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Synthesis of Resveratrol Ester Derivatives Using Selective Enzymatic Hydrolysis.

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by

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ABSTRACT

Synthesis of Resveratrol Ester Derivatives Using Selective Enzymatic Hydrolysis

by

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Resveratrol, a naturally derived stilbene, is an interesting compound mostly talked about recently because for its anti-cancer properties. Unfortunately it has some shortcomings due to its low bioavailability and low solubility in water. For this reason, my research is to overcome resveratrol's drawbacks by improving its bioavailability and hydrophilicity. My research is focused on syntheses of novel derivatives of resveratrol such as 3, 5-di-O-isobutyroyl resveratrol and 3, 5-di-O-hexanoyl resveratrol using lipase catalyzed hydrolysis. Therefore, the tri-acylated resveratrols 3, 5, 4'-tri-O-isobutyroyl resveratrol and 3, 5,4'-tri-O-hexanoyl resveratrol were first synthesized.

3,5,4'-tri-O-isobutyroyl resveratrol and 3,5,4'-tri-O-hexanoyl resveratrol were then hydrolyzed using lipase (C. *Antarctica*) to obtain the products 3,5-di-O-isobutyroyl resveratrol and 3,5-di-O-hexanoyl resveratrol. The four compounds 3,5-di-O-isobutyroyl resveratrol, 3,5-di-O-hexanoyl resveratrol, 3,5,4'-tri-O-hexanoyl resveratrol, and 3,5-di-O-hexanoyl resveratrol were characterized by ^{1}H NMR and ^{13}C NMR.

DEDICATION

This thesis is dedicated to my parents, Dr. & Mrs. Michael Osei – Mensah and my husband Alexander Kamasah.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
COX	Cyclooxygenase
Fig	Figure
UV	Ultraviolet
Hrs	Hours
R_f	Retention factor
Rpm	Rounds per minute
Temp	Temperature
M.p	Melting point
B.p	Boiling point
IR	Infra Red
TMS	Tetramethylsilane
NMR	Nuclear Magnetic Resonance

CHAPTER 1

INTRODUCTION

Resveratrol (3, 5, 4'-trihydroxy-trans-stilbene) occurs in nature as a phytoalexin that is produced by several plants as a result of environmental pressure or pathogenic attack. It can be found in grapes, peanuts, mulberries, and blueberries.[1-3] Considerable amounts of resveratrol can be found in wine as a result of its concentrated amount in grape skin.[4, 5] Research has shown that red wine minimizes the risk of cardiovascular disease. [6-9] Resveratrol has various biological behaviors such as antioxidant,[10] anti-inflammatory ,[11-13] anti-ischemic,[14-16] neuroprotective,[17,18] anti-aging,[19-21] antiobesity,[22] antiviral,[23] cardioprotective,[24-26] anticancer,[27] and cancer chemopreventive effects.[1,28-30] Resveratrol was found to hinder all the three stages of carcinogenesis: initiation, promotion, and progression.[1]

Stilbene derivatives like resveratrol and all other naturally occurring phenolic antioxidants are compounds with two benzene rings connected to each other by ethylene. Currently, more interest has been directed to the development of possible approaches to transform and to improve the functional properties of natural antioxidants without changing the functionality responsible for the biological behavior. For instance, there has been an improvement of the hydrophobicity of antioxidant phenolic acids by esterifying the carboxylic acid moiety of the phenolic acid with a fatty alcohol by enzymatic and chemical methods.[31] Phenethyl alcohols have also been lipophilized by esterifying the alcoholic group with acid chlorides or acid anhydrides.[32] Thus amphiphilic phenolic compounds were acquired and their function is to disseminate between the aqueous phase and the liphophilic phase and interact with an emulsifier at the interface. It has lately been accounted that these compounds are potentially remarkable for probable applications in nanotechnology.[33]

A number of acylated derivatives of resveratrol were found to be of higher cell-growth prevention with regard to DU- 145 human prostate cancer cells than resveratrol itself.[34] However, a property of resveratrol that hinders the therapeutic applications is its limited bioavailability due to its quick metabolism in the liver that converts the resveratrol into less-active 3-sulfate and 3-glucuronide derivatives.[35] As a result, a serum half life of 8-14 min has been reported for resveratrol.[36] An appropriate way to improve bioavailability is to have resveratrol derivatives that cannot be sulfated or glucuronated. In view of that, much attention is noted for the regioselective modification of the 3-OH's.[35]

Resveratrol is a symmetric molecule so the phenolic group at positions 3 and 5 are chemically equivalent and they have very similar reactivity with the phenolic group at position 4, so a special selectivity of enzymes [37] is being used for its regiospecific acylation [34] and oxidation.[38] Regioselective acylation or deacylation catalyzed by lipases in organic solvents have especially been applied to polyphenolic compounds as a biocatalysed reaction to successfully answer many challenging standardized chemical reactions of regioselective derivatisation of polyhydroxylated compounds.[39-43]

History and Discovery of Resveratrol

Resveratrol as shown in (Figure 1) was first discovered in the 1940s in a variety of roots in both Japan and China and these roots were dried and then functioned as medicines. The dried root products were used to cure numerous skin infections such as athlete's foot.

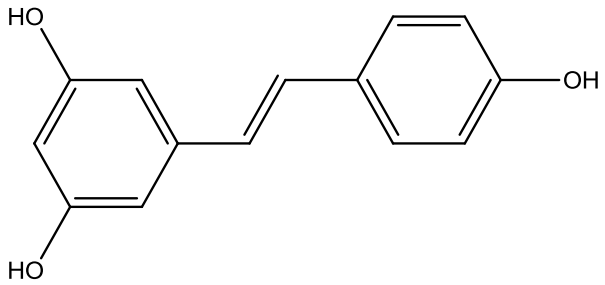


Figure 1. Structure of Resveratrol

During 1976, resveratrol was found in the skin and seeds of grapes but not the flesh. As said by one researcher, the fresh grape skins contain 50-100 mg resveratrol per gram, and the concentration in wine varies from 0.2 mg/L to 7.7 mg/L.

The French paradox gave rise to the research on the advantages of resveratrol because it is in relation to the drinking of wine in France and the minimal rate of cardiovascular problems among the French inhabitants. The outcome of resveratrol in red wines was as a result of this effect.[44]

Biological Activity of Resveratrol

Resveratrol has several major biological activities but here we discuss some of the most recent studies and applications in that field.

Bioavailability is the rate at which a drug or a medication or other substance is absorbed or becomes accessible at the targeted position in the body, and because living organisms response depends on bioavailability the ability of intestines to take up resveratrol was demonstrated over 15 years ago by health benefits. This was acquired by orally administered resveratrol and it was first tested on rats and it prevented liver injury in rats fed peroxidized oil.[26]

Bertelli et al.[45] showed that some of resveratrol in red wine (6.5 mg/L in cis and trans forms) was taken up by rats. Several tests with significant amount of 26 μg resveratrol or ingestion of 13 μg resveratrol on daily basis over 15 days proved that the compound rapidly went into the blood stream and may possibly be identified in high concentrations in plasma and several organs. The same group of authors advanced their research in plasma kinetics and bioavailability of red wine resveratrol dispensed by an intragastric tube (28 μg per rat). The group explained pharmacokinetics by an open one- or two-compartment structure and found a considerable cardiac bioavailability and a strong affinity for the liver and the kidneys.[46] In a different study, Bertelli et al.[47] demonstrated that a moderate amount of resveratrol was pharmacologically effective in vitro as well as vivo. They therefore proposed that, continuing drinking wine for a long period of time allows the drinker to take in adequate amount of resveratrol which in turn explains the benefits of red wine on the human health.

Anticancer

Cancer is the unrestrained enlargement of irregular cells in the body. Cancer is the main cause of mortality in humans, killing more than 6 million people annually worldwide. It happens when a cell's gene mutations make the cell incapable of correcting damaged DNA and unable to commit suicide.[48] Carcinogens are a group of substances that are accountable for damaging DNA, advancing or supporting cancer. Some examples of carcinogens are tobacco, arsenic, asbestos, radiation such as gamma and x-rays, the sun, and compounds in car exhaust fumes.[48] When our bodies are bare to these carcinogens, free radicals are produced and therefore make an effort to take electrons from several molecules in the body. The free radicals formed however destroy cells and have an effect on their capacity to perform normally.[48]

Age is also another risk for cancer. As we grow older, there is a rise in the cancer-causing mutations in our DNA. Again a number of viruses have been related to cancer such as human papillomavirus (a cause of cervical cancer), hepatitis B and C (causes of liver cancer), and Epstein-Barr virus (a cause of some childhood cancers). Human immunodeficiency virus (HIV) and anything for that matter that restrains or weakens the immune system inhibits the body's capability to fight diseases and enhances the risk of developing cancer.[48] Lastly cancer can be the outcome of genetic trend as a result of hereditary. Cancer can however be classified in five main groups.[48]

1. Carcinomas are represented by cells that cover up the internal and external parts of the body such as lung, breast, and colon cancer.

2. Sarcomas are represented by cells that are found in the bone, cartilage, fat, connective tissue, muscle, and other supportive tissues.

3. Lymphomas are cancers that start in the lymph nodes and immune system tissues.

4. Leukemias are cancers that commence in the bone marrow and often build up in the bloodstream.

5. Adenomas are cancers that happen in the thyroid, the pituitary gland, the adrenal gland, and other glandular tissues.

The treatment of cancer is based on the type of cancer and the stage it has reached and can be put into six categories: surgery, radiotherapy, chemotherapy, immunotherapy, hormone therapy, or gene therapy.

Surgery

It is recognized as the oldest treatment of cancer and it can be used to completely cure a patient by surgically removing the cancer from the body if the cancer has not metastasized. This is frequently observed in the getting rid of the prostate or a breast or testicle. However it is almost never possible to get rid of all the cancer cells after the disease has spread. Surgery possibly will as well be involved in aiding to regulate signs for instance bowel obstruction or spinal cord compression.[48]

Radiotherapy

This is a radiation treatment that damages cancer cells by concentrating high-energy rays on the cancer cells. It results in damaging the molecules that constitute the cancer cells and lead the cells to commit suicide. Radiation treatment makes use of high-energy gamma-rays that are emitted from metals, for example radium, or high-energy x-rays that are produced in a distinctive machine. Initial radiation treatments brought about serious consequences for the reason that the energy beams would destroy normal, healthy tissue; however, technologies have enhanced so that beams can be more precisely targeted. Radiation treatment functions as an individual treatment to minimize a tumor or to damage cancer cells (as well as those related to leukemia and lymphoma) and again used collectively in addition to several cancer treatments.[48]

Chemotherapy

Chemotherapy makes use of chemicals that meddle with the cell separation method – destroying protein or DNA – in order that cancer cells will commit suicide. The treatments aim at any fast dividing cells (not actually cancer cells), but healthy cells more often than not can

recuperate from any chemical-induced damage whereas cancer cells cannot. Chemotherapy is usually employed to cure cancer that has multiplied or metastasized because the medicines move all over the whole body. It is also an essential therapy for various kinds of leukemia and lymphoma. Chemotherapy treatment happens in phases, hence the body has longer period for healing between doses. Nevertheless, there are still familiar consequences such as hair loss, nausea, fatigue, and vomiting. Combination therapies usually comprise several forms of chemotherapy or chemotherapy combined with other treatment preferences.[48]

Immunotherapy

Immunotherapy seeks to make the body's immune system battle the tumor. Local immunotherapy infuses medication into the affected part, for instance, to bring about inflammation that allows a tumor to contract. Systematic immunotherapy treats the entire body by dispensing a drug such as protein interferon alpha that can contract tumors. Moreover, immunotherapy can be regarded as non-specific if it enhances cancer fighting-capabilities by motivating the whole immune system, and it can be regarded as targeted if the treatment purposely informs the immune system to damage cancer cells. These therapies are comparatively new; however, scientists have had achievement with treatments that introduces antibodies into the body that prevent the growth of breast cancer cells. Another type of immunotherapy is bone marrow transplantation for the reason that the contributor's immune cell will frequently attack the tumor that is available in the host.[48]

Hormone Therapy

This is aimed to correct hormone formation in the body in order that cancer cells prevent developing or are destroyed entirely. Several cancer cells have been associated with various types of hormones, particularly breast and prostate cancer. Breast cancer hormone therapies usually concentrate on decreasing estrogen levels (a typical drug generally used is tamoxifen), whereas prostate cancer hormone therapies usually concentrate on lowering testosterone levels. Additionally, some leukemia and lymphoma situations can be cured with the hormone cortisone.[48]

Gene Therapy

Gene therapy's objective is to replace damaged genes with ones that work to tackle the root cause of cancer, damage to DNA. For example, researchers are making an effort to replace the damaged gene that signals cells to stop dividing (the p53 gene) with a copy of working gene. Other gene-based therapies concentrate on further damaging cancer cell DNA to the point where the cell commits suicide. Gene therapy is an extremely new field and has so far not produced any success in treatments.

Three decades of research have revealed that cancer is easier to prevent than to treat. Food consumption provides various nutrients essential for life and good health as a whole. Certain daily diets that include specific nutrients may help decrease the danger of acquiring cancer. Chemoprevention is the prevention of cancer that is achieved by ingestion of chemical agents that lower the danger of carcinogenesis,[49] and it is one of the shortest ways to lessen cancer. Some of chemopreventive agents are nonsteriodal anti-inflammatory drugs (NSAIDs)

for instance aspirin, indomethacin, piroxicam, and sulindac all of which inhibit cyclooxygenase (COX).[50] COX acts as a catalyst in the conversion of arachidonic acid to pro-inflammatory substances such as prostaglandins that can promote tumor cell growth and reduce the immune system.[51] Moreover, COX can trigger carcinogens that destroy genetic material.[52]

The discovery of resveratrol was as a result of the search for new chemopreventive agents. On the basis of bioassay-guided fractionation of plant extract gathered in Peru, resveratrol was known to be a strong COX inhibitor.[1] The technique of the chemical carcinogenesis can be grouped into three main stages and the chemopreventive agents have been classified along with the stage they inhibit.[53] The stages resveratrol inhibits are tumor initiation, promotion, and progression.

Anti-Diabetic Effects

Researchers have revealed that resveratrol has hypoglycemic and hypolipidemic effects in both streptozotocin (STZ)-induced diabetes and STZ-nicotinamide-induced diabetes in rats. Resveratrol improves the frequent diabetes symptoms such as polydipsia, polyphagia, and body weight loss.[54]Another research performed by Sirtris Pharmaceutical Inc. demonstrated that in human clinical trials, resveratrol reduced the blood sugar levels in both Phase Ib and Phase IIa.[55]

Anti-Viral Effects

Studies demonstrate that resveratrol inhibits herpes simplex virus (HSV) types 1 and 2 replication by blocking of an early stage in the virus replication cycle. Research in mice in vivo reveals that resveratrol inhibits or lowers HSV replication in the vagina and as a result reduces extra-vaginal disease. It was evident that animals whose skins were healed with resveratrol

demonstrated no dermal contamination or toxicity such as scaling, erythema, crushing, lichenification, or excoriation.[56] Research also reveals that resveratrol inhibits varicella-zoster virus, specific influenza virus, respiratory viruses, and human cytomegalovirus. Moreover, resveratrol works with and improves the anti-HIV-activity of some anti-HIV drugs.[57]

Mechanism of Action

Resveratrol has numerous beneficial effects, but the mechanism of action is not yet clear, although several direct targets have been discovered for it. Resveratrol exhibits antioxidant properties by the addition of exogenous red wine dilutions in aqueous environment to isolated LDL in a buffer solution.[58] Resveratrol additionally exhibits anti-cyclooxygenase activity by inhibiting COX on the basis of bioassay-guided fractionation.[1] Once more, resveratrol displays a modulating result on lipid and lipoprotein metabolism by the blockage of lipid assimilation and the speeding up of lipid consumption in muscles.[59]

Resveratrol again blocks ribonucleotide reductase, the enzyme that supplies proliferating cells with deoxyribonucleotides necessary for DNA synthesis at its initial S-phase of the cell cycle,[60] and DNA polymerases and hinders the mitogen-activated protein kinase and protein kinase C alleys.[61] Lastly, resveratrol is a powerful activator of sirtuin activity and as a result imitates the health benefits of caloric limitations.[62]Sirturns are a preserved family of NAD^+-dependant deacetylases and resveratrol, as a tiny molecule activator of sirtuin lengthens yeast and other higher organisms existence on earth.

Derivatives of Resveratrol

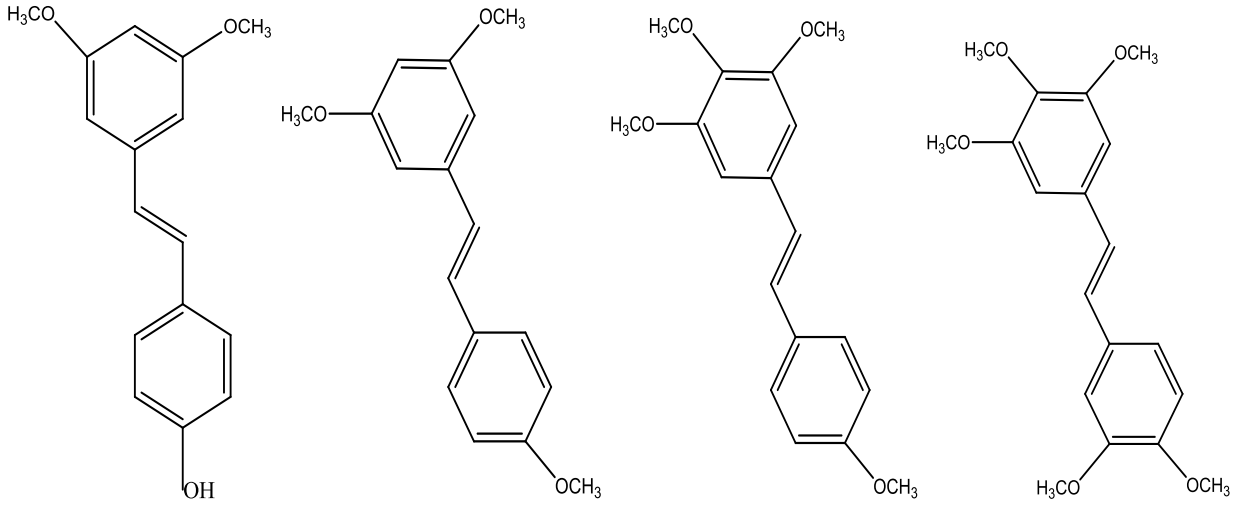
There are several naturally occurring stilbene-like compounds that are similar to resveratrol and also found in food. These compounds are referred to as derivatives of resveratrol

and an insight of the structure-activity creates new possibilities for drug discovery and other applications. For instance, a number of acylated derivatives have revealed higher cell-growth prevention with regard to DU- 145 human prostate cancer cells than resveratrol itself [34] due to resveratrol's limited bioavailability.

Methoxylated Resveratrol Derivatives

Analysis of structure-activity discovered that the substitution of hydroxyl groups of resveratrol with methoxy groups considerably improve its cytotoxic activity.[63] Therefore, some researchers have done a lot of studies on series of methoxylated analogs of resveratrol with the aim of increasing the antitumor activity of resveratrol. Several other stilbenes besides resveratrol, that are similar in structure to resveratrol, occur naturally in food. An example of this is pterostilbene (Figure 2), a dimethylated analog of resveratrol is found in blueberries that has been studied broadly. The substitution of hydroxyl with methoxy groups increases the lipophilicity of pterostilbene over resveratrol, resulting in better bioavailability.[64]

Examples of methoxylated resveratrol derivatives shown in (Figure 2) include;



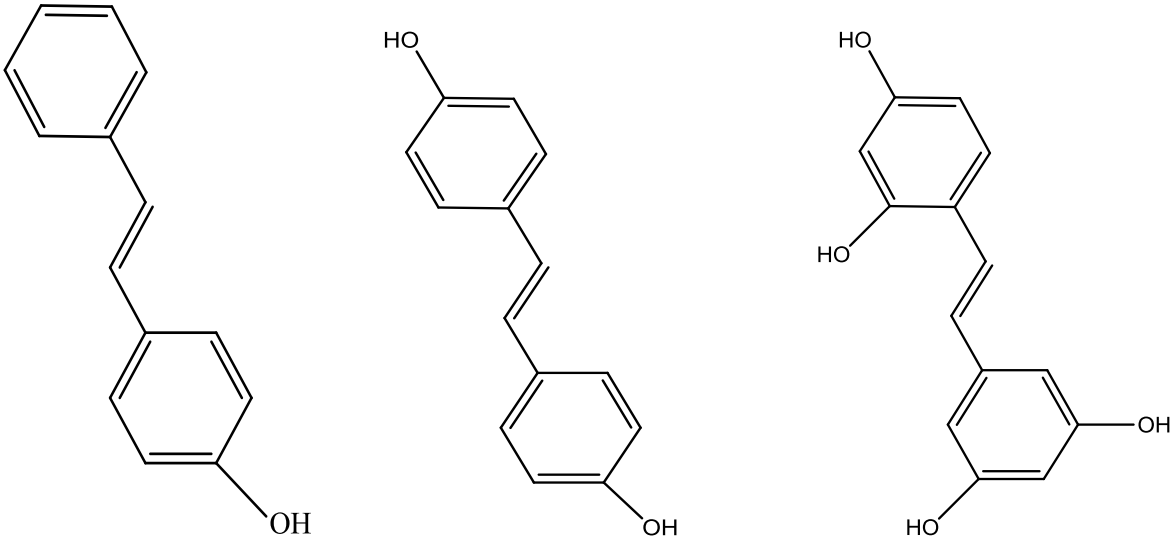
Pterostilbene Trimethoxystilbene Tetramethoxystilbene Pentamethoxystilbene

Figure 2. Structure of selected methoxylated derivatives of resveratrol

Hydroxylated Derivative of Resveratrol

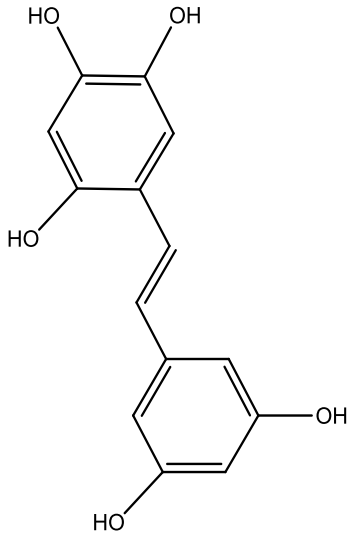
A common example of a hydroxylated derivative of resveratrol is Piceatannol (3, 4, 3', 5'-tetrahydroxystilbene Figure. 3), the metabolite of resveratrol found in red wine. LMP2A, a viral protein-tyrosinekinase linked to leukemia, Non-Hodgkin's lymphoma, and other diseases related to Epstein-Barr virus (EBV) also called human herpes virus4 (HHV4), was discovered to be blocked by piceatannol in vivo. These introductory studies on piceatannol have attracted a great deal of research interest as a potential anti-cancer and anti-EBV drug.[65]

Examples of hydroxylated derivatives of resveratrol shown in (Figure 3) are;

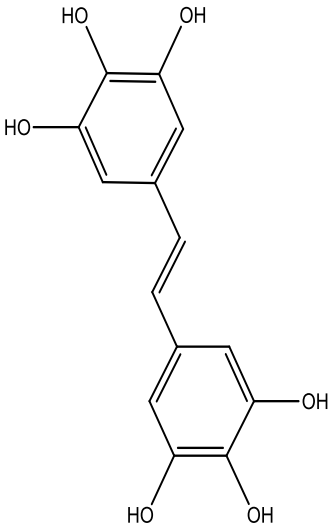


Hydroxystilbene Dihydroxystilbene Tetrahydroxystilbene

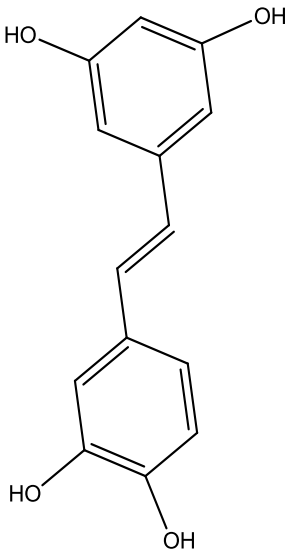
Figure 3. Structure of selected hydroxylated derivatives of resveratrol continued on next page



Pentahydroxystilbene



Hexahydroxystilbene

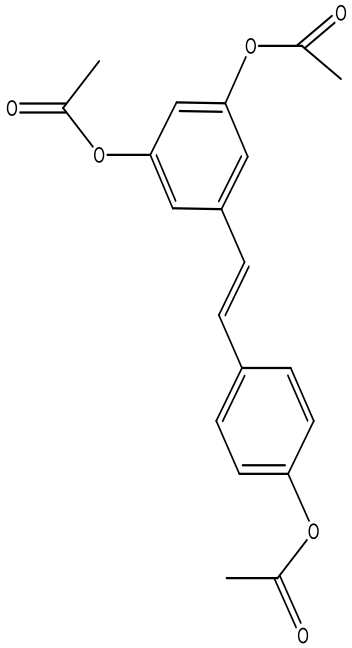


Piceatannol

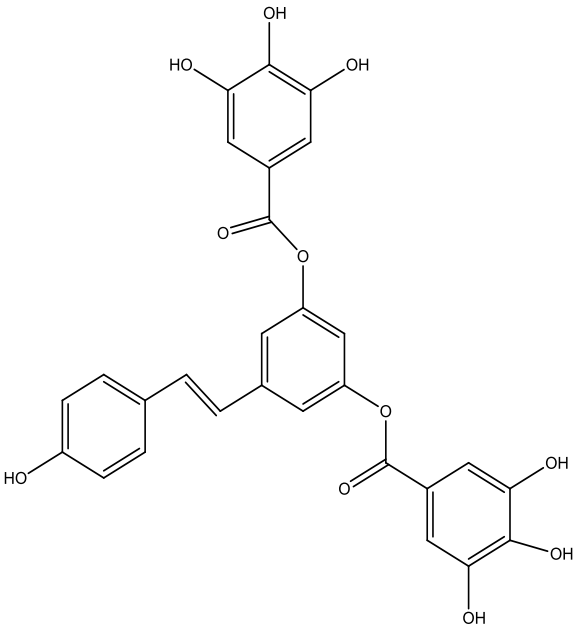
Figure 3. Structure of selected hydroxylated derivatives of resveratrol

Other Resveratrol Derivatives

The derivatives of other resveratrol as shown in (Figure 4) include;



Resveratrol triacetate



Digalloylresveratrol

Figure 4. Structure of other derivatives of Resveratrol

Adverse Effects and Unknowns of Resveratrol

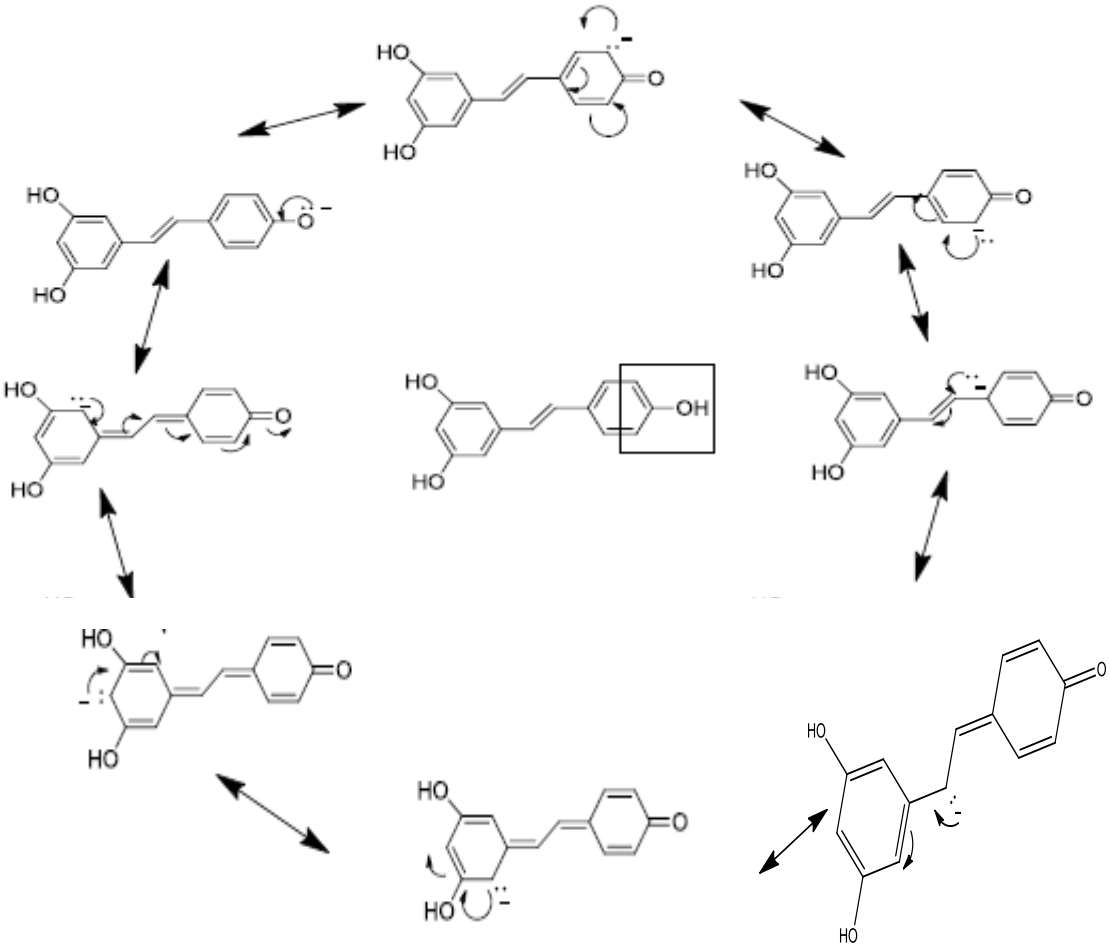
Until now the long-term effects of using resveratrol is not known.[66] Although the health benefits of resveratrol are remarkable, research has come out with a concept that it may promote the development of human breast cancer cells, probably for the reason that the chemical structure of resveratrol and phytoestrogen are alike.[67] On the other hand, that research have shown that ingestion of resveratrol reduces the risk of breast cancer in mice.[68] Other research also found that resveratrol slows down the growth of blood vessels that hold back tumors, although it delays healing.[69] From the indication that resveratrol may be estrogen detrimental, certain sellers of resveratrol suggested that the product may meddle with oral contraceptives and that pregnant women or women looking forward to be pregnant must not take the compound, yet a different group also suggested that children or teenagers under 18 should not take resveratrol because research have not shown its influence on human development. A small research work showed that one dosage equal to 5 g of trans-resveratrol has no severe bad consequences in healthy volunteers.[70]

Lipase Protection of Resveratrol Derivatives

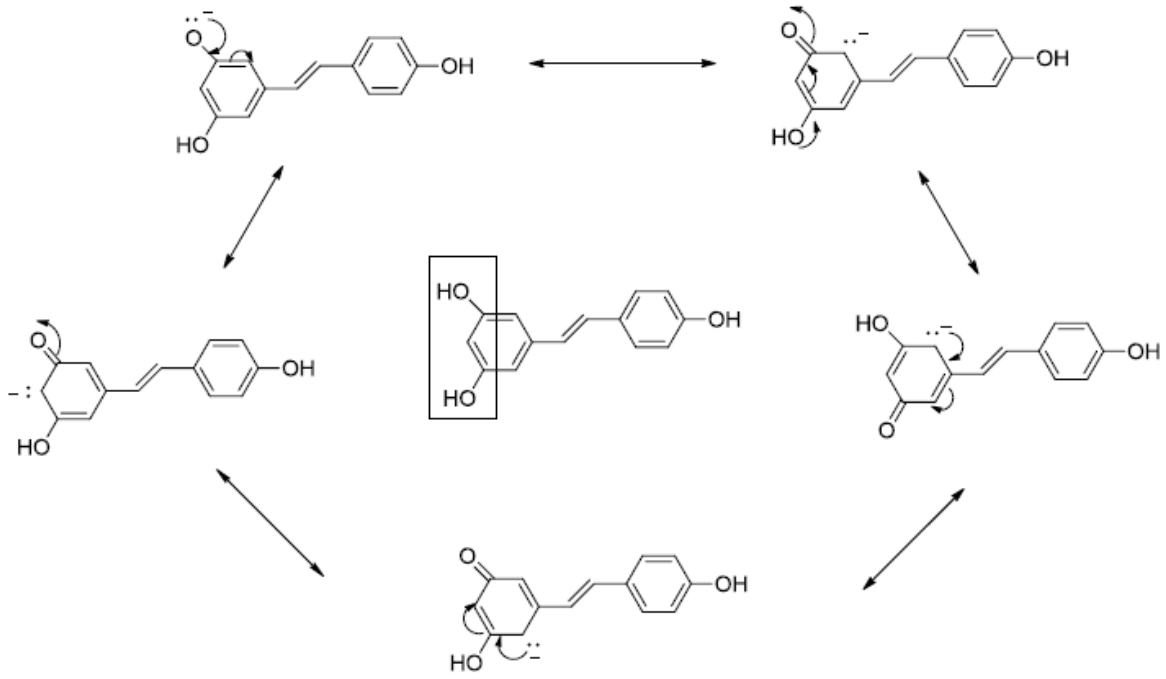
Selectivity in the modification of many functional groups on one compound is particularly an important objective in organic synthesis and because of that a lot of attention is paid to research of new selective methods. It has been discovered that lipases are capable of functioning in organic solvents, so this brings about a wide application of these biocatalysts in organic synthesis.[71-72] Lipases are enzymes that have high molecular identification potentials and as a result can be used in the selective modification of polyhydroxylated aromatic and aliphatic substrates. Research shows that C. *Antarctica* is highly specific for the phenolic group at 4'-OH, [34] because it is less sterically hindered than the 3-OH.[73]

Functional Group Activity

The OH group at position 4' (para) on resveratrol is more stable than the OH groups at position 3 and 5 (meta) because the para position has more resonance structures than the meta. This is shown in Scheme 1 and Scheme 2.



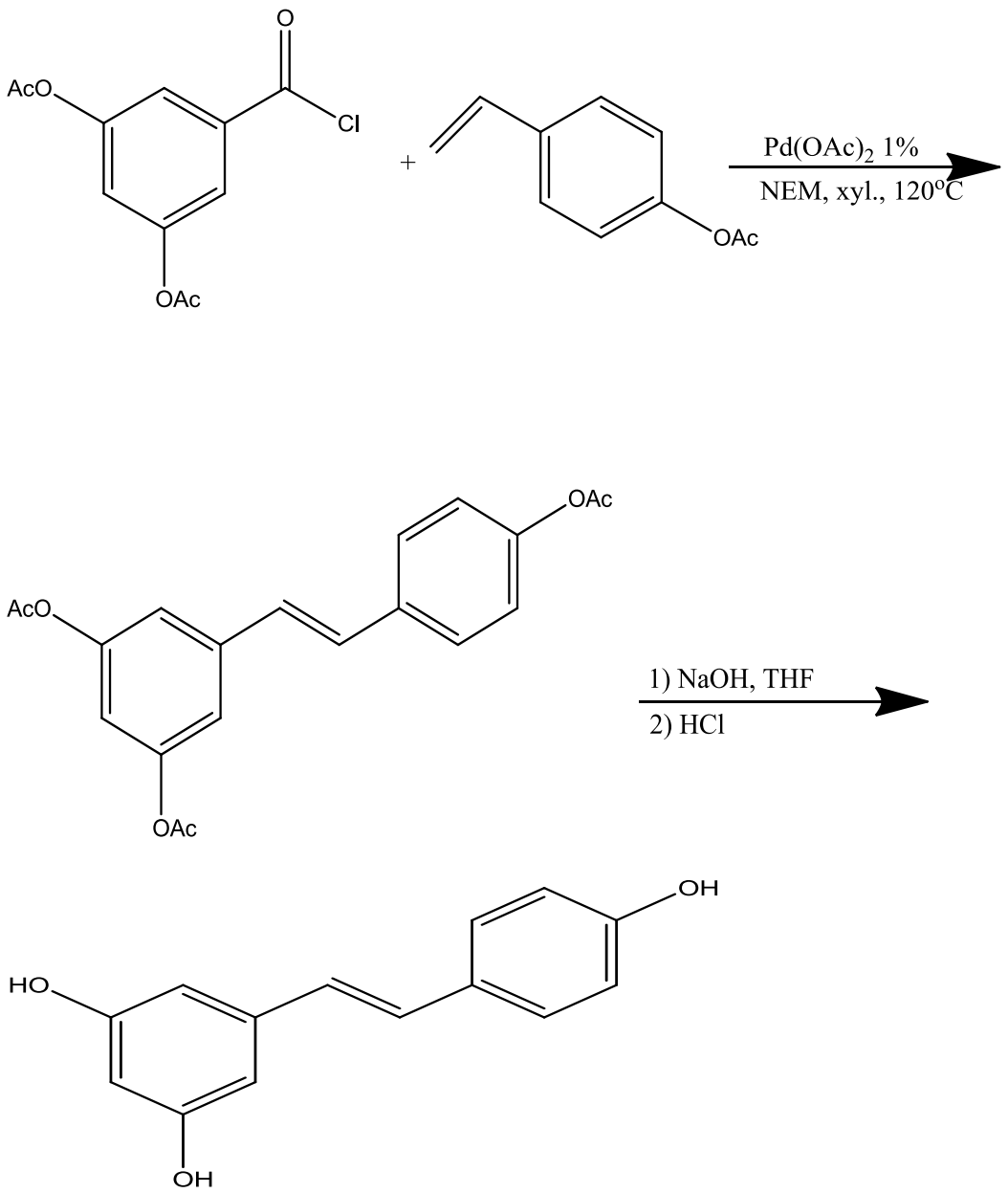
Scheme 1. The resonance structures of resveratrol involving abstraction of a proton from the para-hydroxyl group



Scheme 2. The resonance structures of resveratrol involving abstraction of a proton from the meta-hydroxyl group

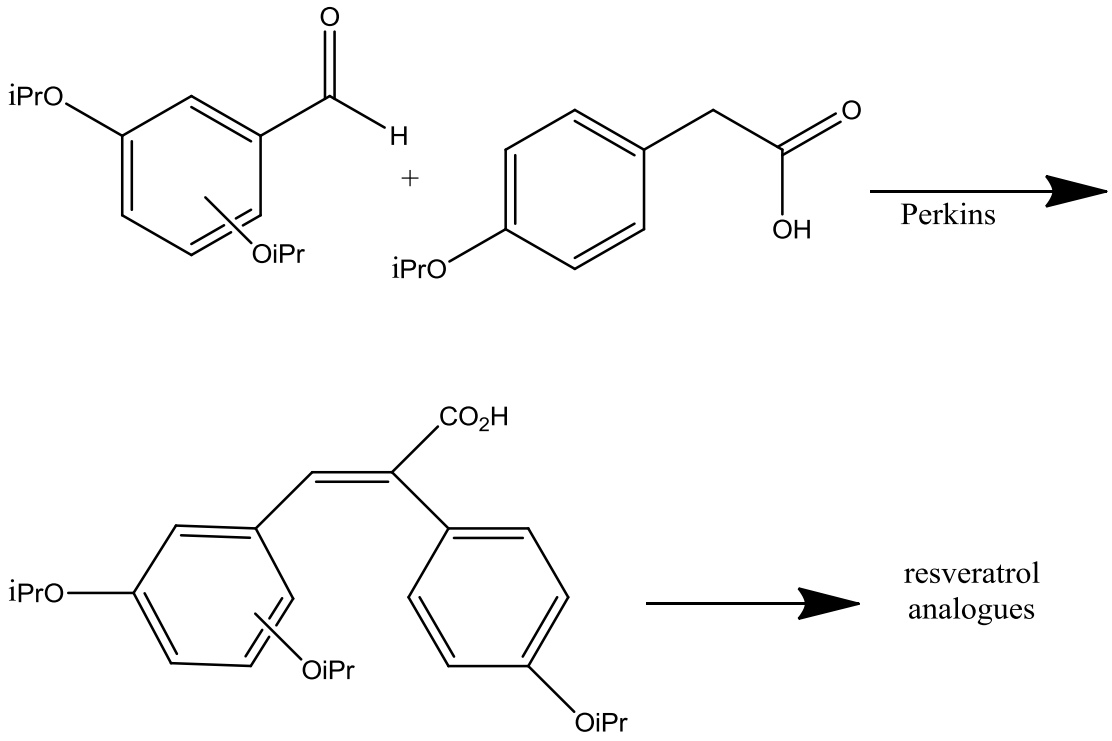
Chemical Synthesis of Resveratrol

There are various reactions used to synthesize resveratrol, but the reaction mainly used is the decarbonylative Heck reaction. This reaction involves the chemical reaction of an unsaturated halide with an alkene and a strong base in the presence of a palladium catalyst to form a substituted alkene. The resveratrol acetate formed is then reacted with sodium hydroxide in THF followed by washing with brine and water to produce resveratrol. The Heck's reaction is outlined in Scheme 3.



Scheme 3. Synthesis of resveratrol using Heck's reaction

Apart from Heck's reaction, Perkin reaction is also another commonly used organic reaction. The Perkin reaction is carried out by the aldol condensation of aromatic aldehydes and acids in the presence of an alkali salt of the acid. This reaction is believed to involve fewer steps with good percent yield (~70%). The Perkin's reaction is outlined in Scheme 4.



Scheme 4. The Perkins Reaction

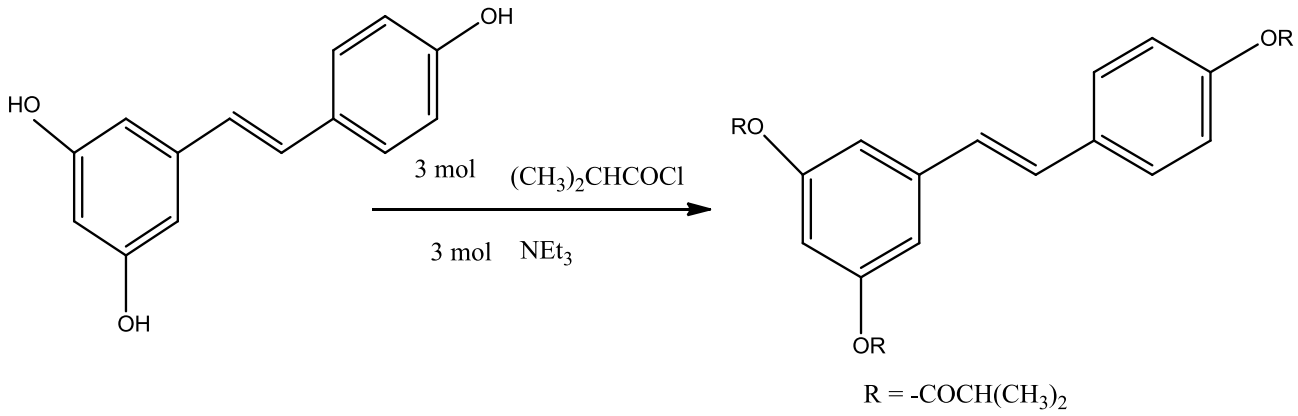
Wittig's reaction can also be used to synthesize resveratrol; however, it the produces a mixture of olefin isomers.

Purpose of Research

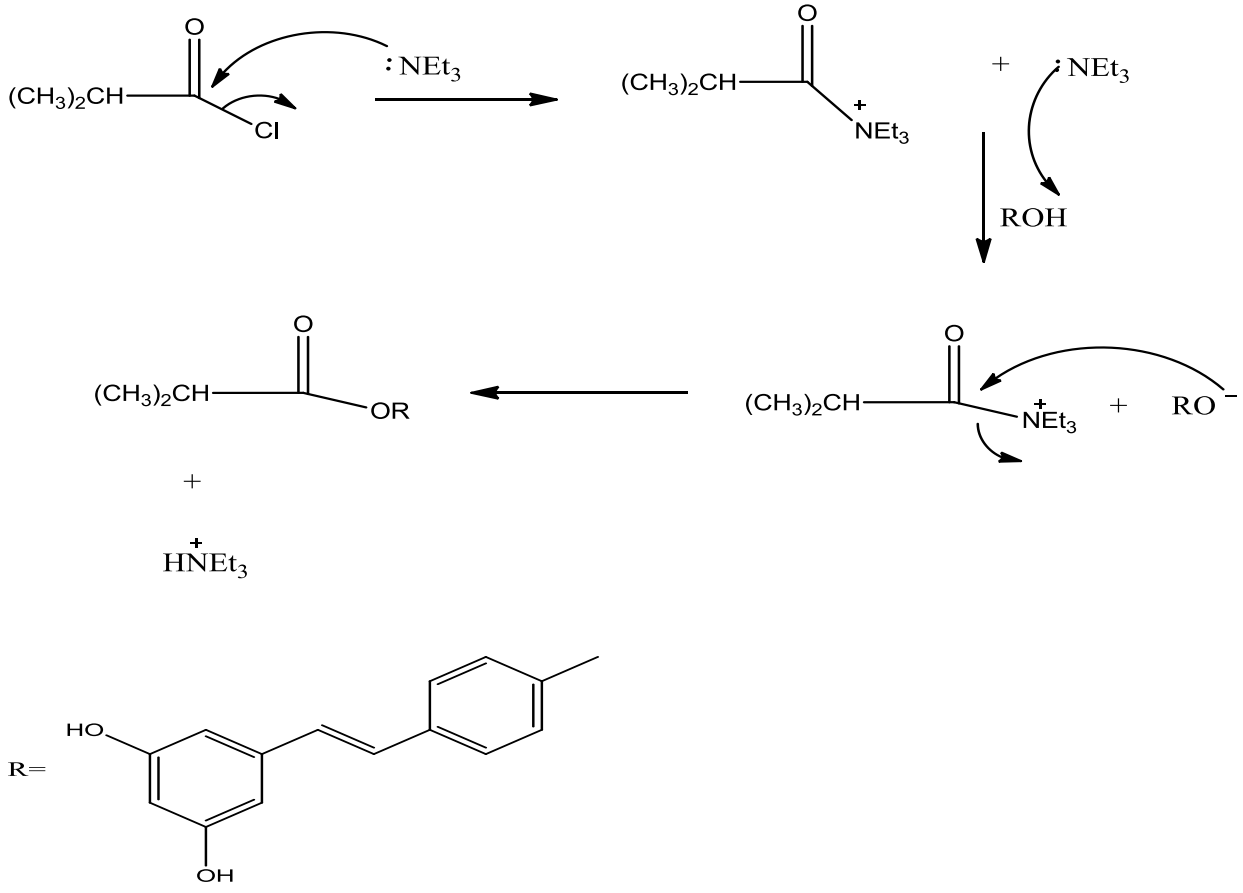
The main aim of this research is to synthesize resveratrol derivatives, 3,5-di-O-isobutyroyl resveratrol and 3,5-Di-O-hexanoyl resveratrol using lipase catalyzed hydrolysis. This research is to modify resveratrol to improve its low bioavailability and solubility in water.

Proposed Synthetic Pathway for Synthesis of 3,5,4'-tri-O-isobutyroyl Resveratrol

The synthesis of 3,5,4'-tri-O-isobutyroyl resveratrol was an esterification synthesis of resveratrol as outlined in Scheme 5 and its mechanism is outlined in Scheme 6.



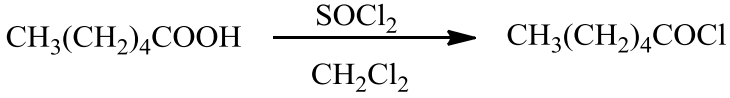
Scheme 5. Synthesis of 3,5,4'-tri-O-isobutyroyl resveratrol (Esterification)



Scheme 6. Mechanism of 3,5,4'-tri-O-isobutyroyl synthesis resveratrol using esterification of reseveratrol

Proposed Synthetic Pathway for Synthesis of Hexanoyl Chloride

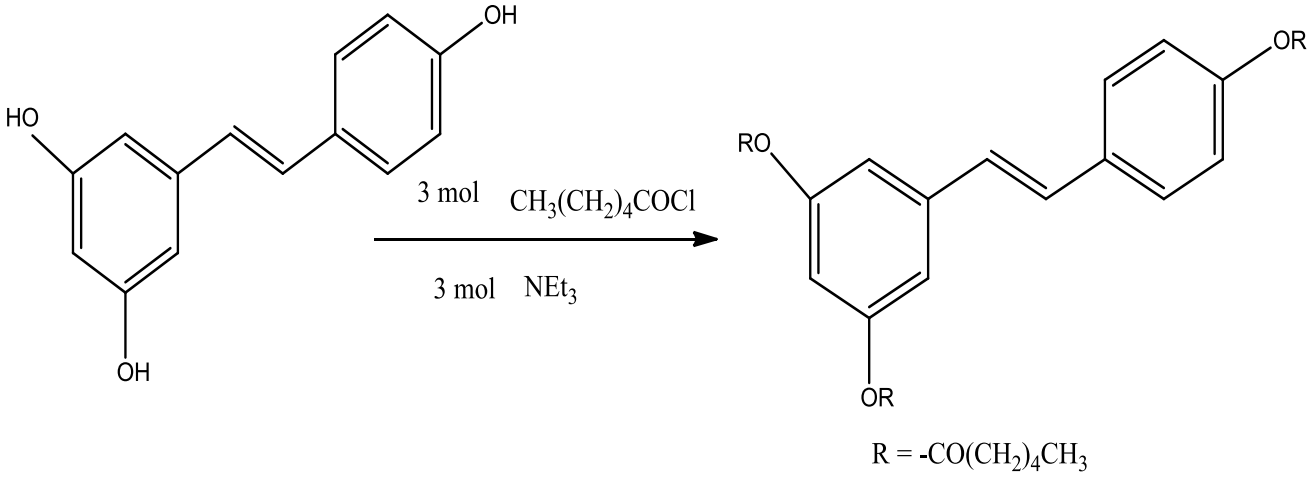
Compound 3 was synthesized by the reaction between hexanoic acid and thionyl chloride as shown in scheme 7. Acyl chlorides (hexanoyl chloride) are generally prepared by replacing the equivalent hydroxy substituents with chlorides. In this reaction, the sulfur dioxide (SO_2) and hydrogen chloride (HCl) generated are both gases that can evaporate from the reaction vessel. Excess thionyl chloride can also evaporate because the boiling point is 79°C.



Scheme 7. Synthetic pathway for hexanoyl chloride

Proposed Synthetic Pathway for Synthesis of 3,5,4'-tri-O-hexanoyl Resveratrol

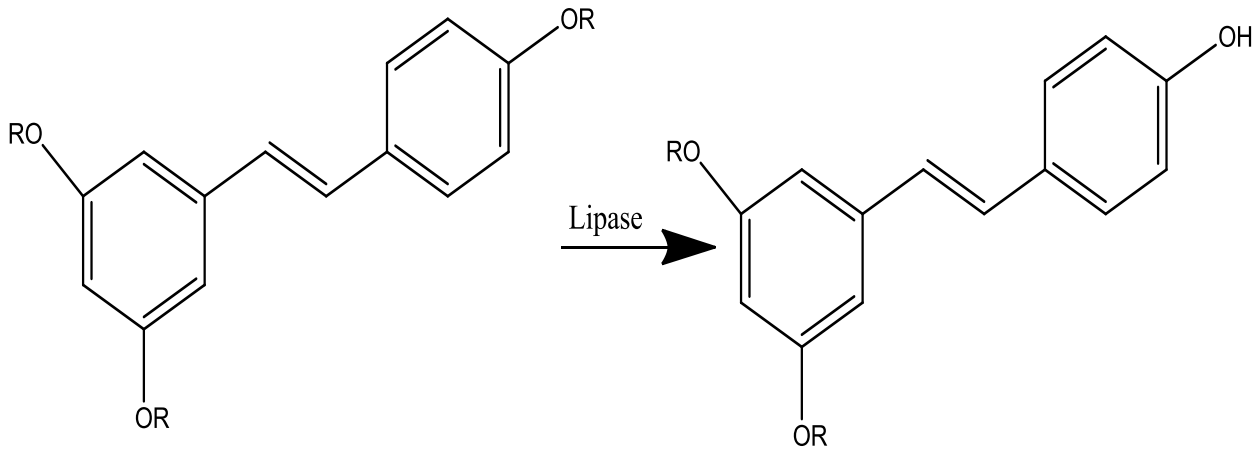
The synthesis of 3,5,4'-tri-O-hexanoyl resveratrol was an esterification synthesis of resveratrol as outlined in Scheme 8.



Scheme 8. Synthesis of 3,5,4'-tri-O-hexanoyl resveratrol (Esterification)

Proposed Synthetic Pathway for Synthesis of 3,5-di-O-isobutyroyl Resveratrol and 3,5-di-O-hexanoyl Reseveratrol

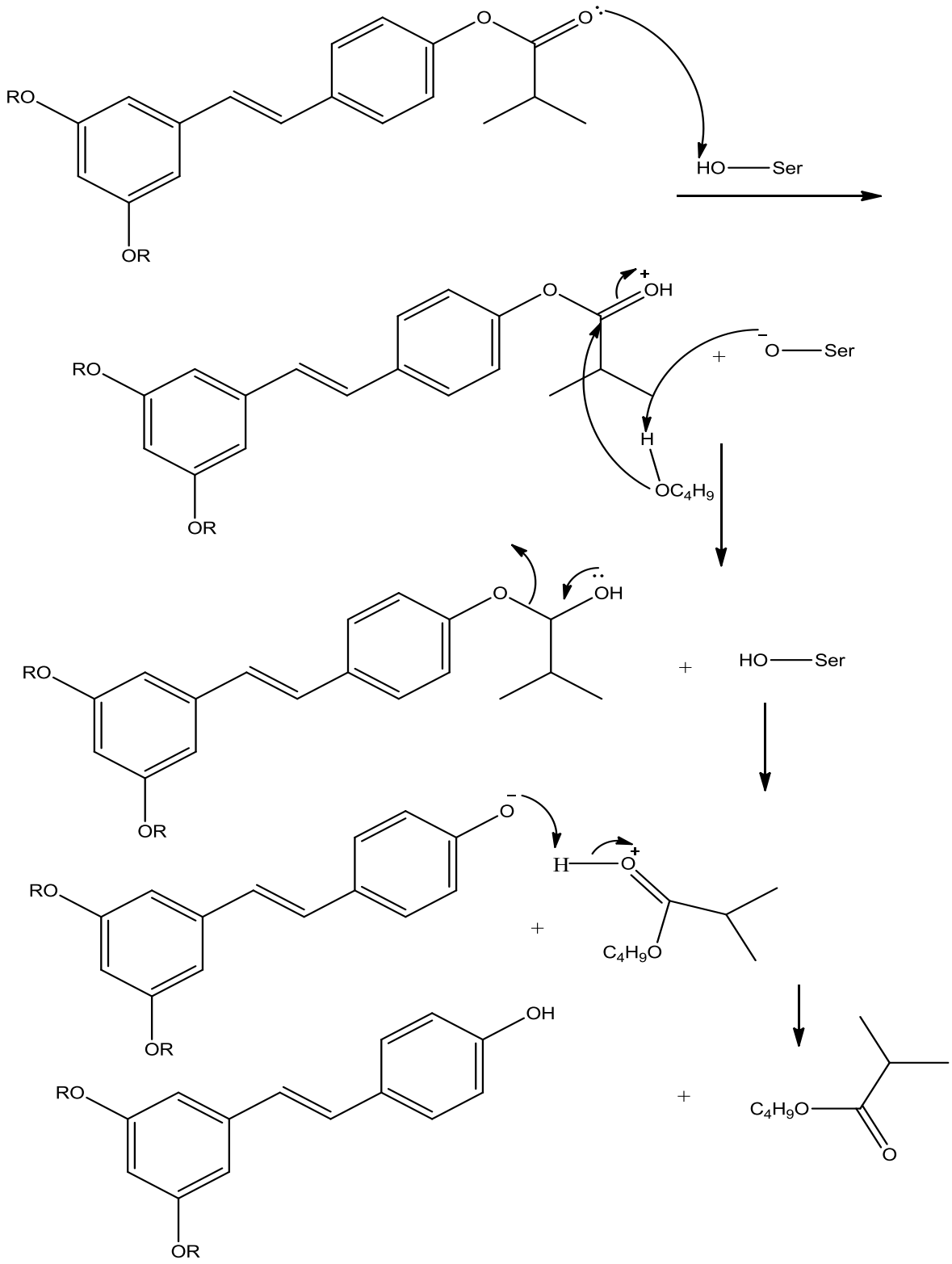
Lipase (C. *Antarctica*) used in this reaction is highly specific for the phenolic group at position 4' because it is less sterically hindered, Scheme 9. The mechanism of this reaction is outlined in Scheme 10.



$R = -COCH(CH_3)_2$

$R = -CO(CH_2)_4CH_3$

Scheme 9. Enzymatic synthesis of 3,5-di-O-isobutyroyl resveratrol and 3,5-di-O-hexanoyl resveratrol

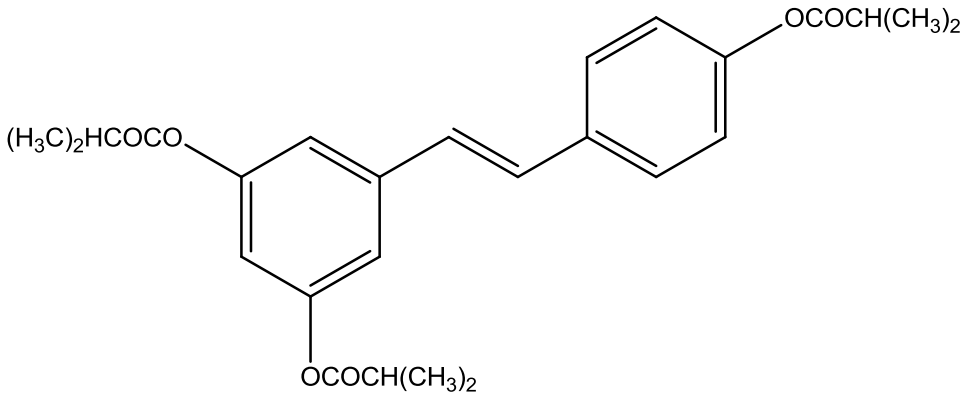


Scheme10. Mechanism of 3,5-di-O-isobutyroyl resveratrol and 3,5-di-O-hexanoyl resveratrol

CHAPTER 2

RESULTS AND DISCUSSION

Synthesis of 3,5,4'-tri-O-isobutyroyl Resveratrol, 2



2

A high yield of 90% of compound 2 was obtained as white crystals after purification of the crude product using column chromatography. The R_F value for the tri-ester was 0.67. The triethylamine acts as a nucleophile and attacks the carbonyl carbon of the isobutyroyl chloride. The carbonyl carbon then forms a bond with the 4' oxygen resveratrol after the chloride ion picks the phenol proton. The reaction continued until the phenol positions at 3 and 5 all acylated as shown in scheme 5.

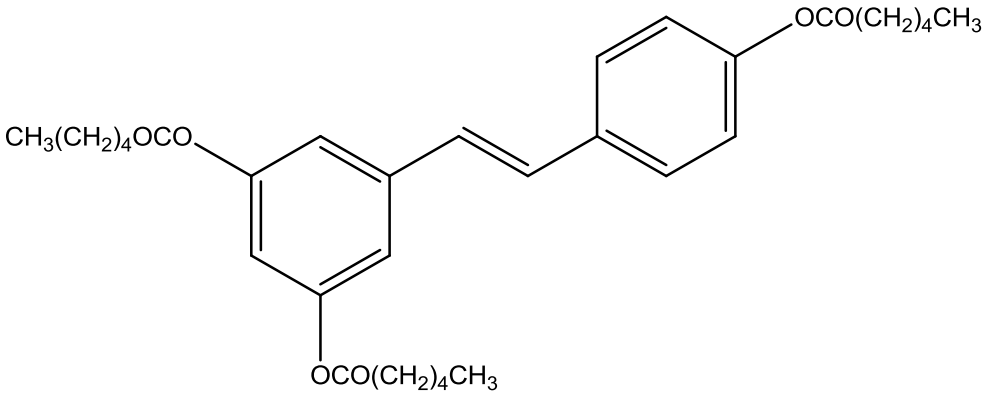
Synthesis of Hexanoyl Chloride, 3

$$CH_3(CH_2)_4COCl$$

3

Pure compound 3 was obtained as a slightly yellow liquid in 90% by reacting hexanoic acid with thionyl chloride in dichloromethane.

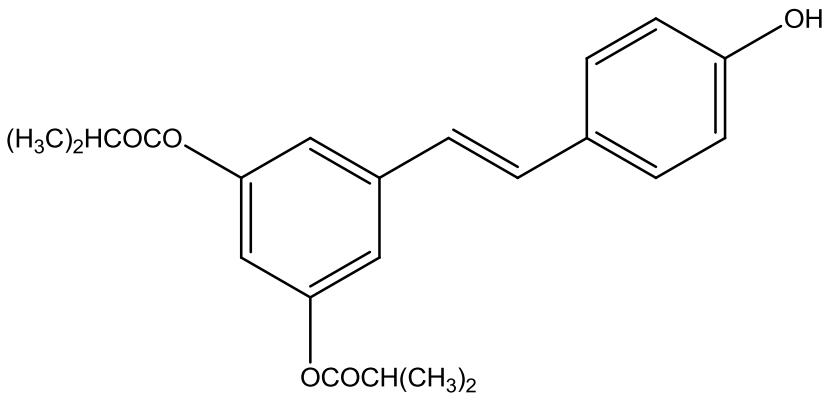
Synthesis of 3,5,4'-tri-O-hexanoyl Resveratrol, 4



4

A high yield of 95% of compound 4 was obtained as a yellow liquid after purification of the crude product using column chromatography. The R_F value for the tri-ester was 0.66. The reason for compound 4 being a liquid is because of the long straight carbon chains on the stilbene. This makes the compound have a large surface area that can accommodate more interactions resulting in higher boiling point because of higher molecular weight.

Synthesis of 3,5-di-O-isobutyroyl Resveratrol, 5



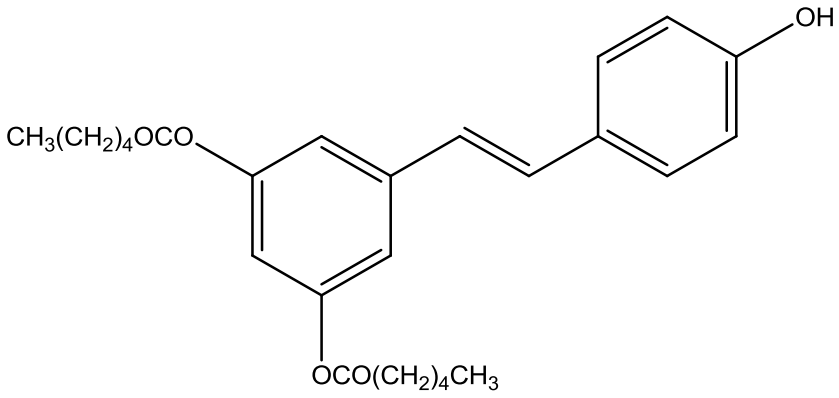
5

Compound 5 was obtained as white crystals with a moderate yield of 25 percent. The R_F value for the di-ester was 0.56. The n-butyl alcohol used in the reaction acted as a nucleophile. The tert-butyl methyl ether was used as a solvent to dissolve the tri-ester. The enzyme reaction was difficult and the low yield produced was due to very difficult separation. The 30 minutes reaction time which was reported could not work for my compound. Several other reaction times were tried using that same method and it became successful when the reaction was run for 24 hours. The reason for the previous unproductive method was because of the large group at the 4'position which needed much time for the reaction to go to completion. Table 1 gives the summary of the reaction results.

Table 1. Summary of Reaction Results

Resveratrol Tri-ester Mass (mg)	**Reaction Time @ 40^{o}C , 280 rpm**	**Resveratrol Di-ester Yield (%)**	**Resveratrol Tri-ester Recovery (%)**
100 mg	30 min	None	100
100 mg	1hr	None	100
100 mg	3hrs	None	100
100 mg	9hrs	None	100
100 mg	12hrs	< 1	98
100 mg	24 hrs	25	75

Synthesis of 3,5-di-O-hexanoyl Resveratrol, 6



6

Compound 6 was obtained as white crystals after purification of the crude product using column chromatography with a moderate yield of 41%. The R_F value for the di-ester was 0.49.

Conclusion

The objective of this research was fulfilled. The compounds, 3,5,4'-tri-O-isobutyroyl resveratrol and 3,5,4'-tri-O-hexanoyl resveratrol have been synthesized with pure yield of 90% and 95%, respectively. Moreover, 3,5-di-O-isobutyryl resveratrol and 3,5-di-O-hexanoyl resveratrol, that was my main research objective, have also been successfully synthesized with pure yields of 25% and 41%, respectively. All the compounds, 3,5,4'-tri-O-isobutyroyl resveratrol, 3,5,4'-tri-O-hexanoyl resveratrol, 3,5-di-O-isobutyroyl resveratrol, and 3,5-di-O-hexanoyl resveratrol are new, publishable, and may be evaluated in biological systems.

The main synthetic routes used in my research were chemical and enzymatic esterification of phenolic compounds. The disadvantage with the chemical synthesis is that a lot of side reactions occur making it difficult to separate because it is relatively unselective. With the enzymatic synthesis, because it is selective it has an advantage over the chemical synthesis in that there is a minimization of side reactions making the purification steps very few. The only problem with the enzymatic synthesis is that the yield is not as high as that of the chemical synthesis.

CHAPTER 3

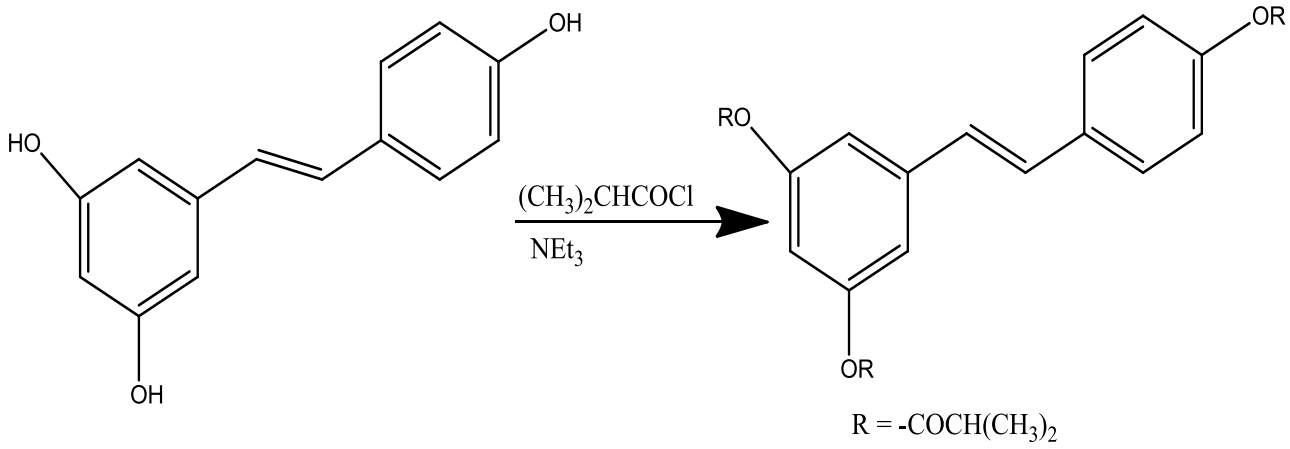
EXPERIMENTAL

General Methods

Resveratrol and other commercial reagents were used without extra purification and they were acquired from sigma (St. Louis, MO, USA). The data of all proton (^{1}H) and (^{13}C) NMR spectra were taken from JEOL-NMR Eclipse spectrometer operating at 400 MHz and 100 MHz for proton and carbon nuclei respectively. The solvents used for the spectra were CD_3COCD_3, CD_3OD, and $CDCl_3$ unless otherwise stated. Chemical shifts were recorded as delta values in parts per million (ppm) relative to TMS. From the spectra, the multiplicity of the signals was documented as s (singlet), d (doublet), t (triplet), h (hextet), hept (heptet), and m (multiplet). Silica gel and suitable solvents were used in carrying out the column chromatography. Thin layer chromatography (TLC) was done with silica gel plate using suitable solvent mixtures and viewed under UV Fluorescent indicator. Melting points for the crystals were recorded using Cambridge MEL-TEMP instrument. All weighing was performed on Mettler PJ360 Delta Range scale unless otherwise stated.

Experimental Procedures

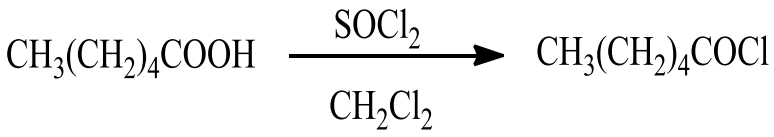
Synthesis of 3,5,4'-tri-O-isobutyroyl Resveratrol



Resveratrol (1.04 g, 4.56 mmol) was dissolved in acetone (35 mL) acting as the solvent, and isobutyroyl chloride (1.46 g, 13.68 mmol) and triethylamine (1.38 g, 13.68 mmol) were added to it and stirred in a 50-mL round bottom flask for 12 hours. The solution was then acidified (≤5) with hydrochloric acid (2N). The crude product was extracted in the organic layer by means of ethyl acetate (2×35 mL). The excess hydrochloric acid was removed by washing with sodium hydrocarbonate (35 mL). The product was dried by means of magnesium sulfate followed by gravity filtration. The crude product was purified again using column chromatography. Melting point of pure compound was $85^{o}C - 87^{o}C$.

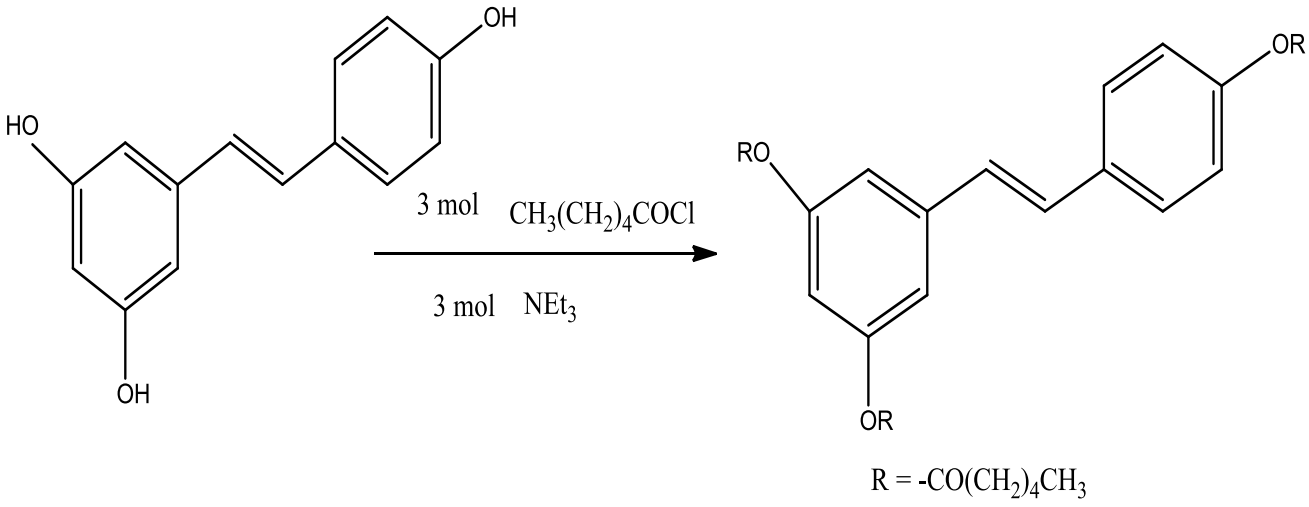
^{1}HNMR (CD_3OD/CD_3COCD_3, 400MHZ, ppm) δ1.27 (d_1, 18H, CH_3); δ 2.84 (m, 3H, CH); δ 6.71 (dd_1, 1H, Ar-H); δ 6.78 (d_1, 1H, CH=C); δ 7.26 (dd_1, 2H, Ar-H); δ 7.00 (d_1, 1H, CH=C); δ 7.12 (ddd_1, 2H, Ar-H); δ 7.41 (ddd_1, 2H, Ar-H) ^{13}CNMR (CD_3OCD_3,100 MHz, ppm) δ 18.32, 34.01, 114.87, 116.99, 122.15, 127.06, 127.70, 129.60, 134.60, 139.74, 151.07, 151.98, 174.67, 174.89

Synthesis of Hexanoyl Chloride



Hexanoic acid (4 mL, 32 mmol) was put in a 25-mL round bottomed flask in addition with 5 mL dichloromethane. Thionyl chloride (4.64mL, 64 mmol) was added drop wise and stirred for an hour for the gases to escape. The mixture was put in an oil bath at 50^{o}C for additional hour to allow all the gas to evaporate. The mixture was then distilled to first collect the dichloromethane and then excess thionyl chloride (boiling point, 79^{o}C) and lastly hexanoyl chloride (boiling point, 151-153^{o}C). A dark brown liquid residue was left behind. ^{1}HNMR (400MHz, $CDCl_3$, ppm) δ 0.81 (t_1, 3H, CH_3); δ 1.23 (h, 2H, CH_2); δ 1.23 (tt1, 2H, CH_2); δ 1.59 (tt1, 2H, CH_2); δ 2.77 (t1, 2H, CH_2)

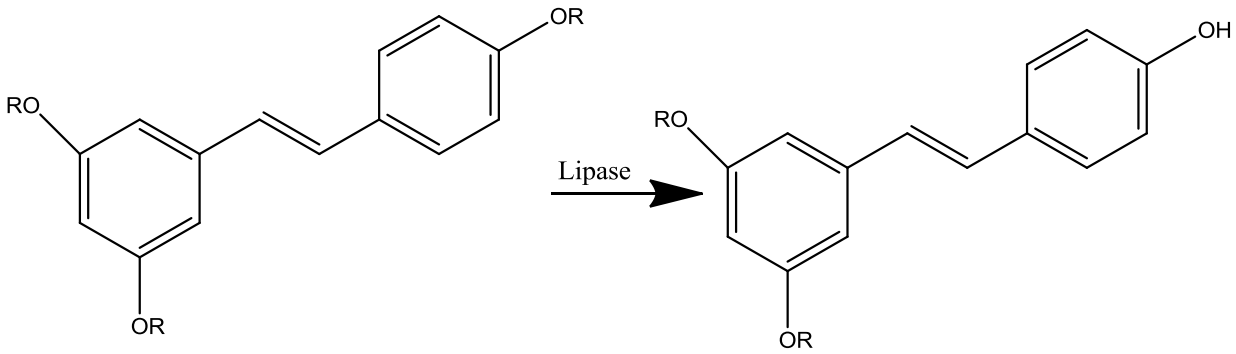
Synthesis of 3,5,4'-tri-O-hexanoyl Resveratrol



Resveratrol (1.00 g, 4.386 mmol) was dissolved in acetone (33 mL) and hexanoyl chloride (1.771 g, 13.158 mmol) and triethylamine (1.331 g, 13.158 mmol) were added and stirred in a 50-mL round bottom flask for 12 hours. The solution was then acidified with

hydrochloric acid (2N). The crude product was extracted with ethyl acetate (2×33 mL). The excess hydrochloric acid was removed by washing the organic layer with saturated sodium hydrocarbonate (33 mL) and drying over magnesium sulfate followed by gravity filtration. The crude product was obtained by evaporation of solvent and was purified using column chromatography. ^{1}HNMR (CD_3COCD_3, 400MHz, ppm) δ0.90 (t_1, 9H, CH_3); δ 1.37 (m, 6H, CH_2); δ 1.37 (tt_1, 6H, CH_2); δ 1.71 (tt_1, 6H, CH_2); δ 2.57 (t_1, 6H, CH_2); δ 6.84 (t_1, 1H, Ar-H); δ 7.23 (d_1, 1H, CH=C); δ 7.14 (dd_1, 2H, Ar-H); δ 7.24 (d_1, 1H, CH=C); δ 7.25 (ddd_1, 2H, Ar-H); δ 7.65 (ddd_1, 2H, Ar-H) ^{13}CNMR ($CDCl_3$,100 MHz, ppm) δ 13.61, 22.01, 25.00, 31.50, 34.10, 115.14, 117.22, 122.40, 127.27, 127.90, 129.78, 134.77, 139.92, 151.17, 152.06, 171.60, 171.79

Synthesis of 3,5-di-O-isobutyroyl Resveratrol

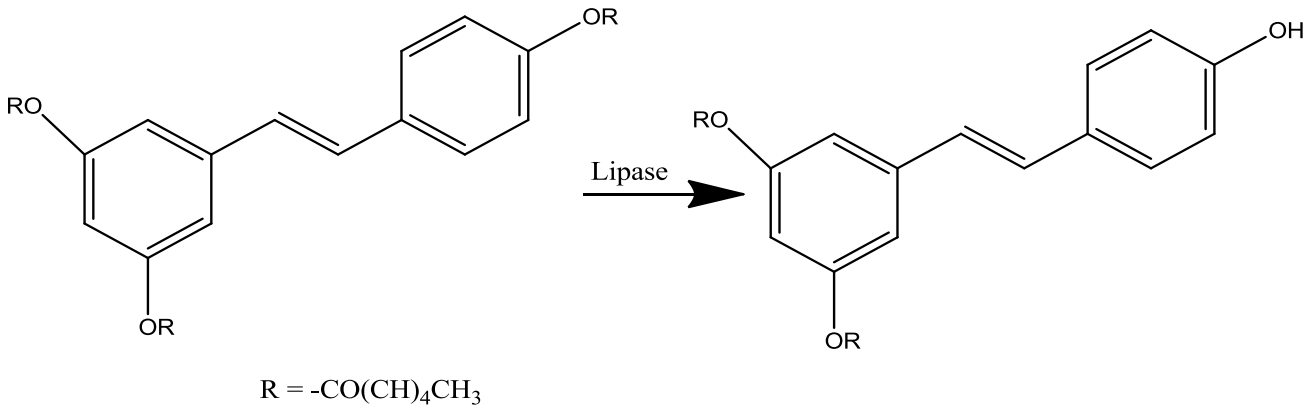


R = -$COCH(CH_3)_2$

3,5,4'-tri-O-isobutyroyl resveratrol (100 mg, 0.023 µmol) was dissolved in t-butyl methyl ether (5 mL, 42 mmol) in 25-mL round bottom flask. A mixture containing *C. Antarctica* (100 mg) and n-butyl alcohol (0.4 mL) was added to the round bottom flask. The mixture was stirred by the help of a rotovap (40^{o}C, 280 rpm) for 24 hrs. After 24 hrs, the enzyme was filtered and the filtrated was left to evaporate leaving behind the crude product. The crude product was purified by the use of column chromatography. Melting point of pure product was 142^{o}C – 145^{o}C.

^{1}HNMR (CD_3OD 400MHZ, ppm) δ1.29 (d_1, 12H, CH_3); δ 2.81 (m, 2H, CH); δ 6.71 (dd_1, 1H, Ar-H); δ 6.78 (ddd_1, 2H, Ar-H); δ 7.00 (ddd_1, 1H, Ar-H); δ 7.10 (dd_1, 2H, Ar-H); δ 7.11 (d_1, 1H, C=CH); δ 7.12 (d_1, 1H, C=CH) δ 7.41 (ddd1, 1H, Ar-H) ^{13}CNMR (CD_3OD,100 MHz, ppm) δ 17.93, 33.98, 113.50, 115.23, 116.23, 123.53, 127.97, 128.49, 130.49, 151.75, 158.06, 175.58

Synthesis of 3,5-di-O-hexanoyl Resveratrol



R = -$CO(CH)_4CH_3$

3,5,4'-tri-O-hexanoyl resveratrol (0.743 g, 1.423 mmol) was put in a 100-mL round bottom flask together with "t"-butyl methyl ether (37.2 mL, 0.313 mol). A mixture containing *C. Antarctica* (0.743 g) and n-butyl alcohol (3.0 mL) was added to the round bottom flask. The mixture was stirred by the help of a rotovap (40^{o}C, 280 rpm) for 24 hrs. After 24 hrs, the enzyme was filtered and the filtrated was left to evaporate leaving behind the crude product. The crude product was purified by the use of column chromatography. Melting point of pure product was 98^{o}C – 100^{o}C. ^{1}HNMR (CD_3COCD_3, 400MHZ, ppm) δ0.90 (t_1, 6H, CH_3); δ 1.37 (m, 4H, CH_2);

δ 1.37 (tt_1, 4H, CH_2); δ 1.71 (tt_1, 4H, CH_2); δ 2.59 (t_1, 4H, CH_2); δ 6.80 (dd_1, 1H, Ar-H); δ 7.06 (d_1, 1H, CH=C); δ 6.86 (dd_1, 2H, Ar-H); δ 7.19 (d_1, 1H, CH=C); δ 7.46 (ddd_1, 2H, Ar-H); δ 7.48 (ddd_1, 2H, Ar-H); δ 8.59 (s, 1H, OH) ^{13}CNMR ($CDCl_3$,100 MHz, ppm) δ 13.42, 22.00, 24.50, 31.00, 33.50, 114.28, 115.59, 115.68, 116.61, 123.96, 128.30, 128.62, 130.46, 140.33, 151.84, 157.83, 171.41

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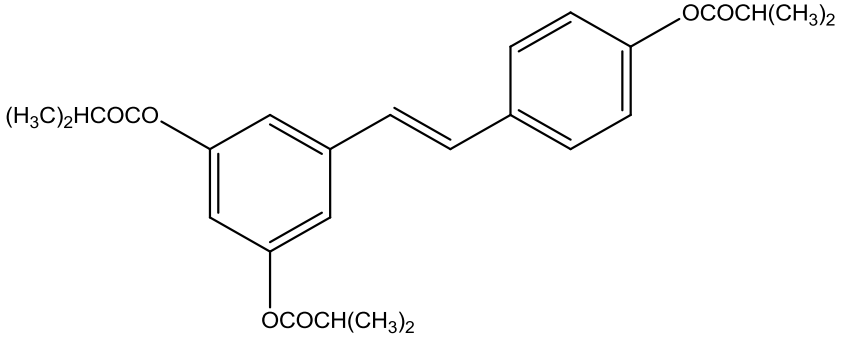
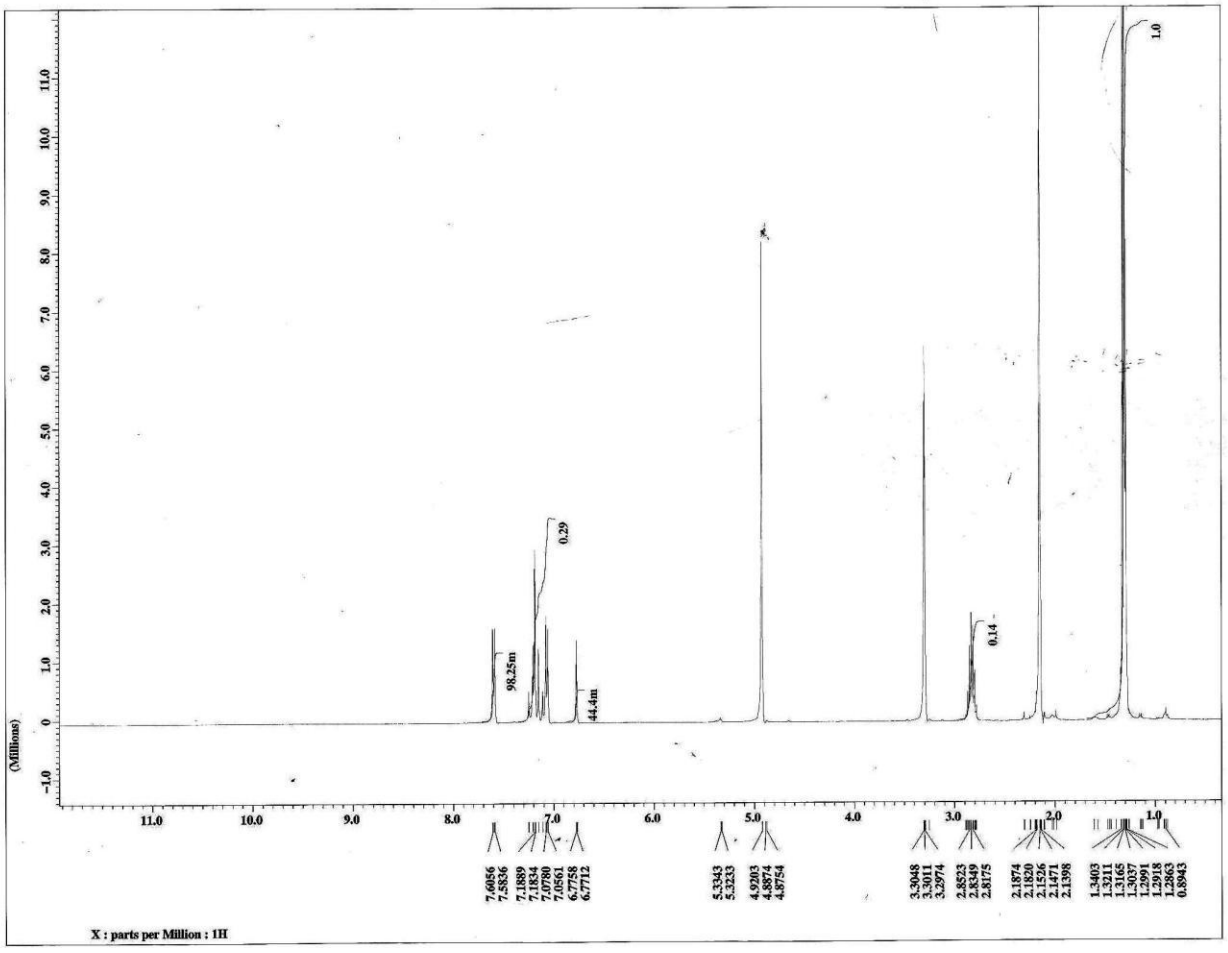
APPENDICES

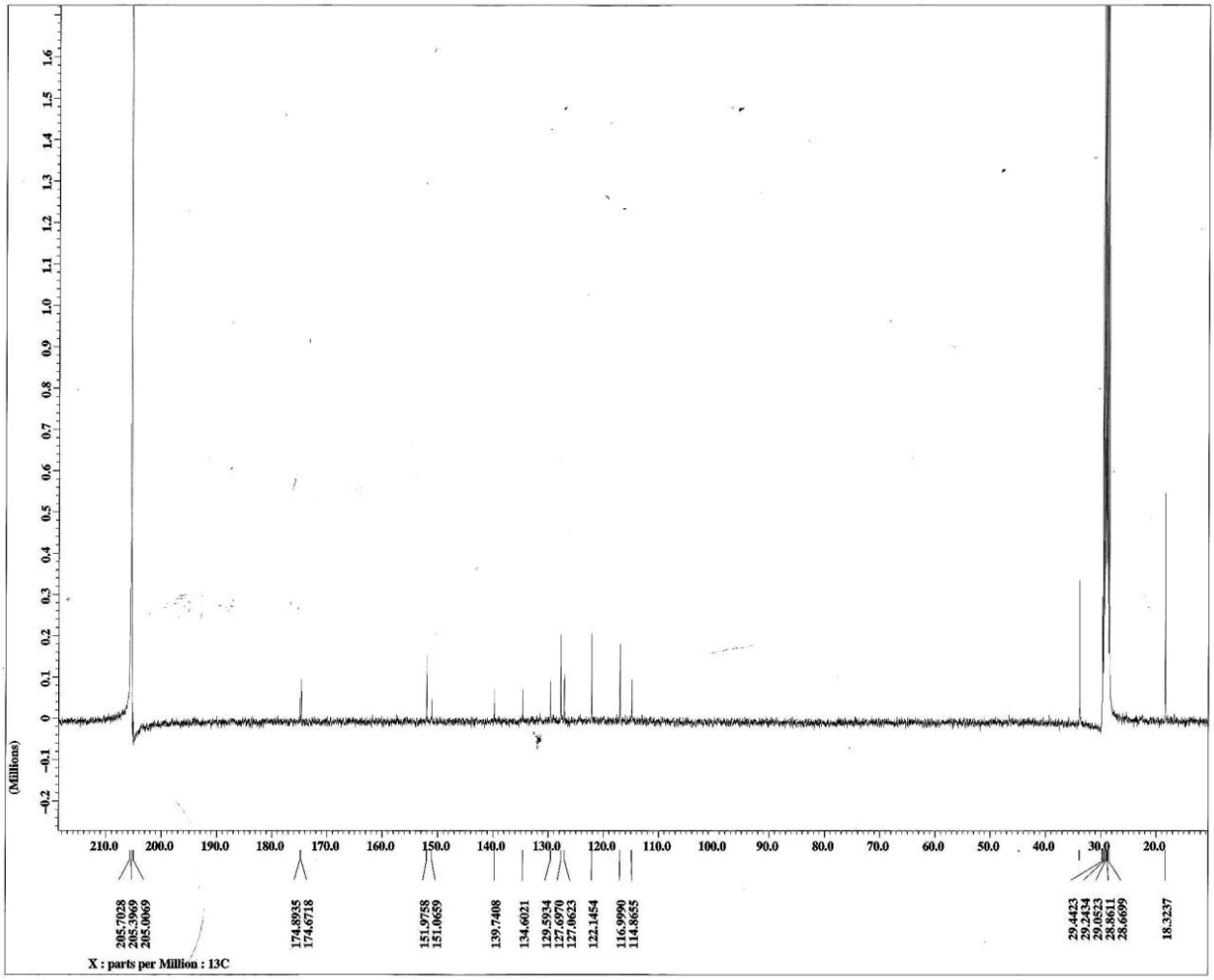
APPENDIX A: ^{1}HNMR Spectrum of Compound 2 in CD_3OD/CD_3COCD_3



2



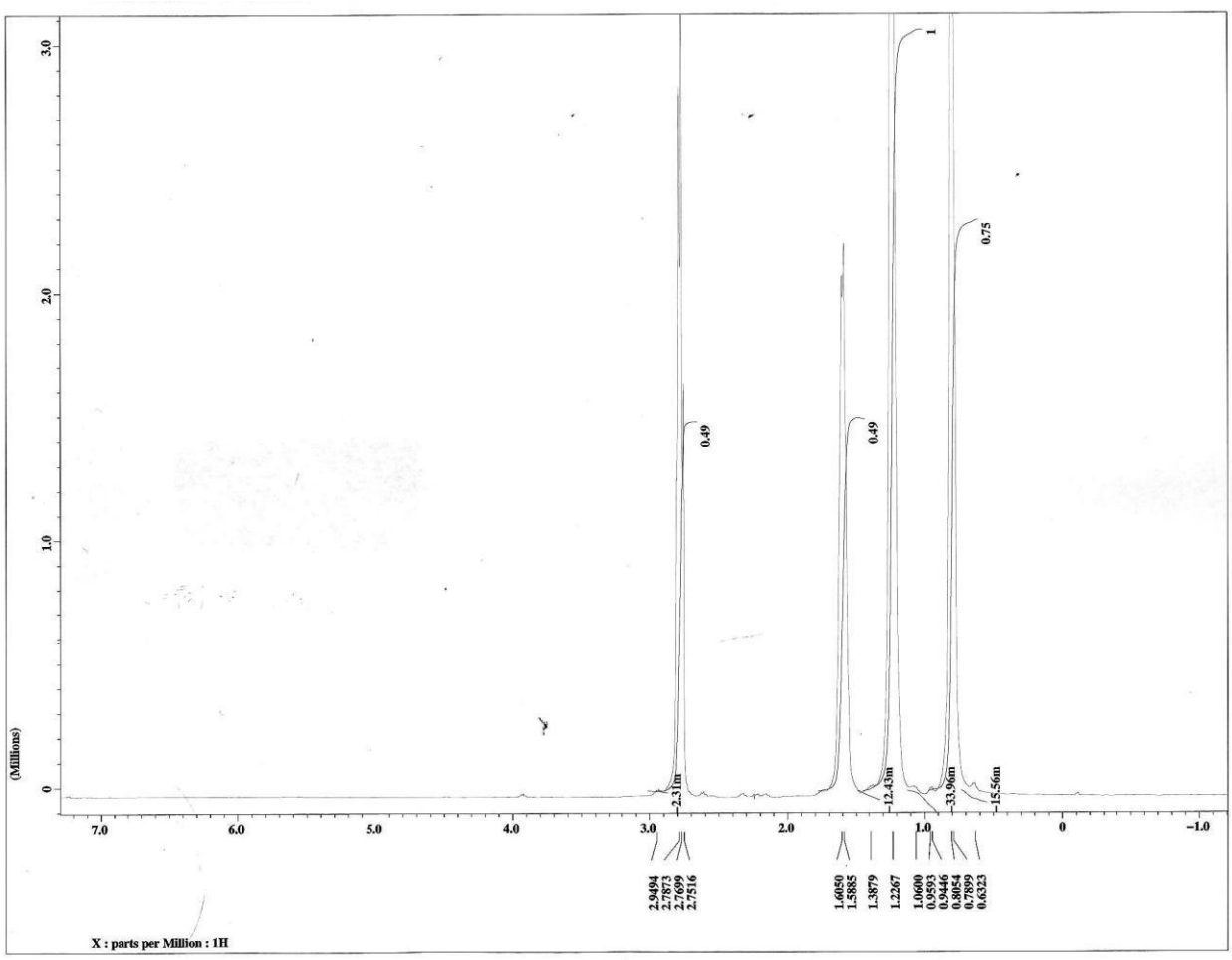
APPENDIX B: ^{13}CNMR Spectrum of Compound 2 in CD_3COCD_3



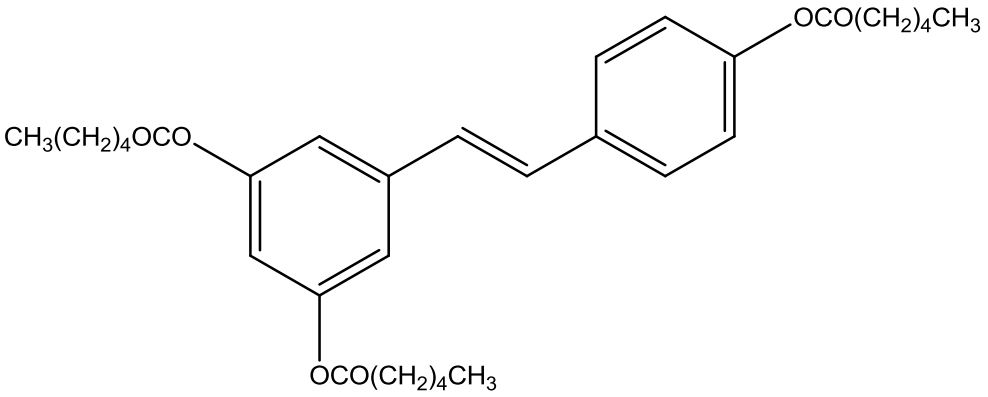
APPENDIX C: ^{1}HNMR Spectrum of Compound 3 in $CDCl_3$

$CH_3(CH_2)_4COCl$

3



APPENDIX D: ^{1}HNMR Spectrum of Compound 4 in CD_3COCD_3



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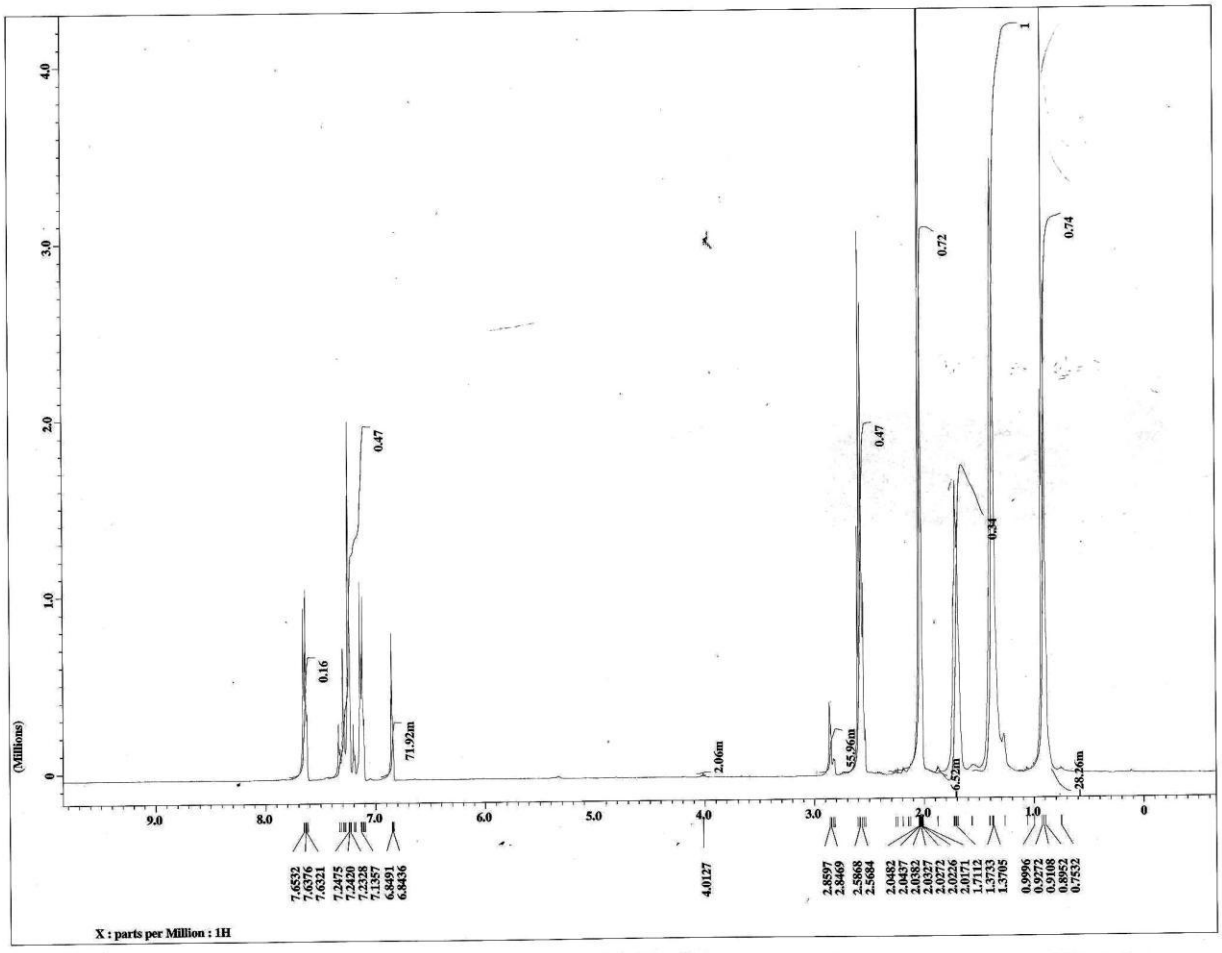
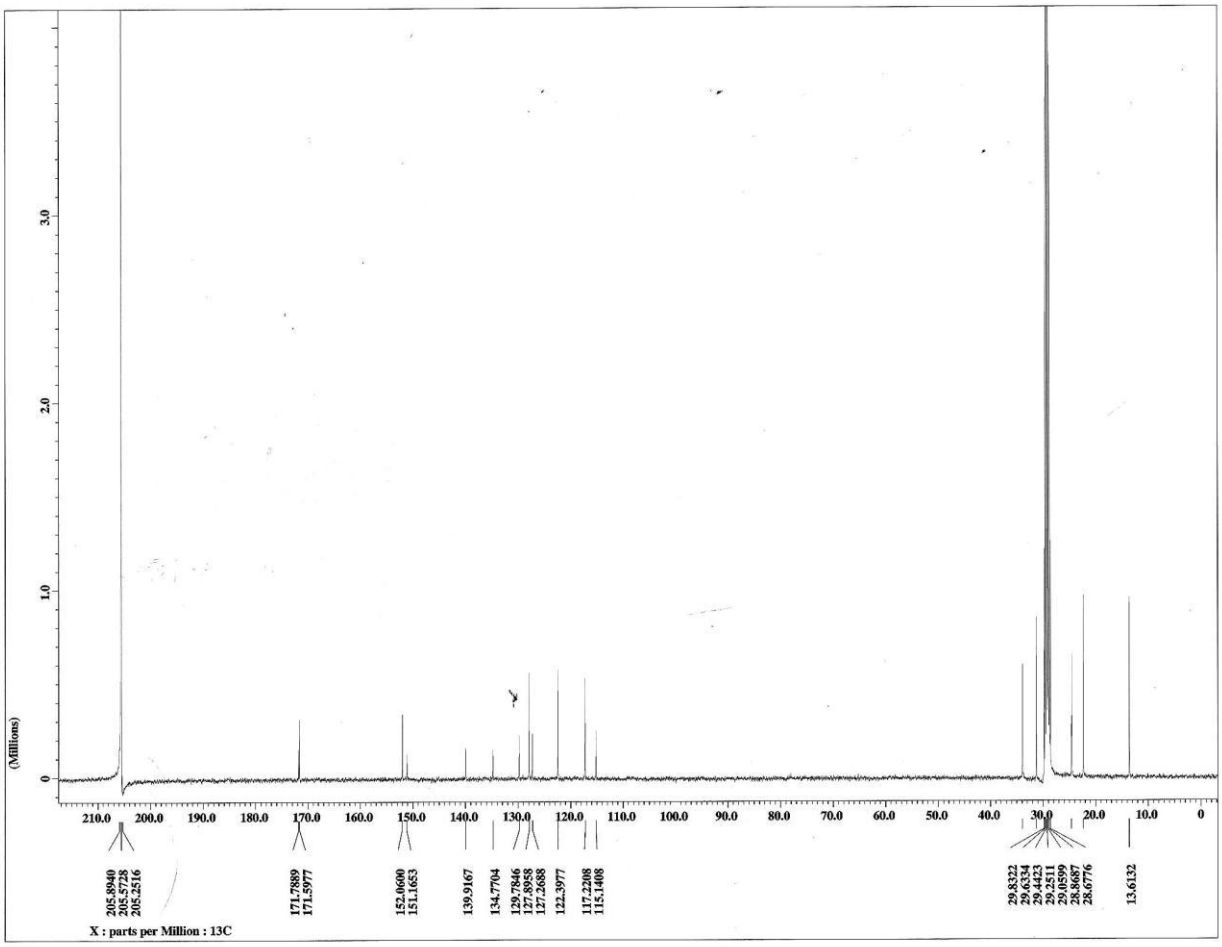
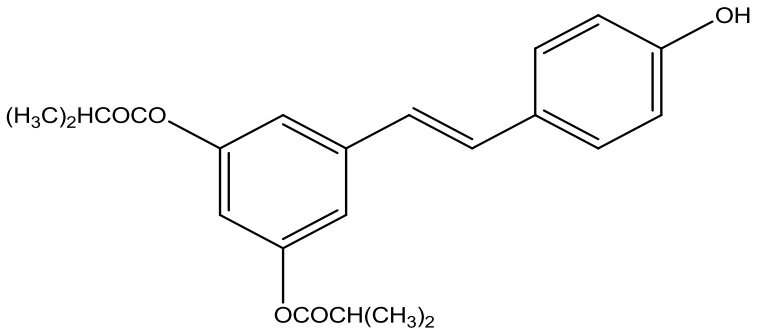


APPENDIX E: ^{13}CNMR Spectrum of Compound 4 in CD_3COCD_3



APPENDIX F: ^{1}HNMR Spectrum of Compound 5 in CD_3OD



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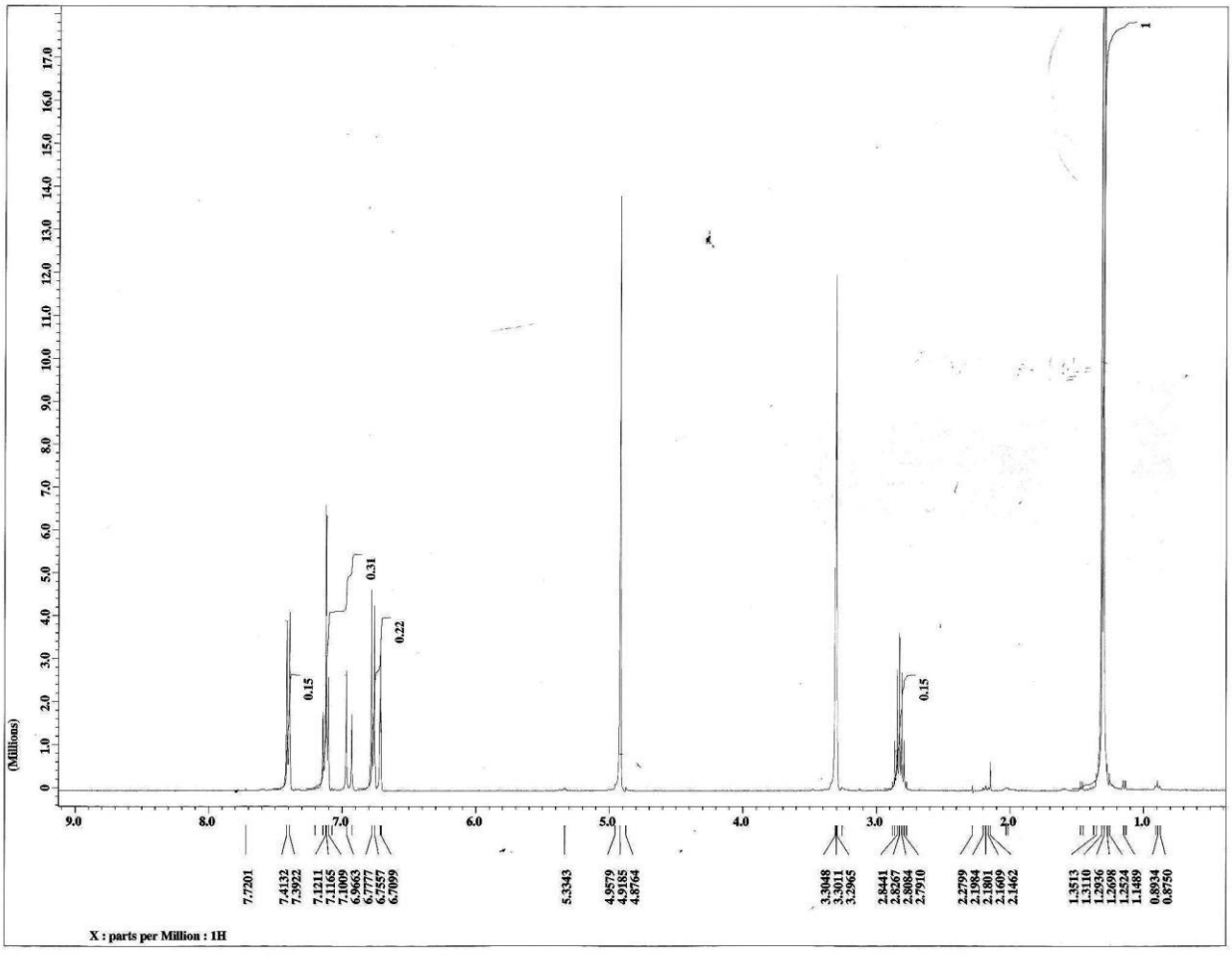
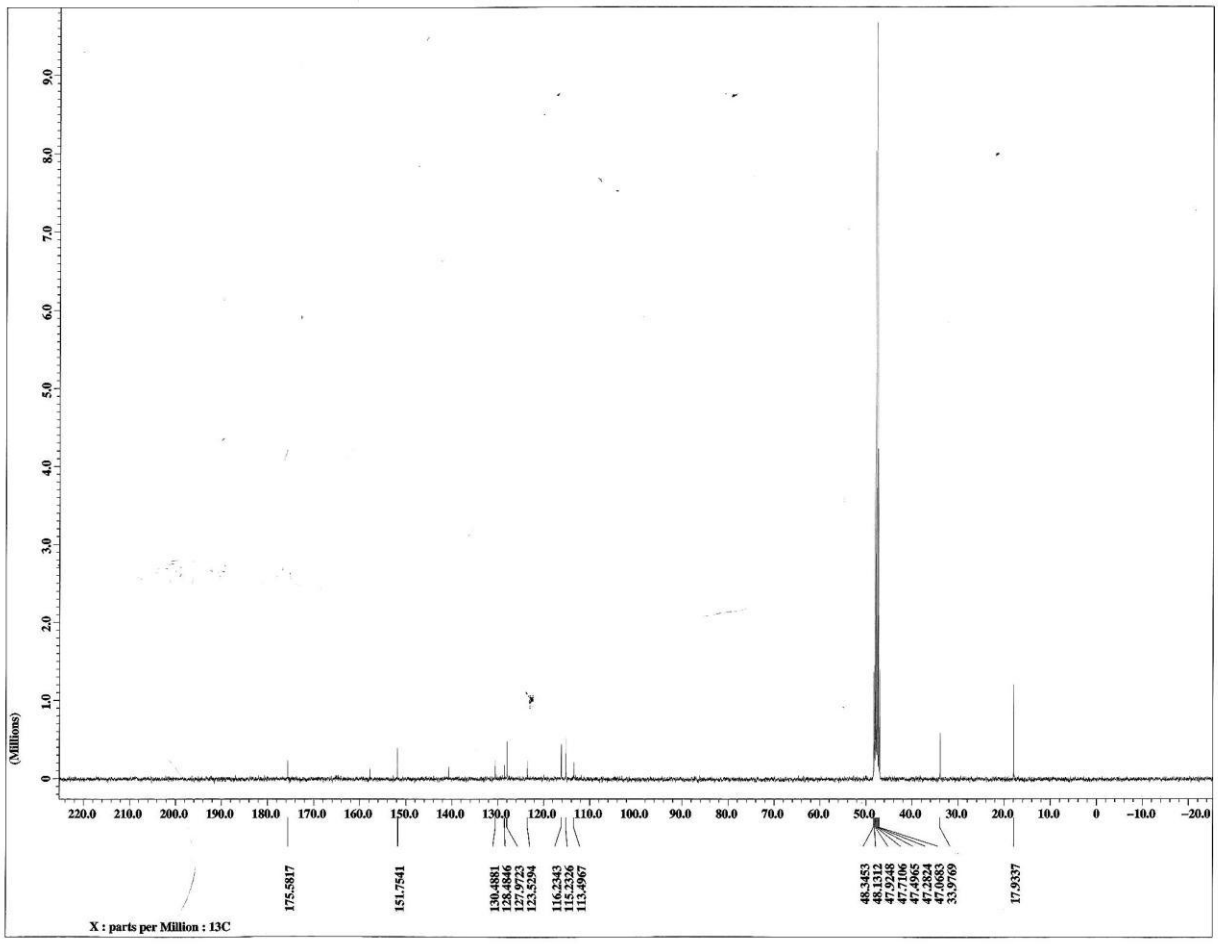
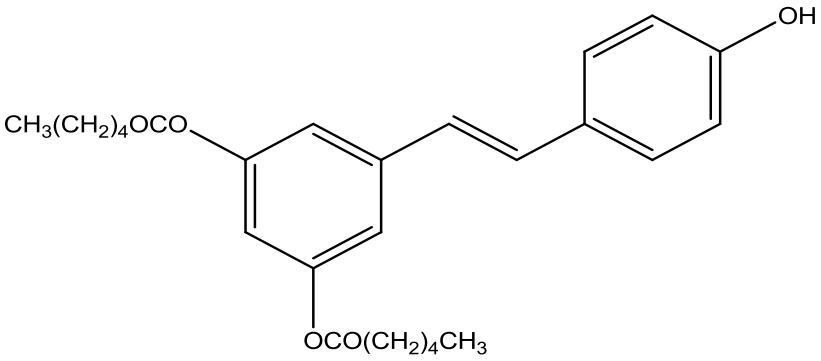


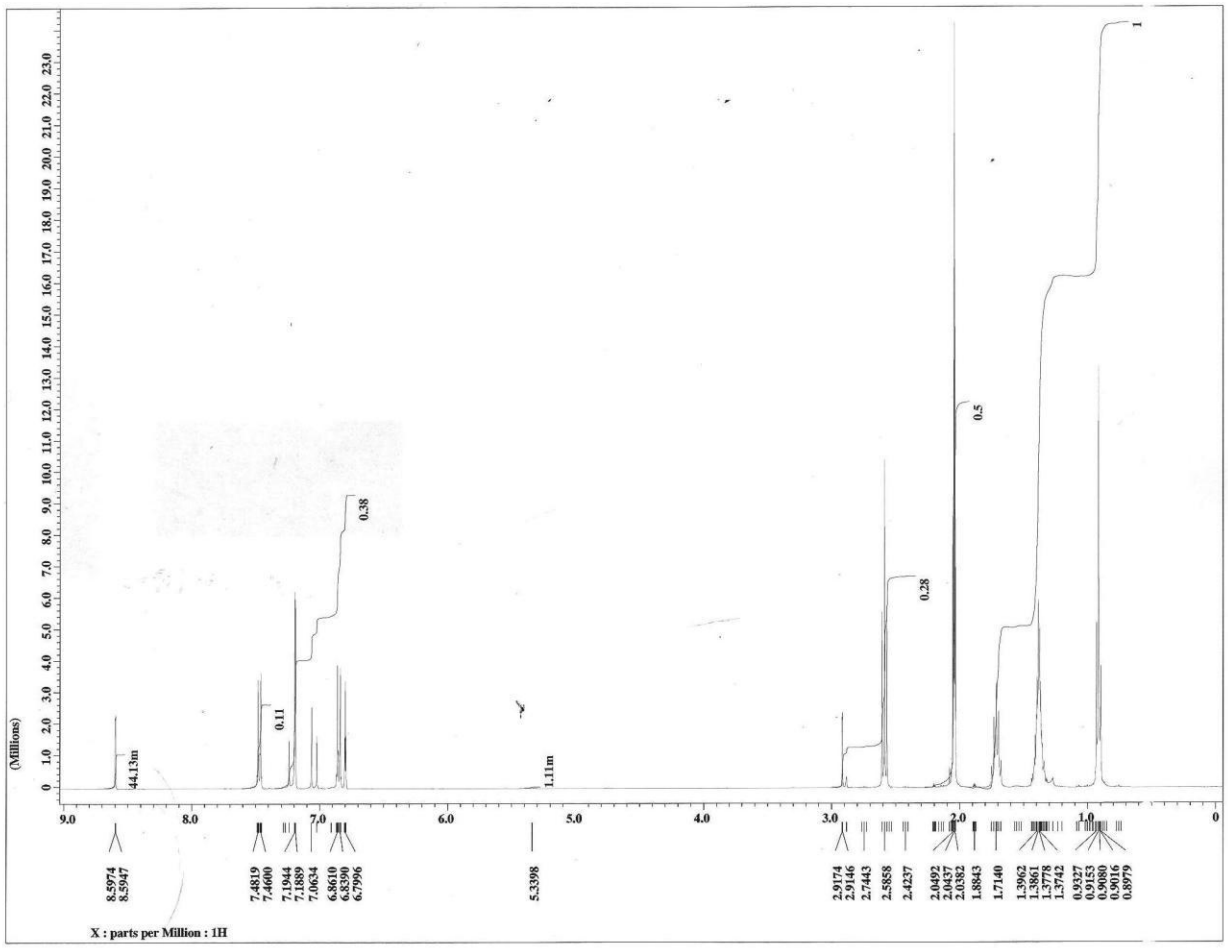
APPENDIX G: ^{13}CNMR Spectrum of Compound 5 in CD_3OD



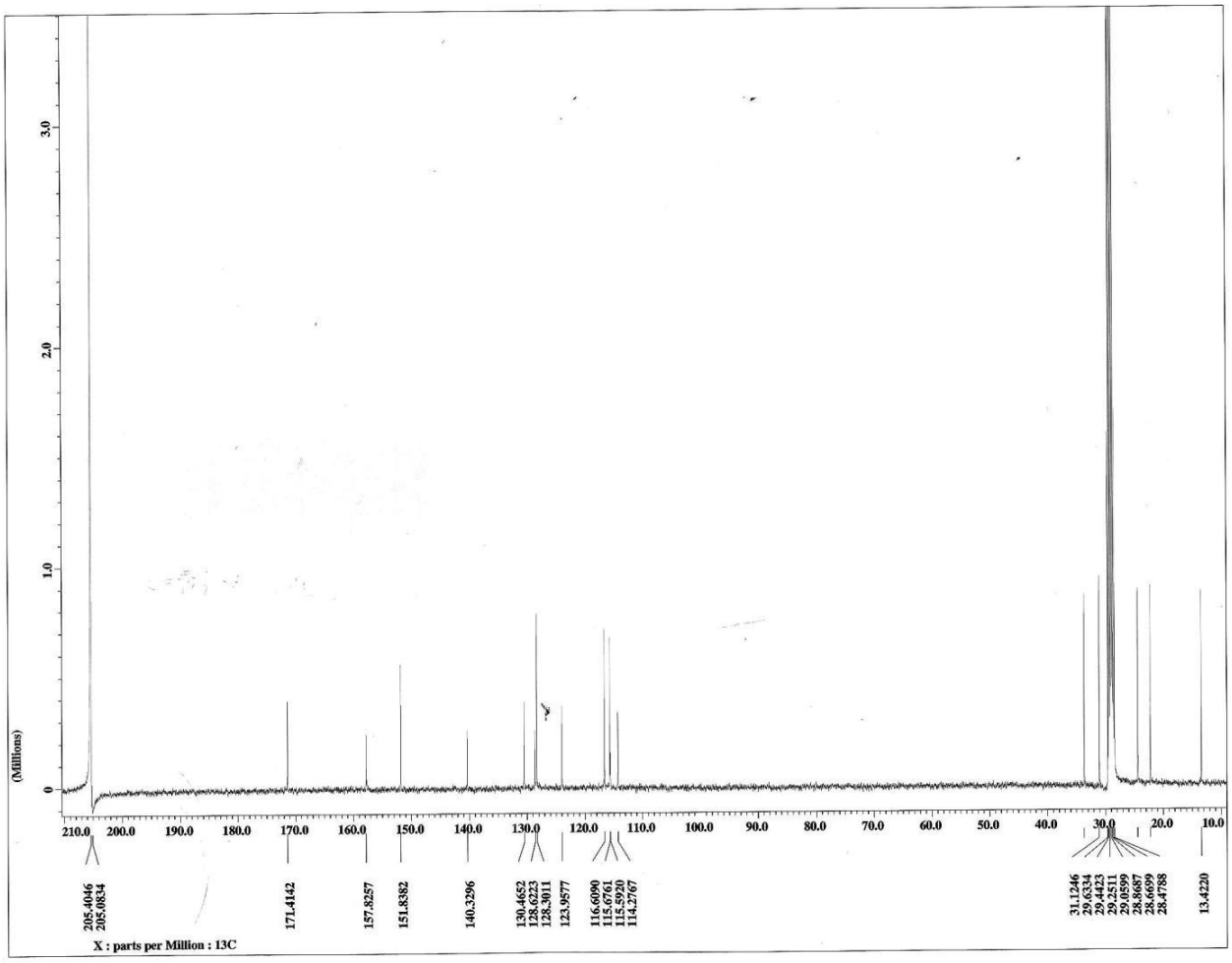
APPENDIX H: ^{1}HNMR Spectrum of Compound 6 in CD_3COCD_3



6



APPENDIX I: ^{13}CNMR Spectrum of Compound 6 in CD_3COCD_3



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