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M.Sc. (Chemistry)	2002
TITRE DE LA THÈSE / TITLE OF THESIS: SYNTHESIS AND KINETIC TESTING OF NOVEL ANTIOXIDANTS	

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FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTDOCTORALES

FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

BABIĆ, Gordana

AUTEUR DE LA THÈSE - AUTHOR OF THESIS

M.Sc. (Chemistry)

GRADE - DEGREE

Chemistry

FACULTÉ, ÉCOLE, DÉPARTEMENT - FACULTY, SCHOOL, DEPARTMENT

TITRE DE LA THÈSE - TITLE OF THE THESIS

Synthesis and Kinetic Testing of Novel Antioxidants

Tony Durst

DIRECTEUR DE LA THÈSE - THESIS SUPERVISOR

EXAMINATEURS DE LA THÈSE - THESIS EXAMINERS

T. Scaiano

J. Wright

J.-M. De Koninck, Ph.D.

LE DOYEN DE LA FACULTÉ DES ÉTUDES SUPÉRIEURES ET POSTDOCTORALES

SIGNATURE

DEAN OF THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

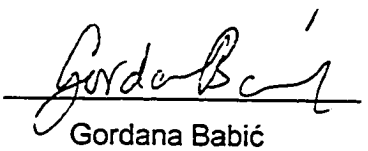
SYNTHESIS AND KINETIC TESTING OF NOVEL ANTIOXIDANTS

Gordana Babić B. Sc.

A thesis submitted to the
School of Graduate Studies and Research
University of Ottawa
in partial fulfilment of the requirements for the degree of
Master of Science in Chemistry
in the
Ottawa-Carleton Chemistry Institute
September 2002

Thèse soumis à la
Faculté des Études Supérieures et Postdoctorales
Université d'Ottawa
En vue de l'obtention de la
Maîtrise dès Sciences
à
L'Institut de Chimie d'Ottawa-Carleton

Candidate



Gordana Babić

Supervisor

Dr. Tony Durst

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"Od svega što čovek u životnom nagonu podiže i gradi, ništa nije u mojim očima bolje i vrednije od mostova. Oni su važniji od kuća, svetiji, jer opštiji, od hramova. Svačiji i prema svakom jednaki, korisni, podignuti uvek smisleno, na mestu na kom se ukrštava najveći broj ljudskih potreba, istrajniji su od drugih građevina i ne služe ničem što je tajno i zlo...
...Tako, svuda na svetu, gde god se moja misao krene ili stane, nailazi na verne i ćutljive mostove kao na večitu i večno nezasićenu ljudsku želju da se poveže, izmiri i spoji sve što iskrsne pred našim duhom, očima i nogama, da ne bude deljnja, protivnosti ni rastanka..."

Odlomak iz teksta "Mostovi" - Ivo Andrić, dobitnik Nobelove nagrade za knjizevnost 1961.

"Of all that a man in the impulse of his life raises and builds, nothing in my eyes is finer and more precious than a bridge. Bridges are more important than houses, holier, since being universal, than places of worship. Belonging to everyone and equal to all, useful, built always very thoughtfully, in a place which is a crossroad of human need, they are more persevering than any other construction and serve nothing which is secret or evil...
...Hence, all over the world, wherever my thought moves or stops, it encounters the faithful and silent bridges as the timeless and eternally insatiable human desire to connect, reconcile and join everything that appears in front of our spirit, our eyes and legs, so that there would be no divisions, oppositions or partings..."

An excerpt from the text "Bridges" - Ivo Andrić, winner of the Nobel prize for literature 1961.

Acknowledgements

To:

my supervisor, Professor Tony Durst, first and foremost, for accepting me to his research group and giving me the opportunity to learn, for his kind and patient guidance over the last two years, and for all the knowledge he was unselfishly willing to share including all the wise stories I enjoyed so much;

Dr. Sasmita Tripathy for welcoming and training me during my first semester;

Dr. Helmi Hussain for synthesizing several target molecules and sharing his ideas with me;

Dr. Jim Wright from Carleton University, who originated the proposal for this project and effectively coordinated all the aspects of it;

Prof. Dr. Keith Ingold from the NRC for allowing me to use the Stopped-Flow Apparatus and the laboratory spaces, as well as for his invaluable advice and gentlemanly kindness;

Dr. Glen Facey and Kim Yach for NMR services and Dr. Clem Kazakoff for MS services;

my fellow graduate students: Evaloni Puniani, Karol Gajewski, Cheryl Enright, Beth Husk, Irina Denisova, Joe Jebreen and Joe Nahra, as well as all the past and present Durst group members, especially Ami Chin and Livia Aumand, for their companionship and help over the past two years we spent together;

all the wonderful people in the department for the fun conversations we shared in the hallways;

my cousin Mike for letting me use his computer, for building mine and helping me heal it a number of times over the last ten years;

my sister, Vesna, for spending these two magnificent loving years in Ottawa with me and allowing herself to get to know me;

my friends all over the globe for continuous support;

and

my fiancé John Atallah for loving me;

I thank you.

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Abbreviations and Symbols

Δ	heat
abs.	absolute
Ac	acetate
AcOH	acetic acid
anh.	anhydrous
aq.	aqueous
ATP	adenosine triphosphate
BDE	bond dissociation enthalpy
Bn	benzyl
br	broad
°C	degrees Celsius
calcd.	calculated
CH_2Cl_2	methylene chloride
$CHCl_3$	chloroform
CH_3I	methyl iodide
cm^{-1}	wave number
^{13}C NMR	carbon-13 nuclear magnetic resonance
d	doublet
dd	doublet of doublets
DHP	3,4-dihydro-2H-pyran
DMAP	N,N-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
DoM	directed *ortho* metallation

e^-	electron
EI	electronic ionization
eq.	equivalents
ETC	electron transport chain
Et_3N	triethylamine
Et_2O	diethyl ether
EtOAc	ethyl acetate
FAD	flavin adenin dinucleotide
FMN	flavin mononucleotide
g	gram
gl.	glacial
h	hour (s)
HAT	hydrogen atom transfer
H_2O	water
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
^1H NMR	proton nuclear magnetic resonance
HRMS	high resolution mass spectroscopy
Hz	Hertz
IP	ionization potential
IR	infrared
J	coupling constant
kcal	kilo calorie
KH	potassium hydride
K_2CO_3	potassium carbonate
LDBS	locally dense basis set

lit.	literature value
LTMP	lithium 2,2,6,6-tetramethylpiperidide
m	multiplet
M	molar
m/z	mass to charge ratio
*m*CPBA	*meta*-chlorobenzoic acid
Me	methyl
$MgSO_4$	magnesium sulphate
MeI	methyl iodide
mg	milligram
min.	minutes
ml	millilitre
mM	millimolar
mmol	millimoles
mol	moles
mp	melting point
Ms	methanesulfonyl
MS	low resolution mass spectroscopy
MW	molecular weight
N	normal
NAD	nicotinamide adenine dinucleotide
O_2	molecular oxygen
Ph	phenyl
ppm	parts per million
q	quartet

ROS	reactive oxygen species
RT	room temperature
s	singlet
sat.	saturated
SET	single electron transfer
S_N2	bimolecular nucleophilic substitution
$SnCl_4$	tin tetrachloride
t	triplet
THF	tetrahydrofuran
TLC	thin layer chromatography
TMP	2,2,6,6-tetramethylpiperidine
TOH	tocopherol
TsOH	*para*-toluenesulfonic acid
water	distilled water

ABSTRACT

A number of antioxidant structures having the 1,2-dihydroxybenzene or catechol motif were designed and synthesized. The design of these compounds was based on calculations that predict the bond dissociation energy of phenolic O-H bonds.

The antioxidant activity of compounds prepared in this thesis (**8** and **59**) and related compounds synthesized in our laboratory (**19**, **33**, and **38**) were compared to α-tocopherol (vitamin E) on the basis of the rate constants for the hydrogen atom transfer to DPPH radical. Fully substituted catechols bearing a *para* alkoxy group were found to be better antioxidants than vitamin E, using the kinetic results criteria. The best compound in this series has a *para* alkoxy as part of a 5-membered ring. The relative order of antioxidant activity (**59** < **78** < **8** ≤ **19** < **33** < **38**) agrees with the theoretical predictions.

Some of the building blocks for the future catechol dendrimers were prepared, such as 4-methyl-benzo[1,3]dioxole-5-carbaldehyde **41** and 3,4-bis-allyloxy-benzaldehyde **52**.

The preparation of carbon-centred antioxidants starting with oxindole or isatin was investigated.

INTRODUCTION

Chapter 1: Introduction

"A moralist, at least, may say that the air which nature has provided for us is as good as we deserve."- Joseph Priestley (1775)

1.1 Oxygen, a "double-edged sword"

The first forms of life on Earth appeared some 3.5 billion years ago.[1] These were anaerobic organisms. They existed for about a billion years until what may be called the biggest environmental pollution ever recorded on this planet occurred. Namely, due to the photosynthetic activity of the blue-green algae, the oxygen gas started accumulating in significant amounts in the Earth's atmosphere. The algae split water to satisfy their need for hydrogen atoms, releasing tonnes of oxygen into the air. Once the oxygen content of the air reached 1% most anaerobes died. Some survived and retreated to oxygen-free areas, and some evolved into more complex organisms capable of reducing O_2 to H_2O. About 500 million years ago the oxygen levels in the atmosphere reached 10% and the ozone layer appeared, effectively shielding the land and allowing the life forms to emerge from the sea. When the man evolved, 5 million years ago, the atmospheric oxygen levels reached 21%, where they remain to date.

Oxygen was first isolated and characterized independently by Scheele and Priestley, the two great European scientists, between 1772 and 1774. Soon after, Lavoisier noted that oxygen gas inhalation had poisonous effects.[2] Further studies over the years demonstrated that oxygen at higher pressures is a powerful poison, but it wasn't until about 1967 that this concept was completely accepted. Today we know that patients should not be exposed to more than 60% oxygen for extended periods of time. Such exposure causes severe pulmonary changes in adults and blindness in newborns.

This dual character of oxygen continues to haunt us. Indeed, we think of oxygen as the fuel of life. Animals (humans included) use it to oxidize foods and produce energy essential for the living processes in the shape of ATP or heat. Since our bodies consist mostly of water, and oxygen is not very soluble in water, we have evolved a transport protein, haemoglobin to carry O_2 into our system. Haemoglobin in the red blood cells, transports about 90% oxygen we breathe to the mitochondria where it is used to oxidize the food materials. The reduced food loses electrons, which are then accepted by the electron carriers, such as nicotinamide adenine dinucleotide (NAD^+) and flavins (FMN and FAD). These now reduced species are re-oxidized in the mitochondria, releasing large amounts of energy. The oxidation is a multi-step process, so the energy is released gradually, using the electron transport chain (ETC) located in the mitochondrial membranes. Oxygen serves as the final electron acceptor in the ETC, adding the four electrons onto one O_2 molecule. This addition proceeds in a stepwise manner, as illustrated in Scheme 1.1:

(1) $O_2 + e^- \rightarrow O_2^{\bullet -}$ (superoxide radical)

(2) $O_2^{\bullet -} + 2H^+ + e^- \rightarrow H_2O_2$ (hydrogen peroxide)

(3) $H_2O_2 + e^- \rightarrow OH^- + OH^\bullet$ (hydroxyl radical)

(4) $OH^\bullet + e^- \rightarrow OH^-$ (hydroxyl ion)

(5) $2OH^- + 2H^+ \rightarrow 2H_2O$

Overall: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

Scheme 1.1: The reduction of oxygen produces two radicals – superoxide and hydroxyl.

Ideally, all of the electrons are consumed by oxygen and all of the intermediates get to be converted to the final product - water. However, the process is not 100% efficient and some of the intermediates manage to escape the system. The most likely intermediates to escape are: superoxide radical ($O_2^{\bullet -}$) and hydroxyl radical ($OH^\bullet$). The excess of these highly reactive oxygen free radicals can be detrimental for the living cells surrounding them, as will be discussed shortly.

1.2 Free radicals

Radicals contain one or more unpaired electrons and are therefore highly reactive. They are also called "free", since they exist independently. Radicals of biological importance can be C-, N-, O-, or S-centred. They are extremely important and useful in biological systems, since they metabolize various chemical compounds foreign to our body.[3] Indeed, the oxygen-centred radicals are probably most useful in breaking down poisons, such as: D-amino acids, drugs, toxins, pesticides, etc. In order for these radicals to function to our advantage, they must work together with a number of enzymes in our body. These enzymes keep the radicals "controlled", i.e. they regulate the free radicals' function by allowing them to react only with specific substrates. If, however, the enzymes are not able to control the radicals completely, some of the radicals escape into the cell matrix and, being the extremely reactive species that they are, attack the important molecules and structural units within the cell. The cell membranes, being made of lipids and proteins, are most likely to be attacked by oxygen radicals, initiating what is called lipid peroxidation (see Scheme 1.3).

Sources of oxygen radicals are various. As it has been mentioned previously, one of the sources of these dangerous free radicals is our basic metabolic pathway. The "leakage" usually happens at early stages of the ETC, hence superoxide radicals, ($O_2^{\bullet -}$), are released (see Scheme 1.1).

Some of our body constituents react directly with oxygen to make radicals[1]; for example hormone epinephrine (adrenaline) oxidizes very easy once exposed to air and makes $O_2^{\bullet -}$. Also, about 3% of all haemoglobin molecules carrying O_2 (oxyhaemoglobin molecules), form methaemoglobin and release $O_2^{\bullet -}$.

Finally, a number of enzymes use oxygen directly to make radicals. About 10% of O_2 we breathe in, not used by the mitochondria, gets to be taken up by oxidase and oxygenase enzymes, and also by non-enzymatic reactions. For example, the enzyme D-amino acid oxidase uses oxygen directly to oxidize the unwanted D-amino acids, producing H_2O_2 (which only needs one electron to make hydroxyl radical, as will be discussed shortly). The cytochromes P450 enzymes, located mostly in liver, are responsible for oxidizing a number of poisons to our body into less dangerous compounds. In fact, these enzymes serve as detoxification system in our body. However, cytochromes P450 can also release some $O_2^{\bullet -}$ radicals.

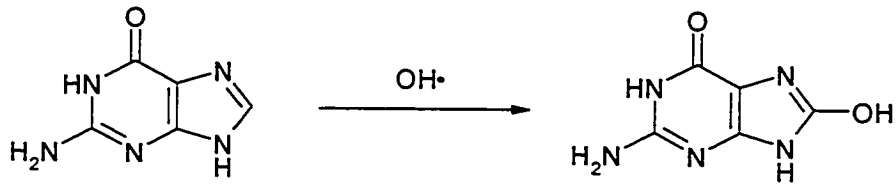
In order to explain the behaviour of hydroxyl (alkoxyl) radical, superoxide radical and hydrogen peroxide, one must look into some of their properties.

Table 1.1: Most common O-centred radicals and reactive oxygen species (ROS)

Name:	Formula:	Radical:	ROS:
superoxide radical	$O_2^{\bullet -}$	+	+
hydrogen peroxide	H_2O_2	-	+
hydroxyl radical	$HO^{\bullet}$	+	+
peroxyl radical	$ROO^{\bullet}$	+	+
peroxide	ROOH	-	+
phenoxyl radical (from vitamin E*)	$E\text{-}O^{\bullet}$	+	-

* For the structure of vitamin E please see Table 1.4.

Hydroxyl radical is considered the most reactive oxygen-centred radical. It attacks all cells, initiating a free radical chain reaction in most cases. It easily attacks all components of the DNA. For example, it can add onto a molecule of guanine, as shown below (Scheme 1.2), and form 8-hydroxyguanine, resulting in an overall mutation of the genetic material.

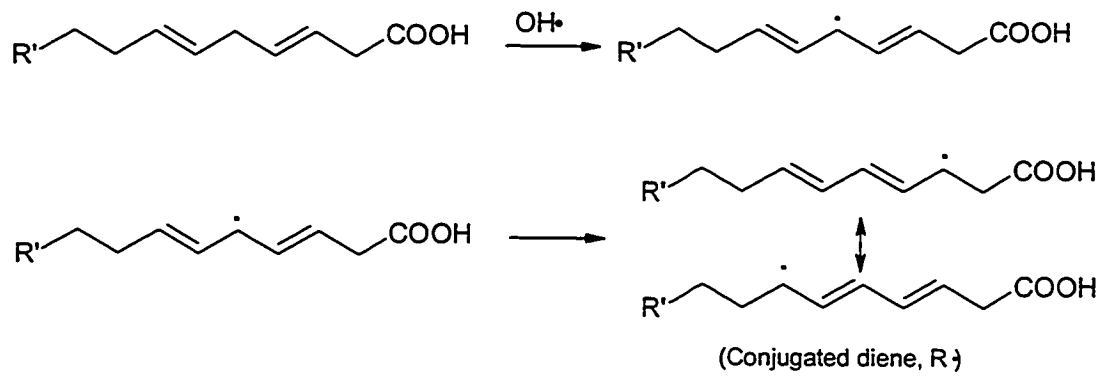


Guanine 8-Hydroxyguanine

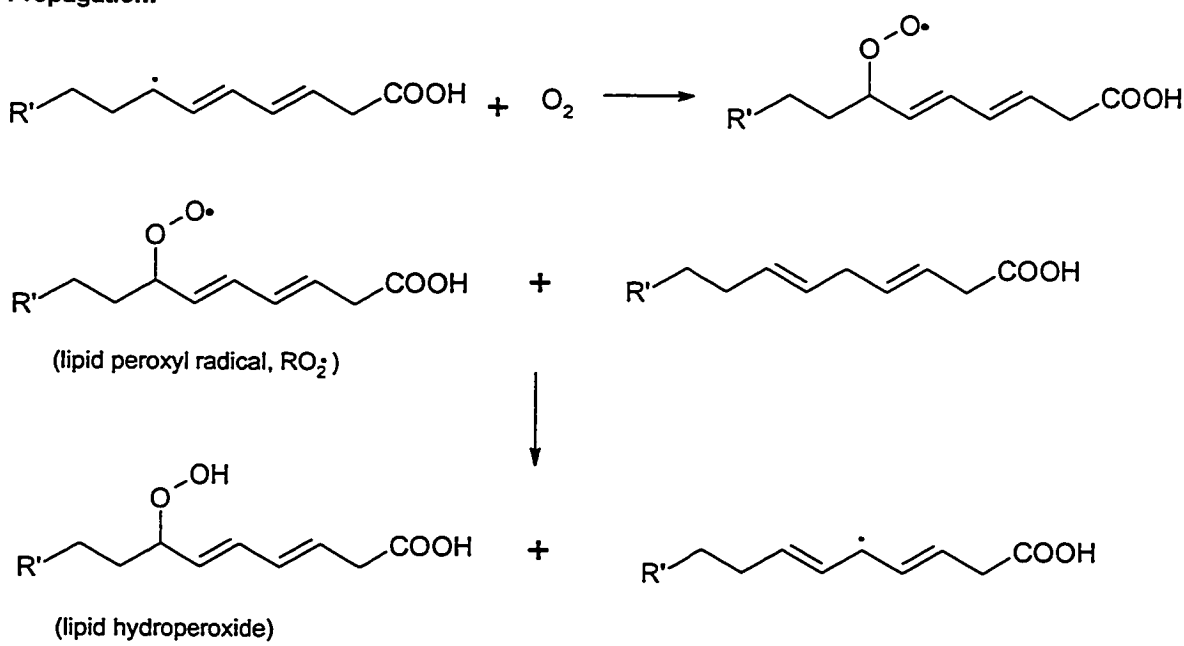
Scheme 1.2: Hydroxyl radical reaction with the genetic material, DNA

Hydroxyl radical can also take up one hydrogen atom and transform into water. This occurs in lipid peroxidation (Scheme 1.3):

Initiation:



Propagation:



Termination:

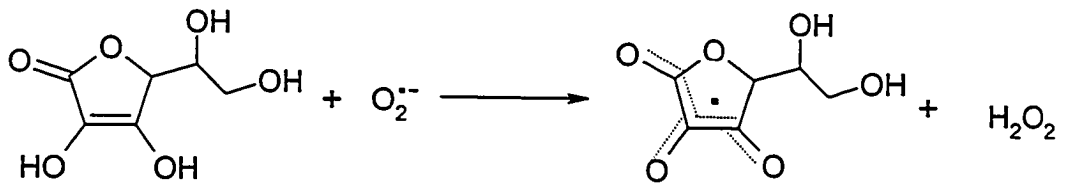
$$2\,R^{\cdot} \longrightarrow RR$$

$$2\,RO_2^{\cdot} \longrightarrow O_2 + ROOR$$

$$RO_2^{\cdot} + R^{\cdot} \longrightarrow ROOR$$

Scheme 1.3: Lipid peroxidation: initiation, propagation and termination of the chain reaction.

Superoxide radical is not as reactive as OH•. In water, it usually acts as a reducing agent, donating its electron to form the stable O_2. It can also oxidize substrates, such as ascorbic acid (vitamin C), as follows:



Ascorbic acid Semidehydroascorbate radical

Scheme 1.4: Vitamin C reaction with superoxide radical produces ascorbate radical and hydrogen peroxide.

In organic solvents, $O_2^{\bullet -}$ is much more reactive and can cause a lot more damage. It readily attacks the phospholipid cell membrane, as outlined in the Scheme 1.3.

Hydrogen peroxide, although not a radical itself, can be thought of as a precursor of a hydroxyl radical. It is completely water soluble, so it readily diffuses into the body and crosses membranes. It is not very reactive on its own, but it can easily accept a free electron from certain metal ions, and form the deadly OH•.

To summarize, a number of intracellular processes produce oxygen-derived radicals, hence they are fairly common in all living cells. Nonetheless, our body has developed a defence mechanism based mostly on various "anti-radical" proteins and enzymes, as mentioned earlier. In addition, human cells are equipped with a number of radical

scavengers. Although nature has given us several fronts of protection against these harmful free radicals, they still manage to cause extensive oxidative damage. Over the years of research free radicals have been associated with a number of diseases including: cancer, arteriosclerosis, stroke, rheumatoid arthritis, Alzheimer's disease, diabetes, emphysema, iron overload, malaria, muscular dystrophy, Parkinson's disease, as well as aging.[1, 2, 4]

1.3 The Free Radical Aging Theory

It is hard to define the process of aging, and equally difficult to explain why it is happening. Scientists agree that this is a molecular or cellular phenomenon. Several theories have appeared in an attempt to rationalize this very complex process.[2]

(i) Stochastic theory sees aging as a random event. We age simply because "our equipment", our bodies deteriorate over time.

(ii) Developmental theory suggests that our neuroendocrine system and our immune system are programmed to slow down as we age. Surely, hormones are involved in regulation of many essential processes in our bodies, such as metabolism, reproduction, immune function, protein synthesis, growth, and behaviour. With age, all of these processes diminish or even shut down their activity. Furthermore, it has been recognized for a long time now that our immune system also breaks down with old age.

(iii) Programmed theory states that every cell in our body is scheduled to self-destruct after a certain time period. In other words, our genes control growth, maturation, deterioration and death.

(iv) Free radical theory was first proposed by Harman in 1956.[2] He suggested that "the oxygen-derived free radicals cause progressive, random damage to enzymes and

other proteins, unsaturated fatty acids and phospholipids in cell membranes, as well as to DNA and RNA, with resultant cell senescence."[2] Nowadays we have abundant evidence to support this theory that aging is basically a result of all free radical damage in our bodies over the years. If free radicals are not completely responsible for aging, they are definitely considered a very important part of the aging process. Numerous studies have been conducted in support of the free radical theory of aging.

Being a very complex process that it is, aging is definitely a consequence of many factors combined, and there is an extensive theory overlap. To be sure, there is enough evidence to support all of the theories described herein, and therefore they are probably all involved, with the free radical one having the most facts and experimental data to support it.

1.4 Antioxidants

As mentioned earlier, our body has several defence mechanisms to fight free radical oxidative damage, either by preventing it or by quenching it. One particular kind of defence involves antioxidants, which are chemical compounds capable of delaying or inhibiting the oxidative damage aimed at a specific target molecule. Antioxidants are found throughout our body, since oxygen-derived free radicals can attack all the molecules in our system: lipids, proteins, nucleic acids and carbohydrates. There are several mechanisms by which antioxidants protect various targets:[1]

(i) oxygen-derived radical scavenging, which happens either by enzymes (as mentioned earlier), or by a direct chemical reaction during which the antioxidant molecule gets to be used up;

(ii) reducing the formation of oxygen-derived species;

(iii) binding metal ions capable of providing free electrons to relatively unreactive species (such as H_2O_2) and making them into very reactive ones ($OH^{\bullet}$);

(iv) repairing damage to the target;

(v) destroying badly damaged target molecules and replacing them with new ones.

Table 1.2: Summary of natural defence systems against free radicals

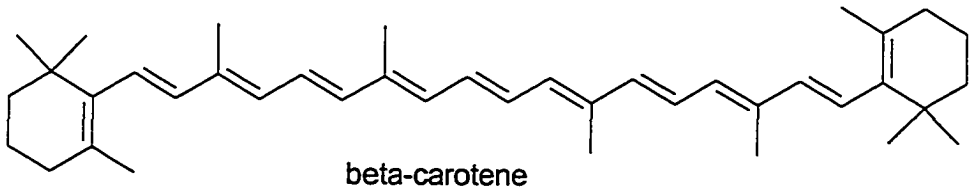
Antioxidant enzymes:	catalaze, glutathione peroxidase, glutathione reductase, superoxide dismutase
Metal-binding proteins:	ceruloplasmin, ferritin, lactoferrin, metallotheinein, transferrin, haemoglobin, myoglobin
Common antioxidants:	bilirubin, carotenoids, flavonoids, uric acid, thiols, vitamins A, C, E
Other antioxidants:	metal ions in antioxidant enzymes (Cu, Mn, Zn, Se)

Table 1.3: Some of the properties of the common antioxidants ("scavengers") [2, 4]

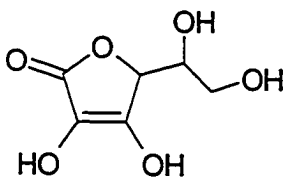
Antioxidant	Solubility	Sources
Vitamin A / carotenoids	fat	Orange-coloured fruits/vegetables, spinach, peas, peppers, broccoli
Vitamin C (ascorbic acid)	water	Citrus fruits, cruciferous vegetables, potatoes, various other vegetables
Vitamin E (tocopherols, tocotrienols)	fat	Nuts, whole grains, vegetable oils, seeds, butter, egg yolk, sweet potatoes
Flavonoids	water	Coloured fruits/vegetables, onions, apples, red grapes, tea, chocolate, potatoes
Uric acid, bilirubin, thiols	water	Common metabolic constituents

Most common scavenger antioxidants are bilirubin, uric acid (see Table 1.2) and dietary antioxidants, such as vitamins A, C and E, as well as carotenoids (beta-carotene and lycopene).

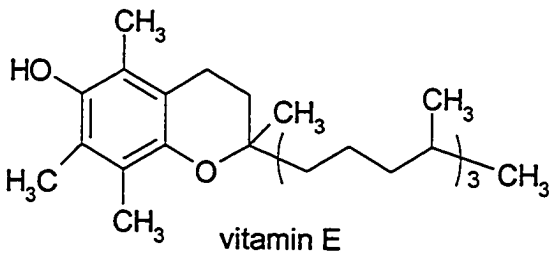
Table 1.4: Chemical structures of various important dietary antioxidants



beta-carotene



vitamin C

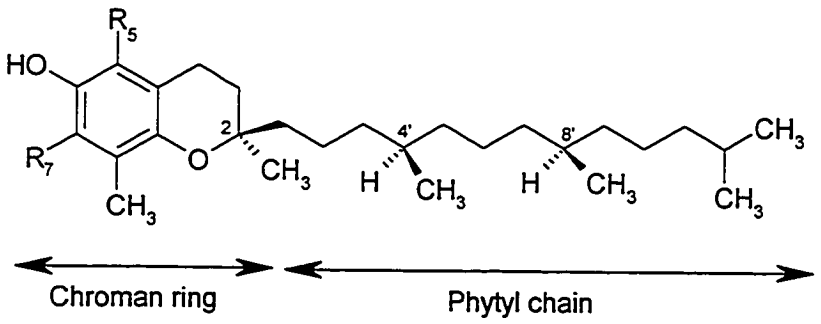


vitamin E

1.5 Vitamin E (α-tocopherol)

Vitamin E (Table 1.4) is the major fat-soluble antioxidant. Just like most phenolic antioxidants, it is a chain-breaking antioxidant. It can be found in all living cells, imbedded into the phospholipid membrane matrix. It was first discovered in 1922 by Evans and Bishop[5], while studying female rat fertility. It was found to be essential for the rat reproduction process, and hence given a name tocopherol, from Greek words *tokos* (childbirth) and *phero* (to bring forth). Vitamin E's chemical reactivity and biological function have been of much interest over the years.

What we generally call vitamin E is essentially a mixture of d-α-, d-β-, d-γ-, and d-δ-tocopherols (Table 1.5), which essentially differ from one another only in the number and position of the methyl groups on the aromatic ring.



	R_7	R_5
α-T	CH_3	CH_3
β-T	H	CH_3
γ-T	CH_3	H
δ-T	H	H

Table 1.5: Structure of α-, β-, γ- and δ-tocopherol (T).

The α-, β-, γ-, and δ-tocotrienols have been found to have some vitamin E activity as well. They are also natural antioxidants and have the same chroman ring as tocopherols, but differ in the phytyl side chain, which contains three double bonds.

The most active form of vitamin E is d-α-tocopherol. It can often be absorbed in a form of an ester, such as acetate or succinate, and then hydrolysed into its active phenolic form. Synthetic vitamin E, dl-α-tocopherol, contains about 12.5% of d-α-tocopherol and the remainder consists of seven other, less reactive tocopherols. Naturally occurring vitamin E does not necessarily contain a great deal more of the most active d-α-tocopherol, but it has been found to be much better absorbed into our blood plasma (about twice as much as the synthetic version).

Tocopherols inhibit lipid peroxidation by scavenging lipid peroxyl radicals (see Scheme 1.3) much faster than they can react with the nearby fatty acids:

α-TOH + lipid-$O_2^{\bullet}$ → α-TO$^{\bullet}$ + lipid-O_2H, where TOH stands for tocopherol.

Tocopherols owe their reactivity to the phenolic OH group. The phytyl side chain favours the insertion into the lipid bilayer (fat solubility). Once produced, the α-tocopheroxyl radical is relatively unreactive, partially due to the steric hindrance caused by the full substitution of the chromane ring. Moreover, the phenoxyl radical is stabilized by conjugation. It generally does not continue the chain reaction but is eventually destroyed by reaction with another peroxyl radical. In some systems the α-tocopheroxyl radical is quenched and α-tocopherol regenerated by a reaction with one of the water-soluble reducing agents. The most common method is regeneration by a reaction with ascorbic acid-vitamin C:

$$\alpha\text{-TO}^{\bullet} + \text{ascorbic acid} \rightarrow \alpha\text{-TOH} + \text{ascorbic acid}^{\bullet}$$

The ascorbic acid radicals are quenched and the resulting molecular products most likely excreted out of the biological system. Hence oxidative damage causes irreversible loss of vitamin C, while tocopherols are recycled.

1.6 Novel antioxidants

Vitamin E's properties have intrigued the scientists over the years. It is commonly known today that vitamin E is "the major (and possibly only) lipid-soluble, chain breaking antioxidant in human blood."[6] However, it is also known that the quantities of vitamin E in the membranes are not large: a ratio of one tocopherol per thousand polyunsaturated fatty acid side chains. Vitamin E's unique antioxidant reactivity inspired scientists to attempt designing a lipid-soluble antioxidant more effective than vitamin E, i.e. one which would be more potent by being more reactive with the free radicals and/or by being more readily adsorbed into the cell's phospholipid bilayer. There have been a number of variations on vitamin E described in the literature. In the last few years, though, the synthesis of novel antioxidants, apparently a very promising field, seemed to slow down a bit.

In terms of the design of novel antioxidants, inspired by a structure of vitamin E, the first people to explore the effect of various ring substituents on the rate of reaction of the designed phenol with the free radicals, were Howard and Ingold.[7] Over forty years ago, they concluded that the maximum rate of reaction was achieved with phenols having a 4-methoxy group and all other four positions fully substituted with four methyl groups. This idea was further explored over the years. The empirical results obtained by synthesizing various tocopherol-type antioxidants and reacting them with free radicals were attempted to be explained by theoretical chemists. The quantification of the experimental results would serve two purposes: it would better explain the existing trend in the tocopherol-type structure reactivity, and it would help design novel, potentially more efficient lipid-soluble antioxidants.

It has been suggested that there are two mechanisms by which the phenolic antioxidants react with free radicals: the hydrogen atom transfer (HAT) and the single electron transfer (SET),[8] as outlined in the Scheme 1.5.

Hydrogen Atom Transfer (HAT):

$$ArOH + ROO^{\bullet} \rightarrow ArO^{\bullet} + ROOH$$

Single Electron Transfer (SET)

$$ArOH + ROO^{\bullet} \rightarrow ArOH^{+} + ROO^{-}$$

$$ArOH^{+} + H_2O \leftrightarrow ArO^{\bullet} + H_3O^{+}$$

$$ROO^{-} + H_3O^{+} \leftrightarrow ROOH + H_2O$$

Scheme 1.5: Hydrogen atom transfer and single electron transfer mechanisms as the main pathways of antioxidant preventive action.

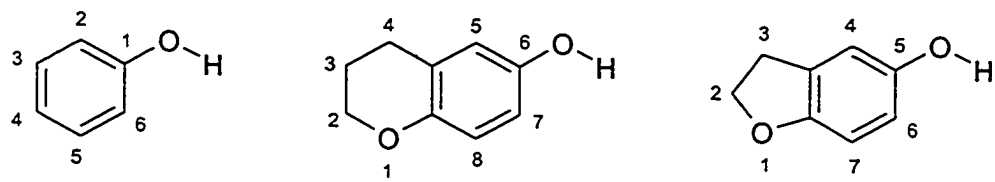
The overall reaction for both mechanisms is the same. The HAT pathway is obviously directly related to the bond dissociation enthalpy (BDE), since the weaker the O-H bond-the faster will be the reaction with free radicals. The SET mechanism, however, depends on the ionization potential (IP) of the phenol substrate. Both mechanisms have been found to occur simultaneously, but with different rates. In addition to the two main mechanisms, a number of other factors may play a role in determining what makes a good antioxidant, such as presence of bulky groups around the OH group and hydrogen bonding affinities of the solvent in question. In biological terms, solubility and transport to specific tissues are probably important to consider when designing a novel antioxidant. Nevertheless, "to do a systematic study of antioxidants from a theoretical prospective, it is desirable to determine accurately both BDE and IP, the former relevant to the atom-transfer mechanism and the latter relevant to electron transfer." [8] Wright et al[9] proposed a density functional theoretical method for calculating gas-phase O-H bond BDE's and IP's for phenols, all with a purpose of attempting to create a lipid-soluble antioxidant superior to vitamin E.

RESULTS AND DISCUSSION

Chapter 2: Results and Discussion

2.1 Theoretical calculations leading to the design of novel antioxidants

Wright et al. have used a density functional method of calculation to determine gas-phase phenolic O-H bond dissociation enthalpies.[9] The process confirmed that different substituents on the aromatic ring influence the strength of the phenolic O-H bond. More specifically, Howard and Ingold's[7] observations were confirmed: the methyl and methoxy substituents on the aromatic phenolic ring weaken the O-H bond. The BDE (gas phase) values directly calculated for the phenols were in agreement with the experimental values. Moreover, the values obtained by additivity, with constants for methyl and methoxy substituents derived using the theoretical calculational method, also agreed closely with the "known" BDE values. The Wright group used this method to determine the effect of substituents on BDE's in the fused 6- and 5-membered oxygenated rings, that is the chromanol (related to vitamin E) and dihydrobenzofuranol systems (Scheme 2.1).



Scheme 2.1: Ring numbering for phenols, chromanols and dihydrobenzofuranols.

The BDE results for these molecules were consistent with the "known" values and the additivity rules proved to hold in these systems. Concurrent to these discoveries, a group of scientists in Italy (Lucarini et al.)[10] used a similar theoretical method to calculate phenolic BDE's, and they confirmed Wright's results. This was reassuring and prompted Wright, Johnson and DiLabio to extend their research by studying BDE's and IP's of several other phenolic antioxidants including: compounds related to vitamin E, commercial antioxidants

used as food additives, flavonoids in tea, stilbenes related to resveratrol and stericaly hindered phenols.[8] Based on the results they were able to predict relative rates of reactions of antioxidants with free radicals, including a comparison of results for hydrogen atom transfer (HAT) mechanism versus single electron transfer (SET) mechanism. They concluded that in almost all cases the dominant antioxidant-radical reaction mechanism is indeed the HAT one, and hence, when attempting to predict the antioxidant potency of certain compounds in question, one should primarily look into BDE's. In the calculations Wright used locally dense basis sets (LDBS) and found that, for example, the BDE for phenol was 87.05 kcal/mol, in agreement with the value obtained by using the full basis set for calculations (87.10 kcal/mol). Hence using the LDBS approximation proved satisfactory. The calculated BDE values were compared with the experimental data by means of a reference standard, 2,4,6-tri-*tert*-butylphenol, for which a BDE of 81.24 kcal/mol was obtained from calorimetric studies.[8] The performed calculations resulted in a set of optimized BDE additivity values (ΔBDE), Table 2.1.

Table 2.1: Recommended additivity values (ΔBDE values) on the OH BDE in phenolic compounds [kcal/mol], relative to phenol 87.10 kcal/mol

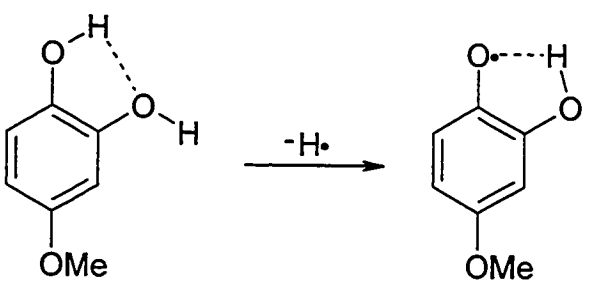
	Substituents		
Group	*Ortho*	*Meta*	*Para*
NH_2	-11.5	-0.2	-9.4
OMe	-1.4	-0.6	-6.1
OH	-9.2	-0.4	-5.9
$CHCH_2$	-4.0	-0.2	-4.7
tert-butyl	-2.7	-0.6	-2.2
CH_3	-2.0	-0.4	-2.5
Cl	+1.0	+1.2	-1.4
CN	+3.6	+2.7	+2.2
CHO	+8.0	+2.2	+2.4
COOH	+8.1	+2.5	+2.6
NO_2	+10.0	+3.4	+4.6

When hydrogen bonding effects are taken into account, the general trend appears to be that the electron donating groups on the aromatic ring lower the overall BDE of the phenolic OH group, while the electron withdrawing increase it. In the molecules with more than one phenolic hydroxy group, the -OH with the lowest BDE is most likely to react with the free radicals. The substituents positioned *ortho* to the phenolic OH bear additional constraints associated with calculation of the BDE. Namely, *ortho* substituents play largest role in potential steric effects and hydrogen bonding, Table 2.2.

Table 2.2: Recommended *ortho*-component additivity values (ΔBDE values) on the OH BDE in phenolic compounds [kcal/mol], relative to phenol 87.10 kcal/mol

Ortho Substituents				
Group	Electronic effect	H-bonding parent	H-bond radical	total
NH_2	-7.5	+4.0	-8.0	-11.5
OMe	-5.4	+4.0	0.0	-1.4
OH	-5.2	+4.0	-8.0	-9.2
$CHCH_2$	-4.0	0.0	0.0	-4.0
tert-butyl	-2.2	+0.5	-1.0	-2.7
CH_3	-2.0	0.0	0.0	-2.0
Cl	-1.0	+2.0	0.0	+1.0
CN	+1.6	+2.0	0.0	+3.6
CHO	+2.0	+6.0	0.0	+8.0
COOH	+2.1	+12.0	-6.0	+8.1
NO_2	+4.0	+6.0	0.0	+10.0

To illustrate how the additivity of the ΔBDE works we can look at compound **13**.



13 parent

13r radical

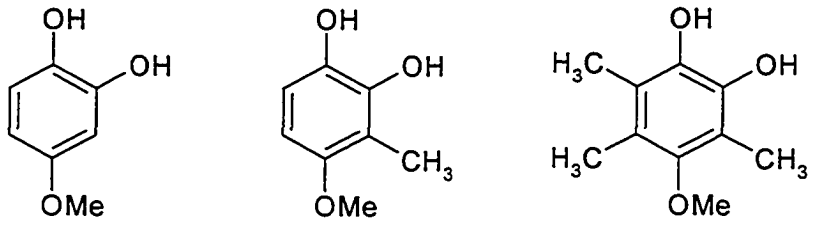
Scheme 2.2: Catechol **13** forming a catechol radical **13r**, according to the HAT mechanism

In order to determine the BDE of the reaction depicted in Scheme 2.2 one must assign the additivity ΔBDE values for all substituents and add them to the basic phenol BDE. From Table 2.1 we note that the *para* methoxy group lowers the OH BDE in greater extent than the *meta* one. Thus, the hydroxy group *para* to the methoxy will more likely loose a hydrogen radical via the HAT mechanism (Scheme 2.2). In the parent catechol, **13**, the *ortho* OH group will decrease the other OH BDE by about 5.2 kcal/mol due to electronic effect (Table 2.2). Both, the parent, as well as the radical, are stabilized by hydrogen bonding between the two hydroxy groups. In the parent, the stabilization effect is 4 kcal/mol while in the radical this effect is much larger, 8 kcal/mol. Finally, the *p*-methoxy group decreases the BDE by 6.1 kcal/mol due to resonance effects. Taking all of these substituent effects into consideration, one predicts: 87.1 (phenol BDE) – 5.2 + 4 – 8 – 6.1 = 71.8 kcal/mol as the BDE of the *para* phenolic OH in **13**. This represents a ΔBDE= -15.3 kcal/mol, which agrees with the experimental value of –15.3 kcal/mol (relative to phenol).[8]

Wright proposed that to create a new antioxidant superior to vitamin E, one must have the BDE lower than 77 kcal/mol (the experimental BDE value of vitamin E). Working under the assumption that the phenoxy radical (from vitamin E) is quenched and vitamin E is regenerated by means of vitamin C, the lower limit of the desired BDE should be 68 kcal/mol (the experimental BDE of vitamin C). A potential antioxidant compound having a lower BDE than that of the ascorbic acid would not be able to be regenerated efficiently. The IP of the designed antioxidant should be around the IP of vitamin E, but not much lower, otherwise the SET mechanism would take precedence over HAT. Using vitamin E as the standard, Wright et al. applied their theoretical predictions to design novel synthetic antioxidants. Based on the low BDE's and relatively high IP's calculated, a particularly promising group of compounds appeared to be catechols. Several of these target molecules were proposed and synthesized.

2.2 Preparation of simple catechols

Based on bond dissociation enthalpy and ionization potential energetical requirements, the following simple catechols were designed:

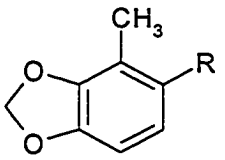


13 **8** **19**

Scheme 2.3: The first designed, simple catechols

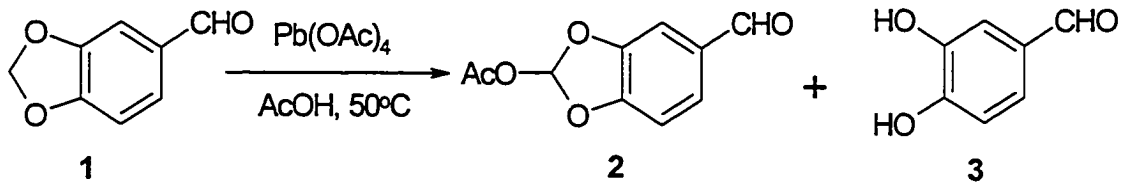
Compounds **13**, **8** and **19** share the catechol structure with the methoxy group positioned *para*. *p*-Methoxy catechol **13** is the simplest. Compound **8** has an additional methyl group positioned *meta*, thus its BDE=71.8kcal/mol (calculated previously for **13**) – 0.4 kcal/mol = 71.4 kcal/mol. Compound **19** is predicted to have BDE of 72.1 kcal/mol. All three compounds have fairly high IP's (as desired) and their BDE's are within the required 68-77 kcal/mol.

The methylenedioxy ring of the many readily available, naturally occurring methylenedioxy benzenes represents a protected catechol. We imagined utilizing the substituents in the available molecules such as piperonal **1**, or sesamol **4**, to help introduce the other substituents in our designed antioxidants.



4-methyl-5-R-substituted-1,3-benzodioxole

Following the introduction of the C-4 methyl group, the methylenedioxy ring could be opened to the catechol either by treatment with a strong acid, or oxidatively with lead tetraacetate. In order to investigate whether the latter process would work, piperonal **1** was heated with lead tetraacetate and acetic acid (Scheme 2.4).[12]



1 **2** **3**

Scheme 2.4: Oxidation and hydrolysis of piperonal.

The *ortho* acetal **2** was isolated in only 13% yield. It was confirmed by the appearance of the acetate methyl group as a singlet at 2.09 ppm in the ^{1}H NMR (Figure 2.1). The methylene dioxy peak of the piperonal (at 6.01 ppm, 2H) disappeared and a singlet appeared at 7.73 ppm (1H), corresponding to the H-2 of **2**. The catechol **3** formed in 17% yield as a result of **2** hydrolysing due to presence of the acetic acid. The ^{1}H NMR of **3** showed the two hydroxy hydrogens present at δ=8.78 ppm. Most of the starting material was recovered (48%). Since the reaction depicted in the Scheme 2.4 was involving a model compound resulting in the desired catechol, the reaction was left unoptimized. It was, however, used in further syntheses and subsequently improved.

The synthesis of 4-methoxy-3-methyl-catechol **8** commenced with the commercially available sesamol **4**, (Scheme 2.5).

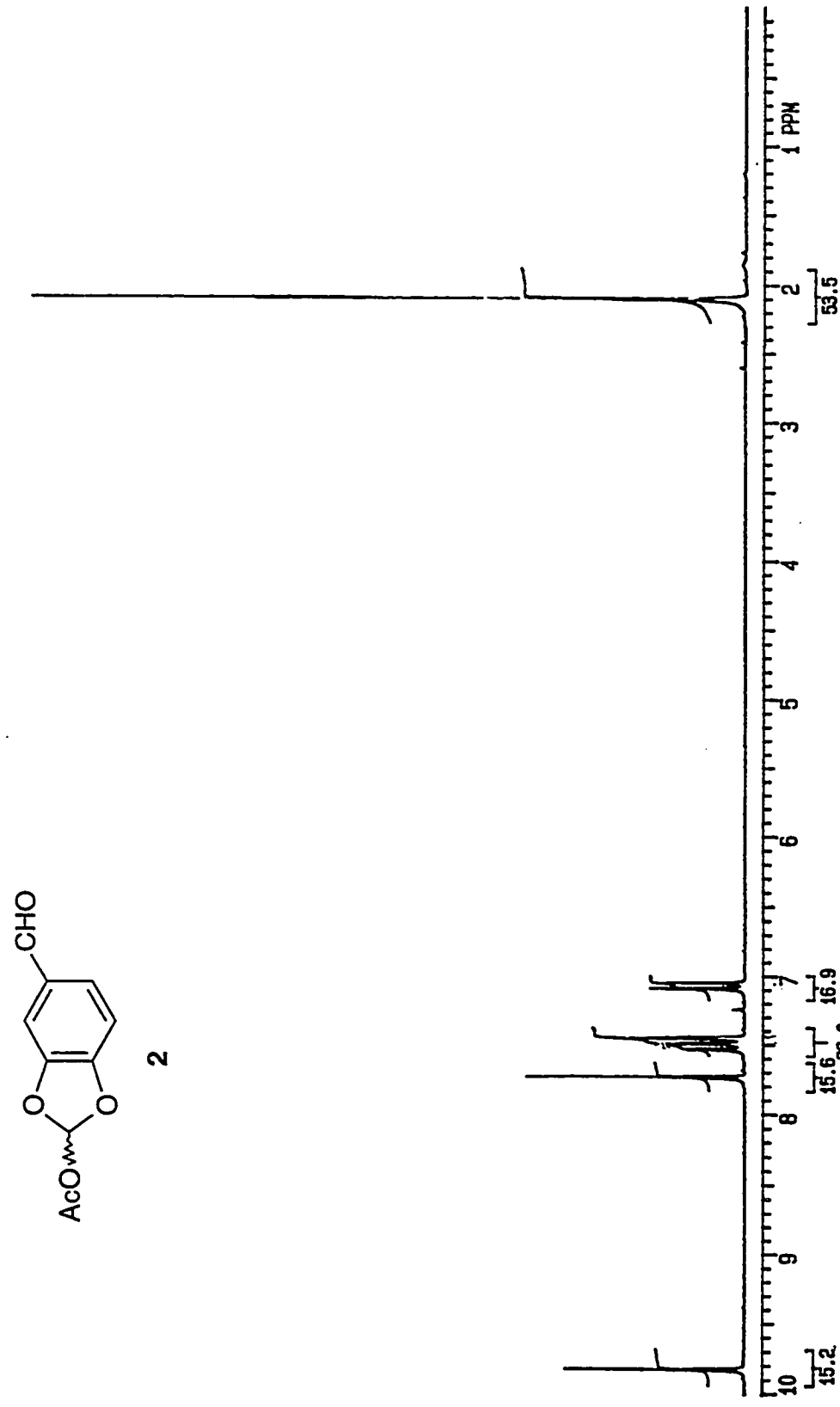


Figure 2.1: ^{1}H NMR spectrum of acetic acid 5-formyl-benzo[1,3]dioxyl-2-yl ester (2)

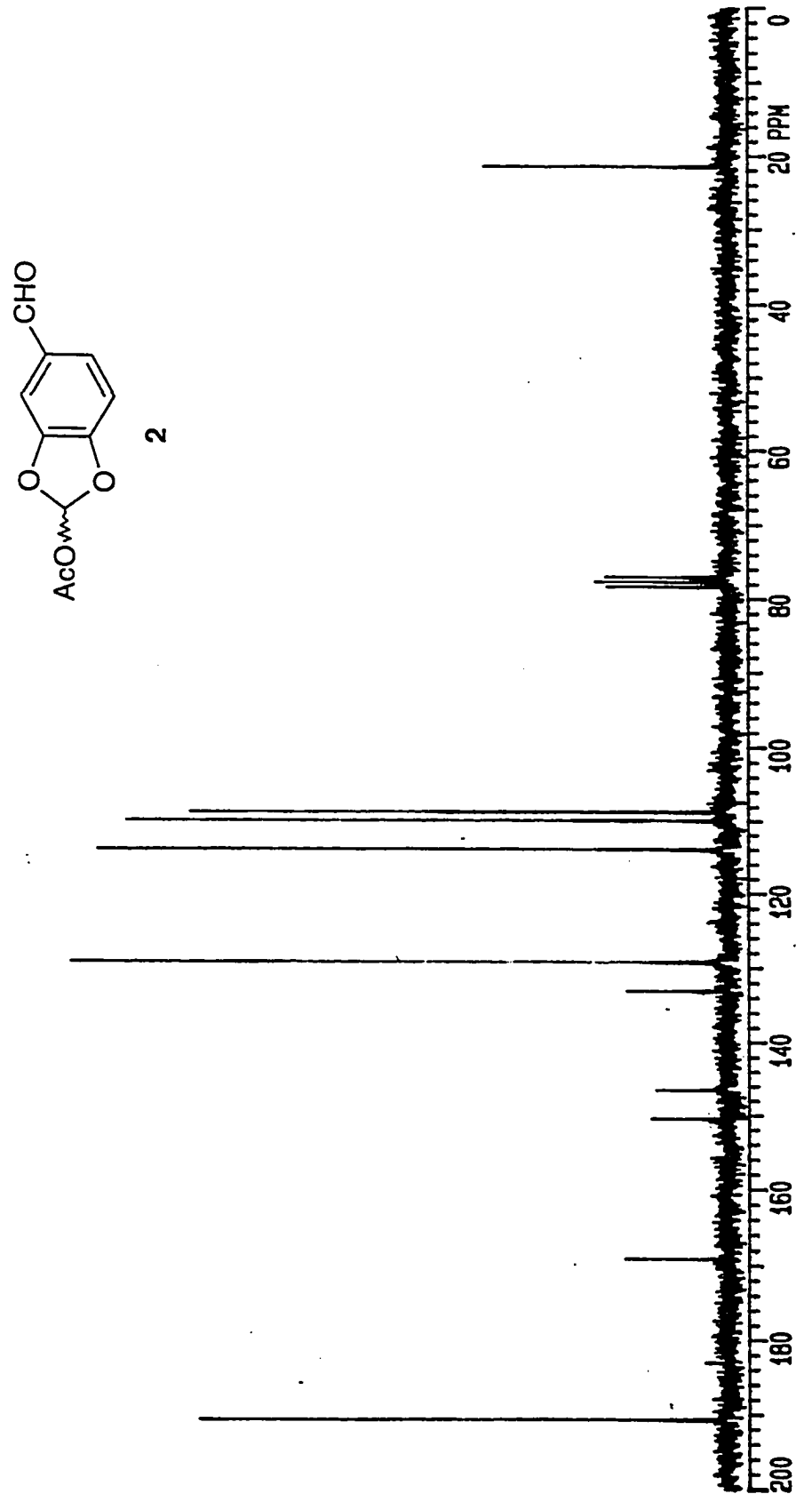
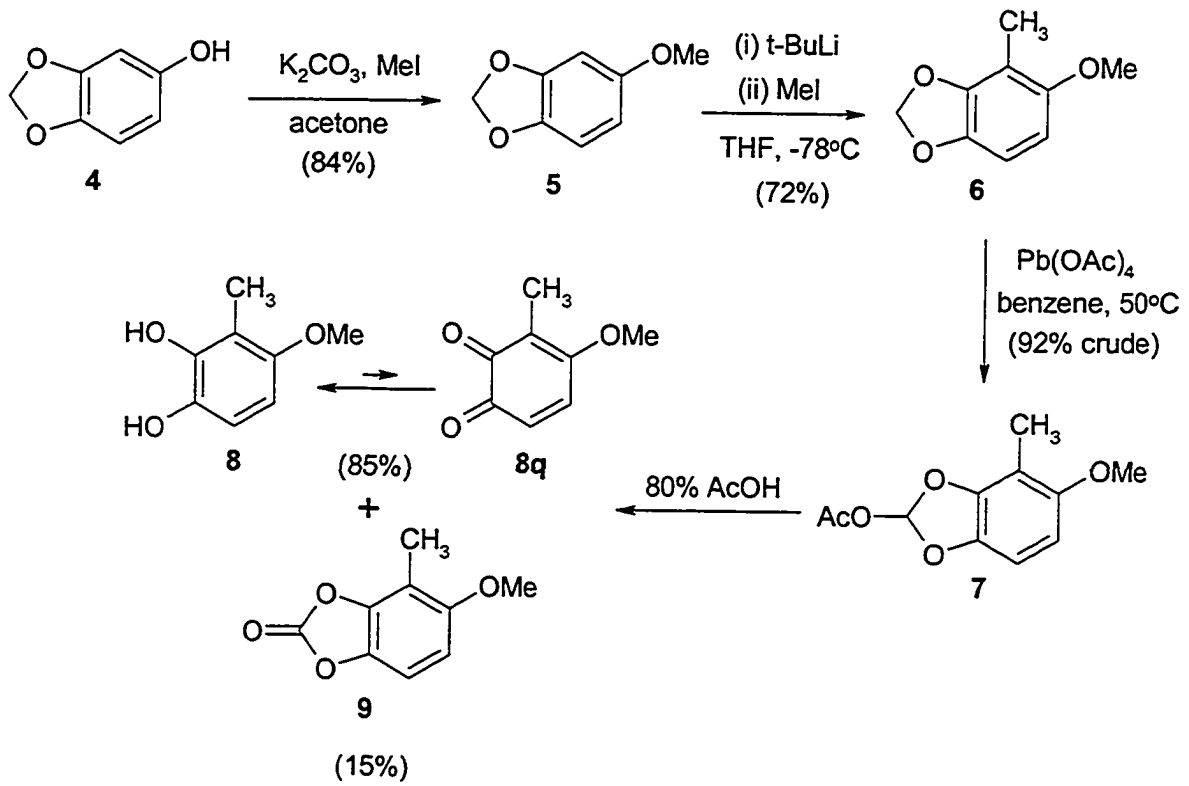


Figure 2.2: ^{13}C NMR spectrum of acetic acid 5-formyl-benzo[1,3]dioxyl-2-yl ester (2)



Scheme 2.5: Synthesis of 4-methoxy-3-methyl-catechol **8**.

The methylation of sesamol was carried out with potassium carbonate and methyl iodide either at room or reflux temperature, for 42 h. A definite advantage of this reaction proved to be a very clean work-up. The unreacted sesamol was simply washed-off with 10% sodium hydroxide aq., leaving a clean product **5**. The presence of a methoxy singlet at 3.73 ppm in the ^{1}H NMR of **5** confirmed the transformation. The IR peaks corresponded to the literature values[13]. The methyl-sesamyl ether **5** underwent a directed ortho-metallation (DoM) reaction with *t*-butyllithium, preferentially at C-3 due to the two adjacent heteroatom coordination sites. The lithiated species was treated with methyl iodide and afforded **6** in 72%. Purified by chromatography, the resulting white crystalline material had a melting point of 39-41°C. The ^{1}H NMR singlet for the methyl group appeared at 2.12 ppm and one of the aromatic hydrogens vanished indicating the conversion. The ^{13}C NMR also showed appearance of the methyl group at 9.13 ppm.

The next step involved the previously discussed oxidative cleavage of the methylene dioxy moiety with lead tetraacetate. The reaction showed optimum conversion and purity when freshly recrystallized lead tetraacetate was used. It was also demonstrated that in order to be able to isolate and characterize the ortho ester **7**, no acetic acid should be used during the reaction. Otherwise, compound **7** quickly hydrolyses to **8**, which is further oxidized to the quinone **8q**. The structure of **7** as an ortho acetate was confirmed by the appearance of the acetoxy methyl singlet at 2.09 ppm and a singlet at 7.64 ppm assigned to the remaining hydrogen on the methylenedioxy ring. The ^{13}C NMR spectrum of **7** showed the expected 11 signals including peaks at 170.16 and 108.76 ppm assigned to the carbonyl carbon and the carbon bearing the three oxygen atoms, respectively. During the first attempts, the solution of 10% sodium hydroxide was used for the hydrolysis that followed, however, this sequence resulted in poor yields. This can be explained by the fact that catechols are prone to further oxidation to *o*-quinones, especially under basic conditions. These quinone products, under the basic conditions, can then undergo a variety of Michael addition type reactions eventually leading to mixtures. In order to minimize the formation of the quinone, the hydrolysis was carried out in 80% aqueous acetic acid. The reaction could be tuned to produce a mixture of **8** and **8q** as exclusive products. An interesting by product **9** was also isolated. This compound, an off-white solid, had a sharp melting point of 110-111°C and it showed a M^+ at *m/z* 180.0392, very close to the calculated value of 180.0422. The ^{13}C NMR of **9** showed a carbonate carbonyl group at 155.54 ppm in addition to the other expected carbon resonances (Figure 2.4). The proton NMR showed only methyl (2.17 ppm), a methoxy (3.80 ppm) and two aromatic hydrogens (Figure 2.3). Based on this data, the compound was assigned the structure **9**. Mechanistically, the formation of **9** can be justified by taking into account that some of the *ortho* acetate **7** probably undergoes a second oxidation with lead tetraacetate to give a diacetate **10**, which readily hydrolyses to **9** (Scheme 2.6).

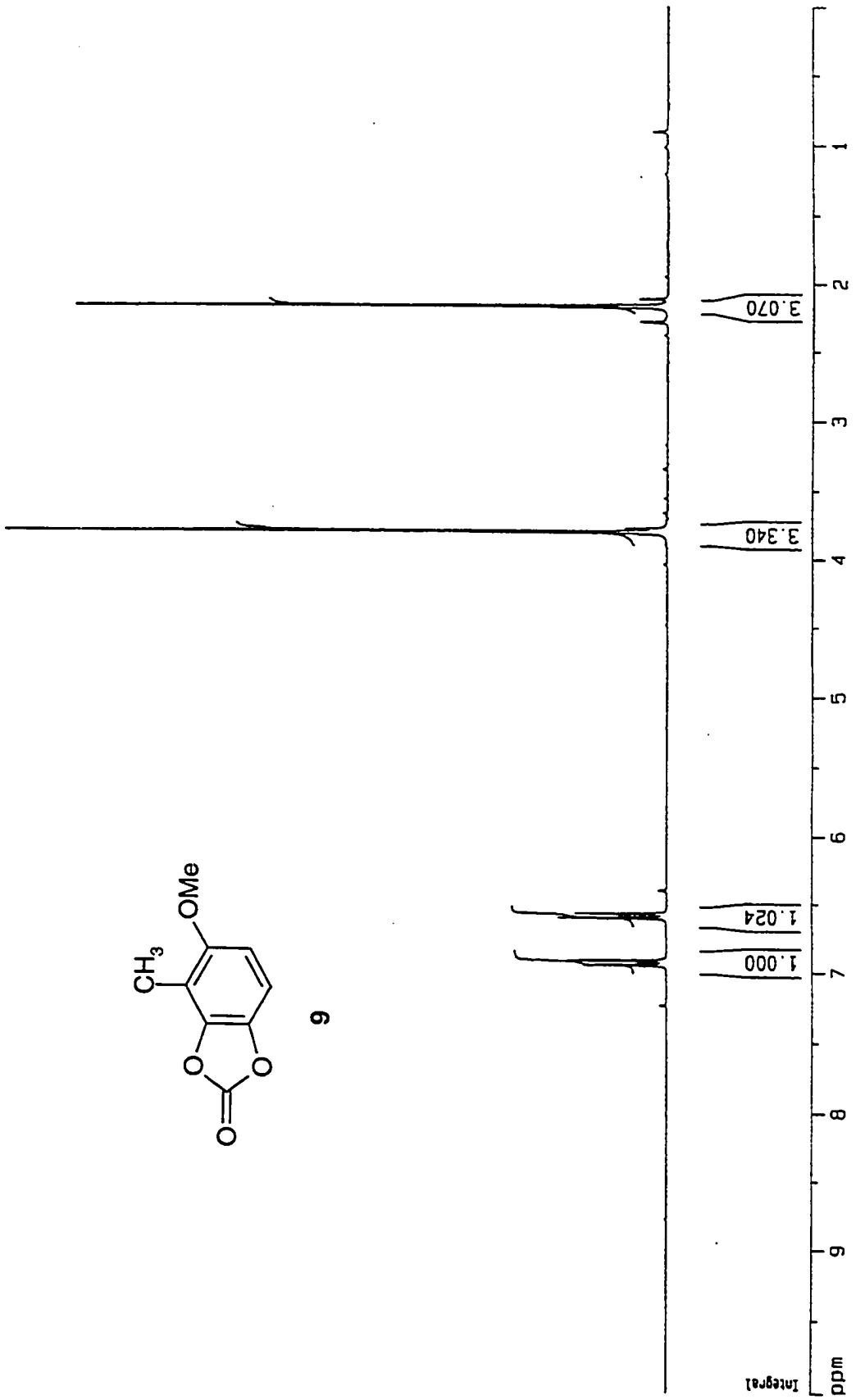


Figure 2.3: ^{1}H NMR spectrum of 5-methoxy-4-methyl-benzo[1,3]dioxol-2-one (**9**)

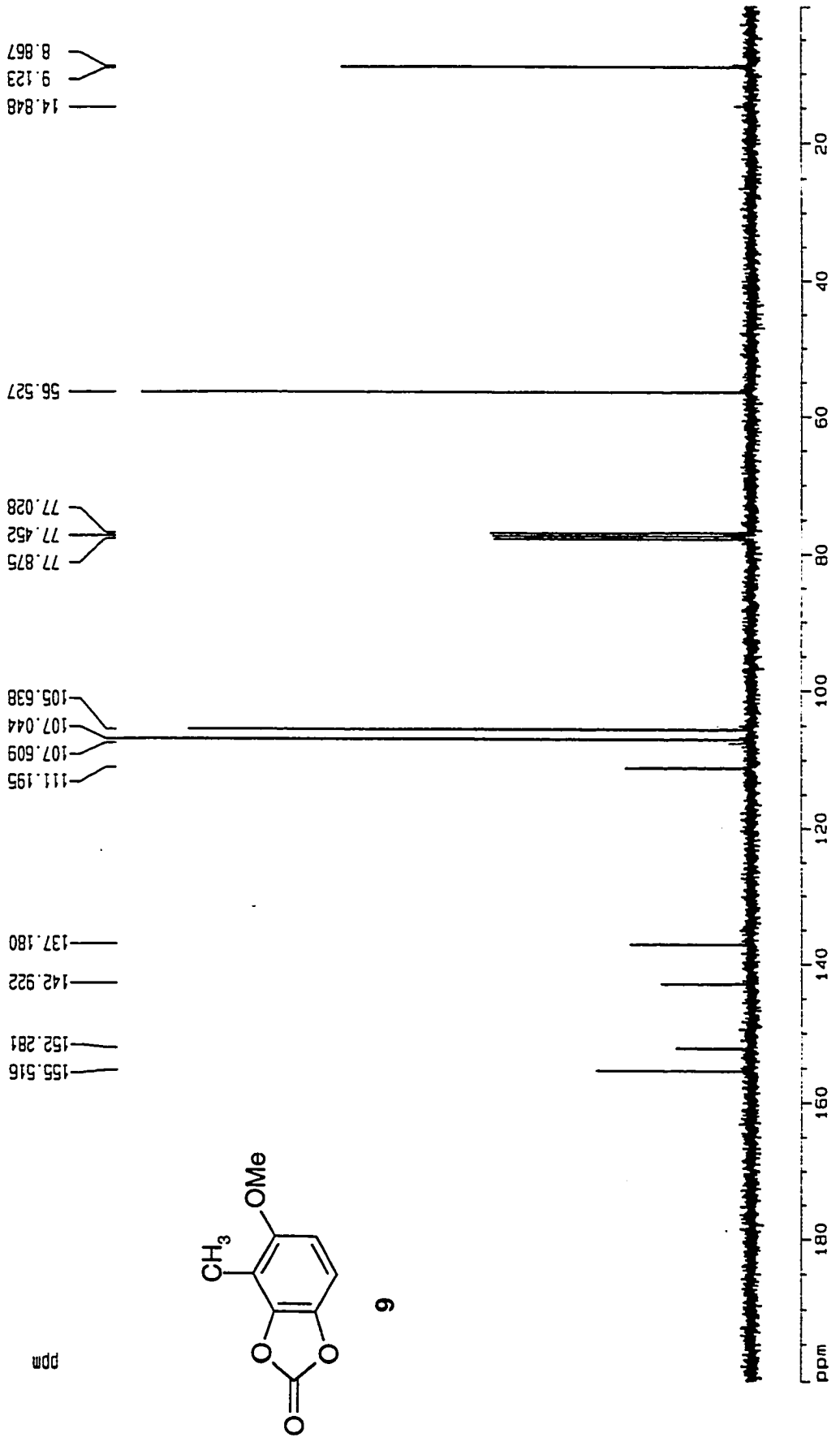
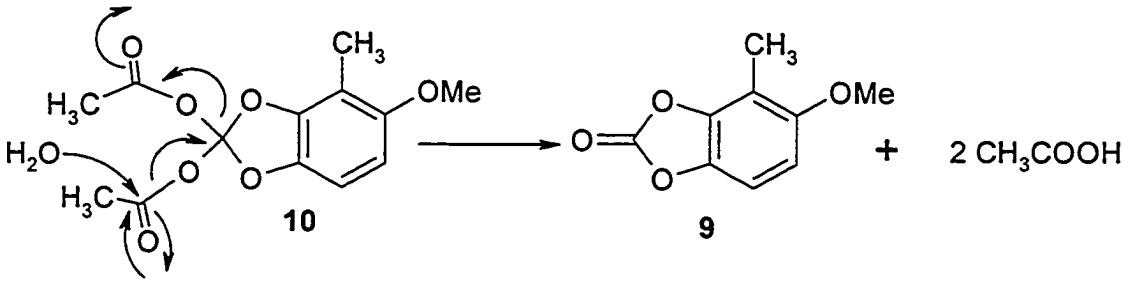


Figure 2.4: ^{13}C NMR spectrum of 5-methoxy-4-methyl-benzo[1,3]dioxol-2-one (**9**)



Scheme 2.6: Formation of intermediate **9**.

The major oxidation product, the mixture of **8** and **8q** (85%) was at first thought to be just **8**, since all the spectral information supported the catechol structure. Yet, the product's appearance as a red oil was consistent with what we empirically know about quinones.[14] Indeed, the product was determined to be greater than 98% catechol **8** with less than 2% quinone **8q** impurity, just enough material to colour all of the product material red. After repeating the hydrolysis reaction numerous times, it was discovered that the catechol **8** product is generally accompanied by 1-2% of quinone, irrelevant of the conditions. We concluded that the catechol **8** is not very stable, since it quickly oxidizes to the quinone form when exposed to air. Luly and Rapoport[15] observed similar trends when dealing with catechol **8** and quinone **8q**. They noticed that the catechol was very unstable, but couldn't infer as to reasons why that was the case. Due to its instability, the authors were not able to isolate **8** and characterize it. Similarly, they were able to observe the quinone **8q** on the TLC plate, but not to isolate it. We also found that, despite numerous attempts, we were unable to isolate and characterize the quinone **8q**. Luckily, the difficulties associated with handling the catechol compounds were recognized early in our synthetic series. Catechol **8** proved to be stable in solution and when stored under the atmosphere of argon at 4°C. Fortunately, an efficient method of converting all of the quinone to the catechol form was available.[11]

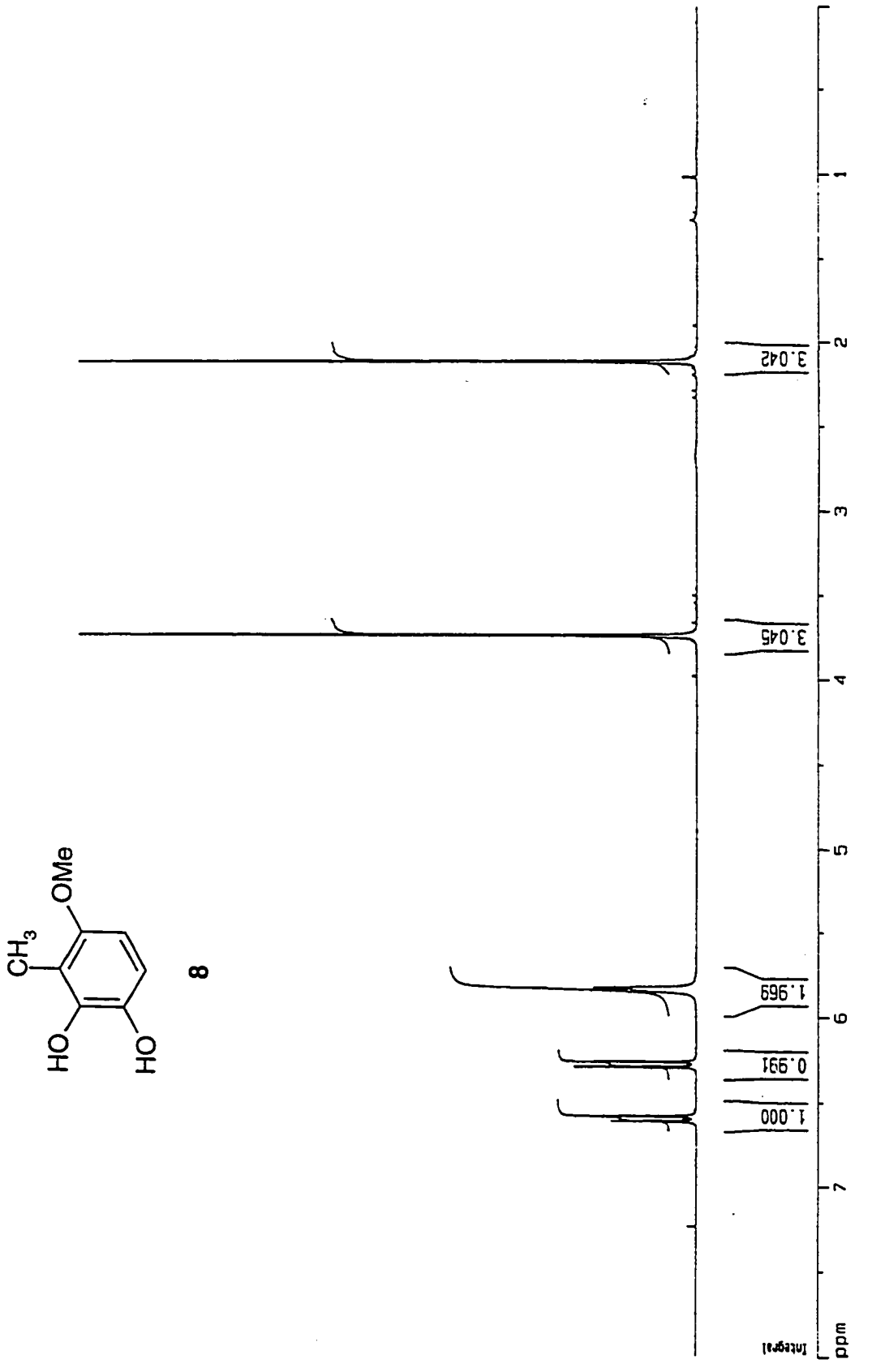


Figure 2.5: ^{1}H NMR spectrum of 4-methoxy-3-methyl-benzene-1,2-diol (8)

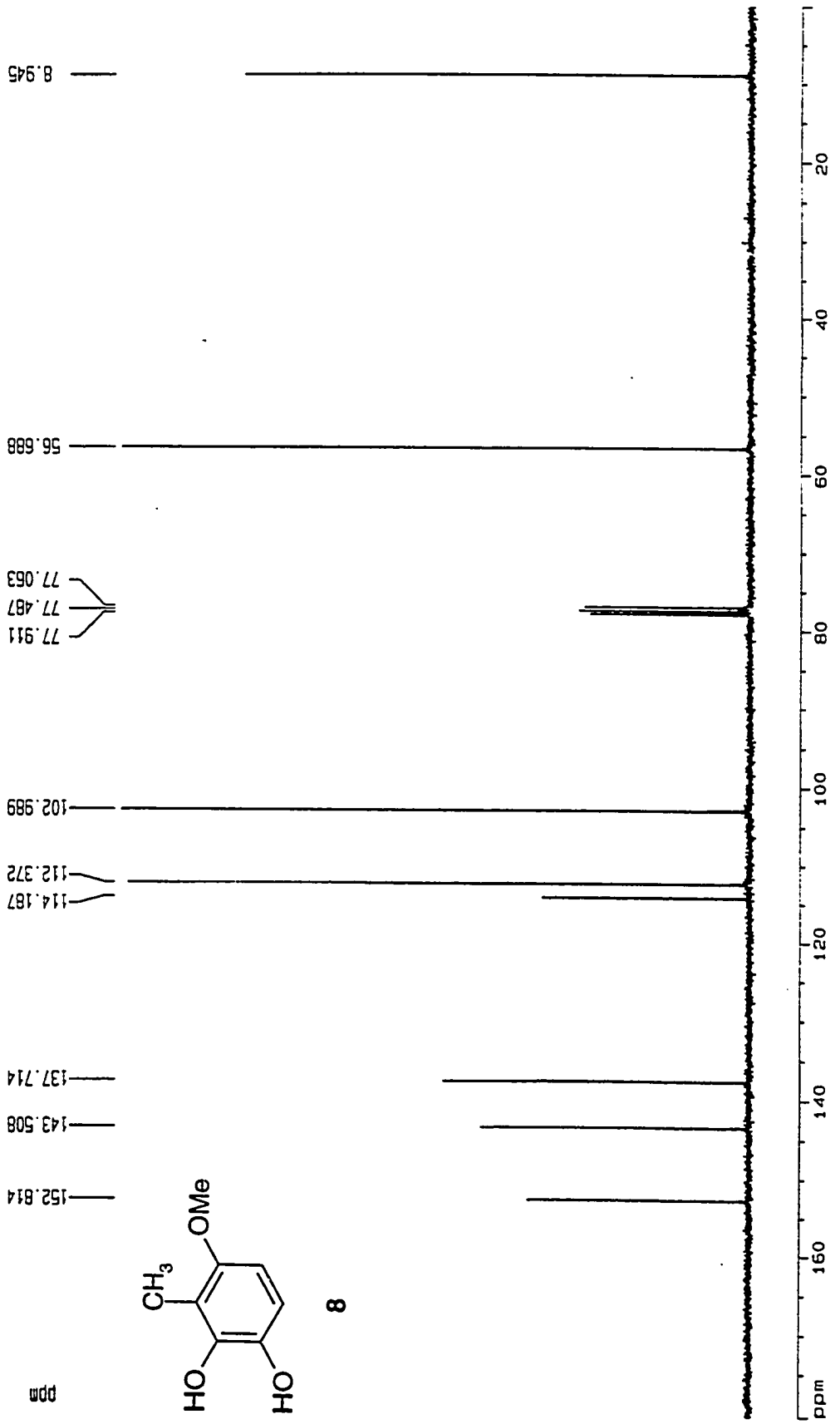
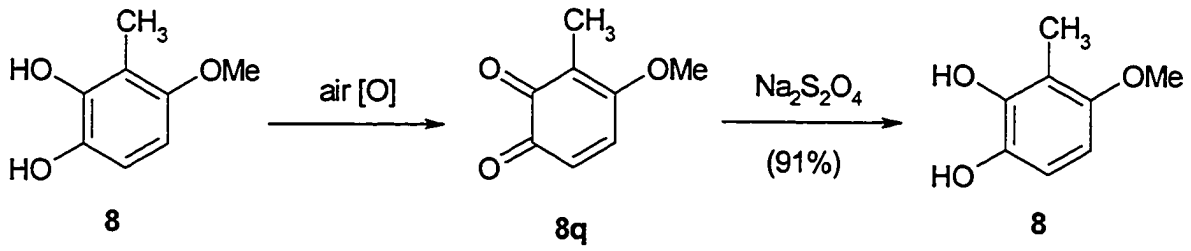


Figure 2.6: ^{13}C NMR spectrum of 4-methoxy-3-methyl-benzene-1,2-diol (8)



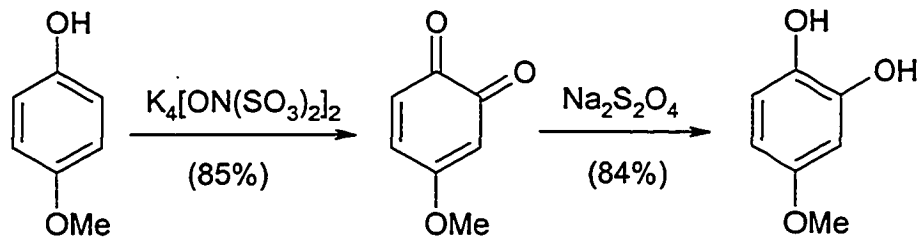
8 **8q** **8**

Scheme 2.7: The oxidation of 4-methoxy-3-methyl-catechol **8** exposed to air and reduction using sodium dithionate.

The catechol-quinone mixture was reduced with sodium dithionate in a biphasic ether – aqueous phase mixture. The experimental observation of the solution colour change from orange to yellow was the only indication of the reaction completion. Upon the workup the product **8** was placed quickly under argon and stored at 4°C. ^{1}H NMR and ^{13}C NMR data (Figure 2.5 and Figure 2.6) authenticated the formation of the desired catechol as a light yellow liquid. The calculated molecular ion for **8** was 154.0630 and the HRMS showed a M^{+} at *m/z* 154.0612. The methylene dioxy cleavage pathway as a means of generating catechols proved to be fast and efficient process. In practice, the acetylation of **6** was immediately followed by the hydrolysis, since the acetylation itself was often accompanied by some hydrolysis products anyway. The pathway from sesamol encompassed five steps, acetylation and hydrolysis being treated as separate steps and the final sodium dithionate reduction included, and yielded **8** in the overall yield of 44%. Due to the simplicity of the reaction conditions used and the availability of the reagents, this particular pathway might prove to be quite convenient to scale up in the future.

Having established an effective method of reducing the quinone **8q** to the corresponding catechol **8**, we hoped to extend the reduction process to encompass a variety of available *o*-quinones. Teuber and Staiger[16] used potassium nitrosodisulfonate, commonly called Fremy's salt, to prepare quinones from phenols. In these reactions a 4-substituted

phenol affords an *o*-quinone, while a phenol which is not substituted in the position 4 gives a *p*-quinone. Since our synthetic target **13** contained a *p*-methoxy substituent and it was an *o*-diol, starting from a readily available *p*-methoxy phenol **11** seemed quite reasonable. The phenol **11** was exposed to the Fremy's salt in the potassium phosphate buffer, resulting in the quinone **12**, 85% yield, as a brick red crystalline material. (Scheme 2.8).

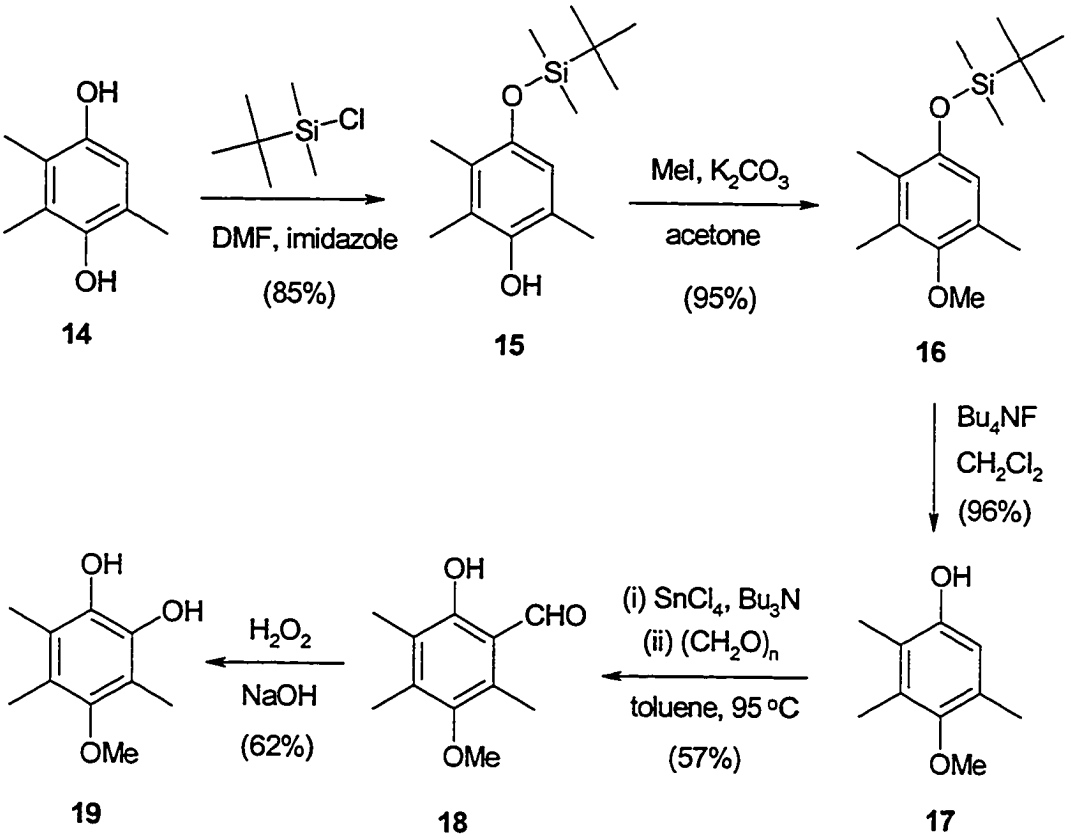


11 **12** **13**

Scheme 2.8: Synthesis of 4-methoxy-catechol **13**.

One of the four aromatic hydrogens of the *p*-methoxy phenol starting material vanished from the resulting ^{1}H NMR, and the H-3 and H-6 of the product **12** were shifted upfield (5.78 and 6.40 ppm respectively), indicating the absence of an aromatic ring. The quinone product was then quickly reduced using sodium dithionate to give the desired catechol **13**, in 84% yield after recrystallization from anhydrous ether (71% over the two steps). The melting point was 45.5-48.0°C, comparable to the literature value of 45-47°C.[17] Cooksey et al.[17] described the same procedures used for preparation of a number of catechols, including **13**. The overall yield reported by Cooksey was only 34%, while our unoptimized yield was 71%. Indeed, other than the cost of the potassium nitrosodisulfonate reagent, the synthetic pathway exhibited no other drawbacks. It proved to be an extremely fast and efficient method of generating the catechol **13**. It is not hard to imagine a possibility of the Fremy's salt oxidation / dithionate reduction sequence used on a variety of potential synthetic targets in the future.

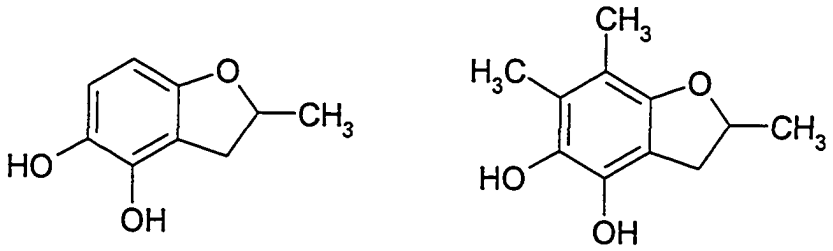
Dr. Helmi Hussain synthesized compound **19**, a fully substituted version of **13** or **8**. His work is herein described, since it pertains directly to the antioxidant catechols' synthesis and also since we measured its antioxidant activity in vitro. The sequence for the preparation of **19** is shown in Scheme 2.9. Details of the synthesis and the spectroscopic properties of **19** and the intermediates are given in the Experimental section, part B.



Scheme 2.9: Synthesis of 4-methoxy-3,5,6-trimethyl-catechol **19**.

2.3 Synthesis of 2,3-dihydro-benzofuran catechols

Based on the predicted bond dissociation enthalpy and ionization potential energy requirements, the following 2,3-dihydro-benzofuran catechols were designed:

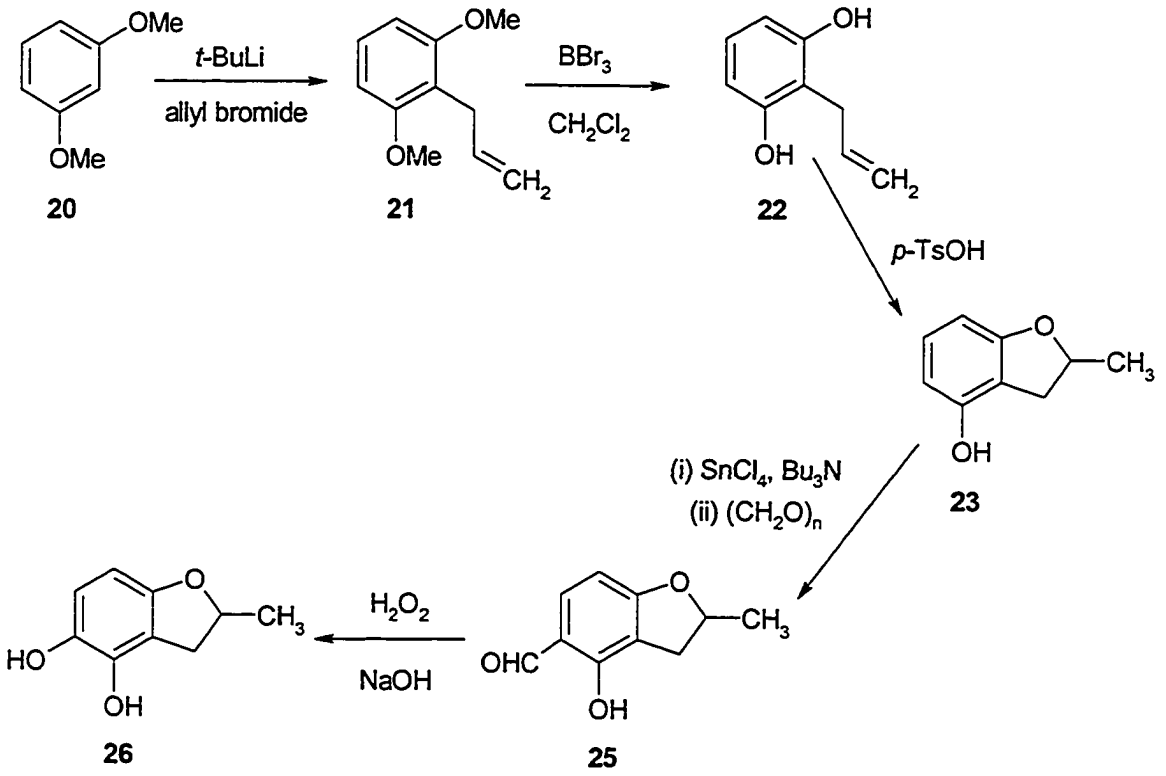


26 **33**

Scheme 2.10: The designed 2,3-dihydro-benzofuran catechols.

The molecule **33** resembles the γ-tocopherol orientation of the methyl groups around the aromatic system (please see Table 1.5 in the Introduction part). Instead of the chroman core the proposed structures have a 2,3-dihydro-benzofuran core. Since both structures are somewhat similar to the structure of γ-tocopherol, and additionally they are catechols, one can reasonably expect these compounds to be better antioxidants than γ-tocopherol itself.

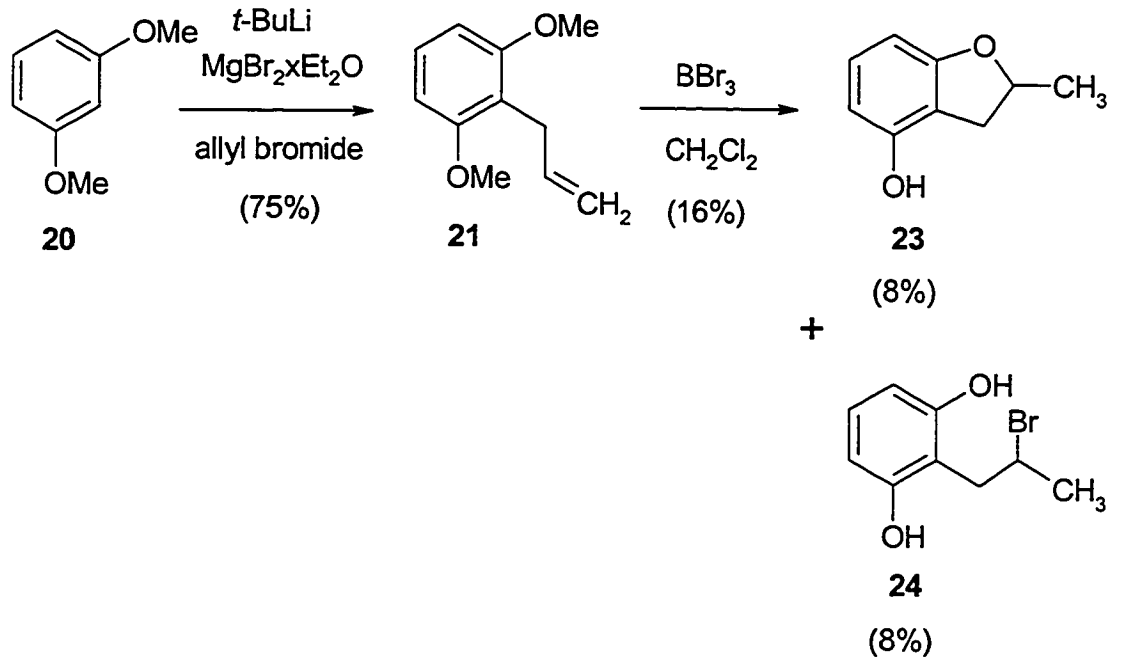
The synthesis of **26** commenced with the commercially available 1,3-dimethoxy benzene **20**. The plan was to allylate the 2-position and make **21**, demethylate the methoxy groups revealing a *meta* diol **22**, create the five-membered ring under acid conditions and hence make **23**, and then formylate the position *ortho* to the remaining hydroxyl group to form **25**. A final Dakin reaction was expected to yield the desired catechol **26** (Scheme 2.11).



Scheme 2.11: The proposed synthetic pathway for the 2-methyl-2,3-dihydro-benzofuran-4,5-diol **26**.

The original attempts to allylate the position between the two methoxy groups on the benzene ring via the 2-lithio derivative were vain. The reaction was tried using *t*-butyllithium as a base and allyl bromide as an electrophile, in THF at –78°C. Judging by the appearance of the anion solution (coloured bright yellow), the formation of the anion was successful. However, when the allyl bromide electrophile was added, no change was observed. Indeed, upon the workup no product was obtained and the unreacted starting material was recovered almost quantitatively. The assumption was made that a stronger electrophile, the one containing a better leaving group, such as allyl iodide, was needed. When the reaction was repeated using the allyl iodide instead of allyl bromide, it proceeded with exactly the same colour changes and it also resulted in full recovery of the starting material. Although convinced that the lithium salt of the starting material formed in solution,

another hypothesis was made. There was a possibility that the lithiated species wasn't a strong enough of a nucleophile to attack the allyl bromide/iodide electrophiles. In order to generate a less basic nucleophile, magnesium bromide was added to form a Grignard reagent. In the event 1,3-dimethoxy benzene **20** was reacted with the *t*-butyllithium base, followed by the $MgBr_2 \times Et_2O$ (3M) addition, at which point the solution turned almost colourless. Allyl bromide was then added. The usual workup afforded the desired product **21** (Scheme 2.12).



Scheme 2.12: Allylation of **20** to afford 2-allyl-1,3-dimethoxy benzene **21** and de-methylation of **21** to afford **23** and **24**.

The allylated product **21** was purified by flash chromatography and isolated as a clear yellow oil in 75% yield. The ^1H NMR peaks, (Figure 2.7) characteristic for the allyl group (methylene doublet of triplets at 3.52 ppm, vinylic methylene doublet of quartets at 5.01 and 5.07 ppm and vinylic methine multiplet in the 6.01-6.09 ppm region), confirmed the formation of **21**. The IR bands matched the literature values.[18] The HRMS of **21** showed a M^+ at *m/z* 178.1012; the calculated value for $C_{11}H_{14}O_2$ was 178.0994.

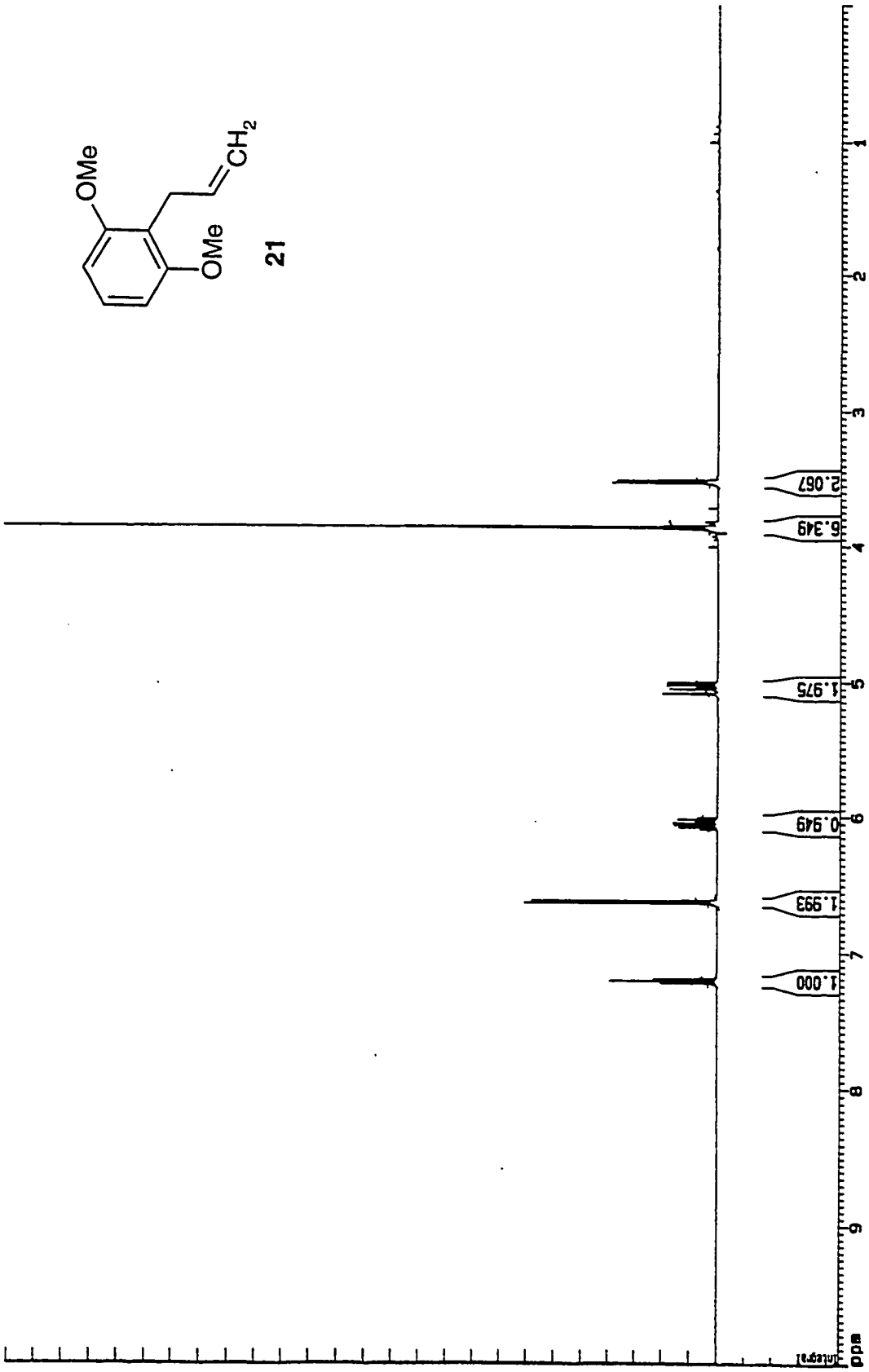


Figure 2.7: ^{1}H NMR spectrum of 2-allyl-1,3-dimethoxy-benzene (**21**)

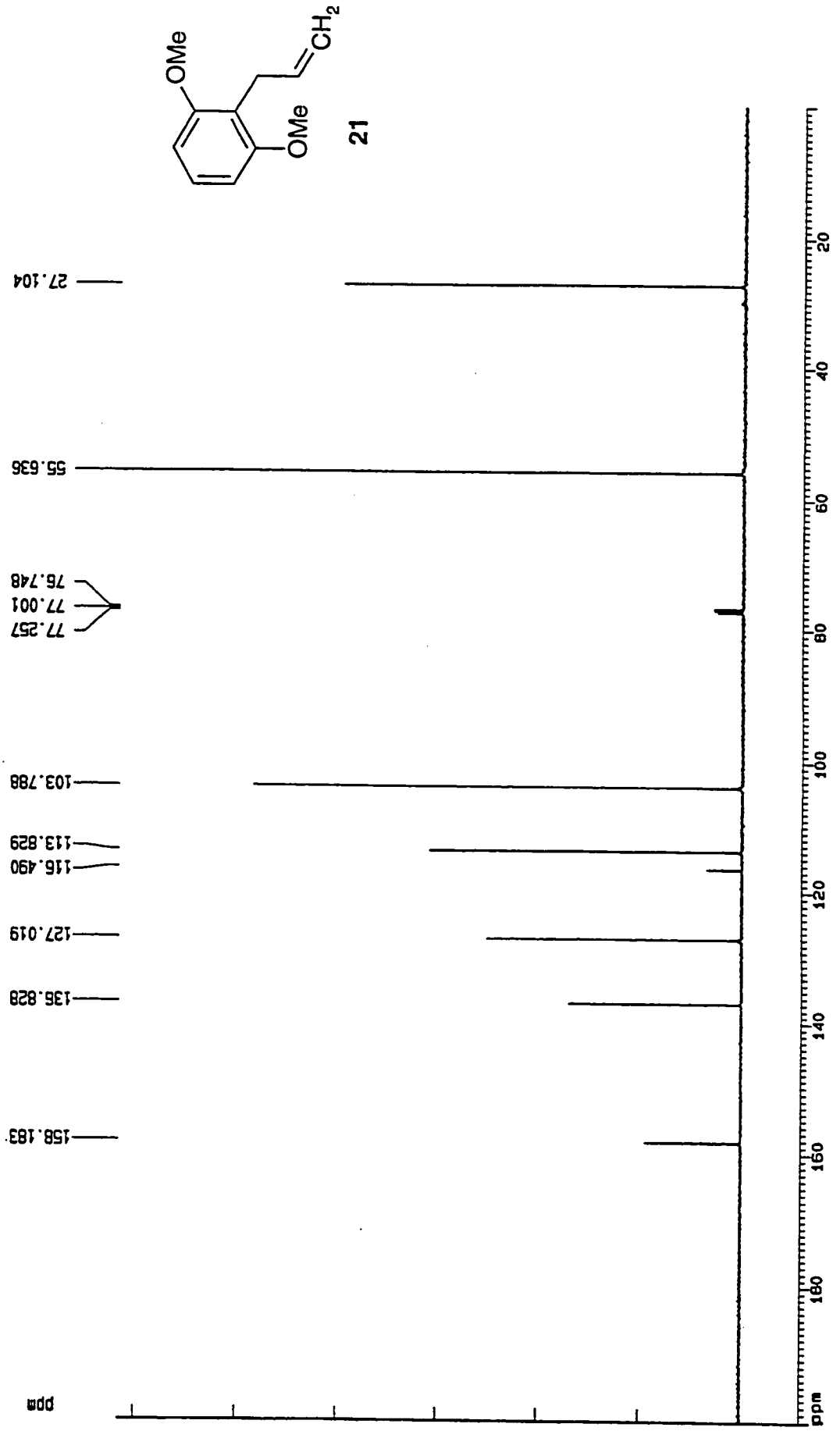
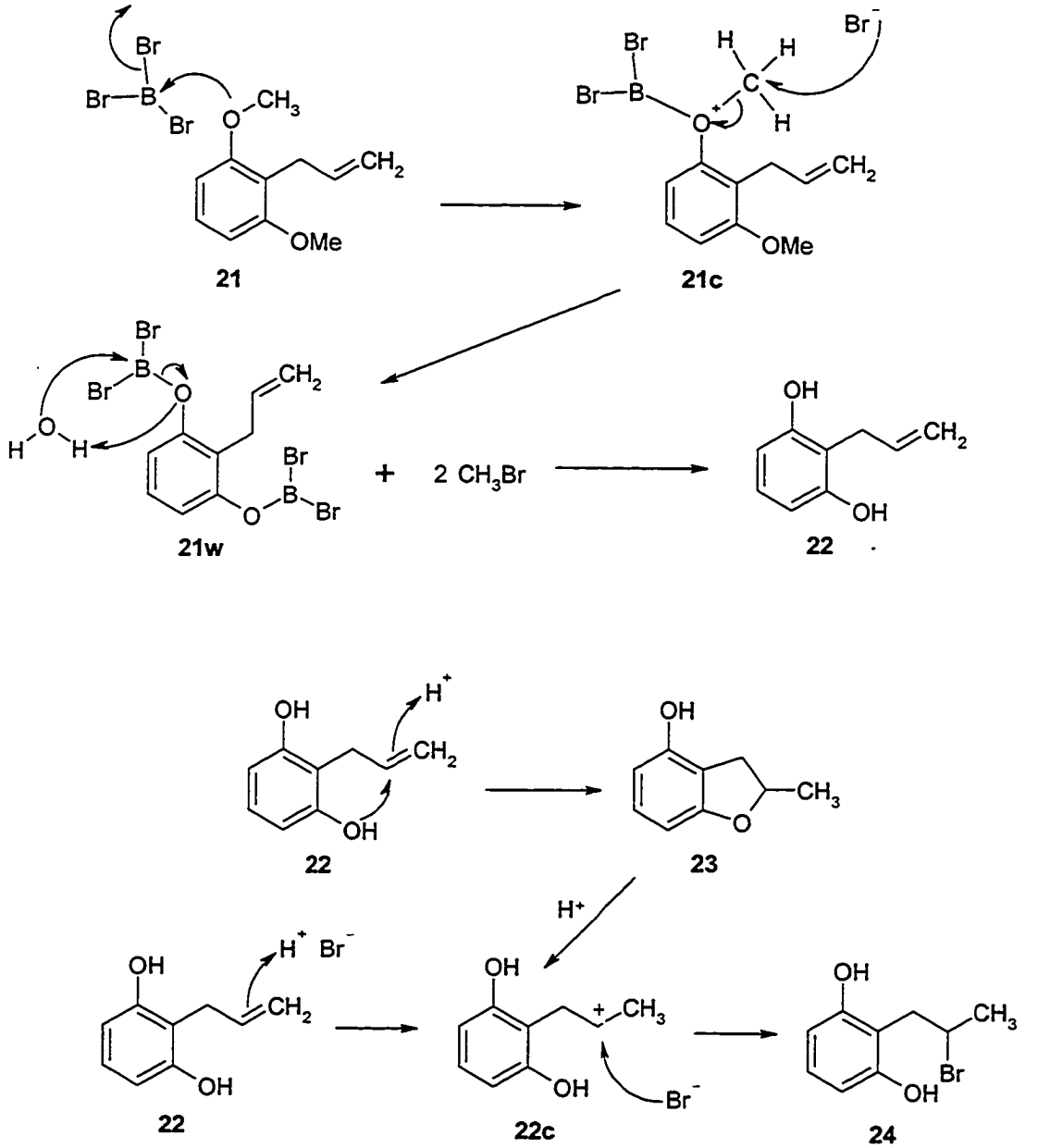


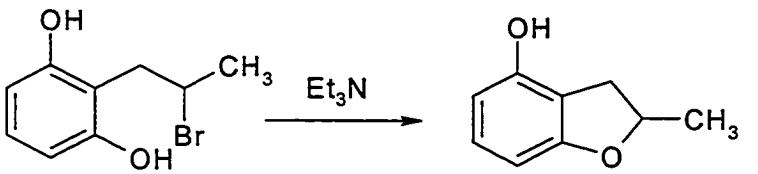
Figure 2.8: ^{13}C NMR spectrum of 2-allyl-1,3-dimethoxy-benzene (**21**)

The next step, de-methylation of the methoxy groups using boron tribromide, was expected to proceed as outlined in the Scheme 2.11, and result in the diol product **22**. However, the reaction resulted in the approximate 1:1 mixture of two products: the already cyclized **23** and brominated **24** in a combined yield of only 16% (Scheme 2.12). The formation of these products can be rationalized by the mechanism proposed in the Scheme 2.13.



Scheme 2.13: The proposed mechanism of the BBr_3 reaction.

After the fact, the formation of both **23** and **24** upon treatment of **21** with BBr_3 was quite reasonable. For instance, either the resorcinol **22** or the dihydrofuran **23** could react with HBr to give **24**. Despite numerous attempts we were not able to isolate a pure sample of **24**. Its existence in the crude reaction product was ascertained by the presence of the ^1H NMR peaks in addition to the ones arising from **23**. For example, a multiplet between 4.43-4.60 ppm due to the hydrogen on the carbon bearing the bromine, a doublet at 3.21 ppm due to the adjacent methylene, and a methyl doublet at 1.75 ppm. Fortunately, treatment of the mixture of **23** and **24** with triethylamine converted all of the brominated species into the desired dihydro-benzofuran, as shown in Scheme 2.14. The yield of the conversion judging by the ^1H NMR seemed to be excellent.



24 **23**

Scheme 2.14: The S_N2 displacement of bromine and formation of **23**.

After a careful gradient flash chromatography, **23** was isolated as a yellow oil. The methyl doublet at 1.48 ppm, methylene doublet of quartets (AB system) at 3.04 ppm, methine multiplet in a range of 4.94-5.02 ppm and the broad phenolic singlet at 6.10 ppm in the ^1H NMR spectrum of **23** , confirmed its structure. The IR spectrum of **23** showed bands which were in agreement with the structure, including: the broad hydroxyl stretch at 3000-3650 cm^{-1}, the methyl C-H stretch at 2974 and 2859 cm^{-1}, the asymmetric C-O-C stretch at 1234 cm^{-1}, symmetric C-O-C stretch at 1010 cm^{-1} and the broad O-H bend at 622 cm^{-1}. The molecular ion of **23** [$C_9H_{10}O_2$] was calculated to be 150.0681 and the HRMS showed M^+ at *m/z* 150.0669.

The formylation of **23** proceeded as outlined in Scheme 2.11. The product **25** was a yellow oil, obtained in 33% yield. The presence of the formyl group in the product was confirmed by the appearance of a singlet at 9.64 ppm in the ^{1}H NMR of **25**, and the carbonyl carbon signal at 194.23 ppm in the ^{13}C NMR (Figure 2.9 and Figure 2.10). Due to intramolecular hydrogen bonding the hydroxy group singlet shifted from 6.10 ppm where it was in the ^{1}H NMR of **23**, to 11.43 ppm in the ^{1}H NMR of **25**. The IR of **25** showed a distinct carbonyl stretch at 1650 cm^{-1}, considerably lower than the usual carbonyl frequency, due to a combination of factors – the conjugation with the phenyl ring and strong hydrogen bonding.

The final step of the synthesis, the Dakin reaction, Scheme 2.11, was attempted three times with no success. The final product could not be isolated, so the sequence was abandoned. No solid explanation can be provided as to why the reaction did not work. It can be only assumed that the product was "lost" possibly during the workup sequence, or that it is not stable and it oxidizes easily to an *ortho*-quinone, which in turn is converted to other products.

This particular sequence proved challenging. The demethylation step using the boron tribromide reagent occurred in a very poor yield (8% directly and 15% after treatment of the mixture of **23** and **24** with triethylamine). Also the BBr_3 reagent proved somewhat difficult to handle, since it is a very harsh Lewis acid. It would be advisable in the future attempts to use a different demethylating reagent, such as pyridine hydrochloride.[19]

Our inability to carry out the Dakin reaction on compound **25** was especially frustrating since Dr. Hussain succeeded in preparing both the fully substituted aromatic ring catechol **33** (Scheme 2.15), as well as the γ-tocopheryl catechol **38** (Scheme 2.16) via the Dakin reaction.

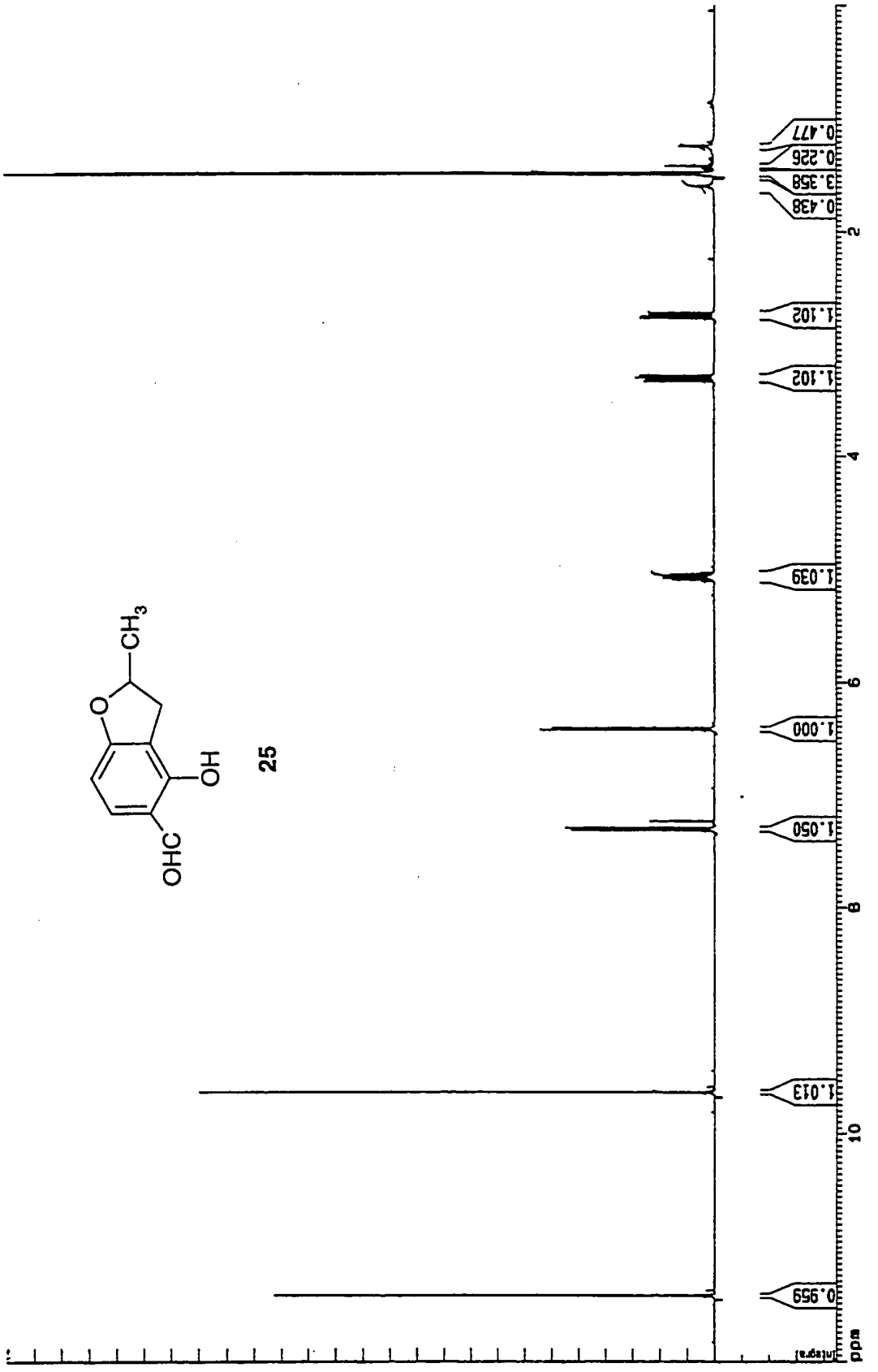


Figure 2.9: ^{1}H NMR spectrum of 4-hydroxy-2-methyl-2,3-dihydro-benzofuran-5-carbaldehyde (25)

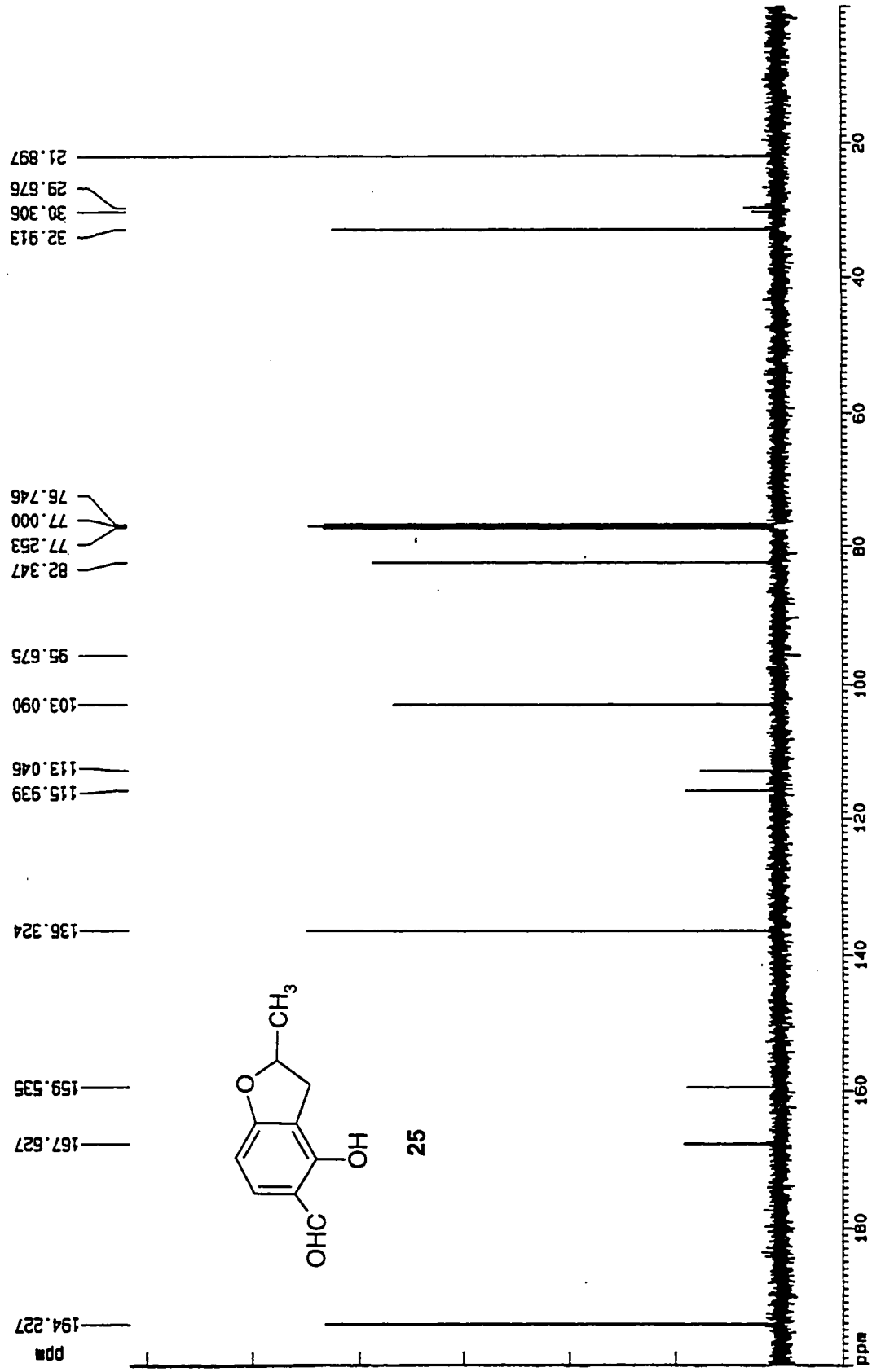
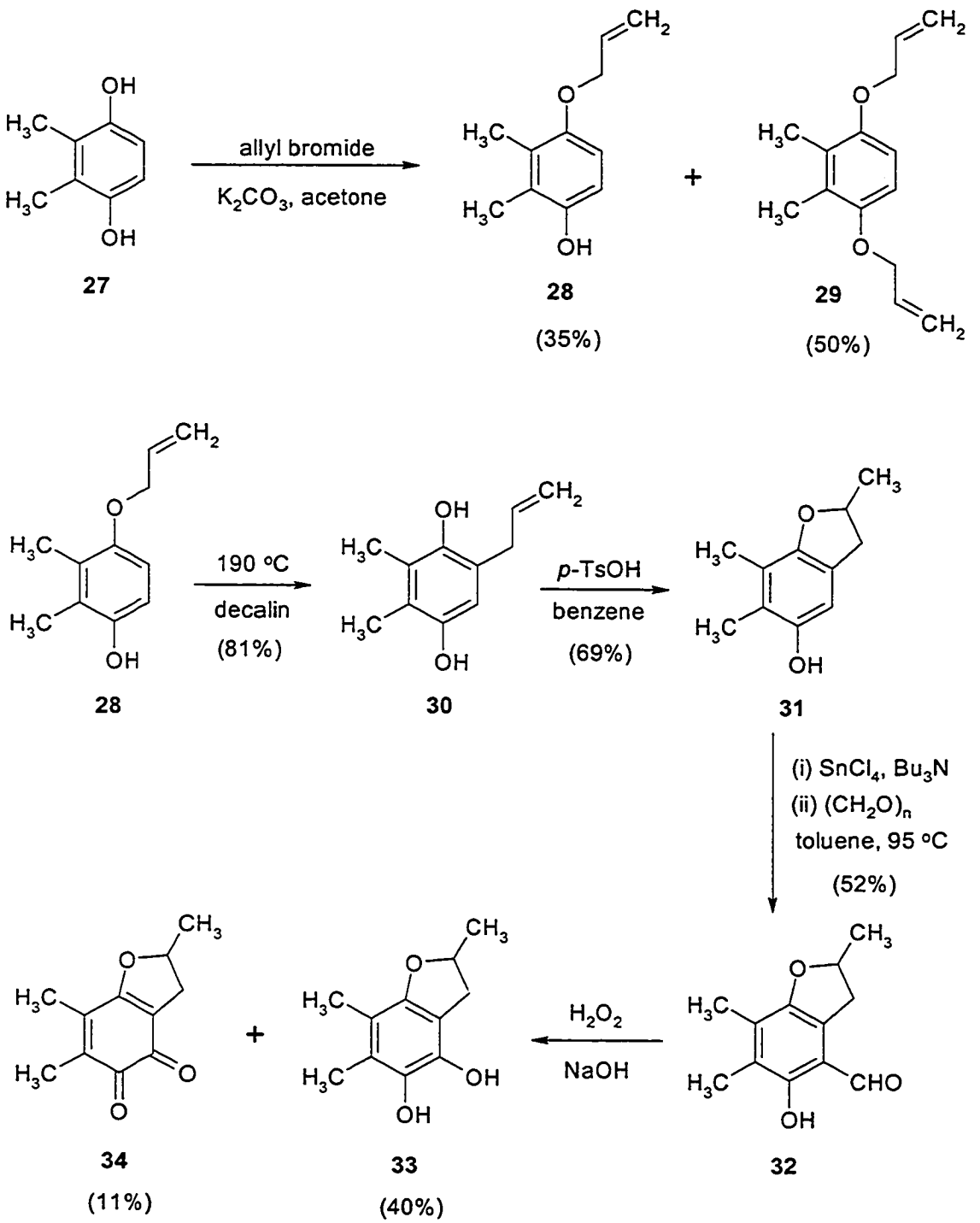


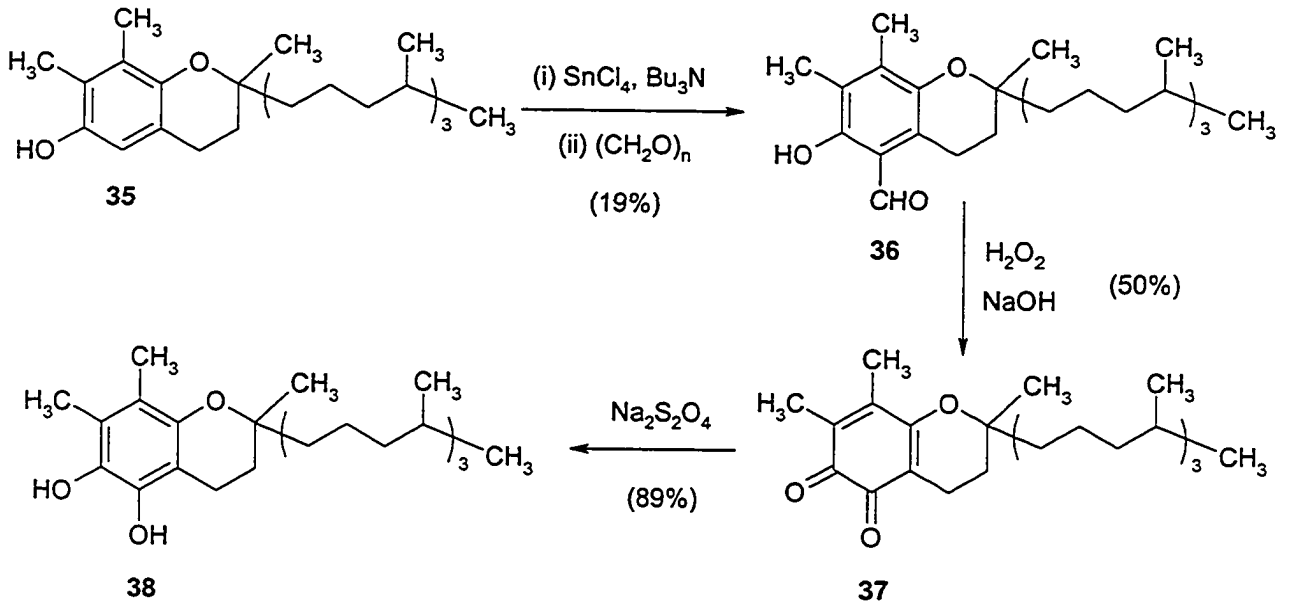
Figure 2.10: ^{13}C NMR spectrum of 4-hydroxy-2-methyl-2,3-dihydro-benzofuran-5-carbaldehyde (**25**)



Scheme 2.15: Preparation of 2,6,7-trimethyl-2,3-dihydro-benzofuran-4,5-diol **33**.

In his synthesis, as in the planned synthesis of **26**, the final step is the conversion of an *ortho*-hydroxy benzaldehyde into a catechol via treatment with sodium hydroxide and hydrogen peroxide. Admittedly, the conversion of **32** to **33** was accompanied by

considerable quinone formation. Nevertheless, a 40% yield of **33** was reported. Similarly, the conversion of γ-tocopherol to the catechol **38** via oxidative deformylation of **36** was also successful.



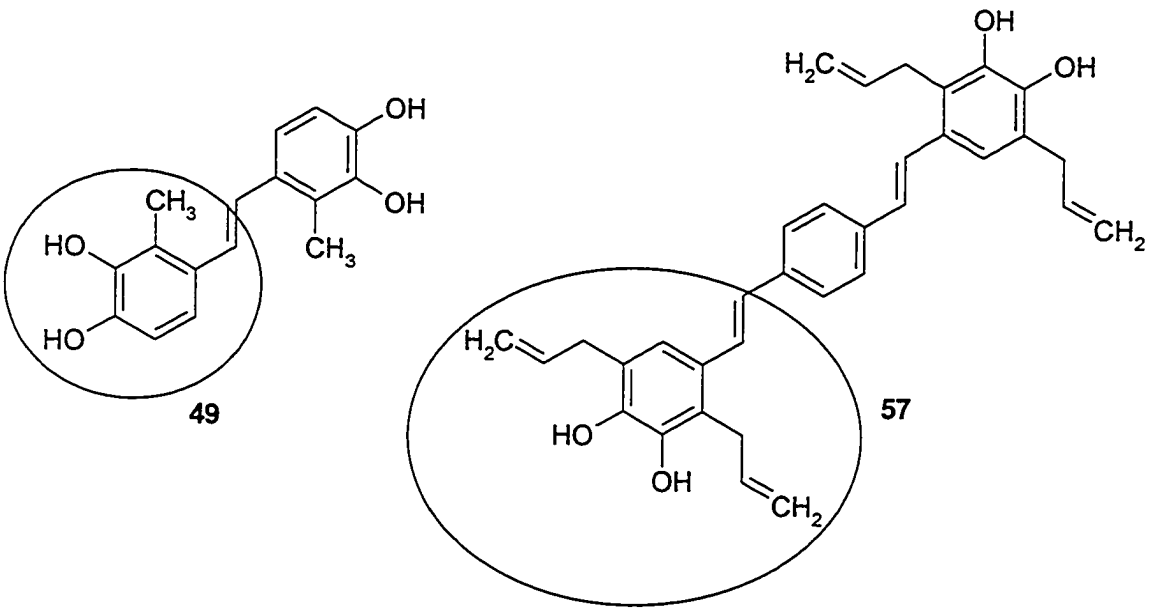
Scheme 2.16: Preparation of γ-tocopheryl catechol **38**.

The structures of these diols were easily deduced based on a combination of ^{1}H and ^{13}C NMR spectroscopy, as well as MS. Details pertaining to the experimental procedures and all the characterization information for these compounds is given in the Experimental section, part B.

It can be seen from the Hussain examples that the Dakin conversion of the formyl to hydroxy group is accompanied by considerable *o*-quinone formation. This adds credence to the rationalization that our inability to obtain **26** from **25** is probably due to oxidation of the initially formed catechol in the basic reaction medium. Perhaps carrying these Dakin reactions under oxygen exclusion would afford the desired products.

2.4 Preparing the building blocks for dendrimeric catechols

The rational behind designing and attempting to synthesize dendrimeric catechols was quite obvious: if one catechol structure is proven to be a good antioxidant, then a number of catechol molecules linked together should logically be a number of times more effective antioxidant. Also, the extended conjugation in molecules such as **49** and **57** would lower the BDE and theoretically increase their antioxidant ability. In order to investigate this hypothesis, we attempted the synthesis of the following structures:

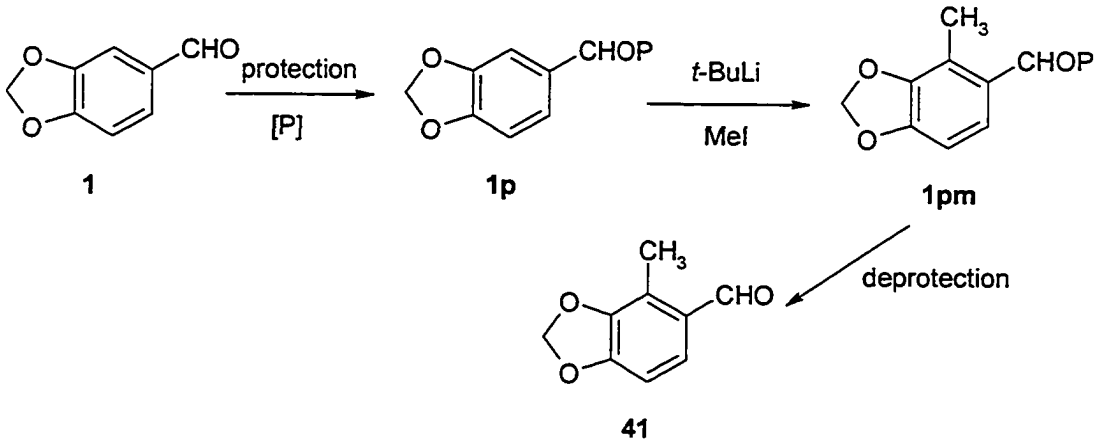


Scheme 2.17: Attempted dimer catechols **49** and **57**.

The circled portions of these molecules would eventually constitute the periphery of an antioxidant dendrimer. The stilbene **49** is somewhat reminiscent of resveratrol, a known antioxidant. It resembles the synthesized compound **8**, with the methyl group in the position number 3. Both compounds **49** and **57** can be envisioned to arise from the Wittig reaction; in both the vinyl group serves as a rigid conjugating spacer.

Commercially available piperonal **1** was the starting point for the preparation of **49**. The initial phases of the plan involved protection of the aldehyde, *ortho*-methylation,

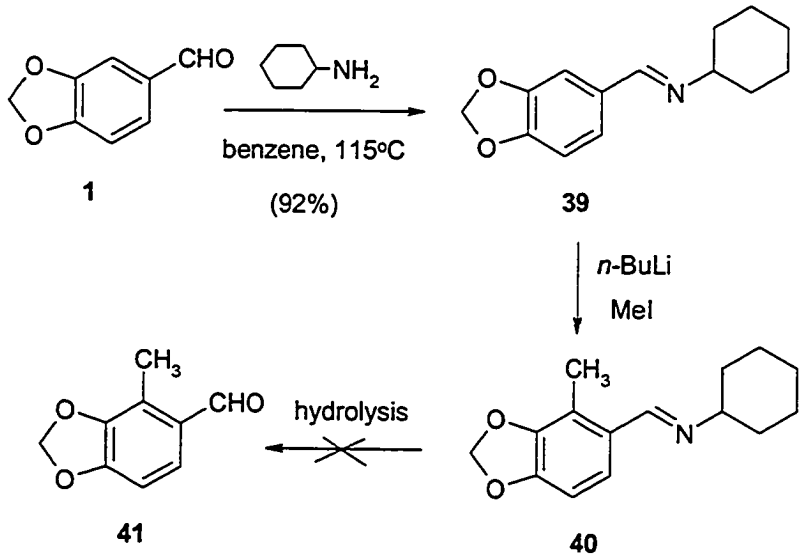
followed by deprotection, as depicted in Scheme 2.18. Ziegler and Fowler[20] were able to protect the piperonal aldehyde group by making it into an imine using cyclohexyl amine. The imine functionality also aids the *ortho* directed metallation process. They found that *ortho* methylation using *n*-butyllithium and methyl iodide, followed by deprotection of the aldehyde group, proceeded smoothly to give 61% overall yield of **41**, Scheme 2.19.



Scheme 2.18: The first steps in the synthesis of **49**.

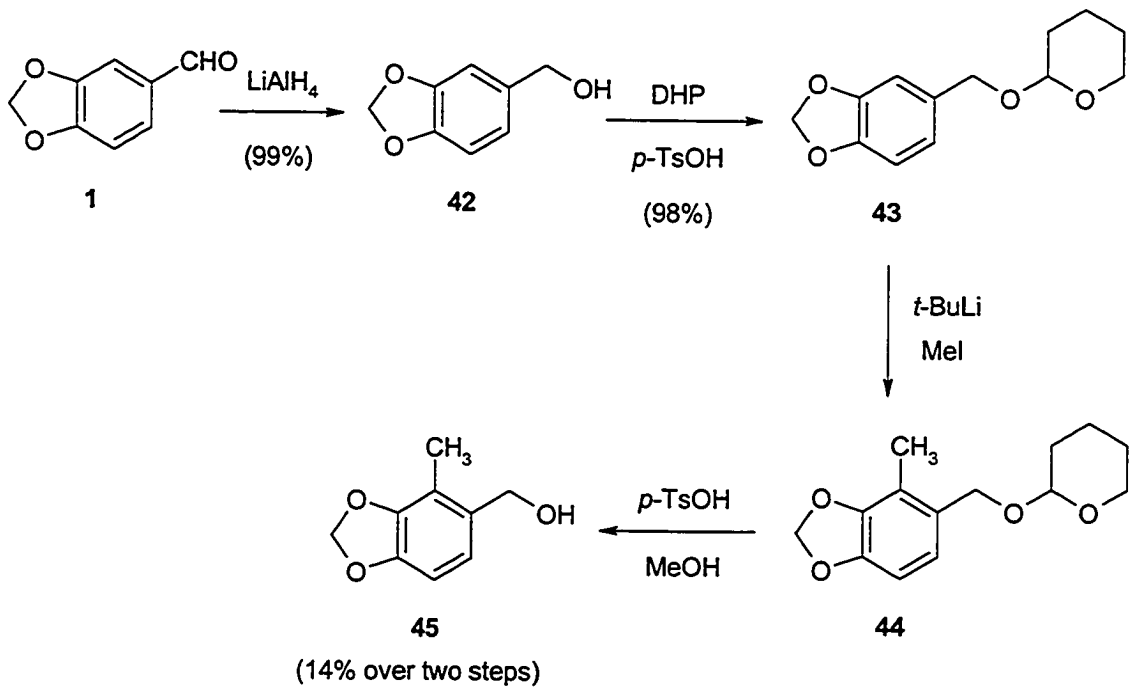
The imine **39** was prepared from piperonal **1** and cyclohexylamine in benzene solution by azeotropic removal of water. Recrystallization from methanol provided **39** as a white solid in 92% yield. The melting point of the imine was found to be 63-65°C, the literature value was 65-66°C.[21] The ^{1}H NMR spectrum showed resonances characteristic of the cyclohexyl group: the multiplet between 1.15-1.95 ppm (10H) and a =NCH- multiplet from 3.05-3.25 ppm. These and the other peaks completely matched the spectra obtained by Ziegler and Fowler[20]. Since the imine **39** was expected to be unstable, it was quickly exposed to the methylating conditions. Instead of *n*-butyllithium, as recommended[20], we used *t*-butyllithium together with methyl iodide. A methyl singlet appeared at 2.19 ppm in the ^{1}H NMR of the crude reaction product. The crude product was then hydrolysed using the 10% aq. hydrochloric acid. Unfortunately, the expected aldehyde **41** could not be isolated. The

above sequence was repeated twice and both times no aldehyde peak was observed in the ^{1}H NMR of the final crude product. The approach was abandoned.



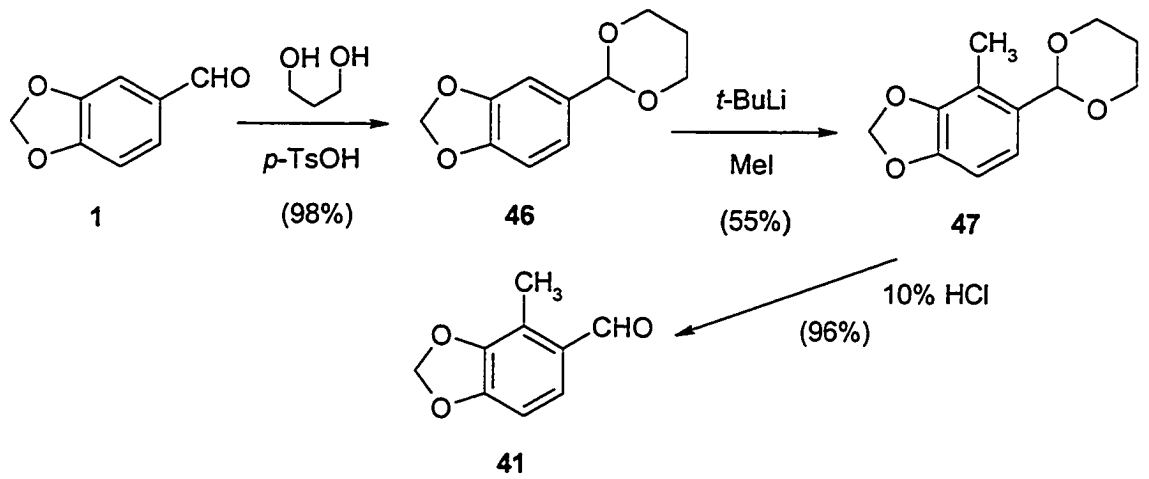
Scheme 2.19: Cyclohexyl amine protection of piperonal, an attempt to make **41**.

A different *ortho* activation methodology was investigated. Piperonal was reduced using lithium aluminium hydride to the benzyl alcohol **42**, as shown in Scheme 2.20.



Scheme 2.20: DHP protection of **42**, an attempt to make **45**.

The reduced alcohol **42** was then protected as its THP acetal **43**. The protected alcohol was methylated using *t*-butyllithium and excess methyl iodide to yield crude **44** as a yellow oil. Hydrolysis and subsequent purification by flash chromatography afforded **45** as a white solid with a mp of 82-83°C in 14% yield over the two steps. The HRMS showed the M^+ at *m/z* 166.0641, which agreed with the formula $C_9H_{10}O_3$ and the calculated value of 166.0630. A broad singlet at 1.59 ppm due to the hydroxy group and a singlet at 2.23 ppm due to the methyl group in the ^1H NMR of **45** corroborated the structure. Although the transformations from piperonal proved to be reasonably successful, the overall yield was not very satisfactory.



Scheme 2.21: A propylene dioxy route towards **41**.

In the final approach to synthesis of **45** the aldehyde group of the starting material piperonal **1** was protected by means of propylene glycol moiety, using the Dean-Stark azeotropic removal of water system (Scheme 2.21). The resulting acetal **46** structure was verified by its ^1H NMR spectrum (see experimental section, part A). Methylation of **46** using *t*-butyllithium and methyl iodide afforded **47**, which was isolated via flash chromatography as a white solid with a mp of 83-84°C in 55% yield. The appearance of the methyl singlet at

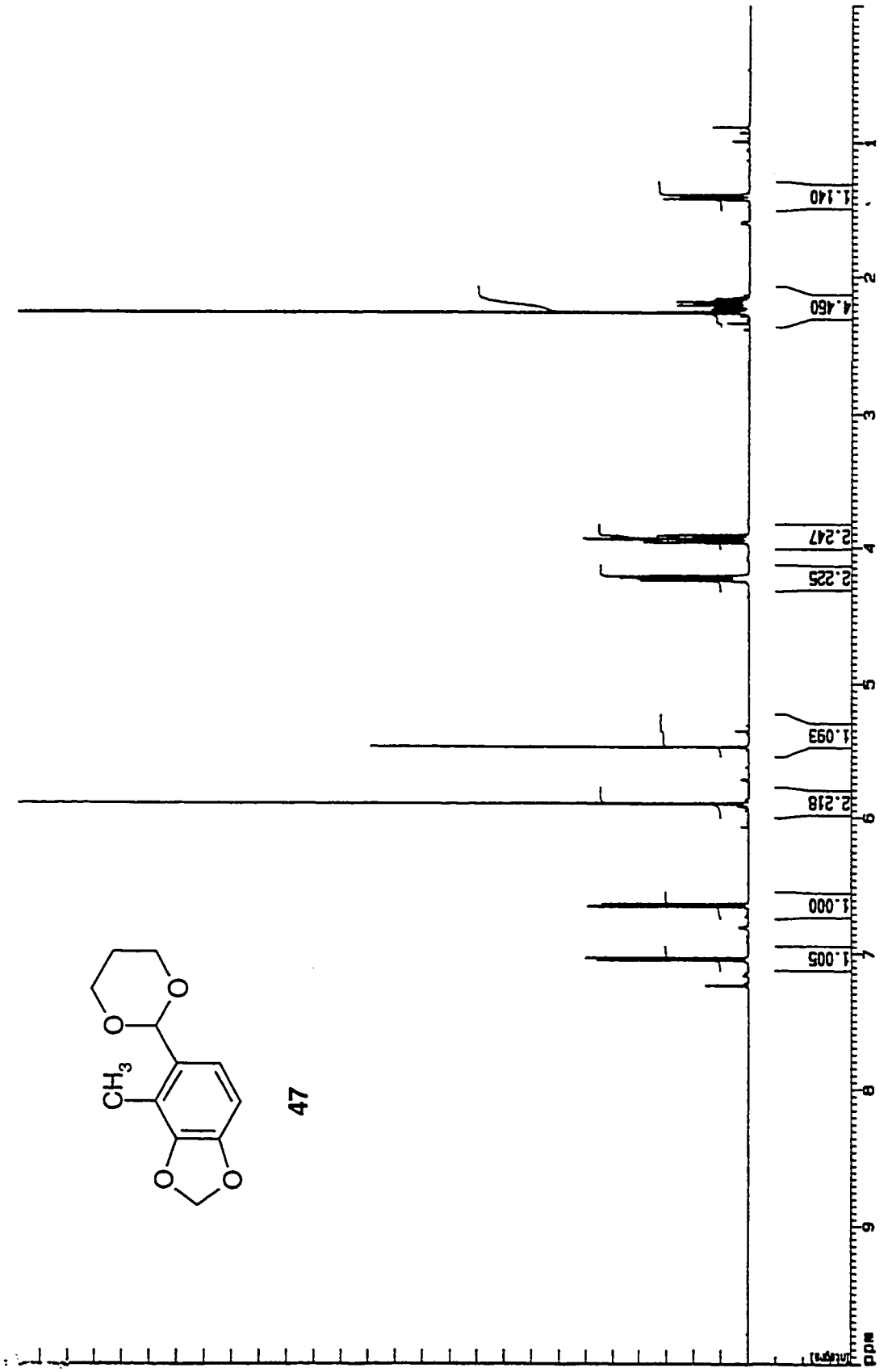


Figure 2.11: ^{1}H NMR spectrum of 5-[1,3]dioxan-2-yl-4-methyl-benzo[1,3]dioxole (**47**)

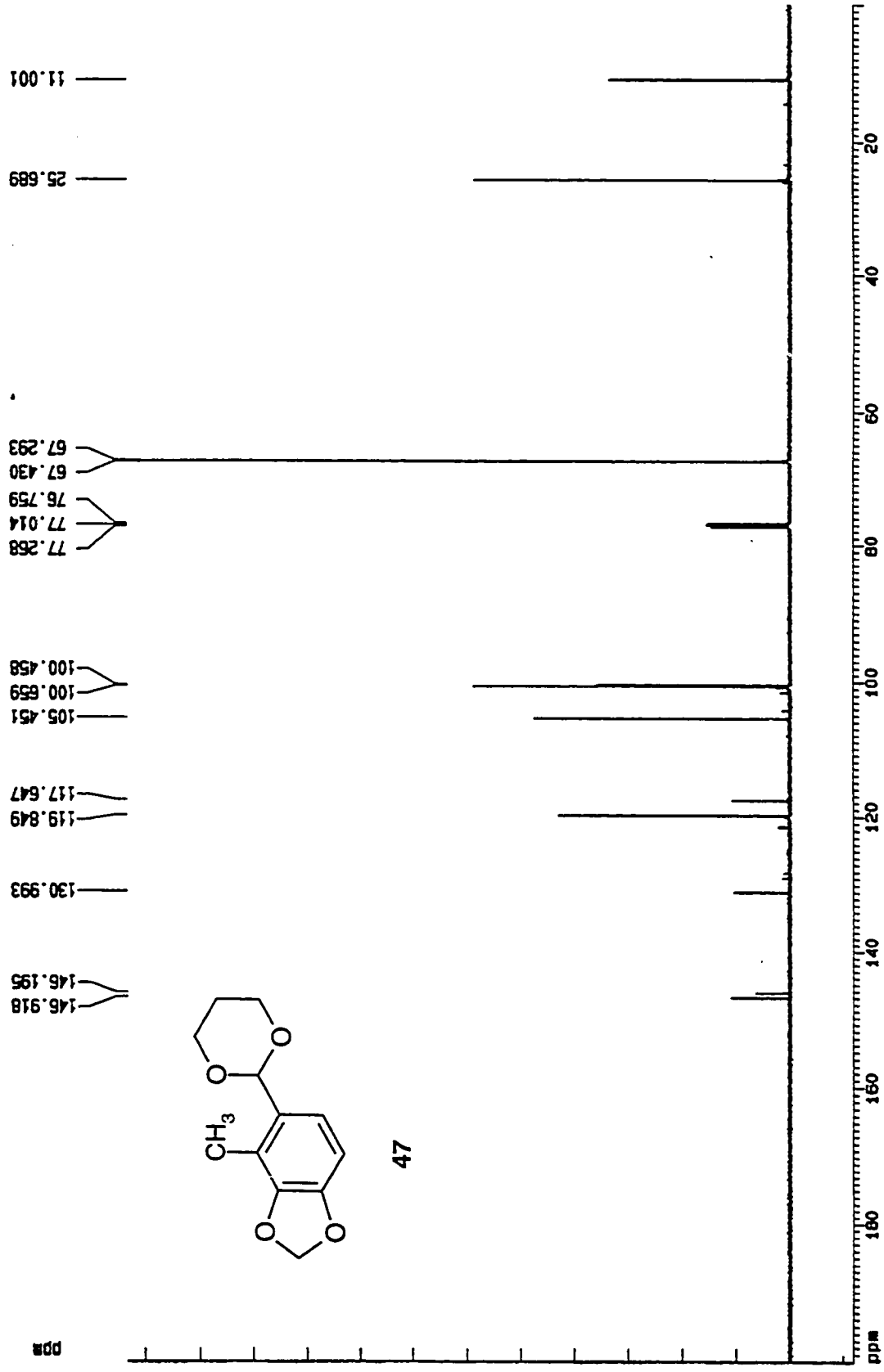
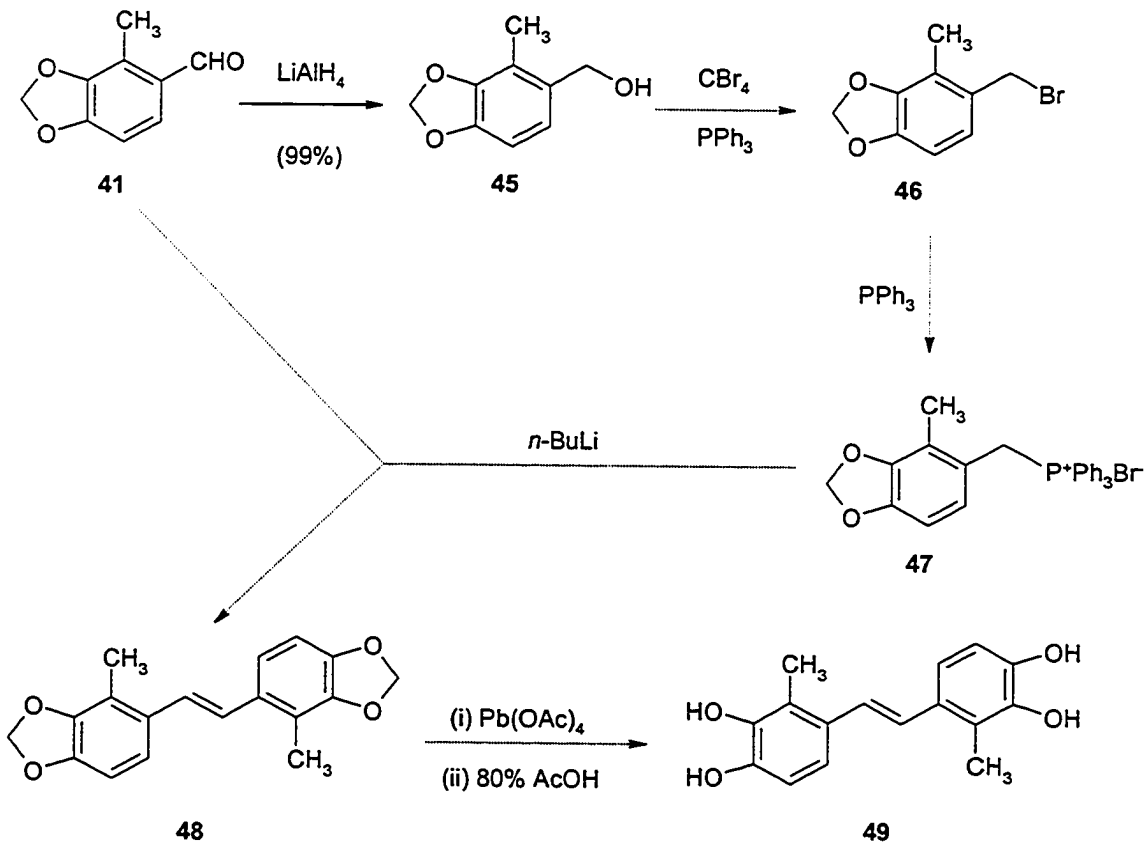


Figure 2.12: ^{13}C NMR spectrum of 5-[1,3]dioxan-2-yl-4-methyl-benzo[1,3]dioxole (**47**)

2.26 ppm in the ^{1}H NMR of **47**, as well as the methyl resonance at 11.36 ppm in the ^{13}C NMR of **47**, confirmed the introduction of the aromatic methyl group (Figure 2.11 and Figure 2.12). The deprotection of **47** to the aldehyde **41**, mp=73-75°C, occurred in 96% yield (52% overall from piperonal **1**). The prominent aldehyde singlet at 9.97 ppm of the ^{1}H NMR of **41** , together with the carbonyl peak at 191.91 ppm of the ^{13}C NMR of **41**, confirmed the structure.

The dimeric structure **49** was envisioned as arising from a Wittig reaction involving the aldehyde **41** and the ylide derived from **47** (Scheme 2.22), followed by the oxidative cleavage of methylenedioxy rings. The alcohol **45** was prepared in 99% yield by reduction of **41** (Figure 2.13 and Figure 2.14). Unfortunately time constraints did not allow us to finalize the preparation of **49** as illustrated in the Scheme 2.22.



Scheme 2.22: Proposed synthesis of **49**.

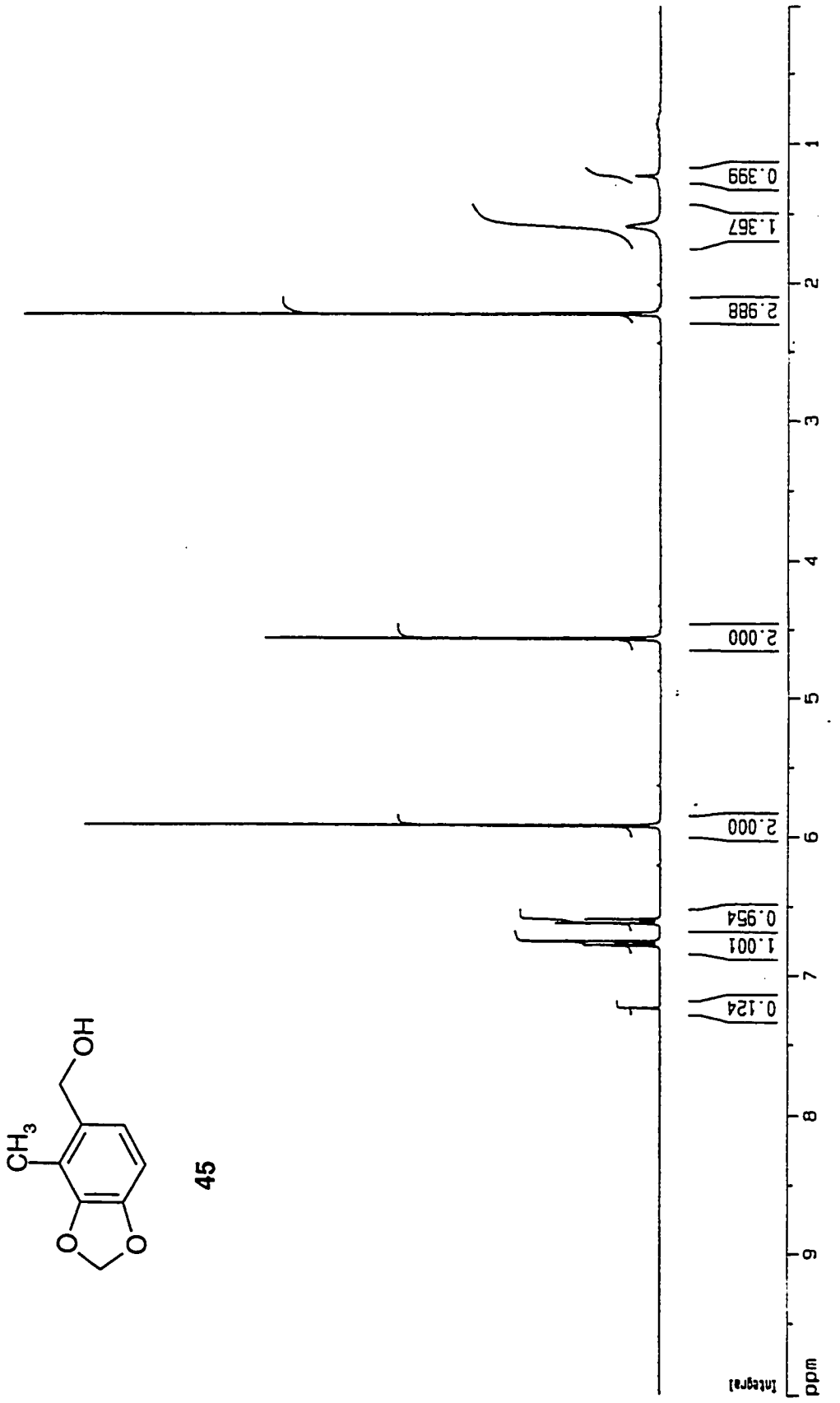


Figure 2.13: ^{1}H NMR spectrum of (4-methyl-benzo[1,3]dioxole-5-yl)-methanol (**45**)

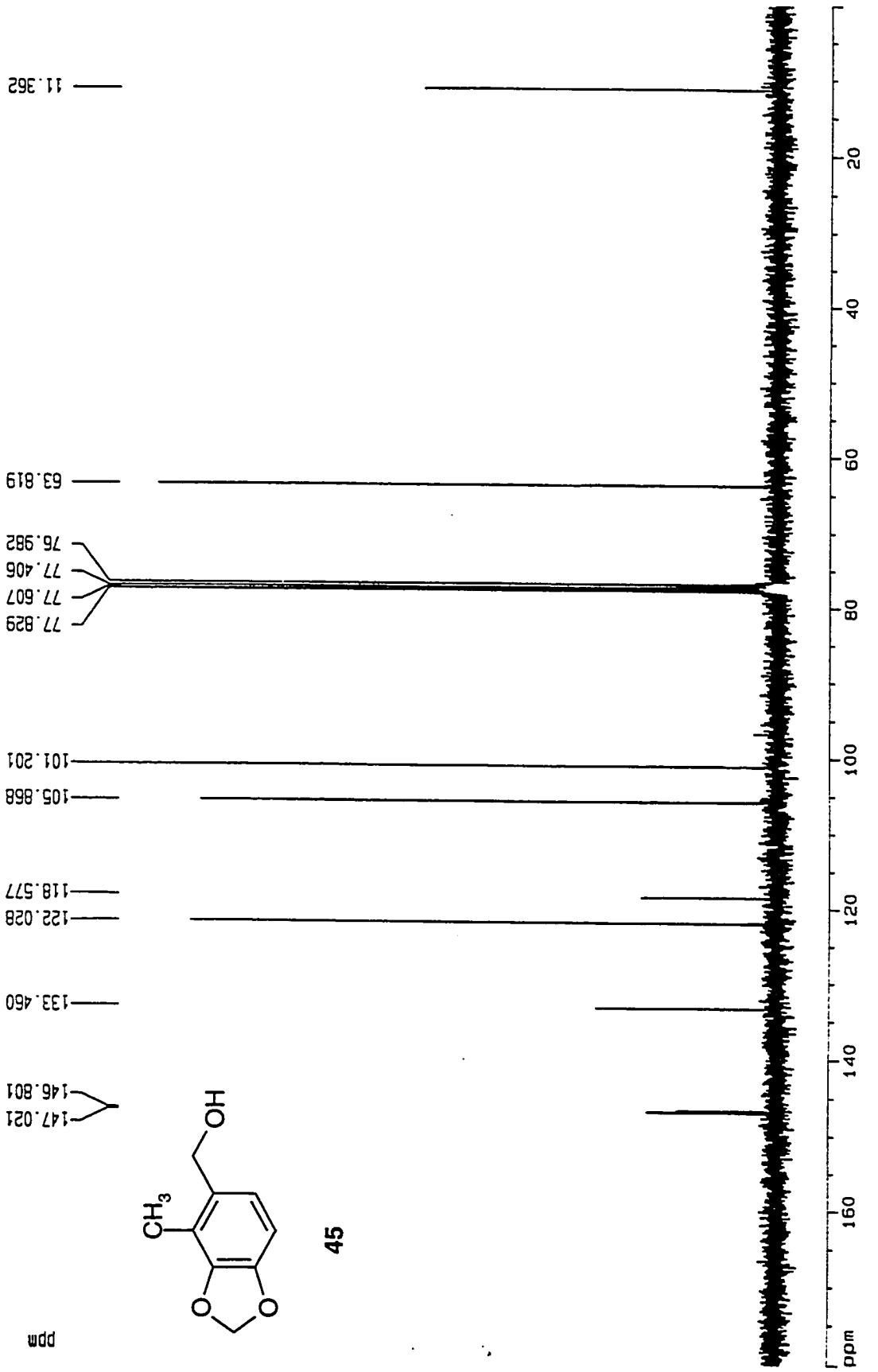
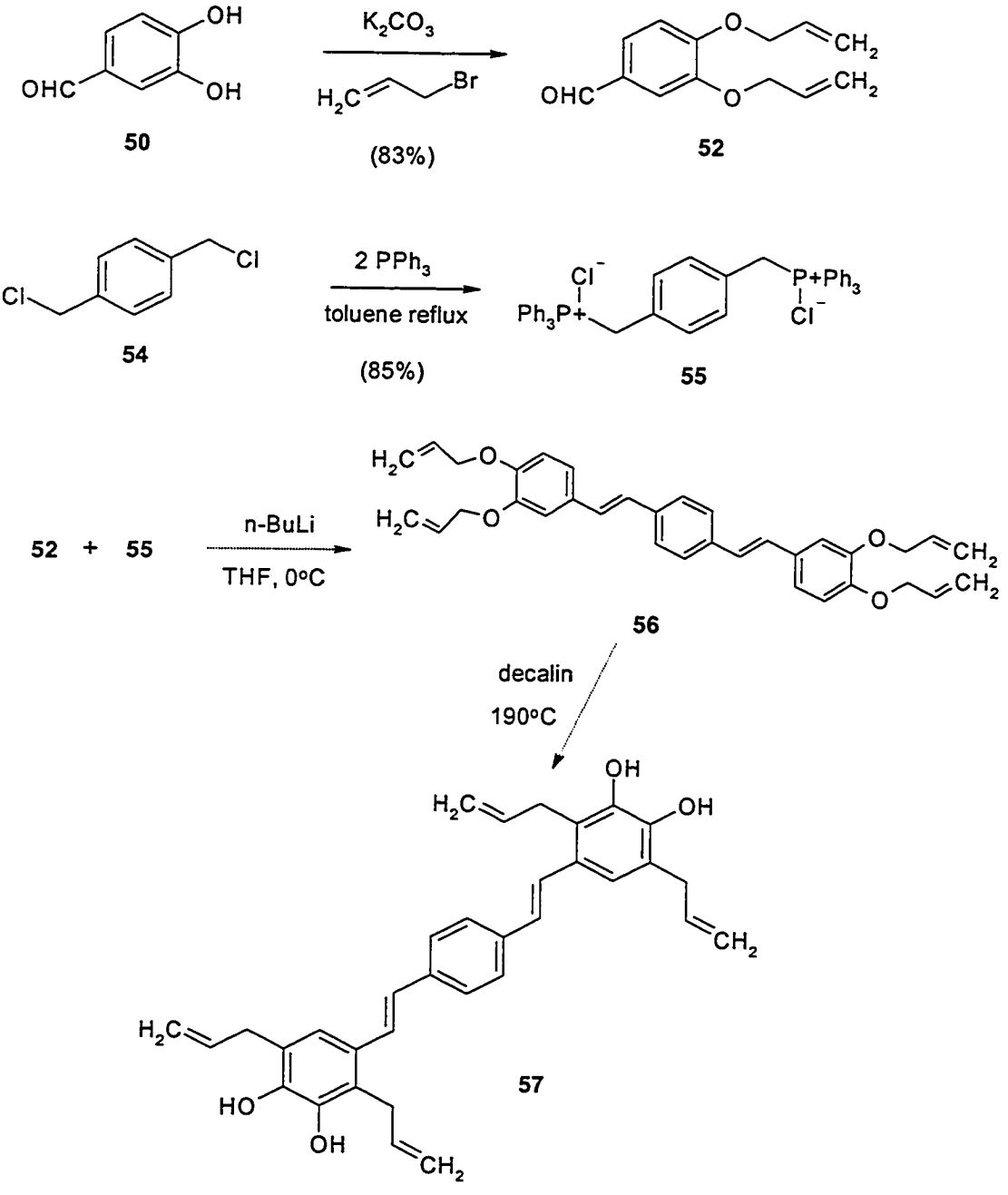


Figure 2.14: ^{13}C NMR spectrum of (4-methyl-benzo[1,3]dioxole-5-yl)-methanol (**45**)

The synthesis of the second "dimer" target **57** (depicted in Scheme 2.23) was initiated with a commercially available 3,4-dihydroxy benzaldehyde **50**. Both hydroxy groups of the aldehyde **50** were allylated to give **52**, the molecular structure of which was confirmed by the spectral data. The characteristic allyl group hydrogens' resonances appeared in the ^{1}H NMR of **52** (Figure 2.15); the ratio of allyl to aryl hydrogens was the expected 2:1.



Scheme 2.23: Preparation of **57**.

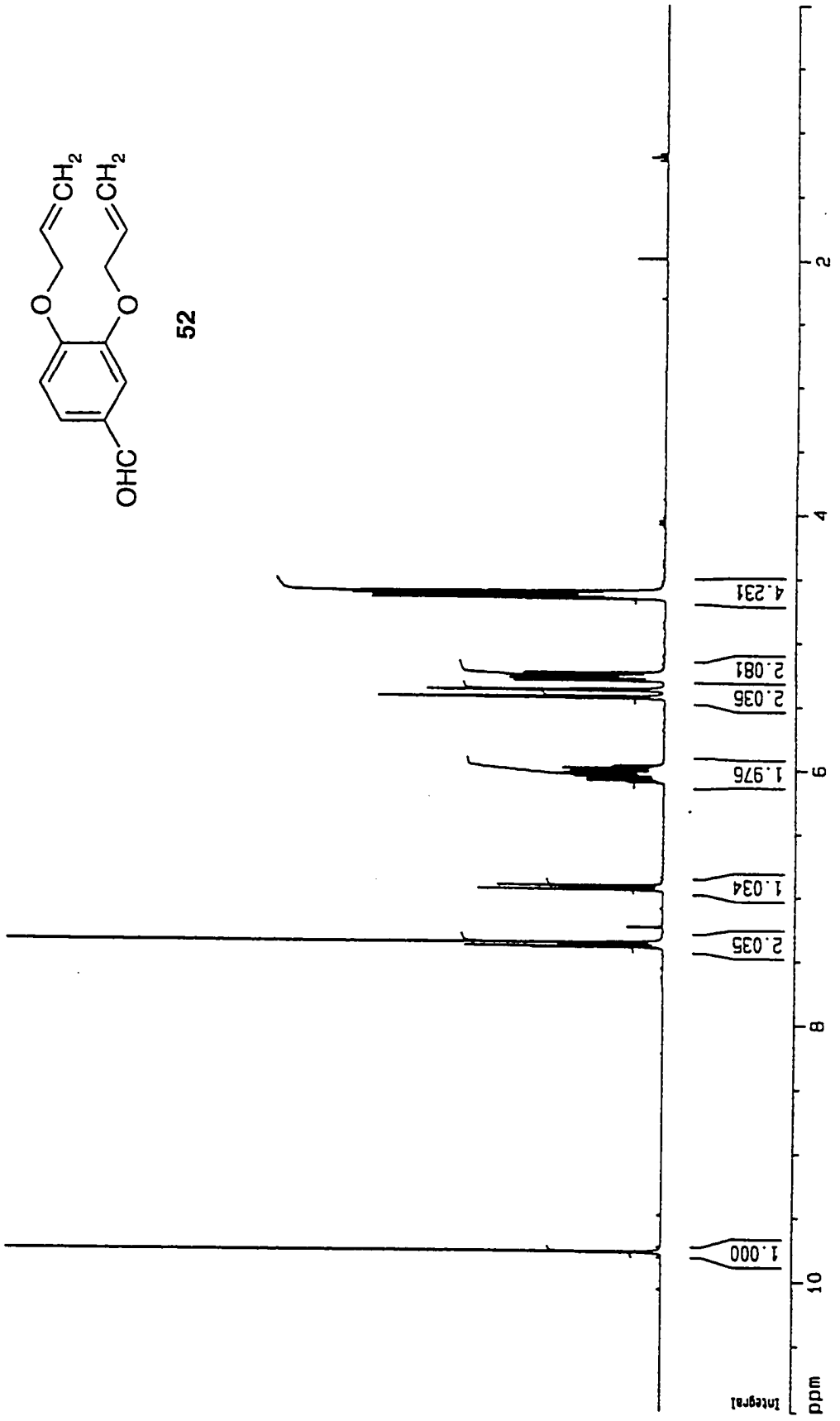
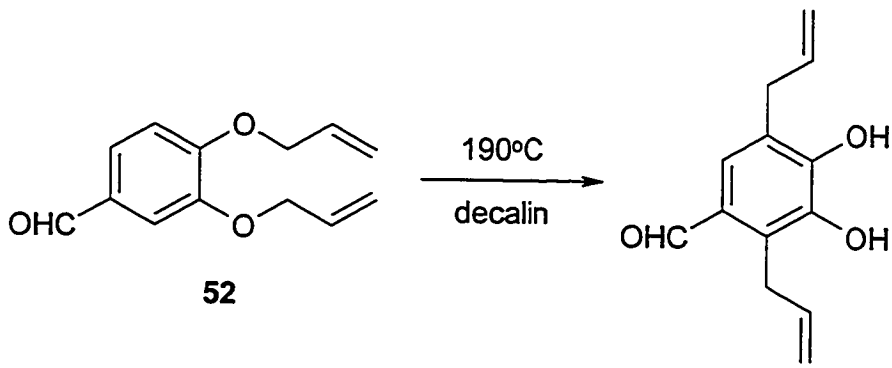


Figure 2.15: ^{1}H NMR spectrum of 3,4-bis-allyloxy-benzaldehyde (**52**)

Commercially available 1,4-bis-chloromethyl benzene **54** was transformed into a fine white powder phosphonium salt **55** in 85% yield. An attempt was then made to combine this salt with the aldehyde **52** under the typical Wittig reaction conditions. Judging by the reaction mixture TLC, a number of products resulted, presumably due to the *E* and *Z* alkene spacer combinations. After a very careful gradient flash chromatography a mixture of two products was isolated in 20 % yield. An attempt to separate and identify the two products failed. The ^{1}H NMR and ^{13}C NMR of the mixture of the two products did not show any aldehyde resonances and both spectra were consistent with what one might expect for the product **56**. However, we cannot claim unambiguously that **56** had indeed been obtained.

The Claisen rearrangement reaction required as the final step in Scheme 2.23 was investigated in the precursor system **52**. After heating **52** at 190°C for 3.5 hours, the compound **53** was obtained in 17% yield after purification by the column chromatography. The transformation was confirmed, since peaks associated with the methylene group showed the expected shifts in the ^{1}H NMR and ^{13}C NMR. The methylene hydrogens α to oxygen in **52** appeared as a multiplet between 4.60-4.66 ppm in the ^{1}H NMR of **52**. In the ^{1}H NMR of **53**, these hydrogens, now adjacent to an aromatic ring, appeared as two doublets at 3.43 and 3.90 ppm each. Similarly, the methylene carbons of **52** at 70.02 ppm (2C) in the ^{13}C NMR, were displaced upfield to 28.86 and 34.20 ppm in the ^{13}C NMR of **53**.

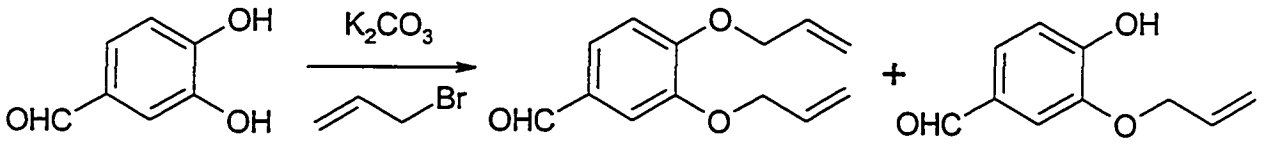


52 **53**

Scheme 2.24: The Claisen rearrangement of **52**.

Given that compounds **52** and **56** both have a diallyl-structure, it is expected that in the future the Claisen rearrangement of **56** might proceed just as smoothly. The yield of **53** was left unoptimized, since this was just a test reaction.

It should be noted that there were two products of the allylation reaction (Scheme 2.23)-mono and di-allylated products **51** and **52**, Scheme 2.25.



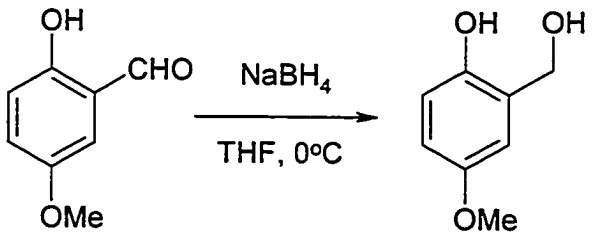
50 **52** (37%) **51** (50%)

Scheme 2.25: Allylation of 3,4-dihydroxy benzaldehyde **13**.

The mono-allyl product **51** was isolated as a white solid in 50% yield. The reaction conditions could be altered to yield up to 75% of **51**, by using 1 equivalent of the base and 1.2 of the allyl bromide. The compound **51** was found to have a melting point of 58-60°C, while the literature value was 58-61°C.[23] Integration of the allylic hydrogens' resonances in the ^{1}H NMR of the **51**, as well as the presence of the free hydroxy singlet at 5.78 ppm, verified the structure.

2.5 Preparation of benzylic alcohols

The hydrogen bonding stabilization effects associated with catechols and catechol-derived radicals were discussed earlier (Section 2.1). For the purpose of comparing the antioxidant activity of the catechols with benzylic alcohol phenols, the compound **59** was prepared by a simple sodium borohydride reduction of the appropriate aldehyde **58**.



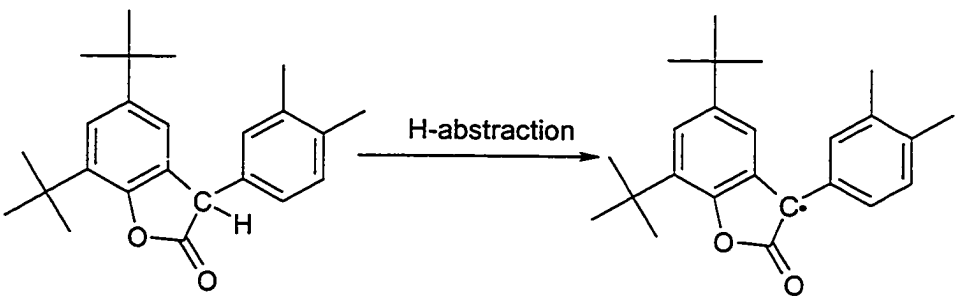
58 **59**

Scheme 2.26: Preparation of 2-hydroxymethyl-4-methoxy-phenol **59**.

The structure of **59** was confirmed by the ^{1}H NMR and ^{13}C NMR spectra. The melting point of the benzylic alcohol **59** was found to be 77-79°C, and the literature value was 78-80°C.[30] The HRMS showed a M^{+} ion at *m/z* of 154.0614 and the calculated value was 154.0630. The compound **59**, unlike catechols, cannot form an *ortho*-quinone structure upon losing a hydrogen atom, hence it would have an advantage over the previously discussed catechols in terms of stability.

2.6 Preparation of N-methyl oxindole substituents

Common chain-breaking antioxidants, as mentioned in the Introduction, often have phenolic structure since phenols have very good hydrogen donating ability and the resulting phenoxyl radicals, just like many other oxygen centred radicals, are unreactive towards oxygen. Carbon centred radicals are usually considered to be poor antioxidants. Although compounds containing weak C-H bonds exist and they can be relatively good hydrogen atom donors (such as toluene for example), most carbon-centred radicals are extremely reactive towards oxygen[33], and hence these compounds cannot be classified as efficient antioxidants. Contrary to the common belief, **HP-136** has been found to be an excellent antioxidant[34] (Scheme 2.27).



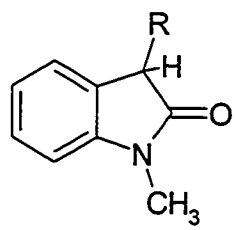
HP-136 **HP-136r**

Scheme 2.27: Formation of the HP-136 radical, **HP-136r**.

As shown in the Scheme 2.27, the benzylic C-H bond is a good hydrogen-donor site. The resulting carbon-centred radical, **HP-136r**, has been proven to be very stable towards oxygen. Scaiano et al. offered an explanation for such stability, after extensive research involving a number of other lactone-derived carbon-centred radicals.[35] They concluded that the radical **HP-136r** is stable towards oxygen due to: (i) benzylic resonance stabilization, (ii) unpaired spin delocalization on oxygen, (iii) favourable stereoelectronic effects, such as the forced planarity caused by the five-membered ring, (iv) electron withdrawing effects, and (v)

steric effects. Indeed, the benzylic resonance stabilization, together with electron withdrawing effects and steric effects, has been proven important in previously discussed phenolic antioxidants as well. However, the planar structure surrounding the radical centre in these lactone systems proved to be a key to the radical inert behaviour towards oxygen. Scaiano et al. suggested that the ring substituents in **HP-136**, as well as the presence of the second aromatic ring in the molecule probably do not play any major role in the lack of reactivity of the radical towards oxygen.

With a goal of making an efficient carbon-centred antioxidant in mind, and taking into consideration all the recommendations and results by Scaiano, we proposed to synthesize a family of compounds having a 1-methyl-1,3-dihydro-indol-2-one (N-methyl oxindole) core structure (Scheme 2.28). This choice was also recommended based on calculations by the Wright group indicating that the benzylic C-H in the oxindole structure was weaker than in the corresponding lactones, eg. **HP-136**.

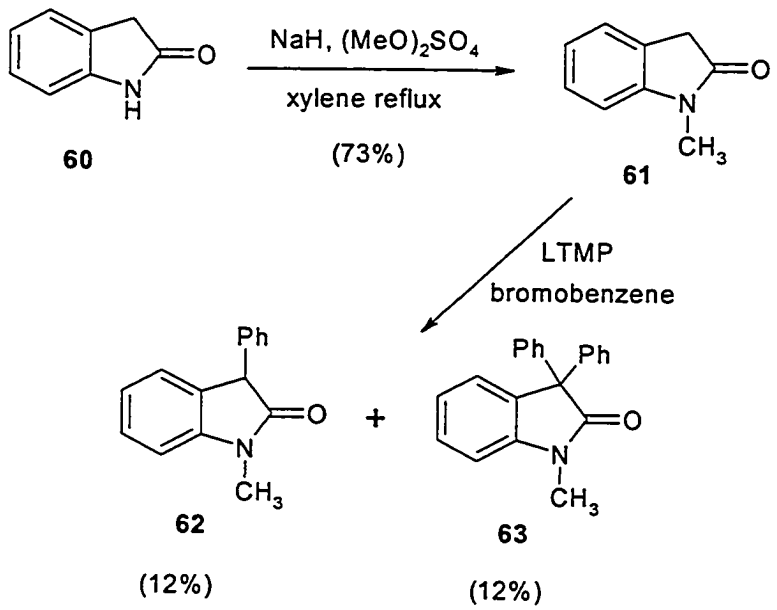


R= -Ph, -OMe, -OR', -NR"$_2$

R', R"= -H, -alkyl

Scheme 2.28: The proposed N-methyl-3-R-oxindole antioxidants.

The synthesis of the first synthetic target, 1-methyl-3-phenyl-1,3-dihydro-indol-2-one **62** commenced with the commercially available oxindole **60**. Using a very specific set of conditions for N-methylation: sodium hydride base, dimethyl sulphate as the electrophile, and xylene as solvent, compound **61** was prepared (Scheme 2.29) in 73% yield. The appearance of the methyl singlet at 3.18 ppm in the ^{1}H NMR of **61**, as well as the resonance at 36.11 ppm in the ^{13}C NMR of **61** confirmed the transformation. The melting point of 86.5-88.5°C was found to be in agreement with the literature value of 86.5-87°C[26].



Scheme 2.29: Preparation of the 1-methyl-3-phenyl-1,3-dihydro-indol-2-one **62**.

For the next step, a typical procedure of generating benzyne in situ, using bromobenzene and lithium tetramethylpiperidide, in THF at 0°C, was followed[36]. To the solution of excess LTMP, the N-methyl oxindole **61** was added, followed by bromobenzene. Upon extensive chromatographic purification the two products, mono-phenyl N-methyl oxindole **62** and di-phenyl N-methyl oxindole **63** were isolated in 12% each. Some of the unreacted starting material (17%) was also recovered. The ^{1}H NMR of **62** showed the new phenyl resonances in the 7.32-7.44 ppm range (6H), as well as the methine hydrogen singlet at 5.14 ppm. The number of carbon resonances and their position in the ^{13}C NMR of **62** also corroborated the structure of **62**. To our regret, we were unable to sufficiently purify the desired product, compound **62**, and obtain a mp or HRMS for it. Compound **63** was characterized using the ^{1}H NMR and MS data. The di-phenyl product was confirmed by integration of the phenolic resonances in the ^{1}H NMR of **63**. In addition, the M^+ ion was found at *m/z* 299.1 (100%) of MS, right where expected, since the MW(**63**)=299.37 g/mol. The fragmentation pattern of the M^+ of **63** in the MS also corresponded to the di-phenyl product.

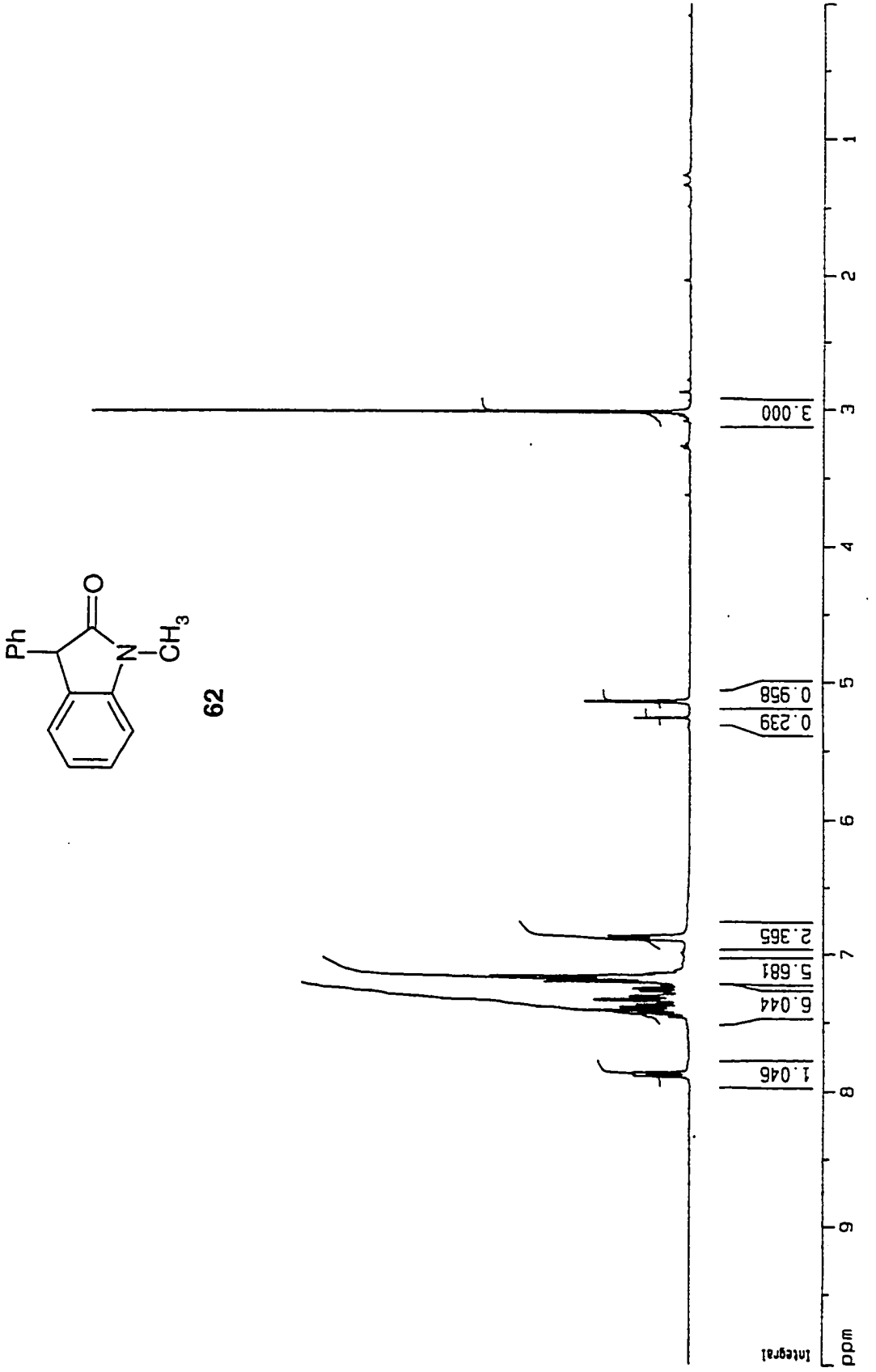
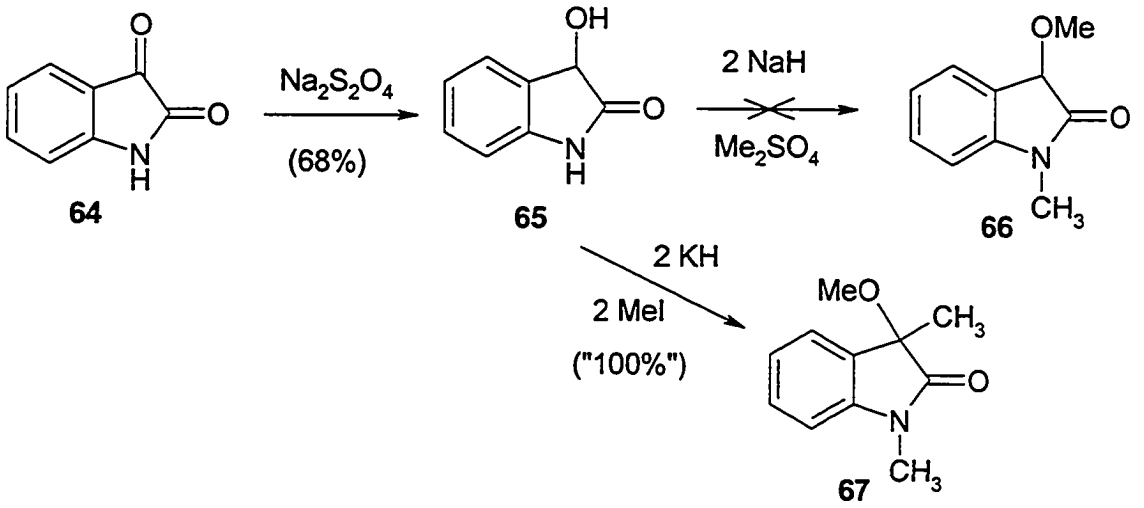


Figure 2.16: ^{1}H NMR spectrum of 1-methyl-3-phenyl-1,3-dihydro-indol-2-one (**62**)

The ^{13}C NMR of **63** was found to be fairly ambiguous due to a very large number of carbon resonances in the aromatic region, and hence it was very hard to interpret. This compound was (same as compound **62**) difficult to purify sufficiently, so the correct assignment of all the other spectral information remained incomplete.

The difficulty in alkylation of oxindoles (α to carbonyl) was recognized back in 1950's, by Wenkert et al.[37] . The authors found that the direct alkylation of 3-unsubstituded N-alkyl oxindoles using alkyl halides and bases, was impossible and made a realization that a 3-acyl or 3-alkyl group was needed in order to promote any further alkylation. Hence, they were unable to isolate any mono-alkylated products, regardless of whether a 1:1 or 2:1 molar ratios were used of methylating agent : oxindole. Once one alkyl or acyl group was introduced into the 3-position, the introduction of another alkyl function was eminent. Wenkert investigated alternate methods of mono-alkylation, such as: (i) acylation via Claisen condensation, followed by reduction, (ii) base-induced alkylation of already 1,3-dialkylated compounds, (iii) Mannich or Michael condensations and (iv) base-induced C-alkylation of 1-alkyl-3-acyl compounds followed by hydrolysis, all of which were at least two-step reactions. In terms of introducing a phenyl substituent, the benzyne approach we adopted seems promising. To be sure, it is a one step reaction and apparently it does work. There is, however, considerable room for the improvement, considering that the isolation and purification of the products proved to be somewhat finicky.

The next synthetic target, compound **66**, was envisioned as being prepared starting from the commercially available isatin **64**. The reduction of the keto carbonyl of isatin was accomplished using the previously established sodium dithionate two-phase reduction process (Scheme 2.30), resulting in a white solid compound **65** in 68% yield.

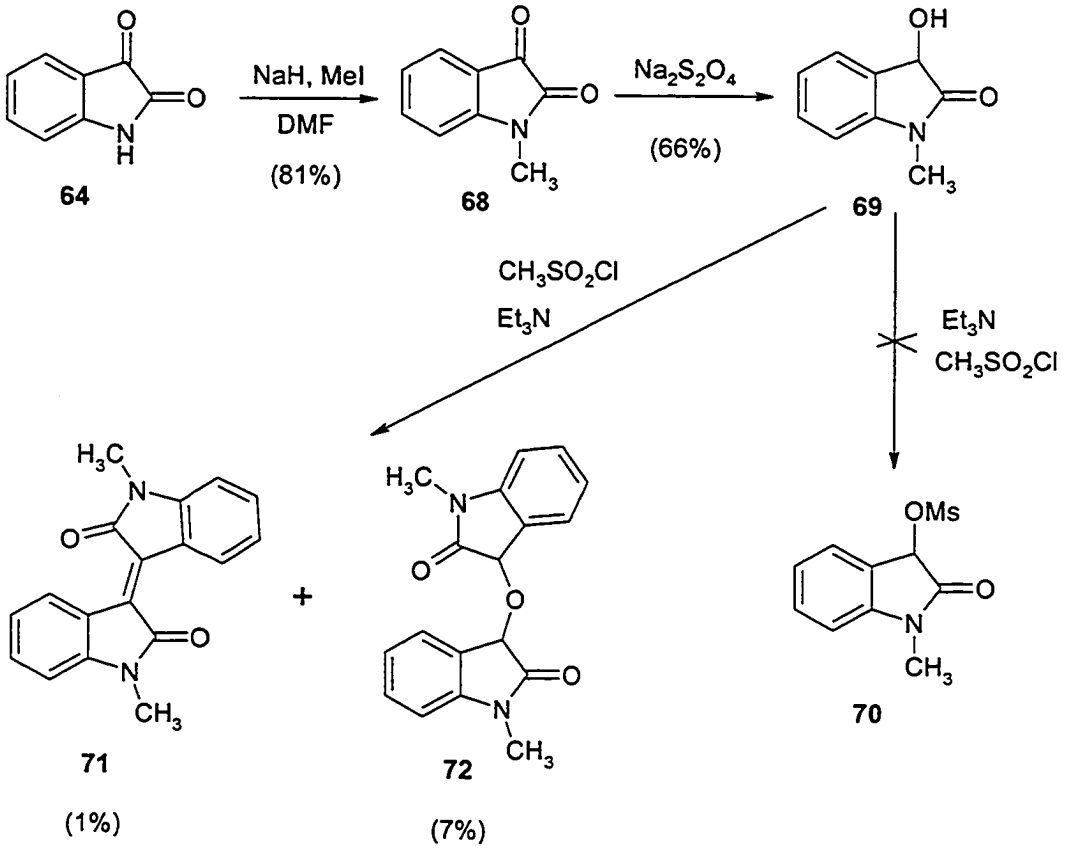


Scheme 2.30: An attempt to make 1-methyl-3-methoxy-1,3-dihydro-indol-2-one **66**.

The appearance of the hydroxy doublet at 4.81 ppm and the methine doublet at 6.17 ppm (both with J=7.6 Hz) in the ^{1}H NMR of **65** confirmed the transformation. The disappearance of the keto carbonyl resonance of isatin and appearance of the methine resonance at 69.99 ppm in the ^{13}C NMR of **65**, also confirmed the transformation. Reduced isatin **65** was found to have a melting point of 187-189°C and the HRMS displayed the M^+ ion at *m/z* 149.0475, while the calculated value was 149.0477. Having a relatively easy access to compound **65**, we were hoping to be able to find a set of methylating conditions, under which both - oxygen, as well as nitrogen, would be methylated at the same time. The use of two equivalents of sodium hydride as base and dimethyl sulphate as the methylating reagent produced 6-7 different products. A different approach - the use of potassium hydride and methyl iodide (two equivalents each), not surprisingly afforded the tri-methylated product **67** in what appeared to be a quantitative yield. The appearance of the methyl singlet at 1.45 ppm, methoxy singlet at 2.99 ppm and N-methyl singlet at 3.20 ppm in the ^{1}H NMR of **67** proved the formation of the "overalkylated" product. The same reaction was repeated, with one equivalent of methyl iodide used instead of two, however only the starting material was isolated at the end. Similarly, one equivalent of base was used with two equivalents of

methyl iodide, and again, only the starting material was observed. Consequently, the predicament of Wenkert et al. was confirmed: the introduction of one alkyl group in the number 3 position of the oxindole prompts further alkylation, regardless of the conditions. Therefore, an alternative approach was adopted.

Starting from isatin, we first methylated the N-position, using sodium hydride and methyl iodide in DMF. The N-methyl isatin **68** was obtained in 81% yield as an orange solid.



Scheme 2.31: An attempt to make 1-methyl-3-mesyl-1,3-dihydro-indol-2-one **70**.

The N-methyl group was obvious in the ^1H NMR (the resonance at 3.19 ppm) and ^{13}C NMR (the resonance at 26.59 ppm) of **68**. The melting point of **68** (129-130.5°C) was in agreement with the literature value of 132-134°C. The N-methyl isatin was then reduced using sodium dithionate to 3-hydroxy-N-methyl oxindole **69**, the structure of which was confirmed by the appearance of the hydroxy doublet at 6.28 ppm and methine doublet at

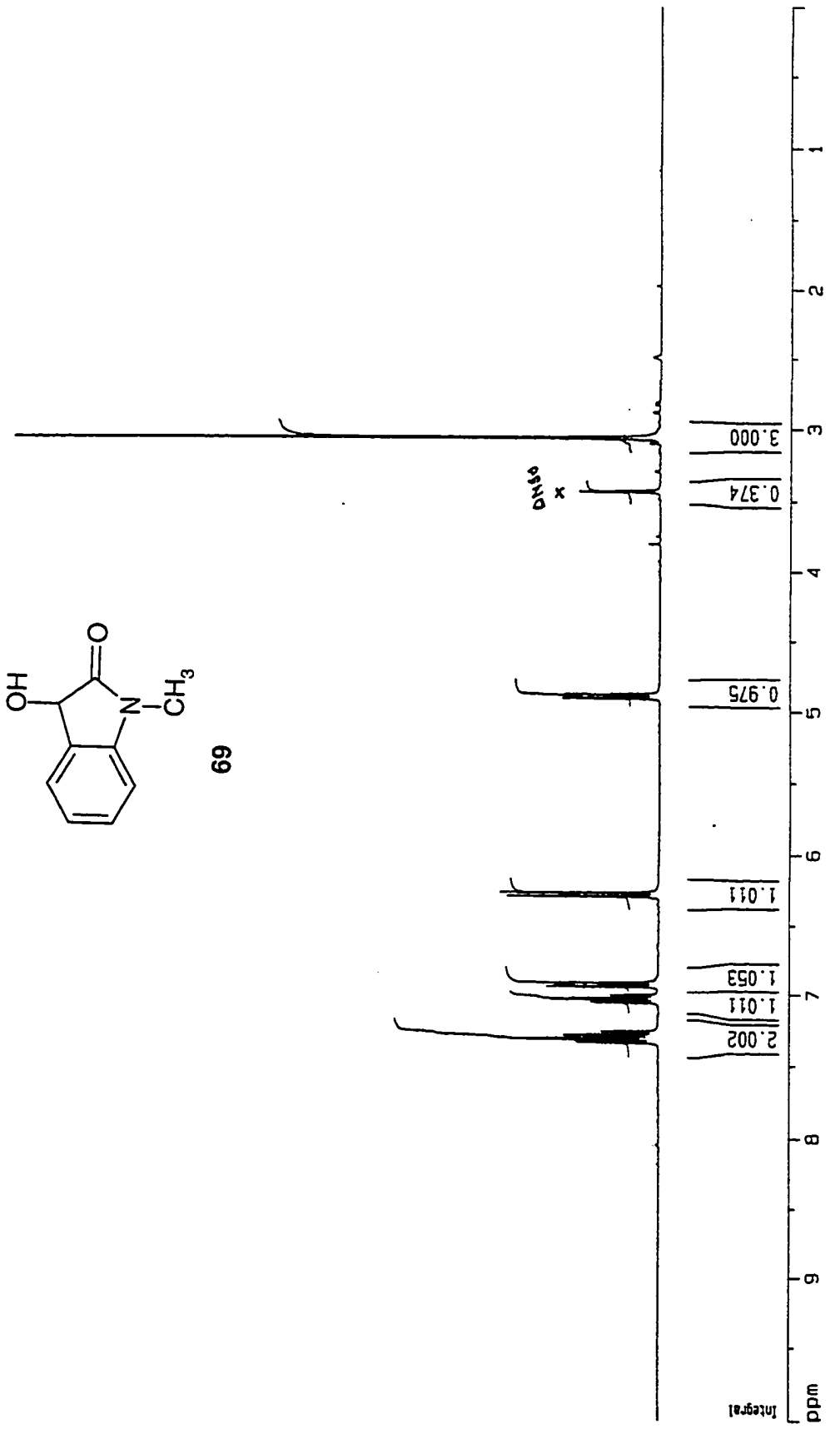


Figure 2.17: ^{1}H NMR spectrum of 3-hydroxy-1-methyl-1,3-dihydro-indol-2-one (69)

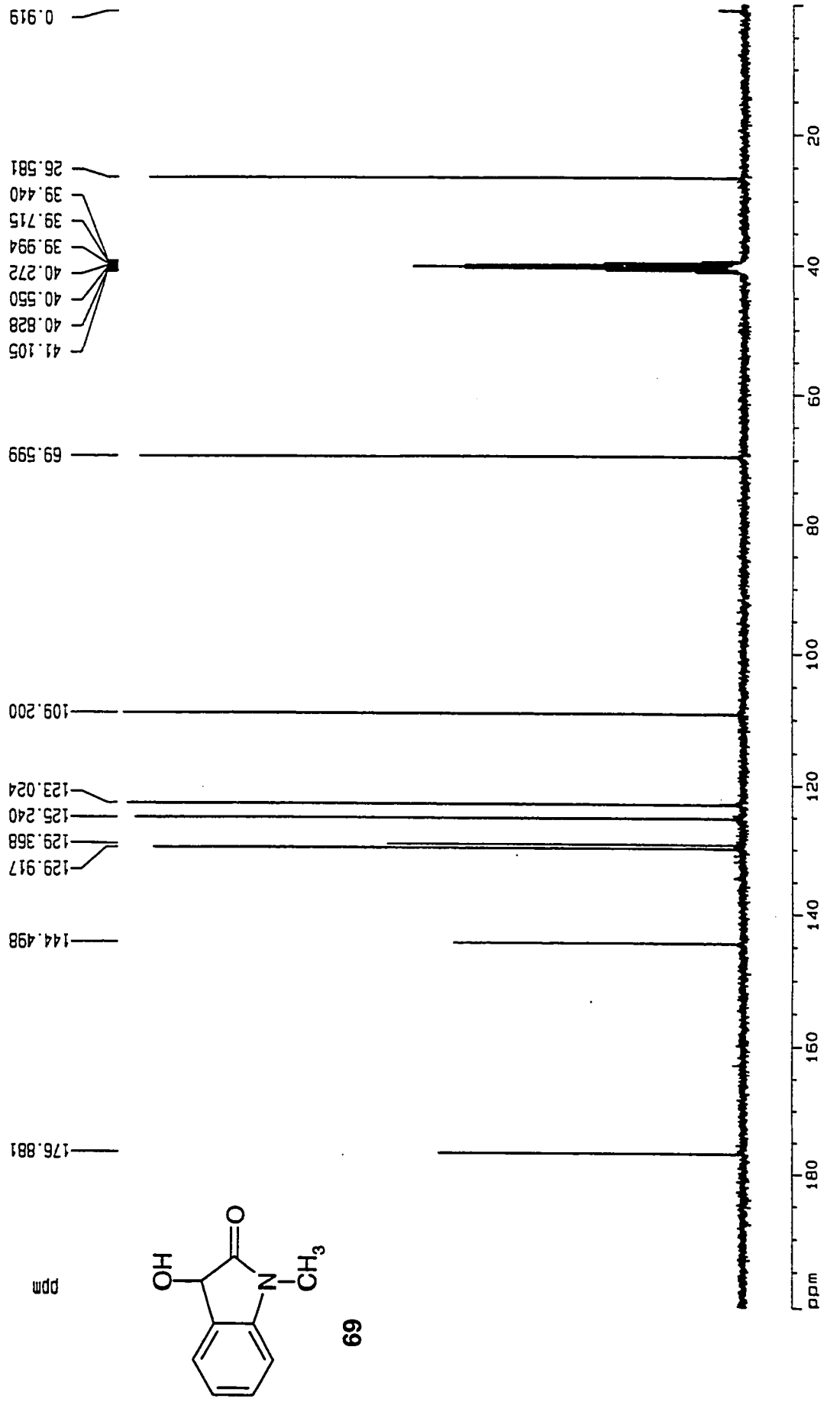
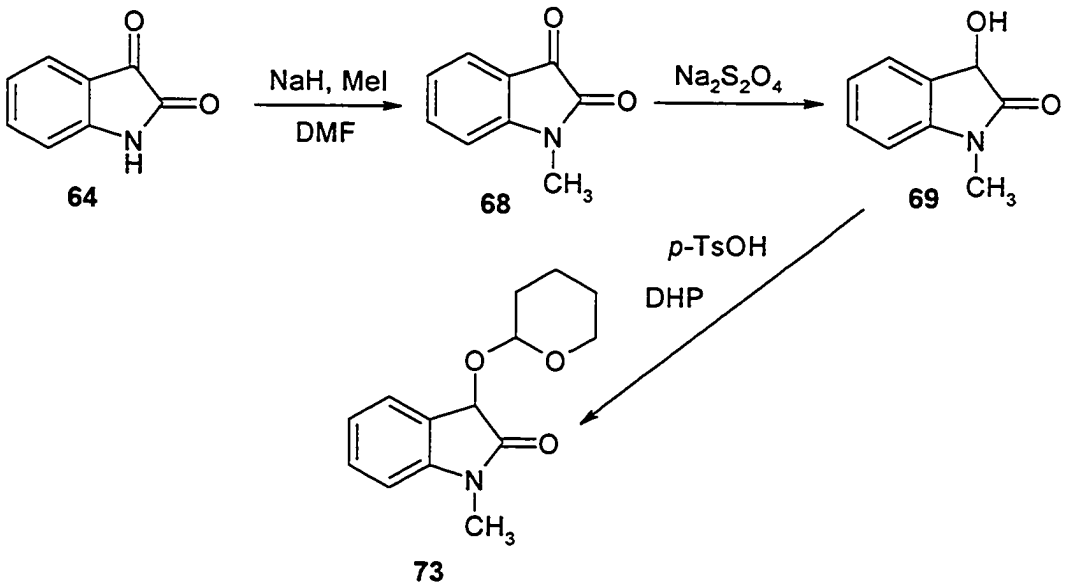


Figure 2.18: ^{13}C NMR spectrum of 3-hydroxy-1-methyl-1,3-dihydro-indol-2-one (**69**)

4.89 ppm in the ^{1}H NMR of **69** (Figure 2.17). ^{13}C NMR showed the methine carbon at 69.60 ppm, while the melting point of **69** was found to be 148-152°C, a few degrees lower than the literature value of 154-155°C.[29] The molecular ion was calculated to be 163.0634 and the HRMS of **69** showed a M^{+} ion at *m/z* 163.0641.

Introducing a mesyl ether group into the 3-position of the N-methyl oxindol-type structures would have allowed us to substitute the mesyl ether with a variety of substituents. However, the mesylation reaction afforded dimers **71** and **72** instead of the expected product **70**. Both compounds are C2 symmetric and hence have 9 carbon resonances showing in their ^{13}C NMR spectra. The ^{1}H NMR spectra of both compounds corroborated their structures.



Scheme 2.32: Preparation of 1-methyl-3-(tetrahydro-pyran-2-yloxy)-1,3-dihydro-indol-2-one **73**.

Due to the difficulty in alkylating the oxygen at C-3 under basic conditions, the attempt was made to alkylate the oxygen of compound **69** by making the acetal **73** (Scheme 2.32) under the acidic conditions. Thus 3-hydroxy N-methyl oxindole compound **69** was

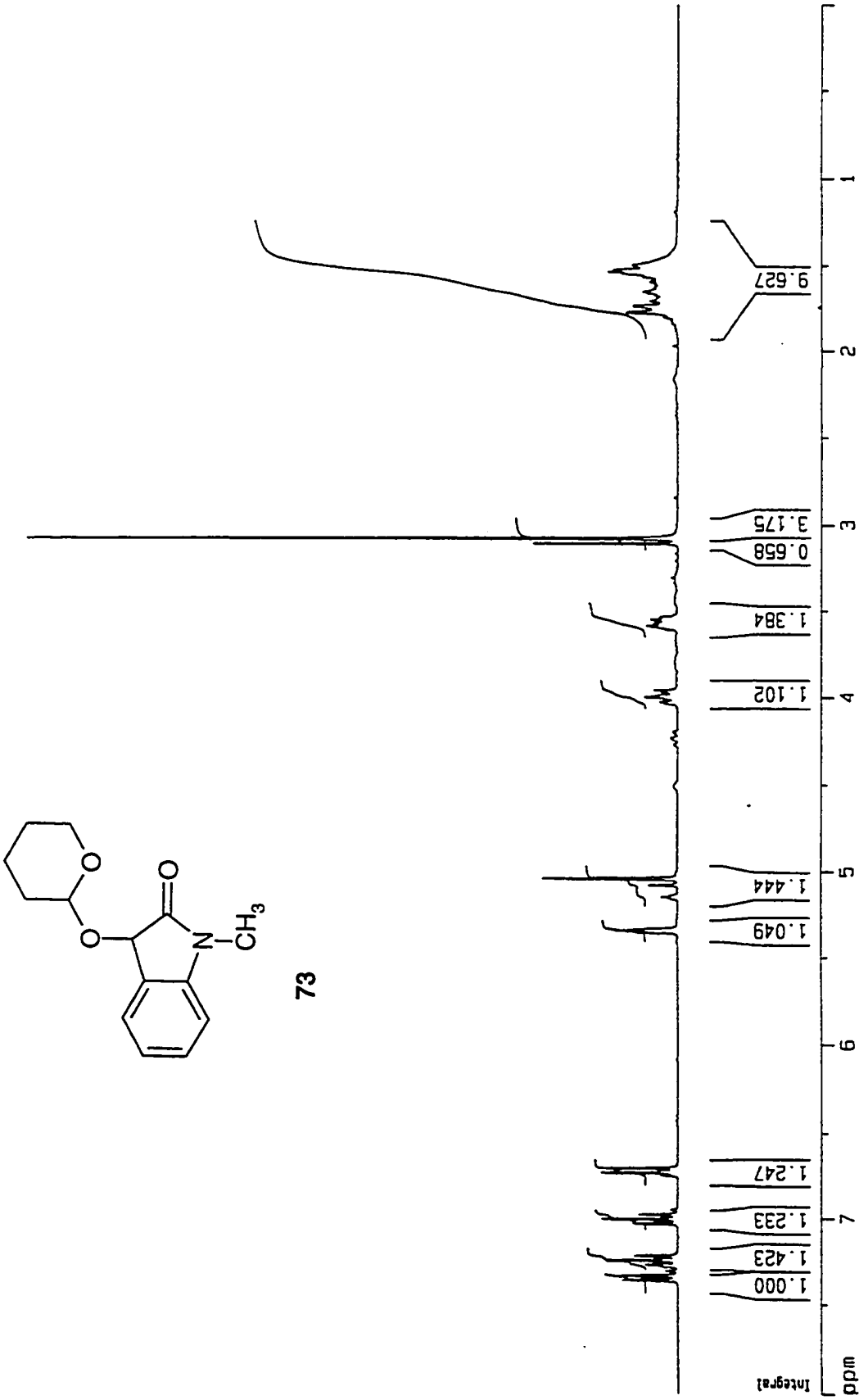


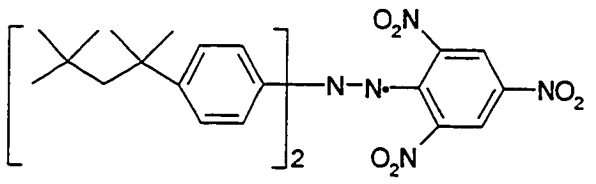
Figure 2.19: ^{1}H NMR spectrum of 1-methyl-3-(tetrahydro-pyran-2-yloxy)-1,3-dihydro-indol-2-one (**73**)

exposed to DHP and *p*-TsOH catalyst to afford, upon a careful gradient flash chromatography, **73** as a yellow liquid in 24% yield. The appearance of resonances characteristic for the DHP methylene peaks (1.38-1.89, multiplet, 6H), the methylene AB system (as a multiplet between 3.50-3.63, 1H and 3.89-4.12, 1H), methine hydrogen (5.30-5.37 multiplet), as well as the H-3 singlet at 5.02 ppm of the ^{1}H NMR of **73**, confirmed the transformation.

2.7 Kinetic testing

Since the main mechanism associated with the reaction of the phenolic antioxidants with free radicals is the hydrogen atom transfer (HAT) one, (Section 1.6 of the Introduction), it is not surprising that one of the established methods of comparing antioxidant activities of various compounds is by measuring the kinetics of abstraction of hydrogen atom from these antioxidants by various radical sources, such as peroxyl or DPPH radicals.[38-41]

In order to determine the H-atom donating ability of the compounds **8**, **19**, **33**, **38** and **59** we measured their reactivity toward the DPPH$^{\bullet}$. We chose the 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl radical, since it is a stable compound (black solid) and hence easy to handle. Also, it colours purple in solution and the colour is visible even in concentrations as low as ~10^{-7}. Therefore, the colour change from the DPPH radical (purple) to $DPPH_2$ (yellow) is visible even by naked eye. DPPH$^{\bullet}$ has two absorption maxima, around λ=330 nm and λ=519-530 nm.



79

Scheme 2.33: Structure of the 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl radical **79**.

We used the stopped-flow apparatus at the National Research Council, provided by Dr. Keith Ingold. For the purpose of ensuring the machine was producing reliable results and to familiarize ourselves with it, we tested two "standard" compounds - natural vitamin E and *p*-methoxyphenol. These compounds were treated as standards, since their rate constants for the reaction with DPPH• are known in literature.[14] The results obtained were in agreement with the literature values (please see Experimental section, part C).

The reaction of the phenolic antioxidants with DPPH• is a second-order reaction:

$$ArOH + DPPH^{\bullet} \rightarrow ArO^{\bullet} + DPPH_2, \text{ (overall rate constant k).}$$

In our experiments, however, we used large excess of the antioxidant in various concentrations at room temperature. Under these conditions, the pseudo-first-order rate can be assumed and the overall rate constant obtained as a slope of the plot of the k_{exp} (which is the observed first order rate constant for the DPPH• decay) versus the various antioxidant concentrations. We calculated the overall rate constants for the antioxidants **8**, **19**, **33**, **38** and **59** (see the Experimental section, part C) and we obtained excellent correlation coefficients in all cases (R^2>0.99). All the results are summarized in Table 3.3 and compared with the α-tocopherol/DPPH• rate of reaction in ethyl acetate.

According to the measured rates, compound **8** was found to have a rate constant of the same order (2.0 x 10^2 $M^{-1}s^{-1}$) as the vitamin E **78** (1.6 x 10^2 $M^{-1}s^{-1}$), indicating that compound **8** might be comparable to vitamin E as an antioxidant. Similarly, the reaction rate

of compound **19** with DPPH$^{\bullet}$ was calculated to be 2.1 x 10^2 $M^{-1}s^{-1}$. This is not surprising, considering that the catechol **8** and catechol **19** structures differ only slightly - compound **19** is fully substituted around the aromatic ring. Kinetic results showed that compound **19** might be a slightly more efficient antioxidant, possibly due to the previously discussed steric factors.

Compound **33** reacted an order of magnitude faster than **78** with the DPPH radicals. The rate constant for **33** was found to be 3.0 x 10^3 $M^{-1}s^{-1}$. Similarly, the catechol prepared from χ-tocopherol, compound **38**, was found to be an order of magnitude more reactive towards DPPH radicals than α-tocopherol. The absolute rate constant for this reaction was 4.5 x 10^3 $M^{-1}s^{-1}$. This result probably gives the closest direct comparison of reactivity towards DPPH radical for the catechol and phenol antioxidant structure we investigated so far. Obviously, the catechol form is more reactive in terms of the hydrogen atom abstraction.

The benzylic phenol **59** was found to be hundred-fold slower in reacting with the DPPH radicals, in comparison with **78** (k=2.9 $M^{-1}s^{-1}$). This result was expected and it convinced us that the catechol moiety is necessary to assure the hydrogen-bonding phenolic radical stabilization (please see Scheme 2.2).

Finally, compound **80**[42] was found to be a true "super"-antioxidant. It reacted with DPPH$^{\bullet}$ at the astonishing rate of 4.1 x 10^5 $M^{-1}s^{-1}$, that is thousand times faster than vitamin E.

We were unable to measure the antioxidant reactivity of the oxindole derivatives **62**, **72** and **73**, mainly due to the fact that the stopped flow apparatus at the NRC was down for several months during the summer of 2002. Time constraints prevented us from completing the measurements once the machine was back in operation.

2.8 Future considerations

Since solvent effects play an important role in determining rates of reactions,[40] it would be advisable to repeat the measurements in a different solvent, such as chlorobenzene or benzene. The hydrogen-bonding effects would thus be minimized and the reaction rates would be expected to be significantly higher. The results of the kinetic testing in different solvents would serve two purposes – they would confirm the original results we obtained and they would confirm the established trends in solvent effects.[40]

Also, we must prove that the radical resulting from a potential antioxidant is unreactive towards oxygen, as discussed earlier. Hence, prior to conducting animal testing, the group[43] suggested that compounds **8**, **19** and **33** be tested in cells producing peroxy radicals. For this purpose the catechols were transformed into more stable di-acetates. The description of the procedures and the spectral data are given in the Experimental section, part A.

Our kinetic results confirmed the BDE and IP predictions. Some of the compounds synthesized might prove to be better antioxidants than vitamin E, providing, of course, that all the other relevant factors allow it. The synthesized compounds must prove to be compatible with the biological systems in terms of solubility, transport, and most importantly toxicity.

EXPERIMENTAL

Chapter 3: Experimental

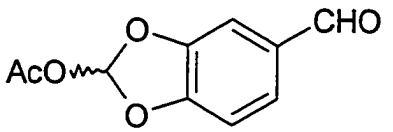
3.1 Part A

GENERAL: Most of the solvents used in the reactions were distilled prior to use. THF was dried by distillation over sodium and benzophenone. Dichloromethane was dried by distillation from calcium hydride. DMF and DMSO were distilled and stored over activated molecular sieves. The starting materials used were commercial samples and were used as received, unless otherwise specified. Purity of starting materials was routinely checked by ^{1}H NMR and thin layer chromatography (TLC). Reactions were followed using the TLC (silica gel on aluminium foil). Visualization of the spots was made possible by means of a UV lamp, and dying of the plate in a molybdate solution, followed by charring using a heat gun. The molybdate solution was prepared by dissolving ammonium molybdate (2.5 g) and cerric sulphate hydrate (1.0 g) in a solution of sulphuric acid (10 ml) in distilled water (90 ml). Flash chromatography[31] used silica gel 270-400 mesh with hexane-ethyl acetate solvent mixtures.

Melting points were measured by a Thomas Hoover Capillary Melting Point Apparatus and were uncorrected. The IR spectra were recorded on a Bomem-Michelson MB-100 FT/IR spectrophotometer. The IR samples were run as thin films on a potassium bromide plate, either dissolved in chloroform, or neat. Mass spectra were obtained by means of a VG 7070E spectrometer and all samples were electronically ionized. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500, Bruker AMX-300 and Varian Gemini XL-200 spectrometers. Most samples were run in spectroscopic grade deuterated chloroform. The ^{1}H NMR peaks were reported using the following abbreviations for their multiplicities: singlet (s), broad singlet (brs), doublet (d), triplet (t), doublet of doublets (dd), doublet of triplets (dt), quartet (q) doublet of quartets (dq) and multiplet (m). For the interpretation of the NMR and IR spectra, the literature resonances were used.[32]

PREPARATION OF ACETIC ACID 5-FORMYL-BENZO[1,3]-DIOXOL-2-YL ESTER (2) AND 3,4-DIHYDROXY-BENZALDEHYDE (3)

Lead tetraacetate (2.67 g, 0.00602 mol), recrystallized from glacial acetic acid, was added to a solution of piperonal **1** (518.0 mg, 0.00345 mol) and glacial acetic acid (7.8 ml) in benzene (35 ml), under nitrogen, and stirred at 50 °C for 23 h. The reaction mixture was cooled, diluted with 60 ml water and extracted with ether (3 x 25 ml). The combined organic extracts were washed with brine (1 x 10 ml), dried over anh. magnesium sulphate, filtered and concentrated in vacuo. Gradient flash chromatography (75 g of silica gel, hexanes-ethyl acetate eluent with 2% polarity increase) afforded **2** (91.1 mg, 13%) as a clear oil and **3** (82.7 mg, 17%) as a yellow oil. The starting material, piperonal **1**, was recovered (0.246 g, 48%).



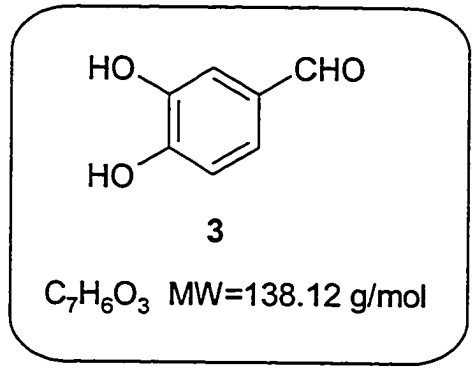
2

$C_{10}H_8O_5$ MW=208.17 g/mol

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 2.09 (s, 3H, methyl), 7.06 (d, J=8.0 Hz, 1H, H-7), 7.45 (d, J=1.5 Hz, 1H, H-4), 7.51 (dd, J=8.0, 1.6 Hz, 1H, H-6), 7.73 (s, 1H, H-2), 9.83 (s, 1H, -CHO).

^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 190.76 (-CHO), 169.17 (acetate -C=O), 150.52, 146.55, 133.11, 129.20, 113.96, 109.91, 108.76, 21.50.

MS [EI, *m/z* (%)]: 208 [MH^+] (20), 180 (21), 166 (33), 149 (97), 138 (79), 119 (11), 91 (5), 43 (100).



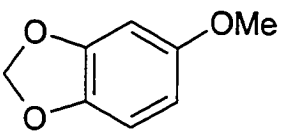
3

$C_7H_6O_3$ MW=138.12 g/mol

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 7.01 (d, J=8.1 Hz, 1H), 7.35 (m, 2H), 8.78 (brs, 2H, -OH), 9.79 (s, 1H, -CHO).

PREPARATION OF 5-METHOXY-BENZO[1,3]DIOXOLE (5)

Anhydrous potassium carbonate (35.0 g, 0.253 mol) was added to the solution of sesamol **1** (5.01 g, 0.0362 mol) in acetone (80 ml) at room temperature under a nitrogen atmosphere. After stirring vigorously for 1 h, methyl iodide (24.8 ml, 0.398 mol) was added dropwise and the reaction mixture was refluxed at 55 °C for 42 h. The resulting slurry was filtered through Celite and the filtrate evaporated in vacuo. The residue was taken up in 40 ml water and extracted with ether (3 x 30 ml). The combined ether fractions were washed with 25 ml of 10% aq. sodium hydroxide solution, followed by 2 x 25 ml of water, dried over anh. magnesium sulphate, filtered and concentrated in vacuo to afford anisole **5** (4.61 g, 84%) as a yellow liquid:



5

$C_8H_8O_3$ MW=152.15 g/mol

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 3.73 (s, 3H, methoxy), 5.89 (s, 2H, methylene dioxy), 6.31 (dd, J=8.5, 2.6 Hz, 1H, H-6), 6.47 (d, J=2.6 Hz, 1H, H-4), 6.69 (d, J=8.5 Hz, 1H, H-7).

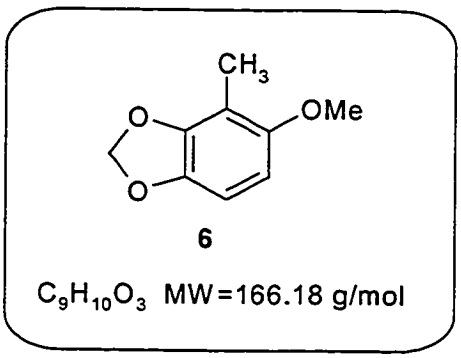
IR (neat) ν(cm^{-1}): 3028, 2953, 2896, 1632, 1489, 1243,1199, 1039, 939.

MS [EI, *m/z* (%)]: 152 [MH^+] (100), 137 (74), 107 (32), 79 (31), 51 (17).

HRMS calcd. for $C_8H_8O_3$: 152.0473; found: 152.0477.

PREPARATION OF 5-METHOXY-4-METHYL-BENZO[1,3]DIOXOLE (6)

A 1.7 M solution of *t*-butyllithium in pentane (27.0 ml, 0.0459 mol) was added dropwise to a solution of 5-methoxy-benzo[1,3]dioxole **5** (4.60 g, 0.0302 mol) in dry THF (20 ml), under nitrogen at 0 °C. After stirring for 30 min, methyl iodide (3.80 ml, 0.0610 mol) was added dropwise and the reaction mixture continued stirring for 1 h at 0 °C, and additional 2 h at RT. The saturated ammonium chloride solution (10 ml) was added, followed by water (15 ml). The resulting mixture was extracted with ethyl acetate (3 x 20 ml). The combined organic layers were dried over anh. $MgSO_4$, filtered and concentrated in vacuo to give 5.29 g of a crude product, brown oil. Graduated flash chromatography (125 g silica gel, hexanes-ethyl acetate eluent, polarity increase by 5%) yielded **6** (3.60 g, 72%) as a white solid:



6

$C_9H_{10}O_3$ MW=166.18 g/mol

mp 39-41°C

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 2.12 (s, 3H, methyl), 3.76 (s, 3H, methoxy), 5.89 (s, 2H, methylene dioxy), 6.25 (d, J=8.4 Hz, 1H, H-6), 6.58 (d, J=8.4 Hz, 1H, H-7).

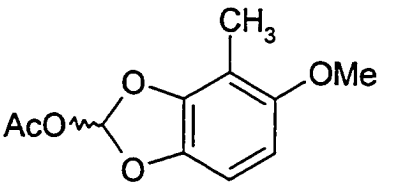
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 153.88 (C-5), 147.07 (C-3), 141.42 (C-1), 109.67 (C-4), 104.62 (C-7), 102.12 (C-6), 101.24 (C-2), 56.46 (methoxy), 9.13 (methyl).

MS [EI, *m/z* (%)]: 166 [MH^+] (98), 151 (100), 121 (47), 93 (16), 65 (17).

HRMS calcd. for $C_9H_{10}O_3$: 166.0630; found: 166.0626.

PREPARATION OF ACETIC ACID 5-METHOXY-4-METHYL-BENZO[1,3]DIOXOL-2-YL ESTER (7)

Lead tetraacetate (14.4 g, 0.0325 mol), recrystallized from glacial acetic acid, was added to a solution of 5-methoxy-4-methyl-benzo[1,3]dioxole **6** (3.60 g, 0.0217 mol) in benzene (60 ml), under nitrogen, and stirred at reflux (75-80°C) for 20 h. The reaction mixture was cooled, diluted with 30 ml water and extracted with ether (3 x 25 ml). The combined organic extracts were dried over anh. magnesium sulphate, filtered and concentrated in vacuo affording a brown oil **7** (4.48 g, 92% crude):



7

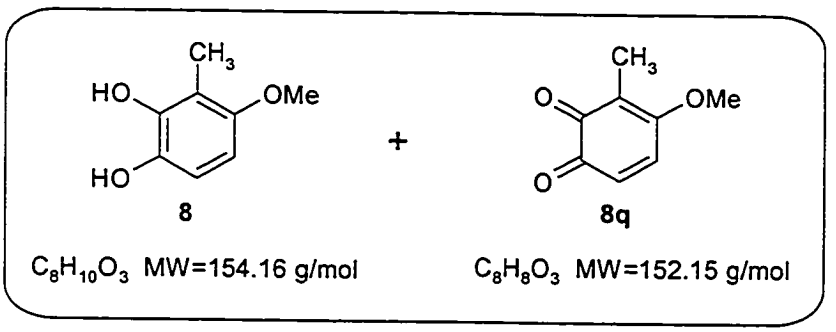
$C_{11}H_{12}O_5$ MW=224.21 g/mol

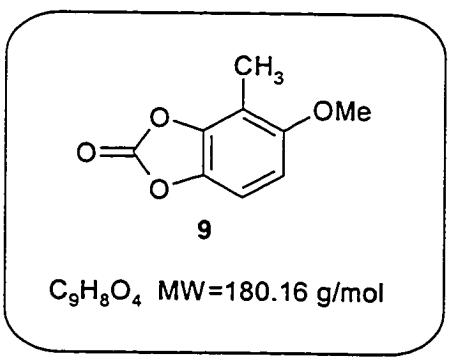
^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 2.09 (s, 3H, acetate methyl), 2.12 (s, 3H, methyl), 3.77 (s, 3H, methoxy), 6.36 (d, J=8.0 Hz, 1H, H-6), 6.70 (d, J=8.0 Hz, 1H, H-7), 7.64 (s, 1H, H-2).

^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 170.16 (acetate -C=O), 151.51, 146.55, 142.79, 113.96, 108.76, 103.14, 102.10, 56.79 (methoxy), 22.32, 9.09 (methyl).

PREPARATION OF 4-METHOXY-3-METHYL-BENZENE-1,2-DIOL (8) AND 5-METHOXY-4-METHYL-BENZO[1,3]DIOXOL-2-ONE (9)

Crude acetate **7** (4.48 g, 0.0200 mol) was dissolved in 80% aq. acetic acid (100 ml) and stirred at RT for 48 h. The solvent was evaporated and the remaining residue washed with toluene (4 x 1 ml) and condensed to dryness in vacuo. Gradient flash chromatography (125 g silica gel, hexanes-ethyl acetate eluent with 5% polarity increase) afforded a mixture of **8** and **8q** (2.62 g, 85%) as a red liquid and **9** (0.540 g, 15%) as an off-white solid:





mp 110-111°C

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 2.17 (s, 3H, methyl), 3.80 (s, 3H, methoxy), 6.59 (d, J=8.8 Hz, 1H, H-6), 6.93 (d, J=8.8 Hz, 1H, H-7).

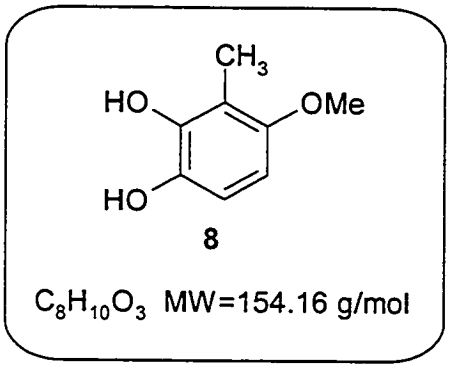
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 155.54 (C-2), 152.34 (C-5), 143.00 (C-1), 137.26 (C-3), 111.30 (C-4), 107.08 (C-7), 105.67 (C-6), 56.56 (methoxy), 9.19 (methyl).

MS [EI, *m/z* (%)]: 180 [MH^+] (100), 135 (12), 121 (84), 93 (44), 65 (17).

HRMS calcd. for $C_9H_8O_4$: 180.0422; found: 180.0392.

PREPARATION OF 4-METHOXY-3-METHYL-BENZENE-1,2-DIOL (8) VIA REDUCTION OF 4-METHOXY-3-METHYL-[1,2]BENZOQUINONE (8q)

A solution of sodium dithionate (20.75 g, 0.119 mol) in water (75 ml) was added to a rapidly stirred solution of the *o*-quinone **8q** / catechol **8** mixture (2.62 g, 0.0172 mol) in ether (25 ml), resulting in the reaction mixture colour change from orange to bright yellow within 3 min. Aqueous 10% hydrochloric acid (10 ml) was added and the reaction mixture extracted with ether (4 x 20 ml). The combined ether extracts were washed with sat. aq. sodium bicarbonate (2 x 10 ml), dried over anh. magnesium sulphate, filtered and concentrated in vacuo to give **8** (2.39 g, 91%) as a yellow oil.



8

$C_8H_{10}O_3$ MW=154.16 g/mol

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 2.12 (s, 3H, methyl), 3.74 (s, 3H, methoxy), 5.83 (brs, 2H, hydroxyl), 6.28 (d, J=8.7 Hz, 1H, H-5), 6.60 (d, J=8.7 Hz, 1H, H-6).

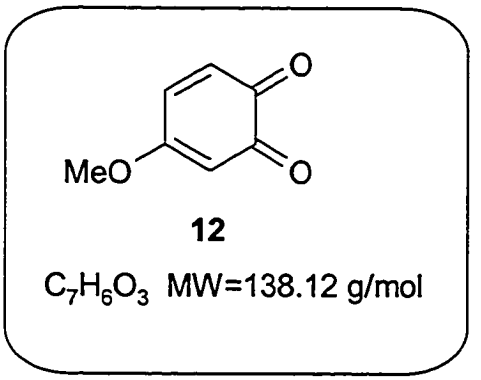
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 152.81 (C-4), 143.51 (C-1), 137.71 (C-2), 114.19 (C-3), 112.37 (C-6), 102.99 (C-5), 56.69 (methoxy), 8.94 (methyl).

MS [EI, *m/z* (%)]: 154 [MH^+] (91), 139 (100), 121 (38), 93 (15), 65 (22).

HRMS calcd. for $C_8H_{10}O_3$: 154.0630; found: 154.0612.

PREPARATION OF 4-METHOXY-[1,2]-BENZOQUINONE (12)

To a stirred 15 mM solution of potassium dihydrogen orthophosphate, KH_2PO_4, (200 ml), cooled to 5°C, potassium nitrosodisulfonate (Fremy's salt) (2.97 g, 0.0111 mol) was added. *p*-Methoxyphenol (0.507 g, 0.00408 mol) was dissolved in 1 ml (plus 4 x 1 ml washes) of ether and added dropwise to the aqueous solution. The resulting reaction mixture was stirred open to air at 5°C for 1 h and afterward extracted with chloroform (4 x 20 ml). The combined organic phases were dried over anh. magnesium sulphate, filtered and the solvents were removed in vacuo to yield a dark red oil which recrystallized from anhydrous ether gave **12** (0.480 g, 85%) as a pink-yellow solid:



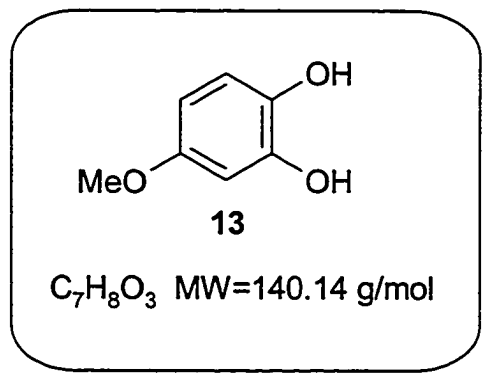
12

$C_7H_6O_3$ MW=138.12 g/mol

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 3.85 (s, 3H, methoxy), 5.78 (d, J=3.5 Hz, 1H, H-3), 6.40 (d, J=11.0 Hz, 1H, H-6), 6.86 (dd, J=11.0, 3.5 Hz, 1H, H-5).

PREPARATION OF 4-METHOXY-BENZENE-1,2-DIOL (13)

A solution of sodium dithionate (2.00 g, 0.0115 mol) in 20 ml of water (plus 2 x 2 ml washes), was added to a solution of 4-methoxy-[1,2]-benzoquinone **12** (0.480 g, 0.00348 mol) in ether (40 ml), and the resulting mixture was stirred open to air at room temperature for 10 min. Aqueous 10% hydrochloric acid (20 ml) was added and the reaction mixture was extracted with ether (3 x 20 ml). The combined ether fractions were washed with sat. aq. sodium bicarbonate solution (10 ml), dried over anh. magnesium sulphate, filtered and rotoevaporated to dryness yielding an orange solid which was then recrystallized from dry ether to give **13** (0.410 g, 84%) as an off-white solid:



13

$C_7H_8O_3$ MW=140.14 g/mol

mp 45.5-48.0°C (lit. mp: 45-47°C)[17]

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 3.72 (s, 3H, methoxy), 4.74 (s, 1H), 5.34 (s, 1H), 6.32 (dd, J=8.6, 2.7 Hz, 1H, H-5), 6.49 (d, J=2.7 Hz, 1H, H-3), 6.76 (d, J=8.6 Hz, 1H, H-6).

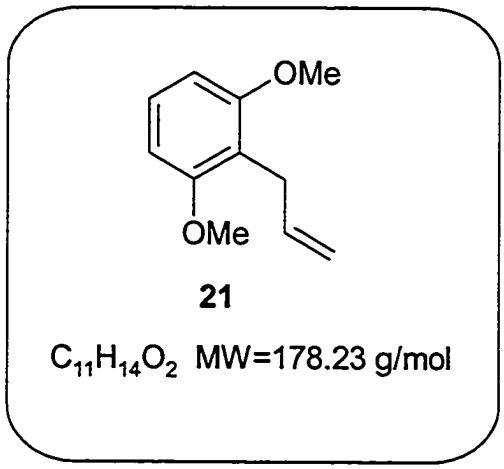
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 154.97 (C-4), 145.05 (C-2), 138.07 (C-1), 116.21 (C-6), 105.63 (C-5), 102.69 (C-3), 56.08 (methoxy).

MS [EI, *m/z* (%)]: 140 [MH^+] (100), 125 (70), 107 (33), 79 (12).

PREPARATION OF 2-ALLYL-1,3-DIMETHOXY-BENZENE (21)

A Grignard solution, (magnesium bromide in anhydrous ether, 3M), was prepared by adding 1,2-dibromoethane (8.50 ml, 0.0988 mol) dropwise, over a 60 min. time period, to magnesium strips (2.01 g, 0.0827 mol), stirred in anh. ether (100 ml), under nitrogen, at room temperature. During the addition of the first 1 ml of 1,2-dibromoethane, the ice bath was used at times to cool the reaction mixture.

A 1.41 M solution of *t*-butyllithium in pentane (30.0 ml, 0.0420 mol) was added slowly to a solution of 1,3-dimethoxybenzene **20** (5.00 ml, 0.0382 mol) in dry THF (50 ml), at -40°C (dry ice/acetonitrile bath), under a nitrogen atmosphere. The reaction mixture was stirred for 15 min., after which time the 3M Grignard solution (14.0 ml, 0.0420 mol) was added and the stirring continued for 30 min. at 0°C. Allyl bromide (4.00 ml, 0.0458 mol) was added dropwise and the reaction mixture allowed to come to room temperature, while stirring for 3.5 h. Saturated aq. ammonium chloride solution (10 ml) and water (15 ml) were added and the mixture extracted with ethyl acetate (4 x 25 ml). The combined organic portions were dried over anh. magnesium sulphate, filtered and concentrated in vacuo affording an orange liquid. Gradient flash chromatography (250 g of silica gel, 10-to-20% ethyl acetate in hexane eluent with 2% polarity increase) yielded **21** (5.10 g, 75%) as a light yellow oil:



21

$C_{11}H_{14}O_2$ MW=178.23 g/mol

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 3.52 (dt, J=6.2, 1.5 Hz, 2H, H-7), 3.86 (s, 6H, methoxy), 5.01 (dq, J=10.6, 1.5 Hz, 1H), 5.07 (dq, J=17.1, 1.7 Hz, 1H), 6.05 (ddt, J=17.1, 10.5, 6.2 Hz, 1H, H-8), 6.62 (d, J=8.3 Hz, 2H, H-4 and H-6), 7.21 (t, J=8.3 Hz, 1H, H-5).

^{13}C NMR ($CDCl_3$, 500 MHz) δ(ppm): 158.18 (2C, C-1 and C-3), 136.83 (C-8), 127.02 (C-5), 116.49 (C-2), 113.83 (C-9), 103.79 (2C, C-4 and C-6), 55.64 (2C, methoxy), 27.10 (C-7).

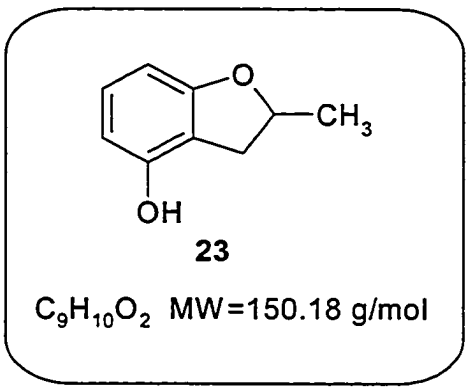
IR (neat) ν(cm^{-1}): 3077, 2836, 1637, 1596, 1495, 1475, 1435, 1259, 1129, 1111, 1080, 1041, 996.

MS [EI, *m/z* (%)]: 178 [MH^+] (100), 163 (21), 149 (47), 135 (21), 121 (18), 103 (27), 91 (54), 77 (24), 65 (16).

HRMS calcd. for $C_{11}H_{14}O_2$: 178.0994; found: 178.1012.

PREPARATION OF 2-METHYL-2,3-DIHYDRO-BENZOFURAN-4-OL (23)

1N boron tribromide solution in dichloromethane was added in 10 ml-portions (total 60 ml, 0.0160 mol) to a solution of 2-allyl-1,3-dimethoxy-benzene **21** (3.80 g, 0.0213 mol) in dry dichloromethane (40 ml) under a nitrogen atmosphere at -78 °C (dry ice/acetone bath). The reaction mixture was stirred for 12 h and slowly quenched with sat. aq. sodium bicarbonate solution (120 ml) at 0 °C. The resulting mixture was extracted with dichloromethane (3 x 50 ml) and the organic extracts were combined, dried over anh. magnesium sulphate, filtered and concentrated in vacuo to give a dark maroon oil. Isocratic flash chromatography (125 g of silica gel, 1:1 hexanes-ethyl acetate) provided **23** (0.270 g, 8%) as a yellow oil:



23

$C_9H_{10}O_2$ MW=150.18 g/mol

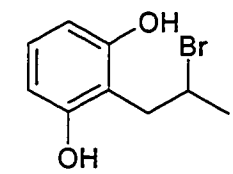
^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 1.48 (d, J=6.3 Hz, 3H, H-10), 3.04 (dq, J=15.2, 8.9 Hz, 2H, H-3), 4.94-5.02 (m, 1H, H-2), 6.10 (brs, 1H, -OH), 6.36 (d, J=8.0 Hz, 1H), 6.42 (d, J=8.0 Hz, 1H), 6.97 (t, J=8.0 Hz, 1H, H-6).

^{13}C NMR ($CDCl_3$, 500 MHz) δ(ppm): 160.68 (C-8), 152.56 (C-4), 128.96 (C-6), 112.59 (C-9), 107.77 (C-5), 102.17 (C-7), 80.17 (C-2), 33.94 (C-3), 21.53 (C-10).

IR (neat) ν(cm^{-1}): 3000-3650 (br), 2974, 1606, 1464, 1234, 1010, 767.

MS [EI, *m/z* (%)]: 150 [MH^+] (100), 135 (48), 123 (16), 107 (39), 91 (6), 77 (22), 65 (6).

HRMS calcd. for $C_9H_{10}O_2$: 150.0681; found: 150.0669.

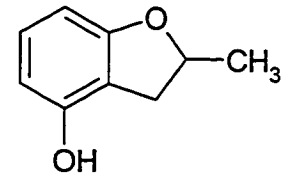


24

$C_9H_{11}O_2Br$ MW=231.09 g/mol

PREPARATION OF 2-METHYL-2,3-DIHYDRO-BENZOFURAN-4-OL (23) FROM 2-(2-BROMOPROPYL)-BENZENE-1,3-DIOL (24)

Triethylamine (0.60 ml, 4.30 mmol) was added dropwise to a solution of 2-(2-bromopropyl)-benzene-1,3-diol **24** (544 mg, 2.36 mmol) in dry benzene (50 ml), under nitrogen, and the reaction mixture was refluxed at 82 °C for 18 h, quenched with aq. 10% hydrochloric acid (15 ml) and extracted with ethyl acetate (3 x 20 ml). The combined organic fractions were washed with sat. aq. sodium bicarbonate (5 ml), then water (10 ml), dried over anh. magnesium sulphate, filtered and concentrated in vacuo to yield an orange liquid. Purification by gradient flash chromatography (25 g of silica gel, hexanes-ethyl acetate eluent, 2.5% polarity increase) resulted in **23** (0.300 g, 85%) as a yellow oil:



23

$C_9H_{10}O_2$ MW=150.18 g/mol

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 1.48 (d, J=6.3 Hz, 3H, H-10), 3.04 (dq, J=15.2, 8.9 Hz, 2H, H-3), 4.94-5.02 (m, 1H, H-2), 6.10 (brs, 1H, -OH), 6.36 (d, J=8.0 Hz, 1H), 6.42 (d, J=8.0 Hz, 1H), 6.97 (t, J=8.0 Hz, 1H, H-6).

^{13}C NMR ($CDCl_3$, 500 MHz) δ(ppm): 160.68 (C-8), 152.56 (C-4), 128.96 (C-6), 112.59 (C-9), 107.77 (C-5), 102.17 (C-7), 80.17 (C-2), 33.94 (C-3), 21.53 (C-10).

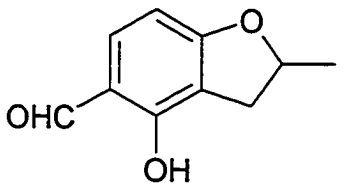
IR (neat) ν(cm^{-1}): 3000-3650 (br), 2974, 2859, 1606, 1464, 1234, 1010, 767, 622.

MS [EI, *m/z* (%)]: 150 [MH^+] (100), 135 (48), 123 (16), 107 (39), 91 (6), 77 (22), 65 (6).

HRMS calcd. for $C_9H_{10}O_2$: 150.0681; found: 150.0669.

PREPARATION OF 4-HYDROXY-2-METHYL-2,3-DIHYDRO-BENZOFURAN-5-CARBALDEHYDE (25)

A solution of 2-methyl-2,3-dihydro-benzofuran-4-ol **23** (560 mg, 3.73 mmol), tin tetrachloride (0.05 ml, 0.427 mmol) and tributylamine (0.36 ml, 1.51 mmol) in anhydrous toluene (30 ml) was rapidly stirred in a 100 ml round bottom flask equipped with a reflux condenser and a side arm, under nitrogen, for 20 min. Paraformaldehyde (756 mg, 8.39 mmol) was added and the resulting yellow suspension heated for 2 h at 90-93°C. After cooling, the reaction mixture was poured into water (15 ml), acidified to pH=2 using 2N aq. hydrochloric acid and extracted with ether (4 x 20 ml). The combined organic layers were washed with water (20 ml), dried over anh. magnesium sulphate, filtered and the solvents were evaporated in vacuo affording a yellow liquid. Isocratic flash chromatography (25 g of silica gel, 1:3 ethyl acetate-hexanes) gave **25** (220 mg, 33%) as a yellow oil:



25

$C_{10}H_{10}O_3$ MW=178.19 g/mol

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 1.47 (d, J=6.3 Hz, 3H, H-10), 3.03 (dq, J=15.5, 7.2 Hz, 2H, H-3), 5.04-5.11 (m, 1H, H-2), 6.41 (d, J=8.4 Hz, 1H, H-7), 7.31 (d, J=8.4 Hz, 1H, H-6), 9.64 (s, 1H, -CHO), 11.43 (s, 1H, -OH).

^{13}C NMR ($CDCl_3$, 500 MHz) δ(ppm): 194.23 (C-11), 167.63 (C-8), 159.54 (C-4), 136.32 (C-6), 115.94 (C-5), 113.05 (C-9), 103.09 (C-7), 82.35 (C-2), 32.91 (C-3), 21.90 (C-10).

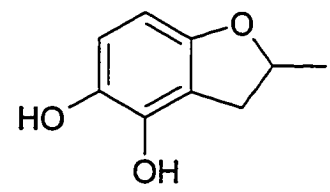
IR (neat) ν(cm^{-1}): 2800-3800 (br), 2360, 1650, 1645, 1489, 1260, 1437, 798.

MS [EI, *m/z* (%)]: 178 [MH^+] (100), 163 (74), 149 (16), 135 (10), 121 (7), 107 (18), 77 (15).

HRMS calcd. for $C_{10}H_{10}O_3$: 178.0630; found: 178.0634.

PREPARATION OF 2-METHYL-2,3-DIHYDRO-BENZOFURAN-4,5-DIOL (26)

To a rapidly stirred solution of 4-hydroxy-2-methyl-2,3-dihydro-benzofuran-5-carbaldehyde **25** (62.9 mg, 0.353 mmol) and sodium hydroxide (14.1 mg, 0.353 mmol) in 10 ml of 9:1 water-THF solvent mixture, under the nitrogen, at 43°C, a solution of hydrogen peroxide (0.14 ml, 30% aq.,1.41 mmol) was added. The reaction mixture was stirred at 43°C for 20 min. and then continued stirring at room temperature for additional 20 min. Hydrochloric acid 10% aq. was added (6 ml) and the reaction mixture extracted with ethyl acetate (3 x 15 ml). The organic portions were combined, washed with water (1 x 20 ml), dried over anh. magnesium sulphate, and concentrated in vacuo. The ^{1}H NMR of the crude material **26** showed only the starting material, **25**.

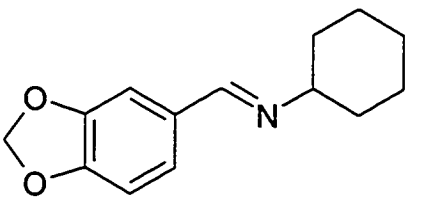


26

$C_9H_{10}O_3$ MW=166.18 g/mol

PREPARATION OF BENZO[1,3]DIOXOL-5-YLMETHYLENE-CYCLOHEXYL-AMINE (39)

Piperonal **1** (0.503 g, 0.00335 mol) was dissolved in 50 ml of dry benzene in a 100 ml round bottom flask equipped with a Dean-Stark apparatus, under nitrogen, at room temperature. Cyclohexylamine (0.50 ml, 0.00437 mol) was added dropwise and the reaction mixture was stirred at 110-115°C for 18 h. The solvent was removed in vacuo to yield, upon recrystallization from methanol, **39** (0.741 g, 92%) as a white solid:



39

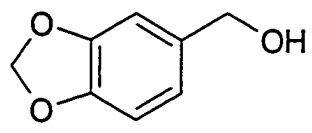
$C_{14}H_{17}O_2N$ MW=231.30 g/mol

mp 63-65°C (lit. mp: 65-66°C)[21]

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 1.15-1.95 (m, 10 H), 3.05-3.25 (m, 1H, =NCH-), 5.97 (s, 2H, methylene dioxy), 6.79 (d, J=8.5 Hz, 1H, H-7), 7.06 (dd, J=8.5, 1.5 Hz, 1H, H-6), 7.34 (d, J=1.5, 1H, H-4), 8.18 (s, 1H, -CH=N-).

PREPARATION OF BENZO[1,3]DIOXOL-5-YL-METHANOL (42)

To a solution of piperonal **1** (1.51 g, 0.0101 mol) in tetrahydrofuran (30 ml), cooled to 0°C, lithium aluminium hydride (0.254 g, 0.00671 mol) was added and the reaction mixture was stirred open to air for 2 h. Water (2 ml) was added, followed by 4 ml of aq. 10% sodium hydroxide and additional 8 ml of water. The mixture was then filtered through a layer of Celite, washed with ethyl acetate, saturated with brine (sat. aq. sodium chloride) and extracted with ethyl acetate (3 x 15 ml). The combined ethyl acetate fractions were dried over anh. magnesium sulphate, filtered and concentrated in vacuo to yield an off-white solid, which recrystallized from hexane:chloroform (approximately 1:1) gave **42** (1.50 g, 99%) as a white solid:



42

$C_8H_8O_3$ MW=152.15 g/mol

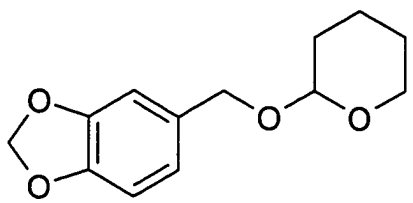
mp 53-55°C

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 2.46 (brs, 1H, -OH), 4.49 (s, 2H, -CH_2O-), 5.90 (s, 2H, methylene dioxy), 6.71-6.77 (m, 2H), 6.80 (s, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 148.11, 147.36, 135.26, 120.85, 108.54, 108.25, 101.36 (C-2), 65.40 (C-8).

PREPARATION OF 5-(TETRAHYDRO-PYRAN-2-YLOXYMETHYL)-BENZO[1,3]DIOXOLE (43)

3,4-Dihydro-2H-pyran (0.95 ml, 0.0104 mol) and *p*-toluenesulfonic acid (7 mg, 0.04 mmol) were added to a solution of benzo[1,3]dioxol-5-yl-methanol **42** (0.495 g, 0.00325 mol) in dry dichloromethane (12 ml), cooled to 0°C, under an atmosphere of nitrogen. The resulting mixture was stirred for 15 h, quenched with sat. aq. sodium bicarbonate (5 ml) and extracted with dichloromethane (4 x 15 ml). The combined methylene chloride fractions were washed with brine (5 ml), dried over anh. magnesium sulphate, filtered and concentrated in vacuo to give a yellow oil. Gradient flash chromatography (13 g of silica gel, hexanes-ethyl acetate eluent, 5% polarity increase) purification resulted in **43** (0.751 g, 98%):



43

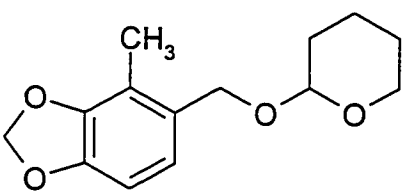
$C_{13}H_{16}O_4$ MW=236.27 g/mol

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 1.40-1.90 (m, 6H), 3.45-3.60 (m, 1H), 3.84-3.96 (m, 1H), 4.39 (d, J=13 Hz, 1H), 4.60-4.70 (m, 2H), 5.92 (s, 2H, methylene dioxy), 6.70-6.90 (m, 3H).

MS [EI, *m/z* (%)]: 236 [MH^+] (19), 152 (18), 135 (100), 85 (43), 77 (23).

PREPARATION OF (4-METHYL-BENZO[1,3]DIOXOLE-5-YL)-METHANOL (45)

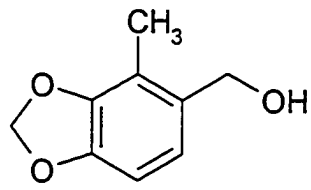
A 1.5 M solution of *t*-butyllithium in pentane (4.20 ml, 0.00630 mol) was added to a solution of 5-(tetrahydro-pyran-2-yloxymethyl)-benzo[1,3]dioxole **43** (0.751 g, 0.00318 mol) in 16 ml of dry THF, at 0 °C, under the nitrogen and the resulting mixture was stirred for 30 min. Methyl iodide (0.80 ml, 0.0128 mol) was added dropwise and the reaction mixture stirred for 3 h, quenched with sat. ammonium chloride (10 ml), diluted with water (10 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic portions were dried over anh. magnesium sulphate, filtered and the solvents evaporated in vacuo giving a crude yellow oil, **44**, which was immediately hydrolysed as follows:



44

$C_{14}H_{18}O_4$ MW=250.29 g/mol

To a solution of 4-methyl-5-(tetrahydro-pyran-2-yloxymethyl)-benzo[1,3]dioxole **44** (crude 0.936 g) in 20 ml of methanol, *p*-toluenesulfonic acid (41.2 mg, 0.217 mmol) was added and the reaction mixture stirred open to air at room temperature for 3 h, neutralized with sat. aq. sodium bicarbonate (6 ml), and the methanol rotatory evaporated. The residue was taken up in 10 ml of water and extracted with ethyl acetate (3 x 10 ml). The organic fractions were dried over anh. magnesium sulphate, filtered and concentrated in vacuo to give a yellow oil. Gradient flash chromatography (14 g of silica gel, hexanes-ethyl acetate eluent, 5% polarity increase) afforded **45** (75. 0 mg, 14%) as a white solid:



45

$C_9H_{10}O_3$ MW=166.18 g/mol

mp 82-83°C

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 1.59 (brs, 1H, -OH), 2.23 (s, 3H, $-CH_3$), 4.57 (s, 2H, H-8), 5.92 (s, 2H, H-2), 6.61 (d, J=7.9 Hz, 1H, H-7), 6.77 (d, J=7.9 Hz, 1H, H-6).

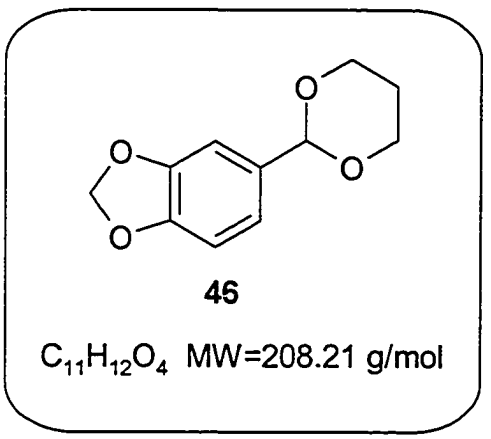
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 147.02 (C-3), 146.80 (C-1), 133.46 (C-5), 122.03 (C-6), 118.58 (C-4), 105.87 (C-7), 101.20 (C-2), 63.82 (C-8), 11.36 (C-9).

MS [EI, *m/z* (%)]: 166 [MH^+] (100), 149 (82), 137 (16), 107 (87), 91 (30), 77 (42).

HRMS calcd. for $C_9H_{10}O_3$: 166.0630; found: 166.0641.

PREPARATION OF 5-[1,3]DIOXAN-2-YL-BENZO[1,3]DIOXOLE (46)

p-Toluenesulfonic acid (10.7 mg, 0.0562 mmol) and propylene glycol (1.20 ml, 0.0166 mol) were added to a solution of piperonal **1** (1.00 g,0.00667 mol) in dry benzene (40 ml) in a 50 ml round bottom flask equipped with a Dean-Stark trap, under a nitrogen atmosphere, at room temperature. The reaction mixture was then refluxed at 110-115°C for 24 h, cooled, diluted with 40 ml of water and extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were dried over anh. magnesium sulphate, filtered and evaporated in vacuo to afford an off-white crude product, which was washed with toluene (3 x 2 ml) and concentrated in vacuo to give **46** (1.36 g, 98% crude) as a white solid:



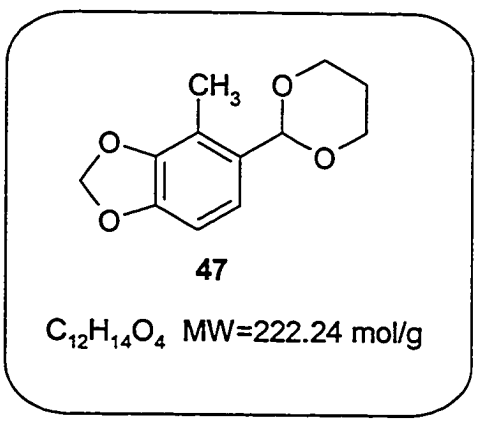
46

$C_{11}H_{12}O_4$ MW=208.21 g/mol

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 1.41 (d, J=15 Hz, 1H), 2.05-2.31 (m, 1H), 3.98 (d, J=15 Hz, 2H), 4.15-4.30 (m, 2H), 5.39 (s, 1H, H-8), 5.91 (s, 2H, H-2), 6.75 (d, J=8.0 Hz, 1H), 6.92 (d, J=8.0 Hz, 1H), 6.97 (s, 1H).

PREPARATION OF 5-[1,3]DIOXAN-2-YL-4-METHYL-BENZO[1,3]DIOXOLE (47)

To a solution of crude 5-[1,3]dioxan-2-yl-benzo[1,3]dioxole **46** (1.36 g, 0.00654 mol) in dry THF (20 ml), cooled to -40°C (dry ice/acetonitrile bath), under nitrogen, a 1.64 M solution of *t*-butyllithium (8.10 ml, 0.0133 mol) was added and the reaction mixture stirred for 20 min. Methyl iodide (1.70 ml, 0.0267 mol) was added dropwise at -40°C and the resulting solution continued stirring for 4 h, during which time the temperature rose to ~5°C. The reaction was quenched with 10 ml of sat. aq. ammonium chloride solution and 10 ml of water, and the reaction mixture extracted with ethyl acetate (3 x 20 ml). The combined organic layers were dried over anh. magnesium sulphate, filtered and concentrated in vacuo to give an amber liquid. Gradient flash chromatography (36 g of silica gel, hexanes-ethyl acetate eluent with 5% polarity increase) afforded **47** (0.798 g, 55%) as a white solid:



47

$C_{12}H_{14}O_4$ MW=222.24 mol/g

mp 83-84°C

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 1.38-1.42 (m, 1H), 2.15-2.29 (m, 1H), 2.26 (s, 3H), 3.91-3.96 (m, 2H), 4.22-4.25 (m, 2H), 5.48 (s, 1H), 5.90 (s, 2H, methylene dioxy), 6.64 (d, J=8.1 Hz, 1H), 7.04 (d, J=8.1 Hz, 1H).

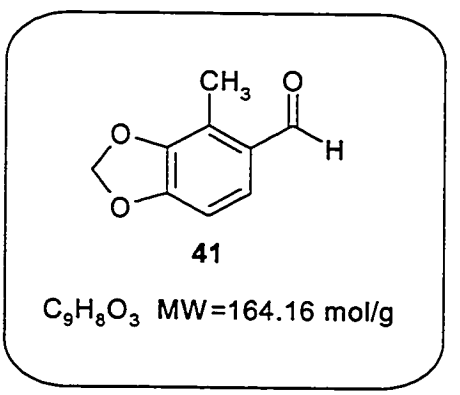
^{13}C NMR ($CDCl_3$, 500 MHz) δ(ppm): 146.92 (C-3), 146.20 (C-1), 130.99 (C-5), 119.85 (C-6), 117.65 (C-4), 105.45 (C-7), 100.66 (C-2), 100.46 (C-9), 67.43, 67.29, 25.69, 11.00.

MS [EI, *m/z* (%)]: 222 [MH^+] (47), 207 (13), 185 (18), 164 (100), 151 (22), 135 (22), 87 (62), 77 (32).

HRMS calcd. for $C_{12}H_{14}O_4$: 222.0892; found: 222.0891.

PREPARATION OF 4-METHYL-BENZO[1,3]DIOXOLE-5-CARBALDEHYDE (41)

Aqueous 10% hydrochloric acid (5 ml) was added to a solution of 5-[1,3]dioxan-2-yl-4-methyl-benzo[1,3]dioxole **47** (97.0 mg, 0.436 mmol) in 5 ml of dichloromethane and the mixture was stirred at room temperature for 65 h. The reaction mixture was extracted with dichloromethane (3 x 10 ml) and the combined dichloromethane portions were concentrated in vacuo to give a yellow solid which was recrystallized from hot hexane to yield **41** (68.4 mg, 96%) as an off-white solid:



41

$C_9H_8O_3$ MW=164.16 mol/g

mp 73-75°C

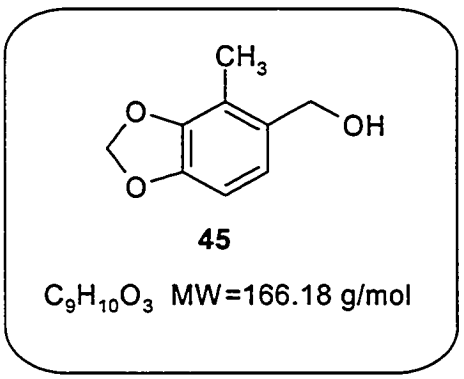
^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 2.50 (s, 3H, methyl), 6.04 (s, 2H, methylene dioxy), 6.78 (d, J=8.1 Hz, 1H), 7.34 (d, J=8.1 Hz, 1H), 9.97 (s, 1H, -CHO).

^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 191.91 (C-9), 151.80 (C-1), 147.59 (C-3), 131.11 (C-6), 130.14 (C-5), 121.44 (C-4), 106.35 (C-7), 102.13 (C-2), 11.98 (C-8).

MS [EI, *m/z* (%)]: 164 [MH^+] (93), 163 (100), 135 (19), 105 (11), 77 (25), 51 (31).

PREPARATION OF (4-METHYL-BENZO[1,3]DIOXOLE-5-YL)-METHANOL (45) VIA REDUCTION OF 4-METHYL-BENZO[1,3]DIOXOLE-5-CARBALDEHYDE (41)

Into a solution of 4-methyl-benzo[1,3]dioxole-5-carbaldehyde **41** (52.9 mg, 0.322 mmol) in 5 ml of dry THF, lithium aluminium hydride (7.30 mg, 0.193 mmol) was added and the reaction mixture was stirred under nitrogen at 0°C for 2.5 h. The reaction was quenched by slow addition of 1 ml of water, followed by 2 ml of 10% aq. sodium hydroxide and additional 2 ml of water. The mixture was then filtered through Celite and extracted with ethyl acetate (3 x 15 ml). The combined organic fractions were dried over anh. magnesium sulphate, filtered and condensed in vacuo. Purification by recrystallization (9:1 hexanes-ethyl acetate) afforded **45** (52.9 mg, 99%) as a white solid:



45

$C_9H_{10}O_3$ MW=166.18 g/mol

mp 82-83°C

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 1.59 (brs, 1H, -OH), 2.23 (s, 3H, $-CH_3$), 4.57 (s, 2H, H-8), 5.92 (s, 2H, H-2), 6.61 (d, J=7.9 Hz, 1H, H-7), 6.77 (d, J=7.9 Hz, 1H, H-6).

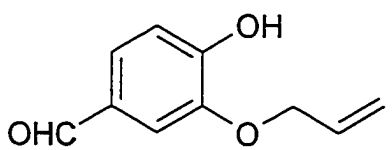
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 147.02 (C-3), 146.80 (C-1), 133.46 (C-5), 122.03 (C-6), 118.58 (C-4), 105.87 (C-7), 101.20 (C-2), 63.82 (C-8), 11.36 (C-9).

MS [EI, *m/z* (%)]: 166 [MH^+] (100), 149 (82), 137 (16), 107 (87), 91 (30), 77 (42).

HRMS calcd. for $C_9H_{10}O_3$: 166.0630; found: 166.0641.

PREPARATION OF 3-ALLYLOXY-4-HYDROXY-BENZALDEHYDE (51) AND 3,4-BIS-ALLYLOXY-BENZALDEHYDE (52)

To a stirred solution of 3,4-dihydroxy benzaldehyde **50** (1.03 g, 0.00748 mol) and anh. potassium carbonate (2.02 g, 0.0146 mol) in dry acetone, under nitrogen, allyl bromide (1.00 ml, 0.0116 mol) was added and the mixture was refluxed at 60°C for 3 h. The acetone solvent was evaporated and the residue taken up in 15 ml of water and 15 ml of ethyl acetate. The mixture was saturated with brine and extracted with ethyl acetate (3 x 15 ml). The collected organic fractions were dried over anh. magnesium sulphate, filtered and concentrated in vacuo to yield a yellow oil. Isocratic flash chromatography purification (35 g of silica gel, 3:1 hexanes-ethyl acetate) yielded **52** (0.60 g, 37%) as a yellow liquid and **51** (0.67 g, 50%) as a white solid. Note: in further preparations the reaction conditions were controlled in such way to yield up to 83% **52** and 75% **51**.



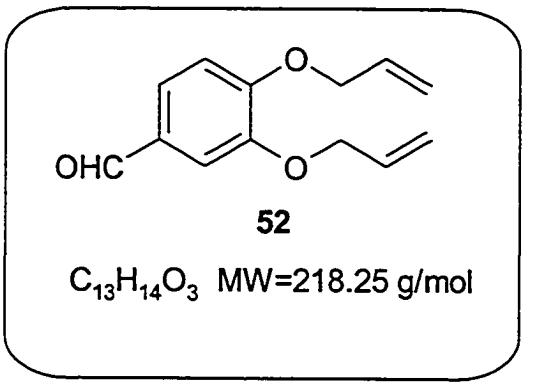
51

$C_{10}H_{10}O_3$ MW=178.19 g/mol

mp 58-60°C (lit. mp: 58-61°C)[23]

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 4.74 (d, J=5.5 Hz, 2H, H-8), 5.34-5.44 (m, 2H, H-10), 5.78 (s, 1H, -OH), 5.98-6.11 (m, 1H, H-9), 6.94 (d, J=8.2 Hz, 1H, H-5), 7.38 (dd, J=8.2, 1.8 Hz, 1H, H-6), 7.43 (d, J=1.8 Hz, 1H, H-2), 9.82 (s, 1H, -CHO)

^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 191.38 (C-7), 151.06 (C-3), 146.60 (C-4), 132.14 (C-9), 131.11 (C-1), 124.68 (C-6), 119.65 (C-10), 114.72 (C-5), 111.74 (C-2), 70.30 (C-8).



52

$C_{13}H_{14}O_3$ MW=218.25 g/mol

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 4.60-4.66 (m, 4H), 5.24-5.30 (m, 2H), 5.37-5.44 (m, 2H), 5.97-6.10 (m, 2H), 6.93 (d, J=8.1 Hz, 1H, H-5), 7.38 (dd, J=8.0, 1.9 Hz, 1H, H-6), 7.36 (d, J=1.9 Hz, 1H, H-2), 9.78 (s, 1H, -CHO).

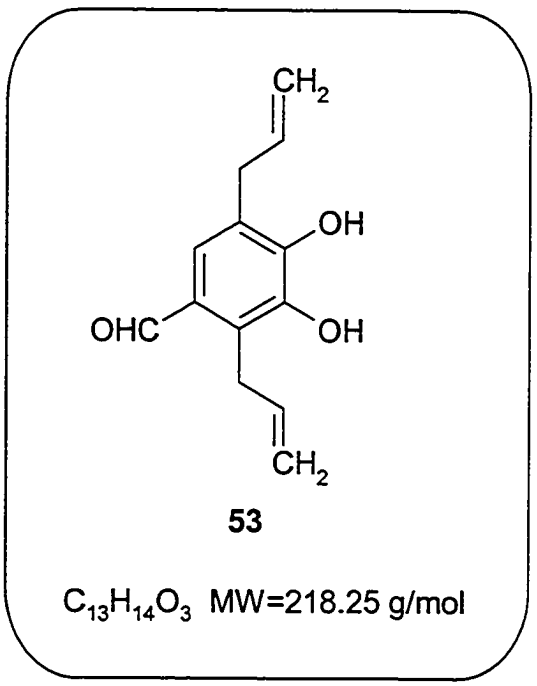
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 191.21 (C-7), 154.22 (C-4), 149.13 (C-3), 133.01, 132.70, 130.42 (C-1) 126.95 (C-6), 118.68, 118.51, 112.65 (C-2), 111.71 (C-5), 70.02 (2C).

MS [EI, *m/z* (%)]: 218 [MH^+] (51), 189 (10), 177 (24), 149 (14), 121 (6), 79 (13), 51 (15), 41 (100).

HRMS calcd. for $C_{13}H_{14}O_3$: 218.0943; found: 218.0952.

PREPARATION OF 2,5-DIALLYL-3,4-DIHYDROXY-BENZALDEHYDE (53)

A solution of 3,4-bis-allyloxy-benzaldehyde **52** (0.600 g, 0.00275 mol) in decalin (1.2 ml) was heated at 190-200°C for 3.5 h, under the atmosphere of nitrogen and then cooled to room temperature. The crude residue was purified by gradient flash chromatography (25 g of silica gel, ethyl acetate-hexane solvent system with 5% polarity increase) to afford **53** (99.8 mg, 17%) as a yellow oil:



53

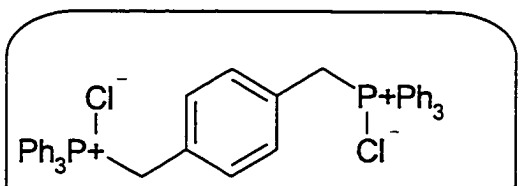
$C_{13}H_{14}O_3$ MW=218.25 g/mol

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 3.43 (d, J=6.5 Hz, 2H), 3.90 (d, J=6.4 Hz, 2H), 4.99-5.20 (m, 4H, $=CH_2$), 5.80 (brs, 1H), 5.94-6.02 (m, 2H, =CH), 6.38 (brs, 1H), 7.24 (s, 1H, H-6), 9.95 (s, 1H, -CHO).

^{13}C NMR ($CDCl_3$, 200 MHz) δ(ppm): 192.12 (C-7), 148.00 (C-4), 142.48 (C-3), 135.94, 135.53, 128.18 (C-6), 126.08 (C-1), 119.13 (C-2), 117.01 and 116.27 ($=CH_2$), 115.65 (C-5), 34.20 and 28.86 ($-CH_2$).

PREPARATION OF TRIPHENYLPHOSPHINE SALT OF 1,4-BIS-CHLOROMETHYL-BENZENE (55)

A solution of 1,4-bis-chloromethyl-benzene **54** (2.01 g, 0.0112 mol) and triphenyl phosphine (6.53 g, 0.0246 mol) in dry toluene was refluxed at 110-120°C under nitrogen for 4 h. The salt **55** precipitated out and was collected, washed with toluene, followed by ether and dried by suction to afford a fine white powder (1.58 g, 20%). Note: the yield increased to 85% if the reaction mixture was refluxed for about 24 h.[24]



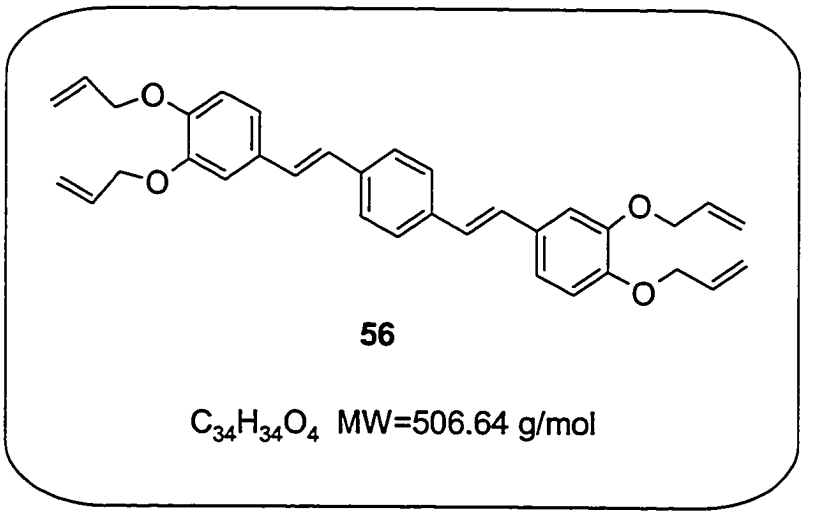
55

$C_{44}H_{38}P_2Cl_2$ MW=699.64 g/mol

^{1}H NMR (D_2O, 200 MHz) δ(ppm): 4.60 (s, 4H), 6.92 (dd, J=3.5, 11.5 Hz, 3H), 7.28 (d, J=12 Hz, 3H), 7.55-7.72 (m, 22H), 7.80-7.93 (m, 6H).

PREPARATION OF COMPOUND (56) VIA THE WITTIG REACTION

To a suspension of the Wittig reagent **55** (0.502 g, 0.000717 mol) in 20 ml of dry THF, stirred under nitrogen at room temperature, a 2M solution of *n*-butyllithium was added dropwise (0.72 ml, 0.00144 mol), resulting in an orange reaction mixture. After 20 minutes of stirring, another 0.72 ml, 0.00144 mol of the *n*-butyllithium was added, resulting in a dark maroon reaction mixture. Diallyl ether compound **52** was dissolved in 4 ml THF (plus 4 x1 ml for rinsing) and added very slowly, over a period of 10 min. to the maroon solution. The resulting mixture was stirred for 1.5 h, quenched with sat. aq. ammonium chloride solution (10 ml) and extracted with ethyl acetate (3 x 20 ml). The combined organic layers were washed with water (4 x 10 ml) and condensed using the rotary evaporator to about a 10 ml volume, which was then passed through a 2-inch layer of silica (30 g), and rinsed with 1:1 hexane-ethyl acetate eluent. The flushed material was dried in vacuo, then purified further by means of the gradient flash chromatography (30 g silica gel, hexane-ethyl acetate eluent with 1% polarity increase), affording a yellow oil (35.6 mg, 20%), which could potentially be characterized as **56**, upon further purification.

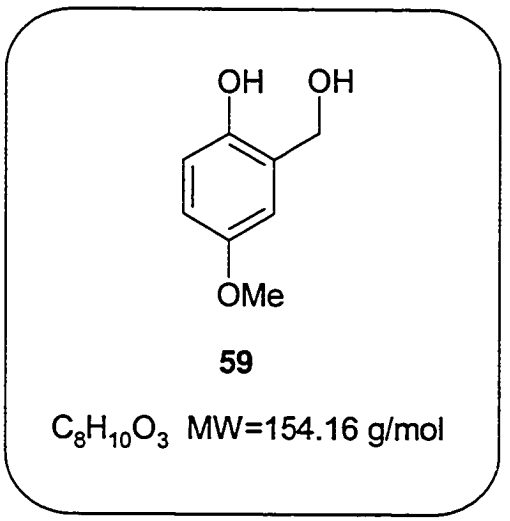


56

$C_{34}H_{34}O_4$ MW=506.64 g/mol

PREPARATION OF 2-HYDROXYMETHYL-4-METHOXY-PHENOL (59)

A solution of 2-hydroxy-5-methoxy-benzaldehyde **58** (1.00 g, 0.00660 mol) and sodium borohydride (0.201 g, 0.00531 mol) in anh. THF (30 ml) was stirred for 3 h at 0°C. Aqueous sodium hydroxide 10% (10 ml) was added and the mixture extracted with ethyl acetate (3 x 20 ml). The combined organic portions were dried over anh. magnesium sulphate, filtered and concentrated in vacuo. The crude product was purified by gradient flash chromatography (30 g silica gel, ethyl acetate-hexane eluent, 5% polarity increase) to afford **59** (0.671 g, 66%) as a white solid:



59

$C_8H_{10}O_3$ MW=154.16 g/mol

mp 77-79°C (lit. mp: 78-80°C)[30]

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 2.75 (brs, 1H, benzylic OH), 3.71 (s, 3H, methoxy), 4.74 (s, 2H, methylene), 6.57 (d, J=2.8 Hz, 1H, H-3), 6.71 (dd, J=8.7, 2.8 Hz, 1H, H-5), 6.77 (d, J=8.7 Hz, 1H, H-6), 7.01 (s, 1H, phenolic OH).

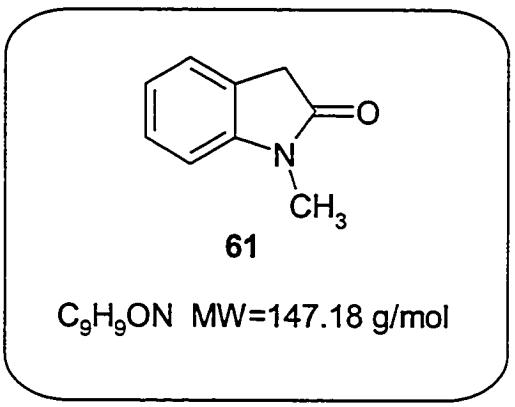
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 153.40, 149.99, 126.00, 117.40, 114.66, 113.91, 64.69, 56.20.

MS [EI, *m/z* (%)]: 154 [MH^+] (14), 136 (100), 108 (39), 93 (7), 78 (39).

HRMS calcd. for $C_8H_{10}O_3$: 154.0630, found: 154.0614.

PREPARATION OF 1-METHYL-1,3-DIHYDRO-INDOL-2-ONE (61)

Stirred solution of sodium hydride (1.26 g, 0.0499 mol), (washed with hexane 3 x 2 ml), in dry xyllene (80 ml) was heated for 30 min at 125°C, under nitrogen. A suspension of oxindole **60** (6.86 g, 0.00500 mol) in 20 ml of xyllene (plus 10 ml for rinsing) was added via an addition funnel over a 10 min time period and the resulting white slurry was refluxed at 150°C for 1.5 h. Dimethyl sulphate (4.78 ml, 0.0500 mol) was added dropwise and the resulting orange clear mixture refluxed for an additional 2 h. The reaction mixture was cooled, washed with water (5 x 20 ml) and concentrated in vacuo to yield an orange crystalline product, which was recrystallized twice from hexane and treated with activated carbon to afford **61** (5.40 g, 73%) as a white solid:



61

C_9H_9ON MW=147.18 g/mol

mp 86.5-88.5°C (lit. mp: 86.5-87°C)[26]

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 3.18 (s, 3H, -NCH_3), 3.49 (s, 2H, H-3), 6.79 (d, J=7.8 Hz, 1H, H-7), 7.01 (t, J=7.5 Hz, 1H), 7.20-7.28 (m, 2H).

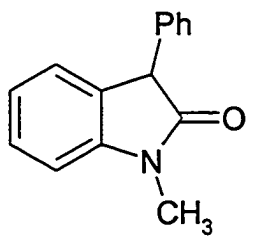
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 175.46 (C-2), 145.56 (C-8), 128.25, 124.86, 124.67, 122.71, 108.44 (C-7), 36.11 (-CH_3), 26.53 (C-3).

MS [EI, *m/z* (%)]: 147 [MH^+] (96), 132 (11), 118 (100), 91 (20), 78 (16).

HRMS calcd. for C_9H_9ON: 147.0685; found: 147.0680.

ATTEMPTED PREPARATION OF 1-METHYL-3-PHENYL-1,3-DIHYDRO-INDOL-2-ONE (62)

A solution of LTMP was generated in situ by adding a 1.29 M solution of *t*-butyllithium (6.2 ml, 0.00800 mol) to a solution of 2,2,6,6-tetramethylpiperidine (1.5 ml, 0.00880 mol) in dry THF (3 ml), under nitrogen at 0°C. N-methyl oxindole **61** was dissolved in 2 ml (plus 3 x 1 ml rinses) of THF and slowly added to the LTMP solution. After stirring the brownish solution for 10 min., bromobenzene (0.40 ml, 0.00376 mol) was added, and the resulting black reaction mixture stirred for 1 h at 0°C and then for additional 4 h at room temperature. The reaction was quenched with sat. aq. ammonium chloride (10 ml), and the mixture diluted with water (10 ml), then extracted with ethyl acetate (3 x 20 ml). The combined organic fractions were dried over anh. $MgSO_4$, filtered and condensed in vacuo. Isocratic flash chromatography (50 g silica gel, 25% ethyl acetate in hexane eluent) afforded starting material **61** (0.085 g, 17%), the desired **62** (0.0891 g, 12%) as a tan solid and diphenyl product **63** (0.120 g, 12%) as a yellow powder. There were three other TLC observable products which could not be isolated.

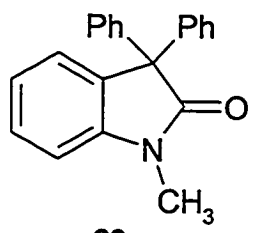


62

$C_{15}H_{13}ON$ MW=223.28 g/mol

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 3.02 (s, 3H, -NCH_3), 5.14 (s, 1H, H-3), 6.88 (d, J=7.9 Hz, 2H), 7.32-7.44 (m, 6H), 7.88 (d, J=7.7 Hz, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 168.34 (C-2), 144.91, 141.86, 132.29, 132.00, 129.78, 128.58, 128.30, 128.28, 128.04, 127.00, 126.74, 124.76, 56.04 (C-3), 37.37 (-CH_3).



63

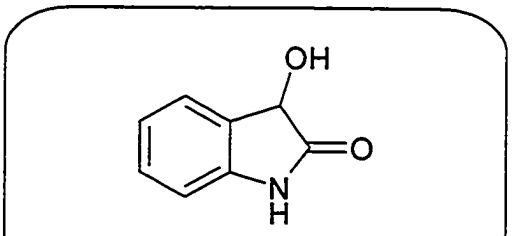
$C_{21}H_{17}ON$ MW=299.37 g/mol

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 3.02 (s, 3H, -NCH_3), 6.61-6.77 (m, 4H), 7.05-7.38 (m, 6H), 7.47-7.64 (m, 3H), 8.30 (d, J=9.1 Hz, 1H).

MS [EI, *m/z* (%)]: 299 [MH^+] (100), 284 (6), 222 (10), 193 (13), 162 (39), 150 (20), 143 (28), 86 (94), 70 (17).

PREPARATION OF 3-HYDROXY-1,3-DIHYDRO-INDOL-2-ONE (65)

A solution of sodium dithionate (7.72 g, 0.0443 mol) in water (10 ml, plus 3 x 2 ml rinses) was added to a solution of isatin **64** (1.06 g, 0.00709 mol) in THF (30 ml) and the resulting mixture was stirred open to air at RT for 10 min. Aqueous 10% HCl was added (30 ml) and the reaction mixture extracted with ether (4 x 20 ml). Combined organic fractions were washed with sat. aq. sodium bicarbonate (2 x 10 ml), dried over anh. magnesium sulphate, filtered and concentrated in vacuo to yield an off-white powder. Recrystallization from acetone-methanol (about 1:1) afforded **65** (0.720 g, 68%) as a white solid:



65

$C_8H_7O_2N$ MW=149.15 g/mol

mp 187-189°C

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 4.81 (d, J=7.6 Hz, 1H, -OH), 6.17 (d, J=7.6 Hz, 1H, H-3), 6.77 (d, J=7.7 Hz, 1H), 6.95 (t, J=7.7 Hz, 1H), 7.18 (t, J=7.7 Hz, 1H), 7.26 (d, J=7.7 Hz, 1H), 10.23 (brs, 1H, -NH).

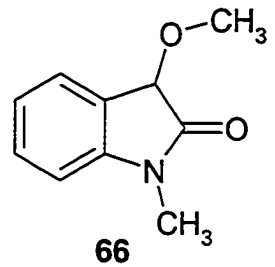
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 178.81 (C-2), 143.01 (C-8), 130.15 (C-9), 129.77, 125.62, 122.34, 110.31 (C-7), 69.99 (C-3).

MS [EI, *m/z* (%)]: 149 [MH^+] (61), 128 (17), 93 (100), 77 (14), 64 (34).

HRMS calcd. for $C_8H_7O_2N$: 149.0477; found: 149.0475.

PREPARATION OF 3-METHOXY-1-METHYL-1,3-DIHYDRO-INDOL-2-ONE (66)- ATTEMPT #1

A solution of sodium hydride (80.4 mg, 60% dispersion in mineral oil, 2.00 mmol) and 3-hydroxy-1,3-dihydro-indol-2-one **65** (150.0 mg, 1.00 mmol) in dry xyllene (10 ml) was stirred at RT under nitrogen for 2 h, until the cloudy solution turned clear. Dimethyl sulphate (0.20 ml, 2.00 mmol) was added dropwise and the reaction mixture stirred for 10 h, then refluxed at 135°C for 4 h. After cooling, the mixture was washed with water (3 x 5 ml) and the xyllene solvent was removed in vacuo. The TLC of the crude mixture showed 6-7 different products.

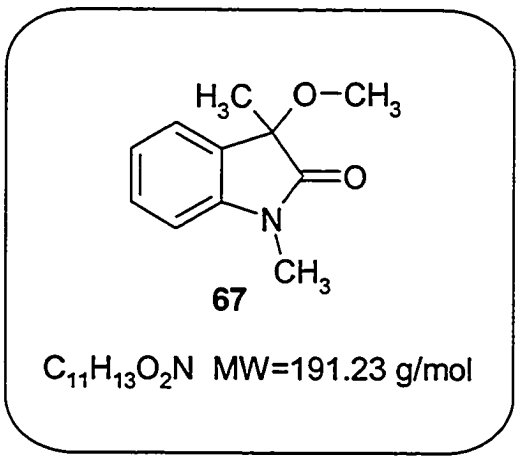


66

$C_{10}H_{11}O_2N$ MW=177.20 g/mol

PREPARATION OF 3-METHOXY-1-METHYL-1,3-DIHYDRO-INDOL-2-ONE (66)- ATTEMPT #2

A solution of potassium hydride (154.0 mg, 35% dispersion in mineral oil, 1.40 mmol) and 3-hydroxy-1,3-dihydro-indol-2-one **65** (100.0 mg, 0.670 mmol) in dry THF (5 ml) was stirred under nitrogen at RT for 30 min. Methyl iodide (0.09 ml, 1.37 mmol) was added dropwise and the resulting mixture stirred for 1 h, quenched with sat. aq. ammonium chloride (5 ml) and extracted with ethyl acetate (5 x 10 ml). The combined organic portions were dried over anh. magnesium sulphate, filtered and concentrated in vacuo to give an orange solid crude product, presumably **67** (193.5 mg, quantitative).



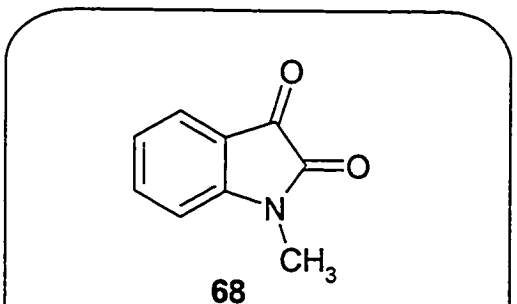
67

$C_{11}H_{13}O_2N$ MW=191.23 g/mol

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 1.45 (s, 3H, $-CH_3$), 2.99 (s, 3H, $-OCH_3$), 3.20 (s, 3H, $-NCH_3$), 6.82 (d, J=8.0 Hz, 1H), 7.10 (d, J=8.0 Hz, 1H), 7.31 (t, J=8.0 Hz, 2H).

PREPARATION OF 1-METHYL-1H-INDOLE-2,3-DIONE (68)

Sodium hydride (1.02 g, 0.0255 mol, 60% dispersion in mineral oil) was added to 10 ml of anh. dimethylformamide and the suspension was stirred under nitrogen at 0°C. A solution of isatin **64** (3.05 g, 0.0203 mol) in 10 ml of dry DMF (plus additional 3 x 1 ml for rinsing) was added dropwise. The resulting mixture was stirred at room temperature for 20 min. Methyl iodide (1.30 ml, 0.0207 mol) was added slowly, over a 15 min period and the reaction mixture continued stirring for 15 h. The reaction was quenched with 4 ml of water and the mixture was extracted with ether (4 x 20 ml). Combined organic layers were washed with water (5 x 10 ml), dried over anh. magnesium sulphate, filtered and concentrated in vacuo to give 2.48 g of an orange solid. To determine the yield and characterize the compound, 101.0 mg was purified by gradient flash chromatography (5 g of silica gel, hexanes-ethyl acetate eluent, 5% polarity increase) to yield **68** (76.1 mg, 81%) as an orange solid:



68

$C_9H_7O_2N$ MW=161.16 g/mol

mp 129-130.5°C (lit. mp: 132-134°C)[28]

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 3.19 (s, 3H, -NCH_3), 6.86 (d, J=7.8 Hz, 1H, H-7), 7.04-7.09 (m, 1H), 7.50-7.59 (m, 2H).

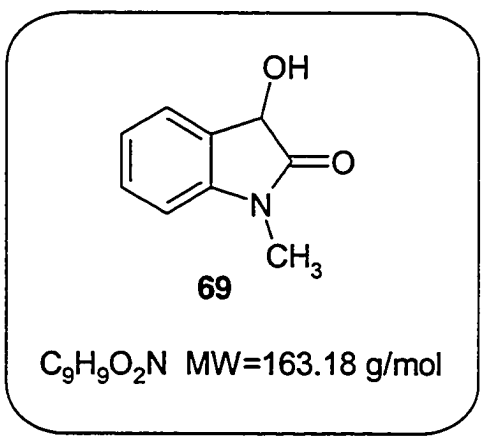
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 183.77 (C-3), 158.59 (C-2), 151.79 (C-8), 138.89 (C-4), 125.56, 124.22, 117.71 (C-9), 110.40 (C-7), 26.59 (-CH_3).

MS [EI, *m/z* (%)]: 161 [MH^+] (100), 133 (34), 104 (69), 92 (14), 78 (37), 63 (15).

HRMS calcd. for $C_9H_7O_2N$: 161.0477; found: 161.0486.

PREPARATION OF 3-HYDROXY-1-METHYL-1,3-DIHYDRO-INDOL-2-ONE (69)

To a solution of N-methyl isatin **68** (2.01 g, 0.0125 mol) in 40 ml of ether, a solution of sodium dithionate (10.2 g, 0.0500 mol) in water (10 ml, plus 2 x2 ml rinses) was added and the resulting mixture stirred for 1 h. Aqueous 10% hydrochloric acid (50 ml) was added and the reaction mixture extracted with ether (4 x 15 ml). The combined ether fractions were washed with sat. aq. sodium bicarbonate solution (2 x 5 ml), dried over anh. magnesium sulphate, filtered and concentrated in vacuo. Gradient flash chromatography (62 g of silica gel, hexane-ethyl acetate eluent, 5-10% polarity increase) afforded **69** (1.35 g, 66%) as a yellow fine powder:



69

$C_9H_9O_2N$ MW=163.18 g/mol

mp 148-152°C (lit. mp: 154-155°C)[29]

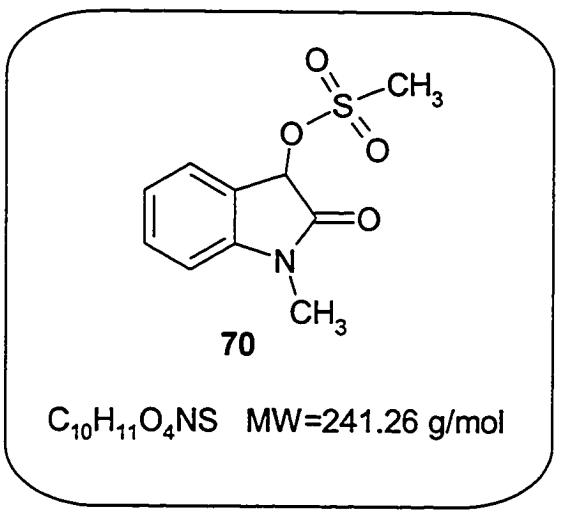
^{1}H NMR (DMSO, 300 MHz) δ(ppm): 3.06 (s, 3H, -NCH_3), 3.43 (s, 0.3H, water in DMSO), 4.89 (d, J=7.5 Hz, 1H, H-3), 6.28 (d, J=7.5 Hz, 1H, -OH), 6.93 (d, J=7.7 Hz, 1H, H-7), 7.01-7.06 (m, 1H), 7.27-7.34 (m, 2H).

^{13}C NMR (DMSO, 300 MHz) δ(ppm): 176.88 (C-2), 144.50 (C-8), 129.92, 129.37, 125.24, 123.02, 109.20 (C-7), 69.60 (C-3), 26.58 (-CH_3).

MS [EI, *m/z* (%)]: 163 [MH^+] (100), 161 (34), 143 (18), 133 (16), 118 (29), 106 (90), 91 (19), 77 (42), 63 (17).

HRMS calcd. for $C_9H_9O_2N$: 163.0634; found: 163.0641.

ATTEMPTED PREPARATION OF METHANESULFONIC ACID 1-METHYL-2-OXO-2,3-DIHYDRO-1H-INDOL-3-YL ESTER (70)

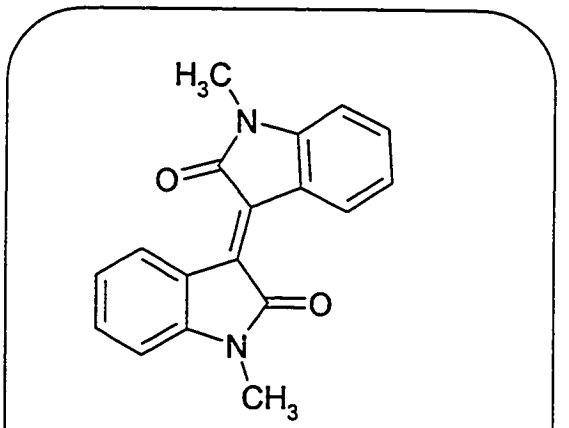


70

$C_{10}H_{11}O_4NS$ MW=241.26 g/mol

To a stirred solution of 3-hydroxy-1-methyl-1,3-dihydro-indol-2-one **69** (500.9 mg, 3.06 mmol) and mesyl chloride (0.30 ml, 3.39 mmol) in 10 ml of dry dichloromethane, at 0°C, under nitrogen, triethylamine (1.30 ml, 9.20 mmol) was added dropwise, over a 10 min time period. The reaction mixture was stirred for 1 h at 0°C, 2 more h at RT, and then it was quenched with water (20 ml) and sat. aq. ammonium chloride (7 ml), and extracted with ethyl acetate (4 x 15 ml). The combined organic fractions were washed with water (3 x 5

ml), dried over anh. $MgSO_4$, filtered and concentrated in vacuo. Isocratic flash chromatography (28 g silica gel, 30% ethyl acetate in hexane eluent) afforded several products, including what appeared to be dimer **71** (7.2 mg, 1%) as a maroon solid and dimer **72** (60.0 mg, 7%) as a red solid. The expected mesyl product **70** could not be isolated.



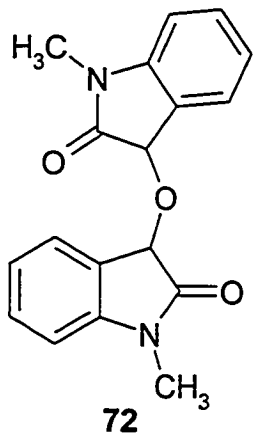
71

$C_{18}H_{14}O_2N_2$ MW=290.32 g/mol

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 3.27 (s, 6H, -NCH_3), 6.76 (d, J=7.8 Hz, 2H), 7.05 (t, J=7.6 Hz, 2H), 7.36 (t, J=7.7 Hz, 2H), 9.19 (d, J=7.9 Hz, 2H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 168.37 (2C=O), 145.57 (2C-8), 132.75 (2C-4), 130.20 (2C-6), 128.46 (2C), 122.78 (2C-5), 121.95 (2C), 108.03 (2C-7), 26.50 ($2CH_3$).

MS [EI, *m/z* (%)]: 290 [MH^+] (100), 275 (21), 262 (21), 247 (9), 233 (18), 204 (9), 181 (32), 146 (85).



72

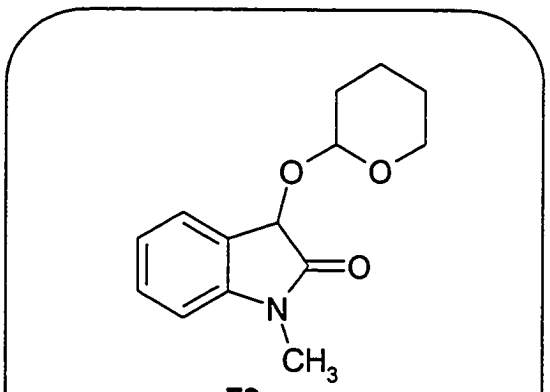
$C_{18}H_{16}O_3N_2$ MW=308.34 g/mol

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 3.19 (s, 6H, -NCH_3), 5.09 (s, 2H, -CH-), 6.81 (d, J=7.8 Hz, 2H), 7.09 (t, J=7.7 Hz, 2H), 7.30-7.39 (m, 4H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 172.42 (2C=O), 144.17 (2C-8), 130.88 (2C-4), 125.97 (2C-6), 123.76 (2C-5), 122.75 (2C), 109.09 (2C-7), 51.87 (2-CH-), 27.04 ($2CH_3$).

PREPARATION OF 1-METHYL-3-(TETRAHYDRO-PYRAN-2-YLOXY)-1,3-DIHYDRO-INDOL-2-ONE (73)

To a stirred solution of 3-hydroxy-1-methyl-1,3-dihydro-indol-2-one **69** (515.5 mg, 3.16 mmol) and *p*-TsOH (59.5 mg, 0.310 mmol) in 40 ml of dry dichloromethane, at RT, under nitrogen, dihydropyran (0.28 ml, 4.60 mmol) was added dropwise, and the resulting reaction mixture stirred for 5 h. Water (20 ml) was added, and the mixture extracted with dichloromethane (3 x 25 ml). The combined CH_2Cl_2 layers were dried over anh. magnesium sulphate, filtered and the solvent removed in vacuo. Gradient flash chromatography (80 g silica gel, ethyl acetate-hexane eluent with 2.5-5% polarity increase) yielded **73** (190.6 mg, 24%) as a yellow liquid.



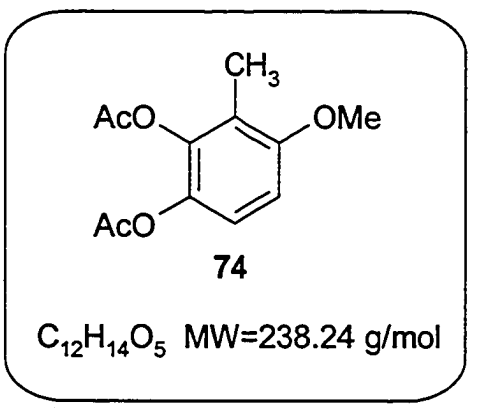
73

$C_{14}H_{17}O_3N$ MW=247.29 g/mol

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 1.38-1.89 (m, 6H), 3.11 (s, 3H, $-CH_3$), 3.50-3.63 (m, 1H, H-6'), 3.89-4.12 (m, 1H, H-6'), 5.02 (s, 1H, H-3), 5.30-5.37 (m, 1H, H-2'), 6.61 (d, J=7.9 Hz, 1H, H-7), 7.01 (t, J=7.8 Hz, 1H), 7.24 (t, J=7.8 Hz, 1H), 7.38 (d, J=7.9 Hz, 1H, H-4).

PREPARATION OF ACETIC ACID 6-ACETOXY-3-METHOXY-2-METHYL-PHENYL ESTER (74)

Triethylamine (3.62 ml, 0.0260 mol) and N,N-dimethylaminopyridine (19 mg, 0.18 mmol) were added to a solution of 4-methoxy-3-methyl-benzene-1,2-diol **8** (0.951 g, 0.00617 mol) in dry dichloromethane (40 ml) at room temperature under the atmosphere of nitrogen, and the mixture was stirred for 5 min. Acetic anhydride (1.85 ml, 0.0196 mol) was added and the mixture was stirred for additional 6 h, then poured into water (20 ml). The organic layer was washed with 5 % aq. hydrochloric acid (20 ml), followed by 20 ml of water, dried over anh. magnesium sulphate and concentrated in vacuo to give a yellow oil. Isocratic flash chromatography (60 g of silica gel, 3:1 hexanes-ethyl acetate eluent) afforded **74** (1.04 g, 71%) as a pale yellow oil:



74

$C_{12}H_{14}O_5$ MW=238.24 g/mol

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 2.04 (s, 3H), 2.27 (s, 3H), 2.32 (s, 3H), 3.82 (s, 3H, methoxy), 6.70 (d, J=8.5 Hz, 1H, H-4), 6.95 (d, J=8.5 Hz, 1H, H-5).

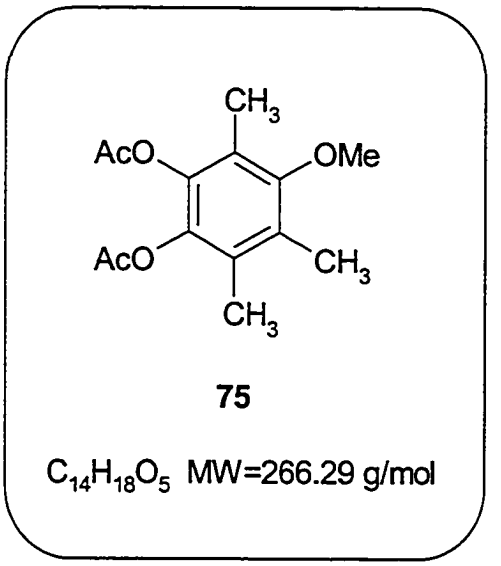
^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 169.37, 168.46, 156.32 (C-3), 141.68 (C-6), 136.35 (C-1), 121.32 (C-2), 120.23 (C-5), 107.94 (C-4), 56.21 (methoxy), 20.98, 20.68, 9.93 (methyl).

MS [EI, *m/z* (%)]: 238 [MH^+] (11), 196 (42), 154 (100), 139 (53), 125 (12).

HRMS calcd. for $C_{12}H_{14}O_5$: 238.0841; found: 238.0833.

PREPARATION OF ACETIC ACID 2-ACETOXY-4-METHOXY-3,4,6-TRIMETHYL-PHENYL ESTER (75)

Same procedure as for preparation of **74** with **19** used as a substrate gave **75** (62%) as a white solid:



75

$C_{14}H_{18}O_5$ MW=266.29 g/mol

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 2.03 (s, 3H), 2.06 (s, 3H), 2.17 (s, 3H), 2.27 (s, 3H), 2.28 (s, 3H), 3.66 (s, 3H, methoxy).

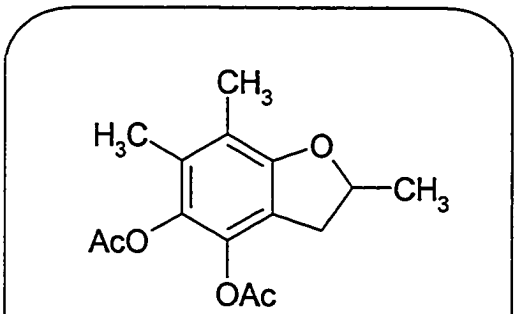
^{13}C NMR ($CDCl_3$, 500 MHz) δ(ppm): 168.51, 168.24, 154.60, 139.35, 137.37, 128.42, 128.23, 122.37, 60.26, 20.32, 20.28, 12.96, 12.64, 9.86.

MS [EI, *m/z* (%)]: 266 [MH^+] (14), 224 (35), 182 (100), 167 (89), 153 (18).

HRMS calcd. for $C_{14}H_{18}O_5$: 266.1154; found: 266.1156.

PREPARATION OF ACETIC ACID 4-ACETOXY-2,6,7-TRIMETHYL-2,3-DIHYDRO-BENZOFURAN-5-YL ESTER (76)

Same procedure as for preparation of **74** with **33** used as a substrate gave **76** (62%) as an off-white solid:



76

$C_{15}H_{18}O_5$ MW=278.30 g/mol

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 1.43 (d, J=6.1 Hz, 3H), 2.01 (s, 3H), 2.09 (s, 3H), 2.24 (s, 3H), 2.29 (s, 3H), 2.65 (dd, J=15.2 Hz, 1H), 3.17 (dd, J=15.2 Hz, 1H), 4.85 (m, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ(ppm): 169.49, 168.20, 156.84, 136.83, 134.24, 130.09, 117.71, 116.81, 80.27, 36.06, 22.14, 20.84, 20.71, 13.12, 12.51.

MS [EI, *m/z* (%)]: 278 [MH^+] (13), 236 (37), 194 (100), 178 (8).

HRMS calcd. for $C_{15}H_{18}O_5$: 278.1154; found: 278.1150.

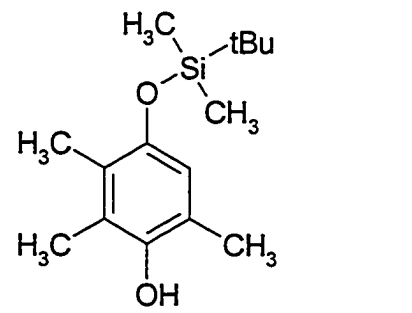
Experimental

3.2 Part B

GENERAL: Please see Experimental section 3.1 for general procedures. Note that following experimental procedures (part B) were performed by Dr. Helmi Hussain and are herein included for completion since we performed the kinetic testing on compounds **19**, **33**, and **38** (synthesized by Dr. Hussain).

PREPARATION OF 4-(TERT-BUTYL-DIMETHYL-SILANYLOXY)-2,3,6-TRIMETHYL-PHENOL (15)

To a solution of 2,3,5-trimethylhydroquinone **14** (1.0 g, 1.0 mol eq.) and immidazole (1.79 g, 4.0 mol eq.) in dry DMF (10 ml) was added a solution of *t*-butyldimethylsilyl chloride (1.19 g, 1.2 mol eq.) in dry DMF (5 ml) at –20°C under nitrogen. The reaction mixture was warmed up to 20°C and after 2h of stirring it was poured into water and extracted with CH_2Cl_2 (3 x 20 ml). The combined organic layers were washed with water (2 x 20 ml), dried ($MgSO_4$) and the solvent was removed under vacuo. The crude product was subjected to a flash chromatography using ethyl acetate and hexane to give a solid product **15** (1.5 g, 85%):



15

$C_{15}H_{26}O_2Si$ MW=266.46 g/mol

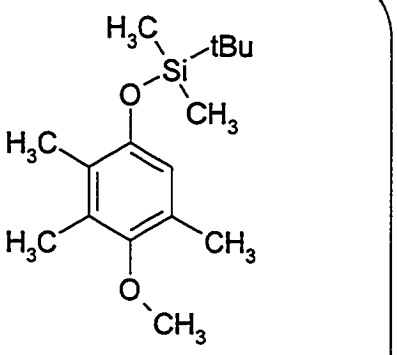
^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 0.23 (s, 6H), 1.07 (s, 9H), 2.16 (s, 3H), 2.19 (s, 3H), 2.21 (s, 3H), 4.46 (s, 1H), 4.50 (s, 1H).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 146.9, 143.5, 125.9, 123.8, 120.5, 118.1, 25.9, 18.3, 16.1, 13.0, 12.4, -4.2.

HRMS calcd. for $C_{15}H_{26}O_2Si$: 266.1702; found: 266.1703.

PREPARATION OF TERT-BUTYL-(4-METHOXY-2,3,5-TRIMETHYL-PHENOXY)-DIMETHYL-SILANE (16)

To a solution of phenol **15** (0.76 g, 1.0 mol eq.) and methyl iodide (0.9 ml, 5.0 mol eq.) in acetone (10 ml), potassium carbonate (0.79 g, 2.0 mol eq.) was added and the mixture was refluxed for 24 h. The solvent was evaporated and water (20 ml) was added to the residue. The resulting mixture was extracted with ethyl acetate (3 x 20 ml). The combined ethyl acetate fractions were washed with water, dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography (ethyl acetate-hexane eluent) yielded **16** (0.76 g, 95%) as a yellow oil



16

$C_{16}H_{28}O_2Si$ MW=280.48 g/mol

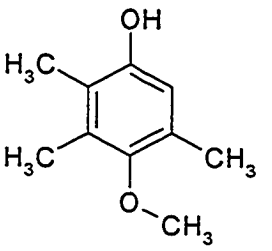
^{1}H NMR ($CDCl_3$, 50 MHz) δ(ppm): 0.18 (s, 6H), 1.00 (s, 9H), 2.08 (s, 3H), 2.16 (s, 3H), 2.20 (s, 3H), 3.63 (s, 3H), 6.42 (s, 1H).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 150.8, 149.2, 130.4, 127.7, 125.8, 118.1, 60.0, 25.8, 18.2, 16.0, 12.8, -4.3.

HRMS calcd. for $C_{16}H_{28}O_2Si$: 280.1860; found: 280.1859.

PREPARATION OF 4-METHOXY-2,3,5-TRIMETHYL-PHENOL (17)

A solution of the protected phenol **16** (0.8 g, 1.0 mol eq.) in dry THF (8 ml) was treated with tetrabutylammonium fluoride (4.27 ml, 1.0M, 1.5 mol eq.) at room temperature, under nitrogen. Water (10 ml) was added and the reaction mixture extracted with ethyl acetate (3 x 20 ml). The combined organic layers were washed with water, dried ($MgSO_4$) and the solvent was removed under vacuo. Flash chromatography using ethyl acetate and hexane gave the pure product **17** (0.45 g, 96%):



17

$C_{10}H_{14}O_2$ MW=166.22 g/mol

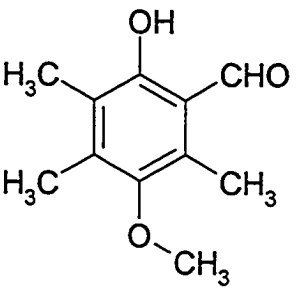
^{1}H NMR ($CDCl_3$, 50 MHz) δ(ppm): 2.11 (s, 3H), 2.19 (s, 6H), 3.65 (s, 3H), 4.98 (brs, 1H), 6.42 (s, 1H).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 150.1, 149.7, 130.6, 128.2, 121.3, 114.5, 60.2, 15.9, 12.7, 11.9.

HRMS calcd. for $C_{10}H_{14}O_2$: 166.0994; found: 166.0996.

PREPARATION OF 2-HYDROXY-5-METHOXY-3,4,6-TRIMETHYL-BENZALDEHYDE (18)

Same formylation procedure as for preparation of **32** (with **17** as a substrate), gave **18** (57%) as a yellow solid:



18

$C_{11}H_{14}O_3$ MW=194.23 g/mol

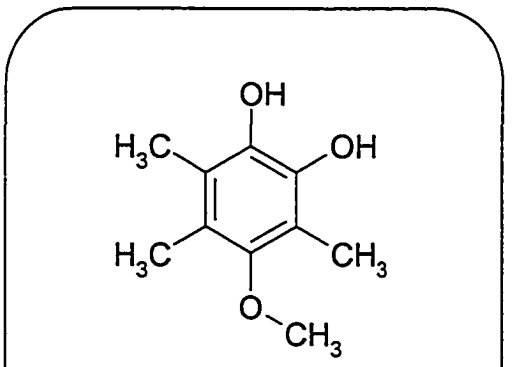
^{1}H NMR ($CDCl_3$, 50 MHz) δ(ppm): 2.07 (s, 3H), 2.20 (s, 3H), 2.44 (s, 3H), 3.58 (s, 3H), 10.14 (s, 1H), 12.13 (s, 1H).

^{13}C NMR ($CDCl_3$, 200 MHz) δ(ppm): 194.5, 157.8, 148.9, 141.7, 129.9, 123.8, 116.1, 60.5, 13.7, 10.9, 10.1.

HRMS calcd. for $C_{11}H_{14}O_3$: 194.0943; found: 194.0953.

PREPARATION OF 4-METHOXY-3,5,6-TRIMETHYL-BENZENE-1,2-DIOL (19)

Same procedure as for preparation of **33** (with **18** as a substrate), gave **19** (62%) as a pink solid:



19

$C_{10}H_{14}O_3$ MW=182.22 g/mol

mp 114-115 °C

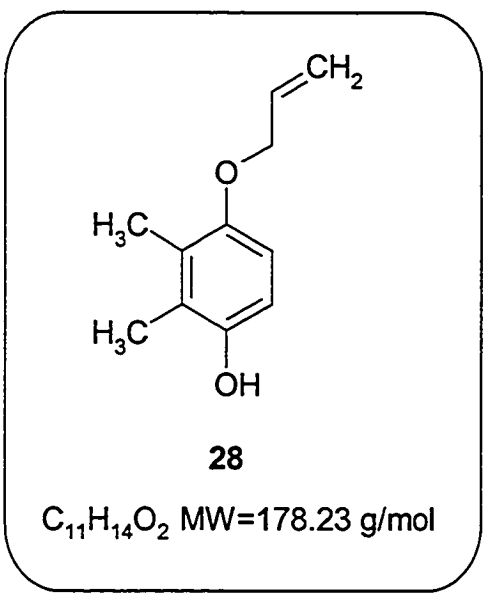
^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 2.13 (s, 6H), 2.16 (s, 3H), 3.62 (s, 3H), 4.81 (s, 1H), 4.93 (s, 1H).

^{13}C NMR ($CDCl_3$, 500 MHz) δ(ppm): 150.2, 140.0, 138.1, 121.3, 120.5, 114.4, 60.4, 12.0, 11.9, 8.9.

HRMS calcd. for $C_{10}H_{14}O_3$: 182.0943; found: 182.0943.

PREPARATION OF 4-ALLYLOXY-2,3-DIMETHYL-PHENOL (28) AND 1,4-BIS-ALLYLOXY-2,3-DIMETHYL-BENZENE (29)

To a solution of 2,3-dimethylhydroxyhydroquinone **27** (1.0 g, 1.0 mol eq.) and allyl bromide (0.94 ml, 1.5 mol eq.) in acetone (10 ml), anh. potassium carbonate (2.02 g, 2.0 mol eq.) was added and the resulting mixture was stirred at reflux for 18 h. The solvent was removed in vacuo and the remaining residue taken up in water and extracted with ether (3 x 30 ml). The combined ether extracts were washed with water (1 x 40 ml), dried over anh. magnesium sulphate, filtered and concentrated in vacuo to give an oil. Flash chromatography of the crude product using ethyl acetate and hexane gave two products: monoallyl ether **28** (0.45 g, 35%) as a brown semisolid and diallyl ether **29** (0.79 g, 50%) as a yellow oil:



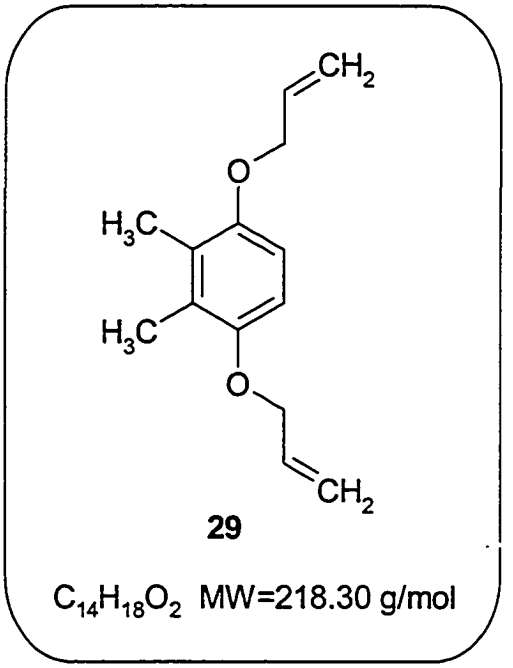
28

$C_{11}H_{14}O_2$ MW=178.23 g/mol

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 2.18 (s, 3H), 2.20 (s, 3H), 4.46 (dt, J=5.2, 1.6 Hz, 2H), 5.02 (brs, 1H), 5.23 (br dd, J=10.4, 1.6 Hz, 1H), 5.46 (br dd, J=17.2, 1.6 Hz, 1H), 6.07 (m, 1H), 6.55 (d, J=9.0 Hz, 1H), 6.61 (d, J=9.0 Hz, 1H).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 150.7, 147.8, 134.0, 127.4, 124.3, 116.9, 112.0, 110.7, 70.2, 12.3, 12.2.

HRMS calcd. for $C_{11}H_{14}O_2$: 178.0994; found: 178.0994.



29

$C_{14}H_{18}O_2$ MW=218.30 g/mol

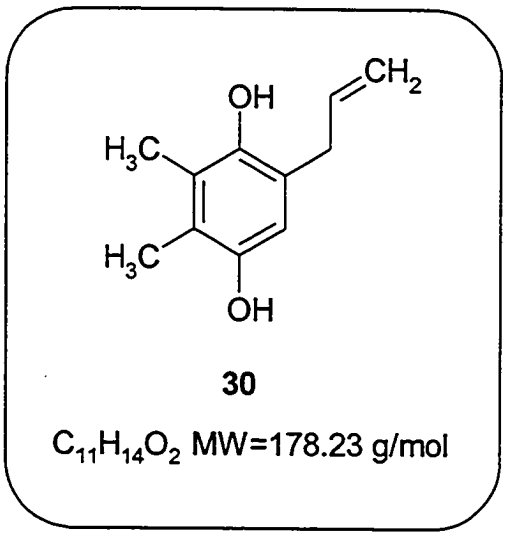
^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 2.21 (s, 6H), 4.48 (dt, J=5.0, 1.6 Hz, 2H), 5.02 (brs, 1H), 5.26 (br dd, J=10.6, 1.4 Hz, 1H), 5.43 (br dd, J=17.2, 1.6 Hz, 1H), 6.08 (m, 1H), 6.64 (s, 1H).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 151.0, 134.1, 127.2, 116.7, 109.5, 69.7, 12.2.

HRMS calcd. for $C_{14}H_{18}O_2$: 218.1307; found: 218.1307.

PREPARATION OF 5-ALLYL-2,3-DIMETHYL-BENZENE-1,4-DIOL (30)

A solution of allyl ether **28** (1.0 g, 1.0 mol eq.) in decalin (10 ml) was heated to 190-195°C for 5 h using the oil bath, and then cooled to room temperature. A solid product was separated by filtration and washed with hexane (20 ml) to afford **30** (0.81 g, 81 %) as a white solid:



30

$C_{11}H_{14}O_2$ MW=178.23 g/mol

mp 124-126 °C

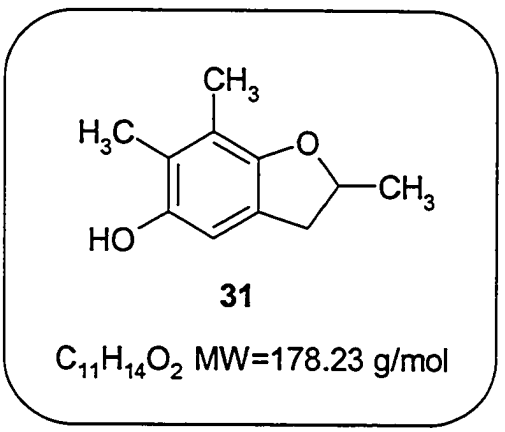
^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 2.14 (s, 3H), 2.16 (s, 3H), 3.31 (br d, J=6.2 Hz, 2H), 4.44 (s, 1H), 4.61 (s, 1H), 5.15 (m, 2H), 5.97 (m, 1H), 6.41 (s, 1H).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 147.1, 146.2, 136.4, 124.7, 122.5, 122.0, 116.5, 116.0, 113.7, 112.5, 35.5, 12.2, 11.9.

HRMS calcd. for $C_{11}H_{14}O_2$: 178.0994; found: 178.0994.

PREPARATION OF 2,6,7-TRIMETHYL-2,3-DIHYDRO-BENZOFURAN-5-OL (31)

p-Toluenesulfonic acid (3.5 g, 1.1 mol eq.) was added to a solution of 5-allyl-2,3-dimethyl-benzene-1,4-diol **30** (3.0 g, 1.0 mol eq.) in benzene (40 ml) and the reaction mixture was refluxed for 8 h. After cooling to room temperature, aq. sat. sodium bicarbonate solution (20 ml) was added and the reaction mixture was extracted with ethyl acetate (3 x 20 ml). The combined organic portions were washed with water (1 x 30 ml), dried ($MgSO_4$), filtered and condensed in vacuo to give a solid. Flash chromatography using ethyl acetate-hexane eluent afforded **31** (2.1 g, 69%) as a white solid:



31

$C_{11}H_{14}O_2$ MW=178.23 g/mol

mp 126-128 °C

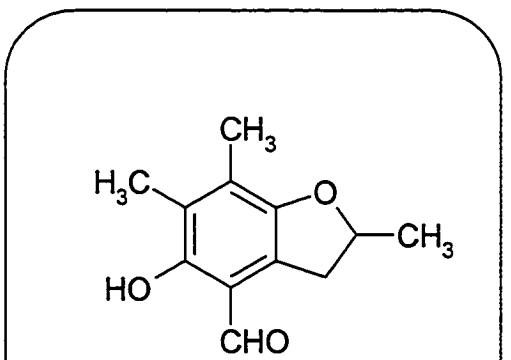
^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 1.43 (d, J=6.2 Hz, 3H), 2.11 (s, 3H), 2.13 (s, 3H), 2.74 (dd, J=14.4, 7.0 Hz, 1H), 3.21 (dd, J=15.0, 8.8 Hz, 1H), 4.33 (s, 1H), 4.82 (m, 1H), 6.49 (s, 1H).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 152.1, 147.2, 123.5, 121.9, 118.9, 109.1, 78.9, 37.8, 21.7, 12.3, 11.8.

HRMS calcd. for $C_{11}H_{14}O_2$: 178.0994; found: 178.0994.

PREPARATION OF 5-HYDROXY-2,6,7-TRIMETHYL-2,3-DIHYDRO-BENZOFURAN-4-CARBALDEHYDE (32)

To a three-neck flask equipped with a reflux condenser, thermometer and a nitrogen source, anhydrous toluene (40 ml), hydroxybenzofuran **31** (3.5 g, 1.0 mol eq.), tin tetrachloride (0.28 ml, 0.12 mol eq.) and tributyl amine (1.87 ml, 0.4 mol eq.) were added. The reaction mixture was stirred at room temperature for 20 min and then paraformaldehyde (1.3 g, 2.2 mol eq.) was added. The resulting yellowish solution was heated for 2 h at 90-95°C. After cooling the mixture to room temperature, it was poured into water (50 ml), acidified to pH=2 with 2N HCl and extracted with ether (3 x 50 ml). The collected organic layers were washed with water (50 ml), dried over $MgSO_4$, filtered and the solvent removed in vacuo. The crude residue was purified by flash chromatography using ethyl acetate and hexane to give a bright yellow solid **32** (2.1 g, 52%):



32

$C_{12}H_{14}O_3$ MW=206.24 g/mol

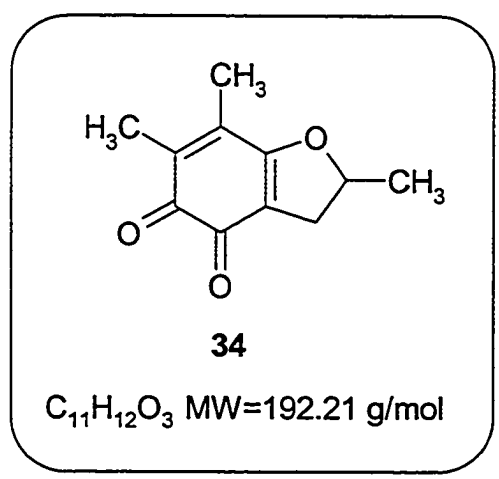
^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 1.47 (d, J=6.2 Hz, 3H), 2.09 (s, 3H), 2.15 (s, 3H), 2.98 (dd, J=15.8, 8.0 Hz, 1H), 3.51 (dd, J=15.6, 8.6 Hz, 1H), 4.91 (m, 1H), 9.84 (s, 1H), 11.06 (s, 1H).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 173.9, 154.3, 151.3, 133.4, 130.2, 124.1, 114.2, 79.3, 35.5, 21.7, 13.3, 10.7.

HRMS calcd. for $C_{12}H_{14}O_3$: 206.0943; found: 206.0943.

PREPARATION OF 2,6,7-TRIMETHYL-2,3-DIHYDRO-BENZOFURAN-4,5-DIONE (34) AND 2,6,7-TRIMETHYL-2,3-DIHYDRO-BENZOFURAN-4,5-DIOL (33)

To a rapidly stirred solution of the aldehyde **32** (1.0 g, 1.0 mol eq.) and sodium hydroxide (0.19 g, 1.0 mol eq.) in 16 ml of water-THF (9:1) solvent mixture, hydrogen peroxide (0.2 ml, 30%) was added at once, under nitrogen, at 28-30°C. After 30 min the solution was cooled to room temperature and stirred for further 30 min. Aqueous 10% HCl was added and the reaction mixture was extracted with ethyl acetate (3 x 30 ml). The combined organic reactions were washed with water (1 x 30 ml), dried ($MgSO_4$), filtered and the solvent was removed in vacuo. Flash chromatography using ethyl acetate and hexane as eluent gave the benzoquinone **34** (0.1 g, 11%) as a deep red liquid and the diol **33** (0.38 g, 40%) as a pink solid:



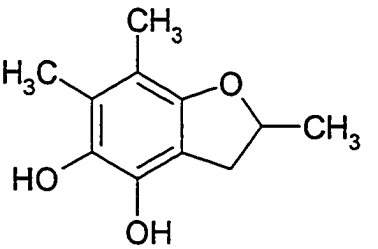
34

$C_{11}H_{12}O_3$ MW=192.21 g/mol

^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 1.45 (d, J=6.4 Hz, 3H), 1.89 (s, 3H), 2.00 (s, 3H), 2.54 (dd, J=15.0, 7.2 Hz, 1H), 3.09 (dd, J=15.0, 10.0 Hz, 1H), 5.04 (m, 1H).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 182.3, 174.6, 171.6, 137.0, 136.8, 111.7, 84.0, 33.0, 21.9, 13.4, 11.8.

HRMS calcd. for $C_{11}H_{12}O_3$: 192.0787; found: 192.0789.



33

$C_{11}H_{14}O_3$ MW=194.23 g/mol

mp 140-142 °C

^{1}H NMR (acetone-d_6, 200 MHz) δ(ppm): 1.35 (d, J=6.2 Hz, 3H), 1.96 (s, 3H), 2.07 (s, 3H), 2.67 (dd, J=15.0, 6.8 Hz, 1H), 3.22 (dd, J=15.0, 8.4 Hz, 1H), 4.76 (m, 1H), 6.50 (s, 1H), 7.51 (s, 1H).

^{13}C NMR (acetone-d_6, 50 MHz) δ(ppm): 152.5, 140.5, 137.5, 123.4, 110.2, 108.9, 79.4, 35.9, 22.0, 12.2, 11.8.

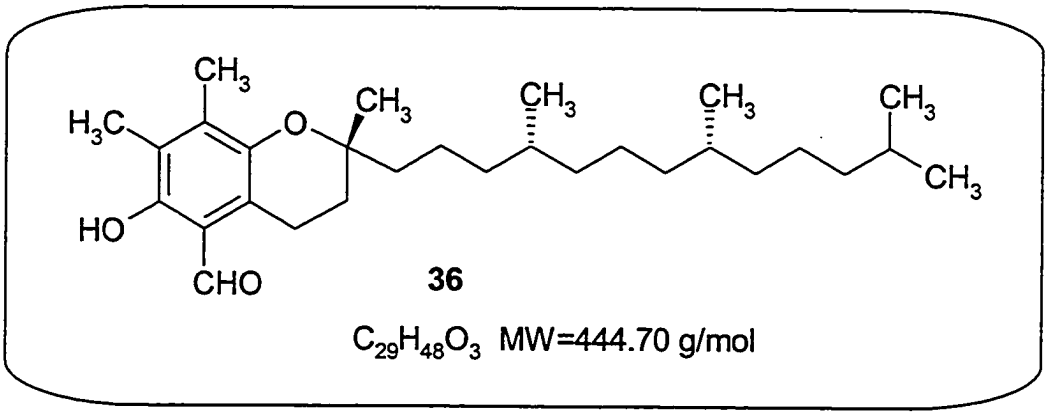
HRMS calcd. for $C_{11}H_{14}O_3$: 194.0943; found: 194.0943.

REDUCTION OF 2,6,7-TRIMETHYL-2,3-DIHYDRO-BENZOFURAN-4,5-DIONE (34) TO 2,6,7-TRIMETHYL-2,3-DIHYDRO-BENZOFURAN-4,5-DIOL (33)

A solution of sodium dithionate (0.53 g, 6.5 mol eq.) in water (2 ml) was added in one portion to a stirred solution of the *o*-quinone **34** (0.09 g, 1.0 mol eq.) in ether (4 ml). The red colour of the quinone was discharged after 3 min. Aqueous hydrochloric acid (10%) was added and the opaque mixture extracted with ether (3 x 15 ml). The combined extracts were washed with sat. aq. sodium bicarbonate (2 x 10 ml), dried over magnesium sulphate and filtered. Evaporation of the solvent afforded the catechol **33** (0.08 g, 90%) as a yellow solid.

PREPARATION OF 6-HYDROXY-2,7,8-TRIMETHYL-2-(4,8,12-TRIMETHYL-TRIDECYL)-CHROMAN-5-CARBALDEHYDE (36)

The same procedure was used as for preparation of **32** with the γ-tocopherol **35** as a substrate. The product **36** is a yellow oil (19%yield):



36

$C_{29}H_{48}O_3$ MW=444.70 g/mol

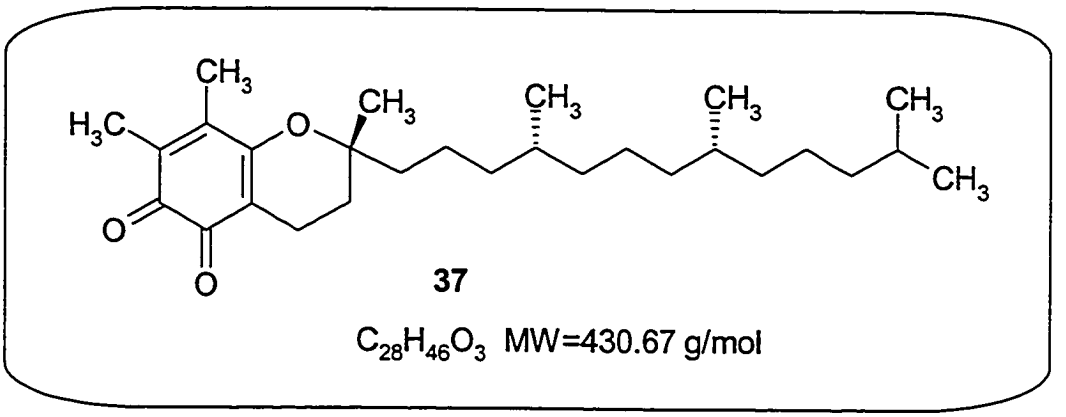
^{1}H NMR ($CDCl_3$, 200 MHz) δ(ppm): 0.86 (m, 12H), 0.96-1.60 (m, 24H), 1.81 (m, 2H), 2.13 (s, 3H), 2.16 (s, 3H), 3.00 (t, J=6.8 Hz, 2H), 10.16 (s, 1H), 12.10 (s, 1H).

^{13}C NMR ($CDCl_3$, 50 MHz) δ(ppm): 193.9, 155.8, 143.9, 138.3, 124.1, 117.4, 114.4, 75.0, 39.5, 39.3, 37.3, 37.2, 32.7, 32.6, 30.7, 27.9, 24.8, 24.4, 23.6, 22.7, 22.6, 20.9, 19.7, 19.6, 18.4, 13.1, 11.0.

HRMS calcd. for $C_{29}H_{48}O_3$: 444.3604; found: 444.3605.

PREPARATION OF 2,7,8-TRIMETHYL-2-(4,8,12-TRIMETHYL-TRIDECYL)-3,4-DIHYDRO-2H-CHROMENE-5,6-DIONE (37) AND 2,7,8-TRIMETHYL-2-(4,8,12-TRIMETHYL-TRIDECYL)-CHROMAN-5,6-DIOL (38)

Same procedure as for preparation of **33** (with **36** as a substrate), gave **37** (12%) as a deep red oil and **38** (50%) as a yellow oil:



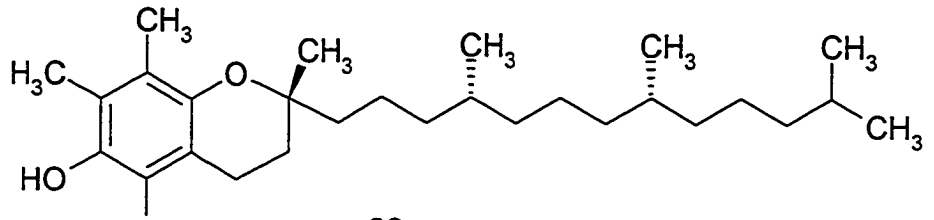
37

$C_{28}H_{46}O_3$ MW=430.67 g/mol

^{1}H NMR ($CDCl_3$, 500 MHz) δ(ppm): 0.81-0.84 (m, 12H), 1.00-1.41 (m, 21H), 1.49 (s, 1H), 1.59 (m, 2H), 1.72 (m, 2H), 1.92 (s, 3H), 2.00 (s, 3H), 2.40 (m, 2H).

^{13}C NMR ($CDCl_3$, 125 MHz) δ(ppm): 180.8, 177.8, 163.2, 143.6, 134.1, 110.2, 81.3, 39.9, 39.3, 37.4, 37.3, 37.2, 37.2, 32.9, 32.7, 32.6, 32.5, 29.7, 29.1, 27.9, 25.6, 24.7, 24.4, 23.8, 22.6, 22.5, 21.3, 20.8, 19.7, 19.5, 15.3, 13.6, 11.5.

HRMS calcd. for $C_{28}H_{46}O_3$: 430.3449; found: 430.3447.



38

$C_{28}H_{48}O_3$ MW=432.69 g/mol

^{1}H NMR (acetone-d_6, 200 MHz) δ(ppm): 0.85-0.89 (m, 12H), 1.11-1.60 (m, 24H), 1.73 (m, 2H), 2.00 (s, 3H), 2.11 (s, 3H), 2.64 (m, 2H), 6.56 (s, 1H), 6.94 (s, 1H).

^{13}C NMR (acetone-d_6, 200 MHz) δ(ppm): 145.9, 141.7, 136.0, 122.9, 115.3, 107.1, 75.3, 40.4, 40.1, 33.5, 33.4, 31.7, 30.6, 30.2, 29.8, 29.4, 29.1, 28.6, 25.5, 25.1, 24.2, 23.0, 22.9, 21.6, 20.1, 17.9, 12.4, 11.5.

HRMS calcd. for $C_{28}H_{48}O_3$: 432.3605; found: 432.3603.

REDUCTION OF 2,7,8-TRIMETHYL-2-(4,8,12-TRIMETHYL-TRIDECYL)-3,4-DIHYDRO-2H-CHROMENE-5,6-DIONE (37) TO 2,7,8-TRIMETHYL-2-(4,8,12-TRIMETHYL-TRIDECYL)-CHROMAN-5,6-DIOL (38)

The same procedure was used as for the reduction of **34** with **37** used as a substrate. The product **38** (89%) corresponds to the above characterization.

Experimental

3.3 Part C: Stopped-flow kinetics testing

GENERAL: The solvents used in kinetic measurements were of the purest grade commercially available and were used as received. The natural vitamin E (2R,4'R,8'R-α-tocopherol), was obtained by hydrolyzing the commercially available tocopheryl-acetate oil **77**. The resulting tocopherol **78** was purified by three consecutive flash chromatography procedures with 0-to-1% of ethyl acetate in hexane used as eluent. *p*-Methoxy phenol was a commercial sample (Aldrich, 98%) and was used without further purification. All the synthesized compounds were purified as described in the Experimental sections A and B. The 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl radical (DPPH•) was obtained from Northern Sources Inc.

The stopped-flow apparatus was equipped with a spectrophotometer with a xenon 150 W arc light source. Some of the readings were taken at λ=330 nm and some in the region of λ=519-530 nm. The amplifier voltage was adjusted as needed (range of 400-625 V). The temperature in the room was 25.4 ± 2 °C.

In a typical procedure a solution of DPPH• (~5×10^{-5} M) in a solvent of choice for a particular analysis was prepared. At least three different concentrations of solutions of the antioxidants were prepared. The lowest concentrations of the antioxidants were always at least two orders of magnitude higher (10^{-3} M) than the concentration of DPPH•. All solutions were deoxygenated by bubbling nitrogen for ~1.5 min. prior to mixing. The DPPH• solution was mixed 1:1 with the solutions of the antioxidants and disappearance of the DPPH• was observed by means of the spectrophotometer. The first part of the decay curve was analyzed to give the k_{exp} results, which were then plotted against the various concentrations to give a linear plot, the slope of which was the overall second-order rate constant k.

(i) Validation of the Stopped-Flow Apparatus

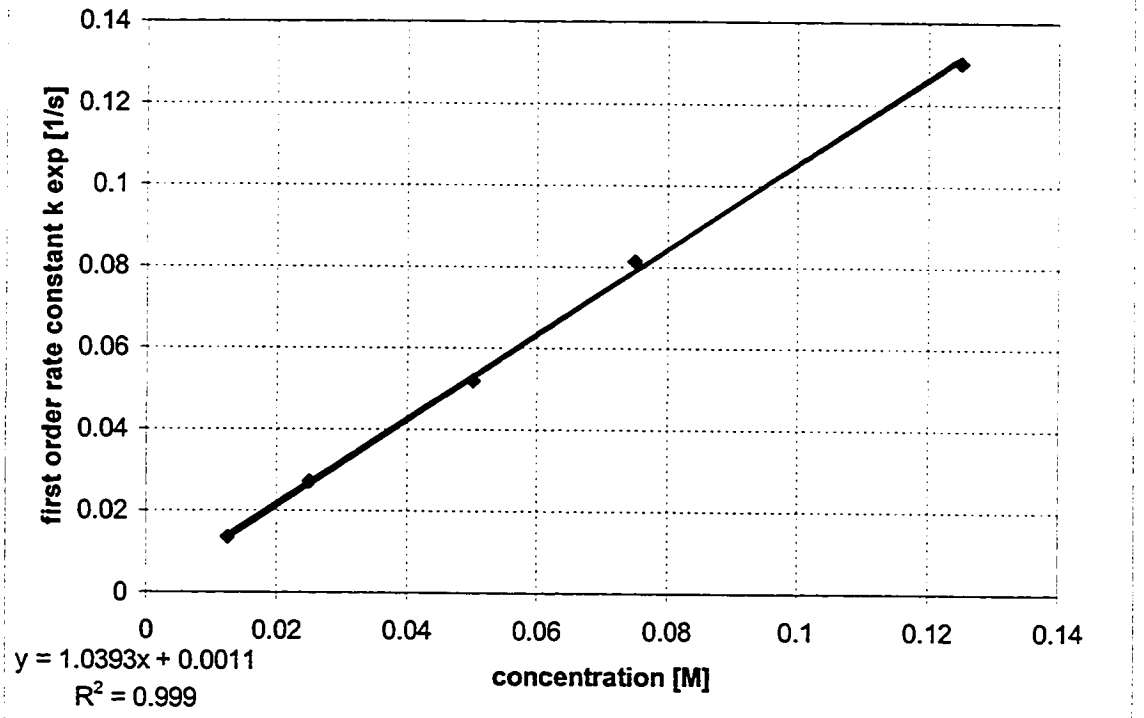
p-Methoxyphenol was tested in ethyl acetate solvent, at λ=330 nm. DPPH• concentration was $5x10^{-5}$ M and it was obtained by dissolving 4.34 mg of DPPH• (MW=394.32 g/mol) in ethyl acetate (100 ml total volume). Please note that the resulting molarity must be divided by two, since this volume of DPPH• solution is mixed 1:1 with the antioxidant solution. The various concentrations of *p*-methoxy phenol and the first order rate constants for the reaction k_{exp} are given in Table 3.1.

Table 3.1: Data obtained from the stopped-flow analysis of different concentrations of *p*-methoxy phenol reacting with DPPH• in ethyl acetate. The disappearance of the DPPH• was measured at λ=330 nm.

p-methoxy phenol concentration [mM]	12.5	25	50	75	125
k_{exp} run 1 [s^{-1}]	0.01301	0.02752	0.05201	0.08309	0.1289
k_{exp} run 2 [s^{-1}]	0.01314	0.02748	0.05182	0.08102	0.1298
k_{exp} run 3 [s^{-1}]	0.01385	0.02717	0.05221	0.08077	0.1299
k_{exp} run 4 [s^{-1}]	0.01389	0.02729	0.05264	0.08140	0.1294
k_{exp} run 5 [s^{-1}]	0.01413	0.02704	0.05132	0.08151	0.1321
k_{exp} average [s^{-1}]	**0.01360**	**0.02730**	**0.05200**	**0.08156**	**0.1300**

Thus obtained k_{exp}s were plotted against the different antioxidant concentrations to give a straight line, the slope of which is the desired second order rate constant k (Figure 3.1), found to be 1.0 $M^{-1}s^{-1}$, which is in agreement with the literature value. [14]

Figure 3.1: Kinetic results of the reaction of *p*-methoxy phenol with DPPH radical



The kinetic measurements of the reaction of α-tocopherol with DPPH• were done in benzene solvent, with the DPPH• solution concentration of $5x10^{-5}$ M, at λ=525 nm. Six vitamin E solution concentrations were prepared: 0.25 mM, 1 mM, 2 mM, 3 mM, 8 mM and 10 mM. The solutions were reacted with DPPH•, the k_{exp} obtained, averaged, plotted against different concentrations and the k second order was calculated to be $2.2x10^3$ $M^{-1}s^{-1}$. The literature value of the absolute rate constant for this reaction in benzene is $1.8x10^3$ $M^{-1}s^{-1}$ [40].

(ii) Testing of the synthesized compounds

All five compounds were tested in ethyl acetate solvent, with DPPH$^{\bullet}$ solution concentration of ~$5x10^{-5}$ M and the readings were taken at λ=519 nm.

Catechol **8** was tested twice in ethyl acetate and once in chloroform. Details of one of the measurements are given in Table 3.2 and Figure 3.2.

Table 3.2: Data obtained from the stopped-flow analysis of different concentrations of 4-methoxy-3-methyl catechol **8** reacting with DPPH$^{\bullet}$ in ethyl acetate.

concentration of **8** [mM]	0.52	1.3	2.6	5.2	7.8
time of reaction [sec.]	50	20	10	5	5
k_{exp} run 1 [s^{-1}]	0.1026	0.2654	0.5279	1.080	1.599
k_{exp} run 2 [s^{-1}]	0.1037	0.2618	0.5759	1.076	1.598
k_{exp} run 3 [s^{-1}]	0.1033	0.2646	0.5208	1.057	1.603
k_{exp} average [s^{-1}]	**0.1032**	**0.2639**	**0.5249**	**1.071**	**1.600**

Figure 3.2: Kinetic results of the reaction of **8** with DPPH radical

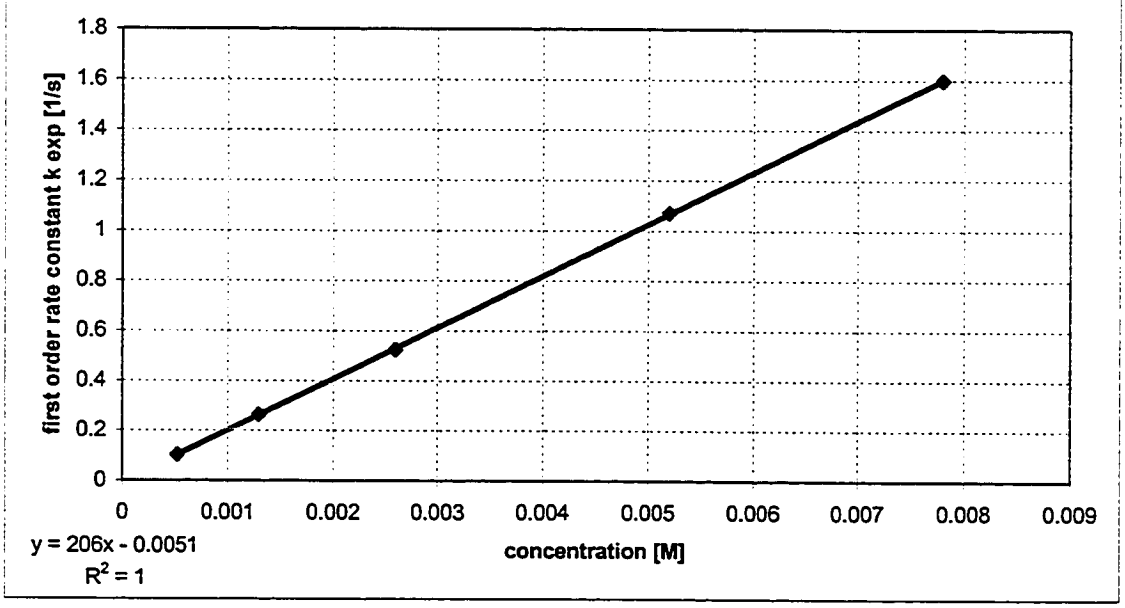
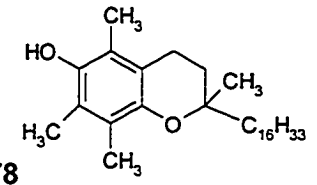
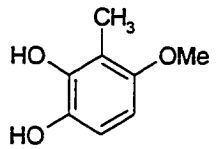
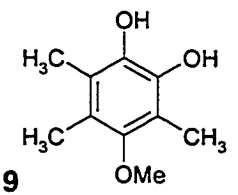
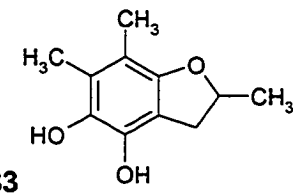
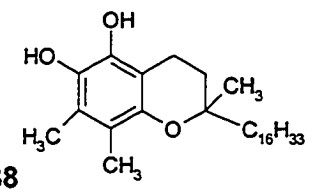
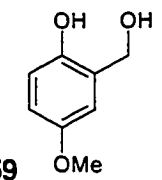
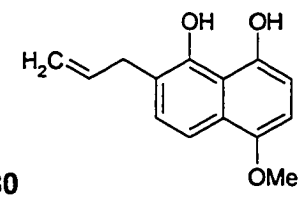


Table 3.3: Comparison of different rate costants (k) for a number of antioxidants reacting with the DPPH$^{\bullet}$ in ethyl acetate. The reaction rates obtained via stopped-flow kinetics testing.

Compound:	k [$M^{-1}s^{-1}$]	R^2
 **78**	$1.6 \times 10^{2*}$	0.9992*
 **8**	(a) 1.9×10^{2} (b) 2.1×10^{2} (c)** 9.4×10^{3}	(a) 0.9995 (b) 1 (c)** 0.9907
 **19**	2.1×10^{2}	0.9993
 **33**	3.0×10^{3}	0.9999
 **38**	4.5×10^{3}	0.9980
 **59**	2.9	0.9942
 **80**	4.1×10^{5}	0.9948

* this is the literature value in ethyl acetate[40]
** done in chloroform

Table 3.3 lists all the results obtained for the synthesized antioxidants **8**, **19**, **33**, **38**, **59** and **80**. Please note that the naphthalene diol **80** was synthesized and tested by Ami Chin.[42]

For compound **8** tested in chloroform, five different concentrations were prepared: 0.267 mM, 0.533 mM, 1.333 mM, 2.667 mM and 5.333 mM.

Tables 3.4-3.7 list the concentration ranges prepared for testing of the comounds **19**, **33**, **38** and **59**. They also give the approximate times of the reactions and the averaged pseudo first order rates of reaction k_{exp}.

Table 3.4: The concentration ranges of the antioxidant **19** in ethyl acetate, k_{exp}s and the approximate times of the reaction with DPPH•.

Compound **19**	Concentration [mM]	k_{exp} [1/s]	Reaction time [sec]
1	0.667	0.1375	20
2	1.67	0.3344	15
3	3.33	0.6484	10
4	6.67	1.4027	5
5	10.0	2.1130	2

Table 3.5: The concentration ranges of the antioxidant **33** in ethyl acetate, k_{exp}s and the approximate times of the reaction with DPPH•.

Compound **33**	Concentration [mM]	k_{exp} [1/s]	Reaction time [sec]
1	0.33	1.035	5
2	0.67	2.141	2
3	1.66	5.261	1
4	3.33	10.013	0.5
5	6.66	20.097	0.2
6	10.0	29.923	0.1

Table 3.6: The concentration ranges of the antioxidant **38** in ethyl acetate, k_{exp}s and the approximate times of the reaction with DPPH•.

Compound **38**	Concentration [mM]	k_{exp} [1/s]	Reaction time [sec]
1	0.154	0.567	10
2	0.308	1.335	5
3	0.770	3.634	1
4	1.54	6.864	0.5

Table 3.7: The concentration ranges of the antioxidant **59** in ethyl acetate, k_{exp}s and the approximate times of the reaction with DPPH•.

Compound **59**	Concentration [mM]	k_{exp} [1/s]	Reaction time [sec]
1	1.65	0.00464	200
2	3.30	0.00921	200
3	6.60	0.01837	150
4	9.90	0.02800	100
5	13.8	0.03637	50
6	20.7	0.06114	20

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CLAIMS TO THE ORIGINAL RESEARCH

(i) The total synthesis of 4-methoxy-5-methyl catechol **8** in 44% yield over five steps starting from sesamol.

(ii) The preparation of 5-methoxy-4-methyl-benzo[1,3]dioxole **6**.

(iii) The preparation and structure elucidation of compound **9**, the by-product of the hydrolysis of acetate **7**.

(iv) The synthesis of 4-hydroxy-2-methyl-2,3-dihydro-benzofuran-5-carbaldehyde **25** starting from 1,3-dimethoxy benzene and hence partial synthesis of 2-methyl-2,3-dihydro-benzofuran-4,5-diol **26**.

(v) The preparation of (4-methyl-benzo[1,3]dioxole-5-yl)-methanol **45**.

(vi) The preparation of 2,5-diallyl-3,4-dihydroxy-benzaldehyde **53**.

(vii) Antioxidant activity measurement of compounds **8**, **19**, **33**, **38** and **59** using the stopped flow kinetics apparatus.

(viii) Preparation of acetic acid 6-acetoxy-3-methoxy-2-methyl-phenyl ester **74**.