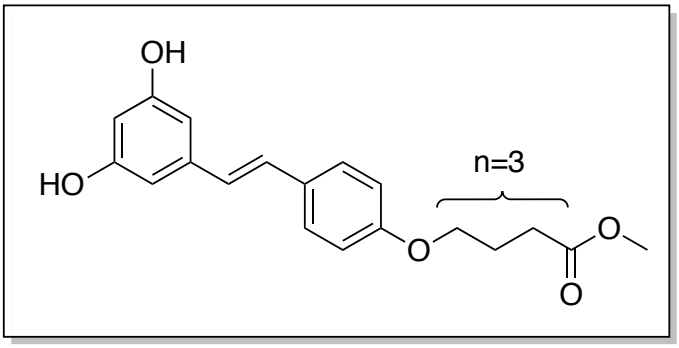


Figure 10. Chemical structure of compound **1** (n =3)

Resveratrol (1.1570 g, 5.0692 mmol, 1 equivalent), potassium carbonate (K_2CO_3) (0.7590 g, 5.492 mmol, 1.0834 equivalent), and methyl-1-4-bromobutane ($C_5H_9BrO_2$) (0.587 mL, 4.65 mmol, .9173 equivalent) were added to a single-neck round bottom flask (rbf). The reaction was placed under nitrogen (N_2), while anhydrous dimethyl sulfoxide (DMSO) (10 mL) was added using a syringe. The reaction was stirred overnight at room temperature under N_2. The solution was poured onto saturated ammonium chloride ($NH_4Cl_{(aq)}$) (40 mL) then extracted with ethyl acetate (EtOAc) (3x30mL). The resulting organic layer was dried with anhydrous magnesium sulfate, filtered to remove the magnesium salt, and solvent was evaporated. From the crude product a 0.1002 g portion was purified by silica column chromatography using a 40% acetone/hexanes as eluent. Fractions were collected from the column. TLC was used to verify the presence of desired product based on UV activity, after evaporation of solvents compound **1** was obtained as a brown powder. (0.0551g, 0.1678 mmol, 55% yield) ^{1}H NMR (400MHz,

DMSO, δ, ppm), 1.5(triplet), 1.7(pentet), 3.6(singlet), 3.9(triplet), 6.1(singlet), 6.4(singlet), 6.7-7.4(stilbene), 9.2(singlet) ^{1}H NMR of compound **1** can be found in appendix A.

Synthesis of (E)-ethyl 6-(4-(3,5-dihydroxystyryl)phenoxy)hexanoate,**2**

Figure 11, below, shows the molecular structure of derivative 2.

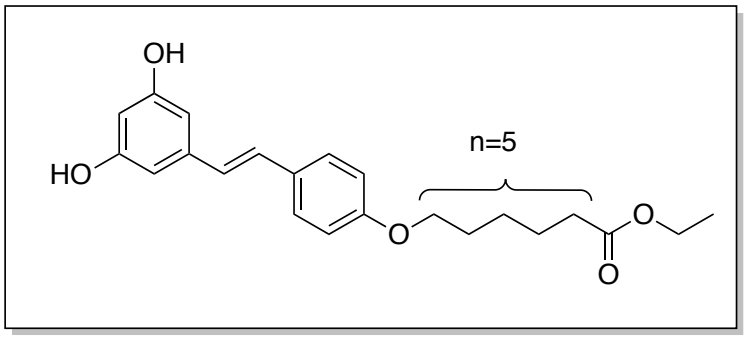


Figure 11. Chemical structure of compound **2** (n=5)

Resveratrol (2.2900 g, 10.033 mmol, 1 equivalent), K_2CO_3 (1.5180 g, 10.984 mmol, 1.0947 equivalent), and 6-bromohexanoic acid ethyl ester (1.660 mL, 9.369 mmol, .9338 equivalent) were added to a single-neck rbf. The reaction was placed under nitrogen (N_2), then dimethyl sulfoxide (DMSO) (20 mL) was added using a syringe. The reaction was stirred at room temperature overnight under N_2. The solution was then poured onto saturated $NH_4Cl_{(aq)}$ (40 mL) then extracted with EtOAc. The resulting organic layer was dried with anhydrous magnesium sulfate, filtered to remove the magnesium salt, and then evaporated. From the crude product a 0.1000 g portion was purified by silica column chromatography using 20% acetone/hexanes, then the gradient was increased to a 30% acetone/hexanes. Fractions were collected from the column. TLC

was used to verify the presence of desired product based on UV activity, after evaporation of solvents compound **2** was obtained. The product, .0721 g, was recrystalized from 1:1 EtOAc/Hexane (7mL/7mL) to give compound **2** as a brown powder (0.0597 g, 0.1661mmol, 60% yield). ^{1}H NMR (400MHz, DMSO, δ, ppm), 1.2(triplet), 1.4(pentet), 1.6(pentet), 1.7(pentet), 2.3(triplet), 3.9(triplet), 4.1(quartet), 6.1(singlet), 6.4(singlet), 6.8-7.0(stilbene), 7.5(doublet), 9.2 (singlet) ^{1}H NMR of compound 2 can be found in appendix B.

Synthesis of (E)-ethyl 8-(4-(3,5-dihydroxystyryl)phenoxy)octanoate, **3b**

Figure 12, below, shows the molecular structure of derivative 3b.

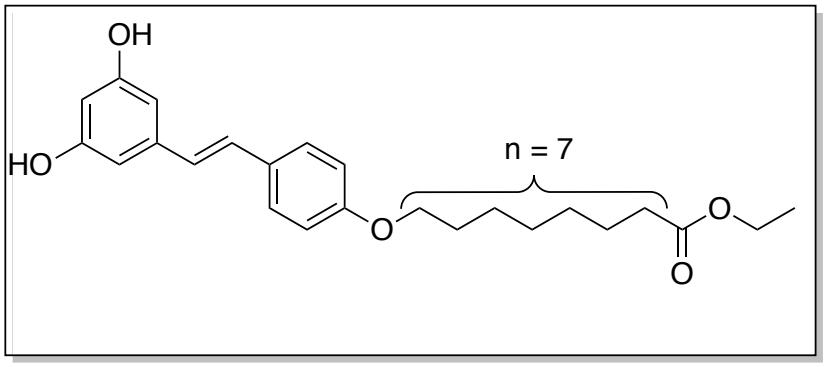


Figure 12. Chemical structure of compound **3b** (n=7)

Procedure for synthesis of compound **3a**:

To a single neck rbf, 8-bromooctanoic acid (2.0000 g, 8.9642 mmol, 1 equivalent) was dissolved in methanol (50 mL), sulfuric acid (1.0 mL, 19 mmol, 2.1195 equivalent) was slowly added to the reaction and allowed to reflux overnight. After 16 hours, the reflux was halted, the rbf was cooled to room temperature, the solution was then concentrated using a rotary evaporator to produce yellowish oil. The yellow oil was

dissolved in methylene chloride (40 mL), and the solution was washed with saturated sodium bicarbonate (2 x 30 mL). Another extraction with methylene chloride (20 mL) was performed on the combined aqueous layers. The combined organic layer was washed with water and dried over magnesium sulfate. The crude product was obtained after evaporation of the organic layer. The resulting product was a yellow oil (0.70 mL, 0.99 g, 4.4 mmol 50% yield). ^{1}H NMR (400MHz, CCl_3D, δ, ppm), 1.3(pentet, 6H), 1.6(pentet, 2H), 1.8(pentet, 2H), 2.3(triplet, 2H), 3.4(triplet, 2H), 3.7(singlet, 3H). ^{1}H NMR (400MHz, DMSO, δ, ppm), 1.2(pentet, 6H), 1.5(pentet, 2H), 1.8(pentet, 2H), 2.3(triplet, 2H), 3.5(triplet, 2H), 3.6(singlet, 3H). ^{13}C NMR (100MHz, CCl_3D, δ, ppm), 25.0, 28.0, 28.3, 28.9, 32.8, 34.0 (2C), 51.6, 170.4 ^{1}H NMR of compound **3a** can be found in appendixes E and F, the ^{13}C NMR spectra can be found in appendix G. Figure 13, below, shows the molecular structure of derivative 3a.

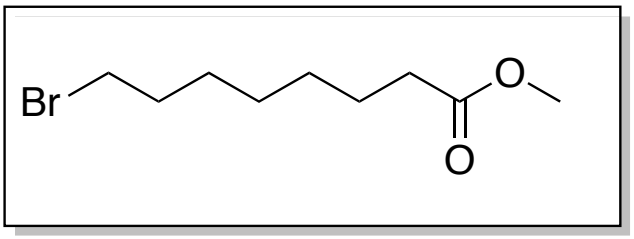


Figure 13. Chemical structure of compound **3a**, $Br(CH_2)_7COOCH_3$

Procedure for synthesis of compound **3b**:

Resveratrol (0.5178 g, 2.269 mmol, 1 equivalent), anhydrous DMSO (5 mL), K_2CO_3 (0.3391 g, 2.453 mmol, 1.08 equivalent), and $Br(CH_2)_7COOCH_3$ (4 mL, 25 mmol) were mixed in a rbf under N_2 atmosphere at room temperature for 16 hours. The solution was then poured onto saturated $NH_4Cl_{(aq)}$ (40 mL) solution and extracted with EtOAc (2x50 mL). The organic layer was dried with anhydrous magnesium sulfate,

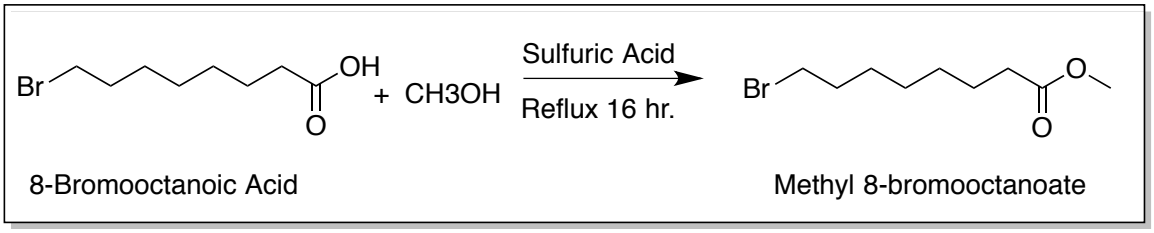
filtered to remove the magnesium salt, and then placed in the hood to dry. A portion of the resulting product (0.1000 g) was purified by column chromatography. The column was run as follows, 700 mL of 20% acetone/hexanes followed by 2100 mL of 30% acetone/hexanes. Fractions were collected from the column after TLC was used to verify the presence of desired product based on UV activity. After evaporation of solvents compound **3b** was obtained as a light brown powder (0.0716g, 0.186 mmol, 72% yield). Mp 182-183 ^{0}C. ^{1}H NMR (400MHz, DMSO, δ, ppm) 1.2(multiplet, 7H)**,** 1.4(pentet, 2H), 1.6(pentet, 2H), 1.7(pentet, 2H), 2.3(triplet, 2H), 3.9(triplet, 2H), 4.1(quartet, 2H), 6.1(doublet, 1H), 6.4(doublet, 2H), 6.8-7.1 (stilbene, 4H), 7.5 (doublet, 2H), 9.2(singlet, 2H). ^{13}C NMR (100MHz, DMSO, δ, ppm), 14.8, 24.9, 25.7, 29.0 (2C), 34.1, 60.3, 67.9, 102.6, 105.0(2C), 115.3(2C), 127.2, 128.1, 128.4(2C), 130.2(2C), 139.8, 158.9, 159.1(2C), 173.5 ^{1}H NMR of compound **3b** can be found in appendix C, the ^{13}C NMR spectra can be found in appendix D and the IR spectra can be found in appendix H.

CHAPTER 3

RESULTS AND DISCUSSION

Synthesis of (E)-ethyl 8-(4-(3,5-dihydroxystyryl)phenoxy)octanoate, **3b**

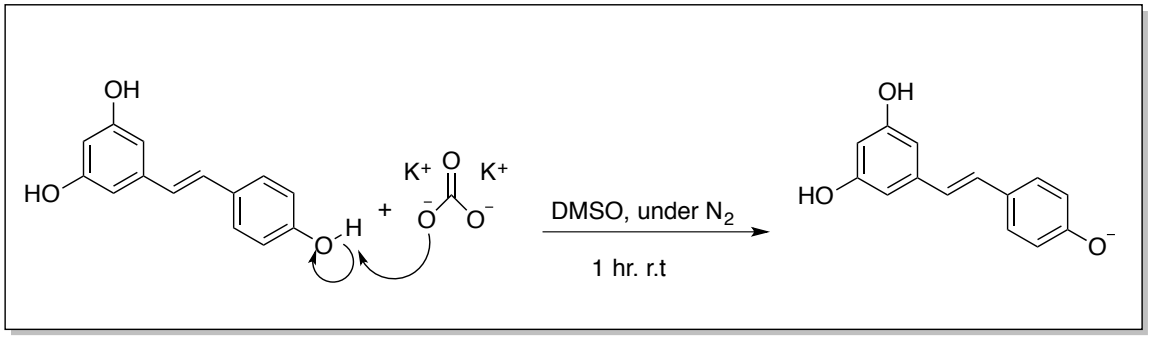
The three resveratrol derivatives were successfully synthesized and verified using NMR spectroscopy; however, only derivative **3b** was pure enough to be tested in vivo. The NMR data are presented in the appendixes A through E. The chemical reaction was a two-step process and is explained below in scheme 6.



Scheme 6. Synthesis of methyl 8-bromooctanoate

`The first part of the synthesis of compound **3a** was a common esterification reaction which converted 8-bromooctanoic acid to methyl 8-bromooctanoate (Scheme 6). The product was isolated as a yellow oil that was purified before it was used in the next step of the synthesis.

Scheme 7. Reaction conditions for compound **3b**



Scheme 8. Acid-base reaction step of compound **3b**

The second part of the synthesis of compound **3** was to replace the desired hydroxyl group with the 8-bromooctanoate chain (Scheme 9). First the hydroxyl group needed to be deprotonated by potassium carbonate to make it a better nucleophile. Literature shows that the three hydroxyl groups of resveratrol, shown in Figure 14, have different pKa values. Deak et al. discovered that, "...pKa values of 9.3, 10.0, and 10.6... The first deprotonation step might be assigned to the 4' -OH' because the charge of the corresponding phenolate ion can be delocalized over the whole molecule in this case only." [39]

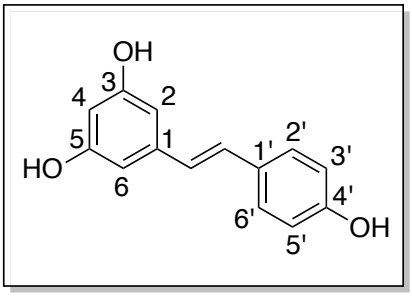
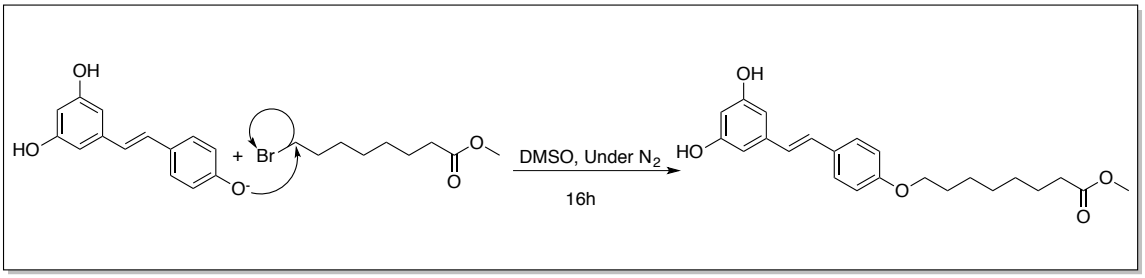


Figure 14. Chemical structure of transresveratrol

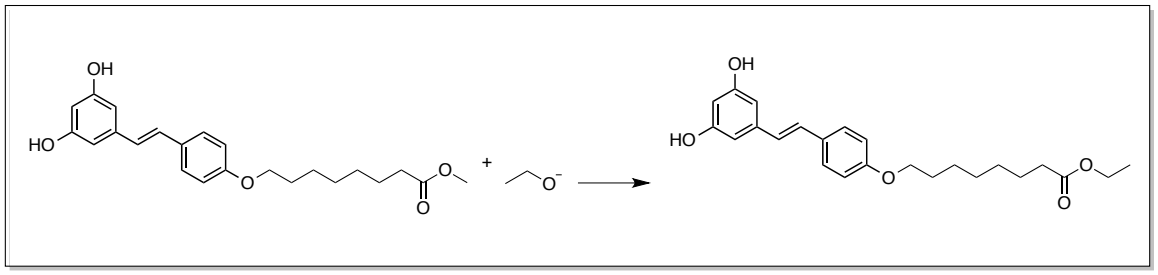
Jiang et al. reported preferential deprotonation of the 4'-OH group with potassium carbonate.[19] However, if deprotonation of all three hydroxyl groups is desired triethylamine is the preferred base because it is a stronger base. Even when potassium

carbonate is used, side products including the di and tri substituted compounds are formed besides the major mono-substituted compound. Once the deprotonation is achieved attaching the 8-bromooctanoate chain is relatively straight forward. DMSO is favored as an aprotic solvent, which is appropriate for the S_N2 reaction



Scheme 9. Synthesis of compound **3b**

The substitution reaction between the alkyl bromide and the resveratrol phenolate ion was optimized to give the mono substituted product. The desired mono-substituted compound was separated from the di- and tri- substituted compounds via column chromatography.



Scheme 10. Transesterification reaction

The purity and structure of compound **3b** were verified by ^{1}H NMR and ^{13}C NMR.

^{1}H NMR of compound **3a** in $CDCl_3$ can be found in appendix A. The protons on this compound are labeled *a* through *e*, (see structure Figure 15).

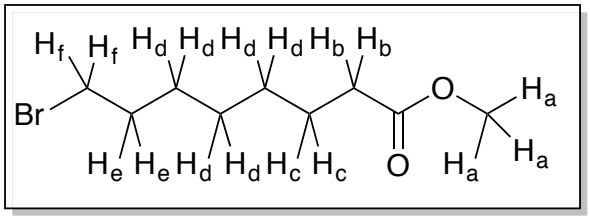


Figure 15. Labeled protons of compound **3a**

Protons (H_a) appear as a singlet at 3.7 ppm. They are shifted downfield from the normal alkyl position due to the deshielding effects of the neighboring oxygen. Because (H_a) protons do not have any vicinal protons, it appears as a singlet peak that integrates to three protons. Protons (H_b) appears as a triplet at 2.3 ppm that integrates to two protons and is shifted slightly downfield due to deshielding by the nearby carbonyl. The triplet splitting for (H_b) occurs because of the two vicinal protons, (H_c). (H_c) Protons gave a pentet at 1.6 ppm that integrates to two protons and is relatively shielded within the alkyl chain. The pentet splitting is a result of the four vicinal protons, (H_b) and (H_d). Protons (H_d) appear as a series of overlapping peaks at 1.3ppm, with the total integration of six protons. Although these protons are bonded to separate carbons, they are all in chemically similar environments. Because they are all located towards the center of the alkyl chain, they are relatively shielded. The pentet for protons (H_e) occurs at 1.8 ppm and integrates to two protons. These protons are slightly deshielded compared to the other alkyl protons because of their proximity to the electronegative bromine atom. The pentet splitting is due to the four vicinal hydrogens, (H_d) and (H_f). Protons labeled (H_f) appear as a triplet at 3.4 ppm with an integration of two protons. These protons are shifted downfield because of the deshielding effects of the neighboring bromine. The splitting pattern for these protons is cause by the two vicinal protons, (H_e). The ^{1}H NMR of compound **3a** in

DMSO can be found in appendix F, the peaks are slightly shifted in this solvent; however, they are relatively similar to those in $CDCl_3$.

The ^1H NMR of compound **3b** can be found in appendix C. The protons are labeled *a* through *o* and the assignments of this compound are shown in Figure 16.

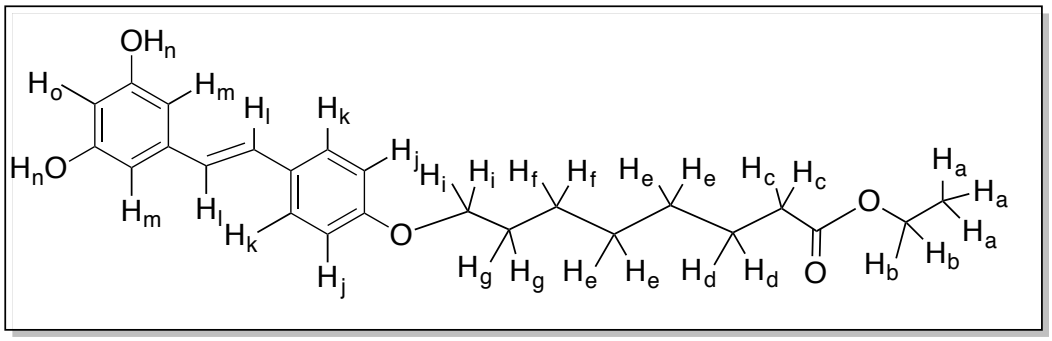


Figure 16. Labeled protons of compound **3b**

Protons (H_a and H_e) showed a multiplet 1.2 ppm, the peaks from H_a form a triplet that overlaps with the peaks generated by H_3. Protons (H_f) show are split into a pentet at 1.4ppm, this splitting is caused by the four neighboring protons (H_g and H_e). Protons (H_d and H_g) are also split into a pentet at 1.6 ppm and 1.7 ppm, perspectively. Protons (H_c) has two neighboring protons that cause it to form a triplet at 2.3 ppm. While (H_i) protons gave a triplet at 3.9 ppm. Protons (H_b) generate a quartet at 4.1 ppm. Protons (H_o and H_m) produce doublets at 6.1 ppm and 6.4 ppm perspectively. The stilbene protons (H_j, H_k and H_m) are found in the aromatic region from 6.8-7.1 ppm. Protons (H_l) generate a doublet at 7.5 ppm and the singlet at 9.2 ppm corresponds to protons (H_n).

^{13}C NMR spectrum of Compound **3b** is shown in appendix D. The carbons are labeled *a* through *s* on the structure below in Figure 17.

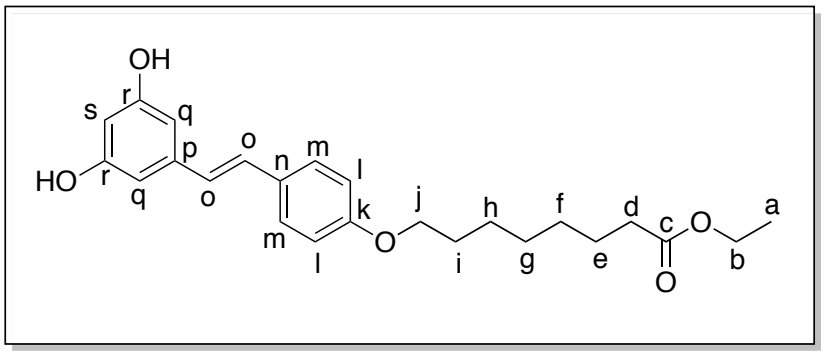


Figure 17. Labeled carbons of compound **3b**

The carbons' signals are shifted from TMS base on to their chemical environments. The ^{13}C NMR spectrum accounts for all the carbons further verifying the molecular structure of compound **3b**. As expected, the carbons for the aromatic rings, (*k, l, m, n, p, q, r* and *s*) can be found from 115-159. The carbons (*o*) can be found around 127, the alkene region. Carbon (*j*) is shifted downfield due to its proximity to the oxygen. The carbonyl carbon (*c*) is found in the ester region around 173.5. The ethoxy carbons, (*a* and *b*) are shifted downfield because of the neighboring oxygen.

Synthesis of (E)-methyl 4-(4-(3,5-dihydroxystyryl)phenoxy)butanoate, **1**

Compound **1** was synthesized with 55% yield and verified by NMR (Appendix A). However, the purity of the compound was not up to the necessary standards to be tested in vivo. Because of the impurities, this spectra was not integrated. Future work with this compound will include purification by column chromatography using a slightly more polar solvent system, which will help separate out the starting material as well as the di and tri substituted compounds. The ^{1}H NMR of Compound **1** can be found in Appendix A. Proton assignments (*a* through *k*) of this compound are shown in Figure 18.

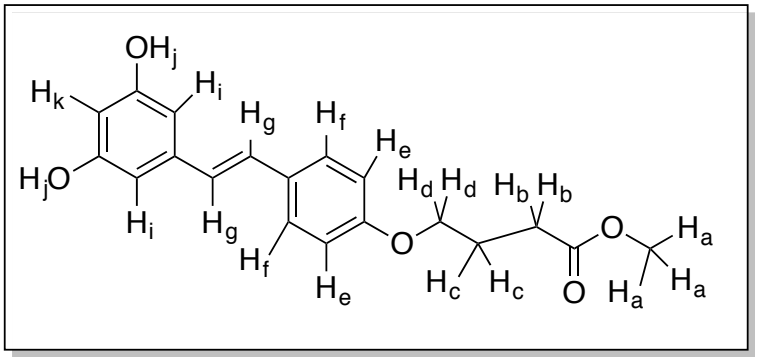


Figure 18. Labeled protons of compound **1**

Proton (H_a) gives a singlet found at 3.6ppm, shifted downfield from the normal alkyl position due to the deshielding effect of the neighboring oxygen. Because (H_a) does not have any vicinal protons, it appears as a singlet. (H_b) appears as a triplet at 1.5 ppm, the splitting of this peak is due to the two vicinal protons, labeled as (H_c). (H_c) is a pentet at 1.7 ppm, split into five due to the four vicinal protons, (H_b and H_d). The protons (H_d) are shifted downfield as well. Due to their vicinity to the electronegative oxygen, this peak can be found as a triplet at 3.9 ppm. Because (H_d) is proximal to two vicinal hydrogens, this peak is split into a triplet. Protons (H_e, H_f, and H_i) are located in the aromatic region ranging from 6.7-7.4 ppm. Proton (H_g) is a singlet found at 6.4 ppm. This remains a singlet as there are no vicinal hydrogens near it. Proton (H_k) is also a singlet found at 6.1 ppm. Finally (H_j) can be found as a singlet at 9.2 ppm. These protons are the most downfield due to their direct attachment to oxygen. There are three main impurity peaks in this spectra, all labeled as "*", the peak at 9.6 is from resveratrol starting material and the peaks at 2.3 and 3.4 are from 8-bromooctanoic acid starting material.

Synthesis of (E)-ethyl 6-(4-(3,5-dihydroxystyryl)phenoxy)hexanoate,**2**

Compound **2** was synthesized with a 60% yield and verified by NMR, (Appendix B). The purity of the compound **2** was not up to the necessary standards to continue and be tested in vivo because of the impurities the NMR spectra was not integrated. Future work with this compound will include purifying it further using column chromatography with a slightly more polar solvent system. Once these two compounds are further purified, tests can be preformed to investigate how the length of the ester chain affects the molecule's ability to enter the cell and be used by the body. The ^{1}H NMR of compound **2** can be found in appendix B. Proton assignments (*a* through *n*) of this compound are shown in Figure 19.

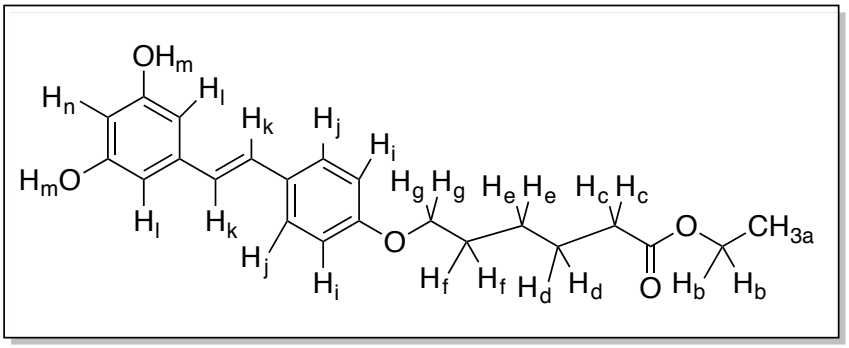


Figure 19. Labeled protons of compound **2**

Protons (H_a) are relatively shielded and occur upfield as a triplet at 1.2 ppm. This triplet splitting occurs because of the two vicinal protons labeled (H_b). (H_b) appear downfield at 4.1 ppm due to the deshielding effects of the neighboring oxygen and the peaks appear as a quartet. The splitting for (H_b) occurs because of the two vicinal protons, (H_a). (H_c) are slightly deshielded by the neighboring carbonyl which results in the triplet at 2.3 ppm. The triplet splitting is a result of the two vicinal protons, labeled (H_d). Protons

(H_d) appear as a pentet at 1.6 ppm. The pentet splitting is due to the four vicinal protons, (H_c and H_e). The pentet for (H_e) occurs at 1.8 ppm, these protons are slightly deshielded, compared to the other alkyl protons because of their proximity to the electronegative bromine atom. The pentet splitting is due to the two vicinal hydrogens, (H_d), as well as the two vicinal hydrogens labeled (H_f). Protons (H_f) appear as a triplet at 3.4 ppm. These protons are shifted downfield because of the deshielding effects of the neighboring bromine. The splitting pattern for these protons is caused by the two vicinal protons, (H_e). The pentet for (H_e) occurs at 1.4 ppm and these protons are shielded compared to the other alkyl protons because of their location towards the center of the alkyl chain. The pentet splitting is due to the two vicinal hydrogens, (H_d) as well as the two vicinal hydrogens labeled (H_f). Protons (H_f) appear as a pentet at 1.7 ppm and these protons are shifted slightly downfield because of the deshielding effects caused by the nearby oxygen. The pentet splitting pattern for these protons is caused by the four vicinal protons, (H_e and H_g). Protons (H_g) are relatively deshielded by the electronegativity of the neighboring oxygen atom and appear as a triplet at 3.9 ppm. These peaks form a triplet because of the two vicinal protons (H_f). The (H_i) and (H_l) protons are in a very similar chemical environment due to the conjugation within the molecule, thereby appearing as a stillbene multiplet between 6.9-7.0 ppm. The (H_i) protons should appear as a doublet in the aromatic region due to (H_j). Protons (H_k) appear as a doublet in the aromatic region because they are vicinal to one another. Protons (H_j) appear as an aromatic doublet at 7.5 ppm and they are identical protons that give a doublet because of the vicinal proton labeled (H_i). Protons (H_k) are identical, aromatic protons that give a singlet peak at 6.4

ppm. This peak occurs as a singlet because there are no vicinal protons. The two protons (H_m) correspond to the identical phenol groups and appear as a singlet at 9.2 ppm. Because these protons are directly bonded to electronegative oxygen atoms, they are extremely deshielded and show up downfield. Protons (H_n) occur as an aromatic singlet at 6.1 ppm due to the deshielding effects of aromatics and the peak is a singlet because there are no vicinal protons. The main impurity in this spectra is labeled with a "*" and it is water, which appears as a singlet around 3.4.

Conclusions

The goal of this research was to synthesize more biologically relevant derivatives of resveratrol. The objective was to adjust the functional groups of a derivative in order to improve its ability to enter the cell as well as improve its binding ability to HSA, therefore, increasing the effectiveness of the derivative as well as its therapeutic potential. Three derivatives were synthesized, (E)-methyl 4-(4-(3,5-di-hydroxy-styryl)-phenoxy)-butanoate, (E)-ethyl 6-(4-(3,5-dihydroxystyryl)phenoxy)hexanoate, and (E)-ethyl 8-(4-(3,5-dihydroxystyryl)phenoxy)octanoate. The same synthesis steps were followed and all three derivatives were purified by column chromatography then verified using NMR spectroscopy. While all three compounds were successfully synthesized, only derivative 3 was pure enough to be used for the next step in the project, which entails testing the binding affinity of the derivative for human serum albumin, (HSA). Derivative 3 was further analyzed through confocal raman spectroscopy; however, there was some laser damage to the sample and a readable spectra could not be generated. The damage to the sample; however, indicated the presence of a conjugated phenyl system that one would

expect from derivative 3. The next aim of this research to investigate derivative 3's ability to enter the cell as well as to to assay the binding ability of derivative 3 with HSA in the hopes of improving resveratrol's anti-tumor properties. Derivatives 1 and 2 need to be further purified through column chromatography using a more polar solvent system and reevaluated using NMR spectroscopy. Once these three derivatives are isolated to the necessary purity, their binding affinities can be properly tested within a biological setting. The protein HSA is fluorescent because of a tryptophan residue and this fluorescence is quenched when HSA binds with resveratrol, because of this the binding affinity can be assessed and calculated.[19] This testing will provide insight into how the length of the alkyl chain affects both the molecule's ability to enter the cell and the binding ability of the molecule to HSA. Future work will also include synthesizing derivatives with different functional groups to determine how these moieties impact the binding affinity with HSA. Such biological studies would provide useful information about the ability of these derivatives to aid in the treatment of cancer, alzheimer's disease, heart disease, as well as many other conditions. Though resveratrol has been shown to be extremely beneficial with regards to many of these diseases and disorders, more biologically available and active derivatives may prove useful as more efficient therapeutics.

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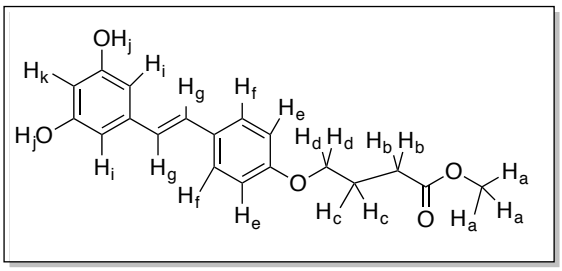
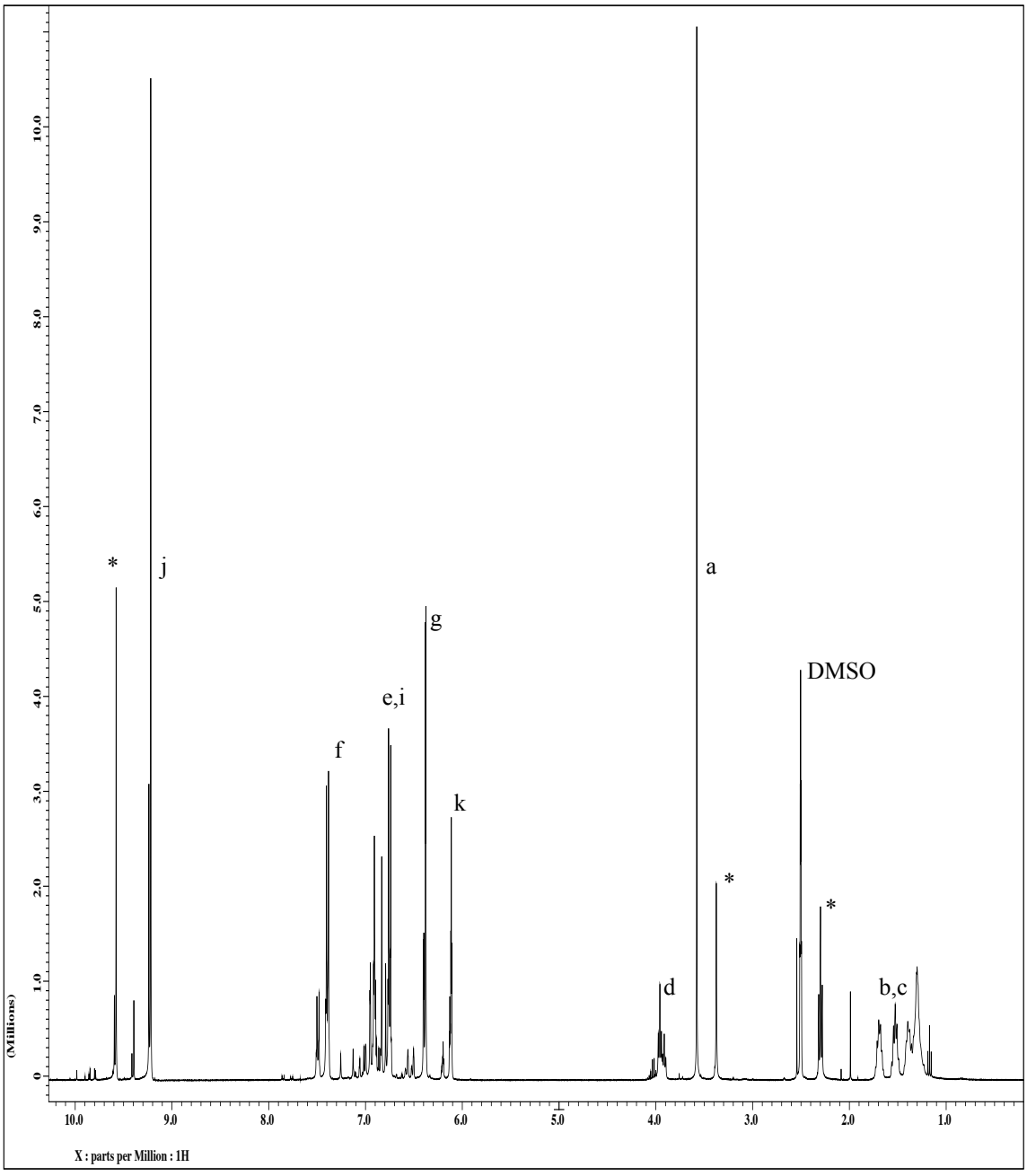
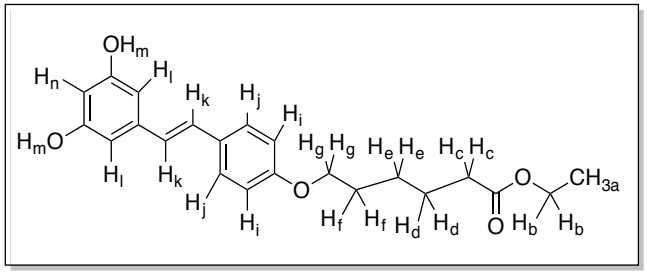
APPENDICES

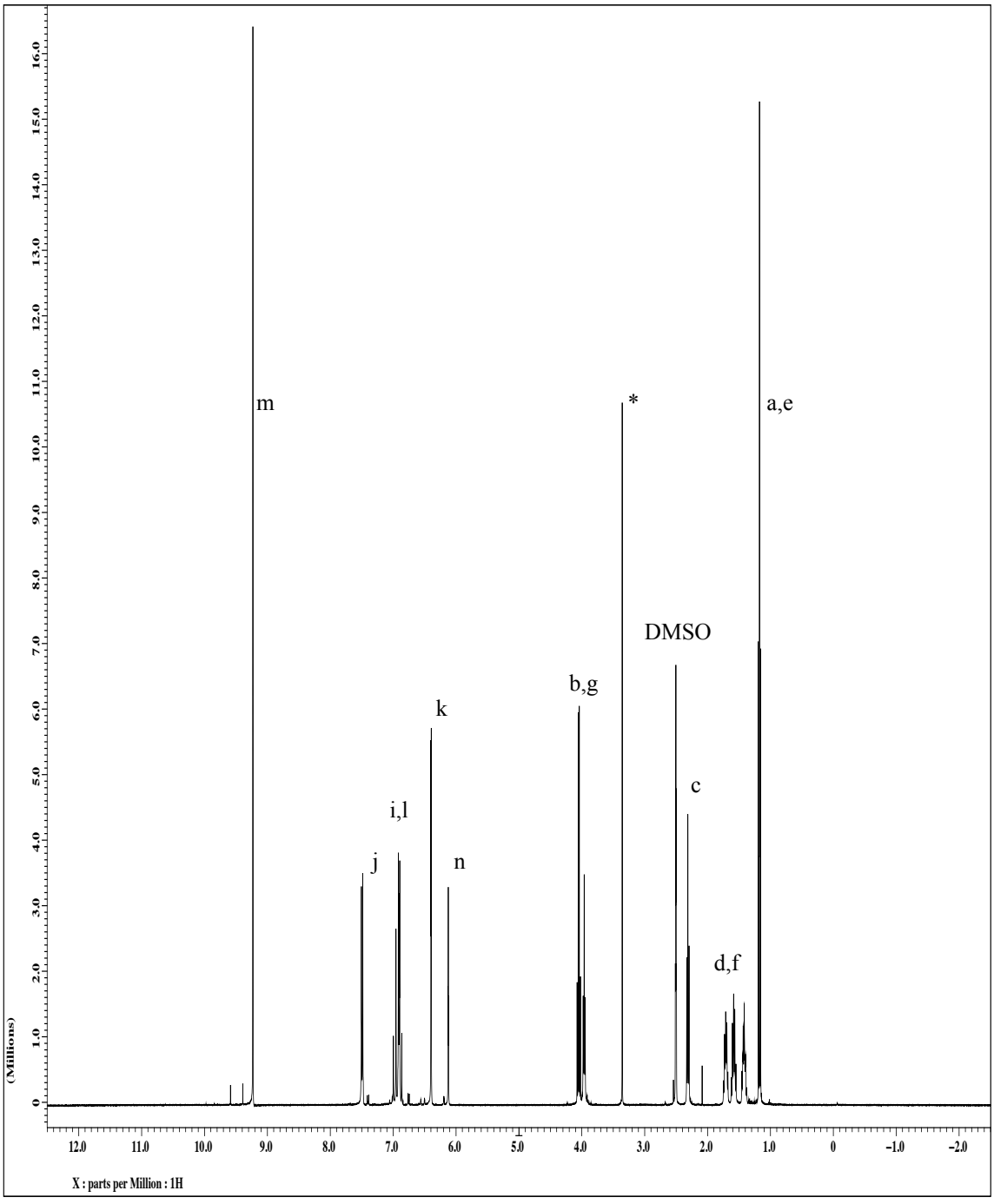
Appendix A: ^{1}H NMR spectrum of compound **1** in DMSO



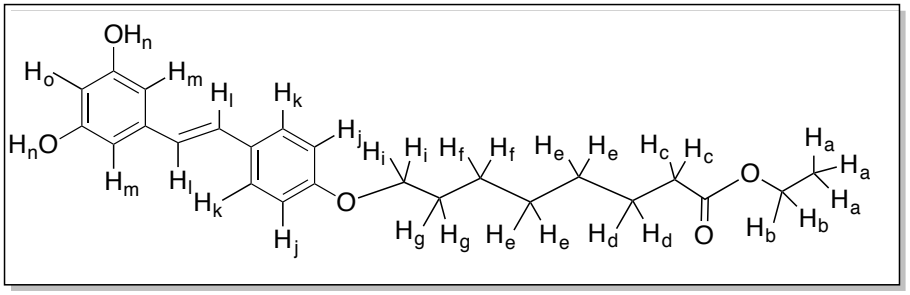


Appendix B: ^{1}H NMR spectrum of compound **2** in DMSO

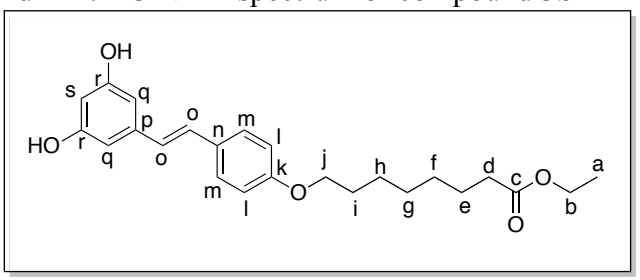


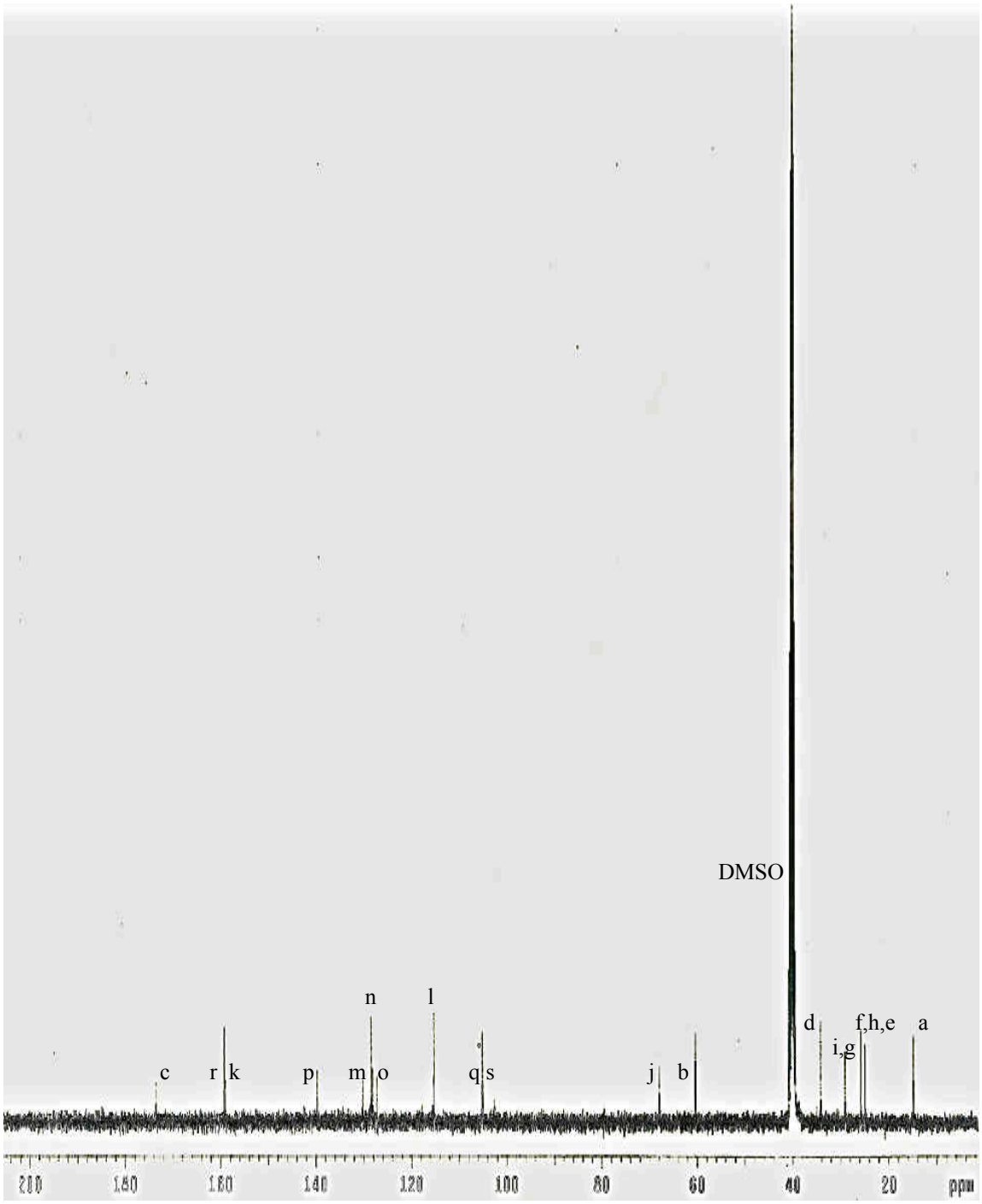


Appendix C: ^{1}H NMR spectrum of compound **3b** in DMSO

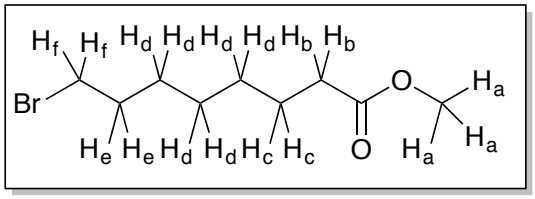


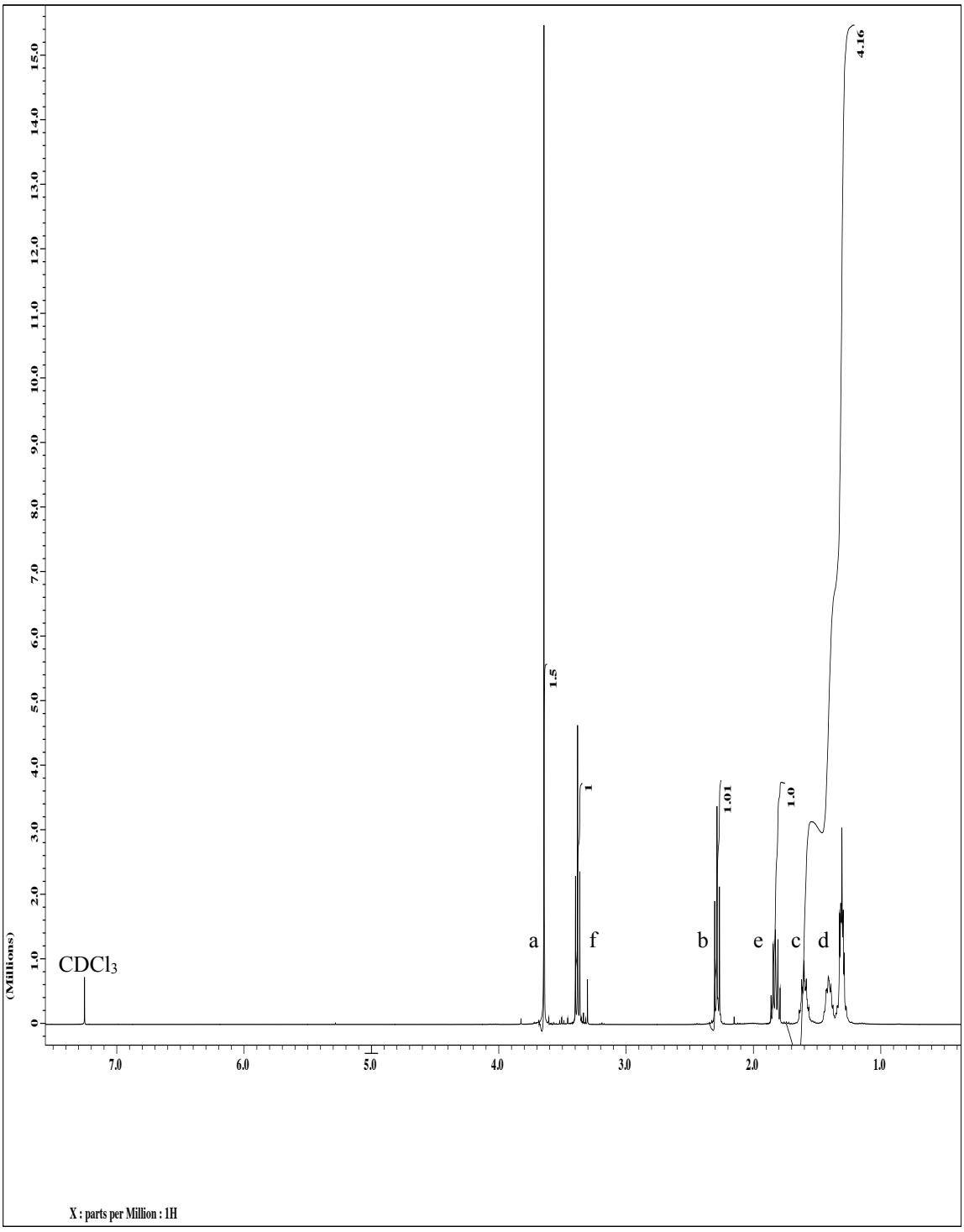
Appendix D: ^{13}C NMR spectrum of compound **3b** in DMSO



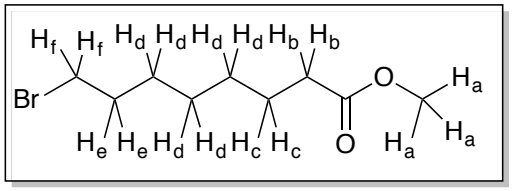


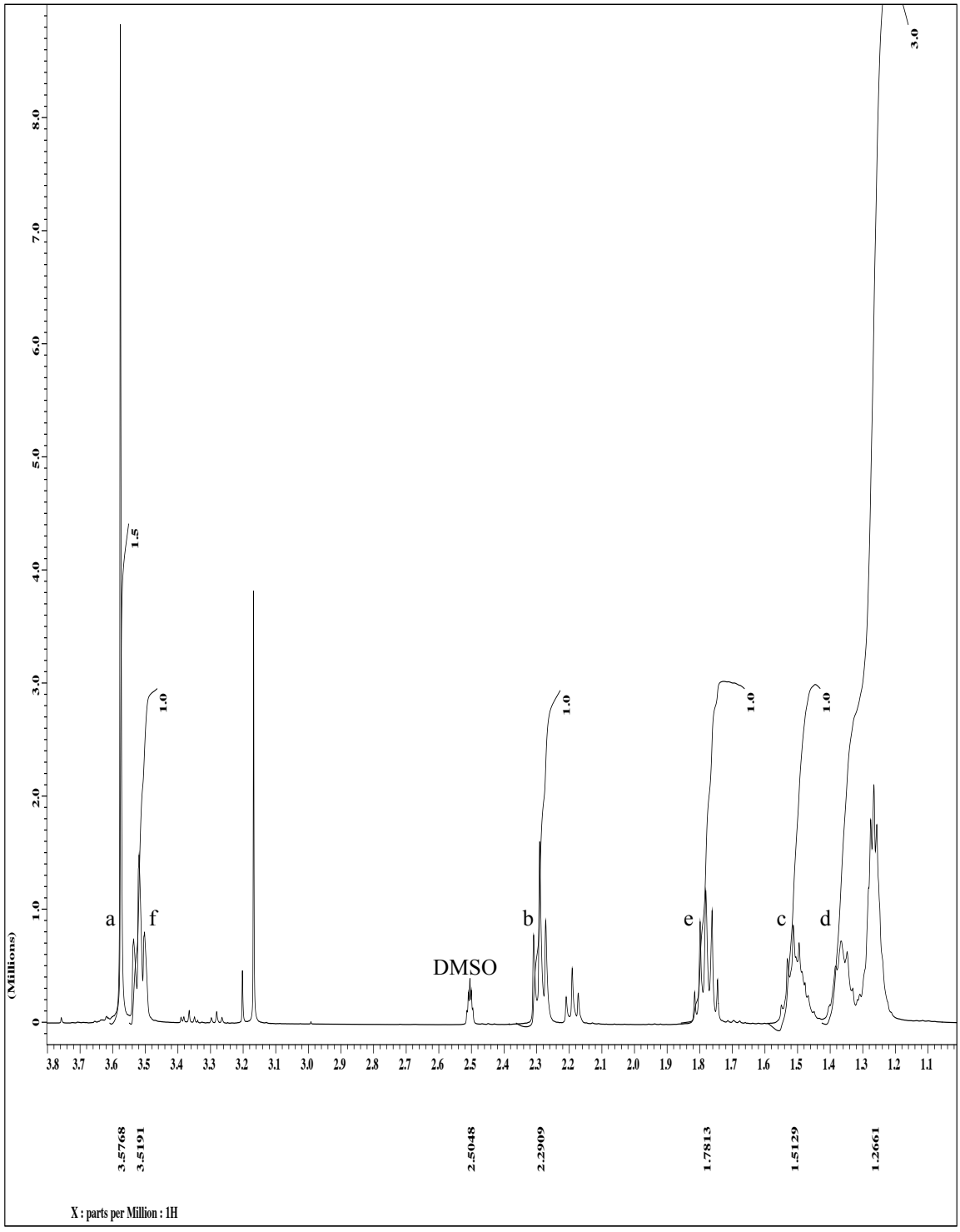
Appendix E: ^{1}H NMR spectrum of compound **3a** in $CDCl_3$



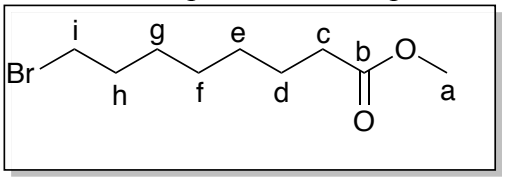


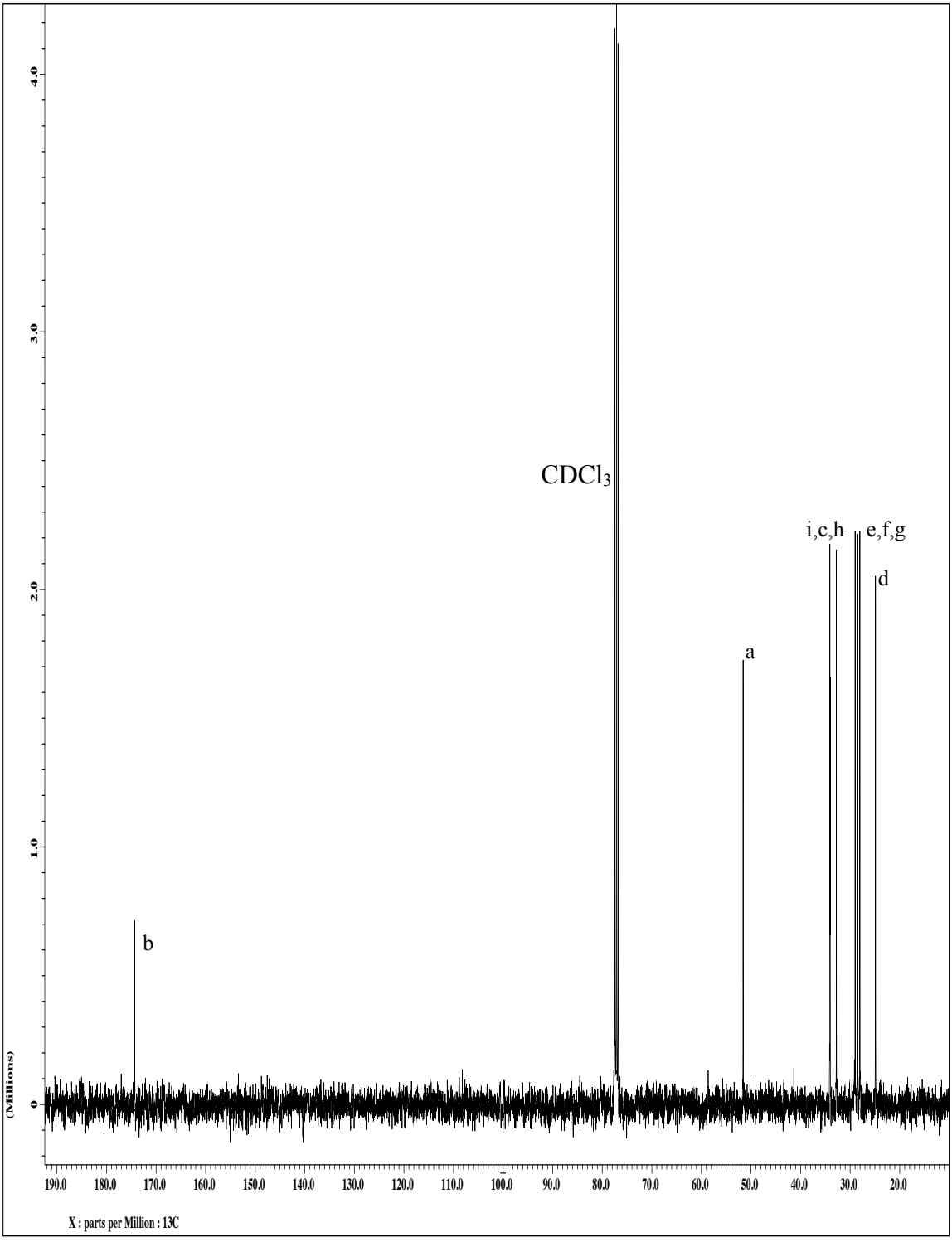
Appendix F: ^{1}H NMR spectrum of compound **3a** in DMSO



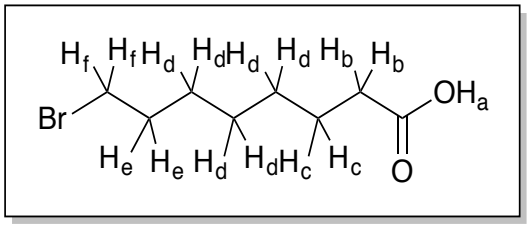


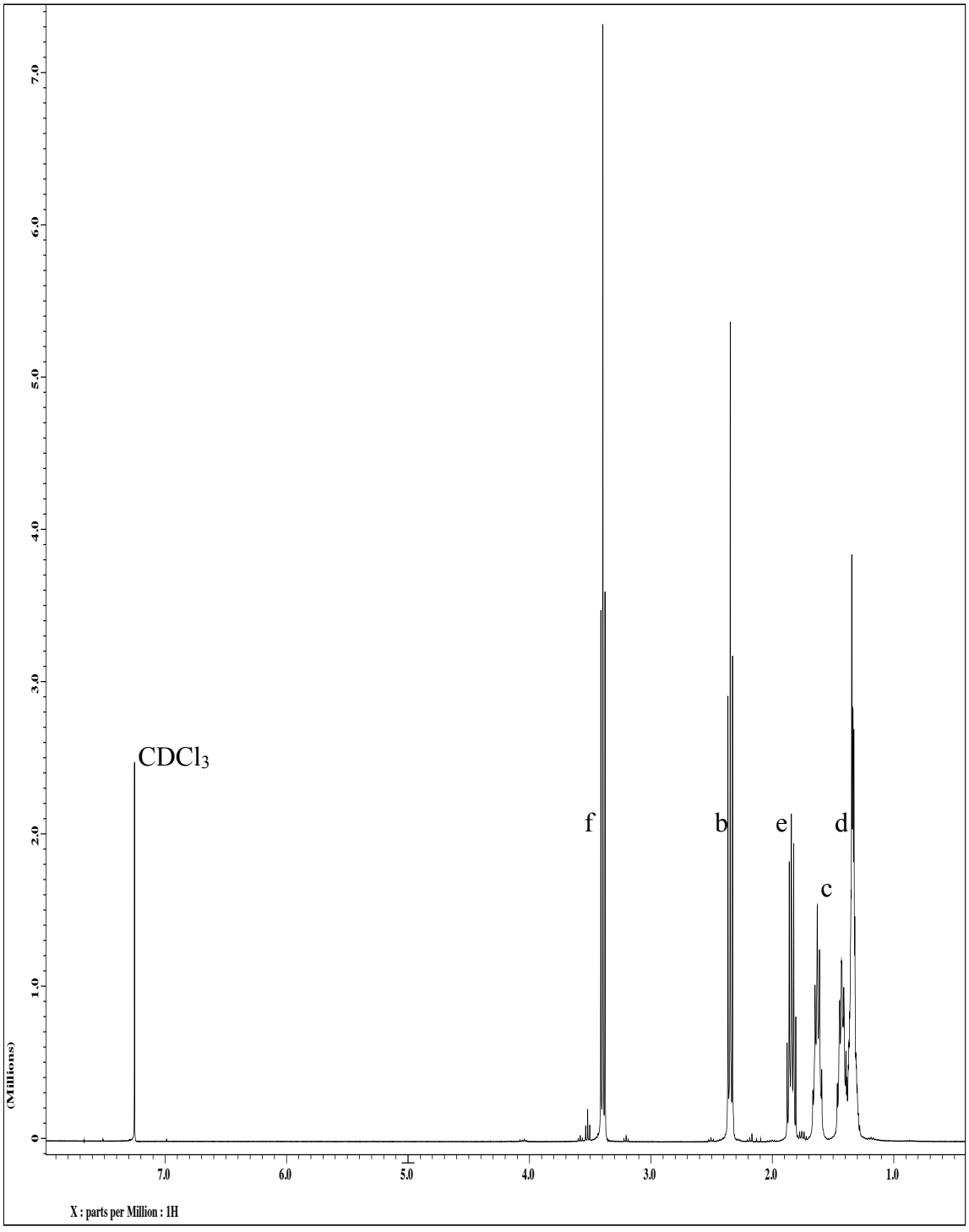
Appendix G: ^{13}C NMR spectrum of compound **3a** in $CDCl_3$



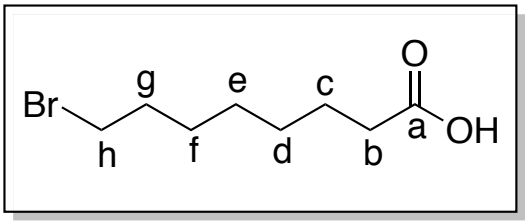


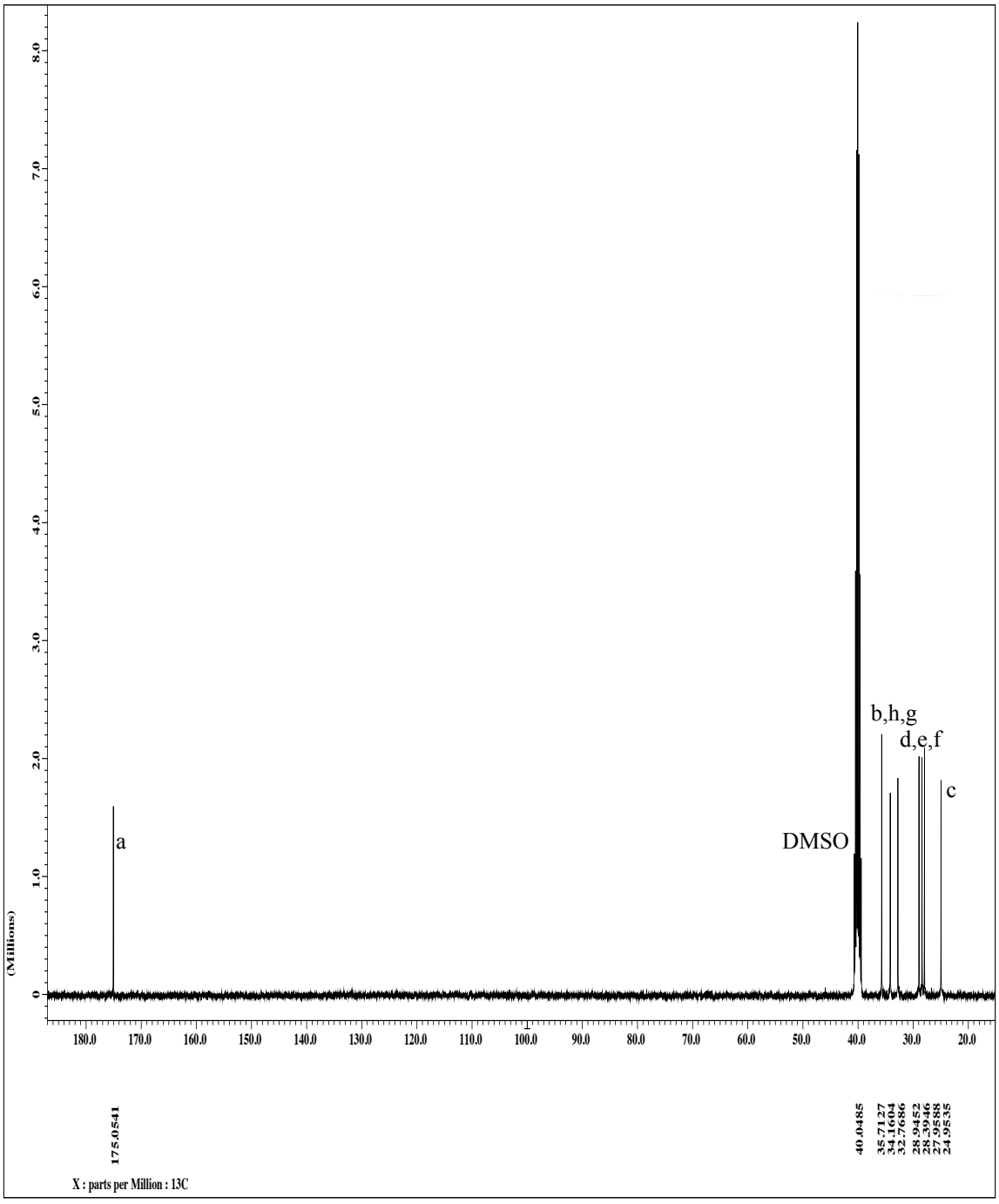
Appendix H: ^{1}H NMR spectrum of 8-bromooctanoic acid in $CDCl_3$



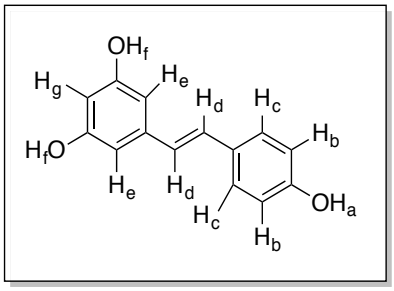


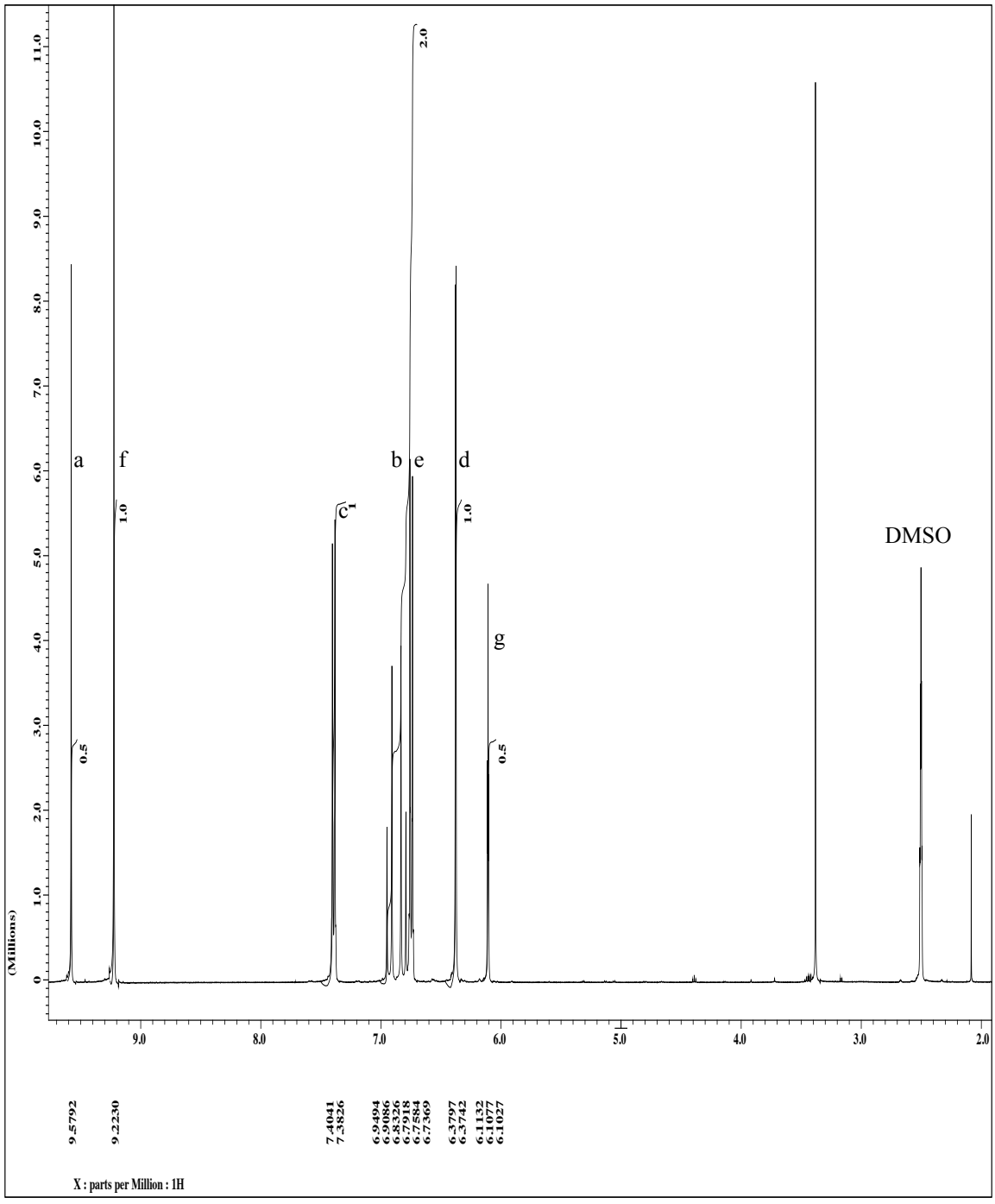
Appendix I: ^{13}C NMR spectrum of 8 bromooctanoic acid in DMSO



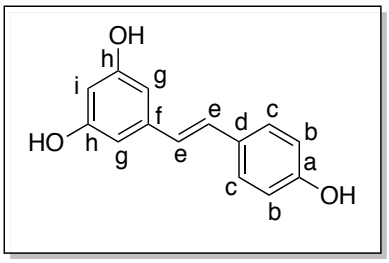


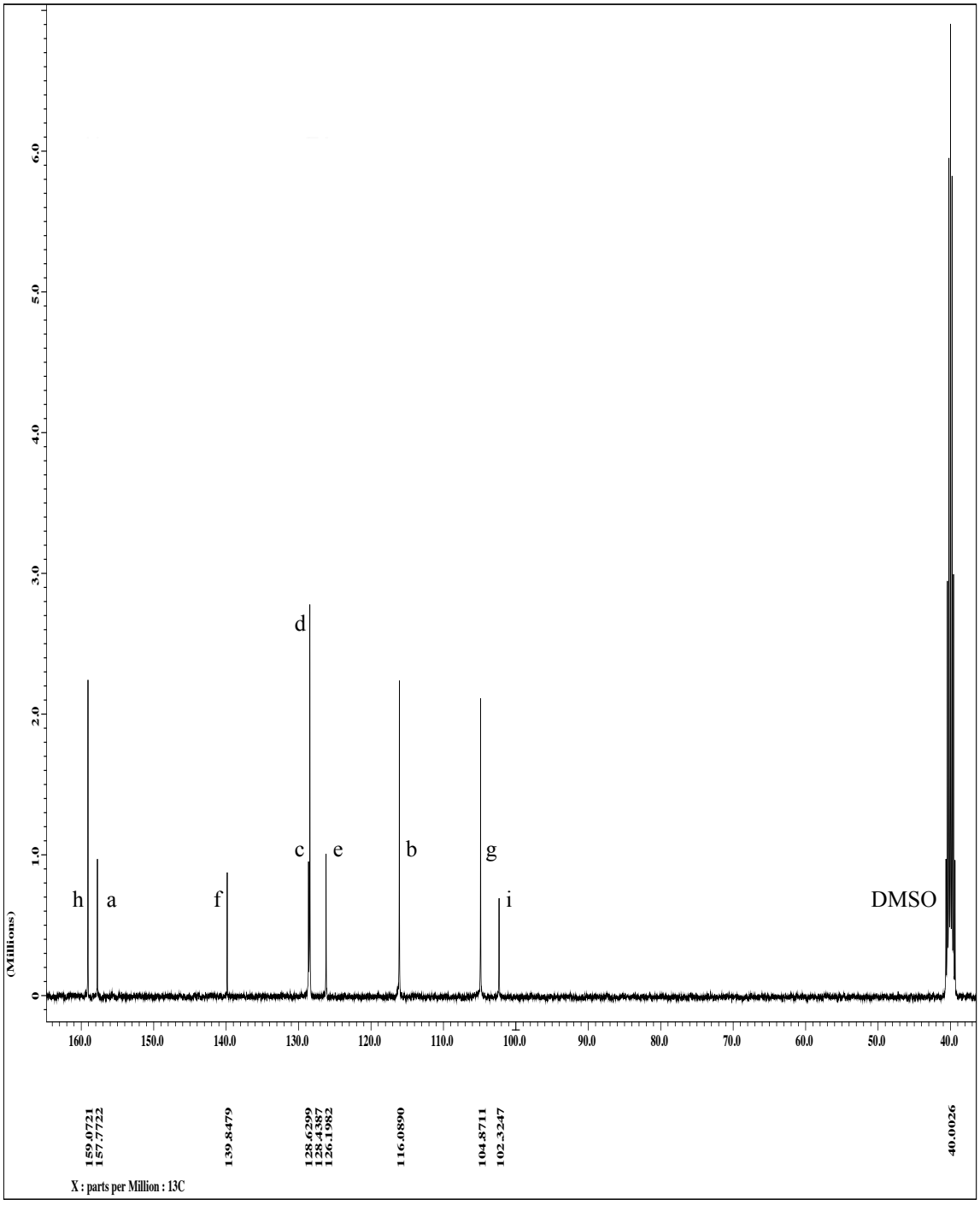
Appendix J: ^{1}H NMR spectrum of resveratrol in DMSO





Appendix K: ^{13}C NMR spectrum of resveratrol in DMSO





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