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A Novel Method for Synthesis of Hydroxytyrosol

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A Novel Method for Synthesis of Hydroxytyrosol

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by

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ABSTRACT

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Hydroxytyrosol, 3,4-dihydroxyphenolethanol, a naturally occurring polyphenol most common in olive tree (*Olea europaea*), is one of the most effective member of the polyphenols family, because of its remarkable antioxidant activity, its ability to inhibit oxidation of low density lipids (LDL), and its protection against DNA oxidative damage. Hydroxytyrosol, which is widely used in cosmetics and food supplements industries, can be purchased as an olive oil extract that contains low concentration of hydroxytyrosol besides other polyphenols. The price and low natural abundance of hydroxytyrosol make alternative synthetic sources very attractive. In this research, a novel method for the synthesis of pure hydroxytyrosol from a commercially inexpensive precursor catechol was developed; this can satisfy the increasing market demand and provide a more economical alternative source for this valuable polyphenol.

DEDICATION

To the entire Onobun's family,

For their encouragement,

For their sacrifice,

For their love

Thank you

ACKNOWLEDGEMENTS

I was forewarned that graduate school would be a test of courage: a devotion of passion that would consume mind, body, and spirit. The truth in these words can never be anticipated; one must simply launch into the storm and fight with every ounce of wit and strength. In the end, I measure my success not by what has been accomplished, nor by what has been learned, but by the character I've gained (perseverance in pursuit of creative goals and education).

I will like to express my utmost gratitude to God Almighty for His wisdom, sustenance, and abundant grace throughout my study. Special thanks to my advisor: Dr. Ismail O. Kady for his insightful role, patience and advise during my research work. I also want to show my appreciation to Dr. Aleksey N. Vasiliev and Dr. Abbas G. Shilabin for serving as my advising committee members and for having open doors when I had questions. Special thanks to Dr. Reza Mohseni for his assistance with instrumentation throughout this work; the chair, graduate coordinator and all faculty members and staff of the Department of Chemistry, ETSU for their help and support. I also thank my parents and my family for their unceasing encouragement, support and attention.

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LIST OF ABBREVIATIONS

BHT	Butylated hydroxytoluene
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DMC	Dichloromethane
DPPH	1,1-diphenyl-2-picryl-hydrazyl
eq	Equivalent
IR	Infra-Red
LDL	low-density lipoproteins
min	Minutes
mmol	Millimoles
mL	Milliliter
NMR	Nuclear Magnetic Resonance
ROS	Reactive oxygen species
Rf	Retention factor
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl

TBDMS	Tert-butyldimethylsilyl

CHAPTER 1

INTRODUCTION

Recently, there has been increasing interest in polyphenols research, primarily due to their wide range of biological activities. Structurally, polyphenols are composed of two or more phenolic rings. The unique chemical structure of polyphenols is believed to be the main factor for their activities as antioxidants, and free radical scavenges. The antioxidant activities of polyphenols are affected by factors such as the number of hydroxyl groups in the phenol, nature of the compound to be reduced, and the degree of methoxylation.[1]

The Mediterranean basin has the lowest overall occurrence of cancer in relations to other parts of the world [2]. Research on animals and in vitro studies together with an enormous body of epidemiological statistics[3] has linked that to the dietary lifestyle in such countries, which involves foodstuff rich in vitamins and polyphenols such as whole grain bread and cereal, fruits and vegetables, fish, and olive oil.[4] Lately there has been increasing focus on products from *Olea europaea* L. (olive), principally olive leaves as a source of a potent polyphenol called hydroxytyrosol (**1**).[2] Although hydroxytyrosol is available commercially as olive leaves extract, the low concentration of the active ingredient, coupled with its high cost and low natural abundance has made an alternative synthetic route of hydroxytyrosol very attractive.

The main purpose of this work is to explore new methods for the synthesis of pure hydroxytyrosol from readily available and relatively inexpensive precursors such as catechol.

Polyphenols

There are over four thousand phenolic phytochemicals widely distributed in the plant kingdom. Dietary phenolic compounds are of three major classes, namely polyphenols, flavonoids and phenolic acids.[5,6] Polyphenols are produced in plants as secondary metabolites to protect plants from ultraviolet radiation and pathogens.[7] According to recent findings, polyphenols play a major role in the prevention of degenerative diseases, including cancers, cardiovascular and neurodegenerative diseases.[8,9] Furthermore, being a strong antioxidant, polyphenols increase the antioxidant activities of vitamins and enzymes in combating oxidative stress that is triggered by excess reactive oxygen species (ROS).[10] The detailed mechanisms of biological activities of some polyphenols are not well understood.[11]

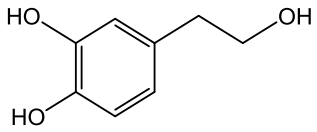
The hydroxyl group of the phenolic compound is bonded to a benzene ring. This makes phenolic compounds more reactive and more acidic than alcohols; this enables them also to form stable phenoxy radicals.[1] This characteristic of polyphenols makes them good reducing agents by donating hydrogen atoms to ROS, thereby acting as an antioxidant by inhibiting harmful chemical processes involving ROS, such as lipid oxidation and DNA damage.

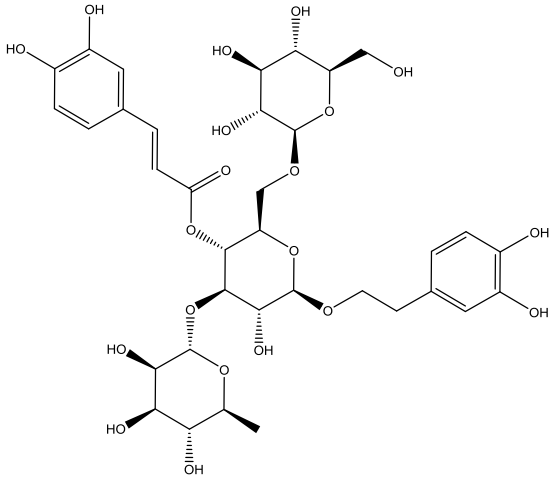
Besides their strong antioxidant properties, polyphenols have other important biological activities. Reports have shown that polyphenols play a role in the prevention of degenerative diseases such as cancers, diabetes mellitus, and neurodegenerative diseases.[12] Polyphenols have been implicated in other biological activities such as antibacterial,[13] antiviral,[14] anti-histamine[15] and anti-inflammatory properties.[16] Several authors[12,17,18] have reviewed epidemiological evidence suggesting the role of dietary polyphenol in prevention of cardiovascular diseases

Polyphenols such as hydroxytyrosol and oleuropein[19] with two adjacent hydroxyl groups on a benzene ring,[20] are more potent antioxidants than polyphenols with one hydroxyl group per benzene ring.[21,22]

Hydroxytyrosol

Hydroxytyrosol or 3,4-dihydroxyphenolethanol was first identified as a component of echinacoside, a phenolic glycoside containing one glucose and two rhamnose moieties, linked to both a dihydroxyphenylethanol residue and caffeic acid. Hydroxytyrosol showed antibiotic activity against *Staphylococcus aureus*, bacteria obtained from the roots of *Echinacea angustifolia*. [26,27]





Hydroxytyrosol **Echinacoside**

It is one of the main bioactive polyphenolic compounds found in small amounts in virgin olive oil,[23,30] but in higher amounts in olive oil mill wastewater.[24] Hydroxytyrosol is produced during the hydrolysis of glycoside oleuropein catalyzed by cellular esterases during olive

processing and storage. Similar hydrolysis takes place also during the debittering process under basic conditions[28,29]

Hydroxytyrosol is a phenylethanoid composed of a catechol moiety and a phenethyl alcohol substituent. Catechol has a lower O−H bond dissociation enthalpy (77.7−80.1 kcal/mol) when compared to phenol (85.1−88.0 kcal/mol).[31] Thus the catechol moiety is capable of stabilizing free radicals via the creation of intramolecular hydrogen bonds[32].

Free Radicals

During certain chemical reactions, weak bonds split in such a way that allows an atom, molecule or ion to have odd, unpaired electron. These species are called radicals or free radicals. .[33] Having unpaired electrons, free radicals tend to be highly unstable and react readily with other compounds where they act as oxidants or reductants.[34] Free radicals can gain electrons from a stable molecule and generate new free radicals. This starts a series of reactions which may eventually lead to damaging living cells.

Free radicals are generated either during metabolic processes by actions of the immune system to neutralize bacteria and viruses or through external sources including exposure to x-ray, air pollutions, industrial chemicals, or tobacco.[34,35] Free radicals can be formed from enzymatic reactions, such as those involved in phagocytosis, the respiratory chain, cytochrome p-450 system, and in the prostaglandin synthesis;[34,35] they can also be formed in non-enzymatic reactions such as reactions between oxygen and organic compounds, and in reactions involving ionization.[34] Among the major oxygen-containing free radicals implicated in many diseases are hydrogen peroxide (H_2O_2), superoxide anion radical ($O2^{\bullet -}$), peroxynitrite ($ONOO^-$),

hypochlorite (NaClO), singlet oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$), and nitric oxide (NO). High concentration of free radicals in the body is harmful. Free radicals target and damage the main components of the cell including cell membrane, nucleic acids, and proteins. The damage, particularly those done on the nucleic acid, may lead to the development of degenerative diseases such as cancer.

The most prevalent types of free radicals generated in living systems are those that contain reactive oxygen elements, called reactive oxygen species (ROS).[36] The unique electronic configuration of molecular oxygen makes it a free radical. Furthermore, superoxide anion radicals ($O_2^{\bullet -}$) are formed when an additional electron is added to molecular oxygen.[37,38] Superoxide anions, which are generated in metabolic processes or from activation of oxygen by physical irradiation, are recognized as the primary ROS's. These primary ROS's can further react with other molecules giving rise to secondary ROS's; this occurs enzymatically or in metal-catalyzed processes.[39] The superoxide, which has been linked pathophysiologically to some diseases,[41,42] is found mainly in the mitochondria;[40] it is produced during the energy transduction process when there is a premature leak of electrons to oxygen. Hydroxyl radical ($HO^{\bullet}$) is also highly reactive and harmful; it has a very short half-life in vivo (10^{-9} s).[37,43] Molecular oxygen is an oxidant, and in the presence of [4Fe–4S] cluster-containing enzymes and under oxidative stress it promotes the production of hydroxyl radicals from hydrogen peroxide.[39, 115]

Oxidative Stress

The concept of oxidative stress first surfaced in the 1950s in the study of free radicals, the toxic effects of both ionizing radiation and of molecular oxygen,[44] and how they can contribute

to the phenomenon of aging.[45] However, it was not until the 1970s that use of the term oxidative stress became frequent.[45] The slow acceptance of free radicals in biology can be attributed to some factors such as the short life of free radicals, the lack of experimental tools for their study and the theoretical and hypothetical nature of its beginnings.[46] In general, the term oxidative stress is used to define the steady state level of oxidative damage in a cell, tissue, or organ, brought about by the ROS. The imbalance between free radical generation and antioxidant defenses that bring about oxidative stress has been implicated in a wide range of damages to molecular species including proteins, nucleic acids, and lipids.[48] Tissues injured as a result of short-term oxidative stress may increase the activities of radical generating enzymes (e.g. cyclooxygenase, lipogenase), increase production of free iron, copper ions, increase activation of phagocytes, or cause a disruption in the electron transport chain of oxidative phosphorylation, leading to the production of excess ROS.[33] Oxidative stress is assumed to play a role in inflammatory condition, certain cancers, the process of aging and anthersclerosis. Furthermore, it is thought to significantly contribute to all inflammatory diseases.[43] Changes in the structure and function of lipids and proteins also have linkage to oxidative stress.[33]

A Recent study of mutation suggests that persistent oxidative stress participates in carcinogenesis.[50] The increased occurrence of gastric cancer and colorectal cancer has been linked to persistent gastritis and ulcerative colitis respectively.[51,52] There are three main stages of cancer development process including initiation, promotion, and progression. During the initiation, there is an inheritable mutation in the cells caused by interaction a chemical with the DNA.[53] ROS is thought to have multiple effects in this stage by facilitating carcinogen activation through hydroxyperoxide-dependent oxidation mediated by peroxyl radicals,[54] causing damage to the DNA and preventing its repair. ROS is also thought to play a crucial role in the clonal

expansion of initiated cell during the promotion stage.[54] Oxidative stress is believed to be directly linked to the development of cancer characteristics including chemotherapy resistance, invasion, metastasis, uncontrolled growth and genomic instability.[54] However, direct scavenging of ROS by antioxidants and inhibition of cell proliferation secondary to the protein phosphorylation can reduce carcinogenesis induced by oxidative stress.[33]

The aging phenomenon is the accumulated result of oxidative damage to the cells and tissues derived mainly from aerobic metabolism.[55] Pathological changes linked to aging is thought to originate from free radical damage to cells. [33,56] The main processes of aging are associated with DNA or the buildup of cellular and functional damage.[57] Research suggest that controlling the amount of free radical damage by increasing the antioxidant defense can significantly delay aging and prolong life.[33] The optimal intake of nutritional antioxidants may significantly reduce oxidative damage related to free radical.

Prevention of DNA Damage by Polyphenols

It is well established that endogenous reactive oxygen species originates from normal cellular processes (usually non-pathogenic), and these cellular processes account for the background levels of oxidative DNA damage observed in normal tissue.[123] These reactive species may also be generated by ionizing or ultraviolet radiation, as well as cell metabolism of certain exogenous chemicals that may produce electrons which can be transferred to molecular oxygen generating superoxide ($O_2^{\bullet -}$). Hydroxyl radicals is also produced by redox-active metal ions such as Fe^{2+} and Cu^{+} in the presence of hydrogen peroxide, a byproduct of respiration.[126]

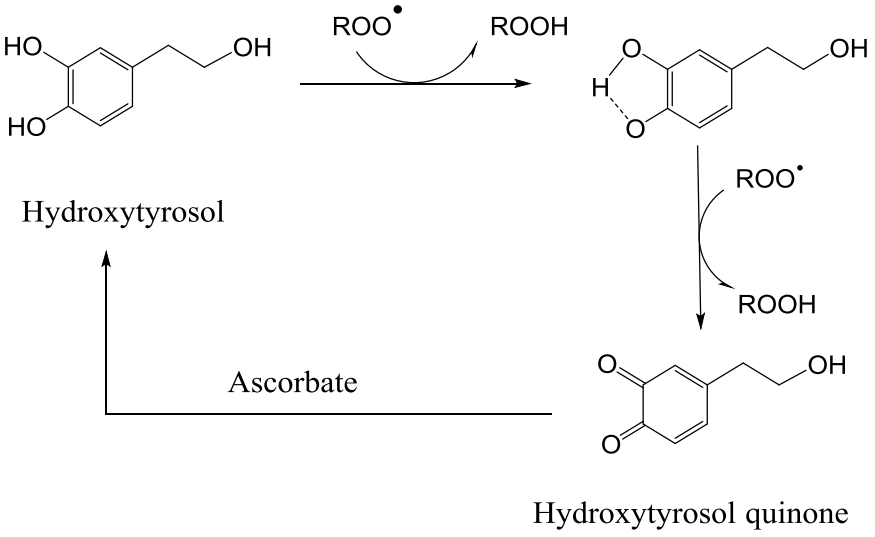
It is reported that oxidative DNA damage by •OH, mediated by iron, is the main cause of cell death given oxidative stress conditions in both prokaryotes and eukaryotes, including humans.[127-129] Reports suggests that Cu^{+} can produce DNA-damaging •OH sixty times faster than Fe^{2+}.[130] Reactive oxygen species may react with cellular biomolecules, such as DNA, leading to modification and potentially serious consequences for the cell regardless of their origin. [123] For instance, the highly reactive •OH may modify DNA either by addition to double bonds of DNA bases or by abstraction of an H atom from the methyl group of thymine and each of the C-H bonds of 2´-deoxyribose.[124]

Certain polyphenols can inhibit oxidizing chain reactions in a number of ways, including direct quenching of reactive oxygen species, inhibition of enzymes, and chelation of metal ions (Fe^{3+}, Cu^{+}).[125] The metal-mediated DNA damage inhibition is directly linked to the metal-binding nature of these polyphenols. Once bound to iron or copper, the polyphenol greatly shift iron oxidation potentials and stabilize Fe^{3+} over Fe^{2+} and Cu^{2+} over Cu^{+}, a component that may inhibit metal redox cycle.[131]

Antioxidant Activity

Usually, the body can effectively manage free radicals generated, but if there are no antioxidants or if there is excessive production of free radical, damage may arise.[58] The phenolic compounds of *Olea europaea* L. have been studied extensively mainly because of its antioxidant property. Hydroxytyrosol, which is one of the major phenolic compound found in *Olea europaea* L., has especially received much attention due to its strong antioxidant activity.[58] Hydroxytyrosol owes its strong antioxidant capability, associated with the presence of the *ortho*-dihydroxyphenyl

moiety, to its capacity for free radical scavenging during oxidation processes and its reducing power on Fe^{3+}.[59,60] Research suggest that the strength of antioxidant activity by these phenolic compounds correlates to the number of hydroxyl substitutions.[22] In particularly, phenolic compounds having *ortho*-dihydroxy substituents confers a high antioxidant ability, while those with a mono-hydroxyl substituent on the benzene ring, such as tyrosol, have no activity. The *ortho*-diphenols act as an antioxidant by forming intramolecular hydrogen bond linking the hydrogen of the hydroxyl group with the phenoxy radical thus, improving the stability of the radical.[19] Hence, the catechol disrupts the chain propagation by donating radical hydrogen to alkylperoxyl radicals generated amid the initiation stage of lipid oxidation (scheme 1).[61] The hydroxytyrosol quinone product formed can either undergo Michael addition reaction with glutathione to give a glutathione conjugate or it be recycled in a redox reaction involving ascorbate to give hydroxytyrosol.[122] Studies on biological activities of hydroxytyrosol have shown its' potent free radical scavenging actions against superoxide radical;[19] its' defense against DNA damage induced by peroxynitrite, produced by reaction between superoxide radical anion ($O_2^{\bullet -}$) and nitric oxide (NO); its' efficiency against oxidative stress caused by *tert*-butylhydroperoxide on human hepatoma HepG2 cells[62] or by free radicals associated with neurodegenerative or other cerebral diseases.[63,64]



Scheme 1: Free radical scavenging by hydroxytyrosol

Free radicals also trigger the oxidation of low-density lipoproteins (LDLs) which is a lipid peroxidation chain reaction. Hydroxytyrosol is known to prevent LDL oxidation occurring in the body owing to its capability for scavenging peroxyl radicals.[65,66] This olive polyphenol also decreases the oxidation of the low-density lipoproteins carrying cholesterol (LDL-C), which is a crucial step in the advancement of cardiovascular diseases including antherosclerosis,[67] and prevents cell activities associated with physiopathological processes, including thrombogenic events, with platelet inhibitory capability similar in magnitude to aspirin.[68,69] Using metal-independent oxidative systems and stable free radicals, like 1,1-diphenyl-2-picryl-hydrazyl (DPPH), the antioxidant activities of hydroxytyrosol was proven to be more efficient when compared with butylated hydroxytoluene (BHT) or vitamin E[70,66] and strongly impede copper sulfate-induced oxidation of LDL in a dose-dependent manner at various concentrations.[70,71] Manna et al., in 1997 demonstrated, using a model of oxidative stress induced in intestinal epithelial cells, the antioxidant property of hydroxytyrosol.[72] These same authors reported the protective effect of hydroxytyrosol against hydrogen peroxide-induced damage on human

erythrocytes in a later publication.[73] Deiana et al., in 1999 indicated that low concentration of hydroxytyrosol (50 μM) is highly potent in scavenging peroxynitrite (NO_3^-) and hence protective against the peroxynitrite-dependent nitration of tyrosine and peroxynitrite-induced DNA damage.[74]

Anticancer Activity

Cancer has significantly impacted the society in the United States and across the globe.[116] In 2015, the National Center for Health Statistics ranked cancer as the second leading cause of death in the United States. [116] Over the past two decades, scientists have continuously sort ways to prevent or remedy this deadly disease. Studies have shown an inverse relationship between consumption of fruits and vegetables, and development of different kinds of cancer.[75,76] A fit lifestyle including ideal nutrition, could be prevented in more than two third of human cancers.[77] Presently, the area of cancer chemoprevention is one with enormous potential in the prevention of cancer.[80] Chemoprevention involves the use of synthetic, natural or biologic chemical agents to suppress, reverse or inhibit the progression of invasive cancer either through blocking the DNA damage that initiates carcinogenesis or by restraining or reversal of the progression of premalignant cells in which those DNA damage has already occurred.[78,79] The chemoprevention by dietary polyphenols is gaining more grounds due to their capacity to interfere with multiple signaling pathways, in various types of cancer, by targeting a plethora of cellular molecules and molecular pathways.[81] Given cancer chemopreventive activities, research has shown that hydroxytyrosol prevents DNA damage caused by various genotoxic molecules thereby hindering the initiation of carcinogenesis.[82] Furthermore, hydroxytyrosol prevents the proliferation and inducing apoptosis in various tumors cell lines and in doing so help to prevent the

promotion/progression stage of carcinogenesis.[83] Much awareness has been given to hydroxytyrosol as the chief anti-cancer compound in several in vitro studies, using human carcinoma cells, which shows notable effects in many cancer cell lines, either solitary or in association.[84]

Cardiovascular Protection

Recent reports have established the benefits of olive oil phenolic compounds (like oleuropein and hydroxytyrosol) on protection against cardiovascular diseases.[97-101] The protective action of oleuropein (OL) and hydroxytyrosol (HT) against antherosclerosis was highlighted using prechemical experimental models. [101-105] These compounds have also shown inhibition to copper sulfate-induced oxidation of low-density lipoprotein (LDL).[101-105] Jemai *et al.* in their study revealed the beneficial effects of these phenolic compounds in promoting hypocholesterolemia by lowering LDL plasma levels and the overall cholesterol level. Similarly, the expression of the proteins associated with aging was found to have reduced after the treatment of cardiomyocytes from rats with hydroxytyrosol.[106]

Antidiabetic Activity

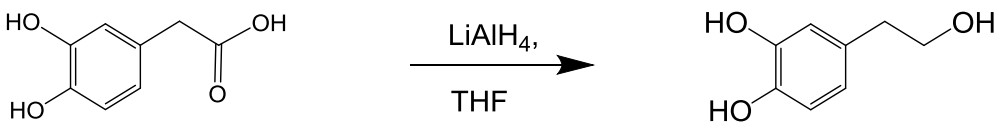
A recent report by the World Health Organization (WHO) shows that the number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014 and that 8.5 % of adults over 18 years had diabetes.[116] Diabetes is a chronic metabolic disease that eventually affects vital body organs in the long run. It occurs in cases where there is insufficient insulin

produced by the pancreas or when the body is unable to utilize the insulin produced adequately.[117] Reports suggested that oxidative stress is a possible contributor to the pathogenesis of the various issues associated with the disease.[107,108] It has also been proposed that hypoglycemia causes oxidative stress, which is conclusive from the features of diabetes which include the altering of glutathione redox state, decrease of individual naturally occurring antioxidants, lipid peroxidation and a decrease in the activity of the antioxidant enzyme.[109-111] Natural antioxidants have been highlighted as a possible means of combating oxidative damage in diabetes, as studies have also suggested that some dietary antioxidants (like vitamins and flavonoids) help to reduce the effects of oxidative stress.[112] The fruits, leaves, and oils of the olive tree have been identified to have the highest antioxidant activity. The olive tree contains well-known antioxidants which include oleuropein and hydroxytyrosol among others.[113] Previous studies have also highlighted the antihyperglycemic effects of oleuropein on diabetic rats. Jemai *et al.* (2009) demonstrated the promising effects of hydroxytyrosol and oleuropein in combating the effects of oxidative stress in diabetic rats, concluding that they have beneficial hypoglycemic effects suitable for managing diabetes.[114]

Synthesis of Hydroxytyrosol

Hydroxytyrosol is naturally available as an extract from extra virgin olive oil. However, the insufficiency of this natural source to meet demand has necessitated the need for synthetic methods of production. Various researchers have reported different methods employed in the synthesis of hydroxytyrosol. One of such methods is based on reduction of 3,4-dihydroxyphenyl acetic acid with Lithium aluminum hydride.[85,86] Although this method is more convenient than

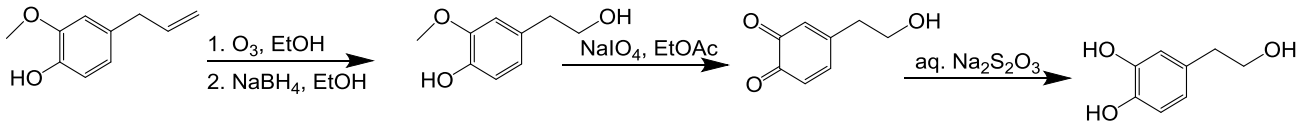
the extraction and purification of hydroxytyrosol, from wastewater generated by olive oil mills,[87] it utilizes an expensive and not readily available precursor.



3,4-dihydroxyphenyl acetic acid **Hydroxytyrosol**

Scheme 2: Lithium aluminum hydride reduction of 3,4-dihydroxyphenyl acetic acid.

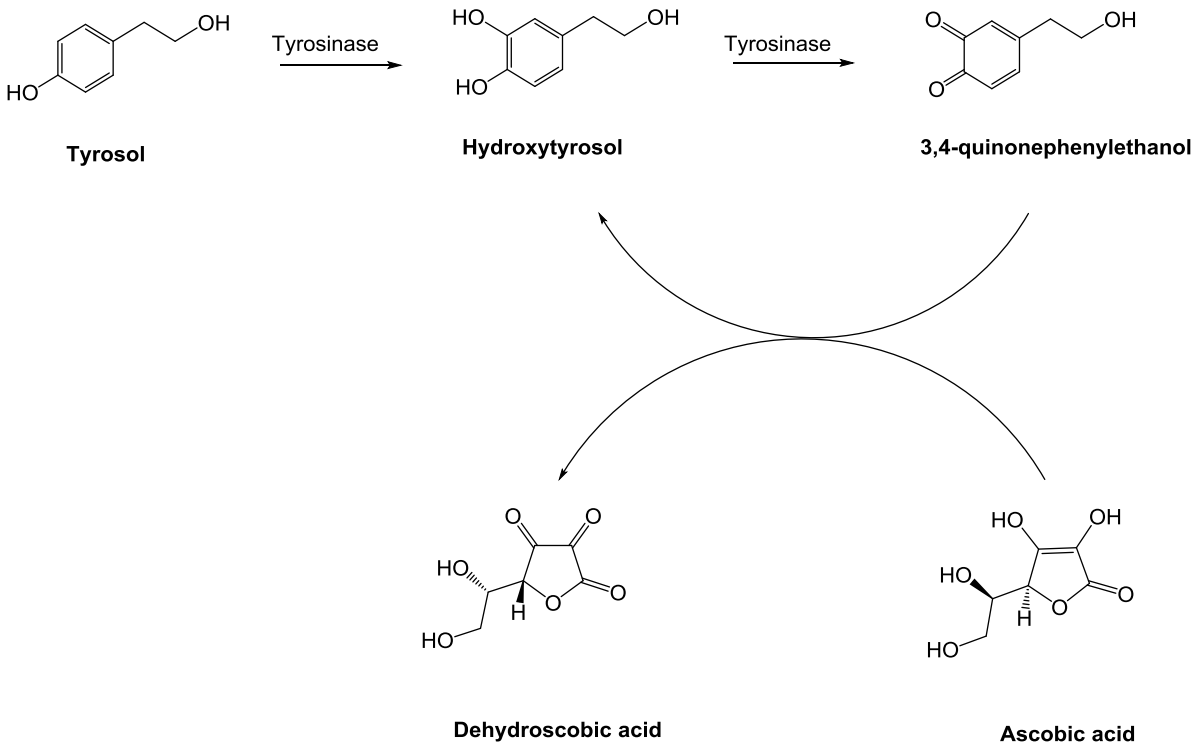
Denis et al., synthesized hydroxytyrosol using eugenol (4-allyl-2- methoxyphenol), a commercially available major constituent of clove oil [88], as a precursor. This procedure involved conversion of eugenol to ozonide intermediate which upon reduction produces homovanillyl alcohol (4,4-hydroxy-3-methoxyphenethanol).[89] This is followed by demethylation by sodium periodate ($NaIO_4$) and reduction (Scheme 3).



Eugenol **4-hydroxy-3-methoxy-phenylethanol** **Hydroxytyrosol**

Scheme 3: Synthesis of 4, 4-hydroxy-3-methoxyphenethanol from eugenol and demethylation process of homovanillyl in ethyl acetate. [32]

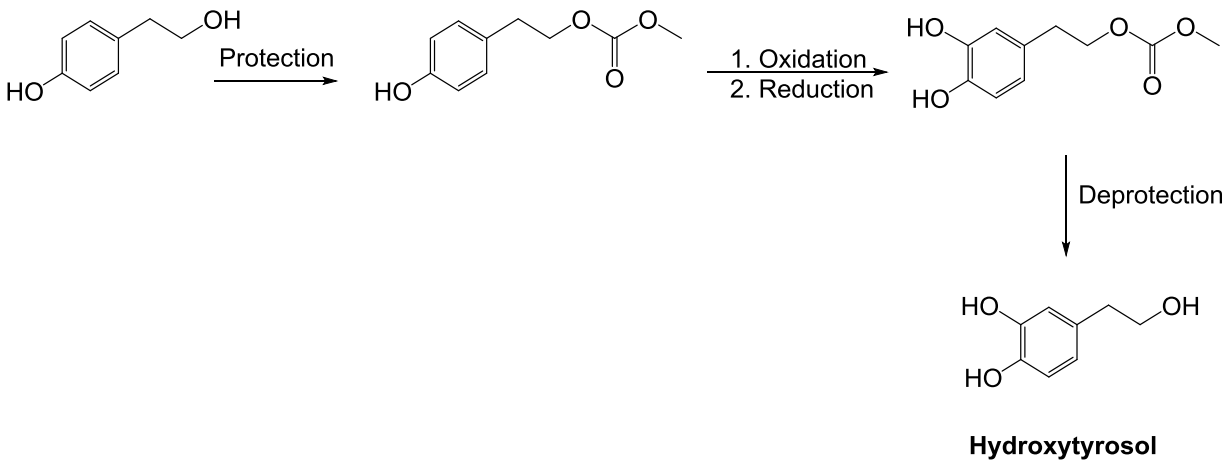
Some other researchers have used biocatalyst in the synthesis of hydroxytyrosol. One of such methods involves tyrosinase-catalyzed process, excess vitamin C, and tyrosol as a precursor, (Scheme 4).[86] The enzyme catalyzes hydroxylation and oxidation of tyrosine.



Scheme 4: Synthesis of hydroxytyrosol from tyrosol precursor in the presence of both mushroom tyrosinase and Ascorbic Acid.[86]

Roberta et al. synthesized hydroxytyrosol from tyrosol, but only after protection of the alcohol hydroxyl group first (scheme 5) by dimethylcarbonate. Oxidation by 2-iodoxybenzoic

acid or Dess-Martin periodinase, followed by reduction with sodium dithionite, then deprotection afforded hydroxyltyrosol.[90]



Scheme 5: Synthesis of hydroxytyrosol from tyrosol.[90]

Research Objectives

Hydroxytyrosol is currently being produced by extraction from natural sources or by synthetic methods. Different procedures have been optimized to isolate hydroxytyrosol from olive oil waste waters.[85] Such methods resulted in isolation of hydroxytyrosol in low concentration, and often in combination with other polyphenols. Several methods for the synthesis of hydroxytyrosol have been described in literature, but they include many steps and use expensive starting materials and some were of low yields.[86,91,92] Hydroxytyrosol is being sold by chemical companies although at a very high price.

Considering the industrial utilization of hydroxytyrosol, together with increasing studies of its biological properties, it is imperative to have more convenient and efficient methods to prepare

this polyphenol. The main objective of this research is to develop a novel method for the synthesis of hydroxytyrosol from relatively inexpensive precursors such as catechol, to provide more economic synthetic alternative routes.

CHAPTER 2

EXPERIMENTAL

Materials and General Methods

The precursor (catechol), as well as reagents and solvents listed for all reactions, were obtained from commercial sources. All chemical reagents and solvent listed were used without further purification unless otherwise stated. Some reagents used in this research include:

Catechol, acetyl chloride, lead tetraacetate, boron trifluoride etherate, 1,2 dibromoethane, bromoethanol, trimethylsilyl chloride, triethylamine, tert-butyldimethylsilyl chloride, Imidazole, lithium aluminium hydride, ethyl acetate, dichloromethane, tetrahydrofuran, silica gel,benzene, aluminium chloride, chloroacetyl chloride,petroleum ether, hexane, liquid bromine, and sodium hydroxide.

The synthesized compounds were characterized by NMR spectroscopy. The NMR spectra were recorded on a JEOL-NMR Eclipse-400 MHz spectrophotometer. The chemical shifts of different peaks are quoted in parts per million (ppm) using the high-frequency position conversion, and the coupling constants value (J) are reported in Hz. The splitting patterns of resonance were also described as follows: singlet (s), doublet (d), doublet of doublet (dd), doublet of doublet of doublet (ddd), triplet (t), doublet of triplets (dt), td = triplet of doublets, quartet (q), and multiplet (m). The melting points of synthesized compounds were measured without calibration of the Cambridge Melt-Temp device. Purification of compounds by chromatographic and recrystallization techniques so as to obtain the pure form of synthesized compounds.

Experimental Procedures

Synthesis of 3,4-dihydroxyacetophenone (**3**)

A Flask containing a suspension of aluminum chloride (0.98 g, 7.4 mmol) in 4 mL 1,2-dibromoethane at 10^0C was stirred for 30 minutes. Powdered catechol (0.32 g, 2.9 mmol) was then added to the reaction mixture in three portions over 4 minutes.The reaction was further stirred at 10^0C for 30 minutes after which acetyl chloride (0.26 mL, 3.2 mmol) in 0.5 mL dibromoethane was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for an additional 20 hrs. After the reaction was complete, the mixture was set on ice and cooled to about 6 ^{0}C before quenching with 1M HCl (10 mL). The mixture was stirred for two hrs and thereafter diluted with CH_2Cl_2 (20 mL), and the organic layer was separated. The aqueous layer was extracted with EtOAc (4x20 mL), and the combined organic layer was washed with saturated sodium chloride (30 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo gave **7** as a reddish solid (0.43 g, 97%).

1H-NMR (400 MHz, CHLOROFORM-D) δ 7.42-7.28 (m, 2H), 6.80 (dd, J = 7.7, 0.7 Hz, 1H), 2.46 (s, 3H).

Synthesis of 3,4-diacetoxyacetophenone (**4**)

To a round-bottom flask containing 6 g (45 mmol) of aluminum chloride in 20 mL dichloromethane, set in an ice bath, was added dropwise6.49 mL (91 mmol) of acetyl chloride in 20 mL dichloromethane. Catechol, 2.00 g (18.2 mmol), was then added to the mixture within a period of five minutes. The mixture was then allowed to warm to room temperature and under

nitrogen atmosphere for seventy-two hours. The resulting solution was poured with mixing into 40 g ice containing 20 mL concentrated hydrochloric acid. The aqueous solution was repeatedly extracted with dichloromethane (3x60 mL). The combined organic layer was washed with 20 mL saturated sodium bicarbonate. After evaporation of the solvent, the product was purified by recrystallization from 1:3 dichloromethane/hexane (v/v) to give 3.39 g (79 %) of the desired product. ^{1}H-NMR ($CDCl_3$): δ 7.89-7.83 (dd,1H), 7.83-7.76 (d,1H), 7.34-7.28 (d,1H), 2.61-2.56 (s,3H), 2.34-2.30 (d,6H). ^{13}C-NMR: δ 196.08 (C=O), 168.20 (ROC=O), 168.84 (ROC=O), 146.10 (ArC), 142.31 (ArC), 135.68 (ArC), 126.99 (ArC), 123.81 (ArC), 108.96 (ArC), 26.69 (CH_3), 20.80-20.70 (CH_3)

Synthesis of Methyl (3,4-diacetoxyphenyl)acetate (**5**)

A mixture of 0.10 g (0.42 mmol) of 3,4-diacetoxyacetophenone, 0.088g (2.75 mmol) methanol, 0.24g (1.67 mmol) boron trifluoride etherate ($BF_3.Et_2O$) was added in one portion to a suspension of 0.198g (0.45 mmol) of lead tetraacetate in 1.11 mL of benzene. The reaction mixture was stirred at room temperature for 5h, after which the reaction was quenched by adding 5mL cold water. The mixture was then extracted 3 times with ethyl acetate (3x10 mL). The combined organic layer was washed with saturated $NaHCO_3$ until the pH is around 7, followed by a second wash with an equal volume of saturated NaCl, and the organic layer was dried over anhydrous sodium sulfate. After evaporation of solvent, 0.102 g of a dark brown viscous oily crude product was obtained. Further purified by column chromatography (dichloromethane/ethyl acetate 3: 1, v/v) gave a yellow viscous oily liquid (86 % yield). ^{1}H-NMR ($CDCl_3$): δ 7.18-7.07 (m, 3H), 3.70-3.63 (s,3H), 3.63-3.55 (s,2H), 2.28-2.22 (s, 6H). ^{13}C-NMR: δ 171.35 (ROC=O),

168.39 (ROC=O), 168.32 (ROC=O), 142.01 (ArC), 141.25 (ArC), 132.71 (ArC), 127.61 (ArC), 124.41 (ArC), 123.50 (ArC), 52.29 (CH_3), 40.48 (CH_2), 20.74 (CH_3).

Synthesis of Hydroxytyrosol (**1**)

$LiAlH_4$ (0.083 g, 2.18 mmol) was suspended in 10 mL of dry tetrahydrofuran at room temperature. A solution of crude methyl (3,4-diacetoxyphenyl)acetate (0.096 g, 0.36 mmol) in 1 mL of dry tetrahydrofuran was added dropwise. Once addition was complete, the reaction mixture was refluxed for 3 hours, after which the mixture was cooled and treated with 10 mL of ethyl acetate containing 1.5 mL water, the mixture was concentrated under reduced pressure. The white residue was suspended in 10 mL of water and acidified to pH 2-3 with 6 M HCl. The mixture was extracted six times with ethyl acetate(6x 7 mL). The combined organic phase was washed with saturated sodium bicarbonate to pH 7.0–7.5. The combined aqueous phase was then extracted three times with ethyl acetate (3x 7 mL). The combined organic layer was dried over sodium sulfate and the solvent was evaporated under reduced pressure to give 0.065 g of the crude product. The crude product was purified on a silica gel column (dichloromethane/ethyl acetate 3: 1, v/v; 1: 3, v/v) to yield 0.049g (87 %) of the hydroxytyrosol as a light yellow oil. ^{1}H-NMR ($CDCl_3$): δ 6.76-6.67 (d, 1H), 6.67-6.62 (s, 1H), 6.56-6.47 (d, 1H), 3.82-3.65 (t, 2H), 2.69-2.55 (t, 2H). ^{13}C-NMR: δ 130.64 (ArC), 120.61 (ArC), 115.89 (ArC), 115.18 (ArC), 109.04 (ArC), 63.57 (CH_2), 38.35 (CH_2).

Synthesis of (2-bromoethoxy)trimethylsilane (**9**)

To a solution of 1 mL of bromoethanol and 4 mL of triethylamine (2 eq) in 3 mL THF stirred at 0 ^{0}C under a nitrogen atmosphere, was added dropwise 2.6 mL (1.5 eq) trimethylsilylchloride. The mixture was allowed to warm up to room temperature and stirred for eight hours. 3 mL of saturated ammonium chloride was then added to the reaction mixture and the resulting precipitate was filtered. The filtrate was then distilled and the product (a colorless liquid) collected in the range 125 ^{0}C – 135 ^{0}C with 84 % yield. ^{1}H-NMR ($CDCl_3$): δ 4.03-3.69 (t, 2H), 3.51-3.27 (t, 2H), 0.25--0.06 (s, 9H).

Synthesis of 1,2-bis-(trimethylsilyloxy)benzene (**10**)

In a round-bottom flask, 1 g of catechol was dissolved in 6 mL of dry THF containing 5 mL of triethylamine (4 eq), the solution was cooled in ice and under a nitrogen atmosphere. Trimethylsilyl chloride (3.45 mL, 3 eq) was added dropwise. The mixture was allowed to warm to room temperature and stirred for eight hours. 5 mL of saturated ammonium chloride was then added and the precipitate was filtered. The filtrate was then concentrated, and the product was purified using flash column chromatography (silica gel, solvent) to yield 2.04 g of a colorless liquid (79 %). ^{1}H-NMR ($CDCl_3$): δ 6.99-6.78 (m, 4H), 0.41-0.22 (m, 18H).

Synthesis of 1,2-bis-(tert-butyldimethylsilanyloxy)benzene (**11**)

A solution of catechol (0.1 g, 0.91 mmol) and imidazole (0.14 g, 1.99 mmol) 1 mL dichloromethane was stirred at 0^0C under nitrogen atmosphere. tert-Butyldimethylsilyl chloride (0.30 g, 1.99 mmol), dissolved in 1 mL of dichloromethane, was added dropwise. The reaction mixture was allowed to warm up to room temperature, then stirred for four hours. The reaction was quenched with 2 mL water and extracted with dichloromethane three times (9 mL). The organic layer was washed twice with water and once with brine and dried over anhydrous sodium sulfate. The organic layer was concentrated and the residue was purified using flash column chromatography (silica gel, Hexane) to yield a colorless oil (0.239g, 77 %). ^{1}H-NMR ($CDCl_3$): δ 6.82 (dt, J = 9.9, 2.7 Hz, 4H), 0.99 (s, 18H), 0.25-0.13 (12H).^{13}C-NMR: δ 147.01 (ArC), 121.50 (ArC), 121.28 (ArC), 25.93 (CH_3), 18.55 (C).

Synthesis of 2-bromo-1-(tert-butyldimethylsilanyloxy)ethane (**12**)

To a round bottom flask containing 2.00 g (16 mmol) of bromoethanol and 1.20 g imidazole (18 mmol), dissolved in 10 mL dichloromethane and stirred at 0^0C under nitrogen atmosphere, was added dropwise a solution of 2.65 g (18 mmol) tert-butyldimethylsilyl chloride in 10 mL of dichloromethane. The mixture was allowed to warm up to room temperature and stirred for four more hours. The reaction was quenched with 20 mL water and then extracted with dichloromethane (40 mL). The organic layer was washed twice with water (10 mL) and once with brine (10 mL) and dried over anhydrous sodium sulfate then concentrated. The residue was purified using flash column chromatography (silica gel, Hexane/dichloromethane/ 2: 1, v/v) to yield a colorless oil (3.29g, 81%). ^{1}H-NMR ($CDCl_3$): δ 3.87 (t, J = 6.6 Hz, 2H), 3.38 (t, J =

6.6 Hz, 2H), 0.92-0.86 (9H), 0.08 (d, J = 3.3 Hz, 6H). ^{13}C-NMR: δ 63.59 (CH_2), 33.32 (CH_2), 25.90 (CH_3), 18.40 (C).

Synthesis of 1,2-methylenedioxybenzene (**15**)

To a refluxing mixture of DMSO (1 ml), dichloromethane (0.2 mL), and tetrabutylammonium bromide (0.016 g, 0.05 mmol) was added simultaneously a solution of catechol (0.1 g, 0.91 mmol) in DMSO (0.6 mL) and 50 % sodium hydroxide (0.07 g, 1.82 mmol) over a period of two hours. Afterward, the reaction mixture was further refluxed for an additional hour.[119] The reaction was quenched slowly with 100 ml water and the product was separated from the crude by distillation under reduced pressure.[119] The distillates containing **15** and water was separated by a separating funnel and dried over anhydrous sodium sulfate obtained a 61.4 % yield of the colorless liquid.

Synthesis of 4-bromocatechol (**18**)

To a solution of 0.10 g catechol in 1 mL dichloromethane stirring under nitrogen atmosphere at 0 ^{0}C was added dropwise (10 drops per minute) a solution of 0.145 g bromine in 1 mL dichloromethane. The reaction mixture was allowed to warm up to room temperature, then stirred for four more hours. The reaction was quenched with 0.5 mL of distilled water and then extracted three times with 2 mL dichloromethane; the combined organic layer was washed with saturated sodium chloride. The solvent was evaporated in vacuo to give a dark brown crude product (1.92 g). Recrystallization of the product from dichloromethane/petroleum ether (3:2,

v/v) yielded a light gray solid (1.4 g, 82 %). ^{1}H-NMR ($CDCl_3$): δ 7.02 (d, J = 2.2 Hz, 1H), 6.92 (dd, J = 8.4, 2.2 Hz, 1H), 6.74 (d, J = 8.4 Hz, 1H). ^{13}C-NMR: δ 142.79 (ArC), 124.05 (ArC), 118.73 (ArC), 116.74 (ArC).

Synthesis of 4-bromo-1,2-bis-(tert-butyldimethylsilanyloxy)benzene (**19**)

To a solution of 0.10 g (0.5 mmol) of 4-bromocatechol and 0.079 g (1.16 mmol) of imidazole in 1 mL dichloromethane in a round bottom flask, with stirring at 0^0C under nitrogen atmosphere, was added dropwise a solution of 0.173 g (1.16 mmol) of tert-butyldimethylsilyl chloride in 1 mL dichloromethane. The mixture was allowed to warm up to room temperature and stirred for four more hours. The reaction was quenched with 2 mL water and then extracted with dichloromethane (8 mL). The organic layer was washed twice with water and once with brine and dried over anhydrous sodium sulfate. Evaporation of the solvent and purification by flash column chromatography yielded a colorless oil (0.173 g, 78 %). ^{1}H-NMR ($CDCl_3$): δ 6.93 (td, J = 8.5, 2.4 Hz, 2H), 6.69 (d, J = 8.4 Hz, 1H), 1.06-0.92 (m, 18H), 0.29-0.11 (12H). ^{13}C-NMR: δ 147.95 (ArC), 146.47 (ArC), 125.39 (ArC), 124.33 (ArC), 122.27 (ArC), 112.81 (ArC), 25.97 (CH_3), 18.54 (C).

General Procedure for the Esterification of Alcohols Using acetyl chloride

To a flask containing a solution of the corresponding alcohol and triethylamine (1.5 eq. per OH group) in THF, stirred at 0 ^{0}C under a nitrogen atmosphere, was added dropwise acetyl chloride (1.5 eq. per OH). After addition was complete, the mixture was allowed to warm up to

room temperature and stirred for 5 hours. Saturated ammonium chloride was then added and the resulting precipitate was filtered. The filtrate was then extracted three times with dichloromethane. Evaporation of the solvent yielded the corresponding ester.

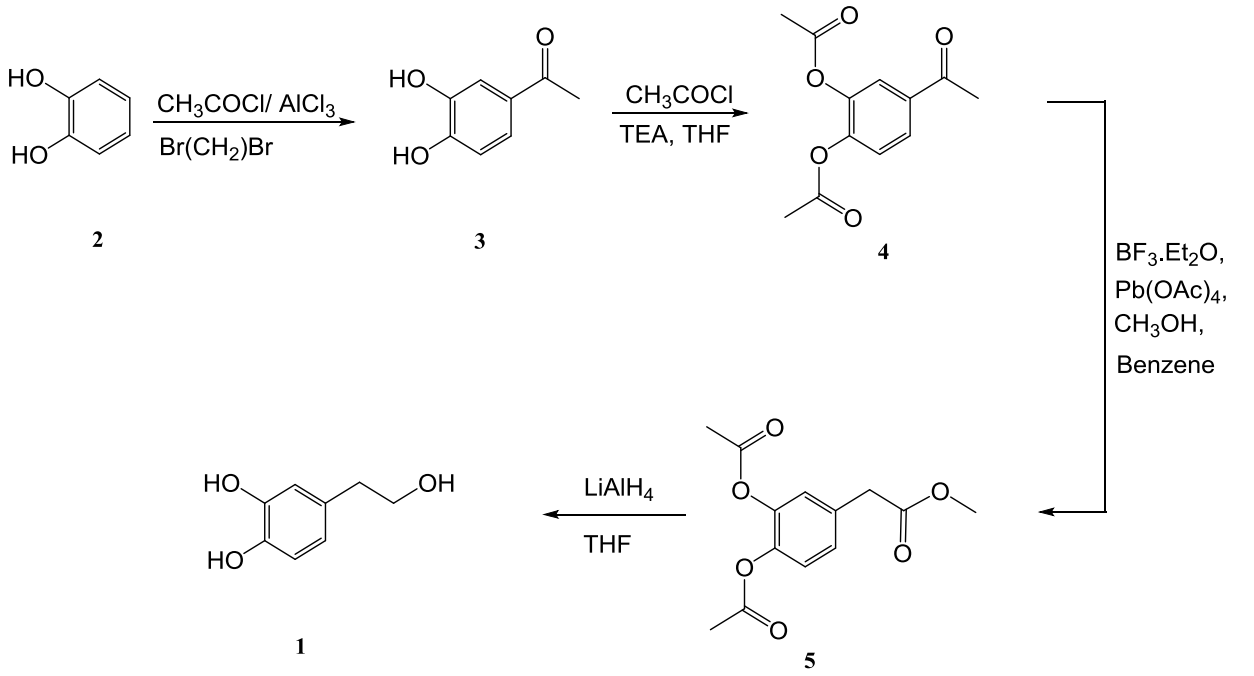
CHAPTER 3

RESULTS AND DISCUSSIONS

Several methods for the synthesis of hydroxytyrosol (**1**) have been reported in literature. However, only a few of these methods are industrially applicable. To begin, several synthetic methods were proposed for the synthesis of hydroxytyrosol. The architecture of the various methods was based on some factors such as the cost and availability of the starting materials, number of reaction steps in the method as well as the overall cost of the entire process.

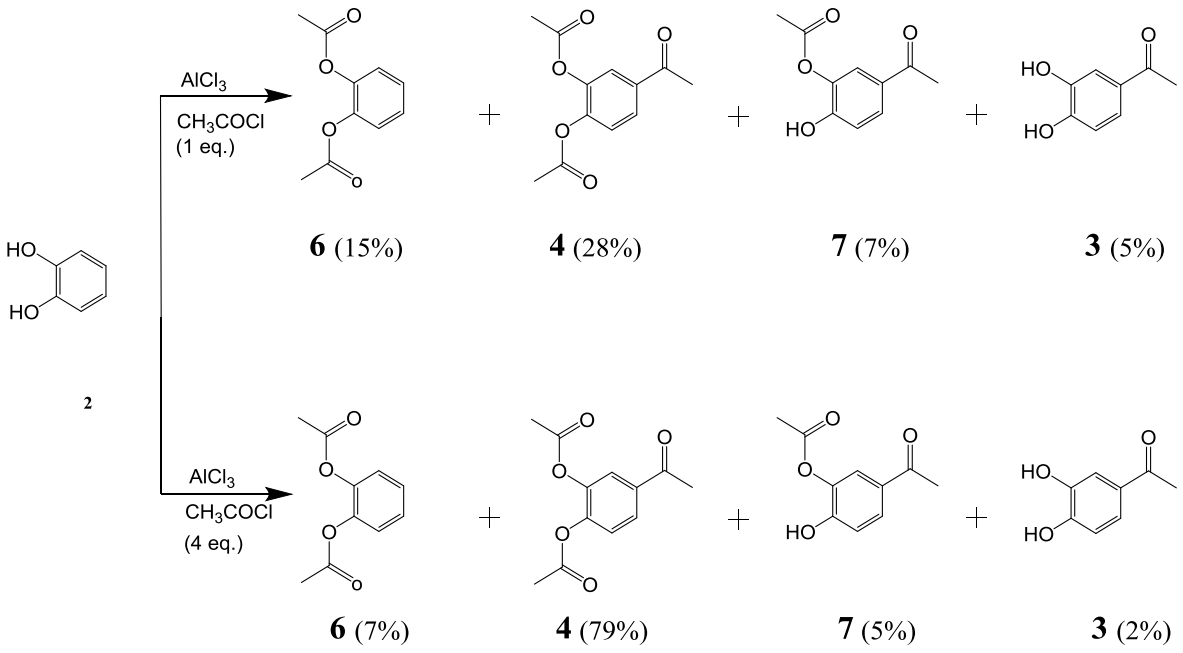
Friedel-Crafts Acylation Approach

The Friedel-Crafts acylation provides a means by which acyl substituents can be added to a benzene ring via an electrophilic aromatic substitution. These substituents can then be further reacted to give desired products. Using this approach, hydroxytyrosol was successfully synthesized from commercially available catechol (**2**) through a four-step process depicted in scheme 6.



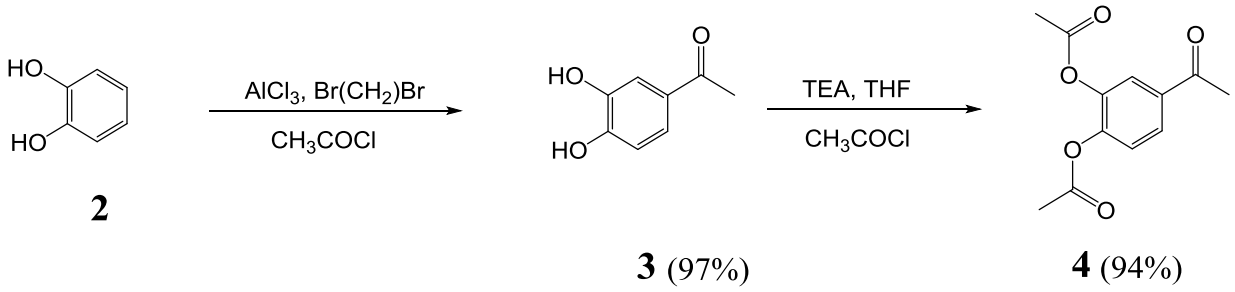
Scheme 6: Synthesis of hydroxytyrosol

The first step involves acylation of **2** using acetyl chloride as the acylating agent in the presence of a Lewis acid catalyst (aluminum chloride). Initial attempt to synthesize 3,4-Diacetoxyacetophenone (**4)** involved using 1:1 molar ratio (catechol: acetyl chloride) gave only low yield of **4** (R_f = 0.79, CH_2Cl_2/EtOAc, 3:1 v/v) in addition to **6** (R_f = 0.85), **7** (R_f = 0.47), and **3** (R_f = 0.39). The low yield may be due to insufficient the acylating agent. Using excess amount of the acylating agent improved the percent yield of the reaction**.** The crude brown product was purified by recrystallization using CH_2Cl_2/hexane (1:4, v/v) to give **4** as brownish white crystals (79% yield). The presence of **6** among the products formed may be an indication that the rate of addition of the catechol during the reaction may have been high causing an excess of the catechol in the reaction mixture which got esterified before a change of acylation.



Scheme 7: Acylation of catechol.

To improve the yield of **4** and reduce the diversity of products formed, a different approach based on a procedure described by Payne et al.[120] was used in the synthesis of **7.** In this procedure, a mixture of aluminum chloride and 1,2-dibromoethane was stirred for 30 minutes after which catechol was added and further stirred for 30 minutes before the introduction of the acylating agent (acetyl chloride).

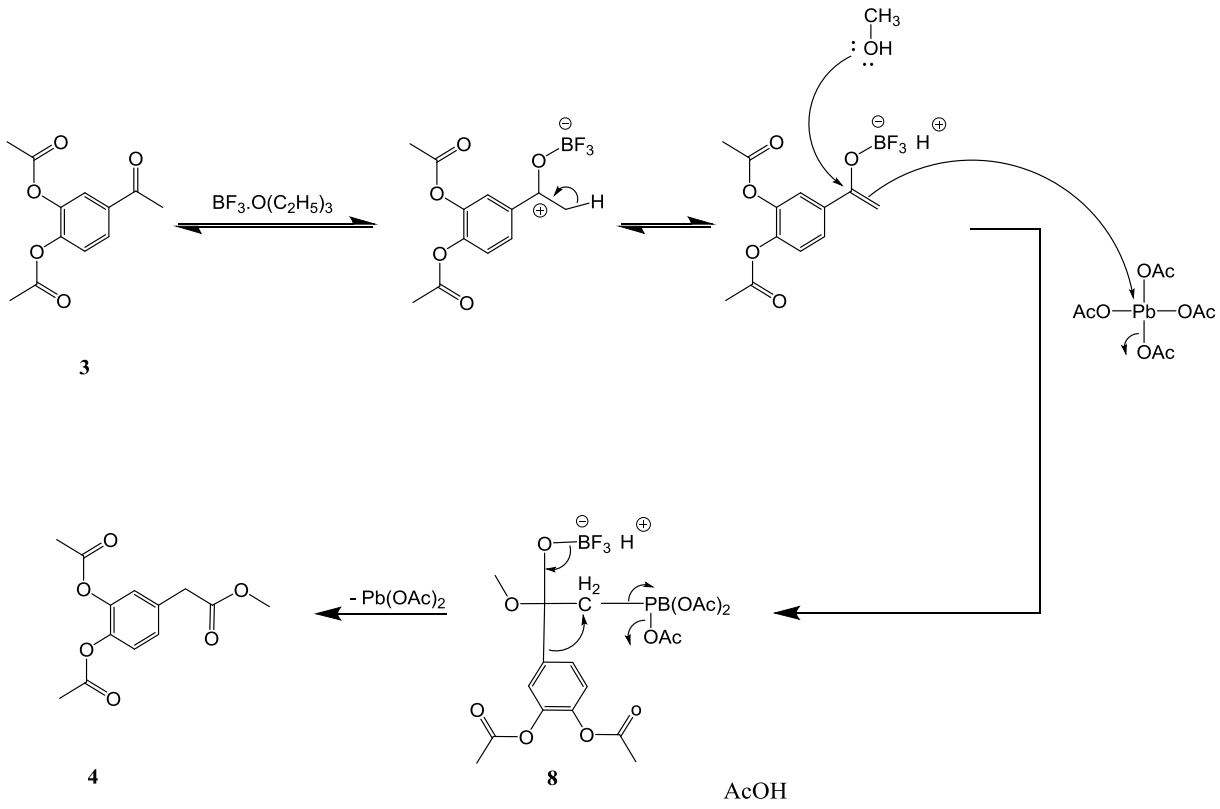


2 **3** (97%) **4** (94%)

Scheme 8: Synthesis of 3,4-diacetoxyacetophenone.

This initial reaction may have offered partial protection from the 1,2-dibromoethane, to the hydroxy groups on the catechol, thereby reducing the chances of esterification of the groups which may lower the nucleophilicity of the ring towards the acylium ion. Using this procedure **3** was synthesized in high yield (97 %) which was further esterified to give **4** with a yield of 94%.

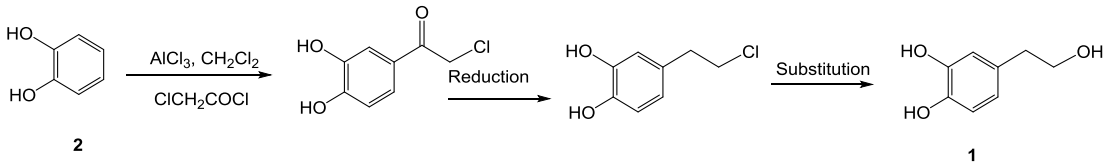
The second step involves converting **4** into **5** (Scheme 9)**,** using a procedure reported in literature by Myrboh et al.[118] The proposed reaction mechanism involves enolization of the ketone, assisted by the boron trifluoride etherate, accompanied by oxyplumbation leading to the formation of intermediate **8,** which rearranges to **5**.[118] The dark brown oily crude product was used for the next step without further purification.



Scheme 9: Mechanism for synthesis of methyl (3,4-diacetoxyphenyl)acetate

In the final step, crude **5** was reduced by lithium aluminum hydride to afford 0.049 g (87 %) of **1** after purification by column chromatography as light yellow oil. The physical properties and NMR analysis of **1** were consistent with those reported in literature.

A different synthetic path earlier proposed, in this research for the synthesis of **1,** uses chloroacetyl chloride as the acylating agent.

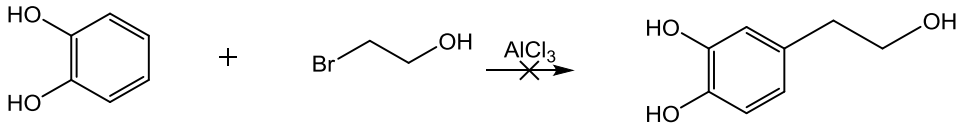


Scheme 10: Proposed route for synthesis of hydroxytyrosol using chloroacetyl chloride as acylating agent.

The first step of this method involves the acylation of **2** using chloroacetyl chloride, this is then followed by reduction of the ketone and subsequent substitution reaction of the halide to give **1**. This method was challenged by a low yield of the desired acylated product in the first step. A reason for the low yield may be due to the formation of a complex during the reaction and as such this method is still under investigation.

Direct Friedel-Crafts Alkylation Approach

Several authors have successfully carried out alkylation on phenolic compounds. In this work, attempts were made to alkylate catechol directly with 2-bromoethanol.



Scheme 11: Friedel-Crafts alkylation of catechol using bromoethanol

The process started by attempting to carry out a Friedel-Crafts alkylation reaction on catechol using bromoethanol as both the alkylating agent and as the solvent in the presence of a Lewis acid (aluminum chloride) catalyst. The presence of reactive hydroxyl groups on both the catechol and bromoethanol was anticipated to pose problems during the reaction, as this group may react with the aluminum chloride to form an aluminum alkoxide. On the other hand, this seemly disadvantageous condition may, in fact, help to mask the hydroxyl groups and allow the reaction proceed to completion upon using excess $AlCl_3$. The reaction mixture almost immediately formed a dark brown precipitate with evolution of white fume (believed to be HCl),

the starting material was recovered after work up. At this point, it was unclear whether or not the reaction would have continued in the presence of a solvent that can dissolve the resulting precipitate. The reaction was repeated in various solvents, such as dichloromethane, tetrahydrofuran, acetonitrile, dioxane, petroleum ether and toluene, but did not yield any measurable amounts of the expected product.

Owing to these unsuccessful attempts, the need to protect the hydroxyl groups seemed more apparent. Several authors have reported the use of different protection groups, such as ethers[93], methylene acetal[94], and esters[95], in masking alcohol groups.

Protection of OH Groups

Several groups for the protection of alcohol compounds are reported in literature.[121] However, there are certain factors that were considered during the selection of protecting groups used in this research. Among these factors, efficiency of protection and deprotection steps and the commercial availability of starting materials. In view of these factors, **9** – **14** were synthesized using various protecting groups.

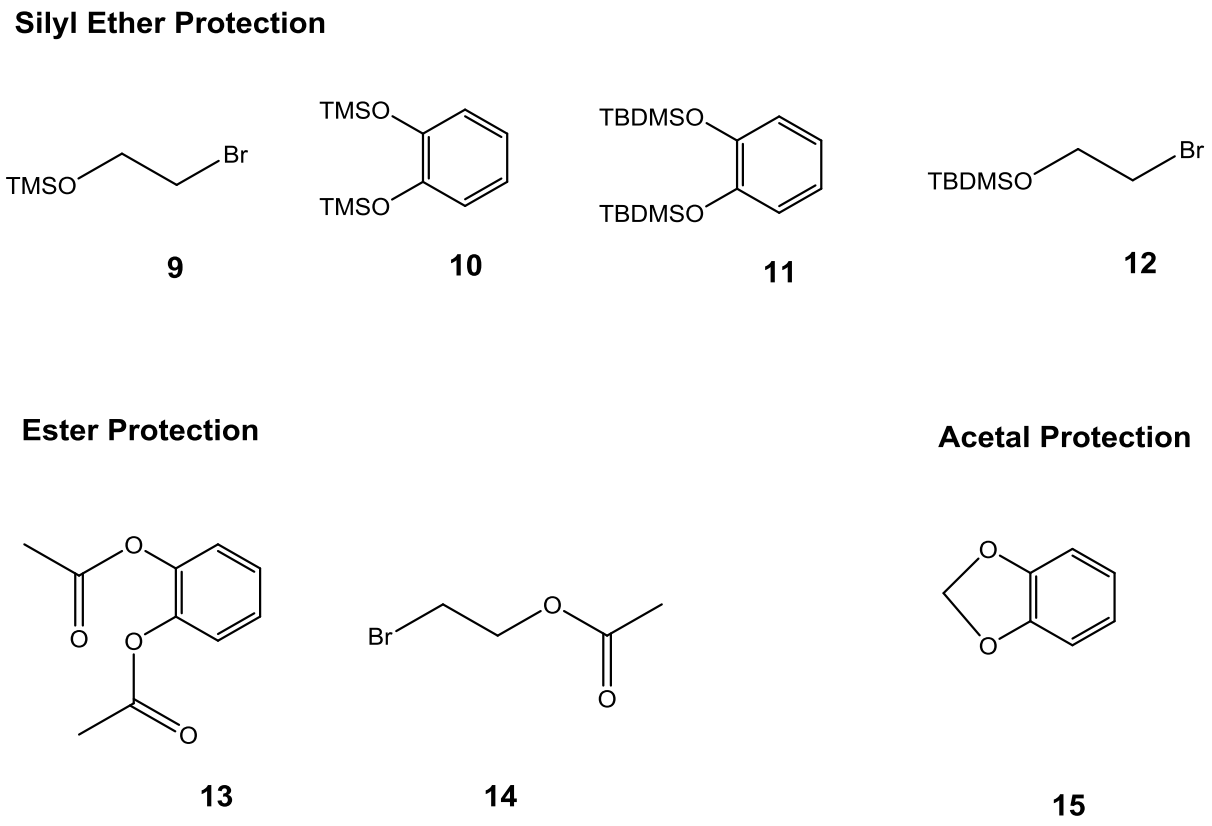


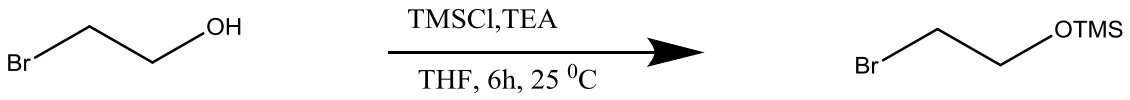
Figure 1: Derivatives of catechol and bromoethanol with different protection groups.

There is a broad range of ether protection documented in the literature for obtaining derivatives of alcohol compounds. However, one of the most common is the use of organosilicon compound. One of the main reasons for selecting this type of protection was because this group can be easily removed by tetrabutylammonium fluoride.

Synthesis of (2-bromoethoxy)trimethylsilane (**9**)

The initial attempt to protect the alcohol group of the 2-bromoethanol involved a reaction containing a mixture of pyridine and trimethylsilyl chloride in dichloromethane. [93] The reaction yielded only 11 % of colorless oil, after18 hours.

The silyl ether derivative of bromoethanol was synthesized in higher yield by using a modified version of a procedure reported by Corey and Snider during the total synthesis of (+-)-fumagilli[96] upon treatment of the alcohol with trimethylsilyl chloride/triethylamine in tetrahydrofuran. **9,** a colorless oil was obtained in 84 % yield, after purification.



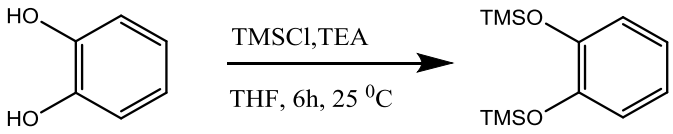
Bromoethanol **9**

Scheme 12: Synthesis of (2-bromoethoxy)trimethylsilane

The initial TLC analysis of this crude product with dichloromethane showed two spots, none of them corresponded to bromoethanol starting material, which indicates that the reaction was complete. After washing of the reaction mixture with saturated ammonium chloride, the product was further purified by distillation to yield 1.97 g (84 %) of colorless oil. ^{1}H NMR analysis of the product shows two triplets and a singlet with integration matching those predicted for the product.

Synthesis of 1,2-bis-(trimethylsilyloxy)benzene (**10**)

The procedure for the synthesis of **3** was similar to the modified procedure used in the synthesis of (2-bromoethoxy) trimethylsilane. The reaction which was carried out under an inert atmosphere and afforded 79 % yield of colorless oil. The low yield may be due to incomplete reaction; TLC analysis showed some unreacted catechol starting material. After washing the crude product with saturated ammonium chloride, and purification by column chromatography, 2.04 g (79 %) of colorless oil was obtained. Proton NMR analysis of the product showed two singlets, and the integration matched those predicted for **10**.



2 **10**

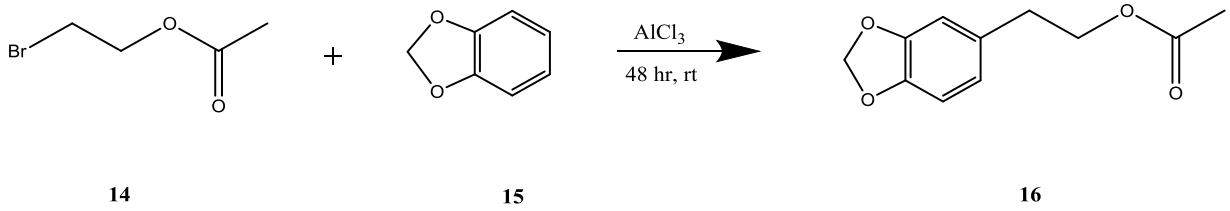
Scheme 13: Synthesis of 1,2-bis-(trimethylsilyloxy)benzene

Friedel-Crafts Alkylation

After protection of the alcohol groups of both bromoethanol and catechol, attempts were made to conduct the Friedel-Craft's alkylation. The reactions showed no significant difference in the yield when the reaction was carried out in the presence or absence of the solvent (dichloromethane).

The methylene acetal derivative **15** was alkylated with the ester derivative of 2-bromoethanol (**14)** in the presence of aluminum chloride. TLC analysis of the crude product showed three spots, both of the starting materials as well as the expected product **16** (3,4-

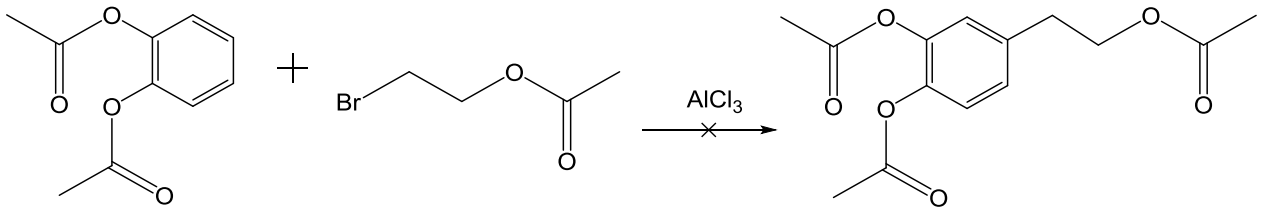
Methylendioxy-phenaethyl -acetate). Although TLC analysis after column chromatographic separation of product **16** showed one spot, the ^{1}H NMR spectrum (Appendix K) however, showed the presence of impurities. This may be an indication that the impurities are invisible under UV light.



14 **15** **16**

Scheme 14: Synthesis of 3,4-Methylendioxy-phenethyl acetate

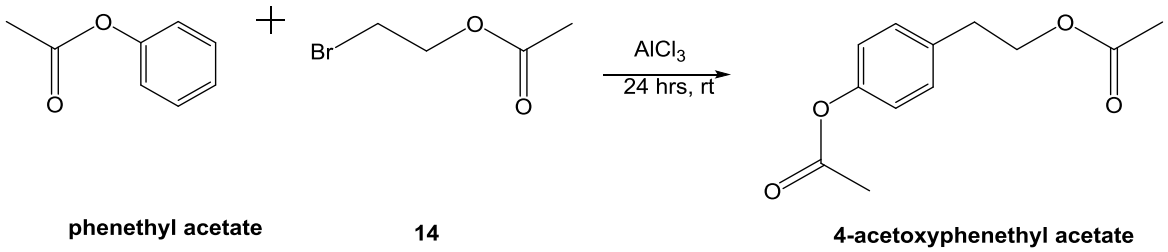
The unsuccessful attempt to carry out the Friedel-Craft's alkylation of **13** with 2-bromoethyl acetate **14** may be due to the presence of the two acetoxy groups on the aromatic compound. This may have caused the delocalization of the lone pair of electron on the oxygen connected to the aromatic ring which may in turn lower the nucleophilicity of the aromatic ring



13 **14** **3,4-diacetoxy-phenethyl acetate**

Scheme 15: Synthesis of 3,4-diacetoxy-phenethyl acetate.

towards the carbocation formed from **14.** This was evident as a similar alkylation reaction involving phenyl acetate and **14** yielded only about 9 % of the alkylated product (4-acetoxyphenethyl acetate).



phenethyl acetate **14** **4-acetoxyphenethyl acetate**

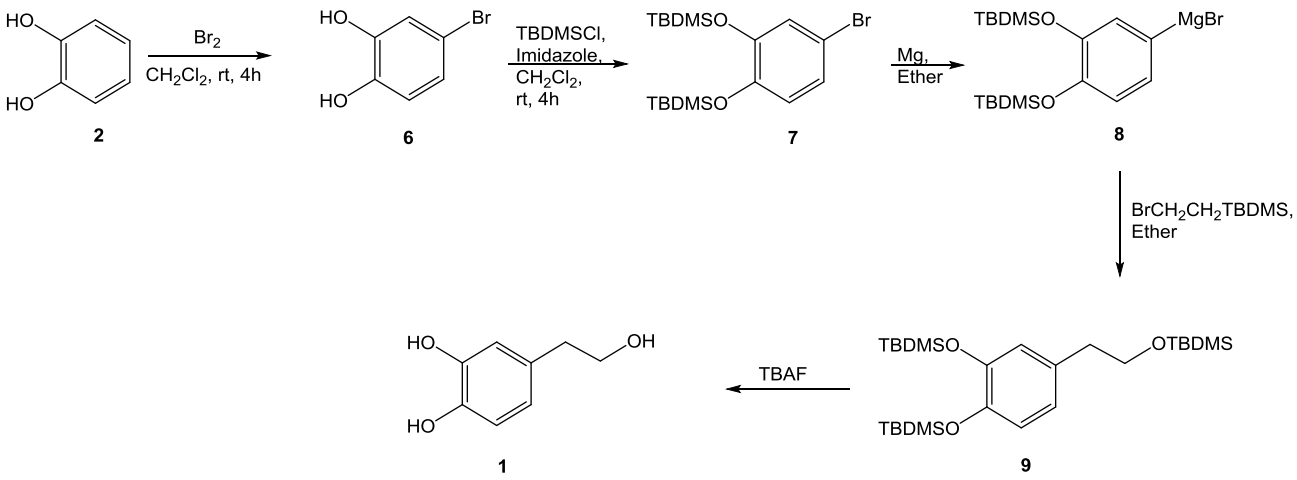
Scheme 16: Synthesis of 4-acetoxyphenethyl acetate.

The reaction between **9** and **10** was performed under similar alkylation conditions. The reaction mixture, which turned orange after 3 hours, was allowed to stir at room temperature for 8 hours. TLC showed four spots, the first two spots were comparable to those of the starting materials, an indication that the reaction was not complete. The slower spots were interestingly comparable to those of the unprotected starting materials which were further confirmed by NMR. The formation of unprotected starting materials suggests that deprotection may have occurred. The trimethylsilyl (TMS) protecting group is known to be somewhat unstable under acidic reaction conditions, this may have resulted in deprotection.

Grignard's Reaction

Grignard reaction provides a good way to alkylate aromatic compounds. Aryl magnesium halides react with alkyl halides to give alkyl aromatic products. In a similar fashion, a Grignard

reagent of the protected bromocatechol can react with protected 2-bromoethanol to give a derivative of hydroxytyrosol which can be converted into hydroxytyrosol by deprotection.



Scheme 17: Proposed Grignard's reaction route for synthesis of hydroxytyrosol.

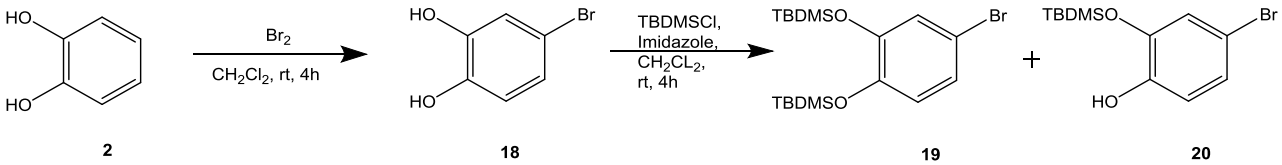
In an earlier approach, attempts were made to first protect catechol with TMS before bromination. These attempts were unsuccessful and resulted in deprotection. Alternatively, bromination of unprotected catechol, followed by protection with tert-butyldimethylsilyl chloride (TBDMSCl) proved to be more successful.

Synthesis of 4-bromo-1,2-bis-(tertbutyldimethyl silanyloxy)benzene **(19)**

Monobrominated catechol upon reaction with bromine in dichloromethane gave 82 % yield of light gray solid after recrystallization from dichloromethane/petroleum ether (3:2, v/v). TLC analysis of the product showed one spot with a R_f value of 0.52 (dichloromethane/petroleum ether 3:1, v/v). ^{1}H NMR showed peaks at δ 7.02 (d, J = 2.2 Hz, 1H),

δ 6.92 (dd, J = 8.4, 2.2 Hz, 1H), and δ 6.74 (d, J = 8.4 Hz, 1H). Integration of the individual peaks gave an approximate ratio of 1: 1: 1 suggesting that the compound had only three aromatic protons as predicted.

Treatment of the bromocatechol (**18**) with TBDMSCl in dichloromethane in the presence of triethylamine afforded **19** as a colorless oil with 78 % yield. TLC analysis of the product showed two spots, none of which correspond to 4-bromocatechol suggesting the reaction was complete;



Scheme 18: Synthesis of 4-Bromo-1,2-bis-(tert-butyldimethylsilanyloxy)benzene.

the spots were separated by column chromatography. Proton NMR analysis of the slower spot showed that the reaction resulted in product **19**, accompanied with about 5 % of the monoprotected bromocatechol (**20)**. Formation of monoprotected bromocatechol may be due to inadequate amounts of TBDMSCl or due to insufficient reaction time.

^{1}H NMR analysis of the product (4-bromo-1,2-bis-(tertbutyldimethyl silanyloxy)benzene showed two singlets at δ 0.97 and δ0.19, two doublets one at δ 6.94 – 6.93 and another at δ 6.70 – 6.67, and doublet of doublets in the range of δ 6.93 - 6.90.

Several attempts to synthesize the hydroxytyrosol derivative from the Grignard reaction of **19** and the protected 2-bromethanol **12** were not successful. It is still unclear why the starting material **19** failed to form the Grignard reagent; this reaction is still under investigation.

CHAPTER 4

CONCLUSION

In this research work, various synthetic routes were explored for the synthesis of hydroxytyrosol (**1**) using commercially available catechol (**2**) precursor. These routes include: Friedel-Craft's acylation, direct Friedel-Craft's alkylation, and Grignard's Reaction. Friedel-Craft's acylation route produced the best result with high overall yield. The method involves four steps wherein the intermediates can be isolated in good yields. On the other hand, Friedel-Craft's alkylation method gave a low yield of **1.** Other attempts were made to synthesize **1** using methods such as Grignard's reaction; however, the synthesis of Grignard reagent **19** was not successful.

Future work will focus on optimization of reaction conditions for synthesis of **1**, as well as an assessment of its ability to inhibit DNA oxidative damage.

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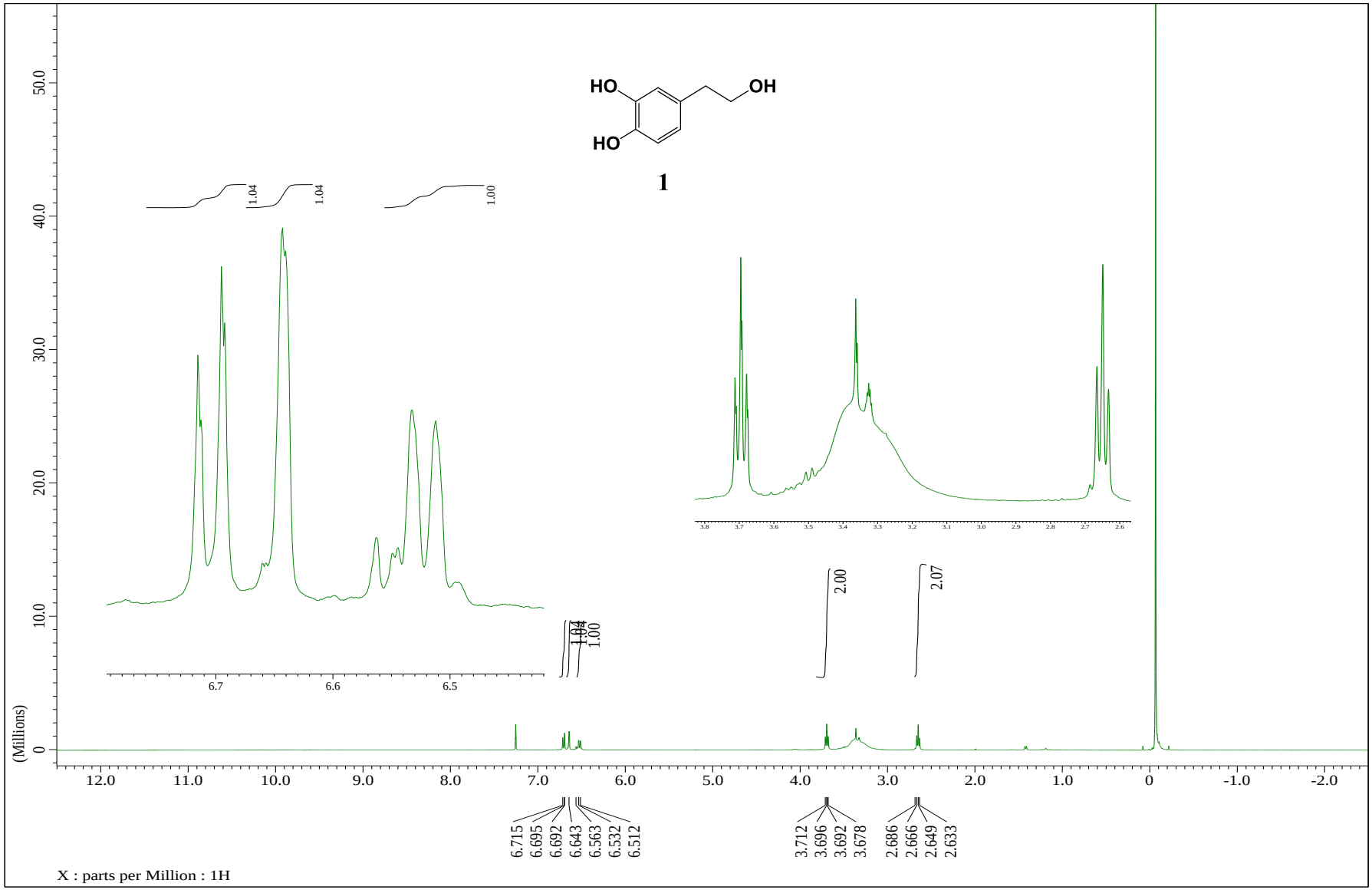
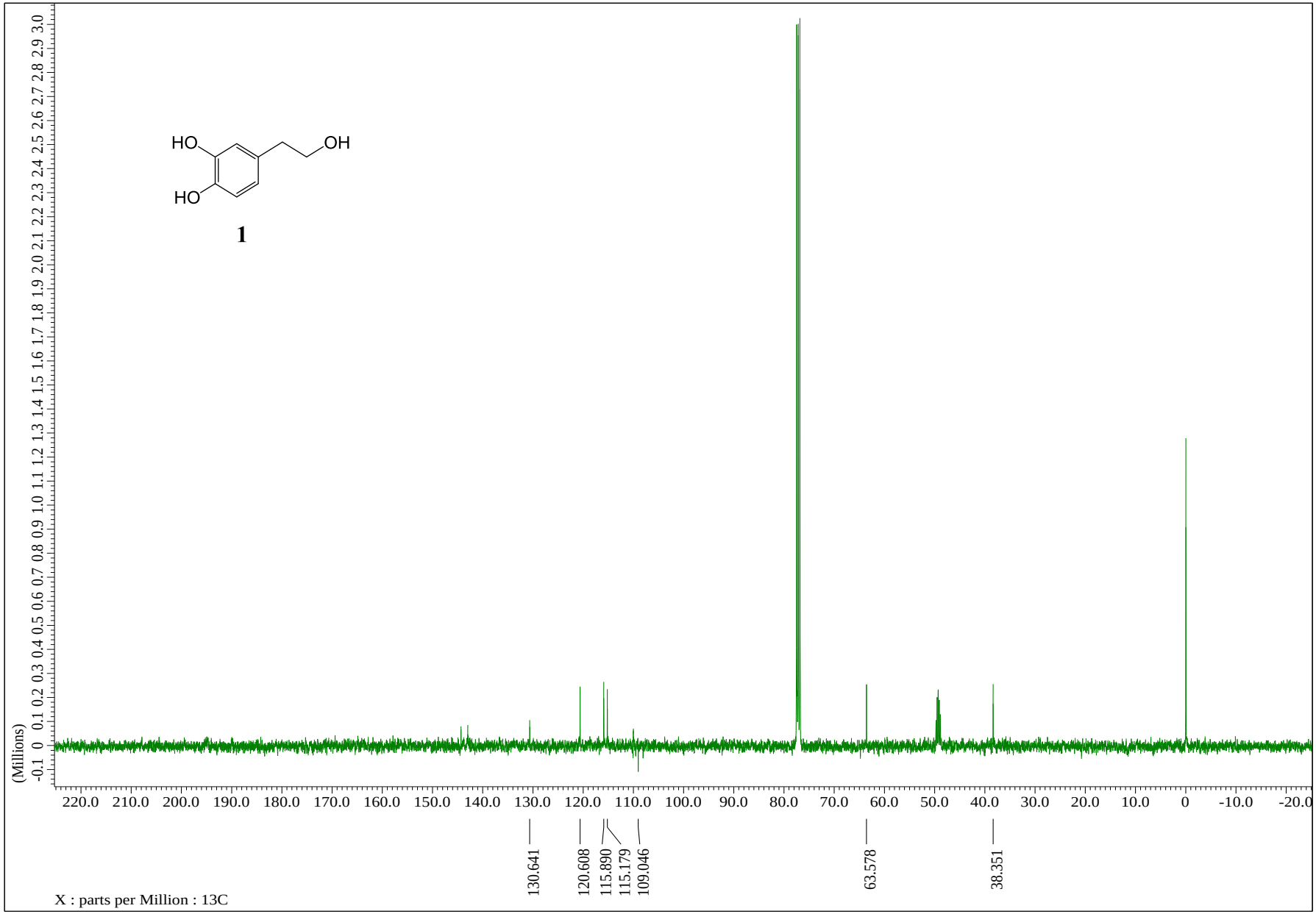
APPENDICES

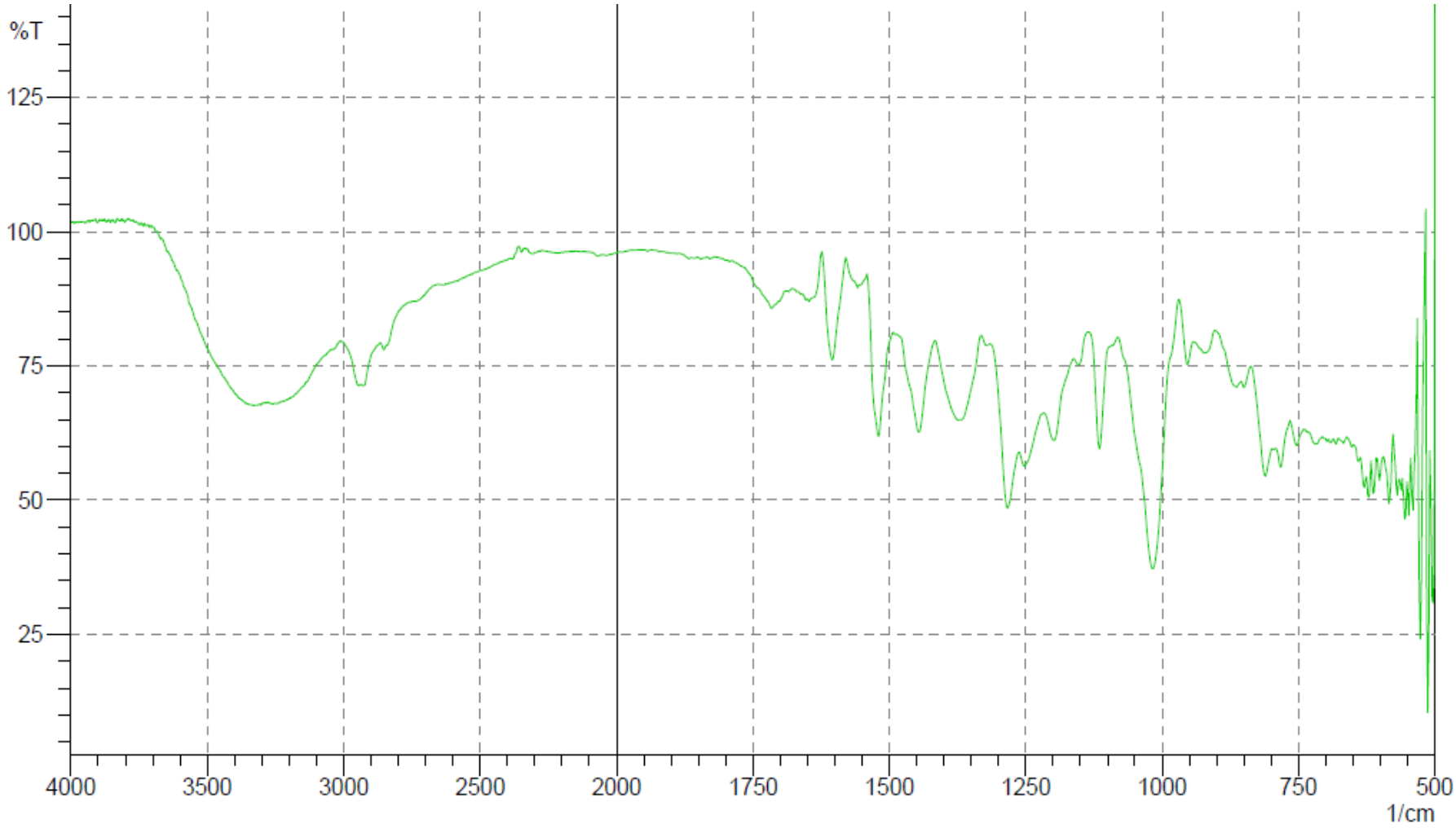
Appendix A1: ^{1}H NMR Spectrum for Compound **1** in $CDCl_3$ and CD_3OD



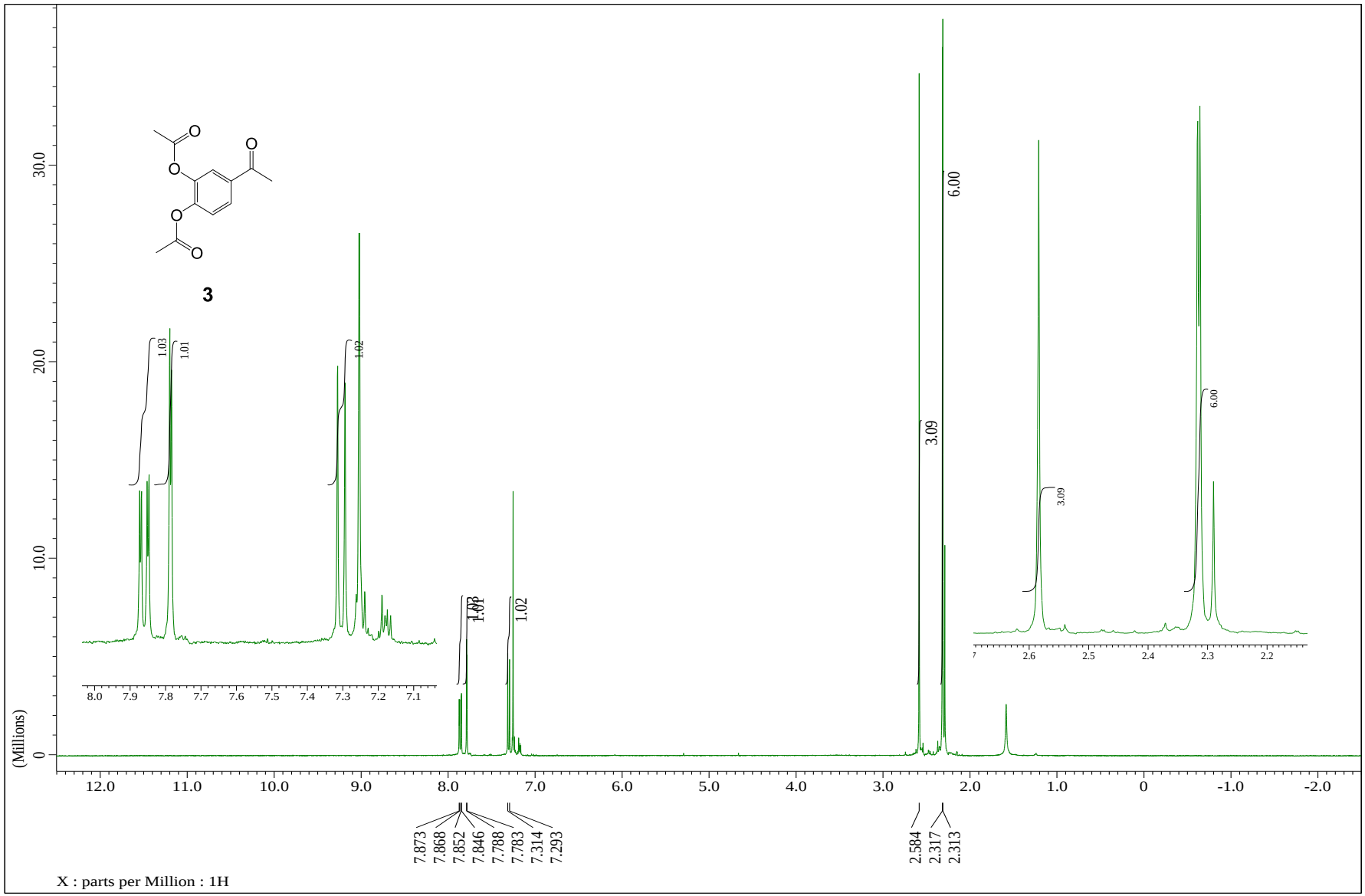
Appendix A2: ^{1}H NMR Spectrum for Compound **1** in $CDCl_3$ and CD_3OD



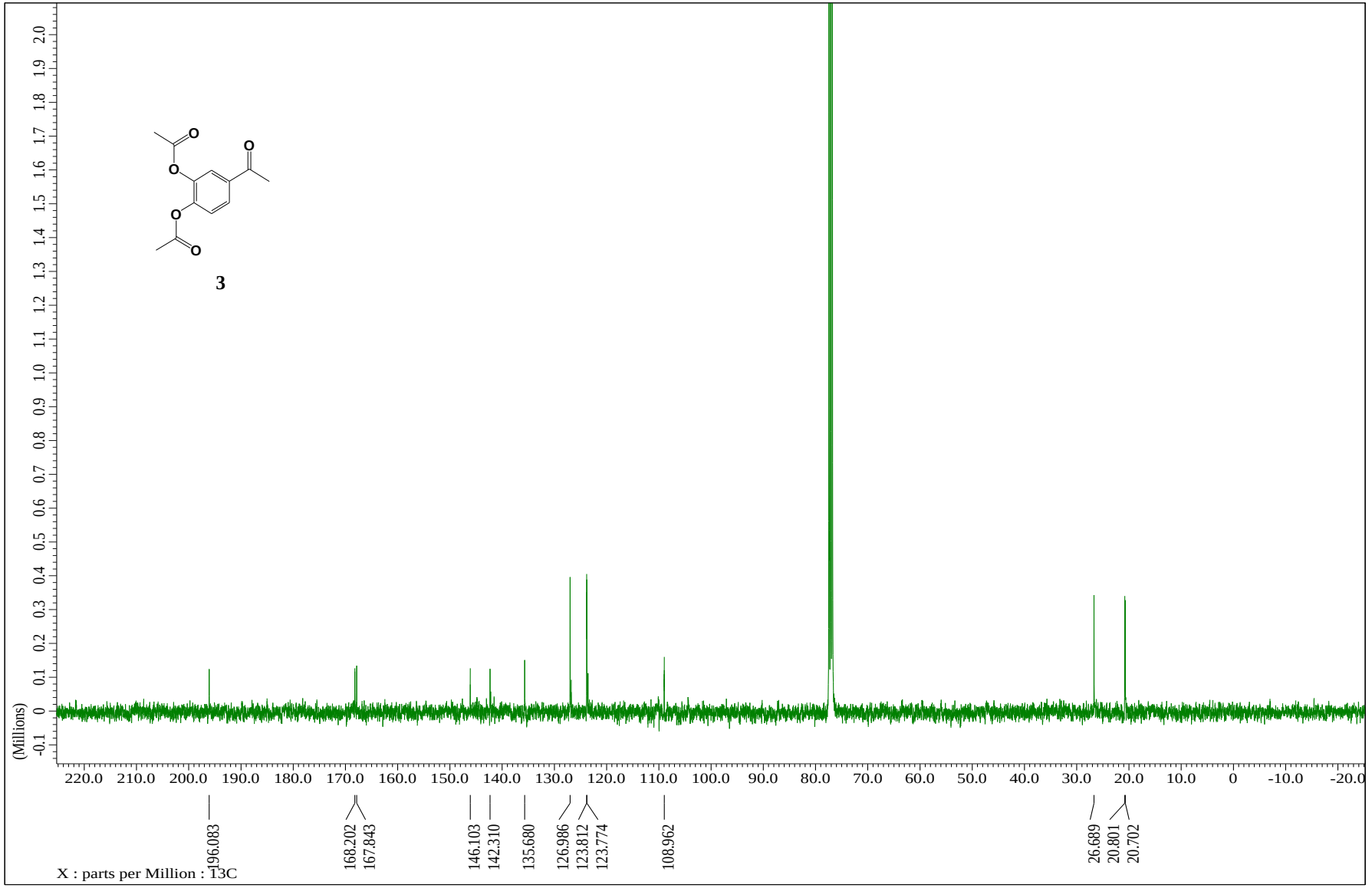
Appendix A3: IR Spectrum for Compound **1**



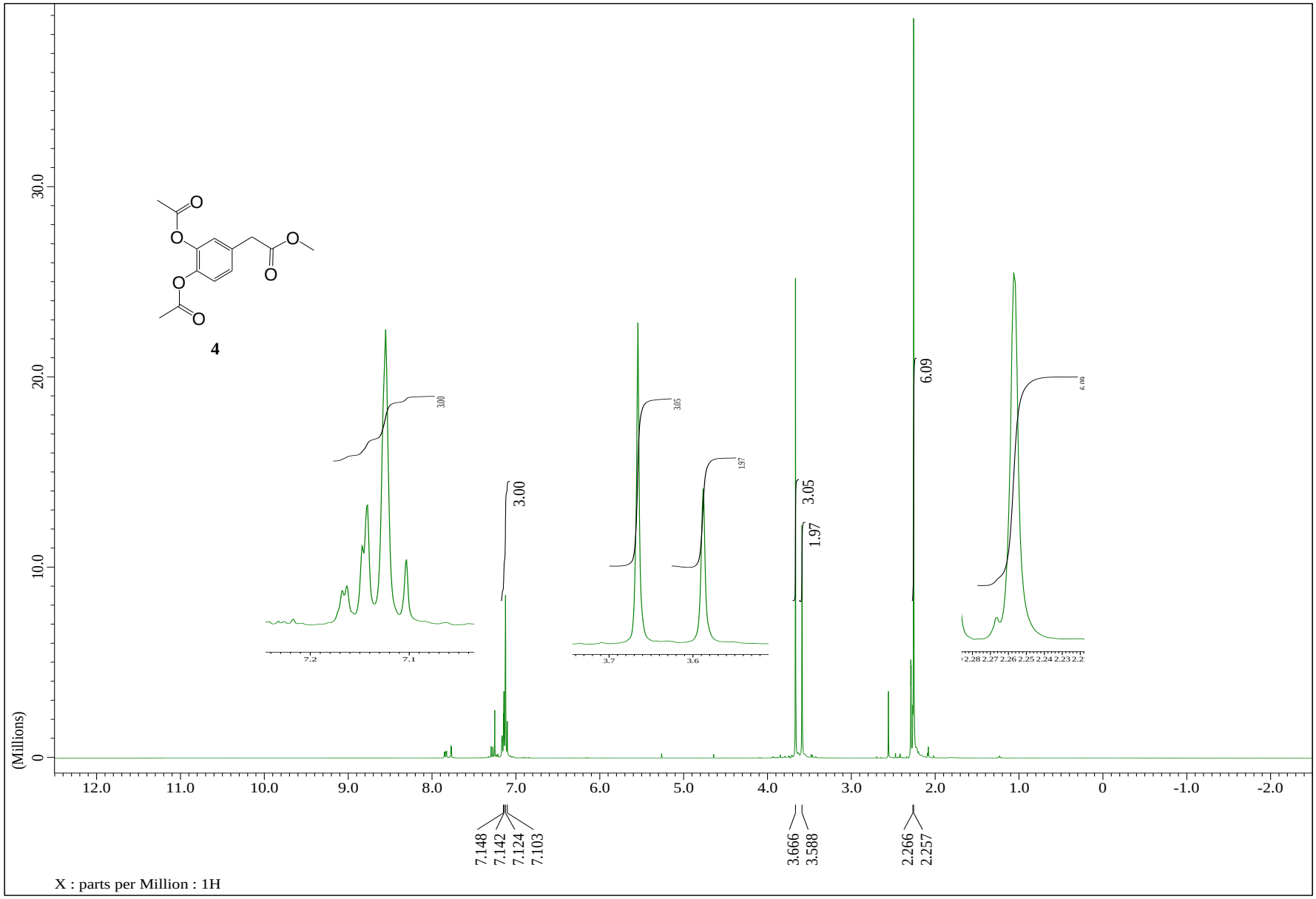
Appendix B1: ^{1}H NMR Spectrum for Compound **3** in $CDCl_3$



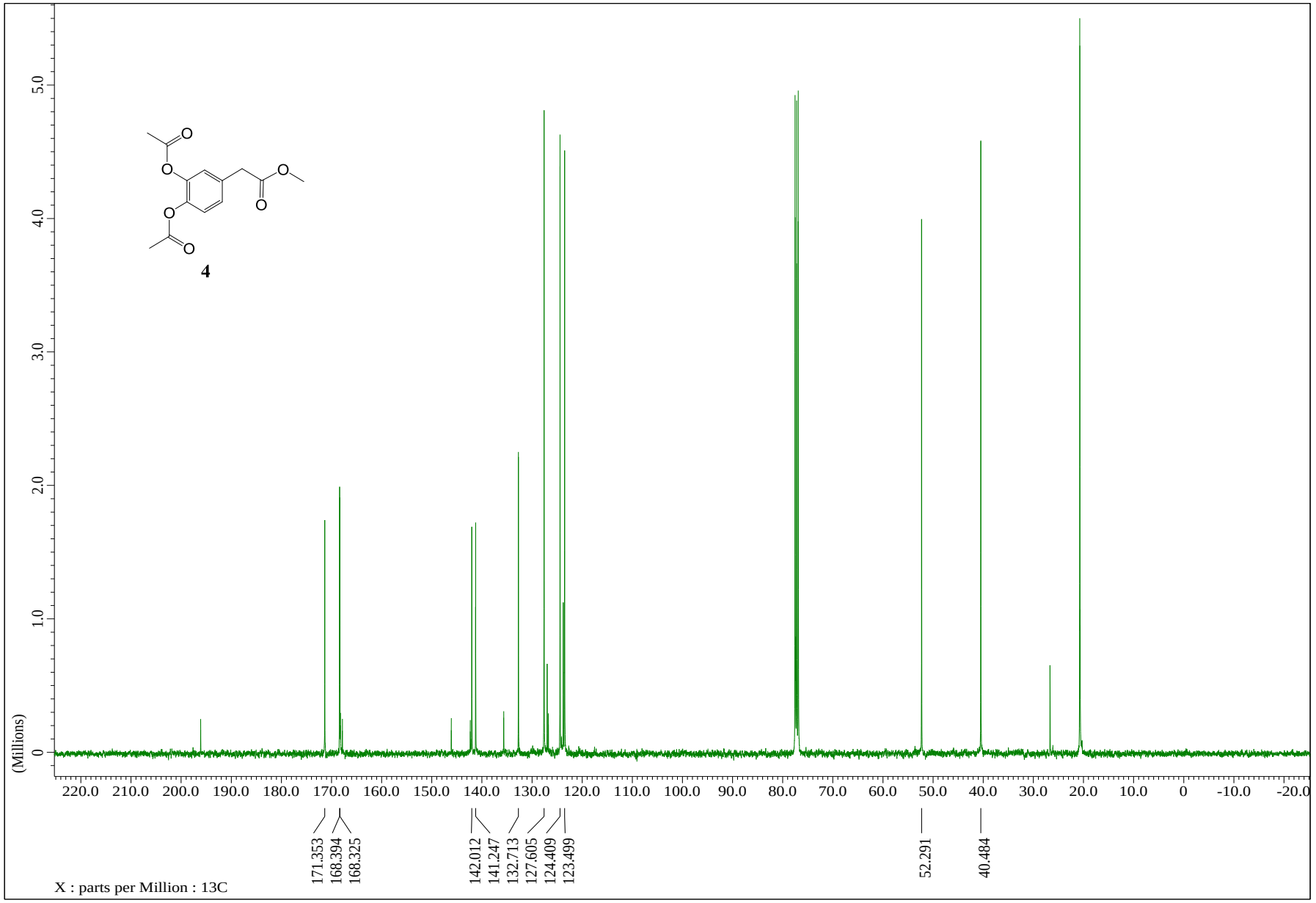
Appendix B2: ^{13}C NMR Spectrum for Compound **3** in $CDCl_3$



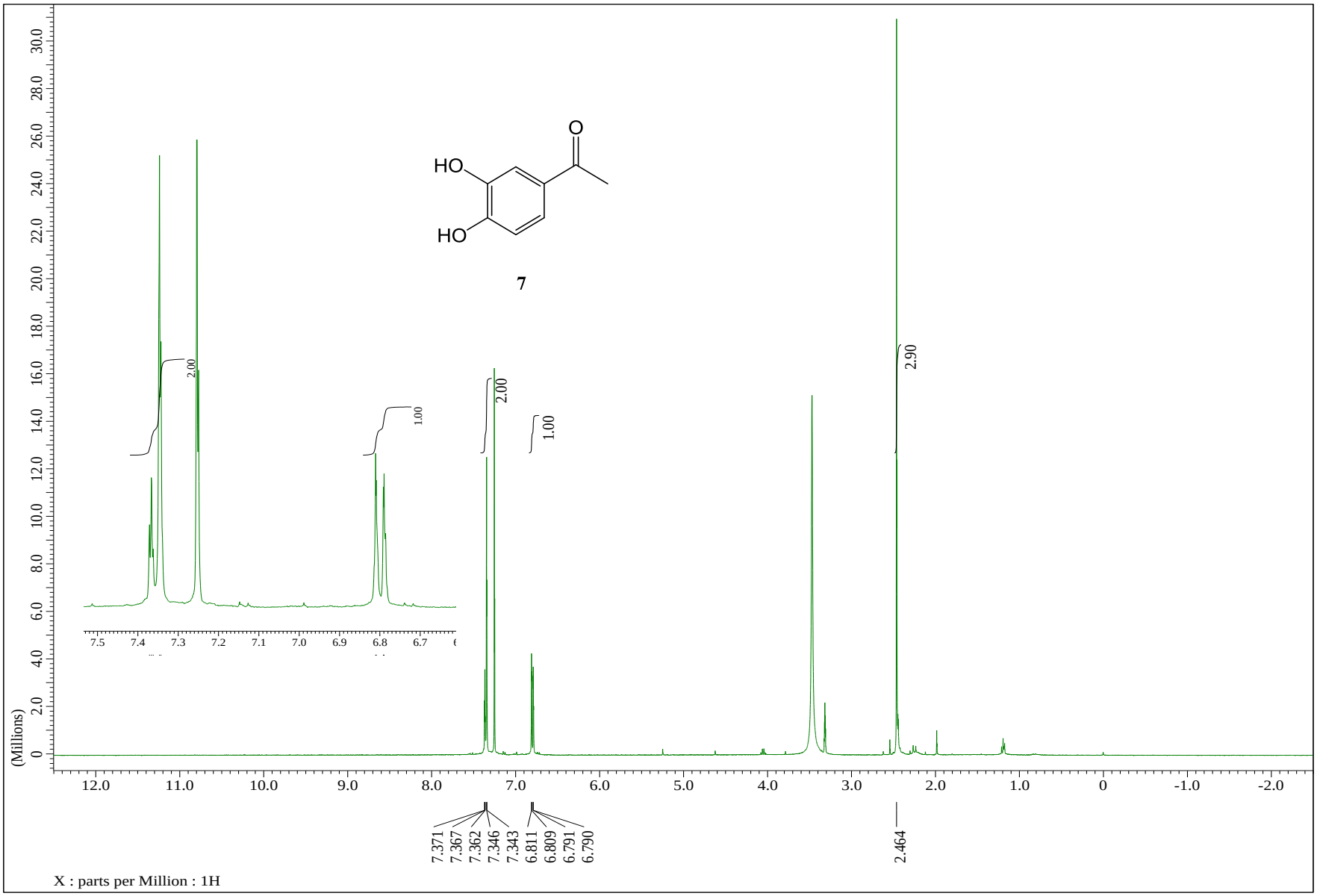
Appendix C1: ^{1}H NMR Spectrum for Compound **4** in $CDCl_3$



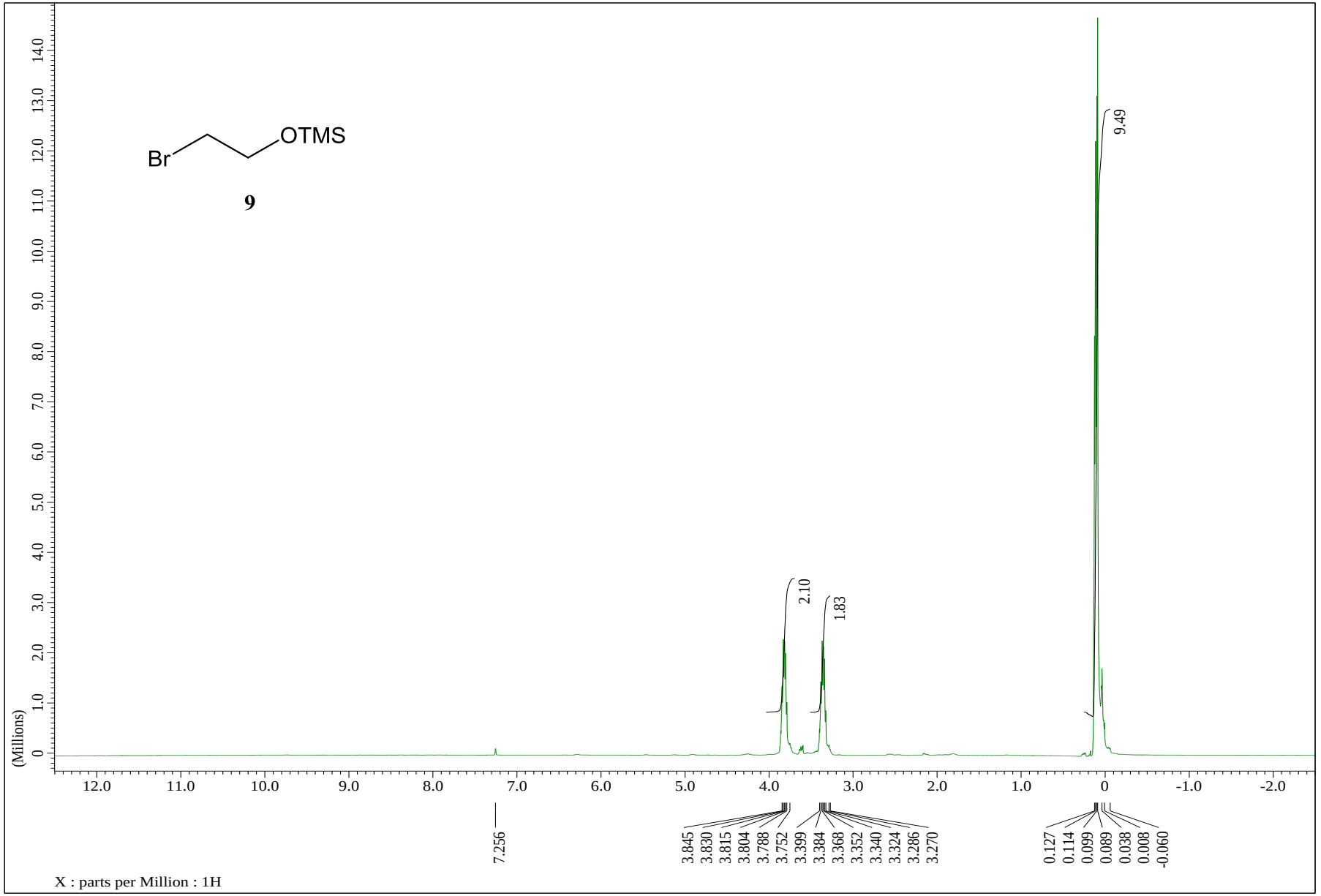
Appendix C2: ^{13}C NMR Spectrum for Compound **4** in $CDCl_3$



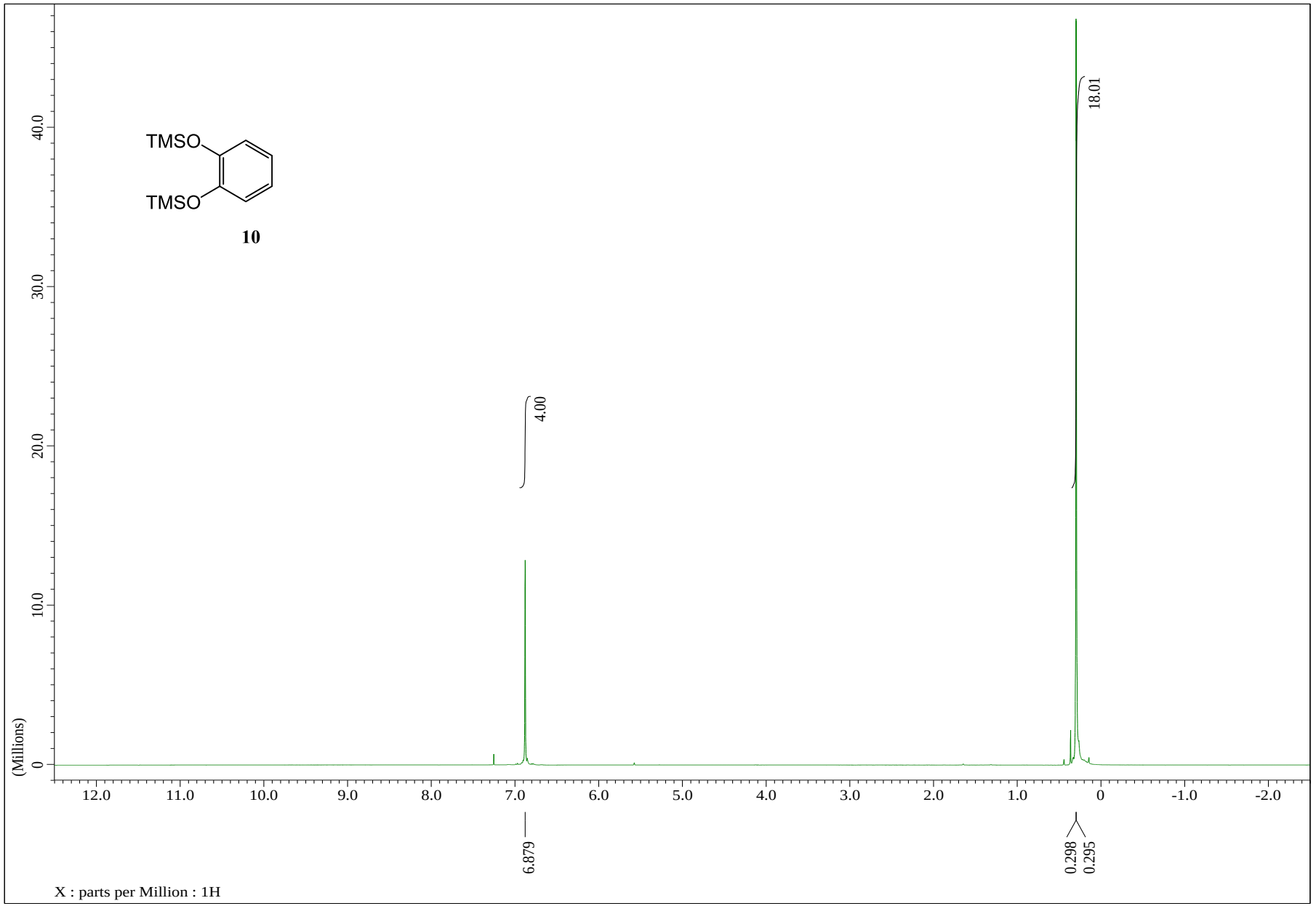
Appendix D: ^{1}H NMR Spectrum for Compound **3** in $CDCl_3$ and CD_3OD



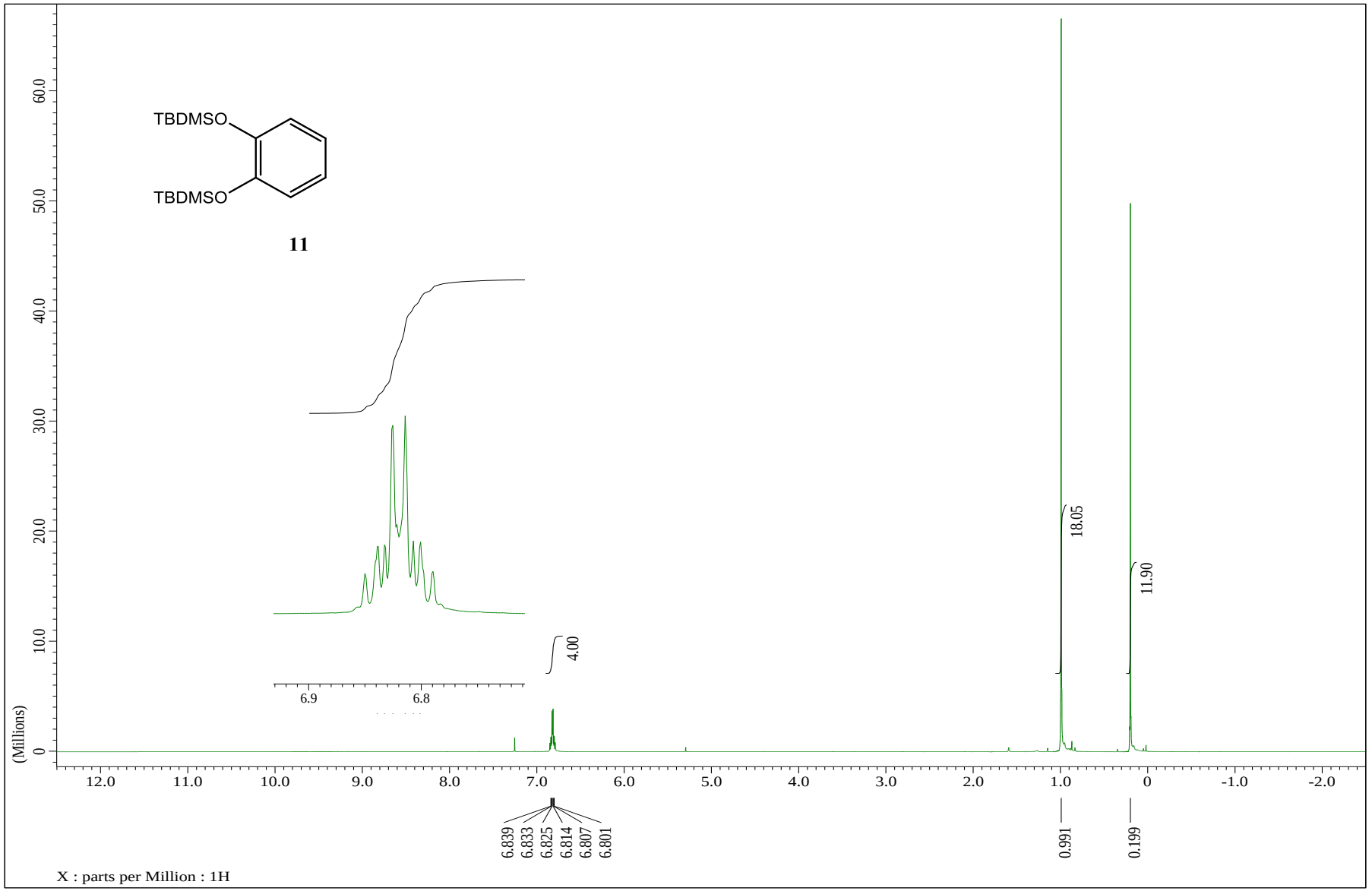
Appendix E: ^{1}H NMR Spectrum for Compound **9** in $CDCl_3$



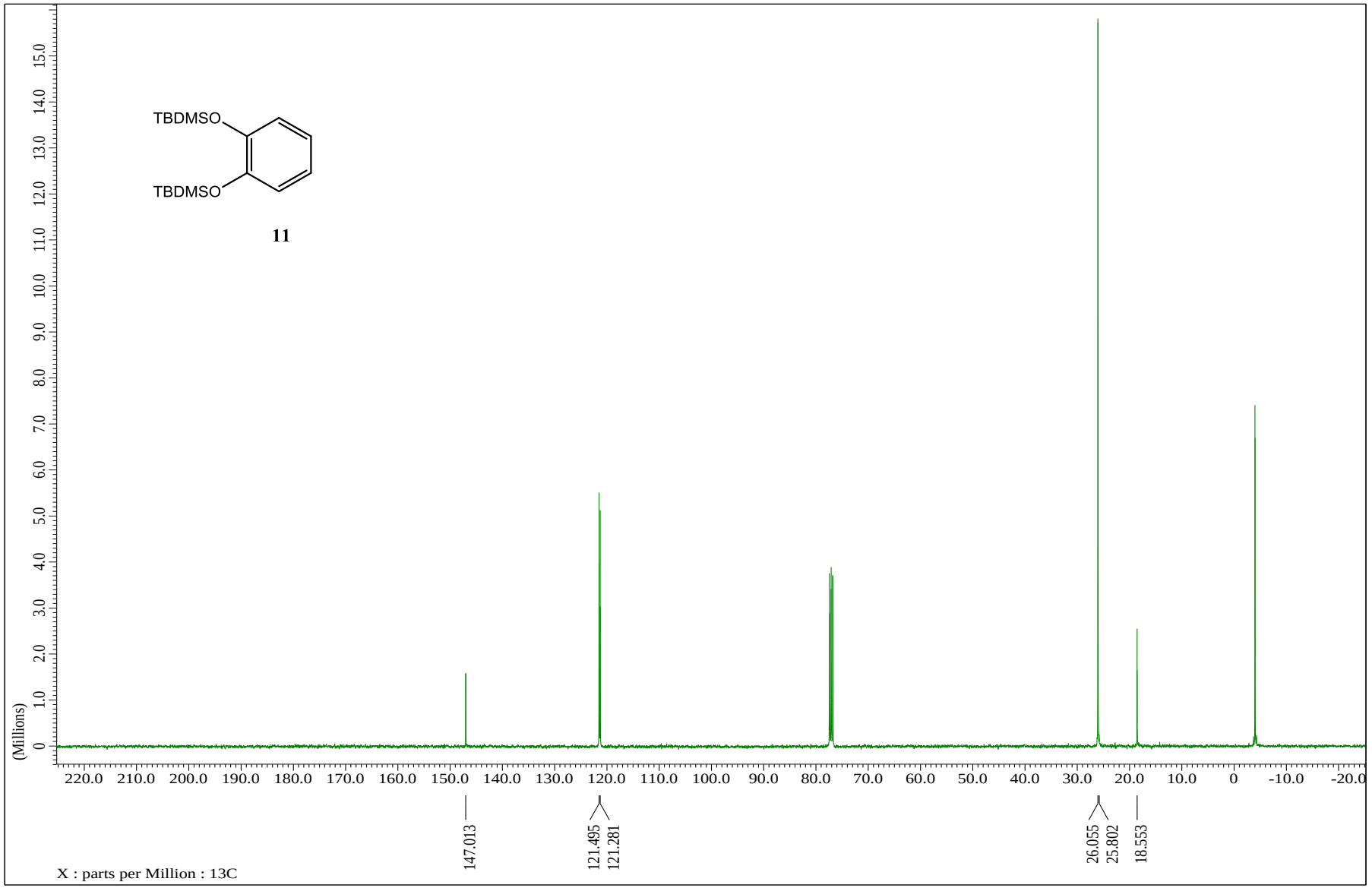
Appendix F: ^{1}H NMR Spectrum for Compound **10** in $CDCl_3$



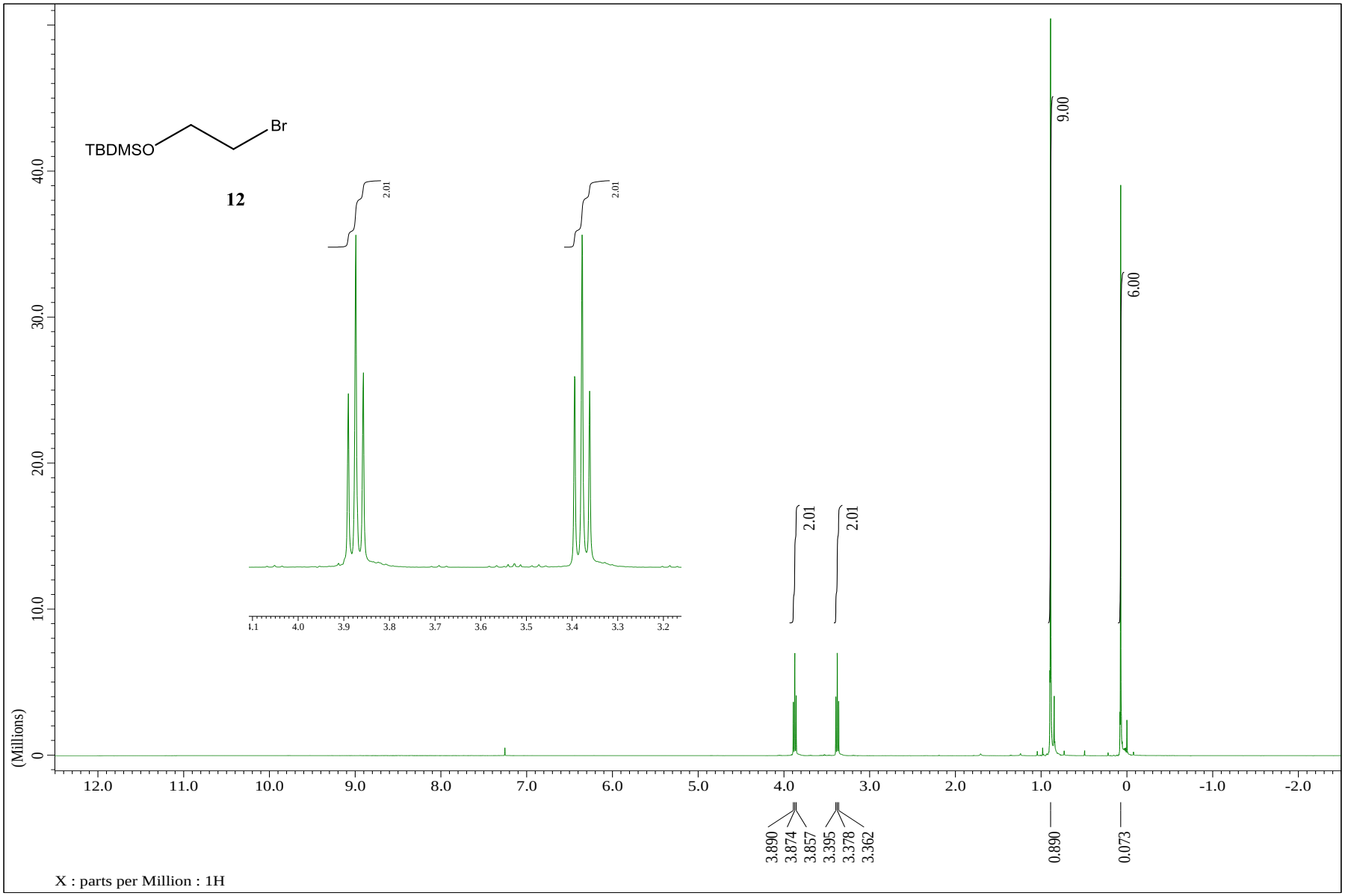
Appendix G1: ^{1}H NMR Spectrum for Compound **11** in $CDCl_3$



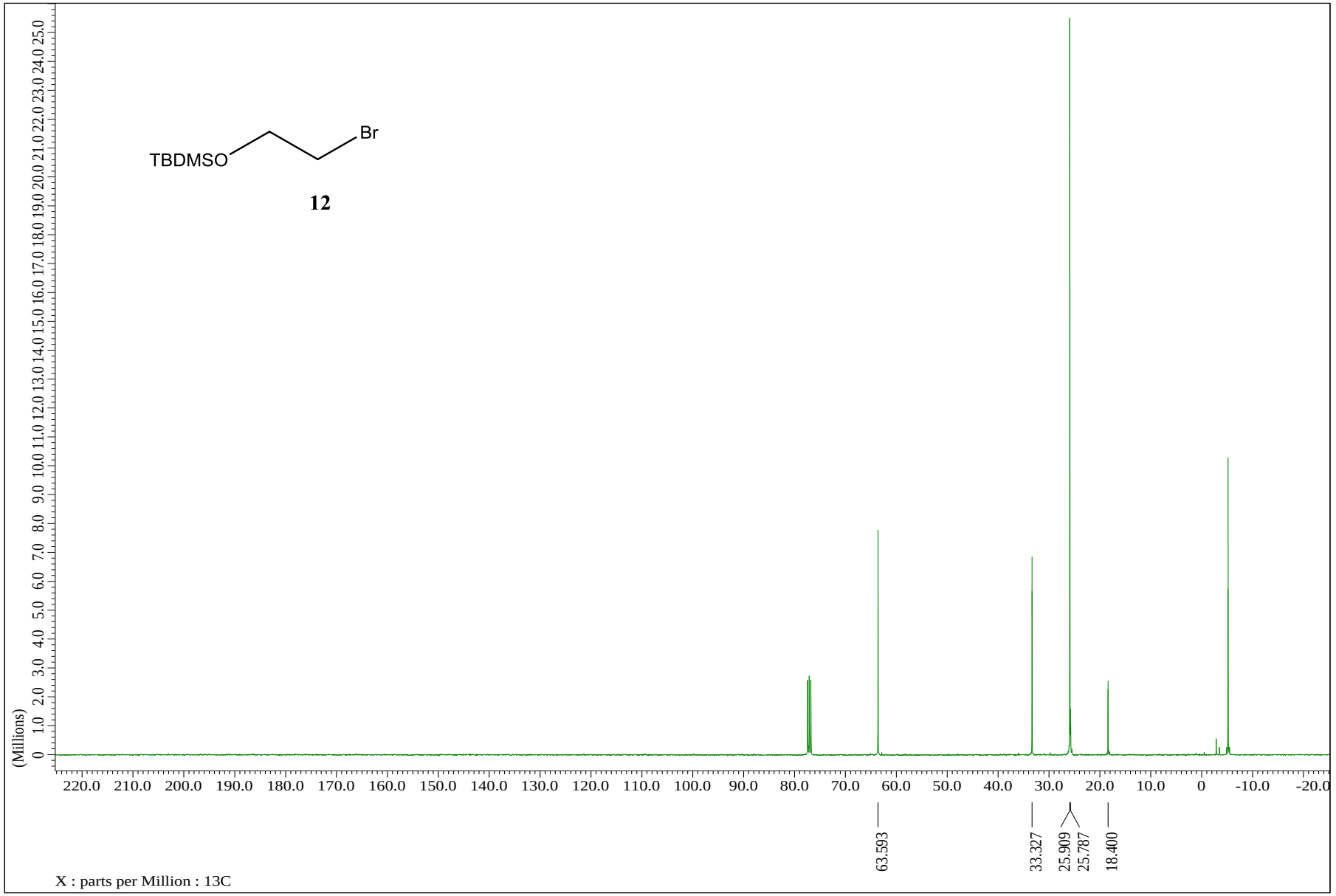
Appendix G2: ^{13}C NMR Spectrum for Compound **11** in $CDCl_3$



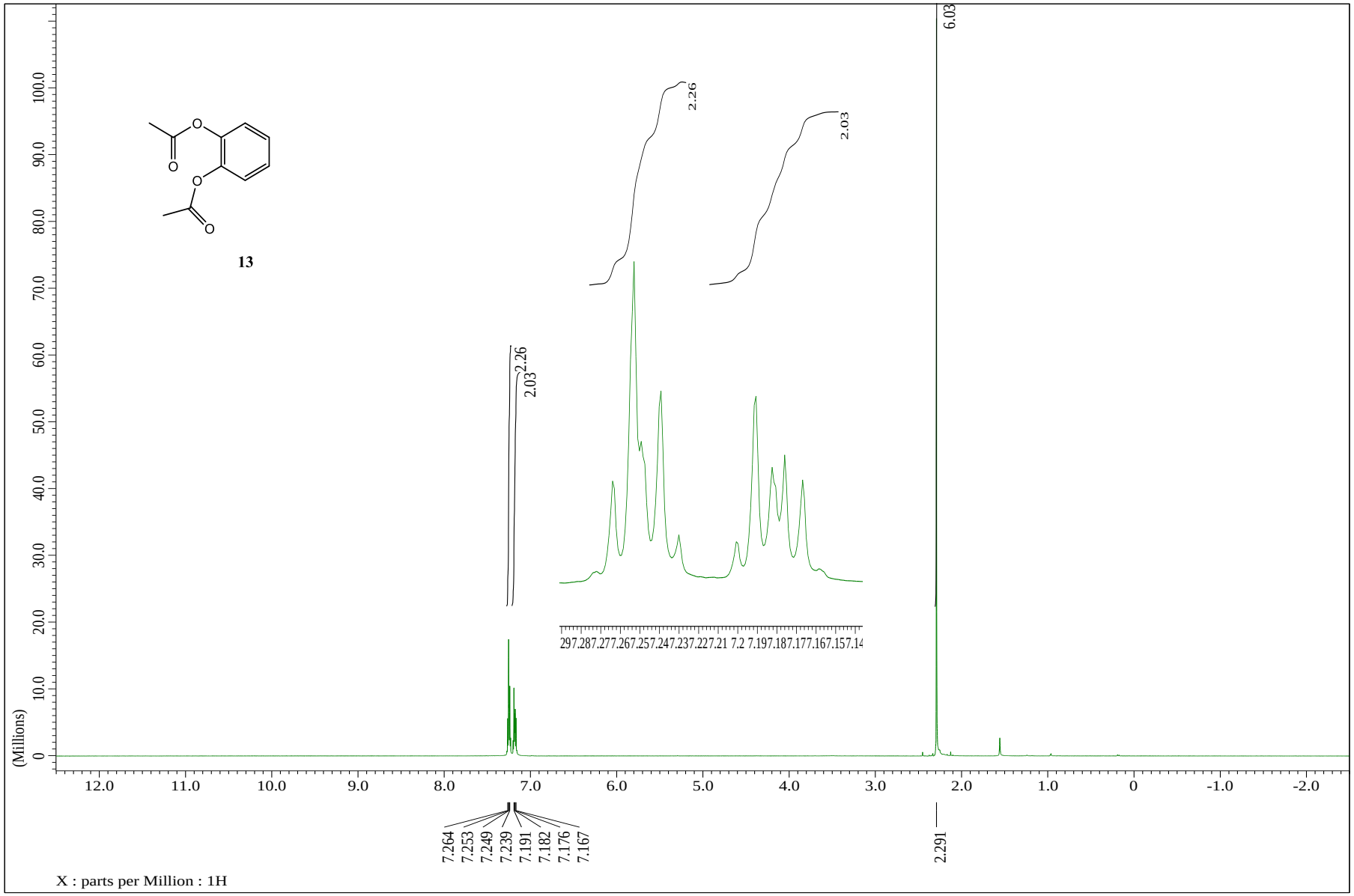
Appendix H1: ^{1}H NMR Spectrum for Compound **12** in $CDCl_3$



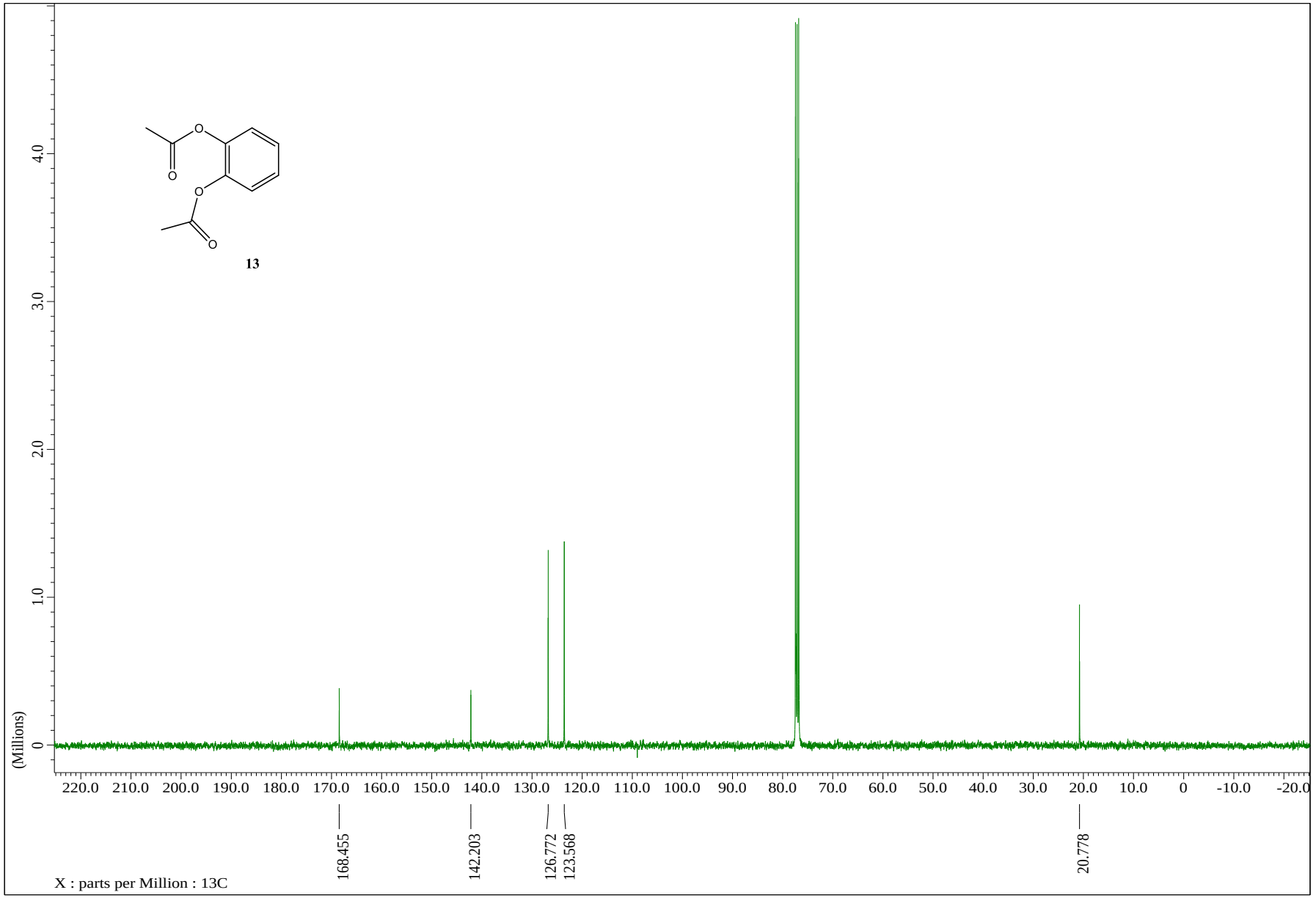
Appendix H2: ^{13}C NMR Spectrum for Compound **12** in $CDCl_3$



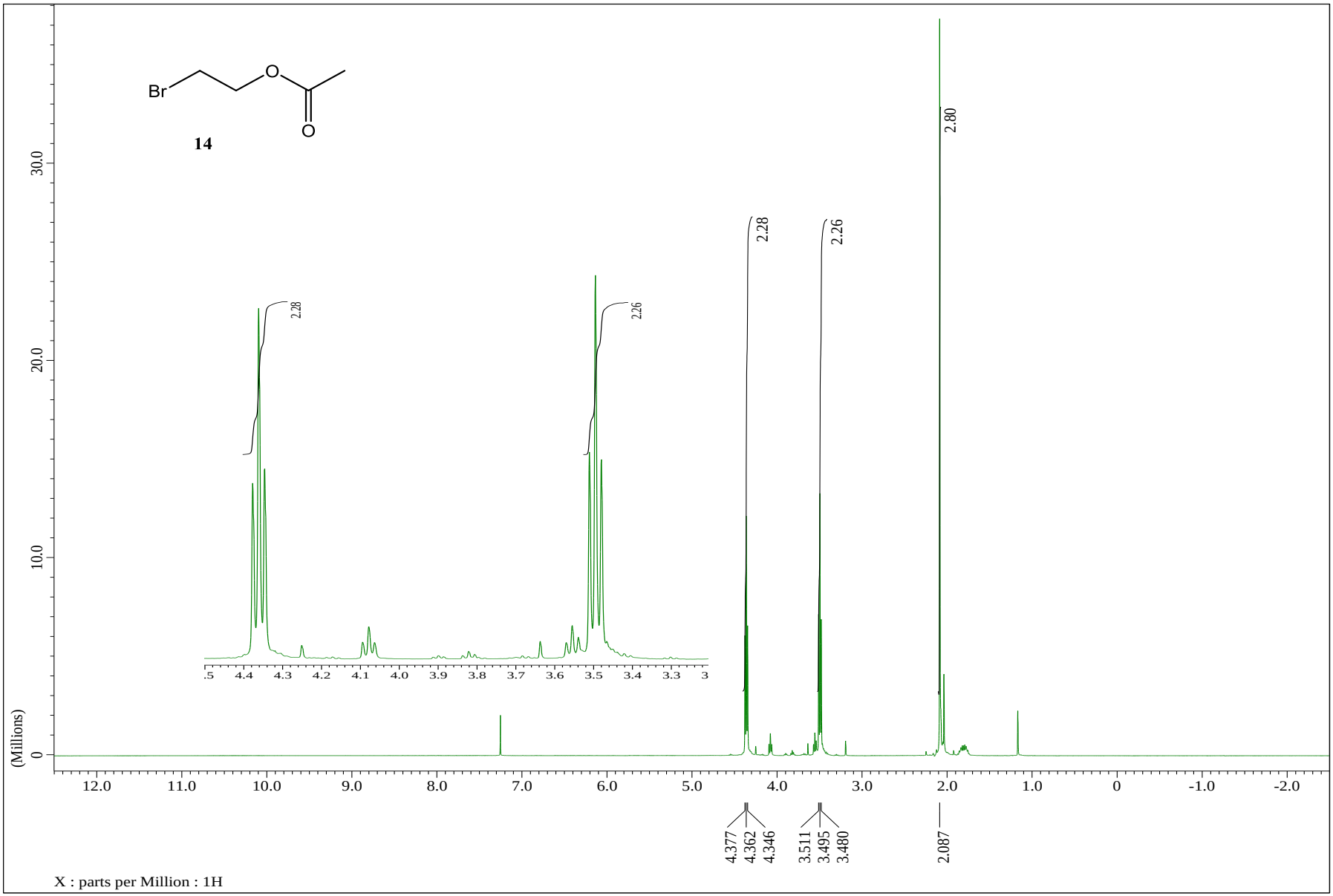
Appendix I1: ^{1}H NMR Spectrum for Compound **13** in $CDCl_3$



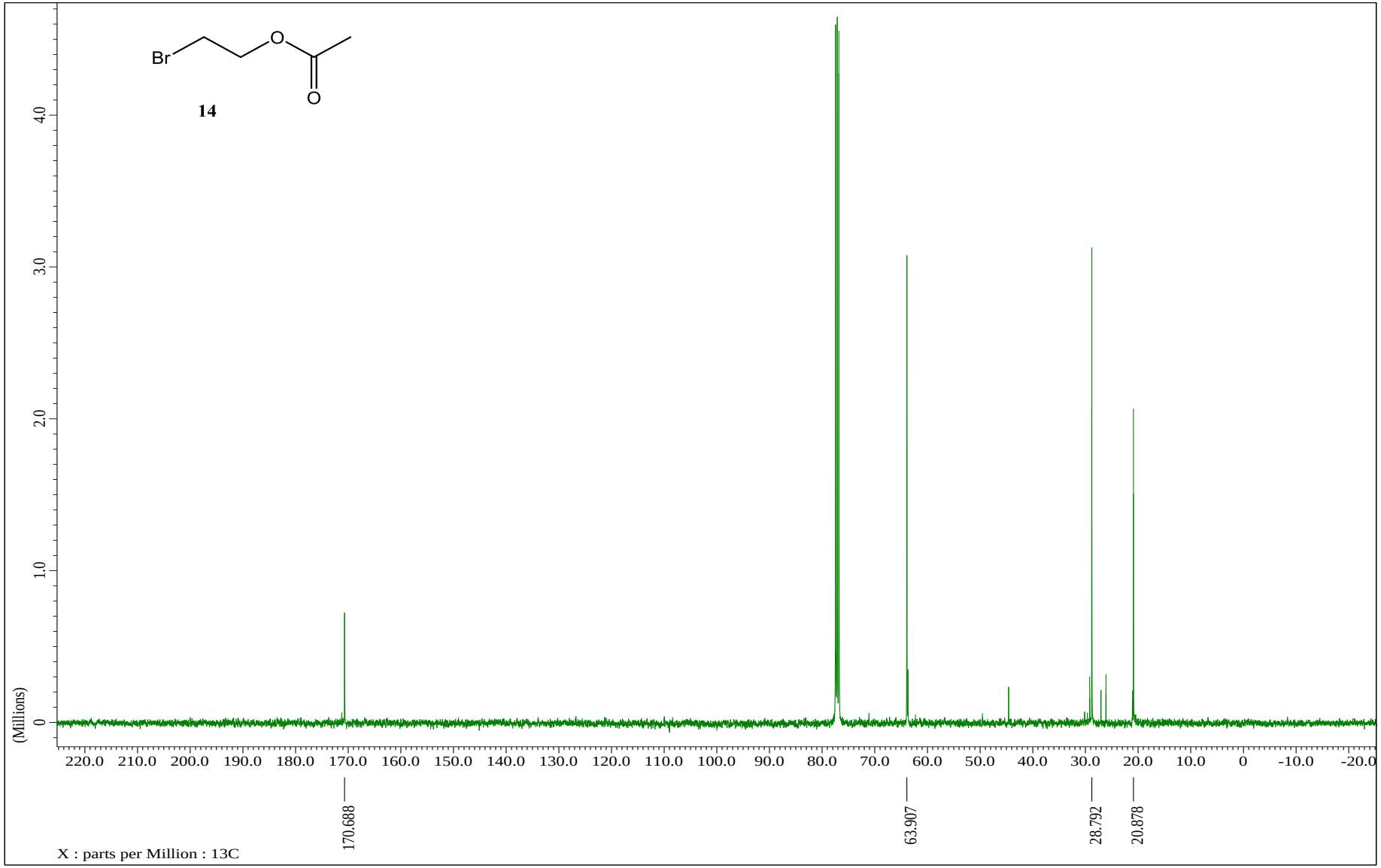
Appendix I2: ^{13}C NMR Spectrum for Compound **13** in $CDCl_3$



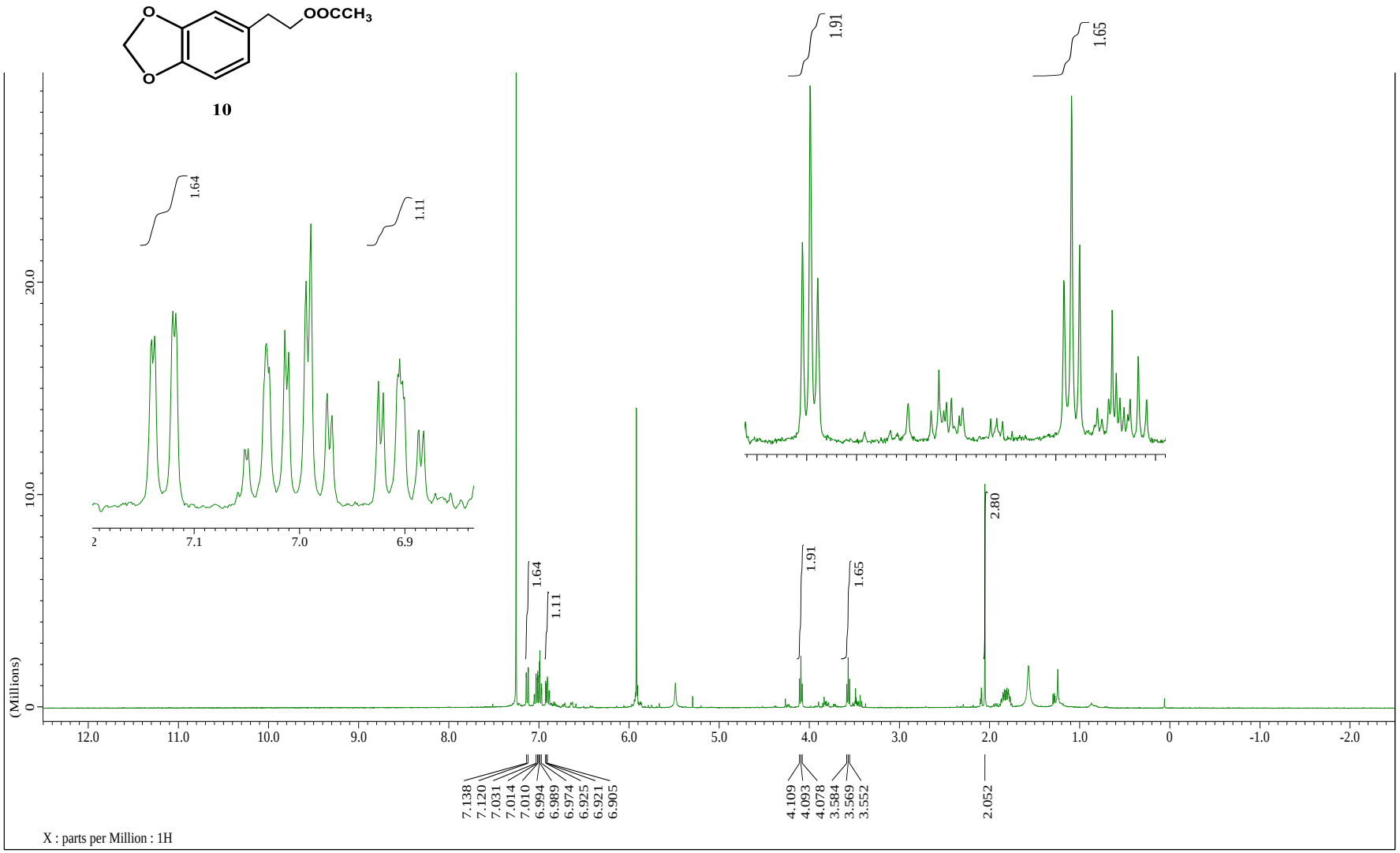
Appendix J2: ^{1}H NMR Spectrum for Compound **14** in $CDCl_3$



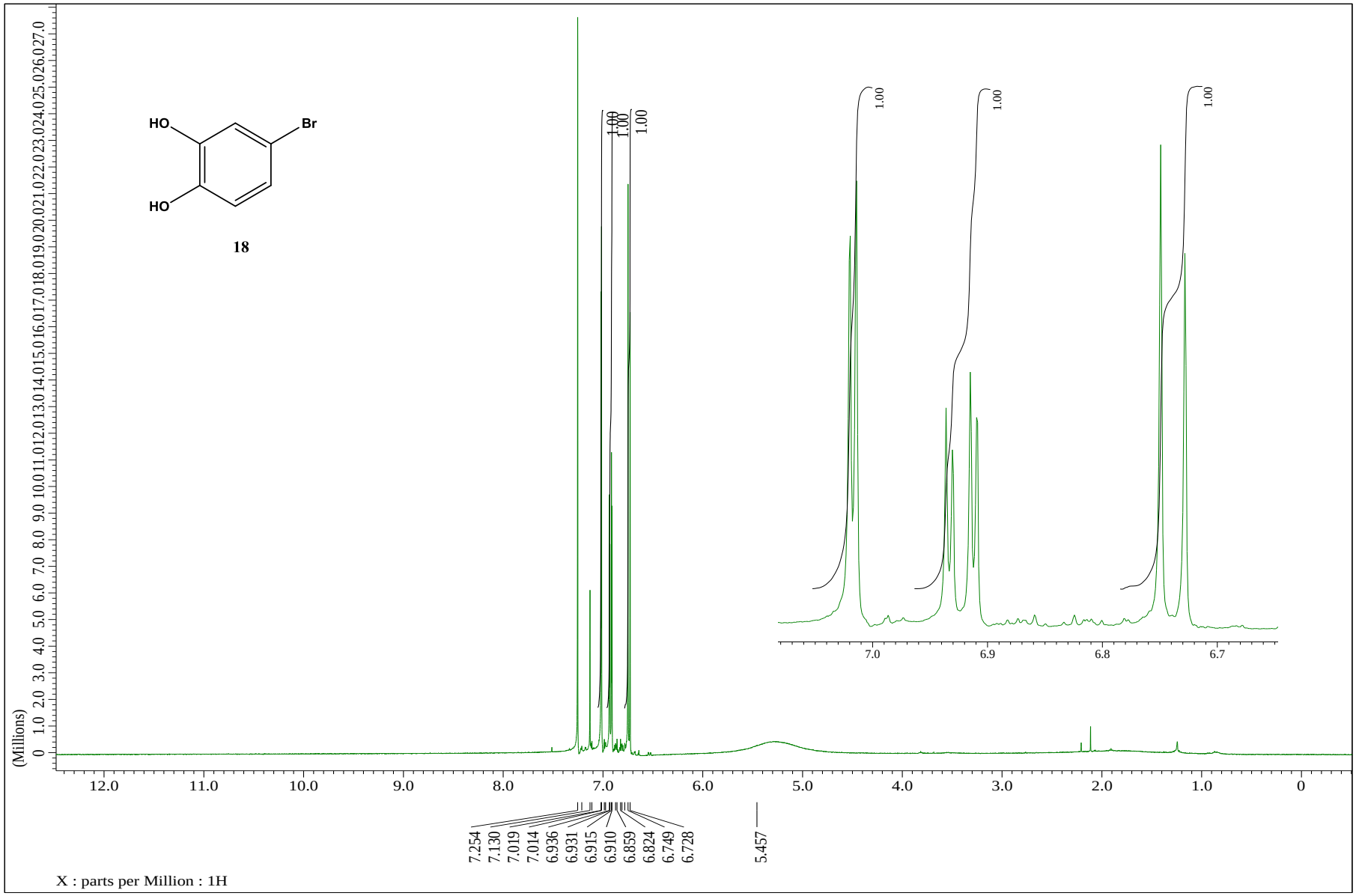
Appendix J2: ^{13}C NMR Spectrum for Compound **14** in $CDCl_3$



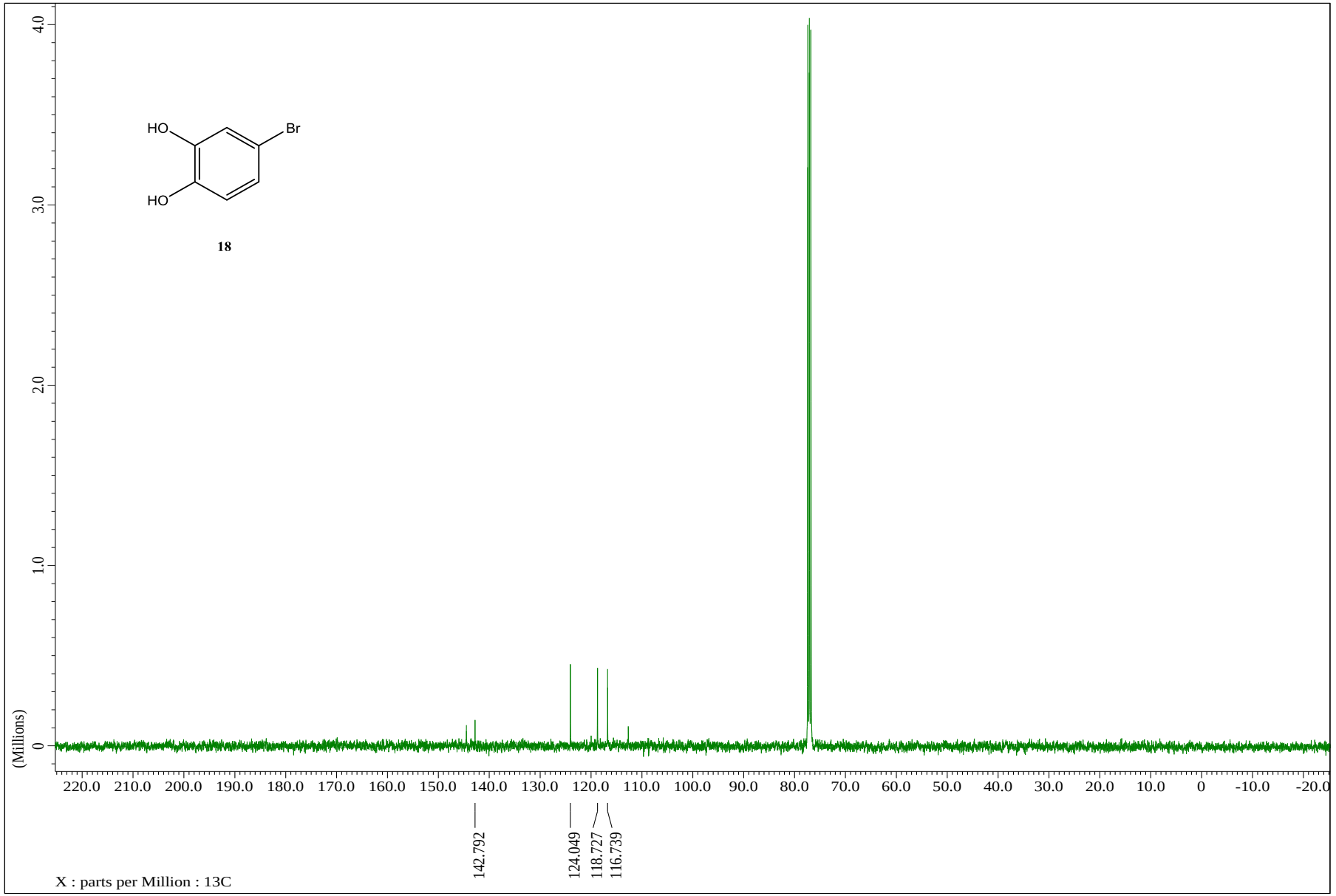
Appendix K: ^{1}H NMR Spectrum for Crude Compound **16** in $CDCl_3$



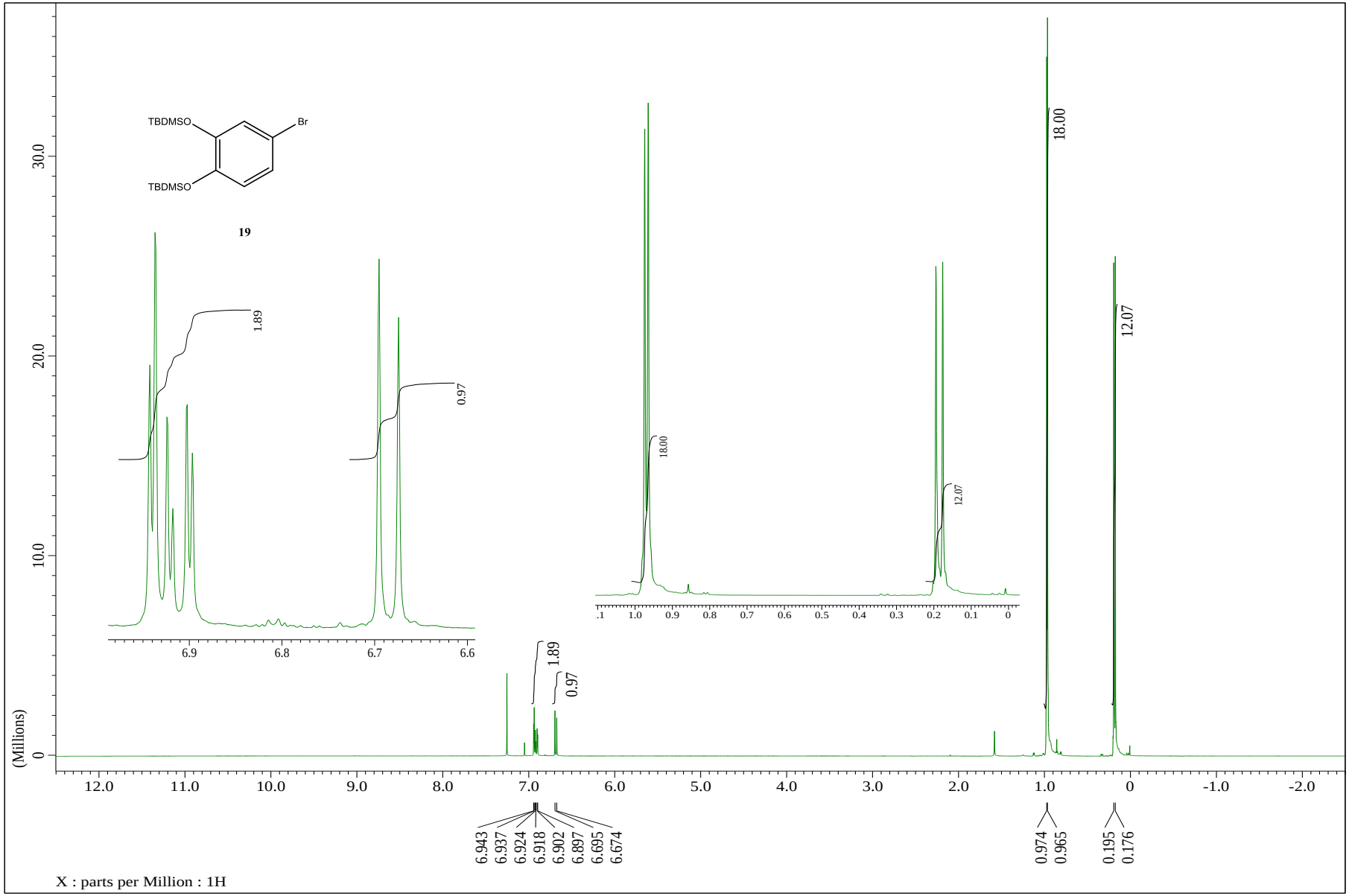
Appendix L1: ^{1}H NMR Spectrum for Compound **18** in $CDCl_3$



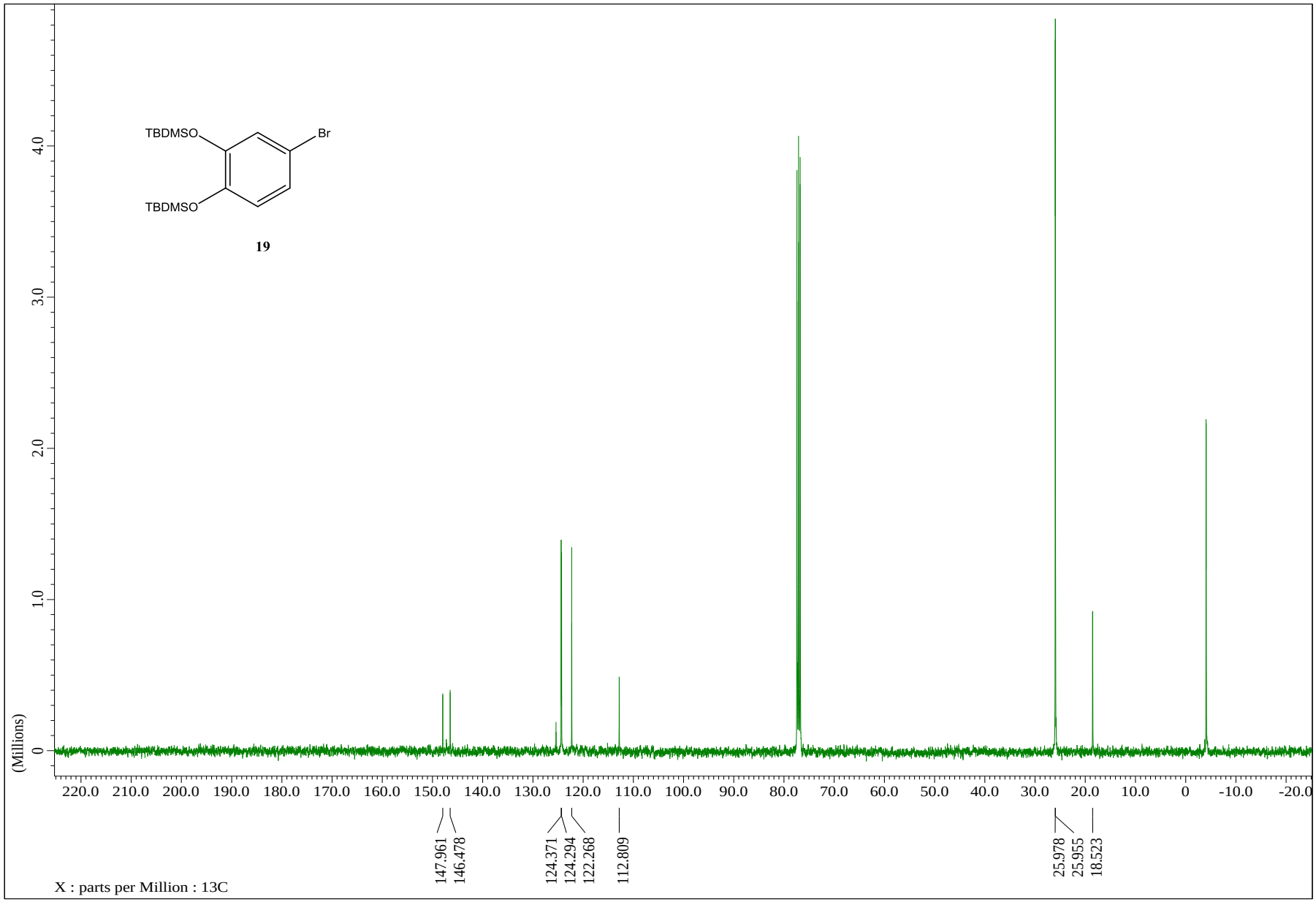
Appendix L2: ^{13}C NMR Spectrum for Compound **18** in $CDCl_3$



Appendix M1: ^{1}H NMR Spectrum for Compound **19** in $CDCl_3$



Appendix M2: ^{13}C NMR Spectrum for Compound **19** in $CDCl_3$



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