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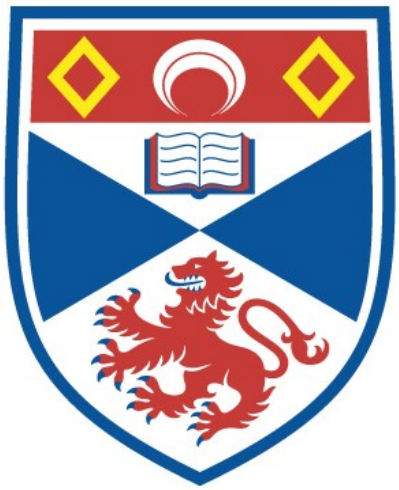

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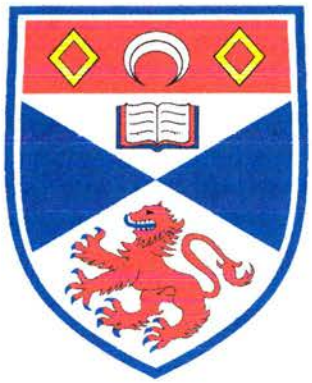

STUDIES ON ALKYLRESORCINOLS AND NOVEL ANALOGUES

A thesis presented for the degree of

Master of Philosophy

to the

University of St. Andrews

in April 2006

by

Anna Elizabeth Talbot

Supervisor – Dr. Nigel P. Botting

DECLARATION

I, Anna E. Talbot, hereby certify that this thesis, which is approximately 14 000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

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Finally, I would like to thank my parents for supporting and encouraging me in all my endeavours. This is for you.

ABBREVIATIONS

Ac	Acetyl
ACP	Acyl carrier protein
9-BBN	9-Borabicyclo[3.3.1]nonane
Bn	Benzyl
BSA	Bovine serum albumin
nBu_4NCl	Tetra-*n*-butylammonium chloride
n-BuLi	*n*-Butyllithium
CI	Chemical ionisation
CNSL	Cashew nutshell liquid
CoA	Coenzyme A
DCM	Dichloromethane
DIBAL	Diisobutylaluminium hydride
DMF	*N*,*N*-Dimethylformamide
DNA	Deoxyribonucleic acid
dppf	1,1'-Bis(diphenylphosphino)ferrocene
EI	Electron impact
ES	Electrospray
Et	Ethyl
Fe(acac)	Iron acetylacetonate
GC-MS	Gas chromatography – Mass spectrometry
HMPA	Hexamethylphosphoramide
IgG	Immunoglobulin G
IR	Infrared

$KSBu^t$	Potassium *t*-butylmercaptide
LC-MS	Liquid chromatography – Mass spectrometry
Me	Methyl
Ms	Methanesulfonyl (mesyl)
MS	Mass spectrometry
NCS	*N*-chlorosuccinimide
NMP	*N*-methylpyrrolidinone
NMR	Nuclear magnetic resonance
Ph	Phenyl
ppm	Parts per million
PTFE	Polytetrafluoroethylene
R-	Alkyl-
RNA	Ribonucleic acid
TEMPO	Tetramethylpiperidine *N*-oxide
THF	Tetrahydrofuran
TMS	Tetramethylsilane
Triflic	Trifluoromethanesulfonic
UV	Ultraviolet

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ABSTRACT

Alkylresorcinols such as 1,3-dihydroxy-5-alkylbenzenes have been found to have a number of biological activites including antiparasitic, cytotoxic, fungicidal and bacteriocidal properties that act against various pathogens. These resorcinols have been found to be present and have been isolated from a range of plants in the *Anarcardiaceae Ginkgoaceae* and *Graminae* families.

Alkylresorcinols have previously been synthesised by various routes, with the attachment of the side chain being the most challenging aspect of the synthesis. For comprehensive studies on their structure-activity relationships, an efficient synthetic route therefore needs to be found. Alkylresorcinols were to be used as standards in GC-MS and LC-MS based analytical methods for the detection and quantification of alkylresorcinols in human fluids. Carboxyalkylresorcinols were required for the development of new automated immunoassays of alkylresorcinols from biological samples.

The work towards the synthesis of a number of novel analogues of resorcinol was achieved, namely 15-(3',5'-dihydroxyphenyl)pentadecane via a Suzuki coupling reaction; 16-(3',5'-dihydroxyphenyl)hexadecanoic acid via an iron(III) catalysed carbon-carbon coupling reaction employing a grignard reagent; 16-(3',5'-dihydroxyphenyl)hexadecane, 17-(3',5'-dihydroxyphenyl)heptadecanoic acid, 21-(3',5'-dihydroxyphenyl)henicosanoic acid and 19-(3',5'-dihydroxyphenyl)nonadecanoic acid employing standard conditions of Wittig reactions.

CHAPTER 1

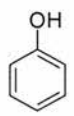
INTRODUCTION

1 INTRODUCTION

1.1 RESORCINOLIC LIPIDS

Phenolic lipids are a class of organic compounds that have a hydroxyl group and an aliphatic chain attached to an aromatic ring. Non-isoprenoid phenolic lipids are a class of long chain phenols, such as phenol **(1)** itself, catechol **(2)**, resorcinol **(3)** and hydroquinone **(4)** and are derived from mono- or dihydroxybenzene structures that have a minimum of one long aliphatic chain attached to the ring.[1] The structure of phenolic lipids results in them being amphiphilic in nature. They have often been depicted as secondary metabolites, but recent research has shown that they are more important in cellular physiology and biochemistry than this name implies.

Resorcinolic lipids are a group of phenolic compounds that are long chain homologues of orcinol (1,3-dihydroxy-5-methylbenzene)[1] **(5)** and often referred to as alkylresorcinols or 5-alkylresorcinols.



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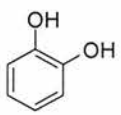

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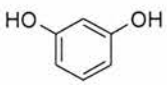

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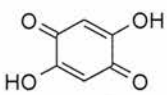

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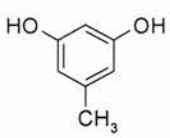

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The occurrence of resorcinolic lipids **(Section 1.2)**, their biosynthesis **(Section 1.3)**, amphiphilic properties **(Section 1.4)**, biological activity **(Section 1.5)** and their dietary benefits **(Section 1.6)** are discussed in this chapter.

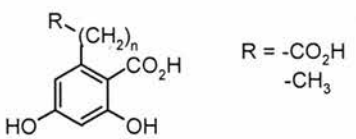
1.2 OCCURRENCE OF RESORCINOLIC LIPIDS

Originally it was thought that phenolic lipids were only present in particular families of plants. Resorcinolic lipids were initially found to be contained in the *Ginkgoaceae (Ginkgo biloba)*,[2,3] *Anacardiaceae (Anacardium occidentale)*[4,5,6] families and the *Graminae* family (cereal grains), observed by Wenkert.[7] However, further studies have shown that resorcinolic lipids can be found in over 15 families of higher and lower plants.[1] As well as the various plant families, resorcinolic lipids, known as leprosols, have been found to be contained in microbial organisms such as the *Actinomycetales (Mycobacterium leprae)*[8] *Pseudomonales (Pseudomonas)* and *Eubacteriales (Azobacter)* families.[9]

The *Anacardiaceae* family is the principal source of phenolic lipids such as alkylresorcinols, alkylphenols and alkylcatechols. These phenolic lipids are of much use in industry. For example, the lipids contained in cashew nut shells (*Anacardium occidentalis*) have been used to make polymerisation surface coatings, modified rubbers, ion exchange resins and waterproofing materials as well as many other products.[10]

Wenkert has carried out a vast amount of research on the *Graminae* family. It has been shown that significant amounts of 5-*n*-alkylresorcinols are present in wheat bran,[7] rye, barley[11] and various other cereal grains. Extensive research has also been carried out on resorcinolic lipids present in fruits, seeds, bacterial cysts, green tissue, leaves, stems and bacterial vegetative cells.[12,13]

Resorcinolic lipids have also been found in *Ononis*, a genus of leguminaceous plants. The homologues include both ring- and chain-derivatives having various lengths of side chain, free or modified hydroxyl and/or keto substituents in the 5-alkyl chain. One of the most common modifications of 5-alkylresorcinols is the presence of the carboxylic acid group in the ring to generate orsellinic acid derivatives or alkylresorcinolic acid **(6)**.[8]



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Research has shown that in microbial sources, such as soil bacteria there is the exclusive occurrence of 5-alkylresorcinol homologues with saturated chains. This therefore suggests a plausible association between microbial and higher plant sources.[14] Due to the rare occurrence of resorcinolic lipids in animal organisms, it is thought that it is possible for the biosynthetic pathway in plant and microbial organisms to be similar.[15,8]

Depending on the source, the amount of resorcinolic lipids differs significantly. Generally resorcinolic lipids occur as mixtures that have various chain lengths and/or degrees of unsaturation.[16,17] In particular, a wide spectrum of homologues have been found in the *Graminaceae*, *Azobacter* and *Pseudomonas* families.

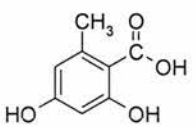
Over one hundred natural resorcinolic lipid homologues have been identified. These include homologues of 1,3-dihydroxy-5-alkylbenzenes with side chains from C_5 to C_{29}, structurally related homologues containing one, two and three double bonds and a variety of derivatives substituted with methyl, dimethyl and hydroxyl groups on the chain.[8]

Various chiral compounds substituted in the side chain with acetoxy, hydroxy and methoxy groups and others with phenyl, 3,5-dihydroxyphenyl or carbonyl groups are also known.[8]

1.3 BIOSYNTHESIS OF RESORCINOLIC LIPIDS

Resorcinolic lipids are biosynthesised in cells via the polyketide (acetate) pathway. As with fatty acids, the biosynthesis of polyketides is characterised by the linear addition of C_2 units derived from acetic acid and the activated forms of acetyl-S-CoA and malonyl-S-CoA. However, unlike the biosynthesis of fatty acids where every C_2 unit is only added to the growing chain after reduction of the previous carbonyl unit to a methylene group, in polyketide biosynthesis, the growth of polyketide chains does not require the reduction of carbonyl units ***(Scheme 1.1)***. This results in the formation of poly-β-ketoacids.

The formation of the phenolic ring structure of the resorcinolic lipids from the linear poly-β-ketoacid chain occurs via Knoevenagel condensation. The methylene groups, which act potentially as nucleophiles, and the carbonyl groups, which potentially act as electrophiles of the linear poly-β-ketoacids, undergo the intramolecular condensation. This leads to the formation of 2,4-dihydroxy-6-alkylbenzoic acids, in which orsellinic acid **(7)** is the first homologue ***(Scheme 1.2)***.



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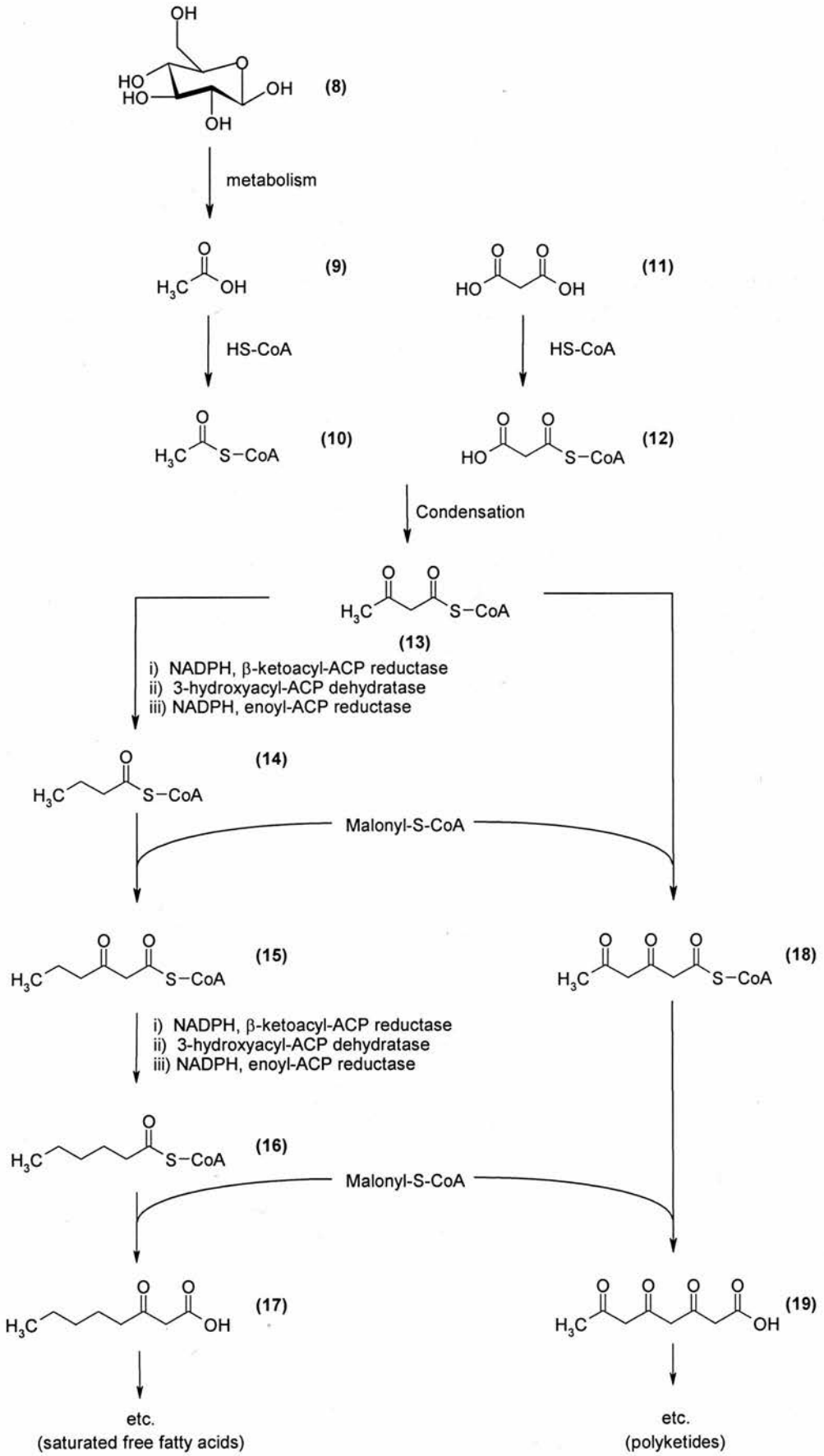
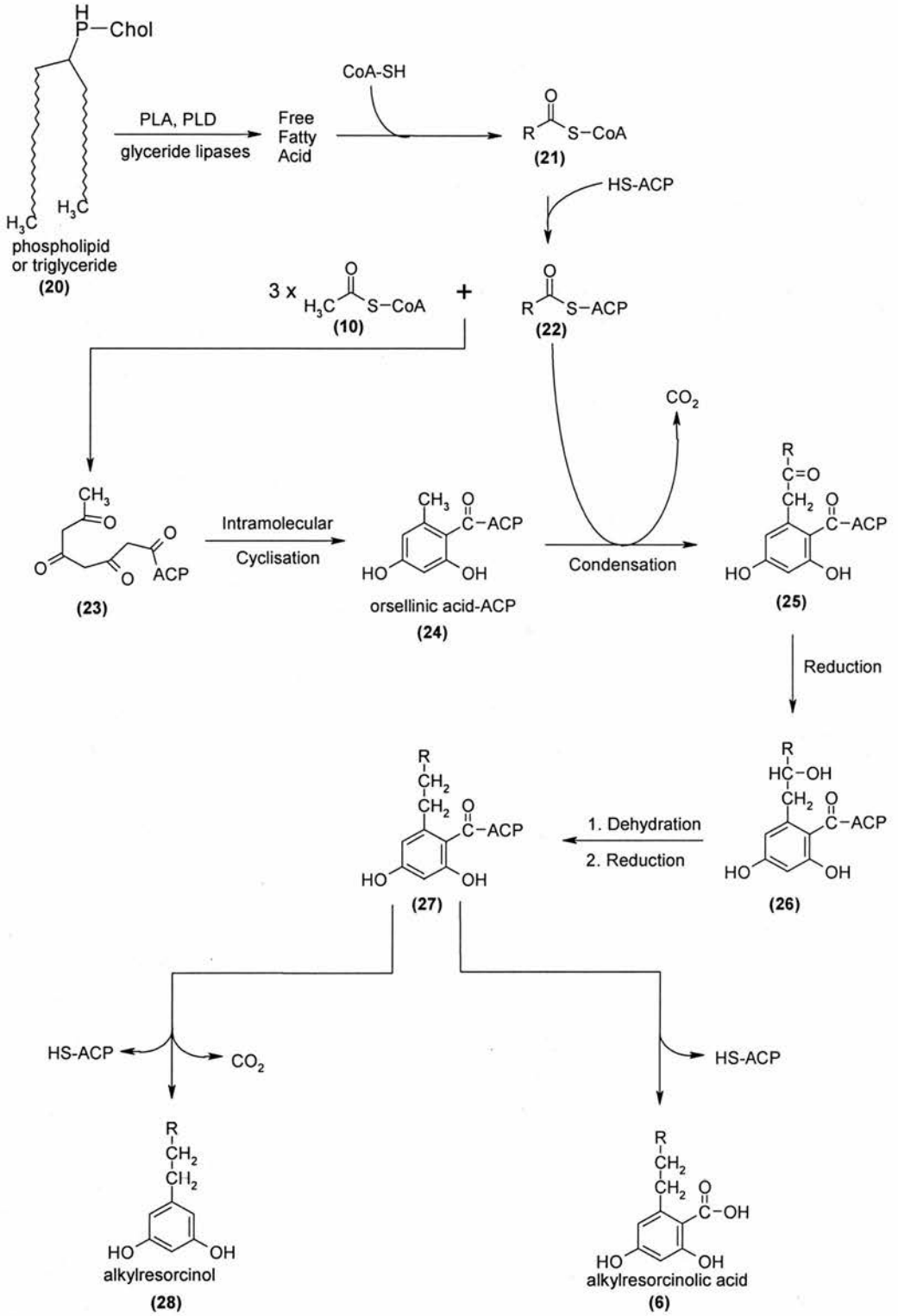
Scheme 1.1 *– The building of acetate units in the biosynthesis of fatty acids and polyketides*[8]

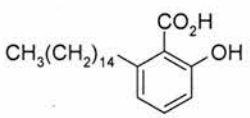


Scheme 1.2 *– Hypothetical scheme of the biosynthesis of resorcinolic lipids*[8]



Gellerman *et al.* have undertaken extensive research into the biosynthesis of long chain phenolic lipids, in particularly how acetate is integrated into the phenolic ring of anacardic

acid **(29)**.[18,10] Their research confirmed the role of acetate in the biosynthesis of long-chain phenolic lipids by using ^{14}C-labelled acetate to show that it was incorporated into the phenolic ring of the phenolic lipids. Similarly ^{13}C-labelling studies of xenognosin **(30)** showed that carbons 1-C and 5-C of the aromatic ring were derived from [1-^{13}C] acetate and carbons 6-C and 4-C were from [2-^{13}C] acetate.[19]



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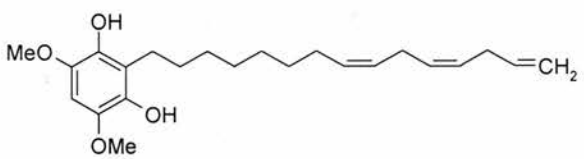

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While the chemical mechanism for the ring formation is understood, little experimental evidence has actually been obtained on how the long aliphatic side chains of alkylresorcinols are formed. If the complete alkylresorcinol (ring and side chain) were synthesised from acetate in a single multicondensation step, this would result in the formation of poly-β-ketoacids that have very long chains (for example C_{34} in the case of C_{27}-alkylresorcinol). However there is no evidence for the formation of such long chains. Research carried out by Gellerman[18,10] suggests that two systems are actually involved in forming the complete alkylresorcinol. Their data suggest that one system is involved in the formation of the ring and another in the formation of the chain. This is supported by studies by Fate and Lynn,[19] which showed that there was no incorporation of [1-^{13}C] acetate in the side chain. It is possible that pre-formed fatty acids are used as precursors of the side chain and are attached once the ring has formed.

To date, the complete biosynthetic pathway of long chain alkylresorcinolic lipids is only hypothesised and therefore the exact pathway is still unclear.

Some alkylresorcinols contain functionalised side-chains. Manitto and Sammes have found that the occurrence of carbonyl or hydroxyl groups in the side chain of alkylresorcinols such as in the 5-(2-hydroxyalkyl)resorcinols, is governed by the number of nonreductive steps in poly-β-ketoacid formation.[20]

Unpublished research by Kozubek and Sokol has shown that glucose is one of the metabolic precursors of 5-*n*-alkylresorcinols as acetate units that are introduced into a phenolic lipid ring can be generated from the metabolism of glucose.[8]

The synthesis of 5-*n*-alkylresorcinols via the polyketide pathway must involve the cyclization of an acyclic precursor, the lengthening of the side chain followed by decarboxylation to generate an alkylresorcinol **(28)** with an odd number of carbons in the chain.[8] There are at least two points in the pathway where decarboxylation of the ring could occur. Firstly enzymatic decarboxylation of alkylresorcinolic acid **(6)** could take place to generate alkylresorcinol **(28)**. Evidence, however, shows that enzymatic decarboxylation of the previously formed alkylresorcinolic acid does not occur. Secondly the alkyl chain can be achieved by attachment to ACP whilst the carboxylic acid groups were also attached to either ACP or CoA. This would result in simultaneous decarboxylation occurring to the alkylresorcinol when the molecule is released from its attachment, as alkylresorcinolic acid would be the product if this were not the case. This therefore shows that an activated state of alkylresorcinolic acid is necessary for alkylresorcinol production.[8]

1.4 AMPHIPHILIC PROPERTIES OF RESORCINOLIC LIPIDS

Biological membrane structures play a vital role in important cellular metabolic processes. It is therefore necessary to determine the effect of resorcinolic lipids on the structure and function of these membranes. Resorcinolic lipids have a polar, water-soluble, i.e. hydrophilic group, namely the dihydroxybenzene ring, which is attached to a non-polar, water-insoluble, i.e. hydrophobic, hydrocarbon chain. This property is known as amphiphilicity. However, research has shown that the hydrophilic ends of the resorcinolic lipids (dihydroxybenzene ring) have a low polarity, and therefore, resorcinolic lipids are virtually insoluble in water.[8]

It has also been reported that in aqueous solutions, resorcinolic lipids form stable monomolecular layers at the air-water interface.[21] The dihydroxybenzene rings of long chain resorcinolic lipids, rest perpendicular to the surface of the subphase.[22] The space taken up by the lipids depend on degree of unsaturation and the length of the hydrocarbon chain. It has been found that analogues with unsaturated chains occupy a larger area than saturated chains at the same surface pressure.[22] Evidently the area occupied increases with the length of the chains. Studies have also shown that the surface area of resorcinolic lipids increases with increasing temperature.[23] However, double bonds inhibit the decrease in size at lower temperatures.[22] Kato reported that the presence of the long aliphatic chain in these molecules increases their pK_a value.[22] It was found by Kieleczawa and Kozubek[8] that the critical micelle concentrations of alkylresorcinols depended on the degree of unsaturation, the length of the aliphatic chains, and the pH.

1.5 BIOLOGICAL ACTIVITY OF RESORCINOLIC LIPIDS

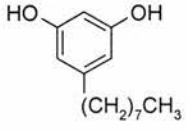
Due to their links with biomedical, biopharmacological and biotechnological areas, a great deal of research on resorcinolic lipids is being carried out.[8]

Resorcinolic lipids are able to interact with not only biological membranes, but also hydrophobic regions of proteins and hence alter their properties and biological activity.[1] Resorcinolic lipids have been found to display several biological activities such as antimicrobial, antiparasitic, cytotoxic and antioxidant effects, growth regulation in the host-parasite relationship and participation in enzymatic activities. They have also been found to have some involvement in contact dermatitis by modifying barrier properties and the structure of biomembranes.[8] It is also possible that resorcinolic lipids could be of use in future biomedical applications. Due to their interaction with nucleic acids they may be able to improve the action of nucleic acid-specific drugs. In addition they may be able to enhance the properties of liposomal drug carriers.[1] Resorcinolic lipids may also have an application in natural plant protection due to the antifungal properties of cereal lipids, increased production and resistance to pathogenic fungi on infection and the stimulation of their production by very low concentrations of herbicides.[1]

1.5.1 Antibacterial, Antifungal and Antiparasitic Activity

Research in the 1920s by Klarmann *et al.* into the antibacterial action of resorcinol derivatives led to homologues of 1,3,4-alkylresorcinol being used to treat infections.[24,25] During the same decade, evidence was found for the bacteriostatic action of 4-hexylresorcinol **(31)** on seven different phytopathological bacterial strains in research carried out by Stapp.[26] Much more recently however, it has been shown that there is a connection between the antibacterial action of resorcinolic lipids and the antibacterial

action of compounds contained in *G. biloba* fruit, *Ardisia japonica* plant, seed husks of *Myristica fragrans* and cashew nut shell liquid.[8] These compounds are very active towards phytopathogenic bacteria[27,26] in addition to pathogenic *Mycobacterium smegmatits* and *Mycobacterium tuberculosis* (acid-resistant Gram-positive bacteria).[28,29] Clinical testing of a mixture of C_{13} monounsaturated alkylresorcinols and their mono-methyl derivatives was carried out on over 200 patients. Over 80% of the cases proved to be successful in the treatment of tuberculosis.[30] Alkylresorcinolic acids and 5-alkylresorcinols extracted from the fungi *Merulius tremellous*, *Phebia radiata*,[31] *Verticicladiella*[32] and *Pulcherricium coeruleum*[33] were also found to demonstrate antibacterial activity. Bacterial growth of *Micrococcus lysodeictius* and *Bacillus subtilis* has been found to be inhibited by resorcinolic lipids isolated from the bacteria *Pseudomonas carboxydoflava*.[34]



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5-*n*-Pentadecylresorcinols with various degrees of unsaturation in their aliphatic chain show antibacterial activity against the bacterium that causes acne (*Propionibacterium acne*) and the bacterium that causes paradonthosis (*Streptococcus mutans*).[35] The least active was the homologue with a saturated aliphatic chain. Studies also showed that the antibacterial activity was increased by the presence of carboxylic acid groups in the ring.[36] Resorcinolic lipids have also been used as active ingredients of antiseptic lotions due to their activity against bacteria.[37, 38, 39, 40, 41, 42]

Resorcinolic lipids also have fungistatic properties, although they inhibit the growth of *Trichophyton mentagrophytes* and *Saccharomyces cerevisiae* less effectively than they do bacteria.[8,43] Mango fruits have a resistance to fungal infections of *Alternaria alternata*, which is due to the presence of the 5-*n*-heptadec-12-enyl- and 5-*n*-pentadecylresorcinols in their peel.[44,45] Research carried out by Reiss[46] has shown that the growth of *Aspergillus parasiticus*, *Aspergillus versicolor*, *Penicillinium chyrysogenum* and *Penicillium roqueforte* is significantly inhibited by 5-*n*-pentadecylresorcinols and 5-*n*-alkylresorcinol. Seed epicuticular wax also contains resorcinolic lipids and it is thought that due to this, barley seeds are resistant to *Aspergillus niger* and *Penicillium chyrysogenum*, both are pathogenic fungi.[47]

The tropical disease schistosomiasis is caused by the parasite *Briomphalaria glabratus* against which 5-*n*-alkylresorcinol homologues from *Anacardium occidentale* have molluscicidal activity.[48] Research showed that the activity of alkylresorcinols was inversely proportional to the degree of unsaturation of the side chains.[8] This was carried out by testing various resorcinolic lipids against a class of worms known as *Filaria*. It was found that 5-*n*-pentadecenylresorcinol was the most active, and at a concentration of only 3.5 μg/mL, it completely eradicated the parasites.[49] This result indicated that the alkylresorcinol was 100 times more active than the drug diethylcarbamazine, usually used for the treatment of parasites.[49]

The pharmaceutical and cosmetic industries have been able to make particular use of alkylresorcinols as basic components for drugs and cosmetics as they are not toxic to higher animals.[49,8] As a result preparations containing resorcinolic lipids are valuable for

various medical treatments such as mouth and gingival infections, acne and fungal infections.[50]

1.5.2 Anti-tumour Activity

Research into the biological activity of phenolic lipids has shown that the growth of chicken embryonic heart cells and the blastogensis of human lymphocytes can be inhibited by 5-*n*-amylresorcinol (olivetol).[51] Other 5-*n*-pentadecenylresorcinols have been found to have a strong antitumour activity against S180 tumour in mice. Tumour cell growth was virtually completely inhibited 24 hours after the active component, 5-*n*-pentadec-8-enylresorcinol (bilobol), was injected into tumour cells at a dosage of 40 mg/kg per day for four days.[52] This kind of activity was also seen for alkenylresorcinols acting against P-338 leukaemia cells.[53] The most active constituents of the plant *Lysimachia japonica*, which acts on the KB cell cultures and the tumour cells B-16, PC-13, L5178Y, P-388 and HEp-2, were the resorcinolic lipids 5-*n*-tridecylresorcinolic acid and 5-*n*-tridecylresorcinol.[53] Research has shown that the most active homologues were those that had between 11 and 15 carbons in the aliphatic chain of the resorcinol. However, it was found that homologues with unsaturated carbon side chains were four times more cytotoxic.[53] It was also found that it is not imperative that the resorcinol ring possesses a carboxyl group for antitumor activity, unlike antibacterial activity in which one is required.[54] These findings support earlier research into the effect of the length and degree of unsaturation of the side chains of 5-*n*-alk(en)ylresorcinols and their interactions with biological membranes.[55]

In 1990 an antitumour drug for melanoma, Cloudman S91, was synthesised containing predominantly 4-*n*-hexylresorcinols that completely inhibited tumour growth in mice when

given in a dosage of 50 μg/Lg of body weight for 10 days.[56] The mice did not experience any toxic side effects. It is thought however that increasing the chain length would increase the therapeutic efficiency of the antitumour drug.[56]

It was found that another resorcinolic lipid, 5-*n*-pentadec-8-enylresorcinol, did not stimulate tumour development when combined with phorbol ester, an inducer for skin tumours.[57] The homologue 5-*n*-pentadec-8-enylresorcinol, did not stimulate carcinogenic effects, even at a dosage of 50 μg twice per week for 30 weeks, although some irritation was exhibited. Phorbol ester on the contrary did stimulate carcinogenic effects and at a dosage that was 20 times lower.[57]

1.5.3 Resorcinolic Lipids as Growth Regulators and in Host-Parasite Relationships

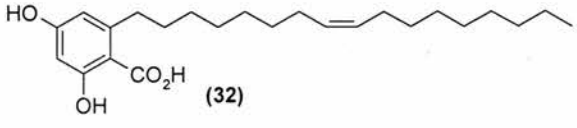
Research by Voblikova has shown that a derivative of alkylresorcinolic acid **(6)** at a concentration of 3 μg/mL inhibits germination of seeds by 50%.[58] This derivative was isolated from a fungal plant pathogen, therefore indicating that resorcinolic lipids could play a role in infections and in the death of organisms infected by fungi.[58] The growth of animals fed with cereal grains was found by Wieringa[59] to be stunted due to resorcinolic lipids in the cereal grains. However, it was found that the actual reason for this stunted growth was due to a decrease in appetite caused in some way by alkylresorcinols **(28)**, the mechanism of which is still currently unknown.[60]

It has been found that seeds are vulnerable to attack by bacteria, fungi or parasites during germination and the formation of shoots. Research has shown that this can lead to the formation of a close mutual relationship between host and parasite.[8] It has been found that this relationship between a parasite such as a parasitic plant, and a host, such as corn, can

result in serious damage to the yield of crops. Following germination of a parasitic seed, the plant can survive up to two weeks without the presence of a host.[61] Studies on xenognosin **(30)**, a derivative of a resorcinol lipid isolated and identified from the roots of the host plant found that its methylated form enhances the activity of the xenognosin-dependent germination signal.[8,19]

1.5.4 Effect of Resorcinolic Lipids on Nucleic Acids

Direct effects on the structure and metabolism of nucleic acids is one possible mechanism of action of resorcinolic lipids in cells. Studies have shown that the synthesis of DNA and RNA can be completely inhibited by heptadec-8-enylresorcinolic acid (merulinic acid) **(32)** at a concentration of 100 μg/mL.[62] This concentration of merulinic acid can also inhibit protein synthesis in *Bacillus brevis* cells.[62] Similarly, studies on 5-*n*-decylresorcinol at 50 μM showed that it too, exhibited similar inhibitory properties towards the synthesis of nucleic acids in isolated rat thymocytes.[63]

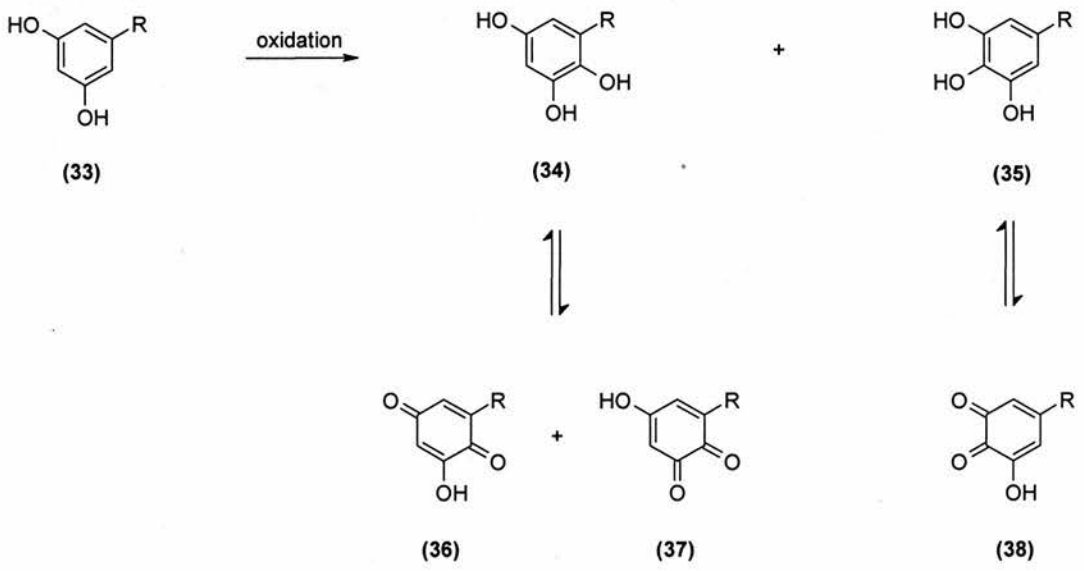


(32)

Resorcinolic lipids have been found to be able to cause DNA strand scission.[64,65,66] It has been found that in the presence of oxygen, alkylresorcinol **(28)** activity is increased. This suggests that the active species involved in DNA scission is a reactive oxygen species generated by initial oxidation of the aromatic ring of the parent molecule.[8] The oxidation of alkylresorcinols results in hydroxyquinone products being produced ***(Scheme 1.3)***. When this is allowed to react further with oxygen and/or hydrogen peroxide, the oxygen species active in DNA scission is generated.[8] An increase in activity was also observed by

Singh *et al.*[67] for homologues with longer aliphatic chains. This suggests the alkylresorcinol molecules interact with the double helix of DNA by intercalation of the alkylresorcinol side chain within the interior of the double helix.

Scheme 1.3 – *Oxidation products of 5-n-alkylresorcinols*[8]



1.5.5 Alkylresorcinols and Allergic Contact Dermatitis

Certain alkylphenolic lipids are known to be linked to allergic contact dermatitis. Contact dermatitis is a medical condition that occurs when someone has an allergic reaction after coming into contact with something that irritates their skin. This allergic reaction results in a bumpy patch of red, itchy, flaky skin, generally known as inflammation.

Cashew nutshell liquid (CNSL) is one source of alkylphenolic lipids and was found to induce dermatitis by Wasserman and Dawson in 1948.[68] Alkylresorcinol-containing plants, such as *Philodendron* and *Rhus toxicodendron* have also been found to induce dermatitis. Reffstrup *et al.* have found that 5-*n*-heptadecenylresorcinols are connected specifically to *Philodendron*-induced dermatitis.[69] It has been found that patients that are sensitive to *Philodendron* may not be sensitive to *Rhus toxicodendron* and vice-versa.[70] However the unsaturated chain alkylresorcinols and alkylcatechols have been found to play a key role in the development of dermatitis.[71]

Studies have shown that the formation of a covalent bond between the *o*-quinone of the catecholic ring and nucleophilic functionalities such as those present in proteins is responsible for bringing more hapten (a molecule that activates B lymphocytes, required for antibody production) to cells in the immunological system. The side chain of the catecholic ring binds and inserts the hapten into epidermal cell cytoplasmic membranes.[72] This results in the allergen being able to react with the membrane proteins and/or be internalised by skin cells for activation.[72]

Recent studies have shown that the oxidative shortening of the side chain is responsible for the activation of the catechol ring.[73] Due to the *meta* position of both the hydroxyl groups

and the chain of alkylresorcinols, *o*-quinones cannot be formed directly. However if dihydroxybenzene is oxidised to 1,2,4-trihydroxyalkylbenzene, and is then oxidised further, this results in the formation of an *o*-quinonic form, which is active for allergy induction (see ***Scheme 1.3***).[67,74] This therefore suggests that oxidised resorcinolic lipids are responsible for the sensitising activity rather than the alkylresorcinols themselves.[8] Also, because urushiols and alkylresorcinols with saturated chains do not exert dermatological activity, this highlights the importance of the direct interaction with cellular membranes in exhibiting biological effects.[8]

1.6 DIETARY BENEFITS OF ALKYLRESORCINOLS

Alkylresorcinols have been found to be present in wheat and rye. A large proportion of the phytochemicals present in these two foodstuffs are alkylresorcinols. In wheat and rye, the concentration of alkylresorcinols is between 300 and 1500 μg/g. It has been suggested that phenolic compounds such as alkylresorcinols have many health benefits. Research has shown that there is a link between eating plant foods and a decrease in the risk of various diseases. For example, the reduced risk of coronary heart disease, some cancers, diabetes and obesity has been associated for many years with the consumption of whole grain cereals.[75,76,77] It is believed that the protective effects of whole grain cereals are largely due to the effects of the phenolic compounds they contain.

It has been found that alkylresorcinols are present in various plants and in some bacteria and fungi.[8] Alkylresorcinols that are found in food are only in high concentrations in wheat and rye. These alkylresorcinols have a saturated alkyl chain that is between 15 and 27 carbons long. Alkylresorcinols constitute between 0.0015–0.3% of whole kernel dry weight which corresponds to a high percentage of the phenolic compounds found in these cereals.[78]

As discussed, research has shown that alkylresorcinols are bioactive in various *in vitro* models, and as such may be essential in food and human nutrition. Alkylresorcinols are also present in barley, maize[79] (in smaller amounts), rice plant shoots,[80] mango latex and peel[81] and in cashew nut shell liquid.[82]

Wheat and rye contain different amounts of various homologues of alkylresorcinols. Wheat contains 90-95% of alkylresorcinols with saturated alkyl chains of length between 17 and 25 carbons. Rye contains alkylresorcinols that have chain lengths between 15 and 25 carbons, with 15-20% of these having a modified alkyl chain, reported to be possibly be unsaturated derivatives or keto and hydroxyl derivatives. The most common homologues in wheat are alkylresorcinols with 21 carbons in the alkyl chains, while the most significant homologue in rye is with 19 carbons.[91,83]

The bran fraction of wheat and rye contain the alkylresorcinols. In white wheat flour no alkylresorcinols are present, however in white rye flour small amounts of alkylresorcinols are present due to contamination from the bran. This is because separating the aluerone layer from the starchy endosperm in rye is very problematic. Alkylresorcinols are not present in the germ of wheat or rye.[84]

Since there is a strong correlation between the amount of bran in flour and the alkylresorcinol content, it has been suggested that alkylresorcinols could be used as markers of bran in flour. Fermentation and baking of sourdough wheat and rye has been found to decrease the amount of alkylresorcinols.[85]

People who regularly consume whole grain wheat and/ or rye are very likely to have a high intake of alkylresorcinols. A single serving of a cereal that is wheat-bran based would contain approximately 70 mg of alkylresorcinols. Similarly a serving of whole grain wheat bread (40 g) would provide the consumer with approximately 10 mg of alkylresorcinols. The exact intake of alkylresorcinols is difficult to calculate due to the content of

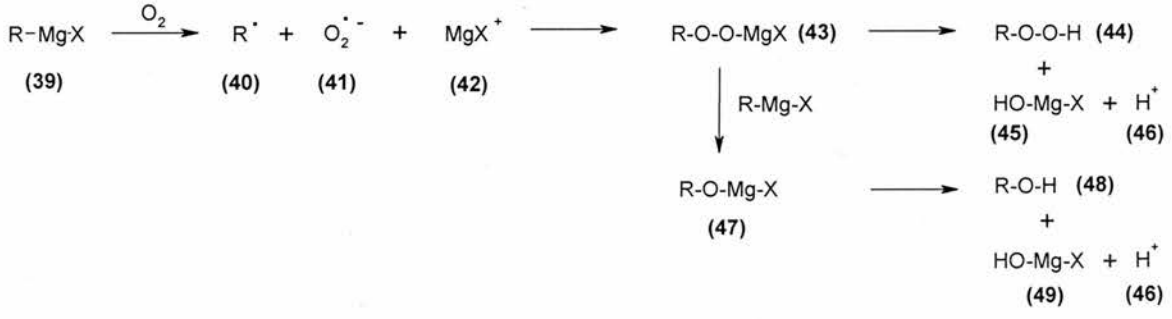
alkylresorcinols varying significantly within wheat and rye grains and also which products contain whole-grain wheat and rye. [86]

Research has shown that rats,[87] pigs[87] and humans[88] all absorb alkylresorcinols. The magnitude of this absorption does depend on the species and the individual. It has been demonstrated that 60-79% of alkylresorcinols had disappeared from pigs' small intestine, the actual value of which depended on the initial intake.[87] A study on humans revealed that approximately 60% of alkylresorcinols were absorbed by the body.[88] This study highlighted that shorter chain homologues of alkylresorcinols were absorbed to a higher extent. This was not seen in the pig study. The study of rats that were fed radiolabelled alkylresorcinols gave 34% radioactivity as metabolised alkylresorcinols in urine and 66% found to be intact alkylresorcinols that were excreted in the faeces (after about 100 hours).[87]

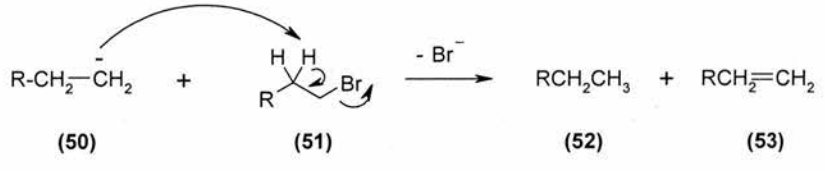
1.7 REVIEW OF CHEMICAL SYNTHESES OF ALKYLRESORCINOLS

The oldest method used to synthesise alkylresorcinols dates back to the 1960s when Wenkert *et al.*[7] employed Grignard chemistry using 3,5-dimethoxybenzaldehyde and *n*-alkylmagnesium bromides as the starting reagents. However the use of *n*-alkylmagnesium bromides in this first step proved to be problematic for two reasons. Firstly, unless the reaction was carried out in a totally inert atmosphere, the Grignard reagent oxidises, resulting in undesired alcohols being formed ***(Scheme 1.4)***. Secondly the use of a Grignard reagent can lead to the Wurtz side reaction taking place, contaminating the desired product ***(Scheme 1.5)***.[15]

Scheme 1.4 – *Oxidation of Grignard reagents*[15]



Scheme 1.5 – *Wurtz side reaction*[15]

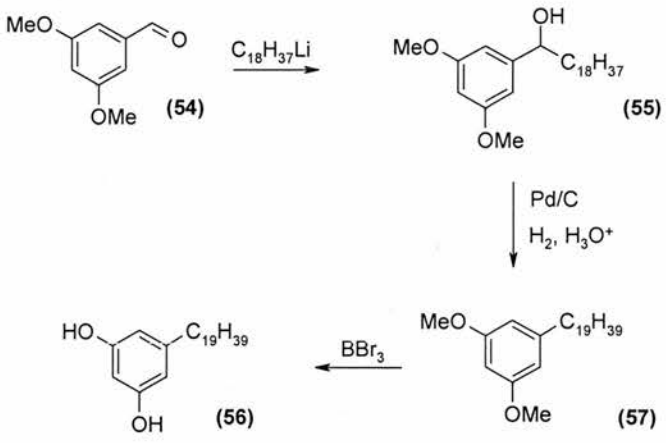


To avoid the complications of Grignard reagents being oxidised and the Wurtz side reaction, alternative methods for the synthesis of alkylresorcinols have been employed.

Ridley *et al.*[89] successfully used 3,5-dimethoxybenzoyl chloride and organic cadmium reagents as starting materials. Occolowitz *et al.*[90] used a diazoketone to synthesise 1,3-dihydroxy-5-tridecylbenzene.

Research[91] reported in 1995 by Kozubek and Tyman showed that organolithium reagents could be used instead of Grignard reagents thus avoiding the complications that they cause. This method is shown in ***Scheme 1.6*** whereby 3,5-dimethoxybenzaldehyde is reacted with *n*-octadecyllithium. The resulting secondary alcohol is catalytically hydrogenated to give the dimethoxyalkylresorcinol and then demethylated using boron tribromide to afford the desired alkylresorcinol, 5-*n*-nonadecylresorcinol. This synthetic route produced overall yields between 14% and 25% for a range of alkylresorcinols with varying lengths of alkyl chain.

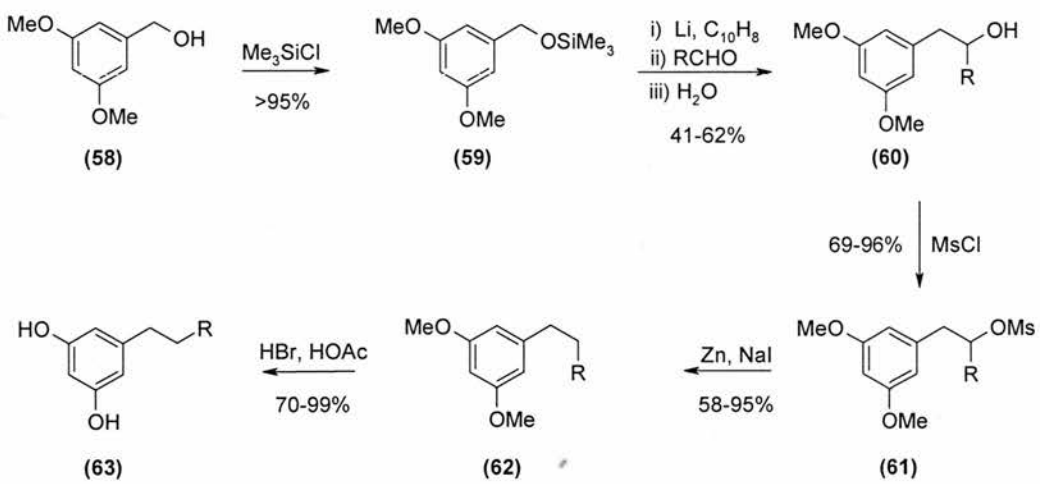
Scheme 1.6 – *Synthetic route to 5-n-alkylresorcinols from 3,5-dimethoxybenzaldehyde*[91]



Alonso *et al.*[92] used an alternative route to synthesise alkylresorcinols using 3,5-dimethoxybenzyl alcohol as the starting material. This method is shown in

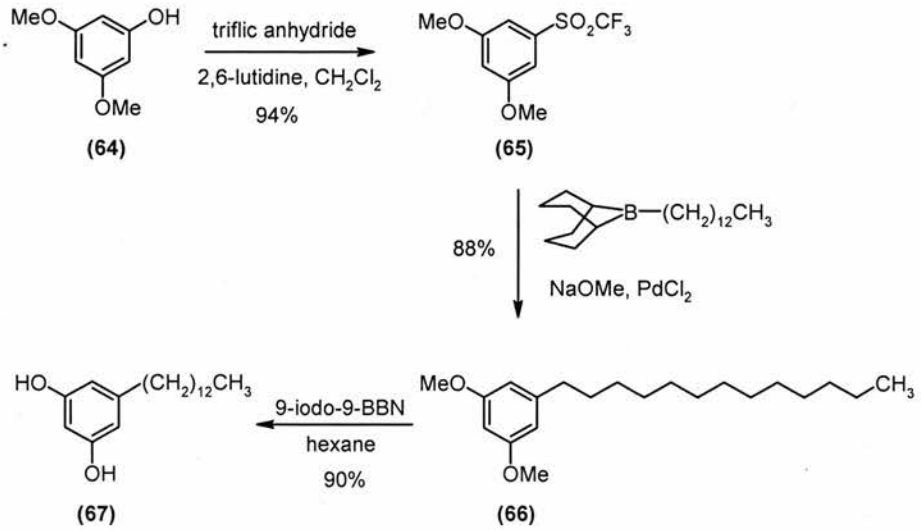
Scheme 1.7 whereby 3,5-dimethoxybenzyl alcohol is reacted with chloromethylsilane and triethylamine in THF to form the *O*-silyl derivative. The *O*-silyl derivative was then treated with excess lithium powder, a catalytic amount of naphthalene and then reacted with aldehyde in THF, which after hydrolysis gave 1,3-dimethoxy-5-(2-hydroxyalkyl)-benzene. This product was then treated with mesyl chloride in THF containing triethylamine and reduced using zinc and sodium iodide in refluxing monoglyme to form the methylated alkylresorcinol. This was finally demethylated via a high-yielding route (70-99%), using 45% hydrobromic acid in acetic acid to obtain the desired alkylresorcinol.

Scheme 1.7 – *Synthetic route to 5-n-alkylresorcinols from 3,5-dimethoxybenzyl alcohol*[92]



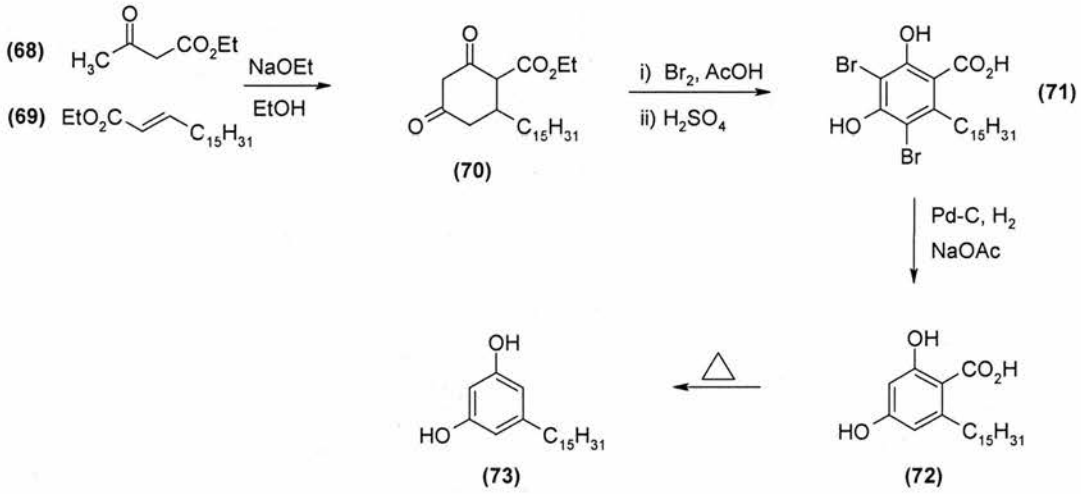
Fürstner and Seidel[93] employed a method ***(Scheme 1.8)*** that used a palladium-catalysed, base-induced cross coupling of 9-alkyl-9-BBN derivatives with aryl triflates, formed from 3,5-dimethoxyphenol and triflic anhydride in the presence of 2,6-lutidine. An 88% yield has been reported[93] for the coupling reaction. The following step of cleavage of the methyl ether was accomplished in 88-90% yield.

Scheme 1.8 *– Synthetic route to 5-n-alkylresorcinols from 3,5-dimethoxyphenol*[93]



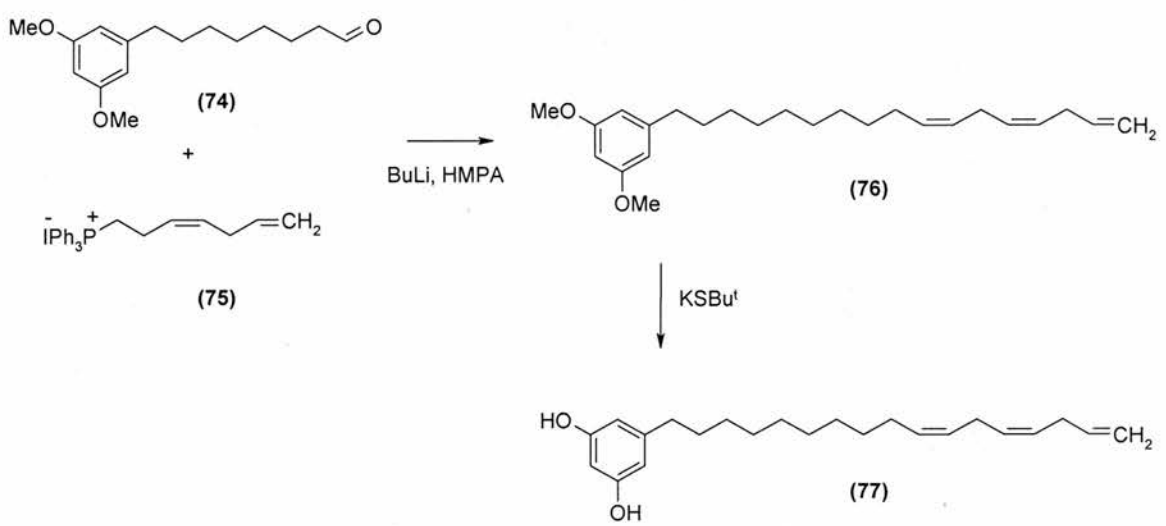
Tyman *et al.*[94] used yet another approach for the synthesis of alkylresorcinols ***(Scheme 1.9)***, namely a Michael addition of ethyl octadec-2-enoate to the ethyl acetoacetate carbanion to form a dione, which was then brominated. The free acid was then obtained by treatment with concentrated sulphuric acid. The desired alkylresorcinol was obtained by debromination using palladium catalysed hydrogenation followed by thermal decarboxylation to give an overall yield of the desired alkylresorcinol between 40-50%.

Scheme 1.9 *– Synthetic route to 5-n-alkylresorcinols via Michael addition*[94]



Several different routes have been used to synthesise alkenylresorcinols. Baylis *et al.*[95] used a procedure based on acetylenic routes to synthesise monoenes and dienes. They used starting materials such as 3,5-dimethoxybenzaldehyde, 3,5-dimethoxybenzoyl chloride and 7-(3,5-dimethoxyphenyl)heptylbromide. Tyman *et al.*[96] synthesised trienes via the Wittig method as shown in ***Scheme 1.10***.

Scheme 1.10 – *Synthetic route to trienes*[96]



Comparative to the study of unsubstituted resorcinolic lipids, there has been less research carried out into the synthesis of substituted resorcinolic lipids such as analogues that have hydroxyl, acetoxy, methyl, methoxy or carbonyl groups as side chain substituents. However it is thought that the current methods employed to synthesise unsubstituted alkylresorcinols could be adapted to synthesise resorcinolic lipids that have side chain substituents.

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CHAPTER 2

RESULTS AND DISCUSSION

2 RESULTS AND DISCUSSION

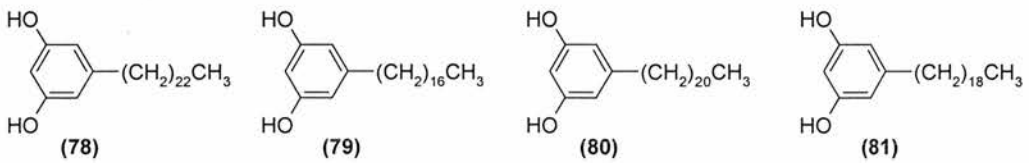
2.1 AIMS

This research project was carried out in association with Professor Herman Adlercreutz (University of Helsinki, Department of Nutrition, Cancer and Preventative Medicine Division of Clinical Chemistry). The project was part of a research programme on alkylresorcinols and their effects on human health. As discussed in the **Introduction**, alkylresorcinols are compounds that are derived from plants and exhibit biological activities such as antimicrobial, antiparasitic, cytotoxic and anti-oxidant effects,[1] regulate growth in the host-parasite relationship and participate in enzymatic activities. The topic requires collaboration between chemists and biologists to gain a complete picture of the biology of alkylresorcinols and their interaction with human cells.

Our role as organic chemists is in the synthesis of alkylresorcinols and therefore to provide compounds that could be tested by medical researchers. The aim of this project was to carry out studies on the synthesis of alkyl resorcinols and various novel analogues.

Figure 2.1 below shows the analogues **(78-81)** that have previously been synthesised in the Botting group.[2] A few of which have been screened for anti-cancer and anti-oxidant properties.

Figure 2.1 *– Previously synthesised alkylresorcinol analogues in the Botting group*



(78) **(79)** **(80)** **(81)**

It was decided to investigate the synthesis of a number of alkylresorcinols, so that they could be used as standards in GC-MS and LC-MS based analytical methods for the detection and quantification of alkylresorcinols in human fluids.

The other synthetic targets were alkylresorcinol derivatives with a carboxylic acid at the end of the alkyl chain. Introduction of this functionality allows the compound to be covalently attached to proteins, or solid supports, via an ester linkage. These derivatives were required for the development of new immunoassays for rapid high through put analysis of alkylresorcinols from biological samples.

The general procedure is as follows: Firstly the alkylresorcinol derivative is covalently attached to bovine serum albumin (BSA), which is used as a carrier protein. Without the BSA as the carrier protein, the small molecule will not be able to pass through plasma membranes as it is too small to be recognised by the plasma membranes. The BSA-alkylresorcinol conjugate is then injected into rabbits. Antibodies are synthesised by the rabbits in response to the presence of the antigen. Several weeks later a sample of blood can be taken from the rabbits to produce a serum which contains the antibody required.

The alkylresorcinols will be used in an immunoassay procedure analogous to that previously carried out using the isoflavone daidzein.[3] The antibody (anti-rabbit IgG) that is specific to the antigen daidzein is attached to a sheet of polymeric support (see ***Figure 2.2***). The anti-rabbit IgG binds the anti-daidzein antibody. Europium-labelled daidzein and sample daidzein compete for the anti-daidzein antibody. The europium ions are freed from the labelled daidzein by the addition of enhancement solution. The ions form highly

fluorescent chelates with the enhancement solution. The concentration of the daidzein in the sample is proportional to the fluorescence.[3]

Figure 2.2 – *Diagram of a competitive immunoassay (taken from reference[3])*

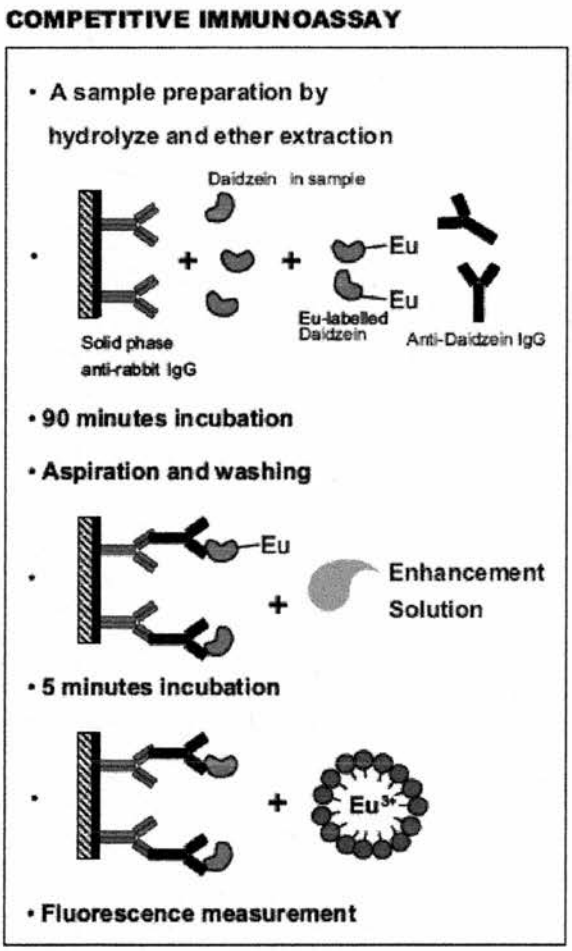
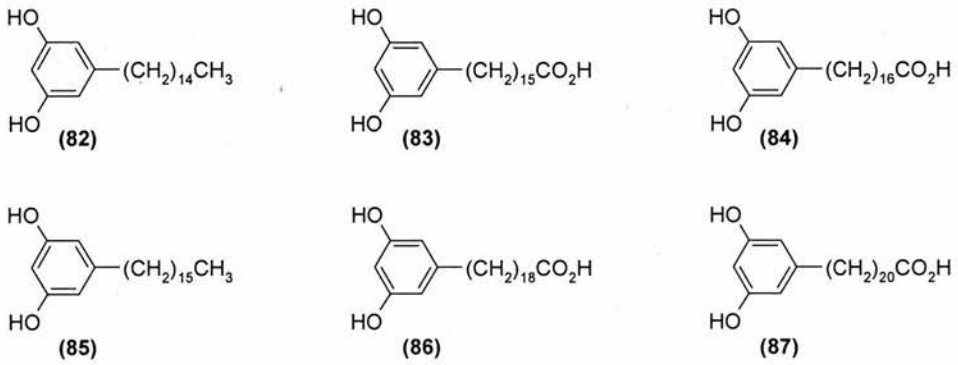


Figure 2.3 below shows the target compounds for this research. The synthetic targets were 15-(3',5'-dihyroxyphenyl)pentadecane **(82)**, 16-(3',5'-dihydroxyphenyl)hexadecanoic acid **(83)**, 17-(3',5'-dihydroxyphenyl) heptadecanoic acid **(84)**, 16-(3',5'-dihydroxyphenyl)hexadecane **(85)**,

19-(3',5'-dihydroxyphenyl)nonadecanoic acid **(86)**, 21-(3',5'-dihydroxyphenyl) henicosanoic acid **(87)**.

Figure 2.3 – *Target compounds*



A range of potential synthetic routes to the alkylresorcinols were to be investigated to see which was most suitable. The methods include Suzuki coupling procedures[4] and Wittig type chemistry.[5] The syntheses of the carboxylic acid analogues were attempted by two routes involving either a Grignard reaction[6] or Wittig chemistry.[5] The suitability and success of these routes are discussed later on in this chapter.

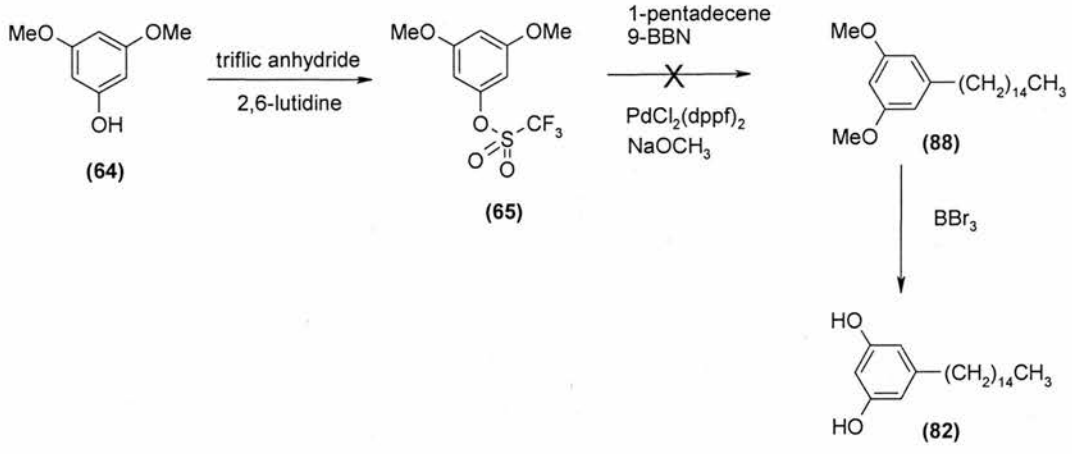
2.2 SYNTHESIS OF ALKYL CHAIN ANALOGUES

As discussed in the **Introduction**, several methods have previously been employed for the synthesis of alkylresorcinols with varying lengths of alkyl chains.

2.2.1 Attempted synthetic route to 15-(3',5'-dihydroxyphenyl)pentadecane

Various alkylresorcinols have been successfully prepared with high yields by Fürstner and Seidel[7] using a palladium-catalysed, base-induced cross coupling of 9-alkyl-9-BBN with aryl triflates. In this project the synthesis of the 15-carbon alkyl chain analogue was attempted by this known route, following the pathway shown in ***Scheme 2.1***, using 3,5-dimethoxyphenol as the starting material **(64)**.

Scheme 2.1 - *Proposed synthetic route for 15-(3',5'-dihyroxyphenyl)pentadecane via a Suzuki coupling*



The first step involved the activation of the phenol **(64)** by generating a good leaving group in preparation for the Suzuki coupling to take place.[7] This was carried out by using triflic anhydride and 2,6-lutidine in dichloromethane. The reaction was stirred for 1 hour at

room temperature and then left in the refrigerator for 66 hours. The product **(65)**, a brown oil, was obtained in 91% yield. The ^{13}C NMR spectrum yielded a quartet at 118.6 ppm attributed to the -$\underline{C}F_3$ group. Since only signals corresponding to the expected product were present in both the ^{1}H NMR and the ^{13}C NMR spectra, it was not necessary to purify the product any further by column chromatography.

The key step was the base-induced cross-coupling of an alkyl-9-BBN with the aryl triflate **(65)** using $PdCl_2$(dppf) as the catalyst.[7,8,9] The alkyl-9-BBN moiety was prepared by stirring a solution of 1-pentadecene and 9-BBN in THF at room temperature for two hours. The starting material, 3,5-dimethoxyphenol trifluoromethane sulfonate, and $PdCl_2$ were added and the reaction was then heated under reflux for 1 hour. The ^{1}H NMR spectrum of the reaction mixture indicated that there was a possibility that some desired product **(88)** had formed. This crude product was then purified by column chromatography isolating three compounds. However, the analysis of the ^{1}H NMR spectra of these three isolated compounds showed that the least polar was a mixture of the alkene (signal at 5.35 ppm) and the 9-BBN (several signals in the aliphatic region between 1 ppm and 2.2 ppm), suggesting that the reduction of the alkene to generate the alkyl-9-BBN reagent had not gone to completion. The ^{1}H NMR spectrum of the second least polar compound revealed a singlet at 3.79 ppm corresponding to the –OC$\underline{H}_3$ groups of the aryl moiety and a triplet peak at 7.1 ppm. The ^{1}H NMR spectrum of the most polar compound revealed a peak at 9.52 ppm corresponding to an aldehyde, most probably the aldehyde derivative of the pentadecane, i.e. 1-pentadecanal. The ^{13}C NMR spectrum confirmed this, as a signal at 201.6 ppm was observed for the carbonyl group. This therefore confirmed that the desired reaction had not taken place and the ^{1}H NMR spectrum of the reaction

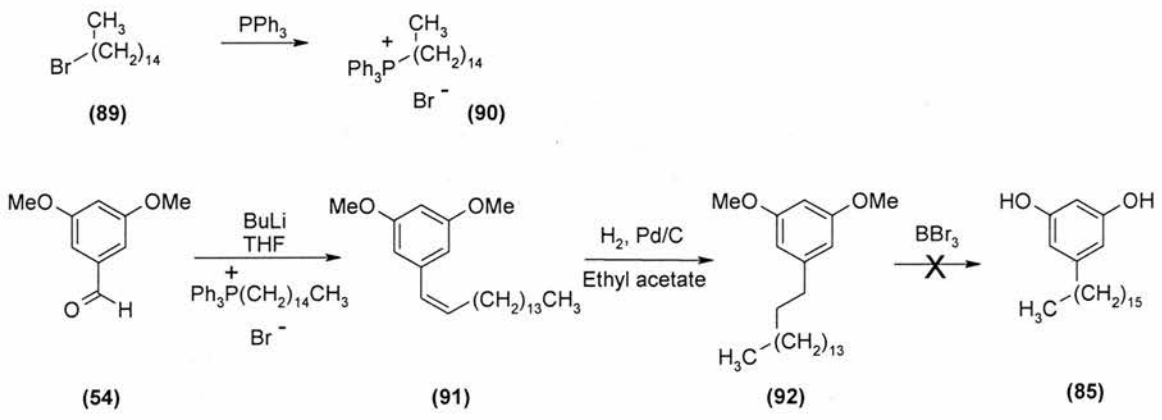
mixture was actually just a mixture of starting materials and small amounts of impurities. This was most likely due to the alkyl-9-BBN precursor not being successfully synthesised.

In our hands, the overall proposed synthetic route for the 15-carbon alkyl chain analogue via the Suzuki coupling method[7] was unsuccessful. However, due to the potential difficulties in removing the 9-BBN moiety we therefore chose to investigate an alternative route to synthesise another alkyl chain analogue, i.e. a synthetic route via the Wittig reaction.[5] As 1-bromopentadecane is commercially available it was decided to use this for the provision of the alkyl chain. The use of suitable starting materials such as 3,5-dimethoxybenzaldehyde, and 1-bromopentadecane would result in a 16-carbon chain being formed. Our synthesis of the 16-carbon alkyl chain **(85)** analogue followed the pathway shown in ***Scheme 2.2*** **(Section 2.2.2)**, using 3,5-dimethoxybenzaldehyde **(54)** as the starting material.

2.2.2 Attempted synthetic route to 16-(3',5'-dihydroxyphenyl)hexadecane

Wittig-type chemistry is a well known route for the formation of carbon-carbon double bonds. The synthesis utilizes this reaction to attach the alkyl chain onto the aromatic head group by forming a carbon-carbon double bond which can then be reduced by catalytic hydrogenation to give the saturated alkyl chain.

Scheme 2.2 *- Proposed synthetic route for 16-(3',5'-dihydroxyphenyl)hexadecane via the Wittig reaction*

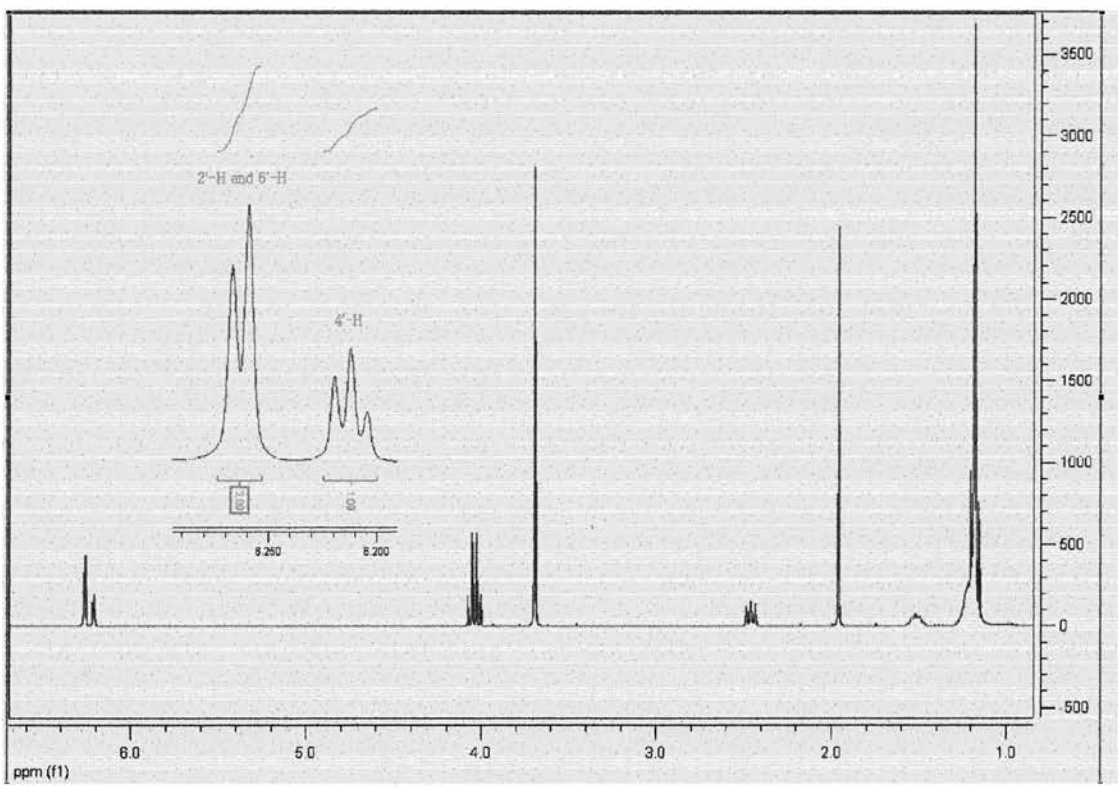


The first step involved the preparation of the phosphonium salt **(90)** from 1-bromopentadecane **(89)**. This was carried out by heating a mixture of the bromoalkane and triphenylphosphine under reflux for 23 hours.[6,8,10] This method is unusual due to solvent not being used, however by allowing the reactants to be more concentrated, it was thought that a higher yield would be obtained than if solvent was indeed present. After washing with diethyl ether, the product, an off-white solid **(90)** was obtained in 52% yield and was then used in the next step without any further purification.

The key step was the Wittig reaction[5] between the ylide formed from the newly prepared phosphonium salt **(90)** and the commercially available 3,5-dimethoxybenzaldehyde **(54)**. The phosphonium salt was deprotonated with *n*-butyllithium and was then reacted with 3,5-dimethoxybenzaldehyde **(54)** to give the desired product **(91)** in 48% yield. The ^{1}H NMR spectrum revealed a multiplet signal corresponding to the carbon-carbon double bond at 5.60-5.70 ppm, with an approximate coupling constant of 12 Hz, thus suggesting a *Z*-double bond. This was to be expected since the ylide used was non-stabilised, and the use of non-stabilised ylides produces *Z*-alkenes whereas the use of stabilised ylides produces *E*-alkenes.[11] Two signals were present in the ^{13}C NMR spectrum at 128.4 and 128.6 ppm confirming the presence of the double bond. Mass spectrometry produced a molecular ion peak at 457 mass units, equivalent to $[M+K]^{+}$. However the ^{1}H NMR spectrum showed that the reaction did not go to completion, as there was a peak at 9.84 ppm corresponding to the unreacted aldehyde. There were also four signals in the aromatic region at 7.39-7.57 ppm 7.60-7.73 ppm and 7.75-7.91 ppm. These corresponded to the aromatic protons of the starting material 3,5-dimethoxybenzaldehyde, triphenylphosphine oxide and some unreacted triphenylphosphine. The ^{13}C NMR spectrum of the crude product also showed a number of aromatic carbons, however the quarternary carbons 1'-C, 3'-C and 5'-C could not be seen in the ^{13}C NMR spectrum. It is possible that these quarternary signals were masked by signals corresponding to impurities. The presence of the many aromatic signals confirmed that more than one compound was present, therefore some of these signals will have probably been due to unreacted aldehyde **(54)** and triphenylphosphine. It was decided to take this material through to the next step without further purification rather than risk losing some of the desired product.

The next step involved the hydrogenation of the double bond.[12] This was carried out using a 10% weight palladium on a carbon catalyst in ethyl acetate under an atmosphere of hydrogen. The crude product was subjected to column chromatography to give the desired product **(92)** in 53% yield. Analysis of the product **(92)** by ^{1}H NMR spectroscopy revealed that the double bond was indeed no longer present as a multiplet signal corresponding to alkene protons could not be seen in the spectrum. In the literature,[13] the 4'-H in the ^{1}H NMR of a similar compound is assigned at a higher chemical shift value than the 2'-H and the 6'-H, however the integrals and the splitting of the signals of the product obtained was in fact the opposite to this; i.e. the signal corresponding to the 2'-H and 6'-H protons were found to be at a higher chemical shift value than the signal corresponding to the 4'-H proton ***(Figure 2.4)***.

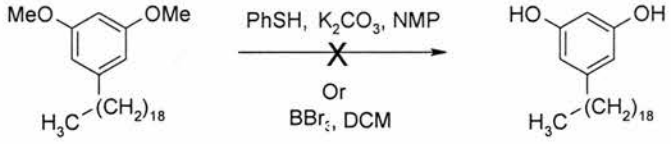
Figure 2.4 *– ^{1}HNMR spectrum of 1-(3',5'-dimethoxyphenyl)hexadecane* **(92)** *in $CDCl_3$ at 300 MHz*



The ^{13}C NMR spectrum confirmed that the double bond had been reduced as signals corresponding to a double bond were no longer present.

In order not to waste vital product from the previous step, 19-(3',5'-dimethoxyphenyl)nonadecane **(93)** was used to test the demethylation step. The demethylation was attempted by two different methods ***(Scheme 2.3)***. The first method employed thiophenol and potassium carbonate in the presence of dry NMP and heating the reaction mixture to 190 °C for 1.5 hours.[14] The second method employed boron tribromide in the presence of dichloromethane and stirring the reaction mixture under nitrogen for 24 hours.[15] Both methods were unsuccessful in demethylating the compound, as the ^{1}H NMR and ^{13}C NMR spectra of the products showed that the $-OCH_3$ groups were still present.

Scheme 2.3 - *Test routes for the demethylation of a methylated alkylresorcinol*



(93) **(94)**

As in our hands the two methods of demethylation[14,15] proved to be unsuccessful, an alternative method was sought. The alternative approach involved the protection of the hydroxyl groups with benzyl groups. Unfortunately this approach was only employed after the synthesis of two of the carboxylic acid analogues (16-(3',5'-dihydroxyphenyl)hexadecanoic acid **(83)**, discussed in **Section 2.3.1**, and 17-(3',5'-dihydroxyphenyl)heptadecanoic acid **(84)**, discussed in **Section 2.3.2**) by the

methyl ether route had already been attempted. The alternative approach of protecting hydroxyl groups with benzyl groups is discussed later in this chapter.

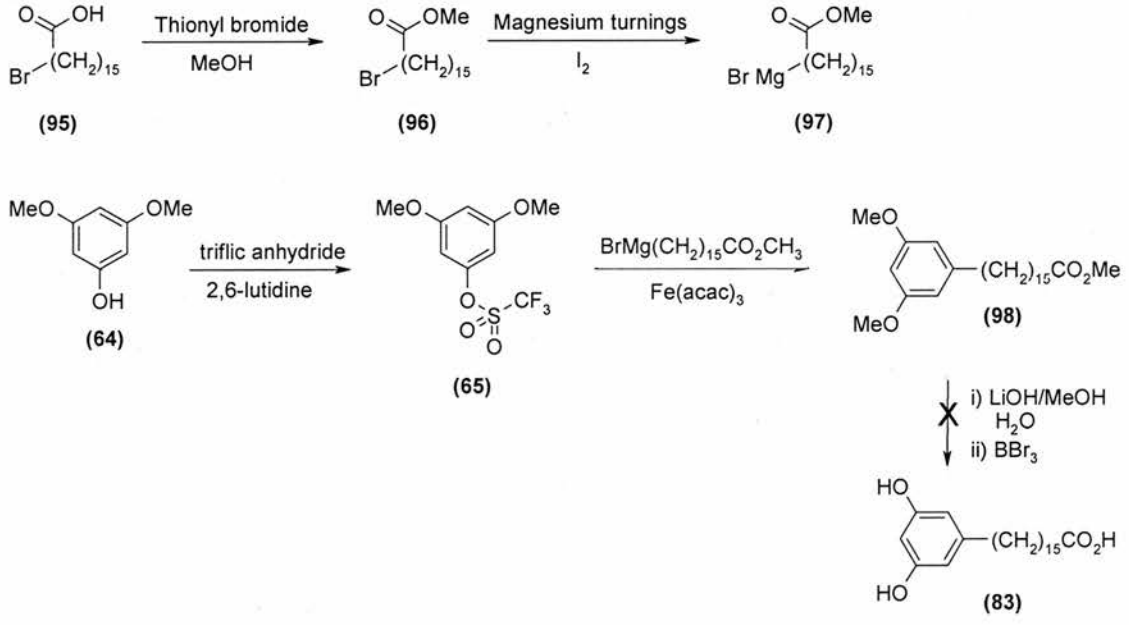
2.3 SYNTHESIS OF CARBOXYLIC ACID ANALOGUES

As discussed in **Section 2.1**, a variety of carboxylic acid analogues that had an alkyl chain between 16 and 21 carbons in length were required for the development of antibodies to set up new immunoassays.

2.3.1 Attempted synthetic route to 16-(3',5'-dihydroxyphenyl)hexadecanoic acid

Various alkylresorcinols have been successfully prepared with good yields by Wenkert *et al.*[16] who utilized Grignard chemistry. In this project the synthesis of the carboxylic acid analogue 16-(3',5'-dihydroxyphenyl)hexadecanoic acid **(83)** was attempted by this known route, following the pathway shown in ***Scheme 2.4***, using 3,5-dimethoxyphenol **(64)** as the starting material.

Scheme 2.4 - *Proposed synthetic route for 16-(3',5'-dihydroxyphenyl)hexadecanoic acid using a Grignard reagent*



The activated phenol **(65)** was successfully synthesised as previously discussed in **Section 2.2.1**. This synthetic route was attempted in parallel with the Wittig type chemistry routes.

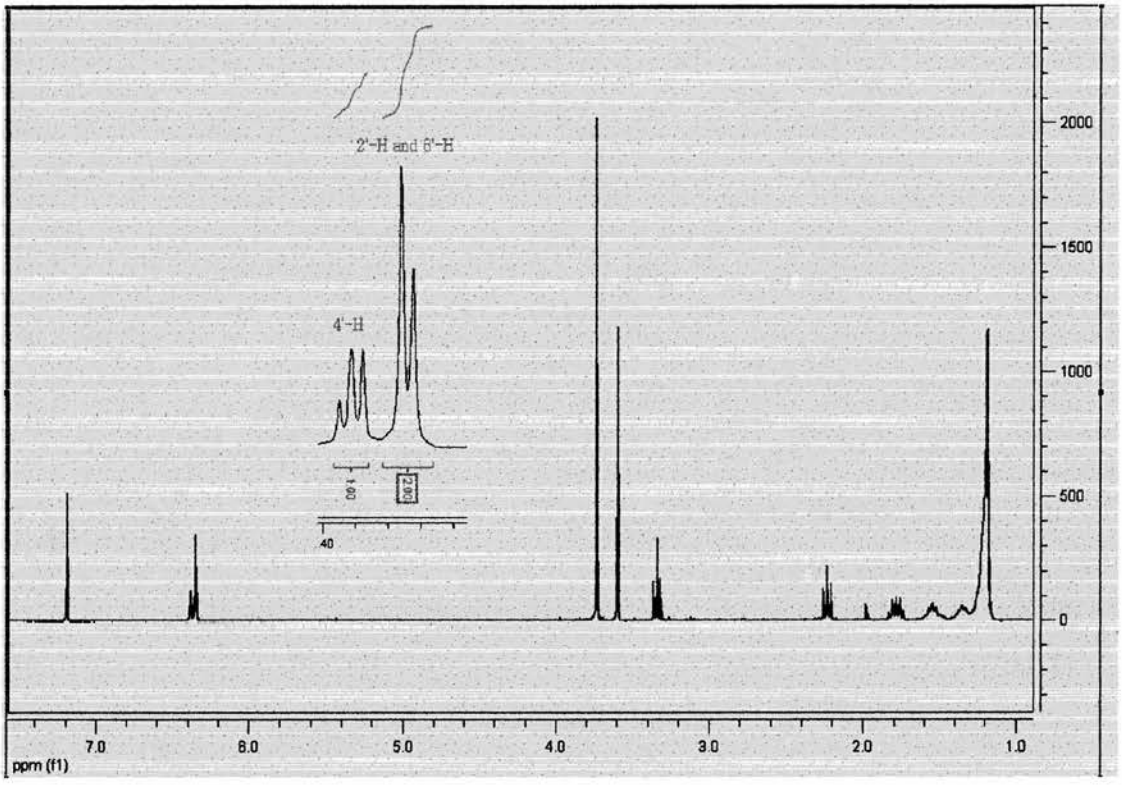
To carry out the key step[6], the iron-catalysed cross-coupling of a Grignard reagent **(97)** with the aryl triflate **(65)**, a bromo ester **(96)** had to be synthesised as a precursor for the Grignard reagent. The ester derivative was required as the acid would destroy the organometallic reagent. We used a bromo carboxylic acid as it contained an alkyl bromide which is required for the formation of a Grignard reagent. The normal way to convert a carboxylic acid to its ester derivative is via the acyl chloride, however, in the presence of thionyl chloride it has been found in similar systems that a mixture of the brominated and chlorinated products is obtained due to reaction of the alkyl bromide. This lowers the yield and the two products are difficult to separate. The use of thionyl bromide prevents the formation of mixtures and gives only the desired product.[17]

The ester was synthesised by dissolving 16-bromohexadecanoic acid **(95)** in methanol and then adding thionyl bromide. The reaction was then heated under reflux with stirring for 3 hours at 110 °C.[17] The product **(96)** was obtained in 99% yield. Analysis by ^{1}H NMR spectroscopy gave a singlet peak at 3.64 ppm attributable to the methyl ester group. The ^{13}C NMR spectrum showed resonances at 51.4 ppm and 174.3 ppm attributable to the $-O\underline{C}H_3$ carbon and the ester carbonyl group respectively. Only peaks corresponding to the bromoester were present in both the NMR spectra, so it was not necessary to purify the product any further. The mass spectrometry data showed that there were two distinctive peaks as expected, due to the two isotopes of bromine, at 373 and 371 mass units corresponding to $[M+Na]^+$ with intensity of 99% and 100% respectively.

The bromoester **(96)** was then treated with magnesium to give the Grignard reagent **(97)**, which was then used *in situ*. The Grignard reagent in diethyl ether was added to a solution of the previously prepared aryl triflate **(65)**, *N*-methylpyrrolidinone, tetrahydrofuran and $Fe(acac)_3$. The resulting mixture was then stirred for 10 minutes. The reaction was quenched by the addition of HCl followed by standard extractive work-up.[6] Analysis of the crude product **(98)** by ^1H NMR spectroscopy gave a triplet peak at 3.41 ppm corresponding to -C$\underline{H}_2$-Ar and a signal at 3.39 ppm attributable to some unreacted bromoester. From integral measurements it was deduced that the product to starting material ratio was approximately 3:2. Interestingly, unlike the spectrum obtained of 1-(3',5'-dimethoxyphenyl)hexadecane **(92)** in which the order of the aromatic protons (4'-H; 2'-H and 6'-H) appeared in the spectrum (the signal corresponding to the 4'-$\underline{H}$ proton was found to be at a lower chemical shift value than the 2'-$\underline{H}$ and the 6'-$\underline{H}$) disagreed with the literature[18] (see ***Figure 2.4*** and **Section 2.2.2**), the signal corresponding to the 4'-$\underline{H}$ proton in the ^1H NMR spectrum of this compound **(98)** was found to be at a higher chemical shift value than the 2'-$\underline{H}$ and the 6'-$\underline{H}$, therefore agreeing with the literature.[18] This was confirmed by the integration and signal splitting ***(Figure 2.5)***. A quartet signal in the region of 118 ppm in the ^{13}C NMR spectrum attributable to the –$\underline{C}F_3$ group could not be seen indicating that the triflate group had been successfully cleaved.

This synthetic route was attempted in parallel to the other routes that required demethylation. The demethylation step was not attempted for this synthetic target as previous approaches[14,15] had been unsuccessful.

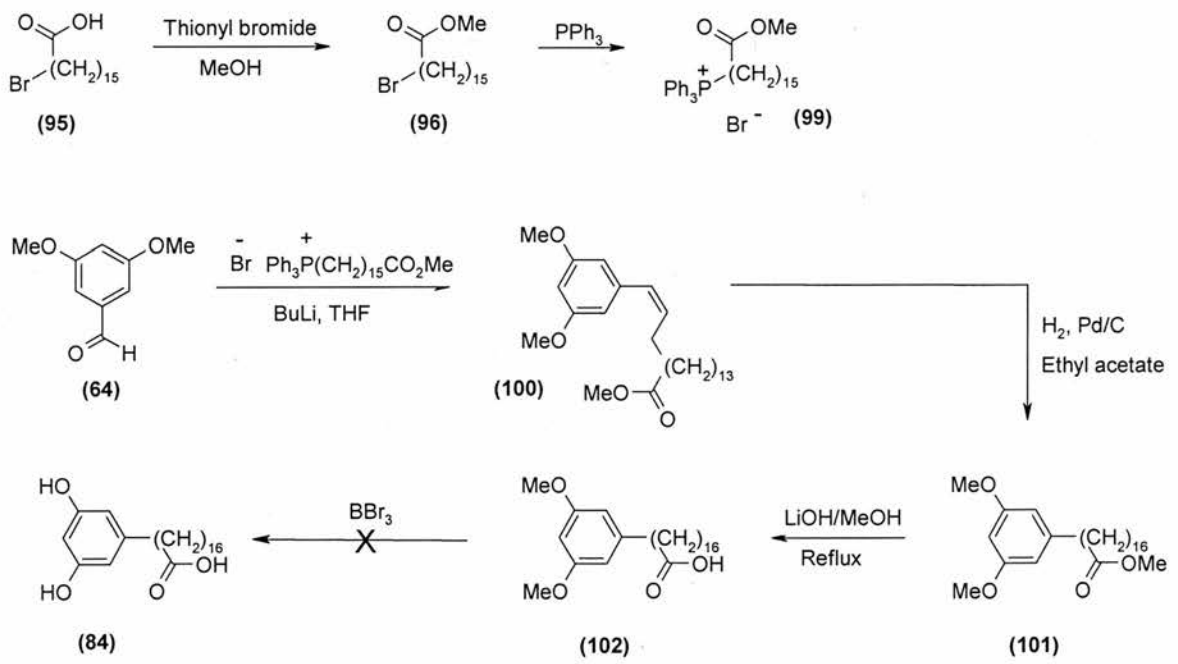
Figure 2.5 *– ^{1}HNMR spectrum of methyl 16-(3',5'-dimethoxyphenyl)hexadecanoate* **(98)** *in $CDCl_3$ at 300 MHz*



2.3.2 Attempted synthetic route to 17-(3',5'-dihydroxyphenyl)heptadecanoic acid

The synthesis of this carboxylic analogue followed the pathway shown in ***Scheme 2.5***, using 3,5-dimethoxybenzaldehyde **(64)** as the starting material. Due to the success of the synthesis of 16-(3',5'-dimethoxyphenyl)hexadecane **(85)**, discussed in **Section 2.2.2**, via Wittig type chemistry[5], it was decided to use a similar route for the attempted synthesis of 17-(3',5'-dihydroxyphenyl)heptadecanoic acid **(84)**.

Scheme 2.5 - *Proposed synthetic route for 17-(3',5'-dihydroxyphenyl)heptadecanoic acid via the Wittig reaction*



The first two steps involved the preparation of the bromoester **(96)** as previously discussed in **Section 2.3.1**, followed by the preparation of the corresponding phosphonium salt **(99)**. The phosphonium salt was synthesised by heating a solution of the bromoester and triphenylphosphine under reflux for four hours,[6,8,9] after which the product was washed

with diethyl ether to remove any unreacted triphenylphosphine. The phosphonium salt was used in the next step without further purification to avoid the loss of valuable salt.

The key step was the Wittig reaction between the ylide formed from the freshly prepared phosphonium salt **(99)** and the commercially available 3,5-dimethoxybenzaldehyde **(64)**.[6,8,9] The phosphonium salt was dissolved in THF, followed by the addition of *n*-butyllithium to deprotonate the salt. This reaction mixture was stirred for 30 minutes at –78 °C, after which, the starting material, 3,5-dimethoxybenzaldehyde was added. The resulting mixture was stirred at –78 °C for one hour and then for a further two hours whilst warming to room temperature. The reaction was quenched by the removal of solvent followed by standard extractive work-up. Analysis of the product **(100)** by ^{1}H NMR spectroscopy gave a multiplet signal at 5.60-5.70 ppm with an approximate coupling constant of 11 Hz attributable to the alkene protons. As expected, due to the use of a non-stabilised ylide, the coupling constant indicates that a *Z*-double bond was obtained. The ^{13}C NMR spectrum confirmed that the carbon-carbon double bond was indeed present by the presence of two signals at 128.6 and 133.8 ppm attributed to the carbons in the double bond environment. The ^{13}C NMR spectrum did not show a signal for a carbonyl carbon. This could be due simply to the weakness of the sample. A signal corresponding to a carbonyl carbon would have confirmed either way whether or not the product had been obtained due to the difference in chemical shifts of a carbonyl ester carbon and a carbonyl aldehyde carbon. However, it was decided to continue with the synthetic sequence. Analysis by infrared proved to be futile due to the sample being too weak and no more sample being available.

The next step involved the reduction of the newly formed carbon-carbon double bond.[12] This was performed using standard hydrogenation conditions (10% weight palladium on a carbon catalyst in ethyl acetate under an atmosphere of hydrogen). The ^{1}H NMR spectrum did show that there was still some unsaturated product still present however the desired product **(101)** was observed by mass spectrometry which gave the expected mass at 443 mass units $[M+Na]^{+}$. The presence of the starting material was confirmed in the mass spectrum at 441 mass units, two units less than the desired product. To avoid the loss of vital desired product, the mixture of desired saturated product and the alkene moiety was used without further purification in the next step. In hindsight the product of this step should have been re-hydrogenated as in the long run it would have been very difficult to separate an alkane from an alkene. Unfortunately the infrared and ^{13}C NMR spectra proved to be too weak to analyse despite using all of the available compound.

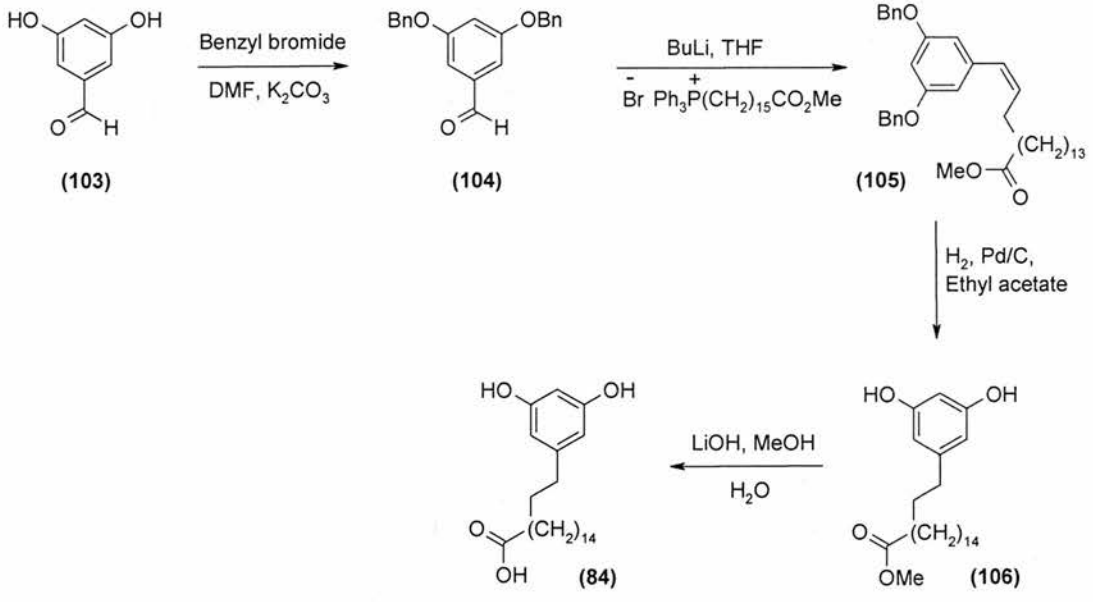
The penultimate step in the synthetic sequence involved the hydrolysis of the ester **(101)** to the carboxylic acid **(102)**.[19] This was carried out by refluxing the ester in methanol, water and lithium hydroxide at 80 °C for 3 hours. The ^{1}H NMR and the ^{13}C NMR spectra showed that the ester group was no longer present. The infrared spectrum confirmed this with signals at 2917cm^{-1} and 1701 cm^{-1} attributable the O-H stretch and the presence of an acid carbonyl group respectively. Mass spectrometry gave a signal at 403 mass units, corresponding to two mass units less than the desired product. This suggests the ester hydrolysis was successful but the alkyl chain was unsaturated. This is consistent with the mass spectrometry data of the starting material, which indicated the presence of a double bond which was confirmed in the ^{13}C NMR spectrum by signals at 127.6 and 128.6 ppm. The desired product could perhaps be obtained by hydrogenation of the unsaturated acid obtained in this reaction.

Again, this synthetic route was attempted in parallel to the other routes that required demethylation. The demethylation step was not attempted for this synthetic target as previous approaches[14,15] had been unsuccessful.

2.3.3 Synthetic route to 17-(3',5'-dihydroxyphenyl)heptadecanoic acid

Due to the demethylation step being unsuccessful using boron tribromide[15] and also using thiophenol,[14] another approach had to be employed and an obvious choice was to protect the hydroxyl groups with benzyl groups which could be removed easily by hydrogenation to regenerate the free hydroxyl groups in the same step that the double bond generated by the Wittig reaction was reduced. The synthetic route for the carboxylic analogue 17-(3',5'-dihydroxyphenyl)heptadecanoic acid **(84)** is outlined below ***(Scheme 2.6)***.

Scheme 2.6 - *Proposed synthetic route for 17-(3',5'-dihydroxyphenyl)heptadecanoic acid via the Wittig reaction*



Prior to the Wittig reaction, 3,5-dihydroxybenzaldehyde **(103)** was protected as the benzyl derivative **(104)**. This was carried out by heating a mixture of 3,5-dihydroxybenzaldehyde, benzyl bromide, DMF and potassium carbonate overnight at 60 °C.[14] After filtration, the solvent was removed under reduced pressure and the product was washed with hydrochloric acid and brine to afford the product **(104)**. The ^{1}H NMR

spectrum showed a resonance at 5.10 ppm attributable to the benzylic methylene group and a multiplet peak between 7.30-7.46 ppm corresponding to the aromatic protons. The ^{13}C NMR spectrum confirmed the presence of the benzylic methylene group with a signal at 70.3 ppm.

The key Wittig reaction was carried out with 3,5-dibenzyloxybenzaldehyde as outlined in **Section 2.3.2**.[6,8,9] This particular reaction produced the product **(105)** in 21% yield. The infrared spectrum revealed peaks corresponding to a carbonyl group, a carbon-carbon double bond and an ester (C-O) group at 1738, 1585, 1151 cm^{-1} respectively. The ^{1}H NMR spectrum gave a multiplet signal at 5.60-5.70 ppm with an approximate coupling constant of 12 Hz attributable to the alkene protons. As expected, due to the use of a non-stabilised ylide, the coupling constants indicate that a *Z*-double bond was obtained. The ^{13}C NMR spectrum confirmed that the carbon-carbon double bond was indeed present by the presence of two signals at 127.4 and 127.9 ppm attributable to the carbons in the double bond environment. Unfortunately a distinct signal could not be seen in the ^{13}C NMR corresponding to 4''-C. The molecular formula of the compound was confirmed by accurate mass measurement.

The penultimate step in the synthetic sequence involved the reduction of the newly formed carbon-carbon double bond and the removal of the two benzyl protecting groups. This was performed using standard hydrogenation conditions (10% weight palladium on a carbon catalyst in ethyl acetate under an atmosphere of hydrogen).[12] The ^{1}H NMR and the ^{13}C NMR spectra showed that the benzyl groups and the double bond had been removed. Unfortunately the carbonyl group, the ester carbon and the quarternary carbons could not

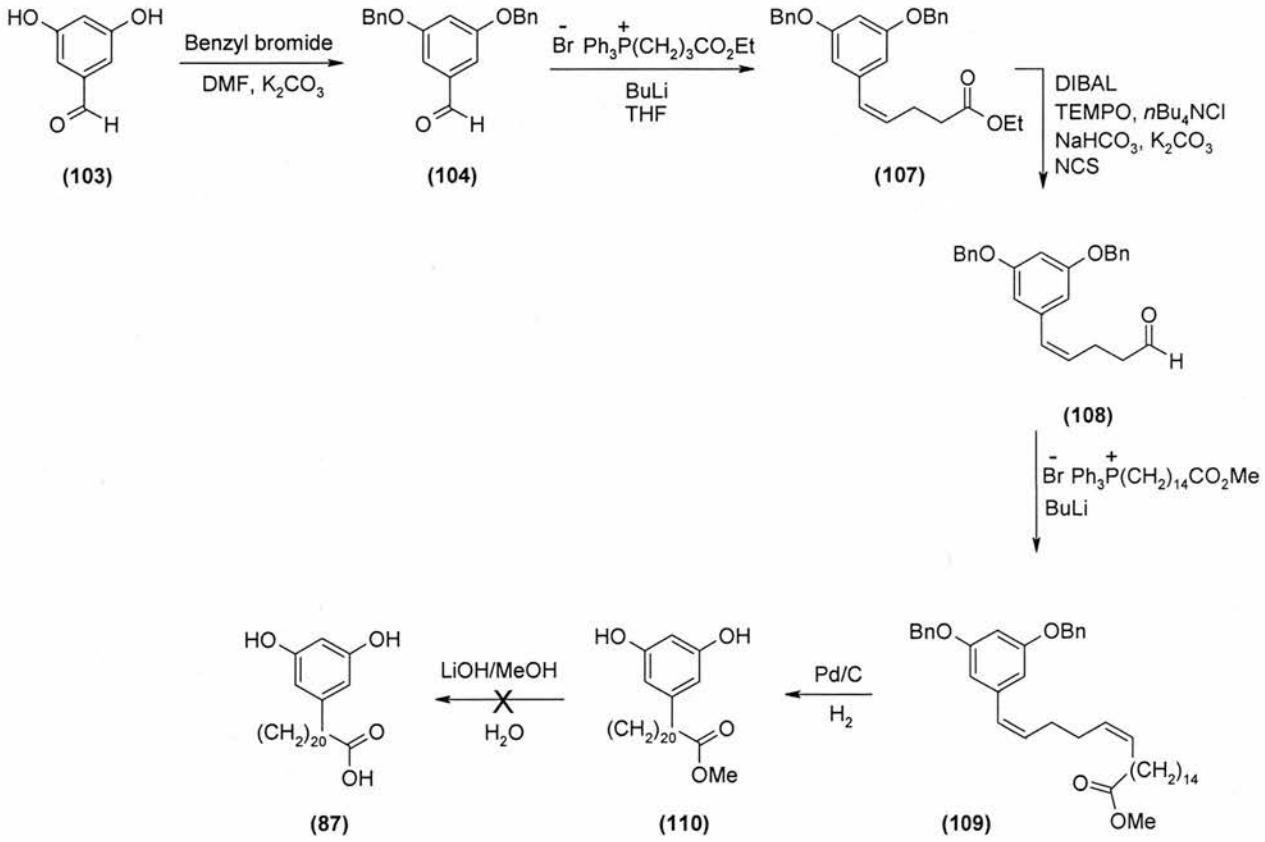
be seen in the ^{13}C NMR spectrum as the sample was too weak. However, the molecular formula of the compound **(106)** was confirmed by accurate mass measurement.

Finally, hydrolysis[19] of the methyl ester yielded the desired alkylresorcinol **(84)**. The product was not purified as it was thought that it would break down on a column rendering it unsuitable for biological testing in Finland. The NMR data were therefore complex but the molecular formula of the compound **(84)** was confirmed by accurate mass measurement.

2.3.4 Attempted synthetic route to 21-(3',5'-dihydroxyphenyl)henicosanoic acid

The synthetic route used to obtain 17-(3',5'-dihydroxyphenyl)heptadecanoic acid **(84)** as discussed in **Section 2.3.3** was also used as a basis to synthesise 21-(3',5'-dihydroxyphenyl)henicosanoic acid **(87)** as outlined below ***(Scheme 2.7)***.

Scheme 2.7 *- Proposed synthetic route for 21-(3',5'-dihydroxyphenyl)henicosanoic acid via the Wittig reaction*



A bromoacid of 21 carbons in length is not commercially available. Therefore as in previous syntheses, the side chain could not be merely attached by a single Wittig reaction. However, because the 16-carbon bromoacid and the 4-carbon moiety ethyl 4-(triphenyl-λ^5-phosphanyl)butanoate were commercially available and also because

in our hands the Wittig reactions had proved to be successful, it was decided that the synthesis of this 21-carbon analogue could be attempted by initially increasing the number of carbons between the aromatic ring and the aldehyde by four carbons using a Wittig reaction with ethyl 4-bromobutanoate. The end of the chain, the ethyl ester, could then be reduced to the aldehyde to allow a second Wittig reaction using the phosphonium ylide from methyl 16-bromohexadecanoate to take place, thus generating a chain of 21 carbons in length.

The previously synthesised 3,5-dibenzyloxybenzaldehyde **(104)** was used in this synthetic route. The synthesis of 3,5-dibenzyloxybenzaldehyde was discussed in **Section 2.3.3.**

The first Wittig reaction was carried out with 3,5-dibenzyloxybenzaldehyde **(104)** and the commercially available ethyl 4-(triphenyl-λ^5-phosphanyl)butanoate using the procedure as outlined in **Section 2.3.2** giving the product **(107)** in a modest yield of 27%.[6,8,9] Analysis of the ^{1}H NMR spectrum revealed a multiplet signal at 5.55-5.70 ppm with an approximate coupling constant of 12 Hz attributable to the alkene protons. As expected, due to the use of a non-stabilised ylide,[11] the coupling constant indicates that a *Z*-double bond was obtained. The ^{13}C NMR spectrum confirmed that the carbon-carbon double bond was indeed present by the presence of two signals at 127.5 and 127.9 ppm attributable to the carbons in the double bond environment. The double bond could also be seen in the infrared spectrum at 1585 cm^{-1}. The ^{13}C NMR spectrum failed to show the carbonyl group signal, however a signal in the infrared spectrum attributable to the carbonyl group could be seen at 1720 cm^{-1}.

The next step involved the reduction of the ethyl ester **(107)** to the corresponding alcohol using DIBAL followed by the oxidation of the alcohol to the aldehyde **(108)** using a catalytic amount of TEMPO.[20] The product was obtained in 60% yield. The ^{1}H NMR spectrum revealed two signals at 9.83 and 9.91 ppm corresponding to aldehydes. Two signals can be attributed to the two isomers, *E* and *Z*. The molecular formula of the compound was confirmed by accurate mass measurement.

The following step in the synthetic sequence was the second Wittig reaction. This was carried out by once again using *n*-butyllithium to deprotonate the previously synthesised phosphonium salt (see **Section 2.3.2**).[6,8,9] The ^{1}H NMR spectrum of the product **(109)** gave two multiplet signals between 5.31-5.43 and 5.60-5.70 ppm attributable to the four alkene protons. The ^{13}C NMR spectrum confirmed that carbon-carbon double bonds were indeed present however the double bond signals had similar chemical shift values to the tertiary aromatic carbon signals; hence it was difficult to identify them individually. The ^{13}C NMR spectrum did not show a carbonyl group, nor the ester carbon. This was most likely due to the sample being too weak. However, the infrared spectrum confirmed that there was a carbonyl group (C=O), and also a carbon-carbon double bond (C=C) and an ester (C-O) present due to peaks at 1727, 1574 and 1275 cm^{-1} respectively.

The penultimate step in the synthesis of 21-(3',5'-dihydroxyphenyl)henicosanoic acid involved the hydrogenation of the two double bonds in the aliphatic chain which were formed as a result of the two Wittig reactions, and the removal of the two benzyl protecting groups.[12] This was carried out using a 10% weight palladium on a carbon catalyst in ethyl acetate under an atmosphere of hydrogen. The ^{1}H NMR spectrum of the product **(110)** showed two multiplet signals between 7.28-7.55 and 7.60-7.93 ppm corresponding to

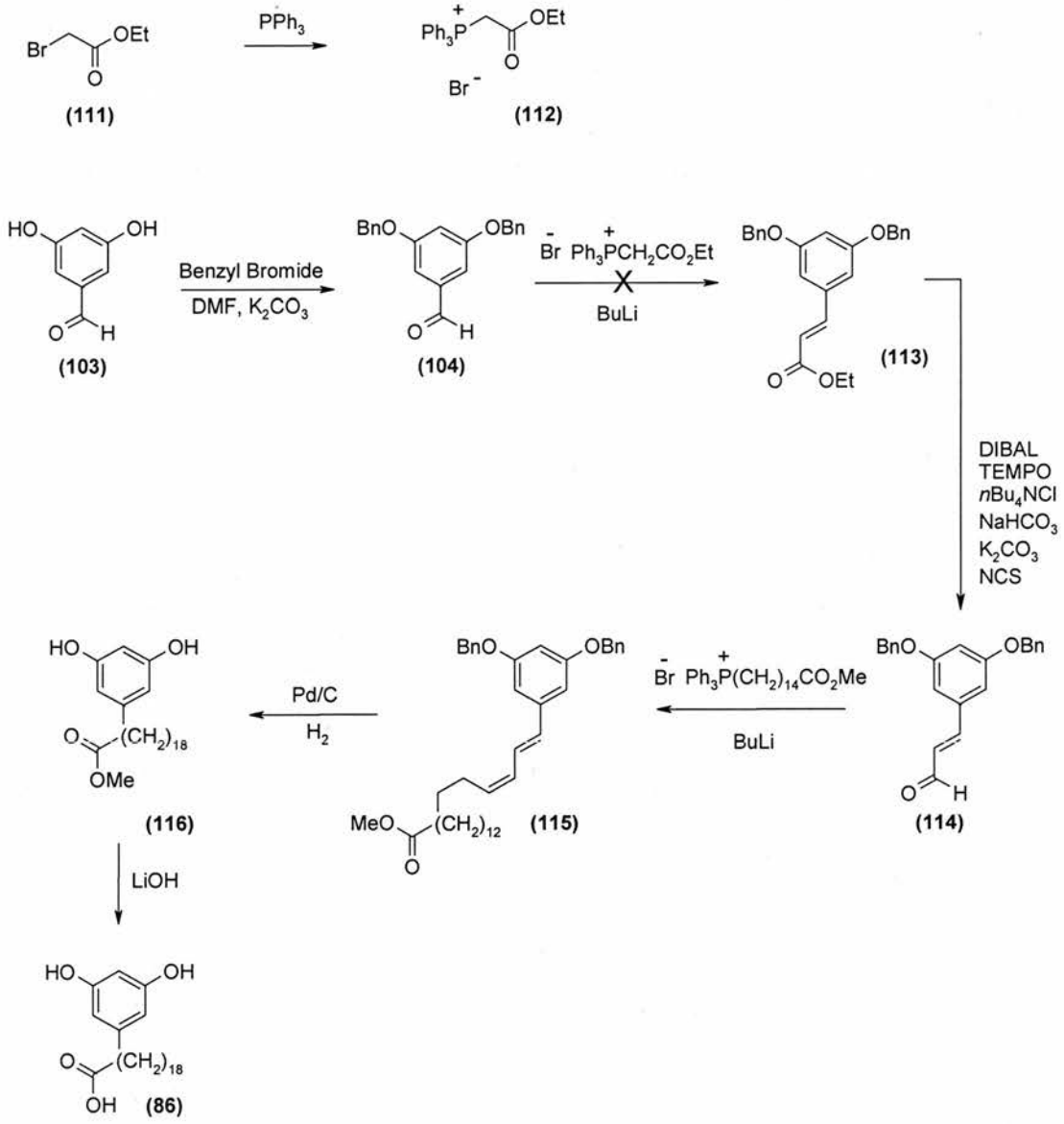
aromatic protons. This therefore suggests that the hydrogenation had not gone to completion. There was also a signal at 70.0 ppm in the ^{13}C NMR spectrum which was probably attributable to the carbons in the benzylic methylene group. Once again, the ^{13}C NMR spectrum was too weak to see the quarternary aromatic carbons and the carbonyl group. The infrared spectrum, did however confirm that there was a carbonyl group (C=O) and an ester (C-O) present due to peaks at 1727 and 1282 cm^{-1} respectively. The mass spectrometry data however did give a signal corresponding to the desired molecular ion at 471 mass units ($[M+Na]^+$) indicating that some of the desired compound had been obtained. To avoid the loss of any valuable desired compound it was decided to proceed to the final step without further purification.

The final step was the hydrolysis[19] of the ester **(110)** to the corresponding carboxylic acid **(87)**. This was carried out by refluxing the product of the previous step **(110)**, methanol, water and lithium hydroxide at 80 °C for 48 hours. While the infrared spectrum confirmed that there was a carboxylic acid functional group present due to an O-H stretch at 3737 cm^{-1} and a hydroxy group present due to an O-H stretch at 2835 cm^{-1}, analysis by NMR spectroscopy proved to be inconclusive as while a signal attributable to the ester protons, as expected, could not be seen in the ^1H NMR spectrum, there was a signal present in the ^{13}C NMR spectrum attributable to an ester carbonyl carbon at 171.2 ppm. The molecular ion of the desired compound could not be seen in the spectrum but a mass at 471 mass units could be seen indicating that the hydrolysis had been unsuccessful.

2.3.5 Attempted synthetic route to 19-(3',5'-dihydroxyphenyl)nonadecanoic acid

The synthetic route that was used to attempt to obtain 21-(3',5'-dihydroxyphenyl)henicosanoic acid **(87)** (see **Section 2.3.4**) was also used as a basis to attempt to synthesise 19-(3',5'-dihydroxyphenyl)nonadecanoic acid **(86)** as outlined below ***(Scheme 2.8)***.

Scheme 2.8 *- Proposed synthetic route for 19-(3',5'-dihydroxyphenyl)nonadecanoic acid via the Wittig reaction*



Like the bromoacid of 21 carbons in length, the bromoacid of 19 carbons in length is also not commercially available. Therefore as in previous syntheses, the side chain could not be merely attached by a single Wittig reaction. Again, because the 16-carbon bromoacid **(95)** and also the 2-carbon bromoester **(111)** were commercially available and as in our hands the Wittig reactions had proved to be successful, it was decided that the synthesis of this 19-carbon analogue could be attempted by initially increasing the number of carbons between the aromatic and the aldehyde by two carbons using the corresponding phosphonium ylide from ethyl 2-bromoethanoate. Again, the end of the chain, the ethyl ester, could then be reduced to the aldehyde to allow a second Wittig reaction using the phosphonium ylide from methyl 16-bromohexadecanoate to take place, thus generating a chain of 19 carbons in length.

The previously synthesised 3,5-dibenzyloxybenzaldehyde **(104)** was used in this synthetic route. The synthesis of 3,5-dibenzyloxybenzaldehyde was discussed in **Section 2.3.3.**

The phosphonium salt **(112)** was prepared from the commercially available ethyl 2-bromoethanoate by treating it with triphenylphosphine as outlined in **Section 2.3.2**, achieving a yield of 91%. The first Wittig reaction was carried out with 3,5-dibenzyloxybenzaldehyde **(104)** and the freshly prepared phosphonium salt **(112)** using the procedure as outlined in **Section 2.3.2**, giving the product **(113)** with a modest yield of 31%.[6,8,9] Since the ylide is stabilised it was expected to form an *E*-alkene. The ^{1}H NMR spectrum failed to show signals with the same coupling constants (between 16-18 Hz for *E*-double bonds) in the region where alkene protons are usually assigned. Both the ^{1}H NMR and the ^{13}C NMR spectra revealed that the aldehyde proton was still present. It

was therefore deduced that the reaction had been unsuccessful and the synthetic route was ceased.

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CHAPTER 3

SUMMARY

3 SUMMARY

The aim of the project was to carry out studies on the synthesis of alkyl resorcinols to provide compounds that could be used as standards in GC-MS and LC-MS based analytical methods for the detection and quantification of alkylresorcinols in human fluids. Studies on the synthesis of various novel analogues of alkylresorcinols, namely carboxyalkylresorcinols, were also to be investigated in order to develop new immunoassays for rapid high through put analysis of alkylresorcinols from biological samples.

The following compounds were successfully synthesised via the Wittig chemistry route 1-(3',5'-dimethoxyphenyl)hexadecane, methyl 16-(3',5'-dimethoxyphenyl)hexadecanoate, 17-(3',5'-dihydroxyphenyl)heptadecanoic acid and 21-(3',5'-dihydroxyphenyl) henicosanoic acid.

The synthesis employing the Wittig reaction utilizes the reaction between a phosphonium ylide and an aldehyde to attach the alkyl chain onto the aromatic head group by forming a carbon-carbon double bond. This carbon-carbon double bond can then be reduced, by catalytic hydrogenation to give the saturated alkyl chain. For the targets where the commercially available bromoester did not have a long enough chain, the chain was lengthened in two steps via two Wittig reactions using bromoesters. After the first Wittig reaction, to lengthen the chain by a few carbons, the terminal ester group was then reduced to the corresponding aldehyde. This aldehyde was then subjected to a further Wittig reaction, resulting in the long chain being formed.

Methyl 16-(3',5'-dimethoxyphenyl)hexadecanoate was successfully synthesised via a reaction employing a Grignard reagent. This synthesis employs an iron-catalysed cross-coupling of a Grignard reagent with the aryl triflate and is a well known route for the formation of carbon-carbon single bonds. The synthesis utilizes this reaction to directly attach the alkyl chain onto the aromatic head group to give a saturated alkyl chain.

The synthetic challenge of alkylresorcinols and carboxyalkylresorcinols lies in the attachment of the side chain. The Wittig route was found to be the most successful in achieving this attachment. Future work would include the optimisation of yields for this route as well as synthesising the 23-carbon carboxyalkylresorcinol and the 25-carbon carboxyalkylresorcinol analogues.

CHAPTER 4

EXPERIMENTAL

4 EXPERIMENTAL

The NMR spectra were recorded on a Bruker Avance 300 spectrometer (^{1}H, 300 MHz; ^{13}C, 75.46 MHz). ^{1}H and ^{13}C spectra were recorded in $CDCl_3$. The spectra are described in parts per million downfield shift from TMS and are reported consecutively as position (δ_H or δ_C), relative integral, multiplicity (s-singlet, d-doublet, t-triplet, q-quartet, quintet-quintet, m-multiplet) and assignment.

Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrometer. The samples were added in solution to PTFE substrate IR cards. Absorption maxima are given in wavenumbers (cm^{-1}).

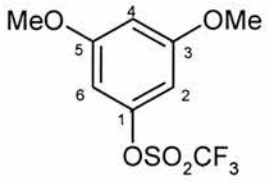
A Micromass GC mass spectrometer was used to obtain Electron Impact (EI) and Chemical Ionisation (CI) mass spectra. A Micromass LCT spectrometer was used to obtain Electrospray (ES) mass spectra and accurate mass measurements. Percentages of the base peak intensity have been given for the major fragments.

Precoated silica gel plates (MN SIL G/UV254) (0.25 mm) were used to carry out analytical thin layer chromatography. Compounds were visualised by U.V. fluorescence or permanganate dip.

Electrothermal melting point apparatus was used to determine the melting points of compounds and are uncorrected.

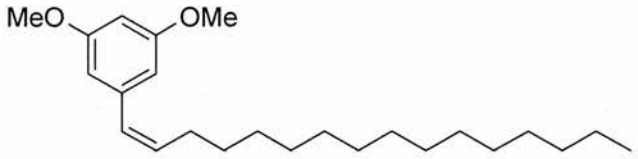
Reagents were obtained from Aldrich, Lancaster, Acros Organics and Strem Chemicals and used without further purification.

Synthesis[1] of 3,5-dimethoxyphenol trifluoromethane sulfonate (65)[1]



A solution of trifluoromethanesulfonic anhydride (triflic anhydride) (5 mL, 0.65 mmol) in dichloromethane (30 mL) was added over a period of 15 minutes to a solution of 3,5-dimethoxyphenol (5.02 g, 0.32 mmol) and 2,6-lutidine (5.26 mL, 0.45 mmol) in dichloromethane (135 mL) at –10 °C. After being stirred at 0 °C for approximately 1 hour then left in the refrigerator for 66 hours, the reaction was quenched with water (100 mL). The organic layer was separated and dried over anhydrous magnesium sulfate and the solvent was removed at reduced pressure. The crude product was filtered through a bed of silica using petroleum ether: ethyl acetate (9:1) as the eluent to afford a brown oil (8.40 g, 91%); δ_H (300 MHz; $CDCl_3$) 3.79 (6H, s, Ar-OC<u>H</u>$_3$), 6.41-6.43 (2H, m, 2-<u>H</u> and 6-<u>H</u>), 6.44-6.46 (1H, m, 4-<u>H</u>); δ_C (75.45 MHz; $CDCl_3$) 55.7 (2 x Ar-O<u>C</u>H$_3$), 100.0 (2-<u>C</u> and 6-<u>C</u>), 100.2 (4-<u>C</u>), 118.6 (q, $J_{C\text{-}F}$ 320 Hz, -<u>C</u>F$_3$), 150.6 (1-<u>C</u>), 161.4 (3-<u>C</u> and 5-<u>C</u>).

Synthesis[2,3,4] of 1-(3',5'-dimethoxyphenyl)hexadec-1-ene (91)



Preparation of the Phosphonium Salt

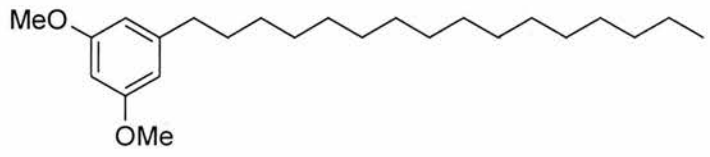
A mixture of 1-bromopentadecane (0.5 mL, 1.7 mmol), triphenylphosphine (0.478 g, 1.82 mmol) and toluene (5 mL) was heated under reflux, with stirring, for 23 hours. The toluene was removed at reduced pressure. The product was washed with diethyl ether to afford an off white solid (0.546 g, 52%). This phosphonium salt was then used without further purification in the next step.

15-(Triphenyl-λ^5-phosphanyl)pentadecane (0.502 g, 0.91 mmol) was dissolved in THF (55 mL). The resulting solution was cooled to –78 °C whilst stirring. *n*-Butyllithium (0.4 mL, 0.99 mmol) was added to the cold solution and stirred for a further 30 minutes. 3,5-Dimethoxybenzaldehyde (0.166 g, 0.99 mmol) was then added and the resulting solution was stirred at –78 °C for a further hour and then warmed to room temperature over a further 2 hours. The reaction was worked-up by removing the solvent at reduced pressure, followed by filtering the product using petroleum ether: ether (8:2) as the eluent. The product was dried over sodium sulfate and then the solvent was removed at reduced pressure to afford a yellow oil (0.193 g, 48%). ν_{max} (ptfe plate)/ cm^{-1} 1279 (C-O-C); δ_H (300 MHz; $CDCl_3$) 0.87 (3H, t, *J* 7 Hz, -C$\underline{H}_3$), 1.15-1.30 (24H, m, -C$\underline{H}_2$-), 1.58-1.66 (2H, m, -C$\underline{H}_2$-), 3.79 (6H, s, Ar-OC$\underline{H}_3$), 5.60-5.70 (2H, m, *J* ~12 Hz, -HC=CH-Ar), 6.38 (1H, t, *J* 2 Hz, 4'-$\underline{H}$), 6.50 (2H, d, *J* 2 Hz, 2'-$\underline{H}$ and 6'-$\underline{H}$); δ_C (75.45 MHz; $CDCl_3$) 14.1

(-$\underline{C}H_3$), 22.7 (-$\underline{C}H_2$-), 25.6 (-$\underline{C}H_2$-), 28.8 (-$\underline{C}H_2$-), 29.3 (-$\underline{C}H_2$-), 29.6 (-$\underline{C}H_2$-), 30.0 (-$\underline{C}H_2$-), 30.5 (-$\underline{C}H_2$-), 31.9 (-$\underline{C}H_2$-), 55.3 (2 x Ar-O$\underline{C}H_3$), 98.7 (4'-$\underline{C}$), 106.8 (2'-$\underline{C}$ and 6'-$\underline{C}$), 128.4 ($\underline{C}$H=C), 128.6 (C=$\underline{C}$H), (1'-$\underline{C}$, 3'-$\underline{C}$ and 5'-$\underline{C}$ not visible); *m/z* (EI^+) 457 ($[M+K]^+$, 6%).

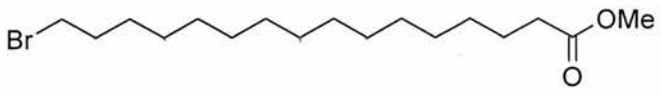
There were also signals in the ^1H NMR spectrum at 7.39-7.57, 7.60-7.73 and 7.75-7.91 ppm corresponding to 3,5-dimethoxybenzaldehyde, triphenylphosphine oxide and some unreacted triphenylphosphine and a signal at 9.84 ppm corresponding to unreacted aldehyde.

Synthesis[5] of 1-(3',5'-dimethoxyphenyl)hexadecane (92)



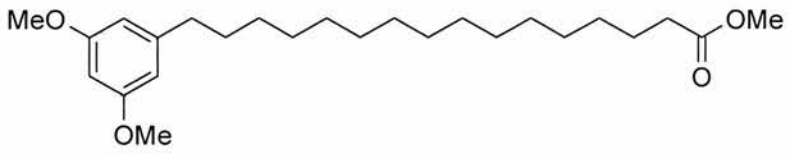
16-(3',5'-Dimethoxyphenyl)hexadec-1-ene (0.193 g, 0.53 mmol) was dissolved in ethyl acetate (20 mL). A catalytic amount of palladium on carbon (10%) was added and the resulting solution was stirred at room temperature under hydrogen at atmospheric pressure for 48 hours. The reaction was filtered through celite and the solvent was removed under reduced pressure. The crude product was purified by column chromatography using petroleum ether: ether (10:1) as the eluent to afford a yellow oil (0.102 g, 53%). ν_{max} (ptfe plate)/ cm^{-1} 1196 (C-O-C); δ_H (300 MHz; $CDCl_3$) 0.88 (3H, t, *J* 7 Hz, $-CH_3$), 1.15-1.38 (26H, m, $-CH_2-$), 1.55-1.64 (2H, m, $-CH_2-$), 2.49-2.58 (2H, m, $-CH_2-$) 3.80 (6H, s, Ar-OCH_3), 6.39 (1H, t, *J* 2 Hz, 4'-H), 6.53 (2H, d, *J* 2 Hz, 2'-H and 6'-H); δ_C (75.45 MHz; $CDCl_3$) 14.1 ($-CH_3$), 22.7 ($-CH_2-$), 29.4 ($-CH_2-$), 29.6 ($-CH_2-$), 30.0 ($-CH_2-$), 30.1 ($-CH_2-$), 30.7 ($-CH_2-$), 31.3 ($-CH_2-$), 31.9 ($-CH_2-$), 36.3 ($-CH_2-$), 55.2 (2 x Ar-OCH_3), 97.5 (4'-C), 106.5 (2'-C and 6'-C), 145.4 (1'-C), 160.6 (3'-C and 5'-C).

Synthesis[6,7] of methyl 16-bromohexadecanoate (96)[8]



To a solution of 16-bromohexadecanoic acid (4.94 g, 15 mmol) in dry methanol (100 mL) was added thionyl bromide (3.33 g, 16 mmol). The resulting solution was heated under reflux, with stirring, for 3 hours at 110 °C under a nitrogen atmosphere then allowed to cool to room temperature. The solvent was then removed under reduced pressure to afford an off-white solid (5.174 g, 99%). m.p. 30-35 °C (Lit.[8] 31 °C); δ_H (300 MHz; $CDCl_3$) 1.15-1.34 (20H, m, -C$\underline{H}_2$-), 1.35-1.43 (2H, m, -C$\underline{H}_2$-), 1.55-1.63 (2H, m, -C$\underline{H}_2$-), 1.83 (2H, quintet, *J* 7 Hz, -C$\underline{H}_2$-), 2.28 (2H, t, *J* 8 Hz, -C$\underline{H}_2$$CO_2Me$), 3.39 (2H, t, *J* 7 Hz, -C$\underline{H}_2$-Br), 3.64 (3H, s, -$CO_2C\underline{H}_3$); δ_C (75.45 MHz; $CDCl_3$) 24.9 (-$\underline{C}H_2$-), 28.2 (-$\underline{C}H_2$-), 28.7 (-$\underline{C}H_2$-), 29.1 (-$\underline{C}H_2$-), 29.2 (-$\underline{C}H_2$-), 29.4 (-$\underline{C}H_2$-), 29.6 (-$\underline{C}H_2$-), 32.8 (-$\underline{C}H_2$-), 34.1 (-$\underline{C}H_2$-), 51.4 (-$CO_2\underline{C}H_3$), 174.3 ($\underline{C}$=O); *m/z* (ES^+) 373 and 371 ($[M+Na]^+$, 99, 100%).

Synthesis[9,10] of methyl 16-(3',5'-dimethoxyphenyl)hexadecanoate (98)



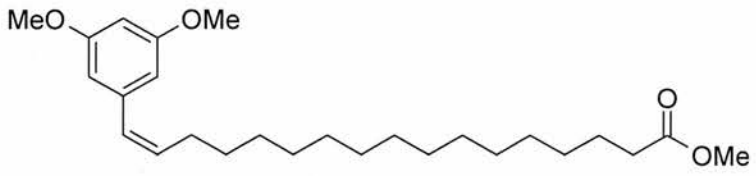
Preparation of the Grignard Reagent

Pure, dry diethyl ether (2.08 mL) and methyl 16-bromohexadecanoate (1.46 g, 4.2 mmol) were added to a flask containing magnesium turnings (0.248 g, 9.1 mmol), a crystal of iodine and diethyl ether (12 mL). The reaction mixture was heated gently to initiate the reaction. The reaction mixture was heated and stirred until only a small amount of the magnesium remained.

A solution of 3,5-dimethoxyphenol trifluromethane sulfonate (0.977 g, 3.5 mmol), [$Fe(acac)_3$] (0.066 g, 0.17 mmol) and *N*-methylpyrrolidinone (1.93 mL) in THF (21 mL) was stirred under nitrogen. A solution of the newly prepared Grignard reagent (2 M in diethyl ether, 2.08 mL, 4.2 mmol) was added to the solution causing an immediate colour change to dark brown. The resulting mixture was stirred for approximately 10 minutes, and was then diluted by addition of diethyl ether (10 mL). The reaction was quenched by addition of aqueous HCl (1M, *ca.* 10 mL). The organic layer was separated and dried over magnesium sulfate, filtered and the solvent was removed under reduced pressure to afford a yellow oil (2.103 g). ν_{max} (ptfe plate)/ cm^{-1} 1738 (C=O), 1245 (C-O); δ_H (300 MHz; $CDCl_3$) 1.21-1.34 (20H, m, -C<u>H</u>$_2$-), 1.36-1.46 (2H, m, -C<u>H</u>$_2$-), 1.56-1.66 (2H, m, -C<u>H</u>$_2$-), 1.85 (2H, quintet, *J* 7 Hz, -C<u>H</u>$_2$-) 2.30 (2H, t, *J* 8 Hz, -C<u>H</u>$_2$$CO_2Me$), 3.41 (2H, t, *J* 7 Hz, -C<u>H</u>$_2$-Ar), 3.67 (3H, s, -CO_2C<u>H</u>$_3$), 3.80 (6H, s, Ar-OC<u>H</u>$_3$), 6.41-6.42 (2H, m, 2'-<u>H</u> and 6'-<u>H</u>), 6.44-6.46 (1H, m, 4'-<u>H</u>); δ_C (75.45 MHz; $CDCl_3$) 24.9 (-<u>C</u>H_2-), 28.1 (-<u>C</u>H_2-), 28.7 (-<u>C</u>H_2-),

29.1 (-$\underline{C}H_2$-), 29.2 (-$\underline{C}H_2$-), 29.4 (-$\underline{C}H_2$-), 29.5 (-$\underline{C}H_2$-), 32.8 (-$\underline{C}H_2$-), 34.0 (-$\underline{C}H_2$-), 51.4 (-$CO_2\underline{C}H_3$), 55.7 (2 x Ar-O$\underline{C}H_3$), 99.9 (4'-$\underline{C}$), 100.2 (2'-$\underline{C}$ and 6'-$\underline{C}$), 150.6 (1'-$\underline{C}$), 161.3 (3'-$\underline{C}$ and 5'-$\underline{C}$), 174.3 ($\underline{C}$=O).

Synthesis[2,3,4] of methyl 17-(3',5'-dimethoxyphenyl)heptadec-1-enoate (100)



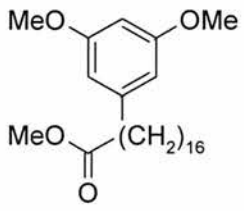
Preparation of the Phosphonium Salt

A mixture of methyl 16-bromohexadecanoate (0.514 g, 1.47 mmol) and triphenylphosphine (0.388 g, 1.47 mmol) was heated under reflux with stirring for 4 hours. The crude product was then washed with diethyl ether to afford methyl 15-(triphenyl-λ^5-phosphanyl) hexadecanoate as a pale yellow oil (0.116 g, 13%). The phosphonium salt was then used in the next step without further purification.

Methyl 16-(triphenyl-λ^5-phosphanyl)hexadecanoate (0.116 g, 0.19 mmol) was dissolved in THF (15 mL). The resulting solution was cooled to –78 °C whilst stirring. *n*-Butyllithium (0.09 mL, 0.21 mmol) was added to the cold solution, which was stirred for a further 30 minutes. 3,5-Dimethoxybenzaldehyde (0.041 g, 0.21 mmol) was then added and the resulting solution was stirred at –78 °C for a further hour and then for an additional 2 hours whilst warming to room temperature. The reaction was quenched by removing the solvent under reduced pressure, and the product was purified by flash chromatography using petroleum ether: dichloromethane (1:1) as the eluent which afforded a yellow oil (0.582 g, 70%). δ_H (300 MHz; $CDCl_3$) 1.22-1.36 (20H, m, -C$\underline{H}_2$-), 1.37-1.48 (4H, m, -C$\underline{H}_2$-), 1.56-1.66 (2H, m, -C$\underline{H}_2$-), 2.25-2.35 (2H, m, -C$\underline{H}_2CO_2CH_3$), 3.66 (3H, s, -$CO_2C\underline{H}_3$), 3.79 (6H, s, Ar-O$C\underline{H}_3$), 5.60-5.70 (2H, m, *J* ~11 Hz, –CH=CH-Ar), 6.39 (2H, d, *J* 2 Hz, 2'-$\underline{H}$ and 6'-$\underline{H}$), 6.50 (1H, t, *J* 2 Hz, 4'-$\underline{H}$); δ_C (75.45 MHz; $CDCl_3$) 23.3 (-$\underline{C}H_2$-),

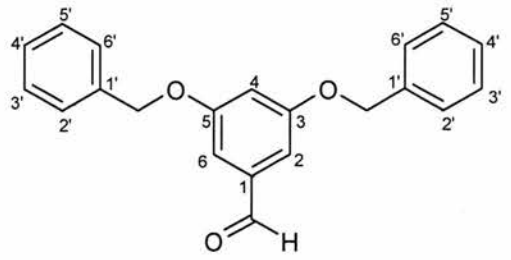
23.4 (-$\underline{C}H_2$-), 25.0 (-$\underline{C}H_2$-), 25.7 (-$\underline{C}H_2$-), 28.8 (-$\underline{C}H_2$-), 29.2 (-$\underline{C}H_2$-), 29.7 (-$\underline{C}H_2$-), 30.0 (-$\underline{C}H_2$-), 30.3 (-$\underline{C}H_2$-), 34.1 (-$\underline{C}H_2$-), 39.0 (-$\underline{C}H_2$-), 51.4 (-$CO_2\underline{C}H_3$), 55.3 (2 x Ar-O$\underline{C}H_3$), 98.7 (4'-$\underline{C}$), 106.8 (2'-$\underline{C}$ and 6'-$\underline{C}$), 128.6 ($\underline{C}$H=C), 133.8 (C=$\underline{C}$H), 160.4 (3'-C and 5'-C).

Synthesis[5] of methyl 17-(3',5'-dimethoxyphenyl)heptadecanoate (101)



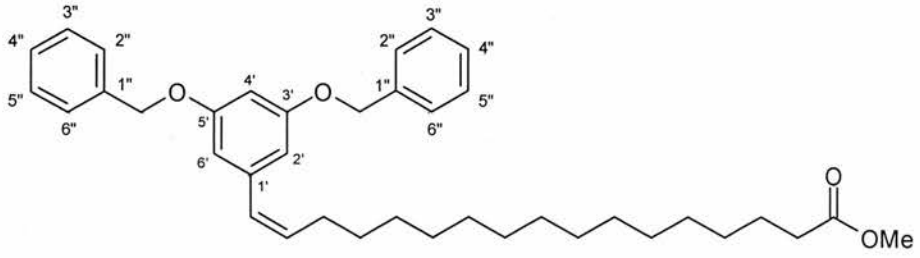
Methyl 17-(3',5'-dimethoxyphenyl)heptadec-1-enoate (0.582 g, 1.39 mmol) was dissolved in ethyl acetate (50 mL). A catalytic amount of palladium on carbon (10%) was added and the resulting solution was stirred at room temperature overnight under hydrogen at atmospheric pressure. The reaction was filtered through celite and the solvent was removed under reduced pressure to afford a yellow oil (0.643 g). This was used in the next step without further purification. δ_H (300 MHz; $CDCl_3$) 1.20-1.36 (26H, m, -C<u>H</u>$_2$-), 1.37-1.45 (2H, m, -C<u>H</u>$_2$-), 1.55-1.65 (2H, m, -C<u>H</u>$_2$-), 2.36-2.38 (2H, m, -C<u>H</u>$_2$$CO_2CH_3$), 3.66 (3H, s, -$CO_2$C<u>H</u>$_3$), 3.79 (6H, s, Ar-OC<u>H</u>$_3$), 6.43 (2H, d, *J* 2 Hz, 2'-<u>H</u> and 6'-<u>H</u>), 6.71 (1H, t, *J* 2 Hz, 4'-<u>H</u>); *m/z* (ES^+) 443 ($[M+Na]^+$, 4%), 441 (100).

Synthesis[11] of 3',5'-dibenzyloxybenzaldehyde (104)[12,13]



To a solution of 3,5-dihydroxybenzaldehyde (5.545 g, 40.15 mmol), benzyl bromide (9.86 mL, 90.5 mmol) and acetone (40 mL) was added K_2CO_3 (19.93 g, 144.1 mmol). The resulting mixture was heated overnight at 60 °C. The mixture was then cooled to room temperature. After filtration the remaining solvent was removed under reduced pressure. The resulting residue was dissolved in ethyl acetate (20 mL) which was then washed with 1 M HCl (3 x 20 mL) and brine (2 x 15 mL), dried over anhydrous magnesium sulfate and filtered. The solvent was removed under reduced pressure to afford an off white solid (13.86 g). m.p. 68-74 °C (Lit.[12,13] 72-73 °C); δ_H (300 MHz; $CDCl_3$) 5.10 (4H, s, -OC<u>H</u>$_2$Bn), 6.88 (1H, t, *J* 2 Hz, 4-<u>H</u>), 7.12 (2H, d, *J* 2 Hz, 2-<u>H</u> and 6-<u>H</u>), 7.30-7.46 (10H, m, Bn-<u>H</u>) 9.90 (1H, s, C<u>H</u>O); δ_C (75.45 MHz; $CDCl_3$) 70.3 (2 x -O<u>C</u>H$_2$Bn), 108.3 (2-<u>C</u> and 6-<u>C</u>), 108.6 (4-<u>C</u>), 127.5 (2 x 2'-<u>C</u> and 2 x 6'-<u>C</u>), 128.2 (2 x 4'-<u>C</u>), 127.7 (2 x 3'-<u>C</u> and 2 x 5'-<u>C</u>), 136.2 (1-<u>C</u>), 138.4 (2 x 1'-<u>C</u>), 160.3 (3-<u>C</u> and 5-<u>C</u>), 191.3 (<u>C</u>HO); *m/z* (CI^+) 319 ($[M+H]^+$, 100%).

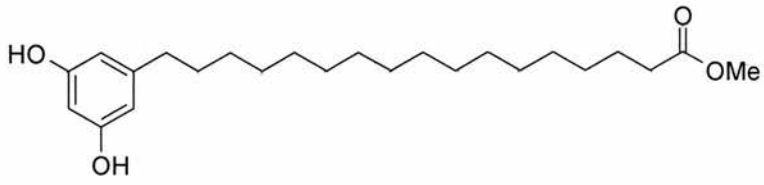
Synthesis[2,3,4] of methyl 17-(3',5'-dibenzyloxyphenyl)heptadec-1-enoate (105)



Methyl 16-(triphenyl-λ^5-phosphanyl)hexadecanoate (0.999 g, 1.63 mmol) was dissolved in THF (35 mL). The resulting solution was cooled to –78 °C whilst stirring. *n*-Butyllithium (0.71 mL, 1.80 mmol) was added to the cold solution and stirred for a further 30 minutes. 3,5-Dibenzyloxybenzaldehyde (0.57 g, 1.80 mmol) was then added and the resulting solution was stirred at –78 °C for a further hour and then for an additional 2 hours whilst warming to room temperature. The reaction was quenched by removing the solvent under reduced pressure and the product was purified by flash column chromatography using petroleum ether: diethyl ether (1:1) as the eluent. The product was dried over anhydrous sodium sulfate and then the solvent was removed under reduced pressure to afford a yellow oil (0.37 g, 21%). ν_{max} (ptfe plate)/ cm^{-1} 1738 (C=O), 1585 (C=C), 1151 (C-O); δ_H (300 MHz; $CDCl_3$) 1.23-1.37 (22H, m, -C$\underline{H}_2$-), 1.38-1.47 (2H, m, -C$\underline{H}_2$-), 1.57-1.68 (2H, m, -C$\underline{H}_2$-), 2.26-2.35 (2H, m, -C$\underline{H}_2$CO_2CH_3), 3.68 (3H, s, -CO_2C$\underline{H}_3$), 5.06 (4H, broad s, -OC$\underline{H}_2$Bn), 5.60-5.70 (2H, m, *J* ~12 Hz, -CH=CH-Ar), 6.51 (1H, t, *J* 2 Hz, 4'-$\underline{H}$), 6.64 (2H, d, *J* 2 Hz, 2'-$\underline{H}$ and 6'-$\underline{H}$), 7.30-7.46 (10H, m, Bn-$\underline{H}$); δ_C (75.45 MHz; $CDCl_3$) 23.3 (-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-), 25.6 (-$\underline{C}H_2$-), 28.7 (-$\underline{C}H_2$-), 29.1 (-$\underline{C}H_2$-), 29.2 (-$\underline{C}H_2$-), 29.3 (-$\underline{C}H_2$-), 29.4 (-$\underline{C}H_2$-), 29.6 (-$\underline{C}H_2$-), 29.9 (-$\underline{C}H_2$-), 30.2 (-$\underline{C}H_2$-), 34.0 (-$\underline{C}H_2$-), 38.9 (-$\underline{C}H_2$-), 39.2 (-$\underline{C}H_2$-), 51.4 (-C$O_2$$\underline{C}H_3$), 70.0 (-O$\underline{C}H_2$Bn), 105.2 (4'-$\underline{C}$), 107.9 (2'-$\underline{C}$ and 6'-$\underline{C}$), 127.4 ($\underline{C}$H=C), 127.9 (C=$\underline{C}$H), 128.5 (2 x 2''-$\underline{C}$ and 2 x 6''-$\underline{C}$), 129.5 (2 x 3''-$\underline{C}$ and 2 x 5''-$\underline{C}$),

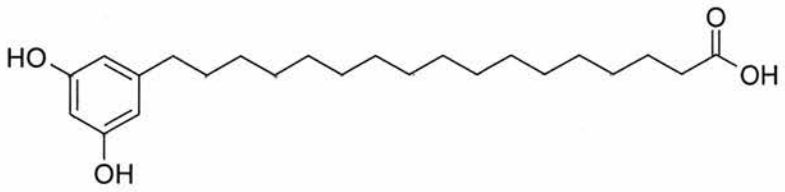
136.9 (1'-C), 159.5 (3'-C and 5'-C), 174.8 (C=O); *m/z* (ES^-) (Found: $[MH^+]$ 571.3748 $C_{38}H_{51}O_4$ requires 571.3787).

Synthesis[5] of methyl 17-(3',5'-dihydroxyphenyl)heptadecanoate (106)



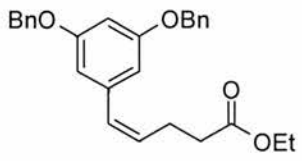
17-(3',5'-Dibenzyloxyphenyl)heptadec-1-ene (0.368 g, 0.64 mmol) was dissolved in ethyl acetate (80 mL). A catalytic amount of palladium on carbon (10%) was added and the resulting solution was stirred under hydrogen at atmospheric pressure for 7 days. The reaction was filtered through celite and the solvent removed under reduced pressure to afford a yellow oil (0.337 g). This was used in the next step without further purification. ν_{max} (ptfe plate)/ cm^{-1} 3556 (O-H), 1742 (C=O), 1267 (C-O); δ_H (300 MHz; $CDCl_3$) 1.20-1.33 (24H, m, -C<u>H</u>$_2$-), 1.36-1.45 (2H, m, -C<u>H</u>$_2$-), 1.52-1.67 (4H, m, -C<u>H</u>$_2$-), 2.26-2.34 (2H, m, -C<u>H</u>$_2$$CO_2CH_3$), 3.66 (3H, s, -$CO_2$C<u>H</u>$_3$), 6.15-6.20 (1H, m, 4'-<u>H</u>), 6.21-6.27 (2H, m, 2'-<u>H</u> and 6'-<u>H</u>); δ_C (75.45 MHz; $CDCl_3$) 25.0 (-<u>C</u>H_2-), 25.7 (-<u>C</u>H_2-), 29.2 (-<u>C</u>H_2-), 29.7 (-<u>C</u>H_2-), 36.6 (-<u>C</u>$H_2CO_2CH_3$), 100.1 (4'-<u>C</u>), 107.9 (2'-<u>C</u> and 6'-<u>C</u>), (1'-<u>C</u>, 3'-<u>C</u> and 5'-<u>C</u> not visible); *m/z* (CI^+) (Found: [MH^+] 393.2997 $C_{24}H_{41}O_4$ requires 393.3005).

Synthesis[14] of 17-(3',5'-dihydroxyphenyl)heptadecanoic acid (84)



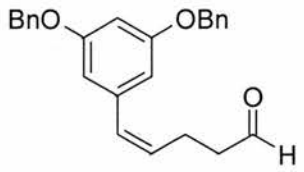
A solution of methyl 17-(3',5'-dihydroxyphenyl)heptadecanoate (0.131 g, 0.33 mmol), methanol (2.5 mL), water (2.5 mL) and lithium hydroxide (0.04 g, 1.67 mmol) was heated under reflux for 3 hours at 80 °C. The reaction was quenched by the solvent being removed under reduced pressure. The product was then extracted into ethyl acetate (20 mL). The organic layer was dried over anhydrous sodium sulfate and then the solvent was removed under reduced pressure to afford a yellow oil (0.113 g). *m/z* (ES^-) (Found: $[MH^-]$ 377.2691 $C_{23}H_{37}O_4$ requires 377.2692). NMR data was complex but it could be seen from the mass spectrometry data that some of the desired compound was obtained.

Synthesis[2,3,4] of ethyl 5-(3',5'-dibenzyloxyphenyl)pent-1-enoate (107)[15]



Ethyl 4-(triphenyl-λ^5-phosphanyl)butanoate (2.01 g, 4.39 mmol) was dissolved in THF (50 mL). The resulting solution was cooled to –78 °C whilst stirring. *n*-Butyllithium (1.92 mL, 4.81 mmol) was added to the cold solution and stirred for a further 30 minutes. 3,5-Dibenzyloxybenzaldehyde (1.532 g, 4.81 mmol) was then added and the resulting solution was stirred at –78 °C for a further hour and then for an additional 2 hours whilst warming to room temperature. The reaction was quenched by removing the solvent under reduced pressure and the product was purified by column chromatography using petroleum ether: diethyl ether (8:2) as the eluent. The product was dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure to afford a yellow oil (0.708 g, 27%). ν_{max} (ptfe plate)/ cm^{-1} 1720 (C=O), 1585 (C=C), 1144 (C-O); δ_H (300 MHz; $CDCl_3$), 1.21 (3H, t, *J* 7 Hz, -$OCH_2\underline{C}H_3$), 1.24-1.34 (2H, m, -$C\underline{H}_2$-), 1.35-1.47 (2H, m, -$C\underline{H}_2CO_2CH_2CH_3$), 4.12 (2H, quartet, *J* 7 Hz, -$OC\underline{H}_2CH_3$), 5.03 (4H, s, -$OC\underline{H}_2$Bn), 5.55-5.70 (2H, m, *J* ~12 Hz, -CH=CH-Ar), 6.49-6.55 (1H, m, 4'-H), 6.63 (2H, d, *J* 2 Hz, 2'-H and 6'-H), 7.30-7.46 (10H, m, Bn-H); δ_C (75.45 MHz; $CDCl_3$) 14.1 (-$OCH_2\underline{C}H_3$), 23.3 (-$\underline{C}H_2$-), 38.8 (-$\underline{C}H_2$-), 65.9 (-$O\underline{C}H_2CH_3$), 70.0 (2 x -$O\underline{C}H_2$Bn), 105.4 (4'-C), 107.9 (2'-C and 6'-C), 127.5 ($\underline{C}$H=C), 127.9 (C=$\underline{C}$H), 128.5 (2 x 2''-C and 2 x 6''-C), 128.6 (2 x 4''-C), 130.0 (2 x 3''-C and 2 x 5''-C), 136.9 (1'-C), 159.7 (3'-C and 5'-C), (C=O not visible); *m/z* (CI^+) 417 ($[M+H]^+$, 53%), 369 (100).

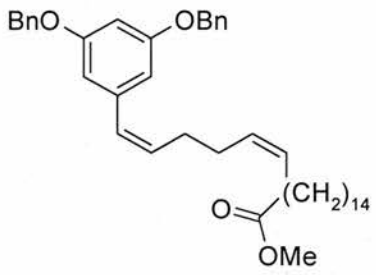
Synthesis[16] of 5-(3',5'-dibenzyloxyphenyl)pent-1-enal (108)



A stirred solution of ethyl 5-(3',5'-dibenzyloxyphenyl)pent-1-enoate (0.37 g, 0.89 mmol) and toluene (4 mL) was cooled to –78 °C under nitrogen. To this solution was added drop wise a 1.0 M solution of DIBAL (8.84 mL, 8.84 mmol). The resulting mixture was stirred for 1 hour followed by hydrolysis with a 1 M solution of HCl (15 mL). Diethyl ether (15 mL) was added to the reaction mixture and this was then warmed up to room temperature. The organic layer was washed with 1.0 M HCl (2 x 20 mL). The combined organic layers were dried over magnesium sulfate, filtered, and then the solvent was removed under reduced pressure. The resulting crude mixture was diluted in DCM (15 mL). TEMPO (0.14 g, 0.09 mmol), nBu$_4$NCl (0.252 g, 0.09 mmol), a buffered solution of $NaHCO_3$ (0.5 M) and K_2CO_3 (0.05 M) and NCS (0.241 g, 1.78 mmol) were successively added to the diluted crude mixture. This two-phase solution was stirred vigorously overnight. The aqueous phase was extracted with DCM (3 x 20 mL) and the resulting organic layer was washed with brine (2 x 20 mL). The organic layer was dried over magnesium sulfate, filtered and the solvent was removed under reduced pressure. The aldehyde was purified by column-chromatography on silica using petroleum ether: diethyl ether (15:1) as the eluent to afford a yellow oil (0.199 g, 60%). ν_{max} (ptfe plate)/ cm^{-1} 1742 (C=O), 1539 (C=C); δ_H (300 MHz; $CDCl_3$) 1.24-1.30 (2H, m, -C$\underline{H}_2$-), 1.54-1.62 (2H, m, -C$\underline{H}_2$CHO), 5.05 (4H, s, -OC$\underline{H}_2$Bn), 5.55-5.70 (2H, m, J ~12 Hz, -CH=CH-Ar), 6.53-6.57

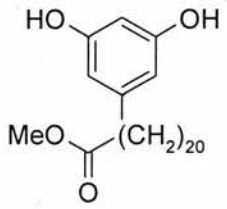
(2H, m, 2'-H and 6'-H), 6.63 (1H, t, *J* 3 Hz, 4'-H), 7.30-7.46 (10H, m, Bn-H), 9.91 (1H, t, *J* 1 Hz, CHO); *m/z* (CI^+) (Found: $[MH^+]$ 373.1808 $C_{25}H_{25}O_3$ requires 373.1804).

Synthesis[2,3,4] of methyl 21-(3',5'-dibenzyloxyphenyl)henicosan-1,5-dienoate (109)



Methyl 16-(triphenyl-λ^5-phosphanyl)hexadecanoate (0.302 g, 0.49 mmol) was dissolved in THF (11mL). The resulting solution was cooled to –78 °C whilst stirring. *n*-Butyllithium (0.8 mL, 2 mmol) was added to the cold solution and stirred for a further 30 minutes. 5-(3',5'-Dibenzyloxyphenyl)pent-1-enal (0.199 g, 0.54 mmol) was then added and the resulting solution was stirred at –78 °C for a further hour and then for an additional 2 hours whilst warming to room temperature. The reaction was quenched by removing the solvent under reduced pressure to afford a yellow oil (0.404 g). This was used in the next step without further purification. ν_{max} (ptfe plate)/ cm^{-1} 1727 (C=O), 1574 (C=C), 1275 (C-O); δ_H (300 MHz; $CDCl_3$) 1.10-1.33 (24H, m, -C$\underline{H}_2$-), 1.36-1.43 (4H, m, -C$\underline{H}_2$-), 1.52-1.66 (2H, m, -C$\underline{H}_2$-), 2.26-2.34 (2H, m, -C$\underline{H}_2CO_2CH_3$), 3.66 (3H, s, -$CO_2C\underline{H}_3$), 5.03 (4H, s, -OC$\underline{H}_2$Bn), 5.31-5.43 (2H, m, -CH=CH-), 5.60-5.70 (2H, m, *J* ~11 Hz, –CH=CH-Ar), 6.50-6.54 (2H, m, 2'-$\underline{H}$ and 6'-$\underline{H}$), 6.59-6.61 (1H, m, 4'-$\underline{H}$), 7.30-7.46 (10H, m, Bn-$\underline{H}$); δ_C (75.45 MHz; $CDCl_3$) 22.8 (-$\underline{C}H_2$-), 23.3 (-$\underline{C}H_2$-), 23.4 (-$\underline{C}H_2$-), 24.9 (-$\underline{C}H_2$-), 25.7 (-$\underline{C}H_2$-), 29.6 (-$\underline{C}H_2$-), 34.1 (-$\underline{C}H_2$-), 38.8 (-$\underline{C}H_2$-), 39.0 (-$\underline{C}H_2$-), 39.3 (-$\underline{C}H_2CO_2CH_3$), 70.1 (2 x -O$\underline{C}H_2$Bn), 105.2 (4'-$\underline{C}$), 107.9 (2'-$\underline{C}$ and 6'-C), 127.4 ($\underline{C}$H=C), 127.9 (C=$\underline{C}$H), 128.4 (2 x 2''-C and 2 x 6''-$\underline{C}$), 128.5 (2 x 4''-$\underline{C}$), 130.3 (2 x 3''-$\underline{C}$ and 2 x 5''-C), (1'-$\underline{C}$, 3'-$\underline{C}$, 5'-$\underline{C}$ and $\underline{C}$=O not visible).

Synthesis[5] of methyl 21-(3',5'-dihydroxyphenyl)henicosanoate (110)



Methyl 21-(3',5'-dibenzyloxyphenyl)henicosan-1,5-dienoate (0.392 g, 0.63 mmol) was dissolved in ethyl acetate (50 mL). A catalytic amount of palladium on carbon (10%) was added and the resulting solution was stirred at room temperature overnight under hydrogen at atmospheric pressure. The reaction was filtered through celite and the solvent was removed under reduced pressure to afford a yellow oil (0.289 g). ν_{max} (ptfe plate)/ cm^{-1} 1727 (C=O), 1282 (C-O); δ_H (300 MHz; $CDCl_3$) 1.20-1.33 (26H, m, -C$\underline{H}_2$-), 1.36-1.45 (8H, m, -C$\underline{H}_2$-), 1.52-1.66 (2H, m, -C$\underline{H}_2$-), 2.26-2.34 (2H, m, -C$\underline{H}_2CO_2CH_3$), 2.50-2.60 (2H, m, -C$\underline{H}_2$-), 3.66 (3H, s, -$CO_2C\underline{H}_3$), 6.49 (1H, t, *J* 2 Hz, 4'-$\underline{H}$), 6.61 (2H, d, *J* 2 Hz, 2'-$\underline{H}$ and 6'-$\underline{H}$); δ_C (75.45 MHz; $CDCl_3$) 23.1 (-$\underline{C}H_2$-), 23.3 (-$\underline{C}H_2$-), 23.4 (-$\underline{C}H_2$-), 24.7 (-$\underline{C}H_2$-), 25.7 (-$\underline{C}H_2$-), 29.6 (-$\underline{C}H_2$-), 31.8 (-$\underline{C}H_2$-), 36.2 (-$\underline{C}H_2$-), 39.0 (-$\underline{C}H_2$-), 39.1 (-$\underline{C}H_2$-), 39.3 (-$\underline{C}H_2CO_2CH_3$), 99.2 (4'-$\underline{C}$), 107.6 (2'-$\underline{C}$ and 6'-$\underline{C}$), 159.9 (3'-$\underline{C}$ and 5'-$\underline{C}$), ($\underline{C}$=O not visible); *m/z* (CI^+) 471 ($[M+Na]^+$, 100).

There was a signal in the ^1H NMR spectrum at 5.03 ppm attributable to the benzyl protons as well as signals between 7.28-7.55 and 7.60-7.93 ppm corresponding to Ar-H. There were also signals in the ^{13}C NMR spectrum at 70.0, 127.5 and 127.9 ppm corresponding to the (-O$\underline{C}H_2$Bn) carbons and the double bonds respectively.

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