DERIVATISATION OF POLYPHENOLS

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Abstract

Polyphenols, such as tannins, offer potential as a bio-derived chemical feedstock. Their present utilisation is limited mainly to leather tanning and wood panel adhesives. However, appropriate derivatisation may alter both the chemical and physical properties and thereby allow further utilisation of polyphenols.

Derivatisation of polyphenols was achieved by esterification and etherification of the phenol groups. Esterification was achieved by alcoholysis of acid chlorides and transesterification with vinyl esters, while etherification was achieved by the ring opening of propylene oxide. The polyphenols used were resorcinol, catechin, *Pinus radiata* bark tannin, and *Schinopsis lorentzii* tannin. The products were characterised using a range of techniques including NMR (^{1}H, ^{13}C and 2D NMR in both the solution and solid state), ESI-MS, GPC, DSC, TGA, and rheology.

The preparation of polyphenolic esters by alcoholysis provided model compounds to establish the key chemical, spectroscopic, and physical features. A range of simple polyphenol esters such as resorcinol dilaurate and catechin pentalaurate were prepared using lauroyl chloride. Furthermore, tannin lauroyl esters were prepared with varying degrees of substitution. A transesterification method was developed for the preparation of polyphenol esters. Ester interchange occurred effectively in the presence of base catalyst in aqueous solution or dimethyl sulfoxide with short or long chain vinyl esters. This included the first report of the base-catalysed transesterification of flavonoids by vinyl esters to give products such as catechin mono- and di-laurate. Transesterification occurred preferentially at the B-ring as shown by NMR spectroscopy. Subsequently, this transesterification procedure was used to prepare tannin esters. The chemical and physical properties of polyphenol esters were assessed using thermal, antioxidant, and UV/VIS light absorption analysis. Thermal analysis indicated melt/flow properties for some of the polyphenol esters. In some cases, the thermal stability was also shown to increase upon esterification. The antioxidant activity was shown to decrease upon transesterification of pine bark tannin with vinyl laurate,

while the UV/VIS absorption was shown to increase. These properties may lend the products towards applications as polymer additives or pharmaceuticals.

Polyphenol ethers were prepared by the Williamson ether synthesis and ring opening of propylene oxide. However, the Williamson ether synthesis, a common route to prepare ethers, proved unsuitable for flavonoids. Catechin and tannin hydroxypropyl ether derivatives of varying substitution were prepared by the ring-opening of propylene oxide in the presence of triethylamine. Upon hydroxypropylation the thermal properties of the polyphenol were altered. For example, catechin hydroxypropyl ethers showed a glass transition, which was dependent upon the molar substitution, while rheology showed melt behaviour for several of the tannin hydroxypropyl ethers.

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This thesis is dedicated to my parents

Jim and Angela Bridson

Structures for all numbered products appear in the fold out section at the rear of this thesis.

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Abbreviations

2D: Two dimensional

ABTS: 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)

Ac: Acetyl

Ar: Aromatic

br: Broad (NMR)

ca: Circa (about)

CoA: Coenzyme A

COSY: Correlation spectroscopy

CP-MAS: Cross polarisation-magic angle spinning

d: Doublet (NMR)

DEPT: Distortionless enhancement by polarisation transfer

DMAP: 4-(Dimethylamino)pyridine

DMF: Dimethylformamide

DMSO: Dimethyl sulfoxide

DNA: Deoxyribonucleic acid

DP: Degree of polymerisation

DS: Degree of substitution

DSC: Differential scanning calorimetry

DW: Dry weight

E2: Bimolecular elimination

ESI-MS: Electrospray ionisation mass spectrometry

Et: Ethyl

EtOAc: Ethyl acetate

FTIR: Fourier transform infrared spectroscopy

G': Elastic modulus

G'': Loss modulus

GPC: Gel permeation chromatography

HMBC: Heteronuclear multiple bond correlation

HPE: Hydroxypropyl ether

HPLC: High performance liquid chromatography

HSQC: Heteronuclear single quantum coherence

IR: Infrared

$J_{x\text{-}x}$: Coupling constant

LR-COSY: Long range correlation spectroscopy

m/z: Mass-to-charge ratio

m: Medium (IR)

m: Multiplet (NMR)

Me: Methyl

MS: Molar substitution

MS^2: Mass spectrometry-mass spectrometry

MW: Molecular weight

NMR: Nuclear magnetic resonance

PBT: Pine bark tannin (*Pinus radiata*)

PLC: Preparative layer chromatography

PMMA: Polymethyl methacrylate

PO: Propylene oxide

ppm: Parts per million

PTFE: Polytetrafluoroethylene (Teflon)

PTSA: *p*-Toluenesulfonic acid

q: Quartet (NMR)

QT: Quebracho tannin (*Schinopsis lorentzii*)

RDA: Resorcinol diacetate

R_f: Retention factor

RMA: Resorcinol monoacetate

s: Singlet (NMR)

s: Strong (IR)

sh: Shoulder (IR)

S_N1: Unimolecular nucleophilic substitution

S_N2: Bimolecular nucleophilic substitution

SPF: Skin protection factor

t: Triplet (NMR)

T_1: Spin-lattice relaxation time

T_2: Spin-spin relaxation time

TEA: Triethylamine

TEAC: Trolox equivalent antioxidant capacity

T_g: Glass transition temperature

TGA: Thermogravimetric analysis

THF: Tetrahydrofuran

TLC: Thin layer chromatography

UV/VIS: Ultraviolet visible (spectroscopy)

UV: Ultraviolet

w: Weak (IR)

δ: Delta scale

ν: Stretching frequency

Chapter 1 - Introduction

This chapter will provide an insight into polyphenols and their derivatisation. First, the context for the thesis is outlined including the forestry industry and natural products. Second, a discussion of polyphenols including flavonoids and tannins highlights their chemistry and applications. Lastly, a review of their esterification and etherification reactions leads into the objectives for this project.

1.1 Forestry

Plantation forestry in New Zealand is predominantly based on *Pinus radiata* and provides timber and fibre for many applications. Common products from *Pinus radiata* wood include lumber, pulp and paper, plywood, particle board, and fibreboards[1-3]. In contrast, the bark from *Pinus radiata* is under-utilised. It is often buried or burnt, contributing towards production costs and environmental impact. However, the utilisation of bio-based resources is becoming popular due to an increasing environmental awareness of the public and governments. The constituents of bark differ greatly from that of wood and may provide a source of new bio-based feedstocks. For example, bark consists of cellulose, hemicellulose, lignin, and solvent-extractable natural products, which include polyphenols (Table 1.1). Therefore, bark and its components may offer a source of potential bio-based starting chemicals for a range of applications[3].

Table 1.1: Approximate chemical composition of radiata pine sapwood and bark.

Component	% Composition	
	Wood	**Bark**
Extractives		
Non-polar	2	3
Lower molecular weight phenols	<1	4
Condensed tannins	-	18
Tannins/phenolic acids	-	40
Subtotal	2	65
Cellulose	40	12
Lignin	27	15
Hemicellulose and other compounds	31	6
Ash	<1	2

Adapted from Kininmonth and Whitehouse[3]

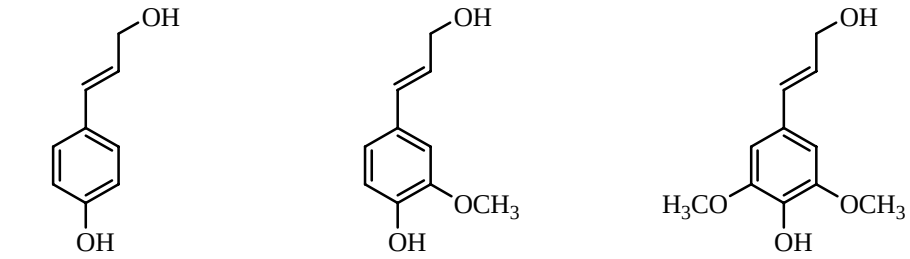
1.2 Natural Products

Metabolites produced by living organisms are classified as either primary or secondary metabolites. Primary metabolites are essential for the survival of an organism. For example, nucleic acids, amino acids, and proteins are found in all organisms. In contrast, secondary metabolites are not essential for survival but usually have an important ecological function. For example, they may provide a chemical signalling agent, protection against radiation, or an aid in reproduction. Accordingly, their distribution varies both within the organism and between species. Three major classes of secondary metabolites include alkaloids, terpenoids, and phenolics[4].

Phenolics are common in plants and they often contain more than one phenol group per molecule, termed polyphenols. Polyphenols occur in all plant tissues such as leaves, stems, heartwood, flowers, and bark. Examples of polyphenols include lignin, flavonoids, stilbenes, coumarins, and tannins[4]. Polyphenols will comprise the majority of this discussion.

1.3 Lignin

Lignin is the most abundant polyphenol occurring in all vascular plants and the second most abundant natural polymer. It comprises *p*-coumaryl, coniferyl, and sinapyl alcohol monomers (Figure 1.1) cross-linked in a three dimensional network by enzymatic polymerisation. The resulting macromolecule is highly complex and variable. Lignin acts as a bonding agent between the cell wall components cellulose and hemicellulose, providing compressive strength and stiffness. In contrast, cellulose provides tensile strength[3, 4].



p-Coumaryl alcohol | Coniferyl alcohol | Sinapyl alcohol

Figure 1.1: The structure of three common lignin monomers.

1.4 Flavonoids

Flavonoids are a diverse subset of polyphenols. Over 3000 different flavonoids exist hence there are variations in their location, structure, and synthesis. Flavonoids occur in most plant tissues including flowers, fruit, foliage, heartwood, and bark. Their structure is a derivative of the basic C6.C3.C6 flavan unit, being two aromatic rings separated by three carbon atoms (Figure 1.2). The flavan rings are labelled A, C, and B respectively with a systematic numbering of carbon atoms[4].

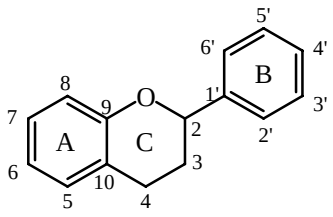


Figure 1.2: A flavan, the basic unit of flavonoids.

Flavonoids are synthesised from the precursors malonyl-coenzyme A (CoA) and *p*-coumaryl-CoA. *p*-Coumaric acid is derived from phenylalanine via the

phenylpropanoid pathway and forms the B- and C-rings. Its condensation with three malonyl-CoA units forms a chalcone, a key intermediate in the biosynthetic pathway[5]. Subsequent reactions such as the chalcone to flavanone interconversion complete the cyclisation process[6, 7]. Further modifications may include: hydroxylation of the A- or B-rings, methylation, acylation, glycosylation, *C*-alkylation, or polymerisation[7]. Consequently, the flavonoids show several common features (Figure 1.3).

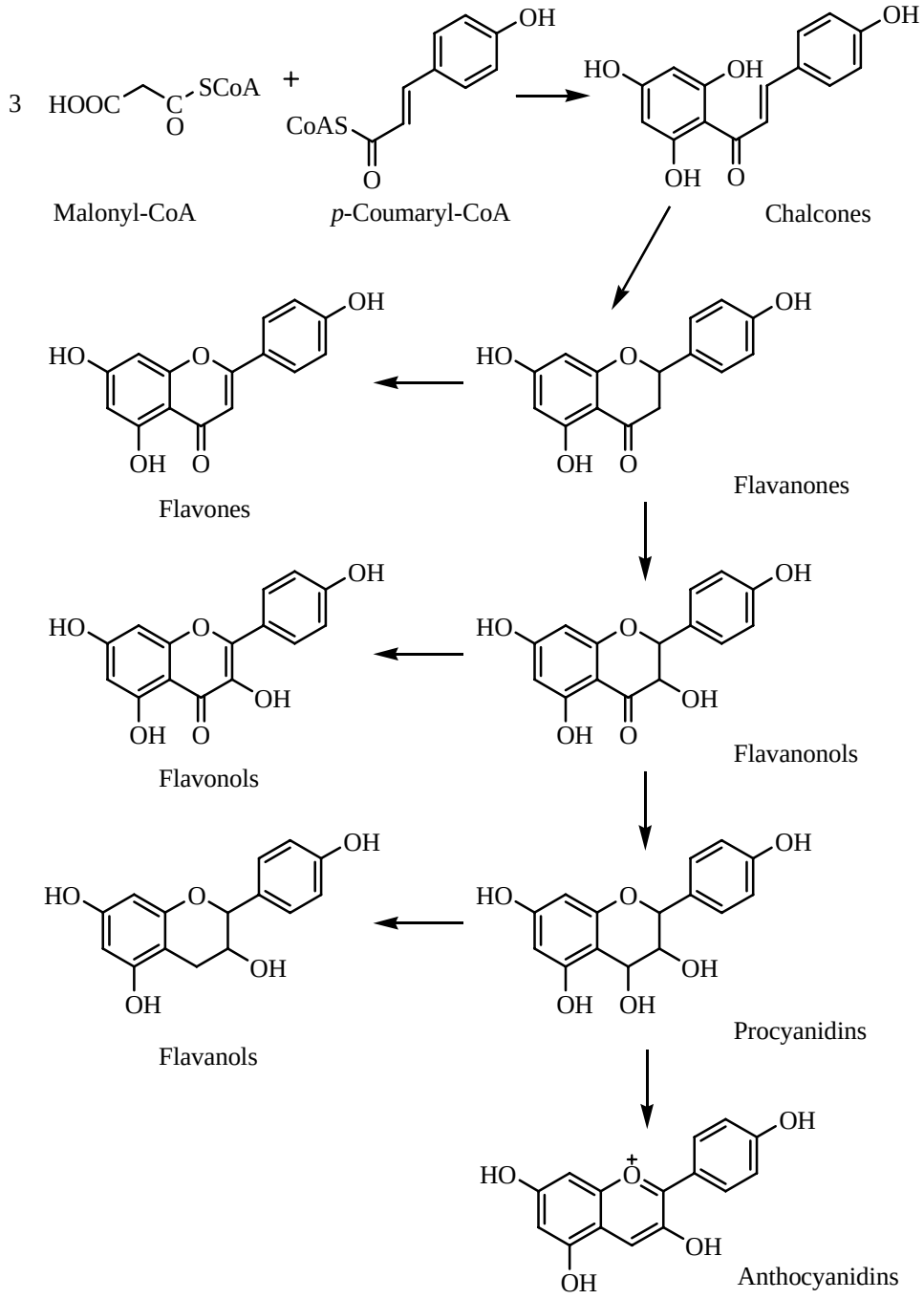
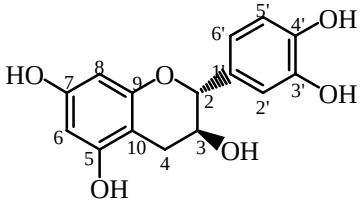


Figure 1.3: The biosynthesis of common flavonoids (adapted from Luckner[7]).

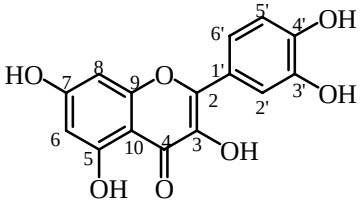
Flavonoids have common features in their hydroxylation pattern and three dimensional structure. The A-ring generally has three oxygen atoms at alternate positions (5, 7, and 9). In contrast, the B-ring has variable oxygenation, however a *para*-hydroxyl, with respect to the C_3 propyl chain, is often present. A second *meta*-hydroxyl is also commonly observed as is further hydroxylation[8]. The molecular conformation of flavonoids may adopt two forms: planar or puckered. For example, the ring systems of flavones, aurones, and chalcones are planar. In contrast the saturated pyran ring of anthocyanins, flavanols, flavanones, isoflavanones, and flavanonol are puckered[9]. These features can often be traced back to their biosynthetic building blocks.

Polymerisation is very common among the flavonoids. Linkage occurs through atoms C-4, C-6, and C-8 via electrophilic substitution. The labile methylene C-4 protons give rise to a carbanion which can attack the nucleophilic centres of the A-ring. The meta hydroxyl pattern of the A-ring encourages the electrophilic attack. Stereoisomerism of oligomers and polymers results around the interflavanoid bonds. These polymers can range from dimers to very large macromolecules[10].

Commonly discussed monomeric flavonoids include (+)-catechin and quercetin (Figure 1.4). Epicatechin, another common flavonoid, is the epimer of catechin. Addition of a third hydroxyl to the B-ring of catechin and epicatechin gives gallocatechin and epigallocatechin respectively.



(+)-Catechin



Quercetin

Figure 1.4: The structure of (+)-catechin and quercetin.

1.4.1 Tannins

Tannins are plant polyphenols, originally defined as a plant extract with the ability to tan leather. However, they are now defined as water-soluble phenolic

compounds with a molecular weight generally between 500 and 3000 Daltons. They give the usual phenolic reactions and have the property of precipitating proteins[11-13].

There are two classes of tannin, those being hydrolysable and condensed tannins. Hydrolysable tannins are polyesters of gallic acid (Figure 1.5). Gallic acid is esterified to a central carbohydrate, commonly D-glucose. Complex hydrolysable tannins have modifications to the galloyl group or carbohydrate[13].

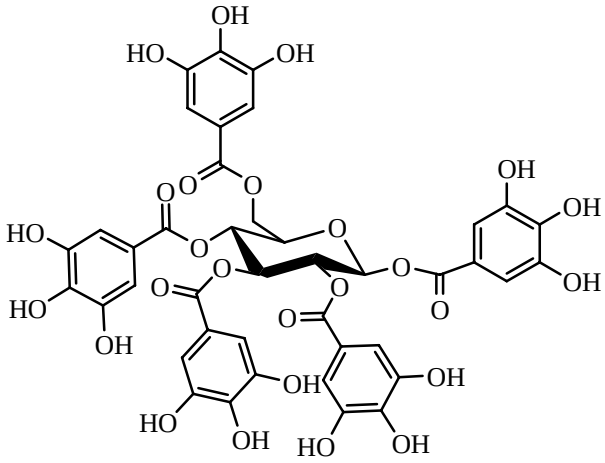


Figure 1.5: Gallotannin (pentagalloyl glucose), an example of a hydrolysable tannin[14].

Condensed tannins are flavanoid oligomers and polymers. More recently they are being termed proanthocyanidins, as they yield anthocyanidins upon oxidative cleavage. Extraction of condensed tannins is common from *Acacia*, *Tsuga*, and *Pinus* bark and *Schinopsis* wood. The total production of tannins from all sources is greater than 200 000 tons per year[15, 16].

Tannin extracted from New Zealand *Pinus radiata* bark consists mainly of procyanidin (3,5,7,3',4'-pentahydroxylation) and prodelphinidin (3,5,7,3',4',5'-hexahydroxylation) units in the ratio 4 : 1[3, 17]. The average degree of polymerisation (DP) varies from 7.3 to 9.2 depending on the method of extraction. However the range of DP is much greater, from 2 to >18. As the molecular weight of tannins becomes higher they are increasingly difficult to study[18]. In pine bark tannin most linkages are assumed to be through atoms 4-8, with some through atoms 4-6 (Figure 1.6)[19].

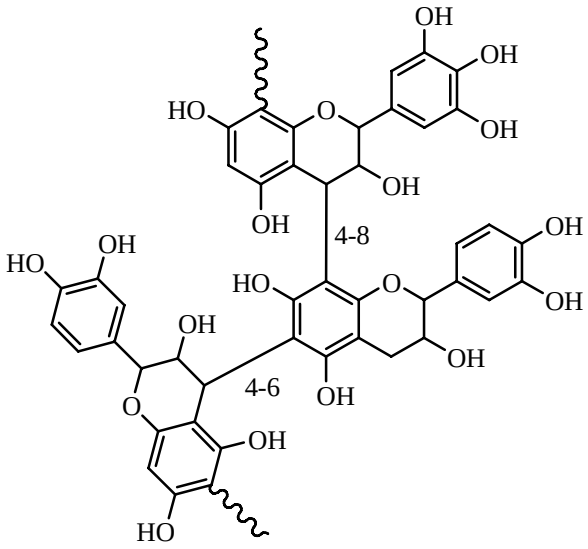


Figure 1.6: An example of a condensed tannin polymer from *Pinus radiata* consisting of procyanidin and prodelphinidin units.

Quebracho tannin is a polyphenol extract from *Schinopsis lorentzii* sapwood. It consists mainly of profisetinidin (3,7,3',4'-tetrahydroxylation) and prorobinetinidin (3,7,3',4',5'-pentahydroxylation) units in the ratio 3 : 1. The polymers chains are mostly linear, linked through atoms 4-6 with an average degree of polymerisation of about 6.5[20].

1.4.2 Biological Properties of Flavonoids

Flavonoids serve many purposes in plants aiding their survival or reproduction. Pest and disease damage is often prevented by flavonoids. The astringent taste of flavonoids is a deterrent to grazing herbivores. This is attributed to the proanthocyanidins, which are found in relatively high concentration within the leaves of many woody plants. Bacterial, fungal, and viral infection is also prevented by the presence of flavonoids[21]. Protection against UV light is another function of flavonoids. Flavonoids absorb light in the 280-315 nm range, this providing protection to leaves against UV-B radiation and preventing tissue damage[22]. Vivid flower colours are often produced by the anthocyanins, ranging from red through to purple and blue. These flower pigments act as visual signals to attract pollinators[4]. The anthocyanins are also common in colouring red fruit such as strawberries, cherries and apples. Many black fruit are actually very dark purple or red due to the high concentration of anthocyanins[23].

1.4.3 Chemistry of Flavonoids

The chemistry of flavonoids is dominated by the presence of multiple phenol groups. As a result, acid and base catalysed rearrangements, nucleophilic substitution, and electrophilic aromatic substitution are common reactions. Furthermore, the phenol groups give flavonoids strong complexing abilities and antioxidant properties.

Nucleophilic substitution occurs at the hydroxyl groups introducing either acyl or alkyl groups. Acylation is commonly achieved by reaction with acid chlorides or anhydrides. Alkylation is commonly achieved through reaction with alkyl halides or epoxides. These reactions form the focus of this project and are covered in detail in the proceeding sections.

Activation towards electrophilic aromatic substitution is due to the multiple phenol groups. The hydroxyl is a powerful electron donor especially under basic conditions where the phenoxide ion is formed, this pushing electron density onto the ring. Under acid conditions the reactivity of the electrophile increases, therefore reaction rate is slowest at neutral pH. Phenol groups are ortho and para directing. Hence, the meta phenol groups commonly on the A-ring both activate the C-6 and C-8 positions (Figure 1.7). In contrast, the vicinal di-phenol groups commonly on the B-ring cause general activation rather than specific activation. Two examples of electrophilic aromatic substitution are halogenation and the reaction with aldehydes. Halogens such as bromine and iodine act as electrophiles and react with flavonoids. For catechin, this is most favourable at the C-8 position followed by the C-6 position. The reaction with an aldehyde results in polymerisation through methylene bridges. The site of reaction is the A-ring and only at the B-ring in very alkaline conditions. This reaction is well studied due to its application in adhesive systems[24, 25].

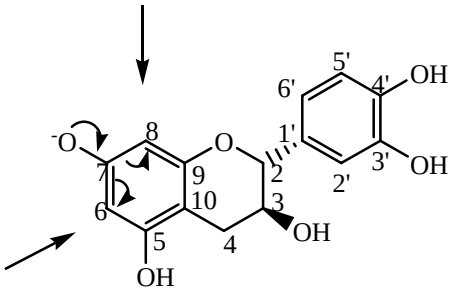


Figure 1.7: Catechin is activated towards electrophilic aromatic substitution at C-6 and C-8, as indicated by the arrows.

In acidic conditions hydrolysis or auto-condensation is common. These are two competing reactions. Polymeric flavonoids can be degraded to their constituent units, alternatively the flavonoid units can condense. The later is thought to occur by hydrolysis of the heterocyclic ring and its electrophilic attack upon a nucleophilic centre of the A-ring[24].

In alkaline conditions epimerisation and rearrangements occur. Epimerisation of catechin to epicatechin occurs in hot water or dilute alkaline solutions (Figure 1.8). This epimerisation involves only a change in stereochemistry at C-2 of catechin. In basic conditions (pH 9-11) this epimerisation reaches equilibrium in several hours. In contrast, epimerisation is much slower in acidic conditions[26]. Rearrangements of catechin to catechinic acid are prevalent in alkaline solutions. However, in acidic conditions this rearrangement is negligible. High temperatures favour rearrangement over epimerisation[27]. Both the epimerisation and rearrangement reactions occur through a quinone methide intermediate[28]. In general, basic conditions and elevated temperatures favour the epimerisation and rearrangements of flavonoids.

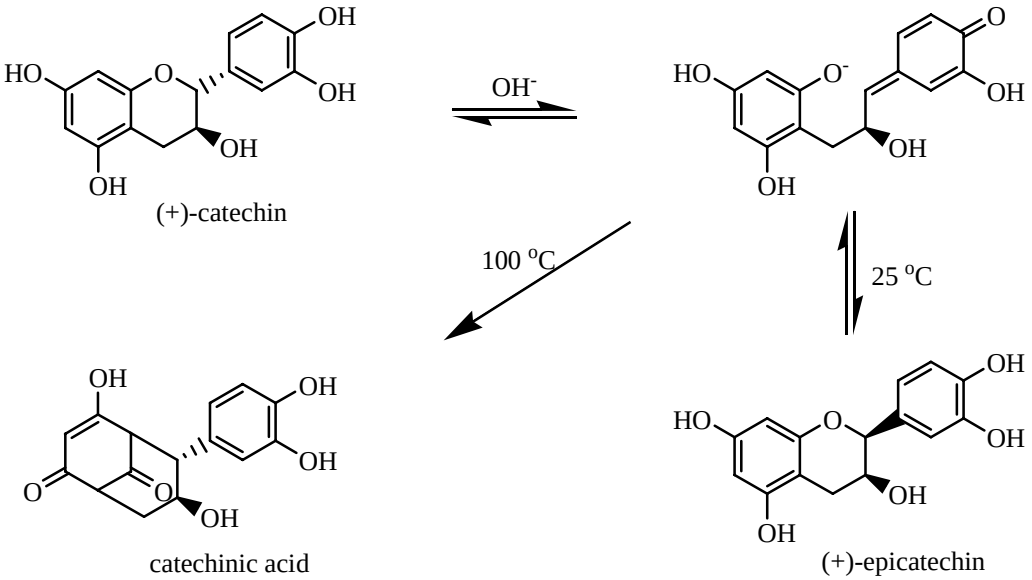


Figure 1.8: Base-catalysed reactions of catechin.

Flavonoids form complexes with a range of substrates including metal ions and macromolecules; this can be both reversible and irreversible. Due to their numerous phenol groups they often act as multidentate ligands. A range of metal ions are reportedly complexed by flavonoids including beryllium, magnesium, aluminium, and boron. A potential site for complexation is the catechol-type B-ring structure[29]. Complexation of macromolecules includes polysaccharides, alkaloids, and proteins. This generally occurs by hydrogen bonding and hydrophobic effects. The complex is generally irreversibly formed, however mild and non-destructive treatments can reverse the complex formation[30].

Flavonoids are effective antioxidants where the phenolics play a large role in the radical-scavenging properties. A range of reactive oxygen species such as superoxide ($O_2^{\bullet -}$), hydroxyl radicals (OH^-) and peroxy radicals are scavenged by flavonoids. Singlet oxygen is also known to be quenched. These properties are attributed to four factors:

- Ortho-hydroxylation of the B-ring
- The presence of a C-3 hydroxyl group
- A carbonyl group at C-4
- A C-2 to C-3 double bond

Hence, flavonoids with these structural features are the best antioxidants. In utilising the antioxidant properties of flavonoids, preservation of some or all of these features is essential[22, 31, 32].

1.4.4 Current Applications of Flavonoids

Extracted tannins have several commercial applications including leather tanning, adhesives additives, dispersants, asphalt additives, vinification, and pharmaceuticals. Leather tanning is the traditional application of tannin. Tannins interact with and precipitate proteins to preserve the hide, hence forming leather[13].

In wood adhesives a phenol-formaldehyde resin is commonly used. The phenol content can be replaced with tannins forming a tannin-formaldehyde resin. In cold setting adhesives, tannins have been used to replace the resorcinol in phenol-resorcinol-formaldehyde resins. This product has found use in wood laminating[33]. Tannin extracts have been used as substitutes for resorcinol in tire cord adhesives, this creating a tannin-formaldehyde-latex adhesive. Tannins were incorporated into the adhesive in varying proportions and offered equal performance compared with the resorcinol-based adhesive[34].

As dispersants, tannins can alter the viscosity of various solids. Addition to clays reduces the viscosity assisting in the manufacture of bricks and pottery. A concrete additive based on tannins provides a more plastic and flowable concrete, improved uniformity, and reduced shrinkage. A tannin drilling fluid additive allows for rheology control in water-based mud. Tannin asphalt additives act as emulsifiers, which improve the performance of hot asphalts. In winemaking tannins are used to clarify the wine and improve the taste after storage[15].

The medicinal properties of polyphenols have more recently become apparent, utilising the biological properties of flavonoids. Potential applications include prevention of chronic diseases[35], antimicrobial uses, antitumor activity, and cardioprotective effect[36]. In summary, tannins are used in a wide range of applications. This is dependent on their chemical and biochemical properties as previously discussed.

1.5 Esterification

Synthesis of esters can be achieved by several methods such as Fischer esterification, alcoholysis of acid halides or anhydrides, or other more novel methods. Fischer esterification is the classic route to prepare esters whereby an acid reacts with an alcohol via nucleophilic acyl substitution. This is straight forward for an aliphatic alcohol. However, with phenols the use of a dehydrating agent is required[37].

$$RCOOH + R'OH \longrightarrow RCOOR' + H_2O$$

Acid halides offer greater reactivity compared with the original carboxylic acid. Therefore, phenols can be easily acylated by this route. The reaction is often carried out in the presence of pyridine or sodium hydroxide. The base assists in activating the alcohol towards electrophilic attack[37].

$$RCOCl + R'OH \longrightarrow RCOOR' + HCl$$

Alcoholysis of anhydrides occurs in a similar manner to that of acid halides. Anhydrides are less reactive than an acid halide but more reactive than a carboxylic acid. Furthermore, they are inefficient on an atomic basis and generate by-products that may be difficult to remove. Both alcoholysis reactions are often affected by steric hindrance. Bulky groups slow down the reaction, resulting in an alcohol reactivity order of primary > secondary > tertiary[38]. These three methods are easy and convenient when used under the appropriate circumstances, however novel methods can be advantageous.

Transesterification is a more novel approach to prepare esters and involves the exchange of an alkoxy group of an ester with another alcohol.

$$RCOOR' + R''OH \longleftrightarrow RCOOR'' + R'OH$$

This reaction is an equilibrium process that is often accelerated by acid or base. The reaction is also catalysed by titanium alkoxides, organotin compounds, and enzymes such as lipase. The equilibrium can be made more favourable by the removal of the product alcohol by distillation or the use of molecular sieves. Primary alcohols are often favoured over secondary or tertiary alcohols however, this is dependent upon the reaction conditions. Transesterification offers the advantage that some carboxylic acids are sparingly soluble in organic solvents whereas their esters are commonly soluble in most organic solvents. It is also

advantageous when the parent carboxylic acid is labile and difficult to isolate. The reaction may also offer better selectivity and control[39]. Some examples of transesterification include the; synthesis of polyesters, synthesis of biodiesel, and the preparation of fatty acid methyl esters for gas chromatography analysis.

Further methods of ester synthesis include the Pinner synthesis, Favorskii rearrangement, and the Baeyer-Villiger oxidation[37]. However, acylation by alcoholysis of acyl halides and transesterification form the focus of the following discussion.

1.5.1 Esterification of Polyphenols

Acylation of phenols is a common procedure. The preparation of resorcinol diacetate by the reaction of resorcinol with acetic anhydride in the presence of pyridine gives high purity resorcinol diacetate[40]. Acetylation of polyphenols offers the ability to derivatise some or all the hydroxyl groups. The methods commonly employed use acetyl chloride or acetic anhydride in pyridine (Figure 1.9)[41-43]. For example, Urano et al.[41] prepared taxifolin pentaacetate with acetic anhydride in good yield. Similarly catechin was acetylated using acetic anhydride to give catechin pentaacetate[44].

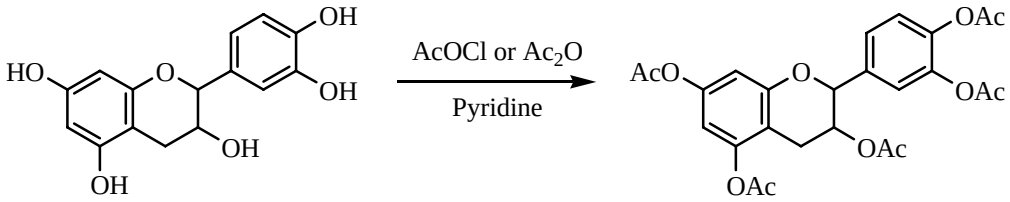
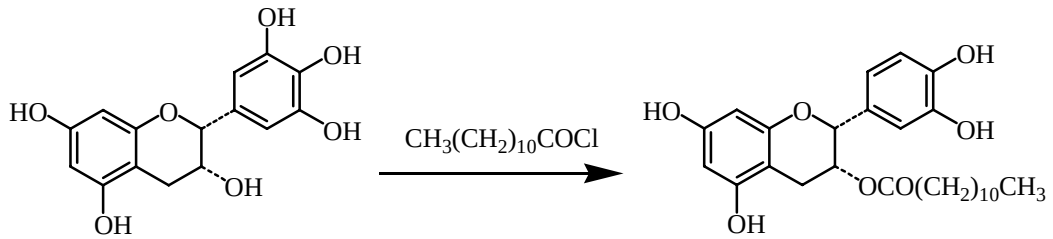


Figure 1.9: Acetylation of catechin with acetyl chloride or acetic anhydride.

Longer acyl chains such as propyl through to octadodecyl or multifunctional groups can be introduced by esterification. Again, acid chlorides or anhydrides are commonly used. For example, catechin, pine bark tannin, and lignin were reacted with various anhydrides, ranging from acetic to butyric. The reactions were conducted at 65 ^{o}C for 16 hours in the presence of a catalytic amount of 1-methylimidazole. Subsequently, the products were assessed as potential plastic modifiers[45]. In the case of catechin and pine bark tannin the degree of substitution was dependent upon the proportion of catalyst used[46]. Tetra-*O*-benzylflavan-3-ol

was esterified using hexanoyl to stearoyl chloride in dichloromethane with triethylamine and 4-dimethylaminopyridine[47]. Nutgall tannin was acylated using propanoyl chloride, hexanoyl chloride, and lauroyl chloride. The reaction was conducted in acetone with pyridine or triethylamine at room temperature for 2 hours. A maximum of 40% of the hydroxyls were acylated when lauroyl chloride was used[48]. A further example is the introduction of dicarboxylic groups to black wattle tannin to reduce the brittleness of tannin-formaldehyde adhesives. Adipoyl chloride was reacted with black wattle tannin at 75 ^{o}C over 2h in the presence of pyridine[49]. Many patents also claim the reactions of polyphenols with anhydrides and acid chlorides of varying chain length. Perrier et al.[50] claim the preparation of pentaacylated quercetin using lauroyl chloride, palmitoyl chloride, and butyric anhydride. Cottman[51] claims the preparation of polyphenolic unsaturated polymerisable esters using acid chloride derivatives.

Selective esterification is often desirable to retain the antioxidant properties of flavonoids. To utilise the antioxidant properties of flavonoids in hydrophobic environments a non-polar group can be introduced to the molecule. Subsequently, the products may find applications as pharmaceuticals. Esterification of catechin at the 3-*O* position achieves this with minimal reduction of its antioxidant capacity, which is mostly due to the ortho hydroxylation of the B-ring. Several groups have reported this. For example, the 1:1 molar reaction of epigallocatechin with acyl chlorides produced 3-*O*-acyl epigallocatechin in moderate yield (Figure 1.10)[52]. The use of preparative HPLC allowed the separation of 3-*O*-lauroyl catechin from a reaction mixture of catechin and lauroyl chloride in a 1:2 and 1:3 molar ratio, again in a moderate yield[53].



(-)-epigallocatechin

3-*O*-lauroyl-(-)-epigallocatechin

Figure 1.10: Preparation of 3-*O*-lauroyl-(-)-epigallocatechin from (-)-epigallocatechin and lauroyl chloride.

Patti et al.[54] described a method whereby the aromatic hydroxyls were protected with acetyl groups followed by the introduction of a palmitic acyl group at the 3-*O* position. Subsequent treatment with *Mucor miehei* lipase removes the protecting acetyl groups. Furthermore, 3-*O*-acyl catechin derivatives were synthesised by Matsubara et al.[47] as previously mentioned. The patent literature also claims methods of producing 3-*O*-acylated catechin by similar methods[55]. In all cases, acid chlorides or anhydrides were the acylating agent. The introduction of longer chain acyl groups either indiscriminately or selectively increases the hydrophobic/lipophilic nature of the polyphenol in question. However in all examples either separation of multi-component products or protection and deprotection steps were required to obtain a selectively esterified product[54, 56, 57].

The acylation of other bio-derived materials is similar to that previously reported. For example lignocellulose was treated with stearoyl chloride in pyridine to increase the hydrophobicity of the lignocellulosic material[58]. *O*-(Carboxymethyl)cellulose was reacted with both stearoyl chloride and a mixed anhydride to afford esterification at the primary and secondary hydroxyls[59].

Transesterification of flavonoids with acid, base, or molecular catalysts has not been reported in the scientific literature. However Roelofsen et al.[60, 61] reported that phenol was esterified by ethyl octanoate and methyl benzoate with a sodium or potassium isopropoxide catalyst over a molecular sieve. Secondly phenol was transesterified with isopropenyl acetate in the presence of a phase transfer catalyst giving excellent yields[62]. More recently, a process for transesterifying a mono-substituted phenol or naphthol with vinyl acetate was claimed[63]. This offers the advantage of lower reaction temperatures, more easily separated product, lower cost of reagents, and greater compatibility with water. The patent showed that sodium or potassium hydroxide are the most efficient base catalysts. The molar ratio of the two reagents has little effect on the percent conversion. Lin and Rei[63] also showed that dimethyl sulfoxide and *N*,*N*-dimethylformamide are effective solvents for the reaction when catalysed by triethylamine. Lastly they showed the successful transesterification of resorcinol with vinyl acetate (Figure 1.11).

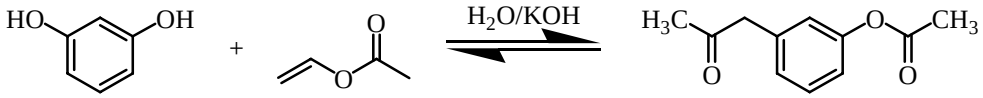


Figure 1.11: Transesterification of resorcinol with vinyl acetate.

The transesterification of cellulose derivatives with fatty acid esters has been reported by Srokova et al.[64]. *O*-(2-Hydroxyethyl)cellulose was reacted with vinyl laurate in DMSO at mild temperatures. The equilibrium was made more favourable by the use of sulphuric acid catalyst and the tautomerism of ethenol to acetaldehyde, hence preventing the reverse reaction. Similarly *O*-(carboxymethyl)cellulose was reacted with vinyl laurate catalysed by *p*-toluenesulfonic acid (Figure 1.12)[59].

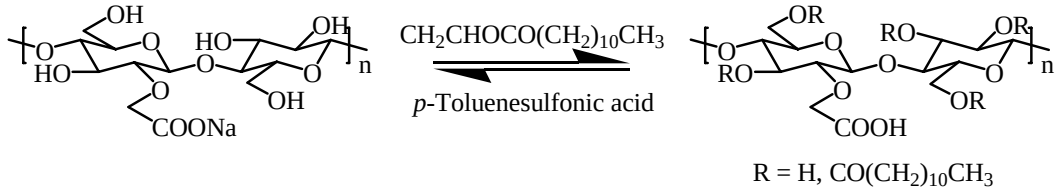


Figure 1.12: Transesterification of *O*-(carboxymethyl)cellulose with vinyl laurate.

Enzyme catalysed transesterification of polyphenols has recently been reported. Rutin, esculin, and hesperidin (glycosylated flavonoids) have been acylated with fatty acids using an immobilised lipase from *Candida antartica*. This occurred at the primary or secondary hydroxyls of the glycoside unit[65, 66]. Passicos et al.[67] acylated rutin and naringin with palmitic methyl ester over *Candida antartica* lipase B. Again this acylation occurred on the glycoside unit. (±)-cis-Flavan-4-ol was treated with lipase AY and vinyl acetate giving the 4-acetyl derivative in 44% yield[68]. None of these examples shows the reaction of phenolic groups with the esters. However, Park et al.[69] reported the lipase-catalysed transesterification of phloroglucinol with vinyl octanoate. Lipase AK from *Pseudomonas fluorescens* was used in acetonitrile solvent. This gave a 70% yield of monooctanoyl phloroglucinol after 3 days reaction.

These alternative esterification methods such as transesterification may offer more selectivity than conventional methods. There is also the prospect of other

interesting reactions such as ring opening of lactones[37], one pot reaction with a triglyceride[70], or incorporation of a polyphenol with polyesters such as polylactic acid or polycaprolactone. The substrates used for transesterification such as vinyl acetate or vinyl laurate are produced on a bulk scale, therefore they may provide a more economical route to acylated polyphenols[64].

Flavonoid fatty acid esters may have many desirable properties as shown in the literature. 3-(*O*-Lauroyl)catechin was assessed for its radical scavenging ability in a liposome system. This was shown to be greater than that of catechin due to its affinity for the membrane[53]. Uesato et al.[52] determined the antitumor activity of 3-(*O*-acyl)-(-)-epigallocatechins. The 3-(*O*-acyl)catechin derivatives were shown to strongly inhibit DNA polymerase, HL-60 cancer cell growth, and angiogenesis[47]. It should also be noted that esters between catechin and fatty acids are enzymatically cleavable. Therefore, acylated catechin will be metabolised in living bodies and is expected to be non toxic[53]. Other desirable properties may include the surfactant capability of mono-acylated flavonoids, plasticiser properties, thermal, and rheological properties.

1.6 Etherification

A simple route to ethers is the process of acid-catalysed dehydration of alcohols. This is limited to symmetric ethers from primary alcohols, such as the industrial production of diethyl ether from ethanol. The Williamson ether synthesis is a better method for preparation of more complex ethers. An alkoxide reacts with an alkyl halide via a S_N2 type reaction[38] as shown below.

$$RO^- + R'X \longrightarrow ROR' + X^-$$

The reaction can be carried out with alcohols and phenols. Alcohols react with sodium to form an alkoxide, which is subsequently treated with the halide. However, phenols are more acidic so a base such as sodium hydroxide or potassium carbonate is adequate to generate the alkoxide[37]. *C*-Alkylation is sometimes a side reaction for phenols, but can be minimised by use of a polar aprotic solvent instead of a protic solvent. This is explained by the ability of the solvent to strongly hydrogen bond to the phenoxide oxygen so that its ability to act as a nucleophile is reduced. Therefore, the carbon atoms *ortho* and *para* to the

phenoxide can compete in the nucleophilic displacement[71]. Alkyl bromides give greater yields as they are more reactive than alkyl chlorides and less susceptible to dehydrohalogenation than alkyl iodides. The reaction proceeds via a S_N2 mechanism therefore primary alkyl halides are best as E2 elimination can occur for secondary substrates. Therefore, unsymmetrical ethers should be synthesised by reaction of the more hindered alkoxide with the less hindered halide[38].

Ethers are generally of low reactivity, hence their common use as solvents. They only undergo a few reactions, one of which is acidic cleavage. Strong acid and elevated temperatures are required to cleave the ether bonds. Cyclic ethers are similar to open chain ethers and are also used as solvents due to their inertness. However, three-membered-ring ethers called epoxides or oxiranes are much more reactive[38].

The ring opening reaction of an epoxide is an efficient route to prepare ethers. Three membered rings are highly strained, this leading to their reactivity compared with larger cyclic ethers. Ring scission can occur under neutral, basic, or acidic conditions, at either of the ring carbons. This is illustrated below where the alkoxide reacts with the epoxide to give two different products (Figure 1.13)[37, 38].

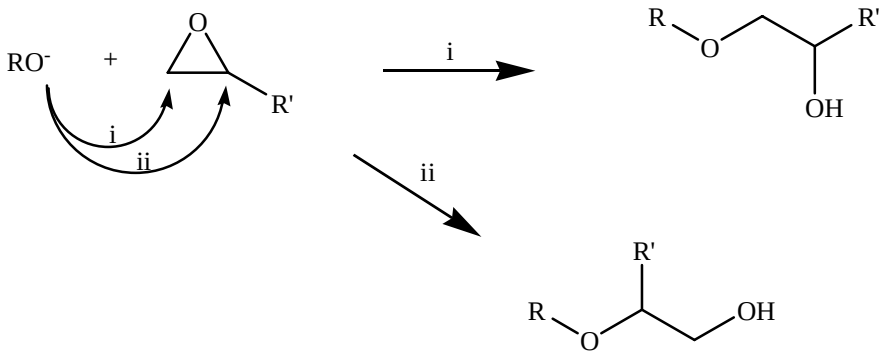
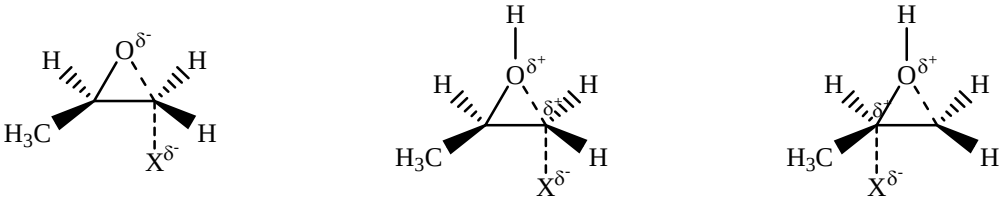


Figure 1.13: Ring opening of an epoxide can occur at either ring carbon to give two different products.

Under basic or neutral conditions monoalkyl-substituted epoxides react predominantly at the less substituted carbon (i), due to steric effects. This reaction is considered as being S_N2 in character (a) (Figure 1.14). In contrast, under acidic conditions attack at the more substituted carbon increases (ii).

However, attack at the less substituted carbon is still predominant. Acid-catalysed ring opening occurs with a partial carbenium ion character on the reaction centre. This is more in agreement with a S_N1 type mechanism (b and c). An alkyl group can stabilise this partial positive charge in (c) but will have little effect in (b). Therefore, the steric disadvantage of attack at the more substituted carbon is balanced by the inductive effect of the methyl group[72].



(a) (b) (c)

Figure 1.14: Comparison of the different epoxide ring opening mechanisms.

The rate constant of alkoxylation (k) greatly depends on the pK_a of the hydroxyl with which the epoxide is reacting[73] whereby:

$$k_{\text{phenolic OH}} > k_{\text{primary OH}} > k_{\text{secondary OH}} > k_{\text{tertiary OH}}$$

Epoxy resins constitute a major utilisation of epoxides. They consist of a prepolymer usually synthesised from bisphenol-A and epichlorohydrin and a hardener or catalyst to cross-link the prepolymers. This requires a multifunctional reagent such as polyamines or polythiols[74].

Other alkylating agents include carbonates such as ethylene carbonate or sulfates such as dimethyl sulphate[75]. However, the use of alkyl halides and epoxides are most common, especially for the synthesis of phenolic ethers.

1.6.1 Etherification of Polyphenols

Polyphenols have been etherified by several methods including the Williamson ether synthesis and alcoholysis of epoxides. For example, gallic acid, resorcinol, flavonoids, tannins, and lignin have all been etherified.

Phenol ethers have been synthesised by their reaction with alkyl halides. Johnstone and Rose[76] describe a rapid, simple, and mild procedure for the

alkylation of phenols. Phenol and methyl iodide were added to powdered potassium hydroxide stirred in DMSO at room temperature. Anisole was formed in greater than 80% yield. Gallic acid was etherified to introduce long chain ethers. The reaction of gallic acid methyl ester with 1-bromododecane afforded methyl 3, 4, 5-tridodecyloxybenzoate[77].

Flavonoids can be alkylated with alkyl halides using a variety of reaction conditions. Firstly, quercetin was alkylated with 1-chloro-2-propanol to give di-, tetra-, and penta-(hydroxypropyl)quercetin (Figure 1.15)[78].

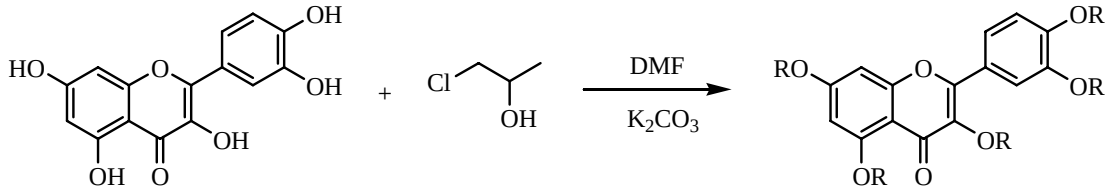


Figure 1.15: Alkylation of quercetin with 1-chloro-2-propanol in DMF over potassium carbonate.

Chrysin, a 5,7-dihydroxyflavone, was methylated at both the 5 and 7 positions using methyl iodide and potassium carbonate[79]. In a similar manner trifluoromethylated 5,7-dihydroxyflavones were alkylated using methyl iodide, ethyl iodide, and propyl iodide. Carbon alkylation at C-3 was a by-product of this reaction occurring in 20-40% yield[80]. Oroxylin A was alkyated using methyl iodide and ethyl bromide with potassium carbonate in acetone under reflux for 3 h[81]. Lastly, flavan-3-ols were reacted with C_{1}-$_{10}$ alkyl halides in the presence of lithium carbonate to give the corresponding ethers[82].

Pine bark tannin and quebracho tannin were carboxymethylated using chloroacetic acid and sodium chloroacetate. Sodium hydroxide or potassium carbonate were used as base in water or acetone, respectively. The best yields were achieved using chloroacetic acid, acetone, and potassium carbonate (Figure 1.16)[83]. Likewise, tannins were reacted with 1-bromooctane in DMF with potassium carbonate at 60 ^{o}C. From 15% to 93% of the hydroxyl groups reacted to give water-insoluble tannins ethers[48]. Furthermore, tannins were etherified to introduce cationic functional groups. Chloroalkane derivatives such as 3-chloro-

2-hydroxypropyltrimethylammonium chloride were used as the alkylating agent with aqueous sodium hydroxide. The reaction was carried out at 48 ^{o}C for 90 minutes using varying ratios of tannin to chloroalkane. The products were assessed for their potential as paint detackifiers and at breaking an oil-in-water emulsion[42].

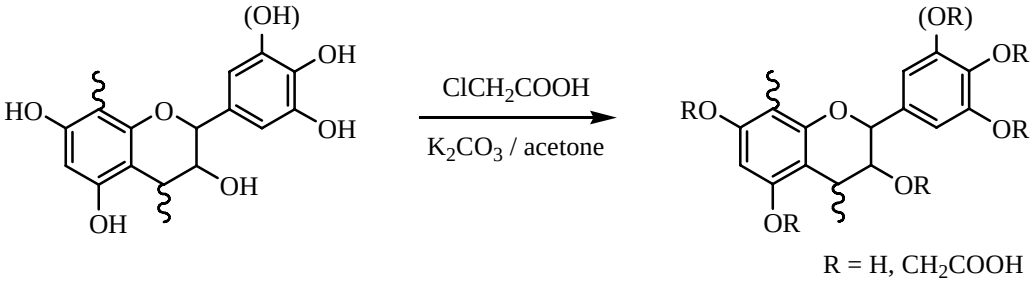


Figure 1.16: Carboxymethylation of pine bark tannin with chloroacetic acid.

Quercetin and rutin were alkylated with dimethyl sulphate and diethyl sulphate in acetone in the presence of an excess of potassium carbonate to give penta derivatives. Rutin was alkylated with dimethyl sulfate and diethyl sulphate in a sodium tetraborate solution containing a catalytic amount of sodium hydroxide to give mono derivatives. Alkylation of rutin at the 7-hydroxyl enhances the radical scavenging ability, however some quercetin derivatives were pro-oxidant, due to the free 3-hydroxyl[84]. Alternatively, hydroxyethylation of quercetin with ethylene carbonate gave the penta-(2-hydroxyethyl) ether. Lesser substituted derivatives were also obtained. However, alkylations using propylene carbonate were unsuccessful[85]

Quercetin pentaacetate can be selectively alkylated at the 7 and 4' positions using methyl iodide and potassium carbonate in anhydrous acetone. The tetra-alkylated product was also synthesised, again using methyl iodide[86, 87]. Partial methylation at other sites was also achieved using methyl iodide, potassium carbonate, acetone, and methanol[88]. Quercetin pentaacetate was also alkylated with alkyl halides from methyl to octyl in acetone in the presence of potassium carbonate to give 7-alkylquercetin tetraacetate[89].

C-Alkylation can compete with *O*-alkylation as shown by Gissot et al.[90]. Phloroglucinol was alkylated with allyl bromide or benzyl bromide. *C*-Alkylation

occurred in the absence of base due to the nucleophilicity of phloroglucinol and *O*-alkylation did not occur.

Alcoholysis of epoxides is a simple method to prepare hydroxyalkyl ethers. Simple phenolic ethers such as resorcinol di-(2-hydroxyethyl) ether have applications as chain-extenders in polyurethanes or as a co-monomer for polyesters with low gas permeability[40]. Hence, there are several recent patents outlining their preparation in high yields. The reaction of resorcinol in aqueous solution with an alkylene oxide in the presence of a catalytic amount of alkali metal hydroxide gives resorcinol di-(2-hydroxyethyl) ether in excellent yield[91]. An alternative method uses ethylene carbonate in the presence of water and an alkali metal carbonate or triorganophosphine compound to etherify resorcinol, giving greater than 90% yield[75, 92].

Simple flavonoid ethers have been prepared from alkylene oxides. Courbat and Valenza[93] prepared hydroxyethyl quercetin derivatives of varying degrees of substitution by the reaction of quercetin with ethylene oxide. Penta- and tetra-hydroxyethylquercetin were obtained by alkylation with an excess of ethylene oxide in water with a catalytic amount of potassium hydroxide (Figure 1.17). Di-hydroxyethylquercetin was obtained by the dealkylation of tetra-hydroxyethylquercetin using aluminium chloride in nitrobenzene. These products were analysed for antioxidant activity. It was shown that alkylation at C-7 enhanced the radical scavenging ability of quercetin[84].

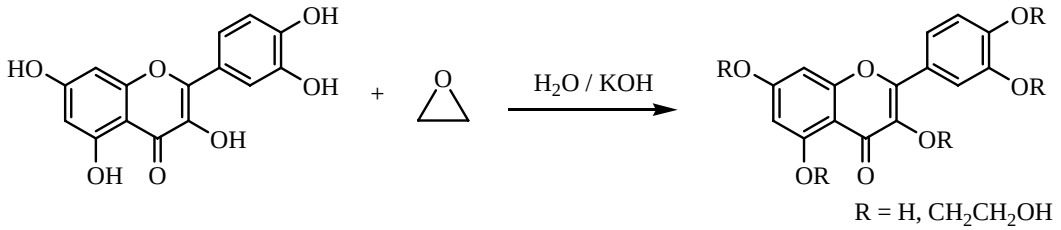


Figure 1.17: Preparation of quercetin hydroxypropyl ether from propylene oxide.

Tannins have been reacted with various epoxies such as epoxy resins and diepoxides. Hydrolysable tannins were reacted with an epoxy resin to form an epoxy-tannin chelating resin for the separation of rare earth elements. The medium was liquid paraffin at 180-190^{o}C and the reaction continued for 20

minutes. The phenolic groups of the tannin were thought to have reacted with the epoxide group of the resin. The product was shown to be an effective chelator of rare elements[94]. Tannin-epoxy compounds were synthesised from di-epoxides. Di-epoxides of diglycidyl ether and polyglycidyl ether type were used to cross-link tannins isolated from *Pinus radiata* bark (Figure 1.18). Various pH values were evaluated, with basic conditions favouring quicker reaction times. Chemical analysis by IR spectroscopy showed the opening of the epoxides was favoured in basic media[95].

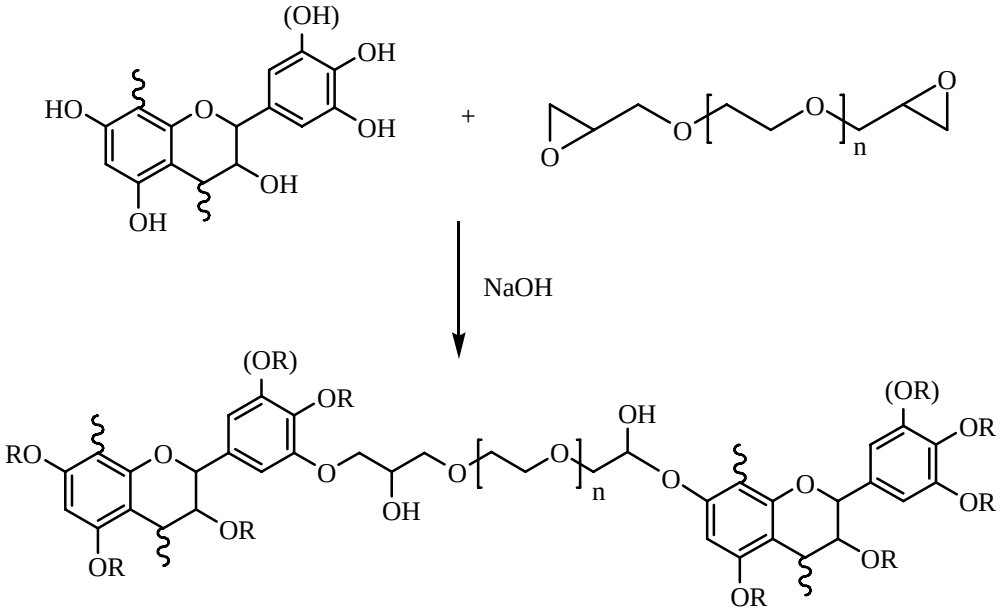


Figure 1.18: The reaction of pine bark tannin with ethylene glycol diglycidyl ether to from an extensively cross-linked polymer.

Polyphenol-epoxy resin prepolymers have been synthesised and analysed. The aim of the research was to replace bisphenol-A with a phenol fraction extracted from *Asclepias syriaca.* The polyphenol extracts were reacted with epichlorohydrin in the presence of sulphuric acid as a catalyst and sodium hydroxide as a co-catalyst. The optimum conditions for the variables studied are a reaction temperature of 70^{o}C for 4-5 hours with 40% sodium hydroxide[96]. These prepolymers were subsequently cross-linked with hardeners such as anhydrides or acids. The products were analysed to determine their suitability as resins[97].

Wood-based epoxy resins were synthesised from resorcinol-liquified wood. The liquefied wood was reacted with excess epichlorohydrin in the presence of sodium

hydroxide at 100 ^{o}C for 2.5 hours. IR spectra showed that the resin possessed epoxy functionality. The resins were subsequently cured using a stoichiometric amount of 4,4'-diaminodiphenylmethane. The wood-based epoxy resins had good strength, flexural, and adhesive properties[98].

The etherification of lignin with alkylene oxides is well studied (Figure 1.19). Ethylene oxide, propylene oxide, and butylene oxide are common alkylating agents. This alkylation offers many benefits including the plasticisation of lignin[99]. Lignin is commonly alkoxylated in a toluene suspension at 110-250 ^{o}C with potassium hydroxide catalyst in a closed pressure reactor. Alternatively, the reaction is performed in aqueous solution at room temperature with potassium hydroxide catalyst. For these products, the molar substitution ranged from 1.4 to 2.2, as determined by integration of the ^{1}H NMR spectra[73, 100-103].

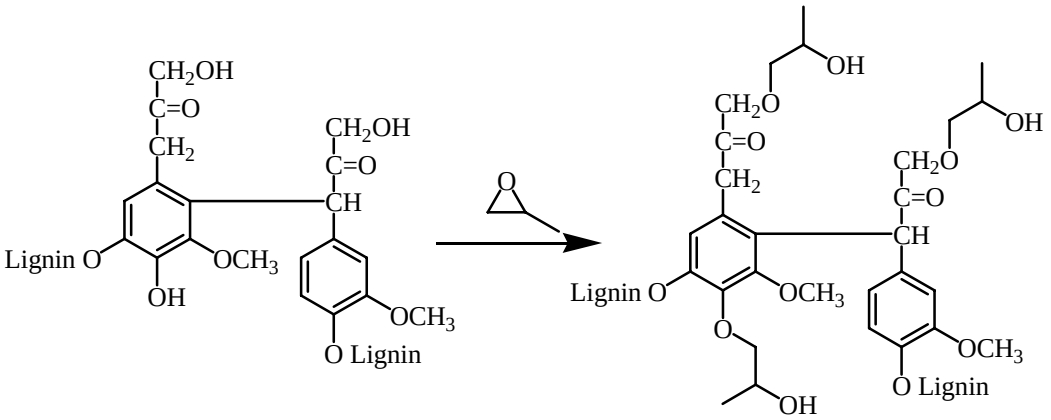


Figure 1.19: The etherification of lignin with propylene oxide.

Chain-extended hydroxypropyl lignin can also be prepared. Hydroxypropyl lignin is reacted with propylene oxide in the presence of potassium hydroxide and 18-crown-6 ether. A molar substitution between 1 and 7 was achieved[104].

The properties of lignin changed upon hydroxyalkylation. The solubility of the derivatised lignin increased in non-polar solvents, with propoxylation being more efficient than butoxylation[103]. The glass transition temperature (T_g) of lignin decreases upon hydroxyalkylation. This decrease in T_g is attributed to two factors. Firstly, intramolecular hydrogen bonding is decreased upon hydroxyalkylation, as phenolic hydroxyl groups are converted to secondary hydroxyls. Secondly, the free volume within the macromolecule increases with

alkoxylation in turn lowering the T_g[73, 100, 103]. By changing the properties, lignin hydroxyalkyl derivatives have found applications in polyurethane foams[99].

It should be noted that alkoxylation of lignin with propylene oxide can be unpredictable and dangerous. The heterogeneous reaction in the presence of solid potassium hydroxide at 140 ^{o}C can lead to an exothermic propylene oxide decomposition or homopolymerisation. Lignin in its solid state was thought to sequester the insoluble potassium hydroxide resulting in an explosion[99].

Lignin-epoxy resins have been synthesised by several methods. Firstly di-epoxides such as polyethylene glycol diglycidyl ether with differing chain lengths were used to cross-link the lignin units. The procedure involved dissolution of Kraft lignin in dilute sodium hydroxide solution at 60 ^{o}C and addition of the epoxy compound. Subsequently an aliphatic amine was added to cure the resin. Evidence for the reaction between lignin and the epoxides was shown by analysis of the gel times and weight change after acetone extraction[105]. Secondly, epichlorohydrin has been used to synthesis lignin-epoxy resins in the presence of sodium hydroxide[106]. Both methods successfully prepared lignin-epoxy polymers via ring-opening of epoxides.

1.7 Objectives

Although the acylation of flavonoids by simple routes is well studied, there is scope for further investigation. Firstly, alternative methods of preparation such as transesterification will be studied. Secondly, the physical characterisation of flavonoid esters using methods such as differential scanning calorimetry, thermogravimetric analysis, or rheology offers room for exploration. The alkylation of polyphenols is also relatively well studied, especially that of lignin. However, there is scope for investigating etherification of flavonoids such as catechin or condensed tannins using simple epoxides.

The overall aim of this project is to modify tannins into value-added products. These products may have utility in various aspects of chemistry including polymers, adhesives, and pharmaceuticals. The objectives of the project are:

- To synthesise catechin and tannin fatty acid esters by standard methods and methods that may be more industrially applicable or environmentally friendly.
- To prepare catechin and tannin hydroxypropyl ethers from alkyl halides and epoxides.
- To physically analyse the flavonoid esters and ethers and thereby investigate their chemistry, thermal behaviours, and applicability for industrial applications.

Chapter 2 - Esterification of Polyphenols

2.1 Introduction

To utilise polyphenols, such as tannins, changes in the chemical and physical properties are required. One means to change the properties is through esterification, which can introduce a range of functionalities such as fatty acids through formation of an ester linkage. The introduction of such esters would be expected to change the chemical and physical properties of the parent polyphenol. Thus, by selectively changing the properties through chemical modification the range of potential utility is increased.

The esterification of polyphenols can be achieved by several different methods. Esterification with acid chlorides and anhydrides is well known, especially for polyphenols. Several papers and patents outline methods for the preparation of catechin derivatives and give detailed analysis of the products[41-43, 46, 47, 53]. However, only a few patents exist outlining the acylation of tannins with fatty acid derivatives[48, 56, 57]. Furthermore, the characterisation of the products is limited to ^{1}H NMR spectroscopy and solubility analysis. Therefore, potential exists to investigate the chemical and physical properties of polyphenol esters prepared from acid chlorides or anhydrides.

Transesterification is one alternative route to prepare polyphenol esters. The transesterification of primary, secondary, and tertiary alcohols is well known[39]. However, the transesterification of phenols is reported only on a few occasions. First, phenol has been transesterified with ethyl octanoate and methyl benzoate catalysed by potassium hydroxide over a molecular sieve[60, 61]. Second, phenol was reacted with isopropenyl acetate over potassium carbonate[62]. In both cases, the equilibrium is moved towards the products by removal of the product alcohol. Therefore, scope exists to adapt transesterification methodologies for the introduction of esters to polyphenols such as catechin and tannin.

This chapter outlines the preparation of catechin and tannin esters by both acid chlorides and transesterification. Polyphenol model compounds are prepared by

the alcoholysis of acid chlorides. These model compounds provide a basis for characterisation and comparison with the transesterified products. A transesterification method is developed to allow the esterification of phenols using vinyl esters. The optimised method is then adapted for flavonoids such as catechin and tannin. Finally, the polyphenol esters are chemically and physically characterised to identify desirable properties for applications such as polymer additives or pharmaceuticals.

2.2 Experimental

2.2.1 Materials and Instrumentation

Solvents and reagents

Pyridine (BDH) was purified by refluxing over potassium hydroxide for 30 min followed by distillation and storage over A4 molecular sieve. Vinyl acetate (Sigma-Aldrich) was distilled, stabilised with 15 ppm hydroquinone and stored in the absence of light. All other solvents were used without further purification.

Resorcinol (BDH) was crystallised from toluene using activated charcoal to remove oxidation products. (+)-Catechin (Fluka) was dried extensively over silica gel in a vacuum desiccator. Pine bark tannin was supplied from a hot water pilot plant extraction of *Pinus radiata* bark (Scion, Rotorua). Quebracho tannin (Colatan GT100) is a natural polyphenolic extract from *Schinopsis lorentzii* and was sourced from Unitán (Buenos Aires, Argentina). Both pine bark tannin and quebracho tannin were dried extensively over silica gel in a vacuum desiccator. All other reagents were used without further purification.

Thin layer chromatography (TLC)

Silica gel 60 F_{254} aluminium TLC plates (Merck) were used with various solvents. Plates were visualised with ultraviolet (UV) light and/or iodine vapour staining.

Preparative layer chromatography (PLC)

Silica gel 60 PF_{254} with gypsum (Merck) was used to prepare PLC plates (20 cm x 20 cm x 2 mm), dried at 105 ^{o}C for 24 h, and equilibrated at room temperature and humidity for >48 h. Plates were developed in a 22 cm x 22 cm x 7 cm tank

and visualised under UV light. Bands were removed from the plate, desorbed with an appropriate solvent, and concentrated by rotary evaporation.

Column chromatography

A silica stationary phase (silica 60 gel, 230-400 mesh, pH 6.5-7.5, Merck) was loaded onto a column (23 mm x 250 mm) in hexane followed by acid-washed sand. The products were loaded onto the column using the least polar solvent, and eluted using various solvents. The collected fractions were analysed by TLC and fractions of the same retention factor (R_f) were pooled, concentrated by rotary evaporation, and weighed.

Nuclear magnetic resonance (NMR) spectroscopy

All solution state NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer (^{1}H: 400.13 MHz and ^{13}C: 100.62 MHz) with a 5 mm inverse broad band probe (Bruker). Chemical shifts were reported in δ ppm with reference to the solvent peak. Carbon multiplicities were determined by DEPT-135 and DEPT-90 (distortionless enhancement by polarisation transfer) experiments. 2D NMR techniques were conducted using standard programs; DQF-COSY (double quantum filtered correlated spectroscopy), LR-COSY (long range COSY), HSQC (heteronuclear single quantum coherence), and HMBC (heteronuclear multiple bond correlation). A gradient was used in all two dimensional (2D) experiments to reduce acquisition time and decrease noise. The proton 90^o pulse was calibrated for all cross polarisation experiments.

Solid state ^{13}C NMR spectra were obtained on a Bruker Avance DRX 200 instrument (^{1}H: 200.13 MHz and ^{13}C: 50.33 MHz) with a 7 mm doubly tuned ^{1}H/X MAS probe (Bruker). Samples were packed into a zirconia rotor fitted with a Kel-F cap and spun at 5 kHz. A CP-MAS (cross polarisation-magic angle spinning) pulse program with a ^{1}H preparation pulse of 5.56 μs, ^{1}H decoupling field of 47 kHz, and an acquisition time of 20 ms were used.

NMR spectroscopy was used to quantify the degree of substitution or molar substitution of derivatised polyphenols. This was routinely achieved by integration of the ^{1}H NMR spectra where the integrals were calibrated against the aromatic protons (6-8 ppm). The average number of aromatic protons for pine

bark tannin was 3.9 and for quebracho tannin was 4.9. Furthermore, NMR spectroscopy of polyphenols often gave broad (br) signals, therefore chemical shifts were often reported as a range.

Fourier transform infrared (FTIR) spectroscopy

Infrared spectra were recorded on a Bruker Vector 33 FTIR instrument. Solid samples were prepared as KBr discs and liquids prepared as neat solutions between two KBr windows. The absorbance was measured between 400-4000 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans.

Electrospray ionisation mass spectrometry (ESI-MS)

ESI-MS spectra were recorded on a Thermo Finnigan LCQ Deca XP ion trap mass spectrometer in negative ion mode. A low flow negative ion tune file was used which had the following settings: sheath gas flow rate, 20 arb; aux/sweep gas flow rate, 4 arb; spray voltage, 4.90 kV; spray current, 78 μA; capillary temperature, 245 ^{o}C; capillary voltage, –16 V; tube lens offset, –20 V; lens voltage, 93 V. Samples were dissolved in methanol, passed through a 0.45 μm Teflon (PTFE) syringe filter and injected into the mass spectrometer by direct infusion. The spectra were recorded on LCQ Tune software and processed using Xcalibur software.

Gel permeation chromatography (GPC)

All GPC was performed on a Polymer Laboratories PL-GPC-50 integrated GPC system with a Var UV detector, WellChrom K-2301 refractive index (RI) detector, and a PL-AS RT GPC autosampler. Results were collected and processed using Cirrus software. Two PLgel mixed-C (300 x 7.5 mm, 5 μm) columns connected in series and protected by a guard column (50 x 7.5 mm) of the same material were used. Samples were eluted using chloroform at 1.0 $mLmin^{-1}$ with a column temperature of 25 ^{o}C and detected by UV (254 nm) and RI. Samples were dissolved in chloroform (1.0 $mgmL^{-1}$), filtered through a 0.45 μm nylon syringe filter, and a volume of 100 μL was injected onto the column. Polystyrene standards (MW 580-7500000 $gmol^{-1}$) were used to generate a calibration curve. However, as the standards were of a different nature to the analytes, the calibration was not absolute and provided only a relative scale of molecular weight.

Differential scanning calorimetry (DSC)

A TA Instruments Q1000 was used for all DSC. This was calibrated from –85 to 450 ^{o}C using indium, tin, lead, and zinc. Standard aluminium pans or hermetic pans were used according to the fluidity of the sample. The general program involved equilibrating at –70 ^{o}C, heating at 10 ^{o}Cmin^{-1} to 110 ^{o}C, cooling at 10 ^{o}Cmin^{-1} to –70 ^{o}C, and heating at 10 ^{o}Cmin^{-1} to 180 ^{o}C. Exothermic processes were shown as positive peaks.

Thermogravimetric analysis (TGA)

A TA Instruments TGA Q500 instrument was used for all thermogravimetric analysis. Platinum pans were used under a nitrogen flow of 50 mLmin^{-1}. The instrument was calibrated via a Curie point calibration with nickel. Samples were heated from room temperature to 800 ^{o}C at a rate of 10 ^{o}Cmin^{-1}.

Antioxidant analysis

Antioxidant testing was performed using an 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) method by Dr Sarah Morrison and Carolyn Lister (Crop and Food Research, New Zealand). The sample (50 mg) was extracted in 80% acetone (10 mL) for 4 h at 4 ^{o}C in the absence of light. The extract was centrifuged at 3220 *g* for 10 min at 4 ^{o}C and the supernatant collected. Antioxidant activity was measured using a modified ABTS radical scavenging assay comparing antioxidant activity to Trolox, a water-soluble vitamin E analogue[107, 108]. Results were expressed as the amount of Trolox equivalent antioxidant capacity (TEAC) per gram of dry weight (μmol TEAC/g DW). Triplicate analyses were undertaken.

In vitro ultraviolet light absorption

Testing of *in vitro* ultraviolet light absorption was performed by Dermatest Pty (Sydney, Australia) using methodology based on that described by Diffey and Robson[109]. The sample was dissolved in aqueous solution at *ca* 10 mg/50 mL. A Shimadzu UV-2450 spectrophotometer fitted with an integrating sphere was used to measure the UV absorbance in the range of 260 to 400 nm. The *in vitro* skin protection factor (SPF) was determined (Equation 1) using values provided by Diffey et al.[110].

$$SPF = {}_{290}\sum^{400} E(\lambda)\sum(\lambda) \,/\, {}_{290}\sum^{400} E(\lambda)\sum(\lambda) \,/\, PF(\lambda) \qquad \text{(Equation 1)}$$

where:

E(λ) = Spectral irradiance of terrestrial sunlight under defined conditions.

Σ(λ) = Erythemal effectiveness of ultraviolet radiation at wavelength λ nm in producing delayed erythema in human skin.

PF(λ) = Protection factor

Polymethyl methacrylate film ultraviolet light absorption

Ultraviolet light absorption was performed on a polymethyl methacrylate (PMMA) film by Dermatest Pty (Sydney, Australia). The sample was prepared in an oil emulsion at 5% content. The sample (30 mg) was spread onto a roughened PMMA plate (5 x 5 cm) using a cot-coated finger. The SPF was calculated using Equation 1.

2.2.2 Synthesis of Model Compounds

Preparation of resorcinol monoacetate and diacetate

Resorcinol (0.56 g, 5.1 mmol) was dissolved in cold sodium hydroxide solution (10 mL, 0.5 $molL^{-1}$). Excess acetic anhydride (1.4 mL, 15 mmol) was added and the mixture was stirred for 5 min. The reaction mixture was poured into ether (20 mL), washed with dilute HCl (0.1 $molL^{-1}$, 2 x 30 mL), water (3 x 30 mL), and the solvent removed by rotary evaporation to yield a yellow oil (0.57 g). Chromatography (PLC) with CH_2Cl_2/EtOAc (4:1) gave two bands.

Band 1 (R_f = 0.79) gave resorcinol diacetate (**1;** 214 mg, 22%) as a yellow oil.

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 7.34 (1H, t, $J_{4,5}$ = 8.2 Hz, H-5), 6.97 (2H, dd, $J_{4,5}$ = 8.2 Hz, $J_{2,4}$ = 2.2 Hz, H-4, 6), 6.91 (1H, t, $J_{2,4}$ = 2.1 Hz, H-2), 2.24 (6H, s, CH_3).

^{13}C NMR (400 MHz, CD_3COCD_3): δ_C 169.3 (CO), 151.5 (C-1, 3), 130.0 (C-5), 119.3 (C-4, 6), 115.8 (C-2), 21.2 (CH_3).

IR (KBr disc): ν(CO) 1770 (s) cm^{-1}.

Band 2 (R_f = 0.59) gave resorcinol monoacetate (**2;** 112 mg, 11%) as a thick golden-yellow oil.

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 7.15 (1H, t, $J_{4,5}$ = 8.2 Hz, H-5), 6.63 (1H, dd, $J_{5,6}$ = 8.6 Hz, $J_{2,6}$ = 1.9 Hz, H-6), 6.59 (1H, dd, $J_{4,5}$ = 8.1 Hz, $J_{2,4}$ = 1.3 Hz, H-4), 6.54 (1H, t, $J_{2,4}$ = 2.2 Hz, H-2), 2.25 (3H, s, CH_3).

^{13}C NMR (400 MHz, CD_3COCD_3): δ_C 170.6 (CO), 157.4 (C-3), 151.7 (C-1), 130.4 (C-5), 113.7 (C-6), 113.6 (C-4), 109.5 (C-2), 21.4 (CH_3).

IR (KBr disc): ν(CO) 1738 (s) cm^{-1}.

*Preparation of resorcinol dilaurate (**3**)*

Resorcinol (0.56 g, 5.1 mmol) was dissolved in pyridine (3.0 mL). Excess lauroyl chloride (3.4 mL, 14 mmol) was added and the solution heated to 40 ^{o}C for 1 h. The reaction was quenched by adding water and the mixture subsequently poured into ether (15 mL). The ethereal layer was washed with water (2 x 20 mL) and the solvent removed by rotary evaporation. Crystallisation of the product from ethanol gave ***resorcinol dilaurate*** (**3;** 1.25 g, 52%) as a white solid, mp 43-44 ^{o}C.

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 7.42 (1H, t, $J_{4,5}$ = 8.2 Hz, H-5), 7.01 (2H, dd, $J_{4,5}$ = 8.2, $J_{2,4}$ = 2.2 Hz, H-4, 6), 6.94 (1H, t, $J_{2,4}$ = 2.2 Hz, H-2), 2.56 (4H, t, J = 7.4 Hz, $OCOCH_2$), 1.72 (4H, tt, J = 7.4 Hz, $OCOCH_2C\underline{H}_2$), 1.30 (32H, m, CH_2), 0.88 (6H, t, J = 6.8 Hz, CH_3).

^{13}C NMR (400 MHz, CD_3COCD_3): δ_C 171.6 (CO), 151.9 (C-1, 3), 129.9 (C-5), 119.3 (C-4, 6), 116.1 (C-2), 34.0 (α-CH_2), 32-29 (CH_2), 25.0 (β-CH_2), 22.8 (CH_2) 14.0 (CH_3).

IR (KBr disc): ν(CO) 1757 (s) cm^{-1}.

Found: C, 76.14%; H, 10.27%.

$C_{30}H_{50}O_4$ calculated: C, 75.90%; H, 10.62%.

*Preparation of catechin pentaacetate (**4**)*

Catechin (0.50 g, 1.7 mmol), excess acetic anhydride (3.6 mL, 38 mmol), and 1-methylimidazole (10% of catechin, 48 μL) were transferred into a flask fitted with a condenser and drying tube and stirred for 16 h at 65 ^{o}C. The reaction was quenched with cold water (100 mL) and the precipitate collected and washed with water until the filtrate was near neutral pH. Crystallisation of the product from acetone/water yielded catechin pentaacetate (**4**; 0.63 g, 73%) as white crystals, mp 125-127 ^{o}C (lit. 125-126 ^{o}C[43]).

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 7.37 (1H, dd, $J_{5',6'}$ = 8.4 Hz, $J_{2',6'}$ = 2.0 Hz, H-6'), 7.32 (1H, d, $J_{2',6'}$ = 2.0 Hz, H-2'), 7.30 (1H, d, $J_{5',6'}$ = 8.4 Hz, H-5'), 6.72 (1H, d, $J_{6,8}$ = 2.2 Hz, H-8), 6.65 (1H, d, $J_{6,8}$ = 2.2 Hz, H-6), 5.35 (1H, dt, $J_{2,3}$ = $J_{3,4}$ = 6.1 Hz, H-3), 5.29 (1H, d, $J_{2,3}$ = 6.5 Hz, H-2), 2.89 (1H, dd, $J_{4,4}$ = 17.0 Hz, $J_{3,4}$ = 6.6 Hz, H-4a), 2.76 (1H, dd, $J_{4,4}$ = 17.0 Hz, $J_{3,4}$ = 6.6 Hz, H-4b), 2.28-2.25 (12H, m, 5, 7, 3', 4'-$OCOCH_3$), 1.95 (3H, s, 3-$OCOCH_3$).

^{13}C NMR (400 MHz, CD_3COCD_3): δ_C 169.7 (3-OCOMe), 169.0 (7-OCOMe), 168.6 (5-OCOMe), 168.3 (3'-OCOMe), 168.2 (4'-OCOMe), 154.8 (C-9), 150.6 (C-7), 150.3 (C-5), 142.9 (C-3', 4'), 136.8 (C-1'), 124.8 (C-6'), 124.2 (C-5'), 122.4 (C-2'), 111.0 (C-10), 109.5 (C-6), 107.9 (C-8), 78.0 (C-2), 68.3 (C-3), 24.3 (C-4), 20.4 (3, 5, 7, 3', 4'-CH_3).

IR (KBr disc): ν(CO) 1767 (s), 1745 (sh) cm^{-1}.

*Synthesis of catechin pentalaurate (**5**)*

Catechin (0.097 g, 0.33 mmol) was dissolved in pyridine (10 mL). Excess lauroyl chloride (0.55 mL, 2.3 mmol) was slowly added with stirring. The reaction flask was fitted with a condenser and drying tube and the mixture heated to 75 ^oC for 2 h. The reaction was quenched with water (50 mL) and the mixture poured into ether (10 mL). Water was separated and the ethereal layer was washed with NaOH (0.5 $molL^{-1}$, 2 x 30 mL), HCl (0.1 $molL^{-1}$, 2 x 30 mL), saturated NaCl (3 x 30 mL), and dried with $MgSO_4$. The solution was concentrated by rotary evaporation to yield a pale yellow solid (0.17 g). The product was further purified by chromatography (PLC) with EtOAc/hexane (1:1) to give one band.

Band 1 (R_f = 0.83) gave ***catechin pentalaurate*** (**5**; 91 mg, 23%) as a white solid, mp 43-46 ^oC.

^{1}H NMR (400MHz, $CDCl_3$): δ_H 7.23 (1H, dd, $J_{5',6'}$ = 8.4 Hz, $J_{2',6'}$ = 1.7 Hz, H-6'), 7.17 (1H, d, $J_{5',6'}$ = 8.4 Hz, H-5'), 7.16 (1H, d, $J_{2',6'}$ = 1.5 Hz, H-2'), 6.63 (1H, d, $J_{6,8}$ = 2.1 Hz, H-6), 6.56 (1H, d, $J_{6,8}$ = 2.2 Hz, H-8), 5.25 (1H, dt, $J_{2,3}$ = $J_{3,4}$ = 5.8 Hz, H-3), 5.12 (1H, d, $J_{2,3}$ = 6.3 Hz, H-2), 2.85 (1H, dd, $J_{4,4}$ = 17.0 Hz, $J_{3,4}$ = 5.0 Hz, H-4a), 2.63 (1H, dd, $J_{4,4}$ = 17.0 Hz, $J_{3,4}$ = 5.0 Hz, H-4b), 2.6-2.5 (8H, m, 5, 7, 3', 4'-$OCOCH_2$), 2.22 (2H, t, J = 7.5 Hz, 3-$OCOCH_2$), 1.8-1.7 (8H, m, 5, 7, 3', 4'-$OCOCH_2C\underline{H}_2$), 1.49 (2H, t, J = 6.8 Hz, 3-$OCOCH_2C\underline{H}_2$), 1.4-1.2 (80H, m, 3, 5, 7, 3', 4'-CH_2), 0.88 (15H, t, J = 6.7 Hz, 3, 5, 7, 3', 4'-CH_3).

^{13}C NMR (400MHz, $CDCl_3$): δ_C 172.1 (3-OCO), 171.5 and 171.2 (5, 7-OCO), 171.2 and 171.1 (3', 4'-OCO), 154.7 (C-9), 150.3 (C-7), 149.8 (C-5), 142.5 (C-3', 4'), 136.4 (C-1'), 124.7 (C-6'), 124.0 (C-5'), 122.1 (C-2'), 110.5 (C-10), 109.1 (C-8), 107.9 (C-6), 78.1 (C-2), 68.5 (C-3), 35-34 (3, 5, 7, 3', 4'-OCO$\underline{C}H_2$), 32-29 (CH_2), 25.2 (3, 5, 7, 3', 4'-OCOCH$_2\underline{C}H_2$), 24.3 (C-4), 23.0 (CH_2), 14.4 (CH_3).
IR (KBr disc): ν(CO) 1763 (s), 1741 (sh) cm^{-1}.
Found: C, 75.16%; H, 10.12%.
$C_{75}H_{124}O_{11}$ calculated: C, 74.96%; H, 10.40%.

Synthesis of catechin laurate with 3 mole equivalents of acylating agent
Catechin (0.40 g, 1.4 mmol) was dissolved in pyridine (10 mL) and heated to 75 ^{o}C under a positive pressure of nitrogen gas. Lauroyl chloride (0.98 mL, 4.2 mmol) was slowly added via dropping funnel with stirring. Samples were taken at regular intervals throughout the reaction to monitor the reaction progress, quenched with ethanol, and analysed by TLC. After 24 h the reaction was quenched with water (50 mL) and the mixture was concentrated by rotary evaporation to yield a pale brown solid (2.2 g). Chromatography (column) with EtOAc/hexane (2:1) followed by EtOAc/MeOH (1:1) yielded many fractions. Analysis by thin layer chromatography with EtOAc/hexane (2:1) allowed their pooling to give four fractions. The component R_f's of each were determined by TLC.

Fraction 1 (R_f = 0.77, 0.64) gave a mixture of catechin laurate derivatives as a yellow solid (**6**; 16 mg).
^{1}H NMR (400MHz, $CDCl_3$): δ_H 7.5-5.8 (5H, m, H-6, 8, 2', 5', 6'), 5.5-3.8 (2H, m, H-2, 3), 3.1-2.3 (8H, m, 5, 7, 3', 4'-OCOCH$_2$, H-4), 2.20 (1H, m, 3-OCOCH$_2$), 1.7-1.5 (10H, m, 3, 5, 7, 3', 4'-OCOCH$_2$C$\underline{H}_2$), 1.5-1.0 (62H, m, CH_2), 0.87 (14H, t, J = 6.6 Hz, CH_3).
IR (KBr disc): ν(CO) 1773 (m), 1739 (sh) cm^{-1}.

Fraction 2 (R_f = 0.77, 0.64, 0.53) gave a mixture of catechin laurate derivatives and lauric acid (60 mg).
^{1}H NMR (400MHz, $CDCl_3$): δ_H 7.4-5.7 (5H, m, H-6, 8, 2', 5', 6'), 5.4-3.8 (2H, m, H-2, 3), 3.0-2.4 (6H, m, 5, 7, 3', 4'-OCOCH$_2$, H-4), 2.33 (1.5H, t, J = 7.5 Hz, acid

α-CH_2), 2.20 (1.1H, m, 3-$OCOCH_2$), 1.8-1.7 (4.5H, m, 3, 5, 7, 3', 4'-$OCOCH_2C\underline{H_2}$), 1.62 (1.6H, m, acid β-CH_2), 1.5-1.0 (61H, m, CH_2), 0.89 (12H, t, J = 6.7 Hz, CH_3).
IR (KBr disc): ν(CO) 1775 (m), 1740 (sh) cm^{-1}.

Fraction 3 (R_f = 0.77, 0.64, 0.53, 0.40) gave a mixture of catechin laurate derivatives and lauric acid (140mg).

Fraction 4 (R_f = 0.64, 0.53, 0.40, 0.34) gave a mixture of catechin laurate derivatives and lauric acid (560 mg).

Chromatography (PLC) with EtOAc/hexane (1:1) of fraction 2 yielded four bands.

Band 1 (R_f = 0.80) gave an isomeric mixture of catechin tetralaurates (**7**; 13 mg, 32%) as a yellow oil, mp –3-0 ^oC.
^1H NMR (400MHz, $CDCl_3$): δ_H 7.4-6.8 (3H, m, H-2', 5', 6'), 6.7-6.1 (2H, m, H-6, 8), 5.4-3.9 (2H, m, H-2, 3), 3.2-2.7 (16H, m, H-4), 2.6-2.5 (6H, m, 5, 7, 3', 4'-$OCOCH_2$), 2.2 (2H, m, 3-$OCOCH_2$), 1.8-1.5 (7H, m, 3, 5, 7, 3', 4'-$OCOCH_2C\underline{H_2}$), 1.5-1.0 (64H, m, 3, 5, 7, 3', 4'-CH_2), 0.87 (14H, t, J = 5.6 Hz, CH_3).
^{13}C NMR (400MHz, $CDCl_3$): δ_C 173-171 (3, 5, 7, 3', 4'-CO), 158-130 (C-5, 7, 9, 3', 4', 1'), 130-100 (C-6, 8, 10, 2', 5', 6'), 85-65 (C-2, 3), 35-34 (3, 5, 7, 3', 4'-$OCO\underline{C}H_2$), 32-29 (CH_2), 25.3 (3, 5, 7, 3', 4'-$OCOCH_2\underline{C}H_2$), 24.3 (C-4), 23.0 (CH_2), 14.0 (CH_3).
IR (KBr disc): ν(CO) 1773 (m), 1740 (sh) cm^{-1}.

Band 2 (R_f = 0.60) gave an isomeric mixture of catechin trilaurates (**8**; 7.4 mg, 19%) as a yellow solid.
^1H NMR (400MHz, CD_3COCD_3): δ_H 7.4-6.8 (3H, m, H-2', 5', 6'), 6.3-5.9 (2H, m, H-6, 8), 5.4-3.9 (2H, m, H-2, 3), 3.2-2.7 (21H, m, H-4), 2.55 (5H, m, 5, 7, 3', 4'-$OCOCH_2$), 2.20 (1H, m, 3-$OCOCH_2$), 1.8-1.5 (5H, m, 3, 5, 7, 3', 4'-$OCOCH_2C\underline{H_2}$), 1.5-1.0 (47H, m, 3, 5, 7, 3', 4'-CH_2), 0.86 (10H, t, J = 5.5 Hz, CH_3)
IR (KBr disc): ν(CO) 1764 (m), 1738 (sh) cm^{-1}.

Band 3 (R_f = 0.39) gave an isomeric mixture of catechin dilaurates (**9**; 5.0 mg, 13%) as a yellow solid.

^{1}H NMR (400MHz, CD_3COCD_3): δ_H 7.2-6.6 (3H, m, H-2', 5', 6'), 6.4-5.8 (2H, m, H-6, 8), 5.4-3.9 (2H, m, H-2, 3), 3.2-2.7 (61H, m, H-4), 2.55 (3H, m, 5, 7, 3', 4'-$OCOCH_2$), 2.20 (2H, m, 3-$OCOCH_2$), 1.7-1.5 (4H, m, 3, 5, 7, 3', 4'-$OCOCH_2C\underline{H}_2$), 1.5-1.0 (40H, m, 3, 5, 7, 3', 4'-CH_2), 0.86 (11H, t, J = 6.4 Hz, CH_3).

IR (KBr disc): ν(CO) 1738 (m) cm^{-1}.

ESI-MS (MeOH): *m/z* 653.4 $[M-H]^-$.

Band 4 (R_f = 0.21) gave an unknown solid (2.9 mg, 7%).

Synthesis of pine bark tannin laurate with excess acylating agent

Pine bark tannin (PBT) (1.1 g, 3.8 mmol of PBT units) was dispersed in pyridine (10 mL) and chloroform (5 mL) at 75 ^oC under a positive pressure of nitrogen. Excess lauroyl chloride (7.1 mL, 30 mmol) was slowly added with stirring. Samples were taken throughout the reaction to monitor reaction progress, quenched with ethanol, and analysed by TLC. After 24 h the reaction mixture was quenched with water (30 mL) and concentrated by rotary evaporation. The reaction mixture was poured into ether (20 mL), washed with saturated $NaHCO_3$ solution (4 x 50 mL), HCl (0.1 $molL^{-1}$, 2 x 50 mL), water (1 x 50 mL), saturated NaCl solution (2 x 50 mL), and dried with $MgSO_4$. The solution was concentrated by rotary evaporation to yield a brown oily product (1.38 g). This extraction was repeated using NaOH (0.5 $molL^{-1}$, 4 x 50 mL) in place of saturated $NaHCO_3$ to yield a brown oily product (**10**; 0.57 g, 24%).

^{1}H NMR (400MHz, $CDCl_3$): δ_H 8.0-6.0 (3.9H, br, Ar-H), 6.0-3.5 (6.1H, br, C-ring), 3.0-2.0 (13H, br, H-4, α-CH_2), 1.9-1.5 (15H, br, β-CH_2), 1.5-1.0 (138H, br, CH_2), 1.0-0.5 (32H, t, J = 6.1 Hz, CH_3).

IR (KBr disc): ν(CO) 1768 (s) cm^{-1}.

Synthesis of PBT laurate with 3 mole equivalents of acylating agent

PBT (0.97 g, 3.3 mmol) was dispersed in pyridine (10 mL) and chloroform (5 mL) at 75 ^oC, under a positive pressure of nitrogen. Lauroyl chloride (2.4 mL, 10 mmol) was slowly added with stirring. Samples were taken throughout the

reaction to monitor the reaction progress, quenched with ethanol, and analysed by TLC. After 24 h the reaction mixture was quenched with water, stirred for 10 min, and concentrated by rotary evaporation. The reaction mixture was poured into ether (20 mL), washed with saturated $NaHCO_3$ solution (10 x 50 mL), HCl (0.1 $molL^{-1}$, 2 x 50 mL), saturated NaCl solution (3 x 50 mL), and dried with $MgSO_4$. The solution was concentrated by rotary evaporation to yield a dark brown oily product (1.6 g). Chromatography (PLC) with EtOAc/hexane (1:1) yielded three bands.

Band 1 (R_f = 0.95) gave a mixed PBT laurate derivative (**11**; 4.8 mg, 10%) as a brown gelatinous oil.
^1H NMR (400MHz, CD_3COCD_3): δ_H 7.8-6.0 (3.9H, br, Ar-H), 6.0-3.5 (5H, br, C-ring), 2.8-2.1 (7.8H, br, H-4, α-CH_2), 1.9-1.5 (19H, br, β-CH_2), 1.5-1.0 (80H, br, CH_2), 1.0-0.5 (16H, t, J = 6.4 Hz, CH_3).
IR (KBr disc): ν(CO) 1773 (m), 1740 (sh) cm^{-1}.

Band 2 (R_f = 0.85) gave a mixed PBT laurate derivative and lauric acid (4.1 mg).

Band 3 (R_f = 0.70-0.00) gave a mixed PBT laurate derivative and lauric acid (14.5 mg).

Synthesis of quebracho tannin laurate with excess acylating agent
Quebracho tannin (QT) (1.0 g, 3.6 mmol of QT units) was dispersed in pyridine (5 mL) and chloroform (7 mL) at 75 ^oC. Excess lauroyl chloride (10 mL, 42 mmol) was added and the mixture stirred for 24 h. Samples were taken at regular intervals to monitor the reaction progress, quenched with ethanol, and analysed by TLC. The reaction was quenched with water (30 mL) and the mixture was concentrated by rotary evaporation. The mixture was dissolved in hexane (20 mL), washed with HCl (2 x 50 mL, 0.1 $molL^{-1}$), water (2 x 50 mL), saturated $NaHCO_3$ (2 x 50 mL), water (2 x 50 mL), and dried with $MgSO_4$. The solution was concentrated by rotary evaporation to yield a brown oily product (2.7 g). Column chromatography with EtOAc/hexane (2:1) yielded many fractions. Analysis by TLC with EtOAc/hexane (2:1) allowed their pooling to give three fractions. The component R_f's of each were determined by TLC.

Fraction 1 (R_f = 0.88-0.92) gave a mixed QT laurate derivative (**12**; 120 mg, 8%) as a brown gelatinous oil.

^{1}H NMR (400MHz, $CDCl_3$): δ_H 7.8-6.0 (4.9H, br, Ar-H), 5.5-3.0 (5.7H, br, C-ring), 2.8-2.0 (11H, br, H-4, α-CH_2), 1.9-1.5 (16H, br, β-CH_2), 1.5-1.0 (131H, br, CH_2), 1.0-0.5 (33H, t, J = 6.7 Hz, CH_3).

IR (KBr disc): ν(CO) 1775 (s), 1740 (sh) cm^{-1}.

Fraction 2 (R_f = 0.86-0.92, 0.63) gave a mixed QT laurate derivative and lauric acid (110 mg).

Fraction 3 (R_f = 0.82-0.92, 0.58, 0.33, 0.16) gave a mixed QT laurate derivative and lauric acid (1.1 g).

Synthesis of QT laurate with 3 mole equivalents of acylating agent

QT (2.3 g, 8.3 mmol) was dispersed in pyridine (5 mL) at 75 ^{o}C. Lauroyl chloride (5.8 mL, 25 mmol) was added and the mixture stirred for 24 h. Samples were taken at regular intervals to monitor the reaction progress, quenched with ethanol and analysed by TLC. The reaction was quenched with water (30 mL) and the mixture concentrated by rotary evaporation to yield a brown oil. Column chromatography with EtOAc/$CHCl_3$/MeOH (8:1:1) yielded many fractions. Analysis by TLC with $CHCl_3$/EtOAc (1:1) allowed their pooling to give three fractions. The component R_f's of each were determined by TLC.

Fraction 1 (R_f = 0.83-0.88) gave a mixed QT laurate derivative (**13**; 380 mg, 19%) as a brown gelatinous oil.

^{1}H NMR (400MHz, $CDCl_3$): δ_H 7.5-6.0 (4.9H, br, Ar-H), 5.5-3.0 (1.9H, br, C-ring), 2.8-2.1 (4.8H, br, H-4, α-CH_2), 1.9-1.5 (8.2H, br, β-CH_2), 1.5-1.0 (48H, br, CH_2), 1.0-0.6 (10H, t, J = 5.7 Hz, CH_3).

IR (KBr disc): ν(CO) 1774 (s), 1739 (sh) cm^{-1}.

Fraction 2 (R_f = 0.83, 0.60-0.80, 0.51) gave a mixed QT laurate derivative and lauric acid (310 mg).

Fraction 3 (R_f = 0.60-0.80, 0.51) gave a mixed QT laurate derivative and lauric acid (290 mg).

2.2.3 Transesterification

Screening acid and base catalysts for transesterification activity

Resorcinol (*ca* 0.10g, 0.9 mmol) was dissolved in water (*ca* 5ml) and 10% catalyst (solution, solid, or neat liquid) was added. A range of common catalysts were used: hydrochloric acid, sulphuric acid, *p*-toluenesulfonic acid (PTSA), potassium hydroxide, triethylamine (TEA), and 4-dimethylaminopyridine (DMAP)[39]. Excess vinyl acetate (*ca* 0.2 mL, 2.1 mmol) was added and the reaction mixture was stirred for 24 hours after which the excess vinyl acetate was removed by rotary-evaporation. ^{1}H NMR spectroscopy was used to determine the ratio of products to starting material.

Transesterification of resorcinol

A general method for the transesterification of resorcinol with a vinyl ester was used. Resorcinol (*ca* 0.50 g, 4.5 mmol) was dissolved in solvent (20 mL) and a catalyst (10% mol/mol of resorcinol) added. Excess acylating agent (*ca* 10 mmol) was added and the reaction heated to temperature with stirring for 48 h (Table 2.1). The reaction was sampled at 0, 1, 2, 6, 24, and 48 h by taking 2 mL of reaction mixture and quenching. ^{1}H NMR spectroscopy allowed the ratio of products (**14a-j**) to be determined.

Table 2.1: Reaction conditions for the transesterification of resorcinol (**14a-j**).

Reaction	Acylating Reagent	Solvent	Catalyst	Temperature (^{o}C)
Aqueous based solvents				
14a	Vinyl acetate	Water	KOH	20
14b	Vinyl acetate	Water/THF (1:1)	KOH	20
14c	Vinyl acetate	THF	KOH	20
14d	Vinyl acetate	Water/THF (1:1)	KOH	40
14e	Vinyl laurate	Water/THF (1:1)	KOH	20
Non-aqueous based solvents				
14f	Vinyl acetate	THF	TEA	20
14g	Vinyl acetate	THF	DMAP	20
14h	Vinyl acetate	DMSO	TEA	20
14i	Vinyl acetate	DMSO	DMAP	20
14j	Vinyl laurate	DMSO	TEA	70

Transesterification of catechin with vinyl acetate in water

Catechin (0.11 g, 0.38 mmol) was partially dissolved in water (25 mL) and potassium hydroxide (10% mol/mol of catechin, 0.38 mL, 0.1 molL^{-1}) was added. Excess vinyl acetate (0.35 mL, 3.8 mmol) was added and the mixture stirred for 48 h at room temperature. The reaction was sampled at 0, 1, 2, 6, 24, and 48 h by taking 2 mL of reaction mixture and concentrating by rotary evaporation. ^{1}H NMR spectroscopy allowed the ratio of products (**15**) to be determined.

Transesterification of catechin with vinyl acetate in water/tetrahydrofuran

Catechin (0.11 g, 0.38 mmol) was partially dissolved in water (10 mL) and potassium hydroxide (10% mol/mol of catechin, 0.36 mL, 0.1 molL^{-1}) was added. Excess vinyl acetate (0.35 mL, 3.8 mmol) was dissolved in tetrahydrofuran (THF) (4 mL) and added to the reaction mixture, which was stirred for 48 h at room temperature. The reaction was sampled at 0, 1, 2, 6, 24, and 48 h by taking 2 mL of reaction mixture and concentrating by rotary evaporation. ^{1}H NMR spectroscopy allowed the ratio of products (**16**) to be determined.

Transesterification of catechin with vinyl laurate

Catechin (0.09 g, 0.31 mmol) was dissolved in dimethyl sulfoxide (DMSO) (5 mL). Triethylamine (10% mol/mol of catechin, 5μL) and excess vinyl laurate (0.90 mL, 3.5 mmol) were added. The flask was fitted with a condenser and drying tube and stirred for 48 h at 70 ^{o}C. The reaction progress was monitored at 0, 1, 2, 6, 24, and 48 h and samples were rotary evaporated to remove triethylamine. Chromatography (TLC) with EtOAc/hexane (1:1) allowed the reaction progress to be assessed. The reaction mixture was poured into ether (10 mL), washed with saturated NaCl (2 x 20 mL), and concentrated by rotary evaporation to yield a yellow oily product (0.29 g). The reaction mixture (0.29 g) was separated by chromatography (PLC) with EtOAc/hexane (2:1) to yield four bands.

Band 1 (R_f = 0.75) gave vinyl laurate (210 mg).
^{1}H NMR (400MHz, $CDCl_3$): δ_H 7.43 (1H, dd, J = 6.4 Hz, J = 14.0 Hz, $CH_2C\underline{H}O$), 4.99 (1H, dd, J = 1.4 Hz, J = 14.0Hz, $C\underline{H}_2CHO$) 4.72 (1H, dd, J = 1.4 Hz, J = 6.4 Hz, $C\underline{H}_2CHO$), 2.56 (2H, t, J = 7.4 Hz, $OCOCH_2$), 1.76 (2H, tt, J = 7.2 Hz, $OCOCH_2C\underline{H}_2$), 1.45 (19H, m, CH_2), 1.03 (3H, t, J = 6.7 Hz, CH_3).

Band 2 (R_f = 0.66) gave an unidentified solid (1 mg).

Band 3 (R_f = 0.53) gave catechin dilaurate (**17**; 9 mg, 3%) as a yellow oil.
^{1}H NMR (400MHz, $CDCl_3$): δ_H 7.5-6.8 (3H, m, H-2', 5', 6'), 6.4-5.8 (2H, m, H-6, 8), 4.69 (1H, d, $J_{2,3}$ = 8.3 Hz, H-2), 4.01 (1H, dt, $J_{2,3} = J_{3,4}$ = 5.8 Hz, H-3), 3.2-2.7 (21H, m, H-4), 2.6-2.1 (5H, m, 3, 5, 7, 3', 4'-$OCOCH_2$), 1.8-1.5 (5H, m, 3, 5, 7, 3', 4'-$OCOCH_2C\underline{H}_2$), 1.5-1.0 (35H, m, CH_2), 0.88 (9H, t, J = 6.1 Hz, CH_3).
IR (KBr disc): ν(CO) 1758 (s) cm^{-1}.
ESI-MS (MeOH): *m/z* 653 $[M-H]^-$.

Band 4 (R_f = 0.34) gave catechin monolaurate (**18**; 37 mg, 10%) as a yellow oil.
^{1}H NMR (400MHz, $CDCl_3$): δ_H 7.4-6.7 (3H, m, H-2', 5', 6'), 6.3-5.8 (2H, m, H-6, 8), 4.7-4.5 (1H, m, H-2), 4.1-3.9 (1H, m, H-3), 3.1-2.8 (6H, m, H-4), 2.6-2.1 (2H, m, 3, 5, 7, 3', 4'-$OCOCH_2$), 1.8-1.5 (3H, m, 3, 5, 7, 3', 4'-$OCOCH_2C\underline{H}_2$), 1.5-1.0 (23H, m, CH_2), 0.88 (4H, t, J = 6.0 Hz, CH_3).

IR (KBr disc): ν(CO) 1742 (m) cm^{-1}.

ESI-MS (MeOH): *m/z* 471 $[M-H]^-$.

Transesterification of PBT with vinyl acetate

PBT (2.0 g, 6.8 mmol) was partially dissolved in water (20 mL) and potassium hydroxide (10% mol/mol of tannin unit, 6.8 mL, 0.10 $molL^{-1}$) was added. Excess vinyl acetate (6.3 mL, 68 mmol) was dissolved in THF (5 mL) and added to the reaction mixture with stirring. After 48 h the reaction mixture was concentrated, poured into water (100 mL), and stirred. The precipitate was filtered, washed with water (4 x 10 mL), and dried under vacuum over silica gel to give a brown solid (**19**; 0.23 g, 7%).

^1H NMR (400 MHz, CD_3SOCD_3): δ_H 7.5-5.5 (3.9H, br, Ar-H), 5.5-3.0 (3H, br, C-ring), 2.4-1.8 (0.5H, br, CH_3).

^{13}C NMR (200 MHz, CP-MAS): δ_C 159-149 (C-5, 7, 9), 148-140 (C-3', 4'), 131 (C-1'), 125-93 (C-6, 8, 10, 2', 5', 6'), 87-63 (C-2, 3), 42-28 (C-4), 24-18 (CH_3).

IR (KBr disc): ν(CO) 1731 (w) cm^{-1}.

Transesterification of QT with vinyl acetate

QT (2.1 g, 7.6 mmol) was partially dissolved in water (20 mL) and potassium hydroxide (10% mol/mol of tannin unit, 7.6 mL, 0.10 $molL^{-1}$) was added. Excess vinyl acetate (7.3 mL, 79 mmol) was dissolved in THF (5 mL) and added to the reaction mixture with stirring. After 48 h the reaction mixture was concentrated, poured into water (200 mL), stirred, and the supernatant removed by aspiration. This washing and aspiration was repeated three times. The precipitate was collected by filtration and washed with water (4 x 10 mL) to give a brown solid (**20**; 0.42 g, 12%).

^1H NMR (400MHz, CD_3SOCD_3): δ_H 7.5-5.5 (4.9H, br, Ar-H), 5.5-3.5 (4H, br, C-ring), 2.4-1.8 (1.0H, br, CH_3).

^{13}C NMR (200 MHz, CP-MAS): δ_C 175-170 (CO), 159-150 (C-7, 9), 148-140 (C-3', 4'), 130 (C-1'), 126-97 (C-5, 6, 8, 10, 2', 5', 6'), 87-63 (C-2, 3), 45-26 (C-4), 25-18 (CH_3).

IR (KBr disc): ν(CO) 1736 (m) cm^{-1}.

Transesterification of PBT with vinyl laurate

PBT (10 g, 35 mmol) was dissolved in DMSO (100 mL) and triethylamine (30% mol/mol of tannin unit, 1.5 mL) was added. Excess vinyl laurate (90 mL, 350 mmol) was added and the mixture heated to 70 ^{o}C with stirring for 48 h. The reaction progress was monitored at 0, 1, 2, 6, 24, and 48 h and samples were rotary evaporated to remove triethylamine. Chromatography (TLC) with EtOAc/hexane (1:1) allowed the reaction progress to be assessed. After 48 h the reaction mixture was concentrated and the immiscible vinyl laurate layer was removed using a separating funnel. To the DMSO fraction, water (1500 mL) and saturated NaCl (200 mL) were added. A precipitate formed, which was collected by filtration through a glass micro-fibre filter paper. The product was washed with water (4 x 40 mL), ether (4 x 40 mL), and dried under vacuum to give a brown solid (**21**; 9.02 g, 21%).

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 7.5-5.5 (3.9H, br, Ar-H), 5.5-3.5 (2H, br, C-ring), 2.7-2.2 (1.8H, br, H-4, α-CH_2), 1.8-1.5 (1.6H, br, β-CH_2), 1.5-1.0 (14H, br, CH_2), 1.0-0.8 (2.5H, br, CH_3).

^{13}C NMR (200 MHz, CP-MAS): δ_C 175 (CO), 160-141 (C-3', 4', 5, 7, 9), 138 (C-1'), 133-92 (C-6, 8, 10, 2', 5', 6'), 87-63 (C-2, 3), 42-17 (CH_2, C-4), 14 (CH_3).

IR (KBr disc): ν(CO) 1731 (m) cm^{-1}.

Transesterification of QT with vinyl laurate

QT (1.0 g, 3.6 mmol) was dissolved in DMSO (20 mL) and triethylamine (30% mol/mol of tannin unit, 160 μL) was added. Excess vinyl laurate (9.9 mL, 38 mmol) was added and the mixture heated to 70 ^{o}C with stirring for 48 h. The reaction progress was monitored at 0, 1, 2, 6, 24, and 48 h and samples were rotary evaporated to remove triethylamine. Chromatography (TLC) with EtOAc/hexane (1:1) allowed the reaction progress to be assessed. After 48 h the reaction mixture was rotary evaporated to remove triethylamine. Subsequently the reaction mixture was poured into ether (20 mL), washed with water (2 x 50 mL), dried with $MgSO_4$, and concentrated by rotary evaporation. The product was separated by chromatography (PLC) with EtOAc/hexane (1:3) to yield two bands.

Band 1 (R_f = 0.29-0.82) gave vinyl laurate (1.7 g).

Band 2 (R_f = 0.12) gave a mixed QT laurate derivative (**22**; 320 mg, 8%) as a brown gelatinous solid.

^{1}H NMR (400MHz, CD_3COCD_3): δ_H 7.5-5.5 (4.9H, br, Ar-H), 5.5-3.0 (4.3H, br, C-ring), 3.0-2.0 (13H, br, H-4, α-CH_2), 1.9-1.6 (8.5H, br, β-CH_2), 1.5-1.0 (78H, br, CH_2), 1.0-0.6 (15H, br, CH_3).

IR (KBr disc): ν(CO) 1743 (s) cm^{-1}.

2.3 Results and Discussion

2.3.1 Synthesis of Model Compounds

Flavonoids esters were prepared by the alcoholysis of acid chlorides and anhydrides for two reasons. Firstly, their preparation allowed synthetic chemistry routes to be established. Secondly, they served as comparisons for the transesterified derivatives in their chemical, spectroscopic, and physical properties. Resorcinol, catechin, and tannin were acylated using standard esterification procedures.

Resorcinol was easily acetylated using acetic anhydride. PLC of the reaction mixture gave resorcinol diacetate (**1**) in moderate yield (22%) and resorcinol monoacetate (**2**; 11%) as the minor product. The ^{1}H NMR spectra of **1** (Appendix 1) and **2** showed downfield shifts of the aromatic protons upon acetylation. The H-5 triplet showed a downfield shift from 7.08 ppm to 7.15 ppm upon mono-acetylation and to 7.34 ppm upon di-acetylation. For the later transesterification reactions, this distinction in chemical shift provided a gauge as to the degree of substitution.

Resorcinol dilaurate (**3**) was prepared using excess lauroyl chloride in pyridine (Figure 2.1). Pyridine acted as a solvent, catalyst, and a medium to remove the hydrogen chloride by-product. Solvent extraction followed by recrystallisation gave **3** (52%) in high purity, as shown by ^{1}H NMR spectroscopy (Appendix 2). As found for resorcinol diacetate, the aromatic protons H-2, -4, -5, and -6 showed downfield shifts upon esterification.

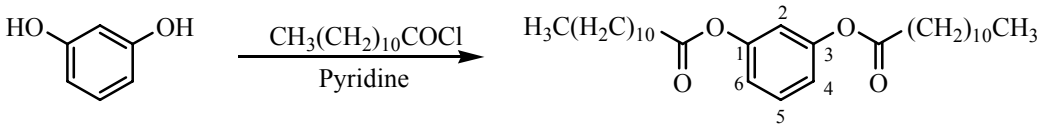


Figure 2.1: Preparation of resorcinol dilaurate using resorcinol and excess lauroyl chloride in pyridine.

Catechin pentaacetate (**4**) was easily prepared from excess acetic anhydride (Figure 2.2) in excellent yield (73%)[46]. Using ^{1}H NMR and ^{13}C NMR spectroscopy the structure was confirmed and the melting point agreed with the literature (125-126 ^{o}C)[43]. In comparison with catechin, downfield shifts for H-2', -5', -6', -2, -3, -6, and -8 were observed in the ^{1}H NMR spectrum (Appendix 3).

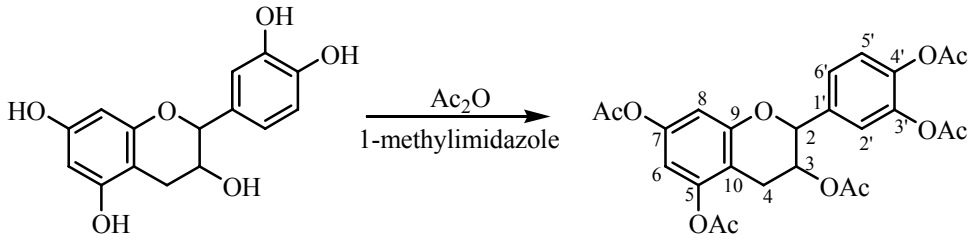


Figure 2.2: Preparation of catechin pentaacetate from catechin and excess acetic anhydride in the presence of 1-methylimidazole.

Catechin pentalaurate (**5**) was prepared by the reaction of catechin with excess acylating agent (Figure 2.3). The exclusion of water was desirable to prevent the hydrolysis of lauroyl chloride. Solvent extraction followed by PLC gave **5** (23%) in high purity. The product was fully characterised by elemental analysis, NMR (Appendix 4), and FTIR spectroscopy.

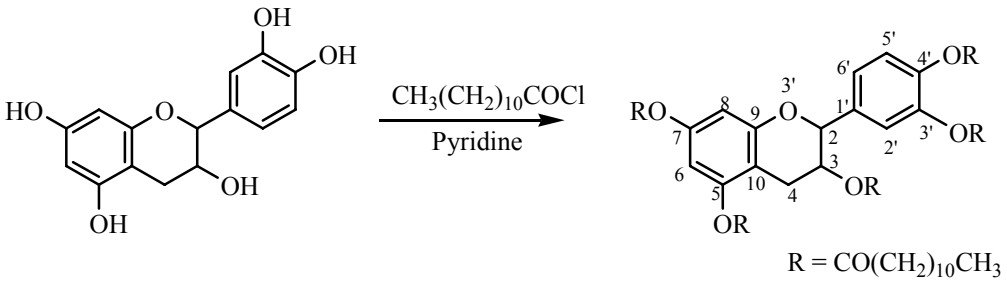


Figure 2.3: Preparation of catechin pentalaurate from catechin and excess lauroyl chloride in pyridine.

A range of NMR experiments allowed the structure of **5** to be fully determined. The unambiguous assignment of the A-ring signals required 2D NMR spectroscopy. Using a HMBC experiment, which shows ^{13}C-^{1}H correlations across multiple bonds (Figure 2.4), the H-6 signal was determined to be downfield of H-8 for two reasons. First, the H-6 signal was expected to be further downfield being adjacent to two esters. Secondly, a $^{4}J_{C-H}$ coupling between C-9 and H-6 was observed. A $^{4}J_{C-H}$ coupling between C-9 and H-6 was more likely than a $^{2}J_{C-H}$ coupling between C-9 and H-8 as the HMBC experiment was optimised for $^{3}J_{C-H}$ and $^{4}J_{C-H}$ couplings. Accordingly, using a HSQC experiment (Appendix 5) the $^{1}J_{C-H}$ coupling from C-8 to H-8 and C-6 to H-6 allowed the assignment of the ^{13}C NMR spectrum. Furthermore, a $^{3}J_{C-H}$ coupling between C-5 and H-4 confirmed that the C-5 signal was downfield of C-7. The unambiguous assignment of the B-ring carbons was aided by a $^{3}J_{C-H}$ coupling observed between H-2 and C-2', -6'.

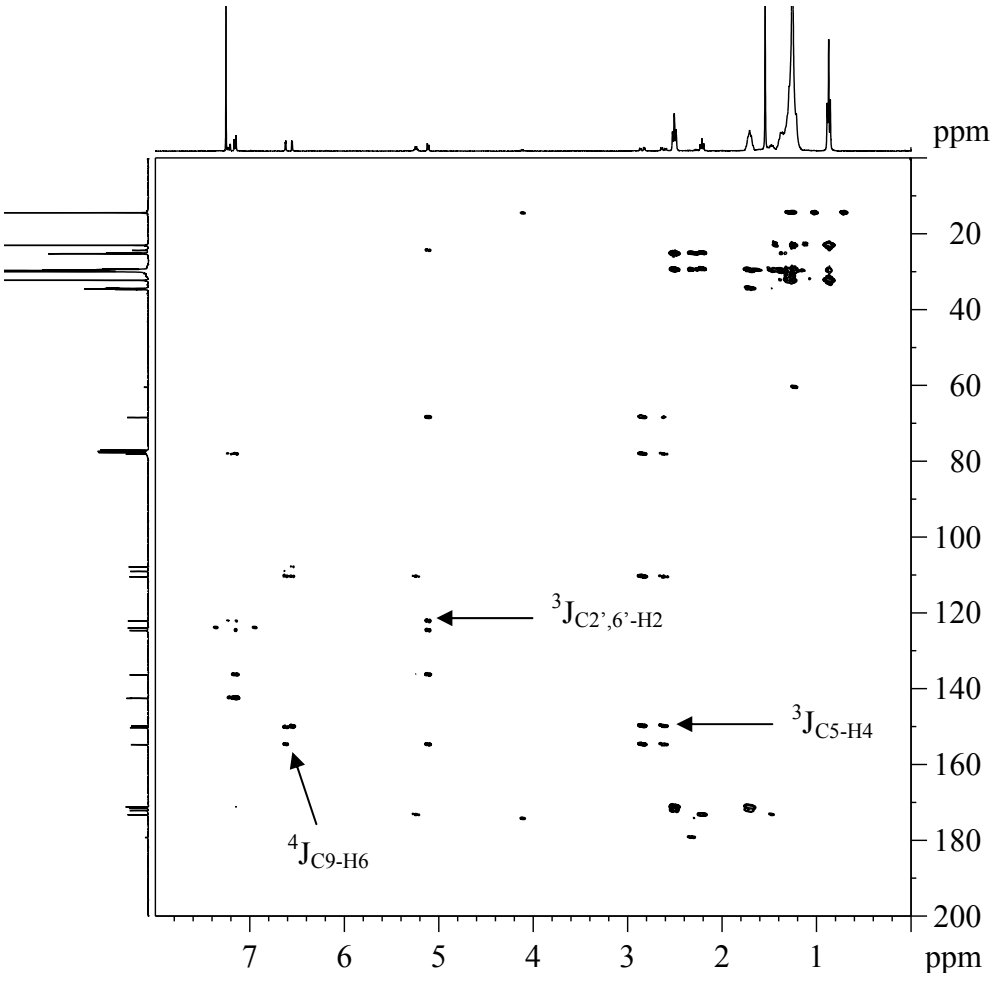


Figure 2.4: HMBC spectrum of **5** which was optimised to observe $^{3}J_{C-H}$ and $^{4}J_{C-H}$ couplings.

To prepare catechin laurate with a lesser degree of substitution, catechin was reacted with three mole equivalents of lauroyl chloride. Analysis by TLC showed three products in the reaction mixture as well as lauric acid and catechin. The amphiphilic properties of the products meant solvent extraction was impractical. Therefore, column chromatography was used to purify the reaction mixture. Analysis of the fractions by TLC allowed their pooling into four fractions of similar constituents. This separation gave one fraction of mixed catechin laurates free of lauric acid. However the resolution of the column chromatography was insufficient to separate the three catechin laurate products. Further separation of these derivatives was achieved using PLC. Three fractions were isolated and identified as isomeric mixtures of catechin tetra- (**7**), tri- (**8**) and di-laurate (**9**). For the purpose of this study, it was not considered necessary to further separate the mixture of isomers.

NMR spectroscopy allowed the products to be identified and provided an insight as to the sites of substitution. ^{1}H NMR spectroscopy of the catechin derivatives showed clear signals due to the aliphatic fatty acid protons (Figure 2.5). Integration of the aliphatic signals versus the aromatic protons gave an indication of the degree of substitution (DS). Catechin tetralaurate (**7**) showed downfield shifts of the A-, B-, and C-ring protons compared with catechin, suggesting that substitution was occurring at all these rings. Catechin trilaurate (**8**) showed downfield shifts of the B- and C-ring protons. However the A-ring protons showed less obvious downfield shifts compared with **7**. Catechin dilaurate (**9**) showed the least downfield shifts of the A-, B-, and C-ring protons as expected. Assigning the specific peaks was impractical due to the number of isomers in each product. In summary, it appeared that substitution occurred preferentially at the B-ring over the A-ring. This agrees with previous research which showed the reaction of catechin with 2 mole equivalents of lauroyl chloride in pyridine gave catechin-3',4'-dilaurate and catechin-3,3',4'-trilaurate[53]. Furthermore, the dissociation constants of catechin's hydroxyl groups decrease in the order 3'-OH > 5-OH ≥ 7-OH > 4'-OH > 3-OH[111, 112]. This explained the initial preference for acylation at the B-ring.

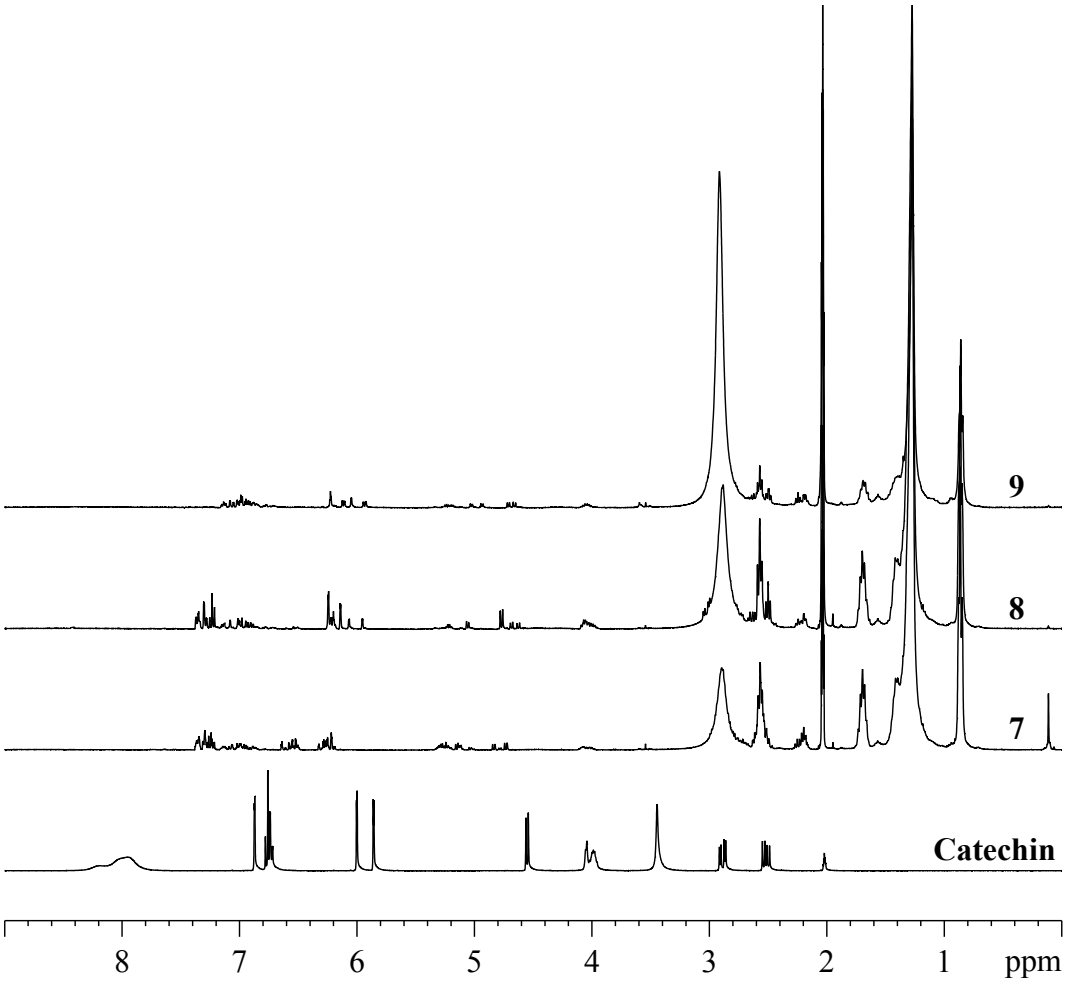


Figure 2.5: ^{1}H NMR spectra of **7**, **8**, and **9** compared with catechin.

Electrospray ionisation mass spectrometry (ESI-MS) of **9** showed the molecular ion, supporting its identification as catechin dilaurate. The molecular ion $[M-H]^-$ was observed at m/z 653, as well as several other fragments and adducts (Appendix 6). A peak at 471 corresponded to catechin monolaurate, which may be present in the sample or from the fragmentation of the 653 ion. Adducts from various combinations of the 471 and 653 peaks were observed at 943, 1125, 1307, 1597, and 1962. Mass spectrometry-mass spectrometry (MS^2) of the 653 peak gave a strong peak at 471 corresponding to loss of one lauroyl group. Furthermore, MS^3 of the 471 peak gave a strong peak at 289 corresponding to further loss of one lauroyl group leaving the bare catechin. Products **7** and **8** were insoluble in the appropriate solvent, hence no ESI-MS were recorded.

Pine bark tannin was acylated in a similar manner to catechin. Varying degrees of substitution were achieved by using an excess of lauroyl chloride (**10**) or three

mole equivalents (**11**). Removing free lauric acid which was identified in the ^{1}H and ^{13}C NMR spectra of **10** proved ineffective using a sodium bicarbonate wash. However, a second extraction using a sodium hydroxide solution gave a product free of lauric acid. Using sodium hydroxide solution during solvent extraction of **11** resulted in the majority of product being lost in the aqueous phase. However, the use of PLC gave a product free of lauric acid.

Integration of the ^{1}H NMR spectra provided a means to determine the average DS, however this required calibration. The aromatic region (6-8 ppm) of PBT was known to consist on average of 3.9 protons. Furthermore, the average number of hydroxyl groups was calculated to be 5.2. These values were determined knowing the characteristics of PBT, such as the average degree of polymerisation (8.9) and the prodelphinidin to procyanidin ratio (1:4)[17]. Accordingly, integration of the methyl and methylene signals relative to the aromatic signals gave an indication as to the DS (Table 2.2). For both samples **10** and **11**, the DS was greater than the number of hydroxyl groups. This may be caused by the acylation of other components in the tannin preparation, which would bias the calculation. Carbohydrates are one such known impurity in the PBT[17].

Table 2.2: The degree of substitution for PBT and QT laurates (**10-13**) determined by integration of the ^{1}H NMR spectra.

Sample	Aromatic signal	Aliphatic signals			Average DS
		β-CH_2	CH_2	CH_3	
PBT laurates					
10	3.9	15	138	32	8.8
11	3.9	19	80	16	5.5
QT laurates					
12	4.9	16	131	33	8.6
13	4.9	8.2	48	10	3.2

Gel permeation chromatography (GPC) provided a method to compare the molecular weight (MW) distribution and consequently the DS. However, as the standards were not of similar nature to the analytes, the results are relative, not

absolute. The MW profiles of **10** and **11** showed marked differences (Figure 2.6). Firstly, the dispersity of **10** was greater than **11**. This was probably due to the different workup procedures used. Secondly, no considerable difference in the average MW was observed, contrary to the DS calculated by ^{1}H NMR spectroscopy. This may be an artefact due to interactions, such as adsorption, with the stationary phase.

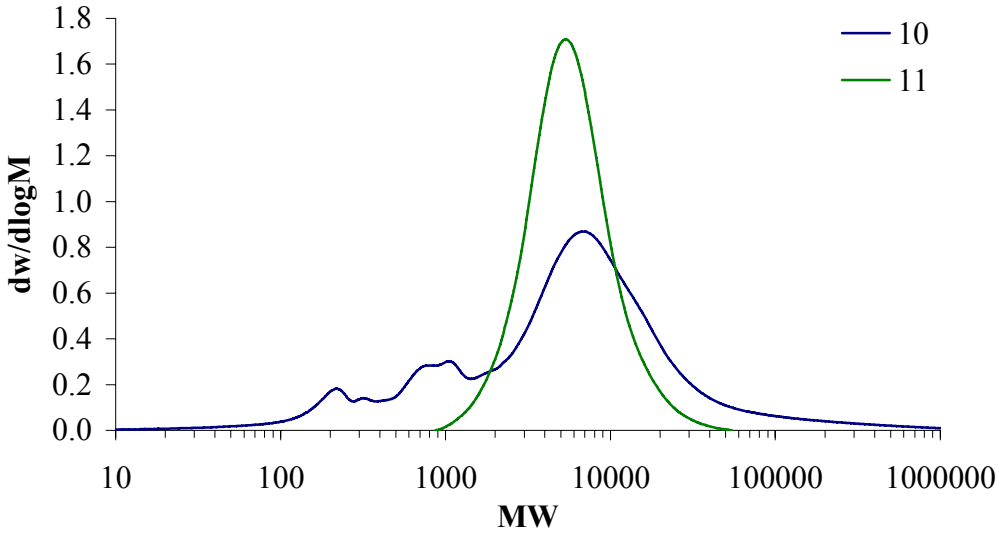


Figure 2.6: Molecular weight distribution of PBT laurates (**10** and **11**).

Solution state ^{13}C NMR spectroscopy of tannin derivatives proved unsuccessful. This may be due to the low molar concentration of samples and/or the short spin-lattice relaxation time (T_1) of the macromolecule. T_1 relaxation times are known to decrease with increasing molecular weight, therefore the T_1 of tannins were expected to be short[113]. The gelatinous nature of the product excluded the use of solid-state ^{13}C NMR spectroscopy. However, using FTIR spectroscopy the presence of a strong carbonyl peak at 1768 cm^{-1} confirmed the presence of ester linkages.

In a similar manner, quebracho tannin (QT) was acylated using excess lauroyl chloride (**12**) or three mole equivalents of lauroyl chloride (**13**). The removal of lauric acid by solvent extraction was again ineffective. Therefore, column chromatography allowed the removal of lauric acid. Of the three fractions obtained, only the first was free of lauric acid. The second and third fractions

contained appreciable amounts of free acid as identified in the ^{1}H and ^{13}C NMR spectra. The yield of product with no lauric acid was low, but sufficient for further analysis.

As previously described, integration of the ^{1}H NMR spectra allowed the DS to be determined. The aromatic region (6-8 ppm) of QT was calibrated to 4.9 protons. Furthermore, the average number of hydroxyl groups was calculated to be 4.3. Knowing the average degree of polymerisation of QT (6.5) and the profisetinidin to prorobinetinidin ratio (3:1) allowed these calculations[20]. Again, the average DS of **12** was greater than the number of hydroxyls, potentially due to reasons previously described. However, **13** showed an average DS similar to that expected when three mole equivalents of lauroyl chloride were used (Table 2.2).

The molecular weight distribution of **12** and **13** showed the former to be of greater MW (Figure 2.7), in accordance with integration of the ^{1}H NMR spectra. The dispersity of the two samples was similar, probably because both were isolated by column chromatography.

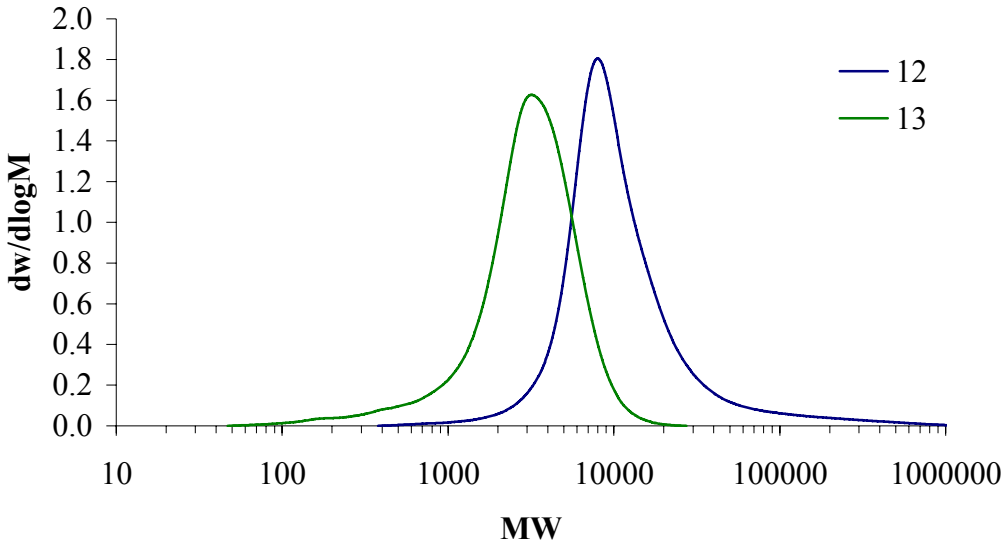


Figure 2.7: Molecular weight distribution of QT laurates (**12** and **13**).

^{13}C NMR spectra were not recorded for the reasons previously mentioned. However, FTIR spectroscopy showed strong carbonyl peaks at 1775 and 1740 cm^{-1} confirming the presence of ester linkages.

As demonstrated, the synthesis of polyphenol esters via acid chlorides and anhydrides was effective. However, there were several disadvantages to this method. Firstly, the reaction was difficult to control due to the high reactivity of the reagents. Water must also be excluded otherwise free fatty acids were produced by hydrolysis. These free fatty acids were difficult to remove as they formed strong emulsions upon solvent extraction. Because of their high reactivity, the acid chlorides were more toxic and hence dangerous to handle. For example, lauroyl chloride causes burns upon skin contact and is extremely destructive to mucous membranes and the upper respiratory tract[114]. Secondly, the price of acid chlorides and anhydrides may contribute significantly to the overall cost of producing polyphenol esters on an industrial scale. Lastly, in the case of anhydrides, the reaction was inefficient on an atomic basis producing large amounts of acid. With this in mind, an alternative method to produce polyphenol esters was sought. Transesterification offers an alternative route to produce polyphenol esters. The reagents are generally less reactive, hence more stable and less toxic. They are generally cheaper, especially vinyl esters that are used in large quantities for polymer synthesis[115]. Accordingly, transesterification of polyphenols was investigated.

2.3.2 Transesterification

The transesterification of phenols was studied using a range of compounds. Resorcinol and catechin were used to model phenolic behaviour and establish the initial chemistry and reaction conditions. The acylating agents used were vinyl acetate and vinyl laurate. The advantage of using vinyl esters was that the by-product alcohol tautomerises to an aldehyde, therefore promoting the equilibrium towards the products and phenolic esterification. The methodology developed using model compounds was then able to be adapted for the transesterification of tannins.

Initial investigations looked at the transesterification of resorcinol with vinyl acetate (Figure 2.8). The percent conversion was determined by integrating the H-5 triplet in the ^{1}H NMR spectra as previously described (Section 2.3.1). The

degree of esterification was used to establish the optimal conditions, such as catalyst and solvent.

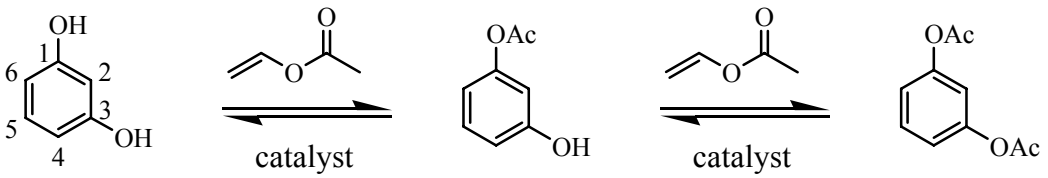


Figure 2.8: The transesterification of resorcinol with vinyl acetate to give resorcinol monoacetate and resorcinol diacetate.

A range of catalysts were screened for their capacity to promote transesterification of resorcinol with vinyl acetate (Table 2.3). Both acid and base catalysis of transesterification is well known[39]. All of the bases showed good transesterification ability. In contrast, none of the acids used showed activity towards the transesterification of resorcinol. Triethylamine and 4-dimethylaminopyridine showed the greatest activity. However, potassium hydroxide, although less active as a catalyst, was advantageous as it was easily available, of low toxicity, and highly water soluble.

Table 2.3: The transesterification ability of some common acid and base catalysts was shown by the percent conversion of resorcinol to resorcinol monoacetate (RMA) and resorcinol diacetate (RDA).

Catalyst	**% Conversion**	
	RMA	**RDA**
Acids		
Hydrochloric acid	0	0
Sulphuric acid	0	0
p-Toluenesulfonic acid	0	0
Bases		
Potassium hydroxide	51	17
Triethylamine	53	22
4-dimethylaminopyridine	63	23

The acetylation of resorcinol using vinyl acetate and potassium hydroxide was studied using polar solvents. Polar solvents were required as flavonoids are

hydrophilic. However, the solubility of vinyl acetate, resorcinol monoacetate and diacetate in water is low[116]. Therefore THF was chosen as a solvent of moderate polarity capable of solvating phenolics, vinyl acetate, and possibly accommodating for potassium hydroxide.

The transesterification of resorcinol with vinyl acetate was performed in water (**14a**), water/THF (**14b**), and THF (**14c**). Using ^{1}H NMR spectroscopy the percent conversion to resorcinol monoacetate (RMA) and resorcinol diacetate (RDA) was monitored throughout the reaction (Figure 2.9). For **14a** and **14b** the maximum conversion to RMA was achieved after 6 h, after which the percentage of RMA in the reaction mixture decreased slightly. This was probably due to the subsequent conversion of RMA to RDA, which was shown to occur throughout the reaction. The conversion of resorcinol to RMA appeared to occur relatively easily, with 40-50% conversion achieved (Figure 2.9). However, the subsequent conversion of RMA to RDA was slow, with less than 10% conversion to RDA. Presumably the pKa of resorcinol was lower than RMA, therefore this poor conversion to RDA may be due to the increased pKa of the second hydroxyl and its decreased reactivity towards the acylating group.

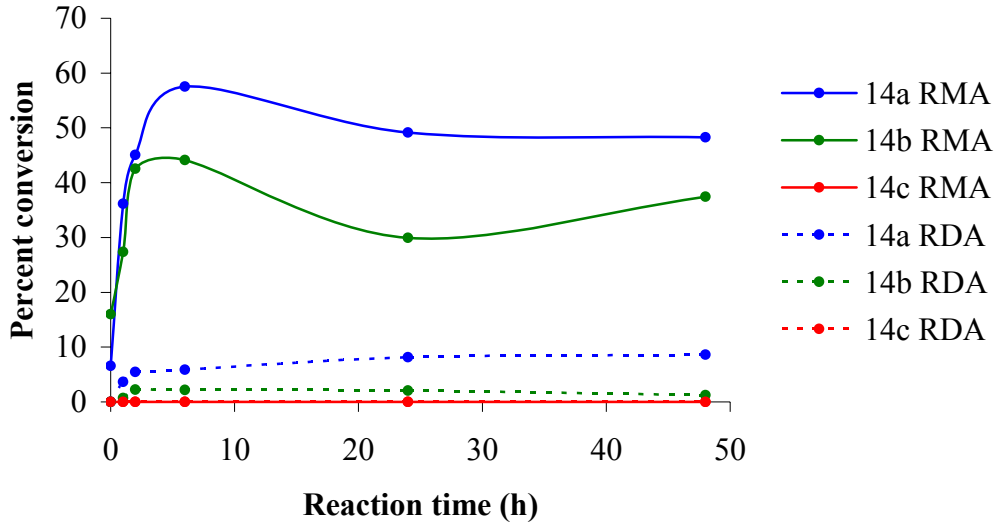


Figure 2.9: Percent conversion of resorcinol to resorcinol monoacetate (RMA) and resorcinol diacetate (RDA) by transesterification (**14a-c**).

The degree of substitution was also shown to vary depending upon the solvent used. As the solvent became more polar, acetylation became more efficient. For example maximum conversion was achieved using water as a solvent. In neat THF solution, a green deposit formed in the reaction flask. Presumably this was associated with the low solubility of potassium hydroxide in THF. This may explain the negligible reaction in THF (Figure 2.9). Furthermore, in water at higher temperatures (**14d**), no increase in the percentage conversion to resorcinol monoacetate was observed. It should be noted that the zero hour samples showed some product formation. This might be due to the time taken to remove vinyl acetate and quench the reaction by rotary evaporation.

With the intention of acylating catechin and tannin with vinyl laurate, a polar solvent such as water was unable to solvate both reagents. This immiscibility rendered the reaction unsuccessful (**14e**) (Table 2.4). In comparison, vinyl acetate was mostly soluble in water, allowing the reaction to proceed as a homogenous mixture. It was thought that an organic solvent of intermediate polarity would be more suitable and aid the miscibility of reagents. Solvents capable of solvating tannins include water, ethanol, THF, DMF, DMSO, glycol, and glycerol[83]. As alcoholic solvents were inappropriate for transesterification, only THF, DMF, and DMSO remained as appropriate choices.

The effectiveness of DMSO and THF as a transesterification solvent was demonstrated using 4-dimethylaminopyridine and triethylamine (**14f-i**) (Table 2.4). Using DMSO and triethylamine, resorcinol was transacetylated with vinyl acetate to give 16% resorcinol monoacetate. In contrast, THF was a poor solvent for the reaction with no appreciable amount of resorcinol monoacetate synthesised. Therefore, the combination of DMSO and triethylamine were chosen for further investigation using vinyl laurate.

Using vinyl laurate, resorcinol was acylated in DMSO catalysed with triethylamine (**14j**). It was observed that vinyl laurate was immiscible with DMSO at room temperature. However, at 70 ^{o}C vinyl laurate and DMSO formed a homogenous solution. The percent conversion with vinyl laurate (**14j**) was slightly higher compared with that for vinyl acetate (**14h**) (Table 2.4). This may

be attributed to the increased temperature or a difference in reactivity of the acylating group.

Table 2.4: Transesterification of resorcinol with vinyl acetate (VA) or vinyl laurate (VL). Two products were observed, resorcinol monoacylate (RMAcyl) and resorcinol diacylate (RDAcyl).

Reaction	Reagent	Solvent	Catalyst	Temp (^{o}C)	Yield (%)	
					RMAcyl	RDAcyl
Aqueous based reactions						
14a	VA	Water	KOH	20	48	9
14b	VA	Water/THF	KOH	20	37	1
14c	VA	THF	KOH	20	0	0
14d	VA	Water/THF	KOH	40	19	1
14e	VL	Water/THF	KOH	20	0	0
Non-aqueous based reactions						
14f	VA	THF	TEA	20	2	0
14g	VA	THF	DMAP	20	2	3
14h	VA	DMSO	TEA	20	16	2
14i	VA	DMSO	DMAP	20	6	0
14j	VL	DMSO	TEA	70	21	0

Development of the method using resorcinol showed the optimal catalysts and solvents for transesterification. For acetylation using vinyl acetate an aqueous system with potassium hydroxide was most preferred. For transesterification with vinyl laurate, triethylamine in DMSO provided a suitable reaction medium, although the yields were relatively lower.

The transesterification procedure was advanced and adapted to catechin using the methodology developed with resorcinol. As previously shown, aqueous systems showed the most promise for transacetylation. Therefore, catechin was acetylated with vinyl acetate in water (**15**) and water/THF (**16**). Transacetylation was determined to occur at both the aromatic and C-ring hydroxyl groups. ^{1}H NMR spectroscopy showed methyl peaks at both 2.24 ppm and 1.86 ppm, corresponding

to the aromatic acetates and C-ring acetates respectively. By integrating the ^{1}H NMR spectra the DS at both the aromatic hydroxyls and C-ring hydroxyls was determined with respect to the reaction time. The reaction proceeded rapidly up to 6 h, after which the degree of substitution changed little (Figure 2.10). The optimal solvent system was water/THF giving the greater total DS (Table 2.5).

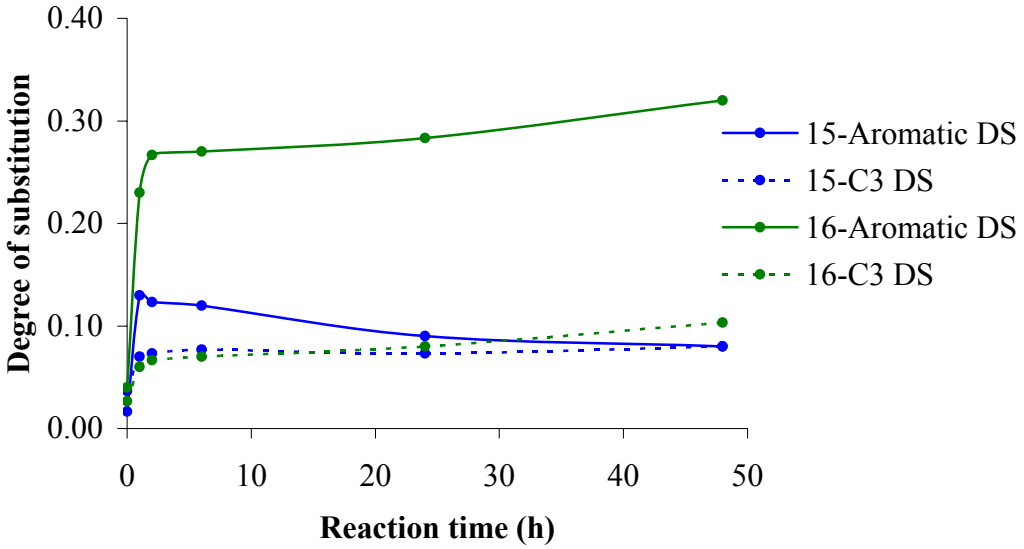


Figure 2.10: Degree of substitution for the acetylation of catechin with vinyl acetate.

Upon further interpretation of the ^{1}H NMR spectrum, the sites of acetylation could be determined (Figure 2.11). A moderate portion of the B-ring signals (6.7-7.2 ppm) showed downfield shifts compared with catechin. Whereas a low portion of the A-ring signals showed downfield shifts. Mono-acetylation of the B-ring was observed, however no di-acetylation was present. This observation suggested that transacetylation occurred preferentially at the B-ring.

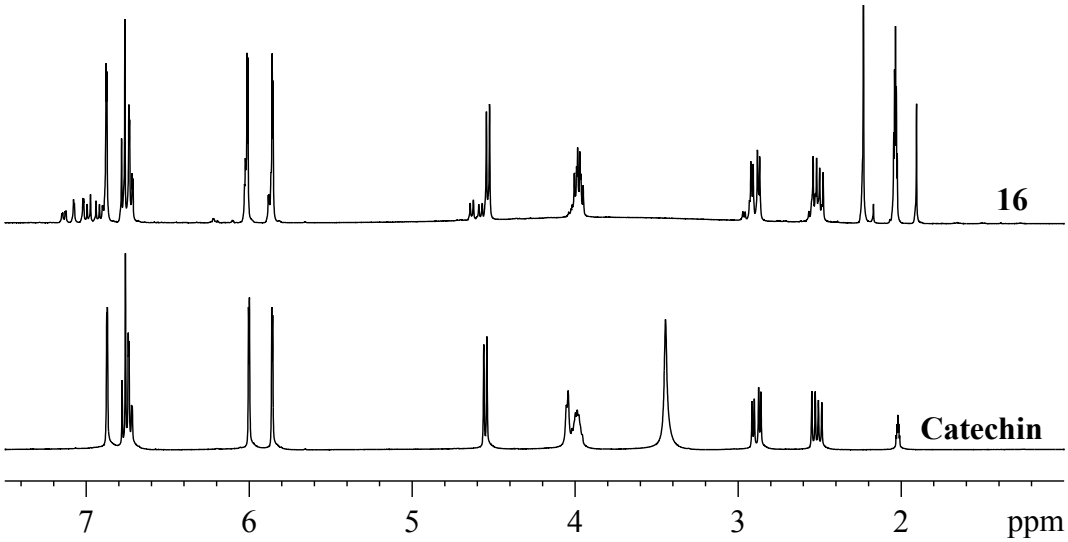


Figure 2.11: ^{1}H NMR spectrum of **16** compared with catechin.

Catechin was acylated with vinyl laurate using the same reaction conditions for resorcinol. TLC of the reaction mixture showed several products, as well as unreacted catechin and vinyl laurate. PLC allowed the removal of vinyl laurate and the isolation of two products, catechin dilaurate (**17**) as the minor product (3%) and catechin monolaurate (**18**) in a low yield (10%). As expected, **17** was more mobile on the PLC plate compared with **18** which only had one lauroyl group.

Using a range of NMR experiments the substitution pattern of both **17** and **18** were elucidated. First however, it should be noted that these products were a mixture of structural isomers. That is the lauroyl group(s) could be located at any of the five hydroxyls. The ^{1}H NMR spectrum of **17** (Appendix 7) showed that most of the substitution occurred at C-3' and C-4' and this is elaborated below. This was indicated by the signals from H-2', -5', and -6', the chemical shifts of which were almost identical to those observed for catechin pentalaurate (**5**). Therefore **17** was almost exclusively catechin-3',4'-dilaurate. In contrast, the ^{1}H NMR spectrum of **18** (Appendix 8) showed a range of substitution patterns about the B-ring. As with vinyl acetate, very little substitution was observed at the A- or C-ring.

2D NMR spectroscopy of **18** gave an insight as to the substitution pattern of this mixture. A COSY experiment showed $^{3}J_{H\text{-}5'\text{-}H\text{-}6'}$ couplings (Appendix 9), while a LR-COSY showed $^{4}J_{H\text{-}2'\text{-}H\text{-}6'}$ couplings (Appendix 10). This allowed the signals from the corresponding catechin-3'-monolaurate and catechin-4'-monolaurate to be assigned (Figure 2.12). The H-2' signal was expected to move further downfield upon acylation at C-3' compared with C-4'. The H-5' signal was expected to move further downfield upon acylation at C-4' compared with C-3'. Therefore the signals labelled in blue correspond to catechin-3'-monolaurate, while the signals in green correspond to catechin-4'-monolaurate, in the approximate ratio of 1:1. The most downfield signals belong to catechin-3',4'-dilaurate. The presence of catechin-3',4'-dilaurate in this isolated mixture showed the resolution from PLC was not sufficient to exclusively isolate the two products.

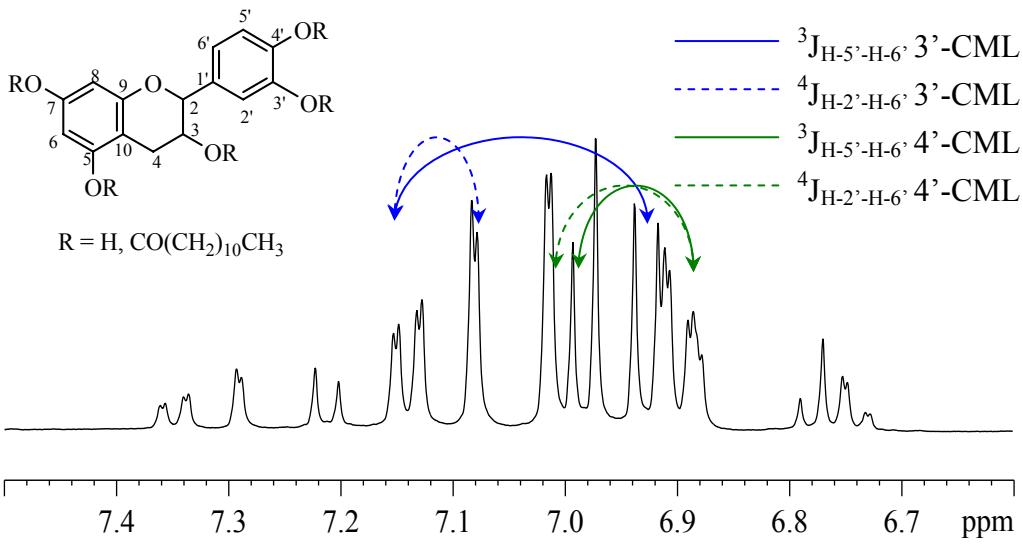


Figure 2.12: ^{1}H NMR spectrum of catechin monolaurate (CML, **18**) showing the ^{3}J and ^{4}J couplings observed in the COSY and LR-COSY spectra.

To corroborate NMR findings of **17** and **18**, mass spectrometry was undertaken. ESI-MS of **17** in negative ion mode showed the molecular ion at m/z 653 while adducts of the $[M\text{-}H]^{-}$ peak were observed at m/z 1307 and 1961 (Appendix 11). MS^{2} of the 653 peak gave loss of one and two lauroyl groups to give fragments at m/z 471 and 298, respectively. ESI-MS of **18** showed the $[M\text{-}H]^{-}$ peak at m/z 471 (Appendix 12). Again adducts were observed at m/z 943, 1415, and 1887. MS^{2} of the 471 peak gave fragmentation to 298 corresponding to the loss of one lauroyl group. These results support the findings by NMR spectroscopy.

In summary, catechin was effectively transesterified with vinyl acetate. However, the degree of substitution was low, with only about 0.4 of the hydroxyls acetylated. Similarly, catechin was acylated with vinyl laurate to give two products. These were identified as catechin monolaurate and catechin dilaurate. The transesterification of catechin using these methods almost exclusively occurred at the B-ring. While, esterification using lauroyl chloride also occurs at the B-ring, the preference to react at the B-ring appears to be more predominant for transesterification.

With a goal to modify polyphenols, tannins were acylated using the transesterificaiton methods developed for resorcinol and catechin. PBT was easily transacetylated with vinyl acetate (**19**), however the isolated yield was very low (7%). The low yield was due to incomplete substitution and perhaps due to product loss during workup and washing of the filter cake. The DS, calculated by integration of the ^{1}H NMR spectrum, was low (0.16) (Table 2.5). This was similar to the DS obtained for the acetylation of catechin (**15**). QT was also easily transacetylated (**20**), again the isolated yield was low (12%), presumably for the reasons given for PBT. However, the DS of **20** (0.34) was greater than for **19**.

Table 2.5: The DS for transacetylation of catechin and tannin. Calculations were based on integration of the ^{1}H NMR spectra.

Reaction	**A-ring signal[a]**	**Aromatic signal[a]**	**Methyl signal**		**Average DS**
			Ar-CH_3[b]	**C-3 CH_3[c]**	
Catechin acetates					
15	2		0.96	0.31	0.42
16	2		0.24	0.24	0.16
Tannin acetates					
19		3.9	0.47		0.16
20		4.9	1.01		0.34

a = Integral of the A-ring or aromatic protons

b = Integral of the C-3',4',5,7-$COCH_3$ protons

c = Integral of the C-3-$COCH_3$ protons

Further spectroscopic techniques aided the chemical characterisation of **19** and **20**. The ^{13}C CP-MAS NMR spectrum of **19** showed a small peak at *ca* 20 ppm due to the acetyl groups (Appendix 13). No obvious carbonyl peak was observed, however FTIR spectroscopy showed the presence of a weak carbonyl stretch at 1731 cm^{-1}. Acetylated QT (**20**) showed a methyl peak (20 ppm) and a carbonyl peak (172 ppm) in the ^{13}C CP-MAS NMR spectrum (Appendix 14). The presence of ester linkages was also supported by FTIR spectroscopy where a carbonyl stretch was observed at 1736 cm^{-1}. The low intensity of the NMR and FTIR carbonyl signals were consistent with the low DS as determined by ^{1}H NMR spectroscopy.

Transesterification of resorcinol and catechin with vinyl laurate showed triethylamine in DMSO to be an effective reaction medium. Therefore, tannins were transesterified using the same method. The product from the reaction of PBT with vinyl laurate (**21**) was easily isolated in relatively moderate yield (21%). Upon cooling of the reaction mixture two immiscible phases formed. This allowed the simple removal of excess vinyl laurate. Washing of the filter cake with a non-polar solvent removed any remaining vinyl laurate. The transesterification of QT with vinyl laurate was not so straightforward. The reaction mixture remained miscible, hence excess vinyl laurate had to be removed by PLC. This gave QT laurate (**22**) in a low isolated yield (8%).

The DS was calculated by integration of the ^{1}H NMR spectra (Table 2.6). It was interesting to note that the DS of **21** was notably less than **22**. This trend was also observed for the transacetylation of tannin with vinyl acetate (**19** and **20**). This suggested that QT was more reactive towards transesterification than PBT. One reason for this increased reactivity towards transesterification may be the greater ratio of B-ring trihydroxylation in QT compared with PBT[17, 20]. As shown for catechin, the B-ring was more reactive towards transesterification, and with more sites for esterification on the B-ring, this would lead to greater transesterification reactivity. However, this alone can not account for the large differences in DS between **21** and **22**. It was also thought that the extraction method of QT may impart a different reactivity towards transesterification compared with the hot

water extraction of PBT. However the methodology for the extraction of QT could not be obtained.

Table 2.6: The average DS of transesterified tannins (**21** and **22**), calculated by integration of the ^{1}H NMR spectra.

Reaction	**Aromatic signal**	**Aliphatic signals**			**Average DS**
		β-CH_2	**CH_2**	**CH_3**	
PBT laurate (21)	3.9	1.6	14	2.5	0.9
QT laurate (22)	4.9	8.5	78	15	4.8

Further spectroscopic techniques assisted the characterisation of **21** and **22**. The presence of ester linkages was confirmed by ^{13}C CP-MAS NMR and FTIR spectroscopy. In the ^{13}C CP-MAS NMR spectrum of **21** a carbonyl signal (180 ppm), methylene, and methyl signals (15-45 ppm) were observed (Figure 2.13). The aromatic carbon signals provided an insight as to the sites of substitution. The C-5, -7, -9 signal at *ca* 160 ppm showed no change in chemical shift after transesterification. In contrast, the C-3', -4' signal at *ca* 150 ppm was split into two peaks, of which one moved upfield by about 5-10 ppm, as expected upon esterification. This suggested transesterification of PBT with vinyl laurate occurred preferentially at the B-ring. This was similar to that observed for the preferential transesterification at the B-ring of catechin. Due to the low yield and gelatinous nature of **22**, a ^{13}C CP-MAS NMR spectrum could not be recorded. However, the FTIR spectrum showed a carbonyl stretch at 1743 cm^{-1} and 1710 cm^{-1} confirming the presence of esters.

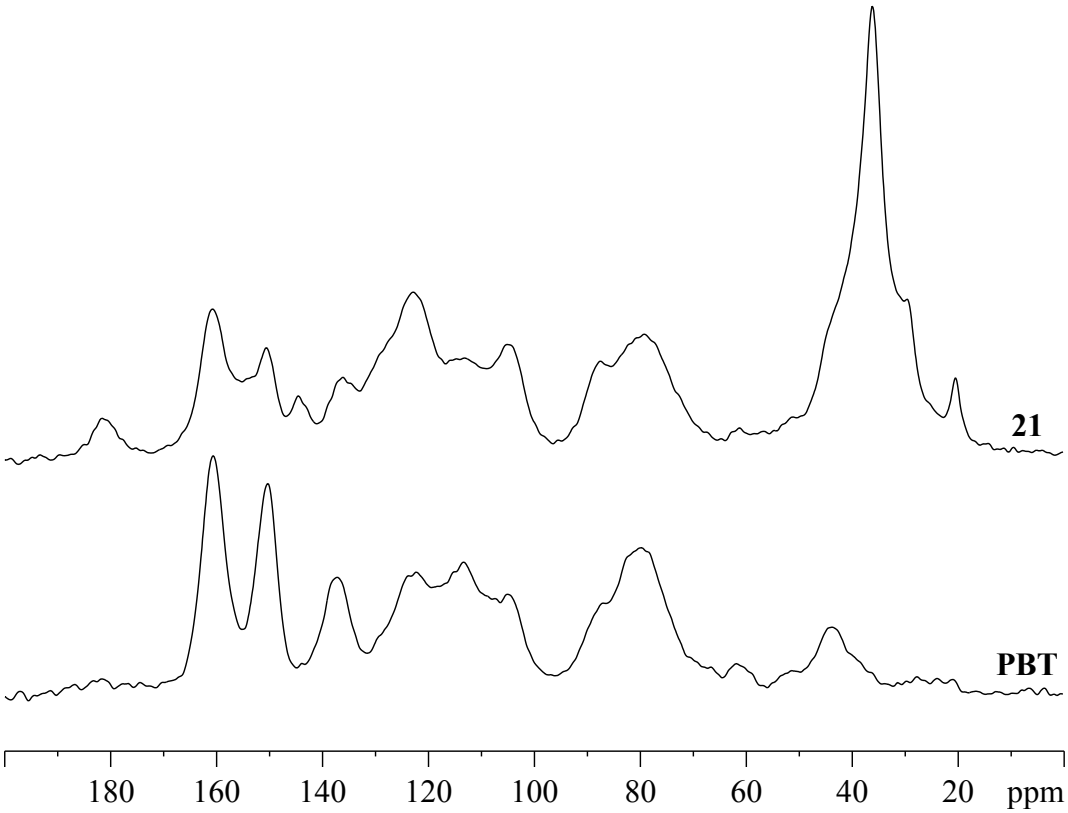


Figure 2.13: Solid state ^{13}C CP-MAS NMR spectrum of **21** compared with unreacted PBT.

GPC was used to understand the effect of the esterification method on the MW profile. Transesterified QT (**22**) was compared with QT laurates prepared from acid chlorides (**12** and **13**) (Figure 2.14). The MW profile of **22** was similar to that of **13**, however there were more low MW products. This agreed with the DS as calculated by ^{1}H NMR spectroscopy where **13** and **22** have similar DS. This also suggested that no major changes to the tannin macromolecule occurred during the esterification process. The MW distribution of **21** could not be compared with that of **10** and **11** as the three products were not mutually soluble in the same solvent.

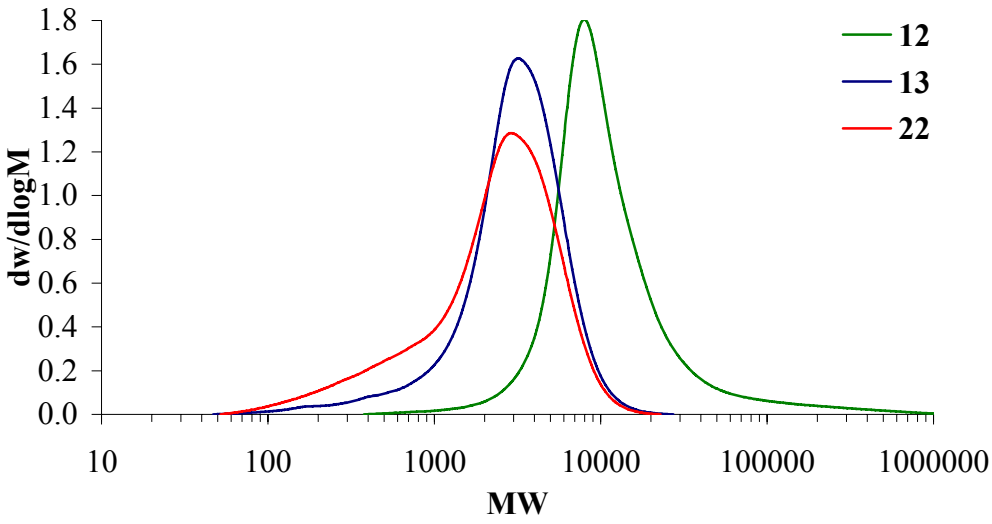


Figure 2.14: MW distributions of QT laurates (**12**, **13**, and **22**).

This transesterification method provided a simple route for the preparation of tannin laurates. In the case of QT laurate, the product was of similar nature to that prepared by the acid chloride. The PBT laurate prepared by transesterification was of a lesser DS compared with that prepared from the acid chloride. However, this method had several advantages. For instance, the reagents and solvents were easy to handle and the product was easy to isolate. The reaction was also more benign and potentially water can be used as a reaction medium.

2.3.3 Thermal Analysis

Thermal analysis is the study of material behaviour as a function of temperature. Both differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were used in this study. DSC measures heat flow to and from the sample during an increase or decrease in temperature, providing information on molecular or polymer transformations such as a phase change. TGA measures mass as a function of temperature, primarily providing information on decomposition and oxidation processes. Together these techniques provide information on how a material behaves under certain thermal conditions.

The thermal properties of catechin laurates were analysed by DSC (Appendix 15). Catechin showed a melting point *ca* 175 ^{o}C. Introducing 4-5 laurate groups (**5**

and **7**) significantly lowered the melting point to near room temperature. Thus, the melt behaviour of the catechin laurates was more like lauric acid which melts at 45 ^{o}C (Appendix 16). Only weak thermal transitions were observed for catechin derivatives with decreasing substitution. This may reflect the low fatty acid to flavonoid ratio or the decreased purity of the samples. However, when the first heating cycle was examined these transitions became more obvious. Especially **17** and **18** which showed thermal transitions at *ca* 78 ^{o}C and 40 ^{o}C respectively (Appendix 17).

The thermal properties of PBT laurates (**10**, **11**, and **21**) were compared by DSC and showed several similarities as well as key differences (Figure 2.15). The properties of **10** and **11** were similar between the first and second heating cycles. However, in the first heating cycle, **21** and PBT showed a broad transition at *ca* 90 ^{o}C which was not present in the second cycle; this may have been the loss of water. All three samples showed a transition near –20 ^{o}C, however for **21** this was less obvious. The more substituted PBT (**10** and **11**) showed strong thermal transitions between 45-50 ^{o}C, presumably melt behaviours. This was similar to the melting point of lauric acid. In contrast, PBT and **21** showed a broad transition with a maximum at about 130 ^{o}C. This suggested that a high degree of substitution was required for the material to adopt thermal characteristics similar to those of lauric acid (low melting point).

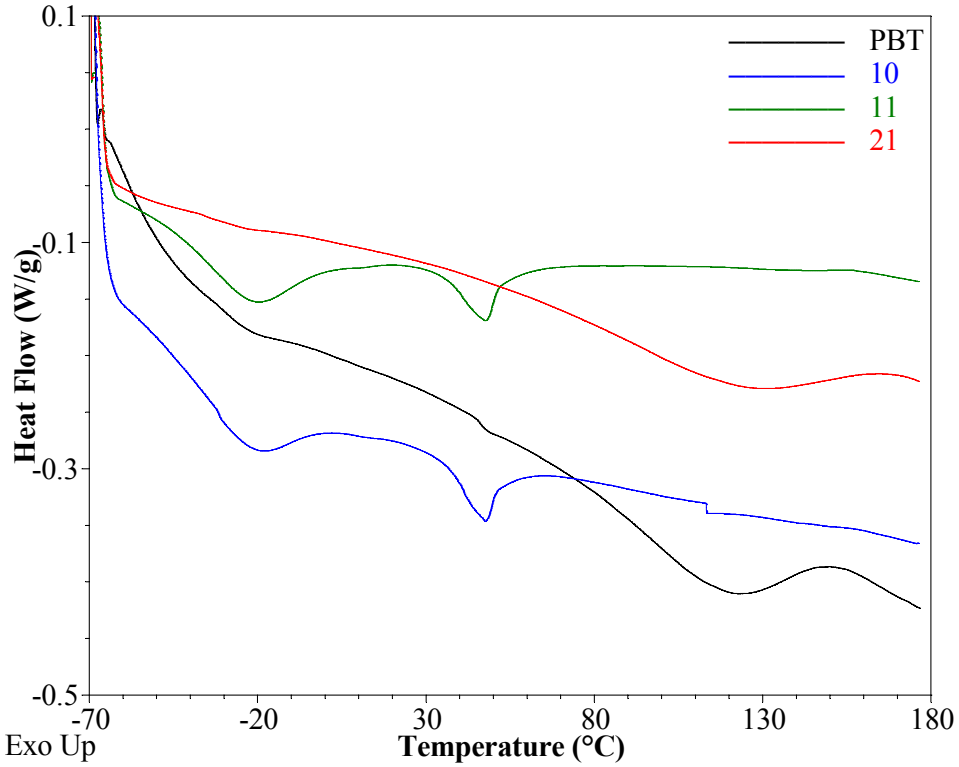


Figure 2.15: DSC thermogram of products **10**, **11**, and **21** compared with unreacted PBT (second heating cycle).

The QT laurates (**12**, **13**, and **22**) were analysed by DSC and showed several thermal transitions (Figure 2.16). For all samples (**12**, **13**, and **22**) a broad endothermic event was observed from –50 to 0 °C. This was also observed for unreacted QT, suggesting this thermal transition was inherent to the tannin polymer. This event was not a melting feature, as tannins were solid at room temperature, rather it may be an increased floppiness or mobility of the tannin macromolecules in the solid state. Only sample **22** showed an obvious melting transition at *ca* 30 °C. Based on PBT results, it was expected that samples **12** and **13** would also show melting behaviour near this temperature, therefore the absence of an obvious endothermic transitions was surprising.

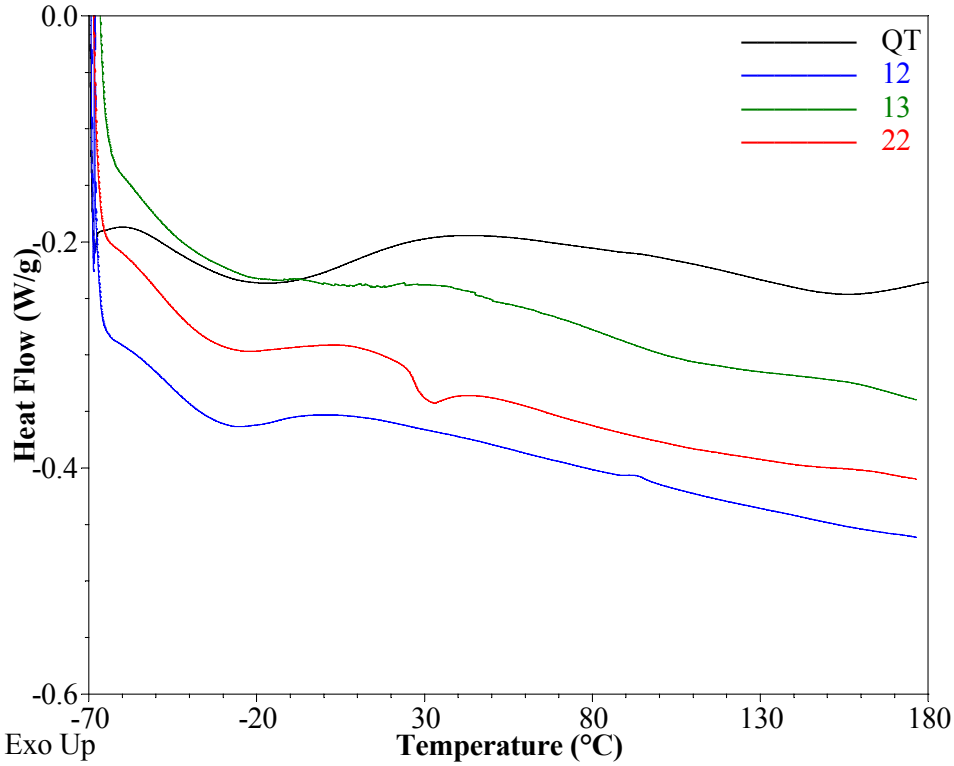


Figure 2.16: DSC thermogram of products **12**, **13**, and **22** compared with unreacted QT (second heating cycle).

The thermal degradation profiles of PBT and QT laurates were analysed by TGA under nitrogen. The PBT laurates showed few differences in the thermal degradation profile compared with unreacted PBT (Appendix 18). The main point of difference was the loss of mass around 80 °C for unreacted PBT. This was most likely water, as tannin extracts are hydroscopic. In contrast, the PBT laurates (**10**, **11,** and **21**) which were more hydrophobic did not show this water loss. The QT laurates showed several differences in thermal degradation compared with unreacted QT (Figure 2.17). Likewise to PBT, unreacted QT showed a loss of mass around 80 °C. In contrast the QT laurates (**12** and **13**) which were more hydrophobic did not show this water loss. Secondly, the onset of degradation was suppressed in both **12** and **13**, by about 50 °C. However the degradation of **12** and **13** occurred much more rapidly once this onset was reached. Increasing the onset temperature of degradation is beneficial for high temperature processing or applications. For instance, the incorporation into thermoplastics which often requires temperatures above 180 °C for plastics such as polyethylene or polypropylene.

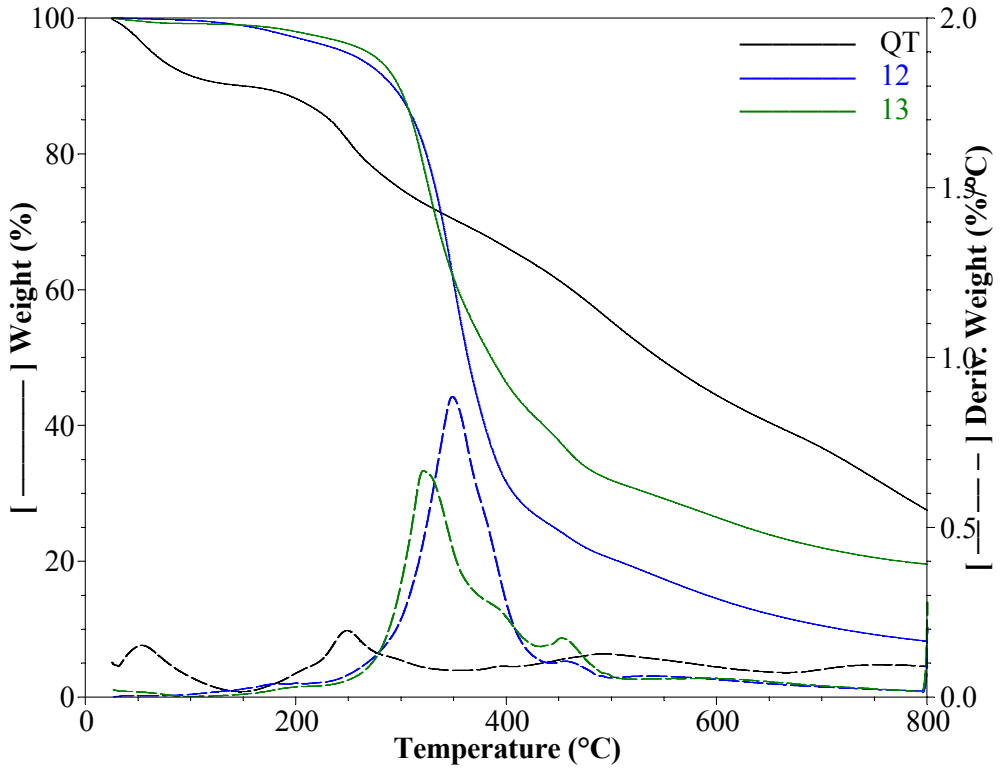


Figure 2.17: TGA thermogram of QT and QT laurates (**12** and **13**). The left axis shows the percent weight, while the right axis shows the weight derivative (rate of weight loss).

In conclusion, the introduction of fatty acid esters to flavonoids changed the thermal characteristics relative to the starting polyphenol. PBT laurates of high DS showed endothermic transitions at similar temperatures to those of lauric acid. In contrast, QT laurates prepared from lauroyl chloride did not show this behaviour, with little distinction in thermal behaviour from unreacted QT. In addition, the onset of thermal degradation was increased by the incorporation of lauric acid esters. Consequently, the products may have applications in areas such as polymer additives, where greater thermal stability and melt/flow properties are desirable.

2.3.4 Antioxidant and Skin Protection Factor Activity

Many studies have shown the high antioxidant and skin protection factor (SPF) activity of tannins[22, 31, 32]. It was of interest to determine the change in antioxidant and SPF activity upon the incorporation of fatty acid esters to tannin. Only PBT

laurate (**21**) was prepared in sufficient quantity for further antioxidant and SPF tests.

The antioxidant activity was measured by a Trolox equivalent antioxidant capacity (TEAC) assay. This showed that **21** had a reduced antioxidant capability compared with unreacted PBT (Table 2.7). This result was expected as most substitution occurred at the B-ring. The ortho hydroxyl groups of the B-ring contribute significantly to the high antioxidant activity of tannins[31, 32]. This was in accordance with Jin and Yoshioka[53] who showed catechin-3',4'-dilaurate and catechin-3,3',4'-trilaurate had decreased radical scavenging ability compared with catechin. However, they also showed that in lipid membranes the catechin laurates performed better compared with catechin due to their greater affinity for the membrane. Therefore, although the total antioxidant activity of **21** was decreased, it may offer better miscibility and hence protection to lipophilic materials.

Table 2.7: The antioxidant and SPF activity of PBT laurate (**21**) compared with unreacted PBT.

Product	**Antioxidant activity**	**SPF**	
	(µmole TEAC/g DW)	***in vitro***	**PMMA film**
PBT	5617	3.0	1.5
PBT laurate (21)	2544	7.4	2.5

The SPF activity was measured in solution (SPF i*n vitro*) and as a polymethyl methacrylate (PMMA) film by UV/VIS spectroscopy (Table 2.7). A PMMA film was used because it is transparent to UV light down to 300 nm. Both tests showed an increase in the SPF of **21** compared with unreacted PBT. In solution **21** gave greater than twice the SPF of unreacted PBT. Perhaps the increased hydrophobic nature of **21** allowed it to disperse better within the solution and the PMMA film, therefore increasing the SPF activity compared with unreacted tannin.

2.4 Summary

The purpose of this research was to prepare and characterise polyphenol fatty acid esters and thereby change the chemical and physical properties of polyphenols. Esterification was achieved through the reaction with acid chlorides and by transesterification. Subsequently, the products were characterised both chemically and physically to identify properties suitable for further application.

Model compounds were prepared from resorcinol, catechin, and tannin using acid chlorides and anhydrides. This included the first report of resorcinol dilaurate and catechin pentalaurate. These products allowed diagnostic features such as ^{1}H NMR and ^{13}C NMR chemical shifts and ESI-MS ions to be identified.

The transesterification of phenols with vinyl esters was developed to provide an alternative route to polyphenol esters. The method was developed using resorcinol. The ester interchange occurred effectively in the presence of a base catalyst, whereas an acid catalyst proved unsuccessful. The reaction was versatile and occurred in both aqueous and DMSO solvents with either short or long chain vinyl esters. These results allowed transesterification methodologies to be adapted for the introduction of acetyl or lauroyl groups to catechin and tannin.

The transesterification of catechin proved successful with both vinyl acetate and vinyl laurate. However, the maximum DS was relatively low in comparison with that obtained by conventional esterification methods. The B-ring of catechin exhibited a preference for transesterification over the A- or C-ring. While, the acylation of catechin with an acid chloride also shows a slight preference for reaction at the B-ring over the A-ring, this preference was more dominant for the transesterification process.

In a similar manner to catechin, tannins were successfully transesterified. The reaction and workup proved simple and effective for PBT. In contrast, the ester preparation using acid chlorides required careful removal of water and extensive purification to remove fatty acid by-products. The B-ring showed a preference for

transesterification over the A-ring as observed for the analogous reaction with catechin.

Changes in the thermal behaviour, antioxidant, and SPF properties of polyphenols was observed upon esterification. The introduction of fatty acid esters generally produced a melting transition near that of the parent fatty acid. However, in several cases no obvious endothermic transitions were observed. For QT, esterification also increased the onset of thermal degradation. The transesterification of PBT (**21**) significantly changed the antioxidant and SPF activities. The antioxidant activity was reduced compared with unreacted PBT, due to capping of the B-ring hydroxyls. However, the SPF activity increased when measured both in solution and as a PMMA film.

In conclusion, many polyphenol fatty acid esters were produced by conventional acylation methods and transesterification. Transesterification proved a successful and potentially advantageous route to generate polyphenolic esters. However, further optimisation of the method is required to achieve full substitution of flavonoids. Thermal and SPF analysis showed polyphenol esters may have desirable properties for several applications.

Chapter 3 - Etherification of Polyphenols

3.1 Introduction

Etherification is a well known route for the derivatisation of lignin[99]. This chemistry can also be mirrored for the derivatisation of other polyphenols such as tannin. As with lignin, etherification of tannin may change the chemical and physical properties of the derivatised materials. Two routes were used to introduce ether linked functionalities to polyphenols: (i) the Williamson ether synthesis and (ii) ring opening of epoxides.

The Williamson ether synthesis has been used on several occasions for the etherification of polyphenols. Most notable are carboxymethylation[83], reaction with bromooctane[48], and the introduction of cationic functional groups to tannins[42]. Both chloroalkane and bromoalkane derivatives have been used in a range of solvents at elevated temperatures. In this study, the Williamson ether synthesis would provide products for comparison to those produced via the ring opening of epoxides.

The hydroxyalkylation of lignin through the ring opening of alkylene oxides has been extensively studied. Furthermore, this process has been carried out on pilot plant scale[99]. Hydroxyalkylation has been found to change the thermal and mechanical properties of lignin. The most studied property is the effect of hydroxyalkylation on the lignin glass transition temperature. The glass transition temperature decreases dramatically upon hydroxyalkylation due to the disruption of hydrogen bonds and increasing the polymer free volume[103]. These products have found application in polyurethane foams to improve their stability at elevated temperatures[99].

When applying these preparation methods to polyphenols, a range of sites for substitution are available. Therefore, a large number of products with varying sites of substitution are possible. Furthermore, for the ring opening of epoxides chain-extension can occur, this leading to a complex array of products. Using NMR spectroscopy an insight to the nature of these products can be gained.

Integration of the NMR spectra allows the average molar substitution to be determined. As a result of the complex nature of the products, numbered products refer to mixtures rather than pure compounds.

Changing the chemistry and properties of tannins through etherification may extend their current utilisation. The modified tannins may be useful in their own right, or provide a bio-derived chemical feedstock for industrial applications. For example, tannin ethers may find application as polymer additives. Furthermore, by changing the phenol groups to secondary hydroxyls may allow previously inaccessible derivatisations, such as Fisher esterification using carboxylic acids, dehydration to yield an alkene or conversion into an alkyl halide[38].

This chapter describes the preparation of polyphenolic ethers via both the Williamson ether synthesis and ring opening of epoxides. Use of simple phenolics such as resorcinol and catechin allowed the chemistry to be established with the method then adapted for tannins.

3.2 Experimental

3.2.1 Materials and Instrumentation

Reagents

1-Chloro-2-propanol (Merck) and propylene oxide (BDH) were used without further purification.

Gel permeation chromatography (GPC)

The instrumentation used was as previously reported (Section 2.2.1). Two PLgel mixed-E (300 mm x 7.5 mm, 3 μm) columns connected in series and protected by a guard column (50 mm x 7.5 mm) of the same material were used. Samples were eluted using DMF containing 1% (v/v) acetic acid, 5% (v/v) water, and 0.15 $molL^{-1}$ lithium chloride. A flow rate of 0.5 $mLmin^{-1}$ was used with a column temperature of 50 ^{o}C and detection by UV (280 nm) and RI. Samples were dissolved in the same solvent used above at approximately 1.0 $mgmL^{-1}$, filtered through a 0.45 μm nylon syringe filter, and a volume of 100 μL was injected onto the column. Polyethylene glycol standards (MW 194-23000 $gmol^{-1}$) and known

constituents of PBT, such as taxifolin, dimers and trimers[17]were used to generate a calibration curve.

Rheology

Rheology measurements were obtained on a TA Instruments AR 2000 rheometer. A 25 mm plate/plate geometry was used with a gap of 300 μm. An oscillation frequency of 1 Hz at constant torque of 2000 μNm^{-1} was used over a temperature ramp from –20 to 80 ^{o}C.

3.2.2 Etherification by The Williamson Ether Synthesis

Screening reactions of resorcinol with 1-chloro-2-propanol

Resorcinol (*ca* 0.1 g, 0.9 mmol) was dissolved in solvent (10 mL). An excess of finely ground potassium carbonate (*ca* 0.4 g, 2.9 mmol) and an excess of 1-chloro-2-propanol (0.25 mL, 2.9 mmol) were added. The reaction mixture was stirred for 8 h with or without heating, after which the solid phase was removed by filtration (Table 3.1). The solution was acidified with HCl (0.1 $molL^{-1}$) and concentrated by rotary evaporation. TLC and ^{1}H NMR spectroscopy allowed assessment of the reaction products (**23a-f**).

Table 3.1: Conditions for screening reactions of resorcinol with 1-chloro-2-propanol.

Product	**Solvent**	**Temperature (^{o}C)**
23a	water	20
23b	acetone	20
23c	DMF	20
23d	water	reflux
23e	acetone	reflux
23f	DMF	105

Preparation of catechin hydroxypropyl ether in water

Catechin (0.09 g, 0.30 mmol) was suspended in water (10 mL) and potassium carbonate (0.04 g, 0.30 mmol) was added. The catechin solution was refluxed for 10 min and allowed to cool. 1-Chloro-2-propanol (30 μL, 0.35 mmol) was added and the mixture refluxed for 60 min. The reaction mixture was

neutralised with HCl (0.1 $molL^{-1}$) and concentrated by rotary evaporation to yield a brown solid (0.11 g), tentatively assigned by NMR spectroscopy as catechinic acid (**24**)[28].

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 6.72 (1H, d, J = 2.2 Hz, H-2'), 6.70 (1H, d, J = 8.1 Hz, H-5'), 6.59 (1H, dd, J = 8.1, 2.1 Hz, H-6'), 5.74 (1H, s, H-3), 4.40 (1H, dt, J = 10.9, 5.3 Hz, H-7), 3.21 (1H, dd, J = 4.1, 1.8 Hz, H-5), 3.13 (1H, m, H-1), 2.99 (1H, dd, J = 10.8, 4.1 Hz, H-6), 2.48 (1H, m, H-8b), 1.87 (1H, m, H-8a).

Preparation of catechin hydroxypropyl ether in acetone

Catechin (0.11 g, 0.38 mmol) was dissolved in acetone (2 mL) and powdered potassium carbonate (0.31 g, 2.2 mmol) was added. The solution was heated to reflux for 10 min, allowed to cool, and 1-chloro-2-propanol (0.31 mL, 3.7 mmol) added. The reaction mixture was refluxed for 24 h, cooled, filtered, and concentrated by rotary evaporation to yield a red liquid (0.44 g). PLC with EtOAc/acetone/MeOH (10:9:1) gave three bands, which were extracted for analysis.

Band 1 (R_f = 0.67) gave a mixed catechin hydroxypropyl ether derivative as a yellow oil (**25;** 39 mg, 9%).

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 7.1-6.7 (3H, m, H-2', 5', 6'), 6.2-5.8 (2H, m, H-6, 8), 4.90 (3H, tq, J = 6.7 Hz, CHOH), 4.6-3.5 (8H, m, CH_2, H-2, 3), 2.50 (1H, dd, $J_{4,4}$ = 15.8 Hz, $J_{3,4}$ = 5.9 Hz, H-4a), 2.90 (1H, dd, $J_{4,4}$ = 16.2 Hz, $J_{3,4}$ = 8.6 Hz, H-4b), 1.42 (9H, d, J = 6.3 Hz, CH_2).

ESI-MS (MeOH): *m/z* 289 $[C\text{-}H]^-$, 325 $[C+Cl]^-$, 347 $[C(HPE)_1\text{-}H]^-$, 383 $[C(HPE)_1+Cl]^-$, 579 $[C_2\text{-}H]^-$.

Band 2 (R_f = 0.30) gave an unidentified brown solid (38 mg).

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 4.9-4.5 (2H, m), 3.94 (1H, m), 3.57 (1H, dd, J = 3.8 Hz, 10.7 Hz), 3.38 (1H, dd, J = 7.5 Hz, 10.7 Hz), 3.20 (10H, s), 1.26 (3H, s), 1.19 (1H, s), 1.13 (3H, d, J = 6.7 Hz), 0.84 (2H, m).

ESI-MS (MeOH): *m/z* 254, 300, 381.

Band 3 (R_f = 0.07) gave an unidentified brown solid (13 mg).

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 8.35 (1H, s), 3.81 (1H, q, J = 6.8 Hz), 3.53 (2H, q, J = 7.2 Hz), 2.01 (3H, m), 1.80 (4H, s), 1.43 (2H, m), 1.17 (12H, m), 1.07 (4H, dt, J = 4.0 Hz, 7.1 Hz), 0.76 (4H, m).
ESI-MS (MeOH): *m/z* 298, 378, 391.

3.2.3 Etherification by Ring Opening of Propylene Oxide

Screening reactions of resorcinol with propylene oxide

Resorcinol (*ca* 0.1 g, 0.9 mmol) was added to a 15 mL Teflon-lined stainless steel pressure reactor and dissolved in varying volumes of water and/or propylene oxide (PO) (Table 3.2). A range of common catalysts were used: potassium hydroxide, sodium hydroxide and triethylamine (TEA). The reactor was sealed and placed in an oil bath at the desired temperatures for 24 h after which it was cooled to room temperature. The products (**26a-l**) were neutralised with HCl (0.1 $molL^{-1}$) and concentrated by rotary evaporation.

Table 3.2: Reaction conditions for the etherification of resorcinol with PO.

Reaction	Volume water (mL)[a]	Catalyst[b]		Charge of PO[c]	Temp (^oC)[d]
		Catalyst	Quantity (%)		
26a	5	KOH	5	2	20
26b	5	KOH	5	2	50
26c	5	KOH	5	2	80
26d	5	KOH	5	2	110
26e	5	KOH	5	4	50
26f	5	KOH	20	2	50
26g	1	KOH	5	2	50
26h	5	NaOH	5	2	50
26i	5	TEA	5	2	50
26j	0	TEA	5	2	50
26k	0	TEA	5	2	80
26l	0	TEA	5	2	110

a = Volume of solvent (water)

b = Catalyst and quantity of catalyst

c = Mole equivalent charge of propylene oxide

d = Temperature (^oC)

Preparation of catechin hydroxypropyl ether with 1 mole equivalent of PO

Catechin (0.20 g, 0.69 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 5 μL) and 1 mole equivalent of PO (49 μL, 0.69 mmol) were added. The reactor was sealed and heated to 110 ^oC for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 $molL^{-1}$), poured into butanol (20 mL), washed with saturated $NaHCO_3$ (2 x 20 mL), HCl (0.1 $molL^{-1}$, 3 x 20 mL), water (1 x 20 mL), saturated NaCl (2 x 20 mL), dried with $MgSO_4$, and concentrated by rotary evaporation to yield a yellow glassy product (**27**; 70 mg, 29%).

^1H NMR (400 MHz, CD_3COCD_3): δ_H 8.5-7.5 (3H, br, OH), 7.3-6.4 (3H, m, H-2', 5', 6'), 6.3-5.8 (1H, m, H-6, 8), 5.0-2.3 (17H, m, CH_2, CHOH, H-2, 3, 4), 1.7-0.6 (5H, m, CH_3).

^{13}C NMR (400MHz, CD_3COCD_3): δ_C 158-154 (C-5, 7, 9), 146-143 (C-3', 4'), 132-130 (C-1'), 122-111 (C-2', 5', 6'), 100 (C-10), 97-93 (C-6, 8), 83-64 (CH_2, CHOH, C-2, 3), 23-15 (CH_3, C-4).
ESI-MS (MeOH): *m/z* 347 $[C(HPE)_1\text{-}H]^-$, 383 $[C(HPE)_1\text{+}Cl]^-$, 405 $[C(HPE)_2\text{-}H]^-$, 441 $[C(HPE)_2\text{+}Cl]^-$, 463 $[C(HPE)_3\text{-}H]^-$, 499 $[C(HPE)_3\text{+}Cl]^-$.

Preparation of catechin hydroxypropyl ether with 3 mole equivalents of PO
Catechin (0.19 g, 0.65 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 5 µL) and 3 mole equivalents of PO (138 µL, 2.0 mmol) were added. The reactor was sealed and heated to 110 ^oC for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 $molL^{-1}$), poured into butanol (20 mL), washed with saturated $NaHCO_3$ (2 x 20 mL), HCl (0.1 $molL^{-1}$, 3 x 20 mL), water (1 x 20 mL), saturated NaCl (2 x 20 mL), dried with $MgSO_4$, and concentrated by rotary evaporation to yield a yellow glassy product (**28**; 81 mg, 27%).
^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 8.5-7.5 (2H, br, OH), 7.3-6.5 (3H, m, H-2', 5', 6'), 6.3-5.8 (1H, m, H-6, 8), 5.0-2.5 (12H, m, CH_2, CHOH, H-2, 3, 4), 1.5-0.8 (7H, m, CH_3).
^{13}C NMR (400MHz, CD_3COCD_3): δ_C 158-153 (C-5, 7, 9), 148-143 (C-3', 4'), 135-130 (C-1'), 123-112 (C-2', 5', 6'), 102-99 (C-10), 97-93 (C-6, 8), 83-65 (CH_2, CHOH, C-2, 3), 25-16 (C-4, CH_3).
ESI-MS (MeOH): *m/z* 347 $[C(HPE)_1\text{-}H]^-$, 383 $[C(HPE)_1\text{+}Cl]^-$, 405 $[C(HPE)_2\text{-}H]^-$, 441 $[C(HPE)_2\text{+}Cl]^-$, 463 $[C(HPE)_3\text{-}H]^-$, 499 $[C(HPE)_3\text{+}Cl]^-$, 521 $[C(HPE)_4\text{-}H]^-$, 557 $[C(HPE)_4\text{+}Cl]^-$.

Preparation of catechin hydroxypropyl ether with 5 mole equivalents of PO
Catechin (0.20 g, 0.69 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 5 µL) and 5 mole equivalents of PO (244 µL, 3.5 mmol) were added. The reactor was sealed and heated to 110 ^oC for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 $molL^{-1}$), poured into butanol (20 mL), washed with saturated $NaHCO_3$ (2 x 20 mL), HCl (0.1 $molL^{-1}$, 3 x 20 mL), water (1 x 20 mL), saturated NaCl (2 x 20 mL), dried with $MgSO_4$, and concentrated by rotary evaporation to yield a yellow glassy product (**29**; 0.19 g, 46%).

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 8.5-7.5 (2H, br, OH), 7.3-6.5 (3H, m, H-2', 5', 6'), 6.4-5.7 (1H, m, H-6, 8), 5.4-2.4 (14H, m, CH_2, CHOH, H-2, 3, 4), 1.7-0.8 (11H, m, CH_3).

^{13}C NMR (400MHz, CD_3COCD_3): δ_C 160-152 (C-5, 7, 9), 150-145 (C-3', 4'), 132-130 (C-1'), 121-112 (C-2', 5', 6'), 101-99 (C-10), 97-92 (C-6, 8), 84-65 (CH_2, CHOH, C-2, 3), 25-13 (C-4, CH_3).

ESI-MS (MeOH): *m/z* 347 $[C(HPE)_1\text{-}H]^-$, 383 $[C(HPE)_1\text{+}Cl]^-$, 405 $[C(HPE)_2\text{-}H]^-$, 441 $[C(HPE)_2\text{+}Cl]^-$, 463 $[C(HPE)_3\text{-}H]^-$, 499 $[C(HPE)_3\text{+}Cl]^-$, 521 $[C(HPE)_4\text{-}H]^-$, 557 $[C(HPE)_4\text{+}Cl]^-$, 579 $[C(HPE)_5\text{-}H]^-$, 615 $[C(HPE)_5\text{+}Cl]^-$.

Preparation of chain-extended catechin hydroxypropyl ether with 10 mole equivalents of PO

Catechin (0.23 g, 0.79 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 5 µL) and 10 mole equivalents of PO (560 µL, 8.0 mmol) were added. The reactor was sealed and heated to 110 ^{o}C for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 molL^{-1}), poured into butanol (20 mL), washed with saturated $NaHCO_3$ (2 x 20 mL), HCl (0.1 molL^{-1}, 4 x 20 mL), water (1 x 20 mL), saturated NaCl (2 x 20 mL), dried with $MgSO_4$, and concentrated by rotary evaporation to yield a yellow oily product (**30**; 0.25 g, 35%).

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 7.4-6.8 (3H, m, H-2', 5', 6'), 6.4-5.9 (2H, m, H-6, 8), 5.1-2.4 (30H, m, CH_2, CHOH, H-2, 3, 4), 1.5-0.7 (19H, m, CH_3).

^{13}C NMR (400MHz, CD_3COCD_3): δ_C 160-155 (C-5, 7, 9), 150-147 (C-3', 4'), 134-133 (C-1'), 122-112 (C-2', 5', 6'), 103-100 (C-10), 95-93 (C-6, 8), 82-64 (CH_2, CHOH, C-2, 3), 25-15 (C-4, CH_3).

ESI-MS (MeOH): *m/z* 1195 $[C(HPE)_{15}\text{+}Cl]^-$, 1253 $[C(HPE)_{16}\text{+}Cl]^-$, 1311 $[C(HPE)_{17}\text{+}Cl]^-$, 1369 $[C(HPE)_{18}\text{+}Cl]^-$, 1427 $[C(HPE)_{19}\text{+}Cl]^-$, 1485 $[C(HPE)_{20}\text{+}Cl]^-$, 1543 $[C(HPE)_{21}\text{+}Cl]^-$.

Preparation of chain-extended catechin hydroxypropyl ether with 20 mole equivalents of PO

Catechin (0.23 g, 0.79 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 5 µL) and 20 mole equivalents of PO (1.09 mL, 16 mmol) were added. The reactor was sealed and heated to 110 ^{o}C for 24 h, after which the reactor was cooled to room temperature. The product was

neutralised with HCl (0.1 $molL^{-1}$), poured into butanol (20 mL), washed with saturated $NaHCO_3$ (2 x 20 mL), HCl (0.1 $molL^{-1}$, 4 x 20 mL), water (1 x 20 mL), saturated NaCl (2 x 20 mL), dried with $MgSO_4$, and concentrated by rotary evaporation to yield a yellow oily product (**31**; 0.34 g, 30%).

^{1}H NMR (400 MHz, CD_3COCD_3): δ_H 7.4-6.8 (3H, m, H-2', 5', 6'), 6.4-5.9 (2H, m, H-6, 8), 5.1-2.4 (30H, m, CH_2, CHOH, H-2, 3, 4), 1.5-0.7 (21H, m, CH_3).

^{13}C NMR (400MHz, CD_3COCD_3): δ_C 160-155 (C-5, 7, 9), 150-147 (C-3', 4'), 134-133 (C-1'), 122-112 (C-2', 5', 6'), 103-100 (C-10), 95-93 (C-6, 8), 82-64 (CH_2, CHOH, C-2, 3), 25-15 (C-4, CH_3).

ESI-MS (MeOH): *m/z* 1253 $[C(HPE)_{16}+Cl]^-$, 1311 $[C(HPE)_{17}+Cl]^-$, 1369 $[C(HPE)_{18}+Cl]^-$, 1427 $[C(HPE)_{19}+Cl]^-$, 1485 $[C(HPE)_{20}+Cl]^-$, 1543 $[C(HPE)_{21}+Cl]^-$, 1601 $[C(HPE)_{22}+Cl]^-$.

Preparation of PBT hydroxypropyl ether with 1 mole equivalent of PO

PBT (0.99 g, 3.4 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 24 μL) and 1 mole equivalent of PO (0.24 mL, 3.4 mmol) were added. The reactor was sealed and heated to 110 ^oC for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 $molL^{-1}$), transferred to a flask, and concentrated by rotary evaporation to yield a brown glassy solid (**32**; 1.17 g, 99%).

^{1}H NMR (400 MHz, CD_3SOCD_3): δ_H 7.8-5.7 (3.9H, br, Ar-H), 5.5-3.0 (10H, br, CH_2, CHOH, C-ring), 1.4-0.8 (5H, br, CH_3).

^{13}C NMR (200 MHz, CP-MAS): δ_C 165-153 (C-5, 7, 9), 153-143 (C-3', 4'), 140-130 (C-1'), 130-96 (C-6, 8, 10, 2', 5', 6'), 93-55 (CH_2, CHOH, C-2, 3), 49-30 (C-4), 30-10 (CH_3).

Preparation of PBT hydroxypropyl ether with 3 mole equivalents of PO

PBT (0.91 g, 3.1 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 22 μL) and 3 mole equivalents of PO (0.66 mL, 9.3 mmol) were added. The reactor was sealed and heated to 110 ^oC for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 $molL^{-1}$), transferred to a flask, and concentrated by rotary evaporation to yield a brown glassy solid (**33**; 1.46 g, 100%).

^{1}H NMR (400 MHz, CD_3SOCD_3): δ_H 7.8-5.7 (3.9H, br, Ar-H), 5.5-3.0 (27H, br, CH_2, CHOH, C-ring), 1.4-0.8 (17H, br, CH_3).
^{13}C NMR (200 MHz, CP-MAS): δ_C 165-153 (C-5, 7, 9), 153-144 (C-3', 4'), 142-130 (C-1'), 130-94 (C-6, 8, 10, 2', 5', 6'), 93-54 (CH_2, CHOH, C-2, 3), 46-31 (C-4), 30-11 (CH_3).

Preparation of PBT hydroxypropyl ether with 5 mole equivalents of PO

PBT (0.96 g, 3.3 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 23 μL) and 5 mole equivalents of PO (1.19 mL, 17 mmol) were added. The reactor was sealed and heated to 110 ^{o}C for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 molL^{-1}), transferred to a flask, and concentrated by rotary evaporation to yield a brown glassy solid (**34**; 1.83 g, 96%).
^{1}H NMR (400 MHz, CD_3SOCD_3): δ_H 7.8-5.7 (3.9H, br, Ar-H), 5.5-3.0 (30H, br, CH_2, CHOH, C-ring), 1.4-0.8 (21H, br, CH_3).
^{13}C NMR (200 MHz, CP-MAS): δ_C 165-154 (C-5, 7, 9), 154-146 (C-3', 4'), 143-131 (C-1'), 130-92 (C-6, 8, 10, 2', 5', 6'), 90-56 (CH_2, CHOH, C-2, 3), 47-31 (C-4), 30-12 (CH_3).

Preparation of chain-extended PBT hydroxypropyl ether with 10 mole equivalents of PO

PBT (0.82 g, 2.8 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 20 μL) and 10 mole equivalents of PO (1.97 mL, 28 mmol) were added. The reactor was sealed and heated to 110 ^{o}C for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 molL^{-1}), transferred to a flask, and concentrated by rotary evaporation to yield a brown oily product (**35**; 2.05 g, 84%).
^{1}H NMR (400 MHz, CD_3SOCD_3): δ_H 8.0-5.6 (3.9H, br, Ar-H), 5.5-2.9 (46H, br, CH_2, CHOH, C-ring), 1.5-0.5 (40H, br, CH_3).

Preparation of chain-extended PBT hydroxypropyl ether with 20 mole equivalents of PO

PBT (0.91 g, 3.1 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 22 μL) and 20 mole equivalents of PO (4.38 mL, 62 mmol) were added. The reactor was sealed and heated to 110 ^{o}C for 24 h, after which the reactor was cooled to room temperature. The product was

neutralised with HCl (0.1 $molL^{-1}$), transferred to a flask, and concentrated by rotary evaporation to yield a brown oily product (**36**; 2.65 g, 58%).

^{1}H NMR (400 MHz, CD_3SOCD_3): δ_H 8.0-5.6 (3.9H, br, Ar-H), 5.5-2.9 (51H, br, CH_2, CHOH, C-ring), 1.5-0.5 (42H, br, CH_3).

Preparation of QT hydroxypropyl ether with 1 mole equivalent of PO

QT (0.98 g, 3.5 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 25 μL) and 1 mole equivalent of PO (0.25 mL, 3.5 mmol) were added. The reactor was sealed and heated to 110 ^{o}C for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 $molL^{-1}$), transferred to a flask, and concentrated by rotary evaporation to yield a brown glassy solid (**37**; 1.10 g, 93%).

^{1}H NMR (400 MHz, CD_3SOCD_3): δ_H 7.4-5.7 (4.9H, br, Ar-H), 5.2-2.9 (24H, br, CH_2, CHOH, C-ring), 1.4-0.8 (5H, br, CH_3).

^{13}C NMR (200 MHz, CP-MAS): δ_C 165-153 (C-7, 9), 153-143 (C-3', 4'), 140-130 (C-1'), 130-100 (C-5, 6, 8, 10, 2', 5', 6'), 90-65 (CH_2, CHOH, C-2, 3), 48-30 (C-4), 29-15 (CH_3).

Preparation of QT hydroxypropyl ether with 3 mole equivalents of PO

QT (0.96 g, 3.5 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 24 μL) and 3 mole equivalents of PO (0.73 mL, 11 mmol) were added. The reactor was sealed and heated to 110 ^{o}C for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 $molL^{-1}$), transferred to a flask, and concentrated by rotary evaporation to yield a brown glassy solid (**38**; 1.45 g, 93%).

^{1}H NMR (400 MHz, CD_3SOCD_3): δ_H 7.4-5.6 (4.9H, br, Ar-H), 5.3-2.9 (39H, br, CH_2, CHOH, C-ring), 1.4-0.8 (15H, br, CH_3).

^{13}C NMR (200 MHz, CP-MAS): δ_C 165-152 (C-7, 9), 152-143 (C-3', 4'), 141-130 (C-1'), 130-100 (C-5, 6, 8, 10, 2', 5', 6'), 90-65 (CH_2, CHOH, C-2, 3), 48-30 (C-4), 30-15 (CH_3).

Preparation of QT hydroxypropyl ether with 5 mole equivalents of PO

QT (0.95 g, 3.4 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 24 μL) and 5 mole equivalents of PO (1.02 mL, 17 mmol) were added. The reactor was sealed and heated to 110 ^{o}C for 24 h,

after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 $molL^{-1}$), transferred to a flask, and concentrated by rotary evaporation to yield a brown glassy solid (**39**; 1.43 g, 79%).

^{1}H NMR (400 MHz, CD_3SOCD_3): δ_H 7.4-5.5 (4.9H, br, Ar-H), 5.0-3.0 (39H, br, CH_2, CHOH, C-ring), 1.4-0.9 (16H, br, CH_3).

^{13}C NMR (200 MHz, CP-MAS): δ_C 166-154 (C-7, 9), 154-143 (C-3', 4'), 141-130 (C-1'), 130-100 (C-5, 6, 8, 10, 2', 5', 6'), 90-65 (CH_2, CHOH, C-2, 3), 48-30 (C-4), 30-15 (CH_3).

Preparation of chain-extended QT hydroxypropyl ether with 10 mole equivalents of PO

QT (0.82 g, 3.0 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 20 μL) and 10 mole equivalents of PO (2.07 mL, 30 mmol) were added. The reactor was sealed and heated to 110 ^{o}C for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 $molL^{-1}$), transferred to a flask, and concentrated by rotary evaporation to yield a brown glassy solid (**40**; 1.19 g, 47%).

^{1}H NMR (400 MHz, CD_3SOCD_3): δ_H 7.5-5.6 (4.9H, br, Ar-H), 5.5-2.9 (59H, br, CH_2, CHOH, C-ring), 1.4-0.7 (19H, br, CH_3).

^{13}C NMR (200 MHz, CP-MAS): δ_C 166-154 (C-7, 9), 154-143 (C-3', 4'), 141-130 (C-1'), 130-100 (C-5, 6, 8, 10, 2', 5', 6'), 90-66 (CH_2, CHOH, C-2, 3), 50-30 (C-4), 30-16 (CH_3).

Preparation of chain-extended QT hydroxypropyl ether with 20 mole equivalents of PO

QT (0.94 g, 3.4 mmol) was transferred to a 15 mL Teflon-lined stainless steel pressure reactor. Triethylamine (5%, 24 μL) and 20 mole equivalents of PO (4.74 mL, 68 mmol) were added. The reactor was sealed and heated to 110 ^{o}C for 24 h, after which the reactor was cooled to room temperature. The product was neutralised with HCl (0.1 $molL^{-1}$), transferred to a flask, and concentrated by rotary evaporation to yield a brown glassy solid (**41**; 1.35 g, 28%).

^{1}H NMR (400 MHz, CD_3SOCD_3): δ_H 7.5-5.6 (4.9H, br, Ar-H), 5.5-2.9 (46H, br, CH_2, CHOH, C-ring), 1.4-0.7 (11H, br, CH_3).

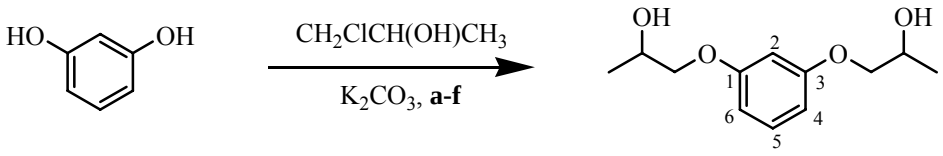
^{13}C NMR (200 MHz, CP-MAS): δ_C 166-153 (C-7, 9), 153-143 (C-3', 4'), 141-131 (C-1'), 131-100 (C-5, 6, 8, 10, 2', 5', 6'), 90-65 (CH_2, CHOH, C-2, 3), 48-30 (C-4), 30-16 (CH_3).

3.3 Results and Discussion

3.3.1 Etherification by The Williamson Ether Synthesis

The synthesis of polyphenol ethers was attempted using the Williamson ether synthesis, whereby an alkoxide was reacted with an alkyl halide. Both resorcinol and catechin were reacted with 1-chloro-2-propanol to produce the corresponding hydroxypropyl ethers. 1-Chloro-2-propanol was used as a reagent in anticipation of preparing products similar to those produced from the ring opening of propylene oxide. Resorcinol was initially used to establish the optimal reaction conditions. Subsequently, catechin was reacted using these preferred reaction conditions.

To determine the optimal procedure for the reaction of resorcinol with 1-chloro-2-propanol a range of reaction conditions were trialled (Figure 3.1). Wurm and Rehn[78] described potassium carbonate as a commonly used base for such a reaction. The use of dipolar aprotic solvents has been shown to favour this S_N2 reaction[78, 81, 83], however the reaction also proceeds in water[42]. A range of temperatures were also trialled for their efficiency to promote the reaction.



a; Water, 20 °C **b**; Acetone, 20 °C **c**; DMF, 20 °C
d; Water, reflux **e**; Acetone, reflux **f**; DMF, 105 °C

Figure 3.1: Screening reactions of resorcinol with 1-chloro-2-propanol (**23a-f**) to determine the optimum reaction conditions.

The reaction products from **23a-f** were analysed using TLC and ^{1}H NMR spectroscopy. TLC showed that **23a-c** consisted mostly of starting material. In contrast, **23d-f** all consisted of several products. This suggested that elevated temperatures were required for the etherification reaction to proceed. The ^{1}H

NMR spectra of reaction mixtures **23d-f** gave an insight as to the products (Figure 3.2). Expected changes to the ^{1}H NMR spectra upon hydroxypropylation of resorcinol include: the presence of signal at *ca* 3-4 ppm due to CH_2 and CHOH groups, the presence of a doublet at *ca* 1.0-1.5 ppm due to the CH_3 group, and a small downfield shift of the H-5 doublet at *ca* 7 ppm. The reaction in water (**23d**) gave the best conversion to resorcinol hydroxypropyl ether. This was determined by the integration of the CH_3 signal compared with aromatic protons. In comparison the reaction in acetone (**23e**) and DMF (**23f**) gave less substitution and more complex products, respectively. In conclusion, the use of water at elevated temperatures provided the best route to resorcinol hydroxypropyl ether.

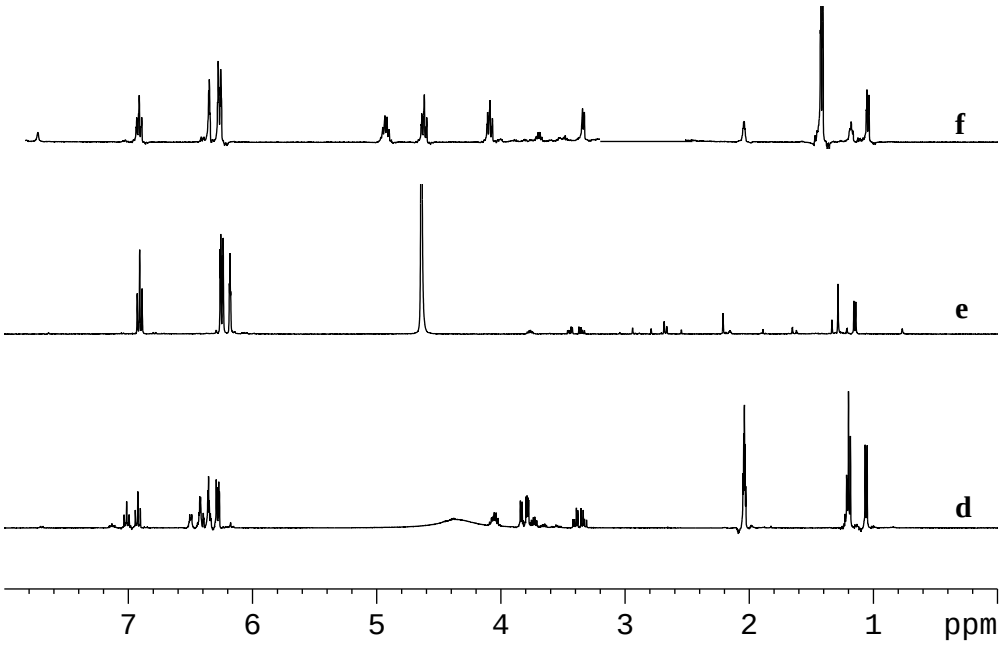


Figure 3.2: ^{1}H NMR spectra of the reaction mixtures (**23d-f**).

Using the optimum procedure for resorcinol, preparation of catechin hydroxypropyl ether was attempted. As the reaction progressed a colour change from yellow to dark brown was observed. Analysis by TLC showed the presence of one major product. Subsequent, analysis by ^{1}H NMR spectroscopy revealed the major product to be catechinic acid (**24**)[28]. This was not surprising as catechin is susceptible to rearrangement in alkaline conditions, forming catechinic acid[27, 28]. This result precluded the use of aqueous alkaline solution at elevated temperatures for the preparation of flavonoid ethers.

It was thought that performing the reaction in acetone over solid potassium carbonate may overcome the problem of rearrangement products. Analysis of the reaction product by TLC revealed three products. PLC allowed their separation for further analysis. The major isolated product (9%) was identified as a mixed catechin hydroxypropyl ether derivative (**25**). The ^{1}H NMR spectrum indicated a molar substitution (MS) of *ca* 3. However, ESI mass spectrometry suggested a lower MS with only the $[C(HPE)_1\text{-}H]^-$ and $[C(HPE)_1\text{+}Cl]^-$ ions observed. Either way the MS was below full substitution.

In summary, obtaining flavonoid hydroxypropyl ethers from 1-chloro-2-propanol via the Williamson ether synthesis was unsatisfactory. Water could not be used due to the rearrangement of catechin to catechinic acid, while preparation in acetone gave a low MS. Therefore, an alternative route to prepare hydroxypropyl ethers was studied.

3.3.2 Etherification by Ring Opening of Propylene Oxide

Polyphenol hydroxypropyl ethers were prepared by ring opening of an epoxide. Resorcinol, catechin, and tannin were reacted with PO to produce hydroxypropyl ether derivatives. Resorcinol was used to establish the optimal reaction conditions and subsequently catechin and tannin hydroxypropyl ethers were prepared.

First, it should be noted that a wide range of products may be produced by the ring opening of PO. This is illustrated for resorcinol in Figure 3.3. Nucleophilic attack is known to occur predominantly at the secondary carbon to produce 2-hydroxypropyl ethers. Alternatively, nucleophilic attack may occur at the tertiary carbon to produce 1-hydroxy-2-propyl ethers[72]. Therefore, the term hydroxypropyl ether was used to encompass both ring opening products. Also, the reaction may occur at some or all of the hydroxyl groups of the polyphenol. Furthermore, chain-extension may occur where PO reacts with the secondary alcohol of a previous hydroxypropyl unit. This culminates in a range of products, potentially making analysis difficult.

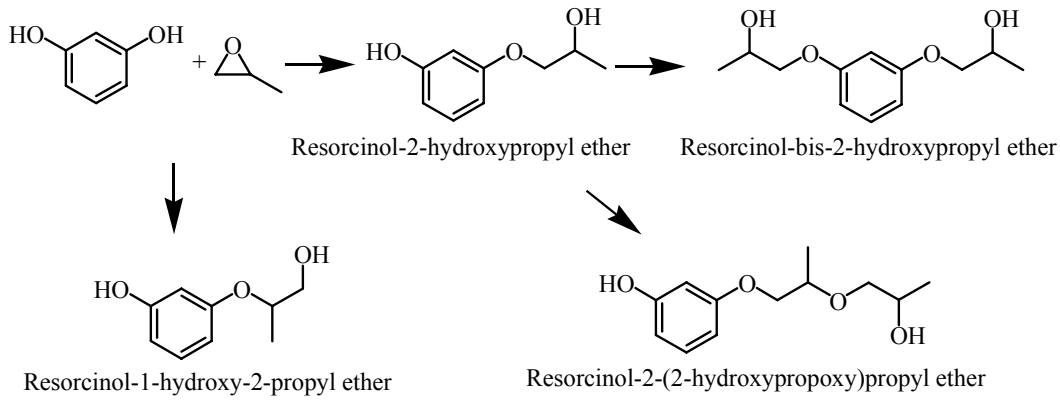


Figure 3.3: Some of the products that may be produced by the ring opening of PO with resorcinol.

The optimal reaction conditions for ring opening of PO were ascertained using resorcinol as a model compound. A common catalyst for the hydroxypropylation of lignin is potassium hydroxide[100, 101, 103], however other alkali metals and organic bases such as triethylamine have been used[117]. For the hydroxypropylation of lignin, common solvents include toluene, acetone, and water at temperatures ranging from room temperature to >200 ^{o}C[100, 101, 103]. Therefore, a range of reaction conditions were trialled (Table 3.2). ^{1}H NMR spectroscopy provided a means to analyse the products (Figure 3.4). The success of the reaction was indicated by signals at 1.0-1.5 ppm and 3.0-4.0 ppm due to the incorporation of hydroxypropyl groups.

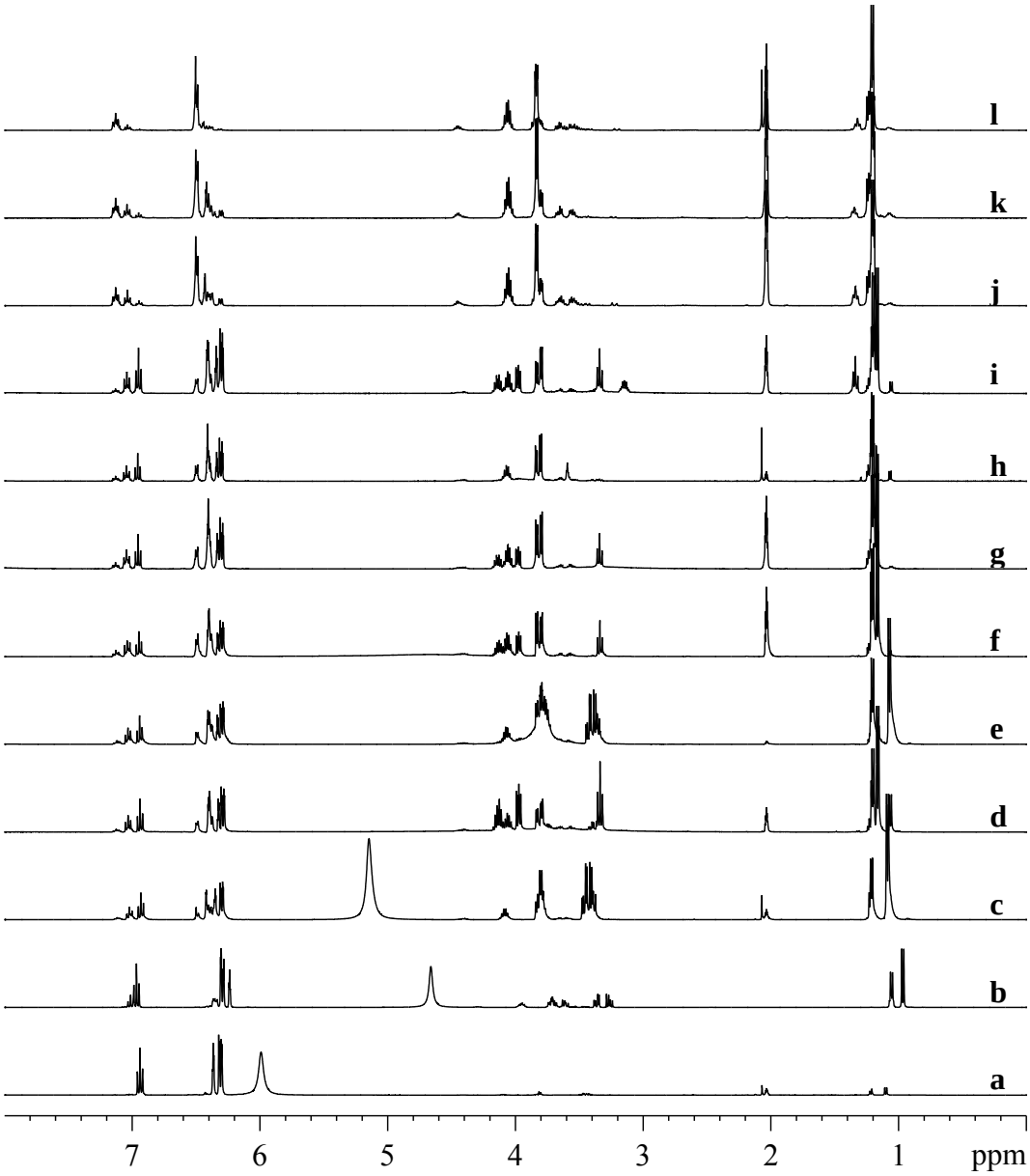
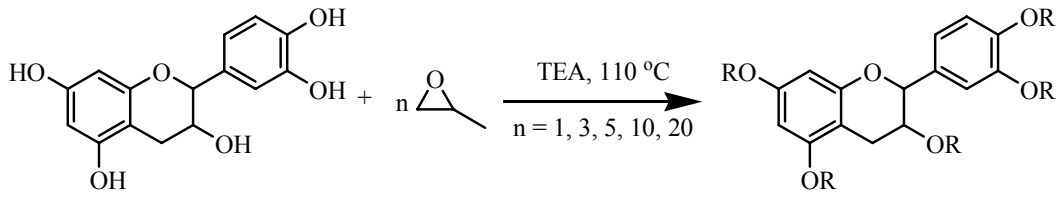


Figure 3.4: ^{1}H NMR spectra of resorcinol hydroxypropyl ether products (**26a-l)**.

The reaction conditions were shown to have a strong influence on the formation of resorcinol hydroxypropyl ethers. Increasing the temperature (**26a-d**) had a positive effect on the reaction. The optimal reaction temperature was shown to be 110 ^{o}C. Increasing the temperature above 110 ^{o}C was not viable as flavonoids are susceptible to thermal degradation. Decreasing the volume of solvent (**26g**) and hence increasing the concentration of reagents, favoured the formation of resorcinol hydroxypropyl ether. Hence, the reaction was performed in the absence of water (**26j-l**). Triethylamine was used as a solvent, to avoid using potassium hydroxide which when insoluble may form an explosive mixture[99]. The best

conversion of resorcinol to resorcinol hydroxypropyl ether was achieved in the absence of water with triethylamine at 110 °C (**26l**).

Catechin was etherified using the reaction conditions most favourable for resorcinol (Figure 3.5). This involved dissolving catechin in PO in the presence of triethylamine and allowing the reaction to proceed at 110 °C in a closed pressure reactor. To achieve various levels of substitution, catechin was reacted with 1, 3, and 5 mole equivalents of PO to give **27** (29%), **28** (27%), and **29** (46%) respectively. Furthermore, chain-extension was attempted using 10 and 20 mole equivalents of PO to give **30** (35%) and **31** (30%) respectively. For **27-31** the yields were moderate to low, possibly due to product loss during solvent extraction. For all reactions, TLC showed multiple products. However, poor resolution excluded the possibility of separating the products by PLC.



$R_{27\text{-}29}$ = H, $CH_2CHOHCH_3$
$R_{30\text{-}31}$ = $[CH_2CH(CH_3)O]_nCH_2CHOHCH_3$

Figure 3.5: Reaction of catechin with 1-20 mole equivalents of PO to produce catechin hydroxypropyl ethers. Where $R_{27\text{-}29}$ were the substituents for products **27-29**, and $R_{30\text{-}31}$ were the substituents for products **30-31**.

The products were characterised using NMR spectroscopy, where both ^{1}H NMR and COSY experiments were used. As previously mentioned, a complex array of products would be expected from the ring opening of PO. This was observed in the ^{1}H NMR spectra, where broad overlapping signals were observed. Therefore the assignment of the aliphatic signals required a COSY experiment, which showed $^3J_{H\text{-}H}$ couplings (Figure 3.6). The methyl signal was easily identified at *ca* 1.1 ppm and provided a benchmark to identify adjacent signals. A $^3J_{CH_3\text{-}CHOH}$ coupling was observed showing the CHOH signal to be at *ca* 4.0 ppm, while a $^3J_{CHOH\text{-}CH_2}$ coupling showed the CH_2 signal to be at *ca* 3.8 ppm.

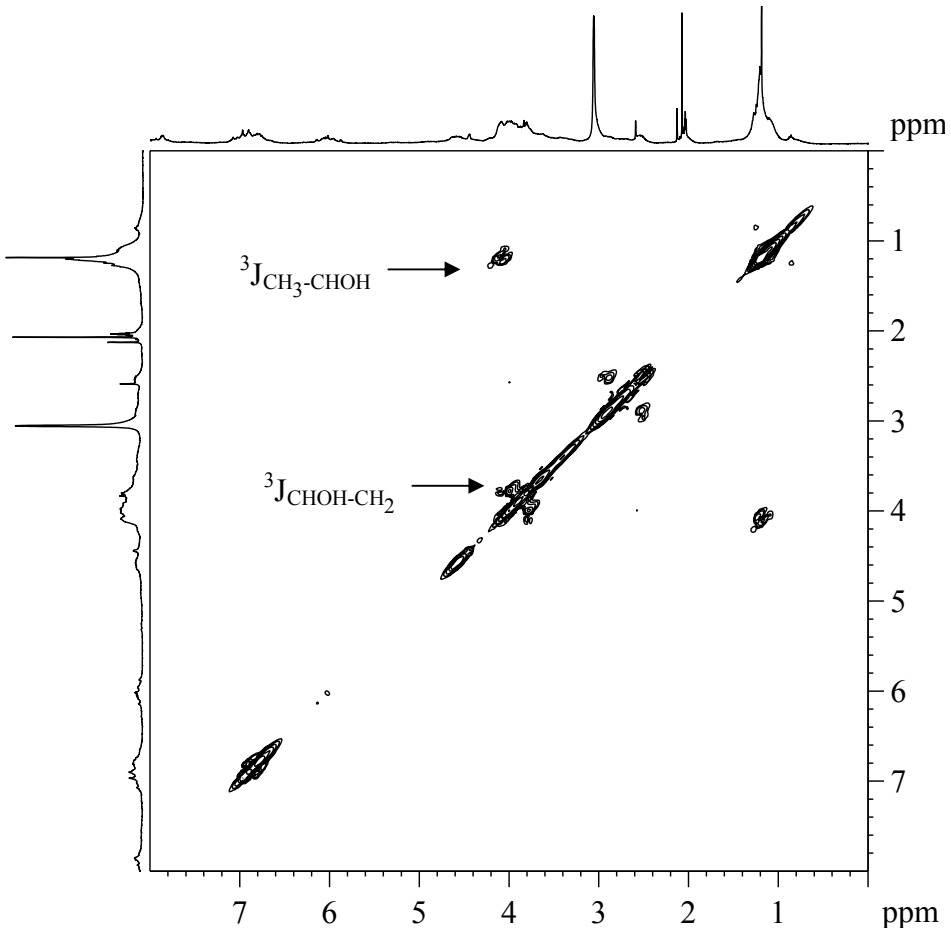


Figure 3.6: A COSY spectrum of **29**, showed $^3J_{H\text{-}H}$ couplings.

Integration of the ^{1}H NMR spectra provided an estimate of the molar substitution (MS) (Table 3.3). The average MS increased accordingly with an increasing charge of PO. However, it was evident that the MS did not exactly match the expected MS. A possible explanation was the loss of PO, which has a low boiling point, either from the closed pressure reactor or while setting up the reaction. Conversely, it may be a distortion in the integration of the ^{1}H NMR spectra. At this point it is appropriate to mention the disproportionate signal from the A-ring of catechin hydroxypropyl ethers. This was attributed to exchange of the H-6 and H-8 protons with deuterium from the solvent. The H-6 and H-8 protons of the catechin A-ring are known to be easily exchanged with deuterium in D_2O[118]. In D_2O at 50 ^oC, the A-ring signal of epigallocatechin gallate decreased to near zero after 500 minutes. Furthermore, in some circumstances deuteroacetone can also exchange with the solute[119]. For this reason only the B-ring protons were used for calibrating the integrals.

Table 3.3: The average MS for catechin hydroxypropyl ethers (**27-31**) calculated by integration of the ^{1}H NMR spectra.

Product	Charge of PO[a]	Integrals of ^{1}H NMR			Average MS[d]
		H-2', 5', 6'[b]	H-6, 8[c]	CH_3[d]	
Catechin hydroxypropyl ethers					
27	1	3.0	1.4	4.9	1.6
28	3	3.0	1.4	7.1	2.4
29	5	3.0	1.1	11.2	3.7
30	10	3.0	1.6	19.1	6.4
31	20	3.0	1.7	20.7	6.9

a = Mole equivalent charge of propylene oxide

b = Integral of the H-2', 5' and 6' protons

c = Integral of the H-6 and 8 protons

d = Integral of the CH_3 protons

e = The average molar substitution

The ^{13}C NMR spectra further supported the identification of catechin hydroxypropyl ethers (**27-31**) and also provided an insight as to the site of reaction (Appendix 19). Firstly, signals at 66 and 75 ppm were observed for the CHOH and CH_2 groups of the hydroxypropyl ether. A DEPT experiment allowed the unambiguous assignment of these peaks. More peaks were located in this region (65-78 ppm) and were thought to be due to 1-hydroxy-2-propyl ethers and/or chain-extended ethers. Through the series **27-31** the signals in the region 65-78 ppm region became increasingly numerous and complex.

Upon hydroxypropylation, the carbon immediately involved in the ether linkage (C-3, -5, -7, -3', -4') and adjacent carbons showed small changes in chemical shift, both upfield and downfield. Therefore, the site of hydroxypropylation could be ascertained. For **27**, peak splitting was observed for the A-, B-, and C-ring signals. This indicated that hydroxypropylation was occurring at all of the hydroxyl groups. Through the series **27-31**, this peak splitting became increasingly predominant. For example, the ^{13}C NMR spectrum of **31** showed all of the peaks had split or shifted when compared with catechin.

The ESI mass spectra of catechin hydroxypropyl ethers (**27**-**29**) [$C(HPE)_n$], exhibited a series of molecular ions sequentially increasing by 58 Daltons (Figure 3.7). The molecular ion of catechin $[C-H]^-$ was observed at m/z 289, and products of increasing molecular weight (58 Da) were observed at m/z 347, 405, 463, 521, and 579. These ions correspond to catechin with 1 to 5 hydroxypropyl groups respectively ($[C(HPE)_{1\text{-}5}\text{-}H]^-$). This series continued past 579, however these ions were identified as adducts of smaller fragments. MS^2 of the 637 ion showed this peak to be predominantly an adduct of $[C+C(HPE)_1\text{-}H]^-$. Such adducts were observed up to *ca* 1 kDa for all catechin hydroxypropyl ethers.

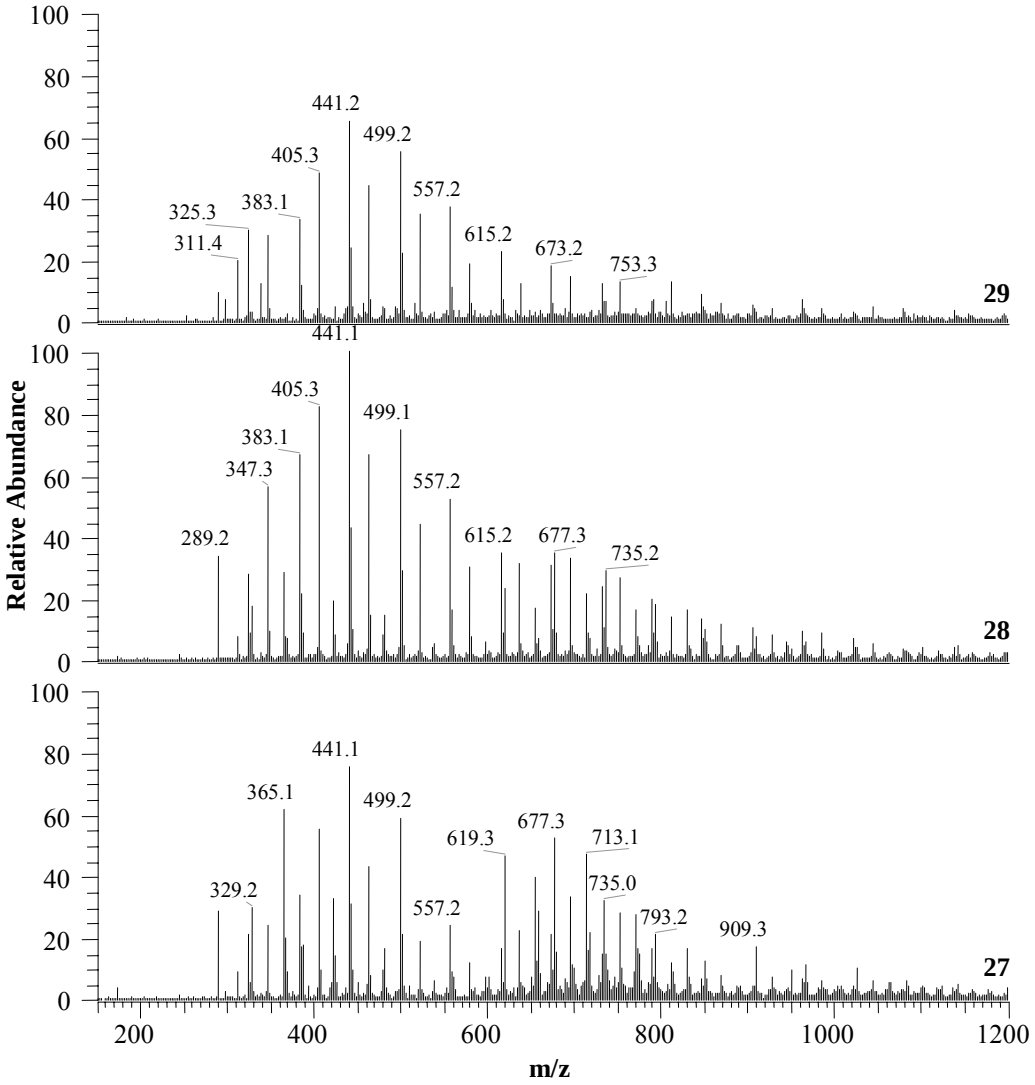


Figure 3.7: ESI mass spectra of **27-29**.

The attachment of chloride anions to form $[C(HPE)_n+Cl]^-$ was also observed. The attachment of chloride anions is known for analytes that lack acidic sites and thus exhibit weak $[M-H]^-$ signals[120]. This sequence of ions was observed at m/z 325, 383, 441, 499, 557, 615. The intensity of the $[C(HPE)_n+Cl]^-$ peaks were almost always greater than the $[C(HPE)_n-H]^-$ peaks. Presumably, chloride anions were present due to the workup, which involved solvent extraction with dilute hydrochloric acid and saturated sodium chloride solution. It could be concluded that catechin hydroxypropyl ethers exhibit a strong preference for the attachment of chloride anions versus proton dissociation.

Comparing the ESI mass spectra of **27**, **28**, and **29** showed differences in the intensity of ions (Figure 3.7). The mass spectrum of **27** showed low intensity of higher molecular weight ions (*m/z* 521, 557, 579, and 615), with the exception of adducts observed at greater than *m/z* 579. In comparison, **28** and **29** showed a greater intensity of high molecular weight ions (*m/z* 521, 557, 579, and 615), corresponding to $[C(HPE)_4]$ and $[C(HPE)_5]$. These results were in accordance with those obtained from integration of the ^{1}H NMR spectra.

The ESI mass spectra of **30** and **31** showed many higher molecular weight ions ranging from m/z 1195 to 1543 (Figure 3.8). These ions may correspond to $[C(HPE)_n+Cl]^-$, n=15-21 or adducts of lower MW ions. MS^2 showed the loss of Cl and one hydroxypropyl ether group, suggesting that the ions were not adducts. However, the average MS calculated by ^{1}H NMR spectroscopy suggested a MS much lower than 15-21.

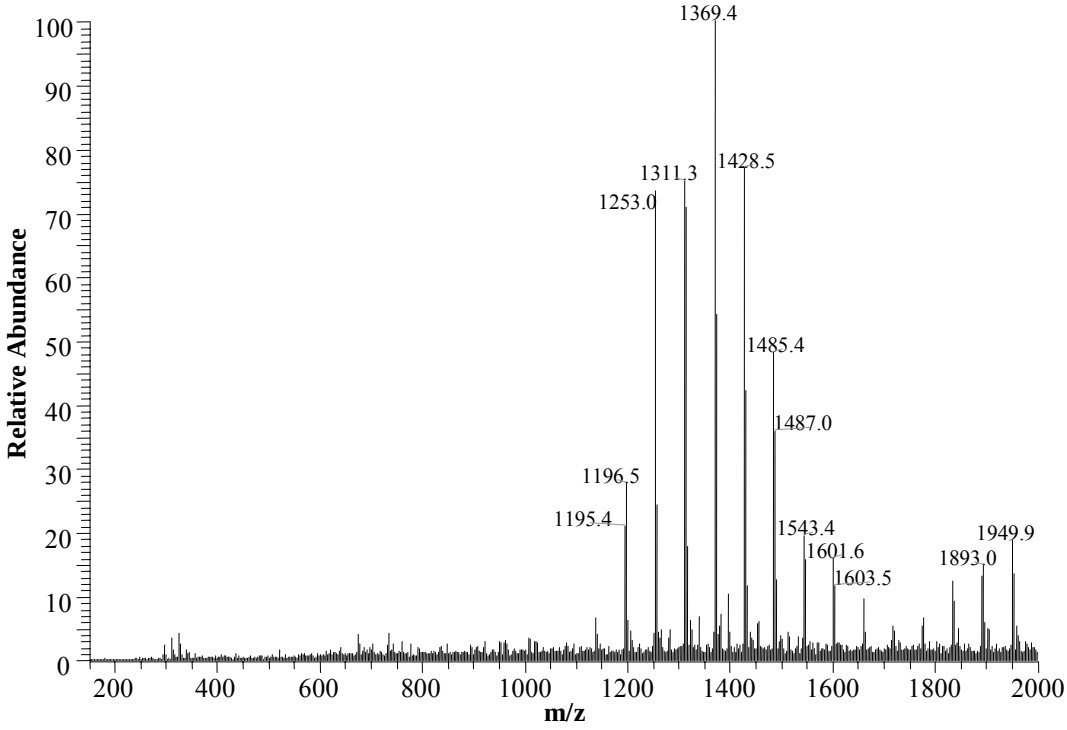


Figure 3.8: ESI mass spectrum of **30** showed many high molecular weight ions.

In summary, catechin hydroxypropyl ethers of varying MS were successfully prepared. When using 1, 3, and 5 mole equivalents of PO the reaction worked efficiently, to give products with approximately equivalent MS. However, attempts at chain-extension were less successful, where the MS was considerably lower than the equivalent charge of PO.

Using methodologies established for catechin, PBT was etherified using 1, 3, and 5 mole equivalents of PO to give **32** (99%), **33** (100%), and **34** (96%) respectively in quantitative yields. Chain-extension was attempted using 10 and 20 mole equivalents of PO to give **35** (84%) and **36** (58%) in good isolated yield. The resulting products were found to be poorly soluble in most common solvents, therefore solvent extraction was impractical. The poor solubility contrasts corresponding lignin derivatives which were found to be relatively soluble[100, 103].

The molar substitution of PBT hydroxypropyl ethers was estimated using both weight gains and NMR spectroscopy (Table 3.4). As the reaction was quantitative the percentage weight gain upon hydroxypropylation provided a good estimate of

the average MS (Appendix 20). For **32-34** the average MS closely matched the mole equivalent charge of PO. However, when attempting chain-extension (**35** and **36**) the MS calculated by weight gain was less than the mole equivalent charge of PO.

Table 3.4: The MS of PBT hydroxypropyl ethers (**32-36**) determined by mass gain and integration of the NMR spectra.

Product	Charge of PO[a]	MS by mass gain[b]	MS by ^{1}H NMR[c]	MS by ^{13}C NMR[d]
PBT hydroxypropyl ethers				
32	1	0.9	1.5	1.8
33	3	3.1	5.8	6.3
34	5	4.6	7.0	7.6
35	10	7.6	13.4	
36	20	9.6	14.0	

a = Mole equivalents of propylene oxide

b = Molar substitution calculated from the mass gain during the reaction

c = Molar substitution calculated from integration of the ^{1}H NMR spectra

d = Molar substitution calculated from integration of the ^{13}C NMR spectra

Integration of the ^{1}H NMR spectra allowed the average MS to be estimated. This was achieved by integration of the methyl group calibrated against the aromatic protons (Appendix 21). The MS was shown to increase accordingly with the increasing charge of PO. However, the average MS did not match the expected MS. As described for catechin, error in the integration of the ^{1}H NMR spectra may be responsible. A second potential source of error was the carbohydrate content known to exist in PBT preparations[17]. Hydroxypropylation of the carbohydrate content would bias the aromatic proton to methyl proton ratio, this favouring a greater than expected MS.

Solid state ^{13}C NMR spectra of products **32-34** assisted in their characterisation (Figure 3.9). Solid state ^{13}C NMR spectra could not be recorded for products **35-36** due to their viscous nature. The CH_3 signal was observed at 10-30 ppm, while the CH_2 and CHOH signals were partially obscured by the broad peak between

55-90 ppm. It was also observed that many signals (especially C-5, -7, and -9 and C-3' and -4') were becoming increasingly broad upon hydroxypropylation, for which two reason were proposed. First, as shown for catechin, small changes in ^{13}C NMR chemical shifts occur upon hydroxypropylation. This would result in the peaks becoming broad as the signal was spread over a wider range of chemical shifts. Secondly, the line width of a signal is proportional to $1/T_2^*$, where T_2^* is dependent upon low frequency motions (molecular mobility)[121, 122]. Hence an increase in molecular mobility will increase the signal width[123].

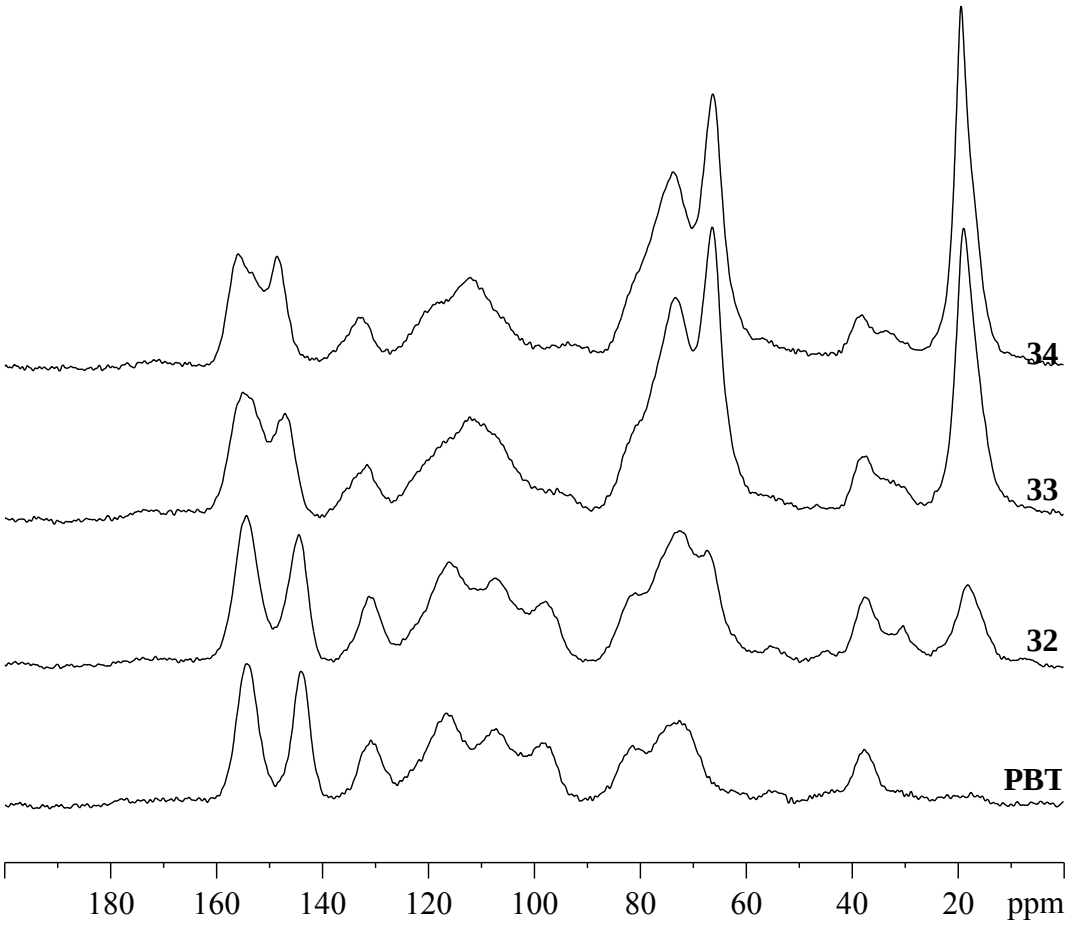


Figure 3.9: Solid state ^{13}C CP-MAS NMR spectra of **32-34** compared with unreacted PBT.

Integration of the solid state ^{13}C NMR spectra provided another method to estimate the MS (Table 3.4). To allow quantification, the Hartmann-Hahn match was optimised[121]. The MS approximated using solid state ^{13}C NMR spectroscopy closely matched those calculated using ^{1}H NMR spectroscopy.

The MW profiles of **32-36** were recorded using gel permeation chromatography (Figure 3.10). The most notable features were the broad peak centred around 10000 Da and the narrow peak centred around 50000 Da. The later was due to high molecular weight products which were excluded from the column and therefore eluted with the void volume. An increase in high MW products (>30000 Da) was observed upon hydroxypropylation. This increase in MW was consistent with the MS calculated using weight gains and NMR spectroscopy (Table 3.4), with the exception of **36**.

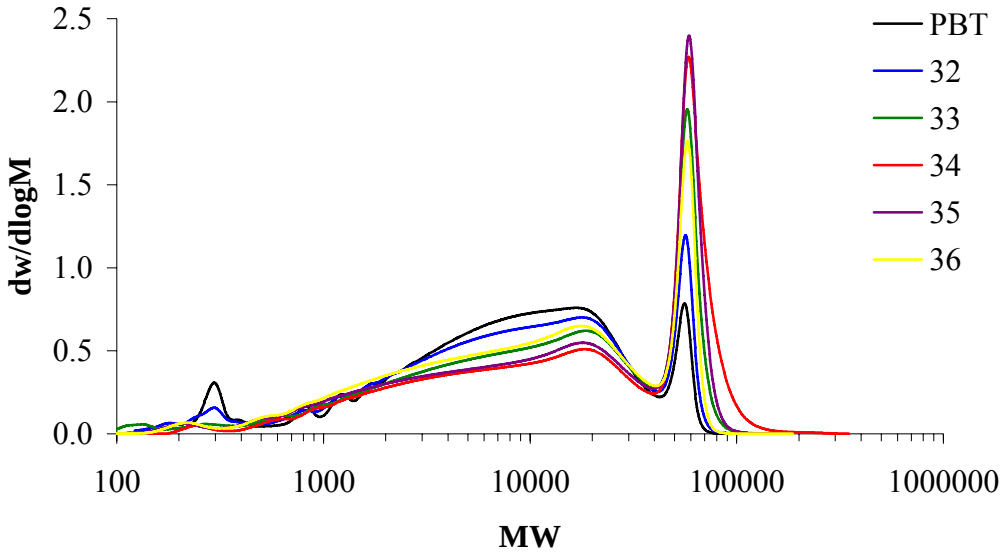


Figure 3.10: Molecular weight profile of PBT hydroxypropyl ethers (**32-36**) determined using GPC.

In summary, PBT hydroxypropyl ethers were successfully prepared as shown by NMR analysis. Quantitative analysis using weight gains and NMR spectroscopy showed the MS increased consistently with the increasing charge of PO. However, chain-extension using 10 and 20 mole equivalents of PO was less successful, with relatively limited chain-extension achieved. This suggested that secondary hydroxyls were less reactive towards hydroxypropylation compared with phenolics, which was in accordance with Glasser et al. (1983)[73].

In a similar manner, QT was etherified using 1, 3, and 5 mole equivalents of PO to give **37** (93%), **38** (93%), and **39** (79%) respectively in excellent isolated

yields. Chain-extension was also attempted using 10 and 20 mole equivalents of PO to give **40** (47%) and **41** (28%) respectively in moderate isolated yields.

As with PBT, the molar substitution of QT ethers was estimated using both weight gains and NMR spectroscopy (Table 3.5). The percentage weight gain upon hydroxypropylation allowed the MS to be estimated (Appendix 22). This indicated that only relatively low levels of molar substitution were achieved, with a maximum MS of 2.4. Integration of the ^{1}H NMR spectra provided another means to estimate the MS. This was achieved by integration of the methyl group calibrated against the aromatic protons (Appendix 23). For **37-40** the MS increased with an increasing charge of PO. However, **41** showed an unexpectedly low MS.

Table 3.5: The MS of QT hydroxypropyl ethers (**37-41**) determined by mass gain and integration of the NMR spectra.

Product	**Charge of PO[a]**	**MS by mass gain[b]**	**MS by ^{1}H NMR[c]**	**MS by ^{13}C NMR[d]**
QT hydroxypropyl ethers				
37	1	0.6	1.7	2.4
38	3	2.4	5.0	4.3
39	5	2.4	5.4	4.5
40	10	2.2	6.5	4.7
41	20	2.1	3.7	3.6

a = Mole equivalents of propylene oxide

b = Molar substitution calculated from the mass gain during the reaction

c = Molar substitution calculated from integration of the ^{1}H NMR spectra

d = Molar substitution calculated from integration of the ^{13}C NMR spectra

Solid state ^{13}C NMR spectroscopy of the products **37-41** also assisted the characterisation and also provided an estimate of the MS (Figure 3.11). Again, the CH_3 signal was observed at 10-30 ppm, while the CH_2 and CHOH signals were partially obscured by the broad peak between 55-90 ppm. A small carbonyl peak at 185 ppm was observed in both the unreacted QT and the products **37-41**. This may have been due to a small proportion of hydrolysable tannin or oxidation

product in the condensed tannin extract. Integration of the solid state ^{13}C NMR spectra provided another method to estimate the MS. The MS approximated using solid state ^{13}C NMR spectroscopy followed similar trends to those obtained using ^{1}H NMR spectroscopy and weight gains.

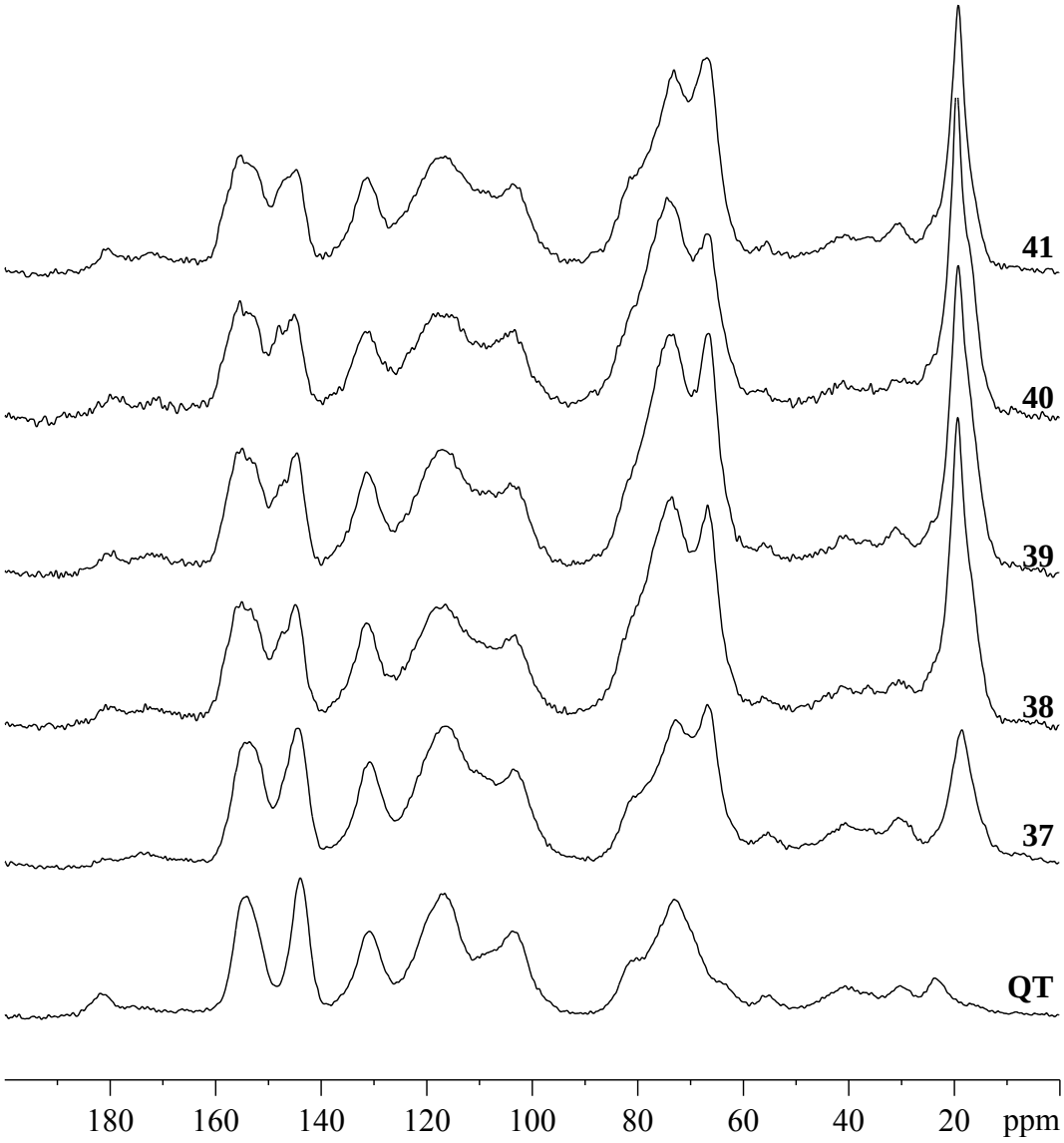


Figure 3.11: Solid state ^{13}C CP-MAS NMR spectra of **37-41** compared with unreacted QT.

GPC allowed further characterisation of the QT hydroxypropyl ethers (**37-41**) (Figure 3.12). Upon hydroxypropylation an increase in MW was expected, as observed for **37**. However, apart from **37** little increase in MW was observed for any of the QT hydroxypropyl ethers. It was thought that upon hydroxypropylation, the conformation of the tannin macromolecules may have changed. This may affect their retention and hence provide inconsistent results.

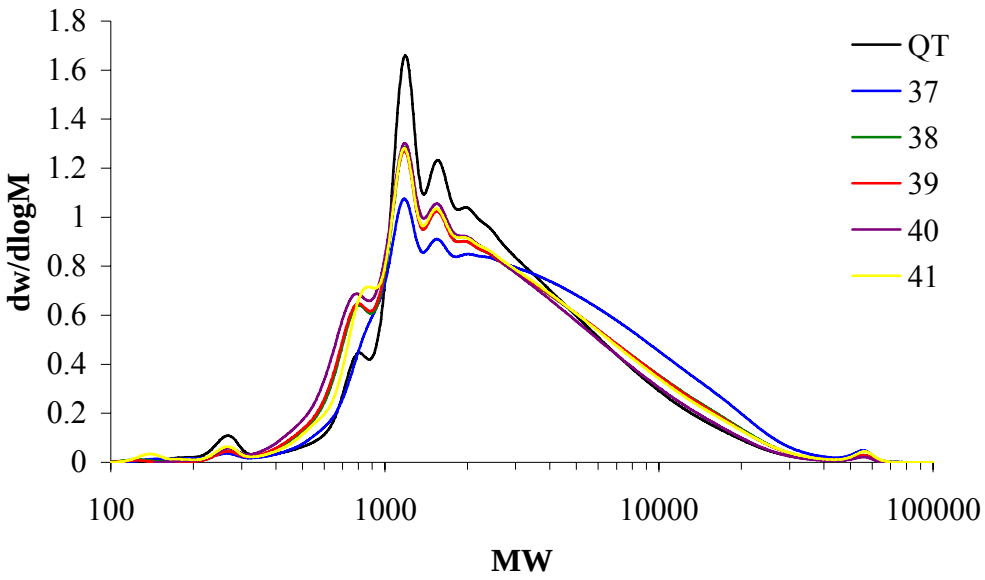


Figure 3.12: Molecular weight profile of QT hydroxypropyl ethers (**37-41**) determined using GPC.

In summary, hydroxypropylation of QT was successful as shown by qualitative characterisation using NMR spectroscopy. However, quantification of the molar substitution proved difficult. Estimates of the MS using weight gains, ^{1}H NMR, and solid state ^{13}C NMR spectroscopy did not provide similar estimates. However, the observed trends were consistent across all three quantification methods.

3.3.3 Thermal Analysis

Thermal analysis of polyphenol hydroxypropyl ethers was performed using TGA and DSC. This provided an insight to the polymeric properties and thermal stability.

Thermogravimetric analysis of PBT hydroxypropyl ethers (**32-36**) provided an insight to the thermal stability (Figure 3.13). It was generally observed that hydroxypropylation decreased the thermal stability compared with unreacted PBT. A product with low molar substitution of hydroxypropyl ethers (**32**) showed a similar thermal degradation profile to PBT. However, with increasing molar substation (**33-36**) the onset of degradation was decreased. For example, **35** and **36** showed significant mass loss between 100-200 ^{o}C. It was also observed that

the percent weight loss at 450 °C mirrored the MS. For example, products with a low MS (**32** and **33**) showed the least weight loss (*ca* 45-55%). In comparison, products with a high MS (**35** and **36**) showed a greater weight loss (*ca* 60-80%). Presumably this represents the proportion of ether groups versus core tannin macromolecules.

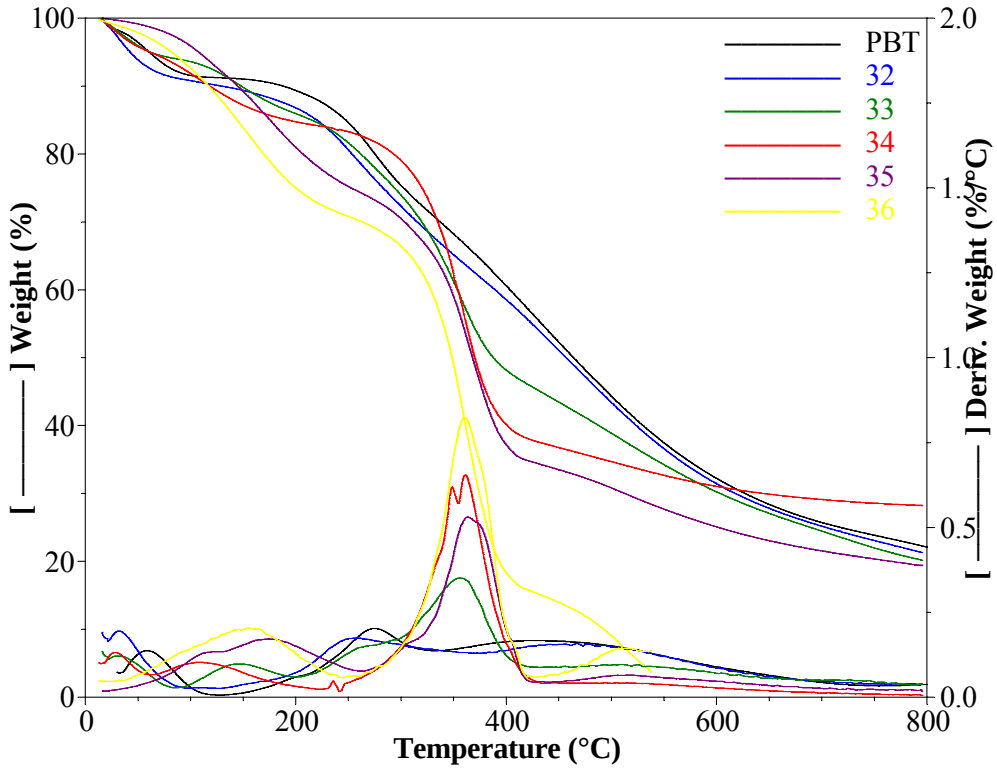


Figure 3.13: TGA thermogram of PBT hydroxypropyl ethers (**32-36**) compared with unreacted PBT.

The thermal degradation profiles of QT hydroxypropyl ethers (**37-41**) were recorded using TGA (Appendix 24). In a similar manner to PBT, QT showed decreased thermal stability upon hydroxypropylation and weight loss which mirrored the MS.

DSC of catechin hydroxypropyl ethers (**27-31**) showed several thermal events including possible glass transition behaviour (Figure 3.14). Most obvious was the endothermic event at *ca* 80-100 °C observed on the first heating cycle of all catechin hydroxypropyl ethers (Appendix 25). This was likely to be water loss, hence was not observed on the second heating cycle. However, the sharp nature of the transition suggested they may be melt behaviours. On the second heating

cycle the signal became unstable at temperatures greater than *ca* 100 °C. As shown by TGA, polyphenol hydroxypropyl ethers have low temperature stability, with degradation starting at temperatures as low at 100-200 °C. Accordingly, this signal instability was attributed to degradation, possibly concurrent with melting.

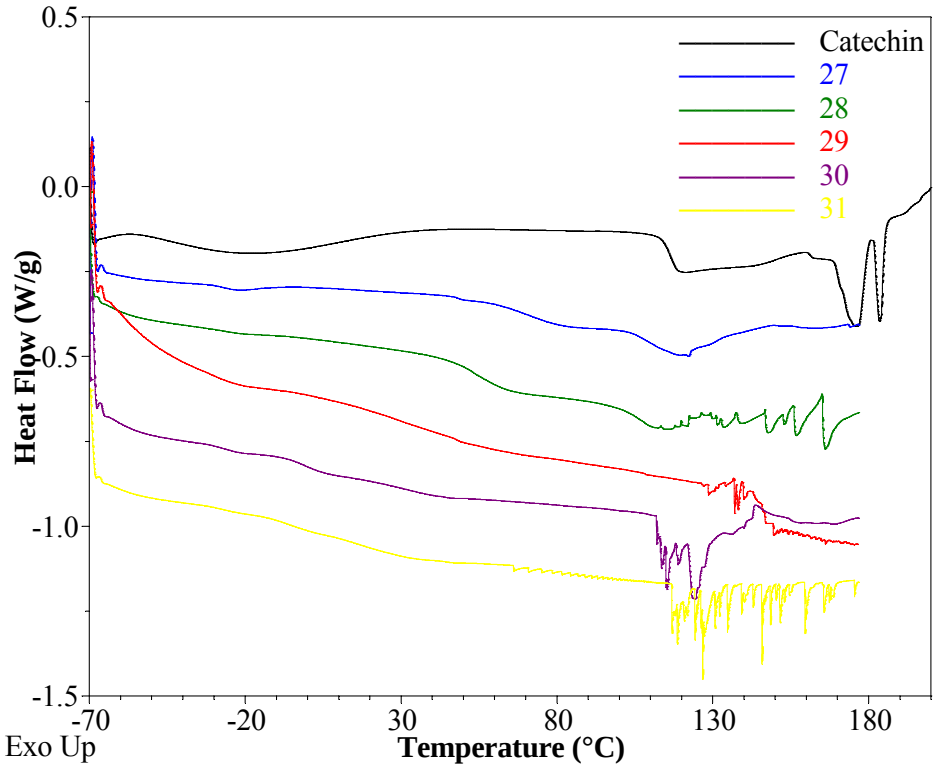


Figure 3.14: DSC thermogram of catechin hydroxypropyl ethers (**27**-**31**) compared with catechin (second heating cycle).

The most significant observation was the introduction of a glass transition (T_g) as a result of hydroxypropylation. Two trends were observed with regard to this T_g feature. First, the T_g changed between the first and second heating cycle. This may be due to the thermal history of the product or the influence of water. After the first heating cycle, all products had the same cooling rates (10 °C/min) and thermal history, therefore measurements of T_g were obtained from the second heating cycle. Secondly, the T_g showed a strong correlation to the molar substitution, with a plot of T_g versus MS having an R^2 value of 0.98 (Figure 3.15). This showed that the glass transition temperature could be directly controlled through altering the molar substitution.

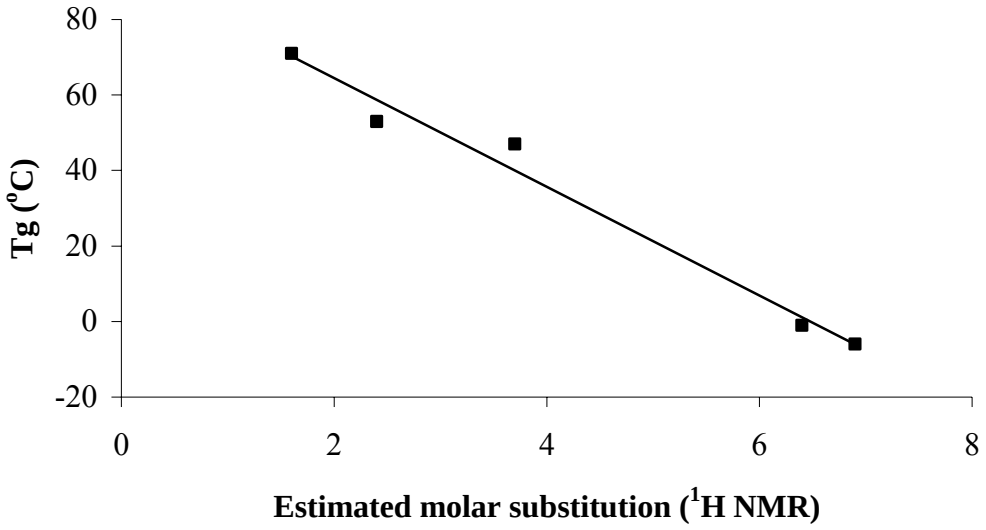


Figure 3.15: Plot of the estimated molar substitution versus glass transition temperature (T_g) for catechin hydroxypropyl ethers (**27-31**).

The introduction of a glass transition to catechin as a result of hydroxypropylation may be explained by two mechanisms, as previously described for lignin[100, 103, 104]. The conversion of phenolic hydroxyls to secondary hydroxyls disrupts the intermolecular hydrogen bonding. Thus, pseudo-cross-links were eliminated allowing increased molecular motion and as a result the T_g was lowered. However, variations of T_g in relation to the MS suggest another, potentially greater, effect. This could be explained in terms of the free volume of the macromolecule. As bulky substituents were added to the macromolecule, the free volume would increase, thereby lowering the T_g[100, 103, 104]. These effects have been reported on many occasions for lignin. The results obtained in this study suggested similar processes were occurring for catechin hydroxypropyl ethers.

DSC of PBT hydroxypropyl ethers (**32-36**) showed few features (Appendix 26). The flow behaviour observed for catechin hydroxypropyl ethers was not observed for PBT hydroxypropyl ethers (i.e. glass transitions were not observed). This was surprising as products **35** and **36** were highly viscous and tacky at room temperature, while in contrast PBT was a brown powder. A broad transition (*ca* 80 ^{o}C) in the first heating cycle was observed for **32-34**, probably due to water loss. This was not observed for PBT hydroxypropyl ethers with a high MS (**35**

and **36**), presumably because no hygroscopic phenol groups remained. Furthermore, **35** and **36** showed unusual behaviour at temperatures greater than *ca* 130 ^{o}C. This may be due to initial degradation of the products.

In contrast to DSC, rheology showed melt/flow behaviour for PBT hydroxypropyl ethers (**35** and **36**). Rheology is the study of the flow of fluids and deformation of solids under stress and strain. Oscillatory measurements over a temperature ramp provided information such as the elastic modulus (G'), loss modulus (G''), and tan(delta) which is a measure of the ratio of the two. The loss modulus showed a maximum at *ca* –3 ^{o}C indicating a melting transition (Appendix 27). However, no glass transition temperature was observed, as would be identified by a maximum in tan(delta).

DSC of QT hydroxypropyl ethers (**37-41**) showed few features (Appendix 28). Again, the flow behaviour observed for catechin hydroxypropyl ethers was not observed for QT hydroxypropyl ethers. However, on the first heating cycle a broad transition (*ca* 80 ^{o}C) was observed. Presumably this was water loss, as it was not present on the second heating cycle.

In summary, hydroxypropylation of polyphenols significantly changed their thermal properties as shown using TGA, DSC, and rheology. TGA showed that it decreased their thermal stability. Furthermore, the percent weight loss was proportional to the molar substitution. The introduction of hydroxypropyl groups to PBT decreased the thermal stability with the degradation onset between 100 and 200 ^{o}C. A glass transition was observed for catechin hydroxypropyl ethers, the temperature of which was dependent upon the molar substitution. This was explained by the disruption of intermolecular hydrogen bonding and increasing the free volume. The presence of a glass transition suggested that the products were behaving like a thermoplastic. However, this effect was not observed for PBT or QT hydroxypropyl ethers, rather melt behaviour was observed for the chain-extended PBT. In conclusion, hydroxypropylation of polyphenols changed the thermal properties such as melt/flow behaviour and thermal degradation. These properties were dependent upon the molar substitution of the product.

3.4 Summary

The purpose of this research was to prepare polyphenol ethers and through etherification change the physical properties of the starting polyphenol. The products may be useful in their own right or as chemical feedstocks for further modification. Two methods to prepare polyphenol ethers were used: the Williamson ether synthesis and the ring opening of epoxides.

The Williamson ether synthesis is a well known route to prepare ethers, hence it was applied to the etherification of polyphenols. The optimal reaction conditions were established for the etherification of resorcinol with 1-chloro-2-propanol. The reaction required elevated temperatures with a stoichiometric amount of base in aqueous solution. Applying this method to catechin was unsuccessful and resulted in rearrangement products. Therefore, the ring opening of epoxides was chosen in favour of the Williamson ether synthesis.

The ring opening of epoxides is a well known route to etherify lignin and modify the chemical and physical properties. This methodology was mirrored for the derivatisation of flavonoids such as catechin and tannins. The optimal reaction conditions for the ring opening of PO were determined using resorcinol. This was shown to be elevated pressure and temperatures in the presence of a base catalyst.

Catechin hydroxypropyl ethers were successfully prepared using the methodology developed for resorcinol. Furthermore, through altering the ratio of PO the molar substitution was able to be controlled. Chain-extension was also achieved, however this reaction was less efficient. Thermal analysis showed that hydroxypropylation of catechin introduced polymeric properties, as shown by the introduction of a glass transition. The glass transitions ranged from *ca* –5 ^{o}C to 70 ^{o}C and were shown to be dependent upon the molar substitution. Water loss or melt behaviours were observed at 80-100 ^{o}C, while the onset of degradation was observed at higher temperatures. These observations suggested that the catechin hydroxypropyl ethers were beginning to adopt thermoplastic characteristics, albeit with a low thermal stability.

PBT and QT hydroxypropyl ethers were prepared in a similar manner to those of catechin. Again, through altering the charge of PO the molar substitution was controlled. Chain-extension was attempted, however this was only partially successful for PBT. Determining the average molar substitution proved difficult. However, using the weight gains, ^{1}H NMR spectroscopy, solid state ^{13}C NMR spectroscopy, and GPC an estimate of the molar substitution was determined. DSC did not show the introduction of a glass transition, as observed for catechin hydroxypropyl ethers. However, using rheology melt behaviour was observed for chain-extended PBT hydroxypropyl ethers.

In conclusion, the etherification of polyphenols such as flavonoids using epoxides proved successful. The molar substitution was able to be controlled through altering the reaction conditions, and chain-extension was possible on some occasions. Upon hydroxypropylation, the physical properties of the polyphenol were altered. Thus, by changing the chemical and physical properties the products may find applications as a bio-derived chemical feedstock or as a precursor for further derivatisation.

Chapter 4 - Concluding Remarks

The aim of this project was to chemically modify polyphenols potentially to create value-added products or materials for further synthetic utility. A review of the literature showed that esterification and etherification were viable routes to derivatise polyphenols, such as tannins. The introduction of long-chain acyl groups was previously shown to increase the hydrophobic nature of polyphenols. Furthermore, the etherification of polyphenols, especially lignin, has been shown to change the physical properties of the lignin material. Esterification and etherification products were shown to have potential applications as chemical feedstocks in the polymer, adhesive, and pharmaceutical industries.

In this work, the esterification of polyphenols was used to introduce long chain fatty acids through an ester linkage. This was achieved using two methods: the alcoholysis of acid chlorides and anhydrides or a novel transesterification procedure. The alcoholysis of acid chlorides and anhydrides allowed the preparation of model compounds, which was a more conventional route to such materials. These model compounds served as comparisons for the later transesterification procedure. Of note was the preparation of resorcinol dilaurate and catechin pentalaurate by acylation of the parent polyphenols with lauroyl chloride. In a similar manner, tannin laurates having varying degrees of esterification were prepared. The preparation of these compounds was primarily to allow the characteristic spectroscopic and physical features to be identified for later comparison with the transesterifed derivatives.

Transesterification provided an alternative route to polyphenol long-chain esters. In this procedure vinyl esters of fatty acids were used as the acylating agent, producing the desired polyphenol ester and vinyl alcohol; the latter tautomerises to acetaldehyde and drives the transesterification equilibrium towards the products. Ester interchange occurred effectively in both aqueous and dimethyl sulfoxide solvents with base catalyst. This versatility allowed both catechin and tannin to be transesterified using vinyl esters. This included the first report of base-catalysed transesterification of a flavonoid with a vinyl ester. Changes in the

thermal behaviour, antioxidant, and SPF properties were observed upon esterification. For example, PBT laurate (**21**) showed decreased antioxidant activity, however this compound also exhibited increased SPF activity compared with unreacted PBT.

Etherification of polyphenols provided a further method to alter the chemical and physical properties. In this reaction, polyphenols were reacted with propylene oxide producing materials which could be isolated with minimal workup in quantitative yields. Furthermore, by altering the reaction conditions, the molar substitution could be controlled. The possibility of chain-extension was also investigated, however formation of poly(hydroxypropyl) ethers showed limited success. Hydroxypropylation of polyphenols was found to change the thermal properties, such as the glass transition temperatures and thermal degradation profiles. For instance, hydroxypropylation of catechin resulted in a glass transition ranging from –5 to 70 ^{o}C depending on the molar substitution. However, polyphenol hydroxypropyl ethers were found to decompose at relatively low temperatures (<200 ^{o}C).

Both the transesterification and etherification methodologies were developed with industrial scale-up in mind. The transesterification of polyphenols proved relatively simple both in synthesis and workup. This offered the advantage over alcoholysis of acid chlorides that hydrolysis products were not present. Etherification of polyphenols proved very simple, with little workup required. The reagents for both methods were affordable and easily handled. These factors would be important if attempting to produce polyphenol ester or ethers on a large scale.

These derivatisation methods allowed the properties of polyphenols to be modified. These modified polyphenols may find application in a range of areas, potentially adding value to bark, a waste product of the forestry industry. For example, polyphenol esters may hold potential as sunscreen additives or associated cosmetics, while the polyphenol ethers showed potential as a thermoplastic material which could find application in a range of products.

However, further work is required to fine-tune the methods, perform further physical analysis, and trial the products in potential applications.

4.1 Future Work

The transesterification approach developed in this study may be further optimised to encourage the equilibrium towards the products. This could be achieved using alternative molecular catalysts such as a titanium alkoxide or organotin compound which are known to be effective transesterification catalysts[39]. Furthermore, the transesterification of polyphenols could be enzyme-catalysed. Simple phenols have been transesterified using lipase in good yield[69]. This may offer better selectivity and control which is important in determining the properties of the final product.

Alternative acylating agents could be used for transesterification of polyphenols. Mono-, di- or tri-glycerides could be used as the acylating agent, as they are in the production of biodiesel[70]. Furthermore, biodiesel itself could be used as an acylating agent. These alternative acylating agents would offer a fully bio-derived product.

The transesterification process may allow the grafting of polyesters onto polyphenols. For example, transesterification of polyphenols with lactide (the cyclic di-ester of lactic acid) may produce dendrimers or star-shaped polymers.

The etherification of polyphenols was studied using propylene oxide. In principle, this could be mirrored using other alkylene oxides such as ethylene oxide or butylene oxide. Here, the use of more or less bulky substituents may have differing effects on the chemical and physical properties of the resulting products.

Through modifying polyphenols by esterification and etherification the chemical, thermal, and physical properties were changed. This may aid their successful incorporation into polymers such as thermoplastics. For example, the hydrophobic fatty acid groups of tannin laurates may enhance the mixing and

hence homogeneity upon incorporation into thermoplastics. These polyphenol additives may offer plasticisation, weathering, or UV stability to the polymers. Products similar to those produced in this thesis could be incorporated into polymers and the mechanical properties and weathering stability tested.

References

1. New Zealand Forest Owners' Association, *New Zealand Forestry Industry Facts & Figures 2005/2006*. New Zealand Forest Owners' Association: Auckland, 2006.
2. Ministry of Agriculture and Forestry, *Forestry Production and Trade: For the September 2006 Quarter*. Ministry of Agriculture and Forestry: Wellington, 2006.
3. Kininmonth, J. A.; Whitehouse, L. J., *Properties and Uses of New Zealand Radiata Pine*. Ministry of Forestry, Forest Research Institute: Rotorua, 1991.
4. Raven, P. H.; Evert, R. F.; Eichhorn, S. E., *Biology of Plants*. 6th ed.; W.H. Freeman: Worth Publishers: New York, 1999.
5. Winkel-Shirley, B., Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiology* **2001,** 126, 485-493.
6. Herbert, R. B., *The Biosynthesis of Secondary Metabolites*. 2nd ed.; Chapman and Hall: New York, 1989.
7. Luckner, M., *Secondary Metabolism in Microorganisms, Plants and Animals*. 3rd ed.; Springer-Verlag: Berlin, 1990.
8. Manitto, P., *Biosynthesis of Natural Products*. 1st ed.; Ellis Horwood: Chichester, 1981.
9. Cody, V., Molecular conformation of flavonoids and their protein binding interactions. In *Polyphenolic Phenomena*, Scalbert, A., Ed. INRA Editions: Paris, 1993.
10. Coombridge, B. A. *Pinus radiata* bark extractives: flavonoids and tannins. PhD, University of Waikato, Hamilton, N.Z., 1997.
11. Bate-Smith, E. C.; Swain, T., Flavonoid compounds. In *Comparative Biochemistry*, Florkin, M.; Mason, H. S., Eds. Academic Press: New York, 1962; pp 705-809.
12. Ferreira, D.; Xing-Cong, L., Oligomeric proanthocyanidins: naturally occurring *O*-heterocycles. *Natural Product Reports* **2000,** 17, 193-212.

13. Hagerman, A. E. Tannin Chemistry. http://www.users.muohio.edu/hagermae (accessed 07/03/2007).
14. Haslam, E., *Plant Polyphenols*. Cambridge University Press: Cambridge, 1989.
15. Unitan. Unitan. http://www.unitan.net/english/index.html (accessed 07/03/2007).
16. Pizzi, A., Recent developments in eco-efficient bio-based adhesives for wood bonding: opportunities and issues. *Journal of Adhesion Science and Technology* **2006,** 20, (8), 829-846.
17. Bogun, B. R. Molecular weight characterisation of *Pinus radiata* bark condensed tannins. MSc, The University of Waikato, Hamilton, N.Z., 2007.
18. Schofield, P.; Mbugua, D. M.; Pell, A. N., Analysis of condensed tannins: A review. *Animal Feed Science and Technology* **2001,** 91, 21-40.
19. Thompson, D.; Pizzi, A., Simple ^{13}C-NMR methods for quantitative determinations of polyflavonoid tannin characteristics. *Journal of Applied Polymer Science* **1995,** 55, 107-112.
20. Pasch, H.; Pizzi, A.; Rode, K., MALDI-TOF mass spectrometry of polyflavonoid tannins. *Polymer* **2001,** 42, (18), 7531-7539.
21. Laks, P. E., Condensed tannins as a source of novel biocides. In *Chemistry and Significance of Condensed Tannins*, Hemingway, R. W.; Karchesy, J. J., Eds. Plenum Press: New York, 1989; p 503.
22. Harborne, J. B.; Williams, C. A., Advances in flavonoid research since 1992. *Phytochemistry* **2000,** 55, (6), 481-504.
23. Britton, G., *The Biochemistry of Natural Pigments*. Cambridge University Press: Cambridge, 1983.
24. Pizzi, A., Tannin-based wood adhesives. In *Wood Adhesives: Chemistry and Technology*, Pizzi, A., Ed. Marcel Dekker: New York, 1983.
25. McGraw, G. W., Reactions at the A-ring of proanthocyanidins. In *Chemistry and Significance of Condensed Tannins*, Hemingway, R. W.; Karchesy, J. J., Eds. Plenum Press: New York, 1989.
26. Kiatgrajai, P.; Wellons, J. D.; Gollob, L.; White, J. D., Kinetics of epimerization of (+)-catechin and its rearrangement to catechinic acid. *Journal of Organic Chemistry* **1982,** 47, (15), 2910-2912.

27. Sears, K. D.; Casebier, R. L.; Hergert, H. L., The structure of catechinic acid: A base rearrangement product of catechin. *Journal of Organic Chemistry* **1974,** 39, (22), 3244-3247.

28. Hashida, K.; Ohara, S., Formation of a novel catechinic acid stereoisomer from base-catalyzed reactions of (+)-catechin. *Journal of Wood Chemistry and Technology* **2002,** 22, (1), 11-23.

29. Laks, P. E., Chemistry of the condensed tannin B-ring. In *Chemistry and Significance of Condensed Tannins*, Hemingway, R. W.; Karchesy, J. J., Eds. Plenum Press: New York, 1989.

30. Hagerman, A. E., Chemistry of tannin - protein complexation. In *Chemistry and Significance of Condensed Tannins*, Hemingway, R. W.; Karchesy, J. J., Eds. Plenum Press: New York, 1989.

31. Chen, Z. Y.; Chan, P. T.; Ho, K. Y.; Fung, K. P.; Wang, J., Antioxidant activity of natural flavonoids is governed by number and location of their aromatic hydroxyl groups. *Chemistry and Physics of Lipids* **1996,** 79, 157-163.

32. Burda, S.; Oleszek, W., Antioxidant and antiradical activities of flavonoids. *Journal of Agricultural and Food Chemistry* **2001,** 49, 2774-2779.

33. Kreibich, R. E., Tannin-based wood adhesives. In *Chemistry and Significance of Condensed Tannins*, Hemingway, R. W.; Karchesy, J. J., Eds. Plenum Press: New York, 1989.

34. Chung, K. H.; Hamed, G. R., Adhesives containing pine bark tannin for bonding nylon cord to rubber. In *Chemistry and Significance of Condensed Tannins*, Hemingway, R. W.; Karchesy, J. J., Eds. Plenum Press: New York, 1989.

35. Bruyne, T. D.; Pieters, L.; Deelstra, H.; Vlietinck, A., Condensed vegetable tannins: Biodiversity in structure and biological activities. *Biochemical Systematics and Ecology* **1999,** 27, 445-459.

36. Singh, B.; Bhat, T. K.; Singh, B., Potential therapeutic applications of some antinutritional plant secondary metabolites. *Journal of Agricultural and Food Chemistry* **2003,** 51, 5579-5597.

37. Smith, M.; March, J., *March's Advanced Organic Chemistry : Reactions, Mechanisms, and Structure*. Wiley: New York, 2001.

38. McMurry, J., *Organic Chemistry*. 5th ed.; Brooks/Cole: Pacific Grove, 1999.
39. Otera, J., Transesterification. *Chemical Reviews* **1993,** 93, (4), 1449-1470.
40. Dressler, H., *Resorcinol: Its Uses and Derivatives*. Plenum Press: New York, 1994.
41. Urano, M.; Kagawa, H.; Harigaya, Y., A total synthesis of optically pure (+)-catechin pentaacetate. *Journal of Heterocycle Chemistry* **1991,** 28, (8), 1845-1847.
42. Mitchell, D. B.; Minnis, R. L.; Curran, T. P.; Deboo, S. M.; Kelly, J. A.; Patwardhan, R.; Tai, W. T. Treatment of aqueous systems using a chemically modified tannin. U.S. Patent 5,843,337, December 1, 1998.
43. Lambusta, D.; Nicolosi, G.; Patti, A.; Piattelli, M., Enzyme-mediated regioprotection deprotection of hydroxyl-groups in (+)-catechin. *Synthesis-Stuttgart* **1993**, (11), 1155-1158.
44. Bonati, A.; Giuseppe, M. Pharmacologically active polyphenolic substances. U.S. Patent 4,166,861, August 4, 1979.
45. Grigsby, W. J.; Steward, D.; Mueller, H., Comparison of lignin and tannin esters as potential plastic modifiers. In *27th Australasian Polymer Symposium*, Adelaide, Australia, 2004.
46. Grigsby, W. J.; Bridson, J. H., Investigating the extent of polyphenolic oligomer esterifications. In *28th Australasian Polymer Symposium*, Rotorua, New Zealand, 2006.
47. Matsubara, K.; Saito, A.; Tanaka, A.; Nakajima, N.; Akagi, R.; Mori, M.; Mizushina, Y., Catechin conjugated with fatty acid inhibits DNA polymerase and angiogenesis. *DNA and Cell Biology* **2006,** 25, (2), 95-103.
48. Nomura, E.; Hosoda, A.; Hashizume, T.; Kimura, K.; Asahi, K.; Yamazaki, M.; Taniguchi, H. Preparation of water-insoluble tannins. Japan Patent 2004307362, November 4, 2004.
49. Barbosa, A. P.; Mano, E. B.; Andrade, C. T., Tannin-based resins modified to reduce wood adhesive brittleness. *Forest Products Journal* **2000,** 50, (9), 89-92.

50. Perrier, E.; Mariotte, A.; Boumendjel, A.; Bresson-Rival, D. Flavonoid esters and their use notably in cosmetics. U.S. Patent 6,235,294, July 10, 1998.

51. Cottman, K., S. Polymer compositions containing esters of polyphenolic compounds as built-in antioxidants. U.S. Patent 3,984,372, July 25, 1975.

52. Uesato, S.; Kitagawa, Y.; Hara, Y.; Tokuda, H.; Okuda, M.; Mou, X. Y.; Mukainaka, T.; Nishino, H., Antitumor promoting activities of 3-*O*-acyl-(-)epigallocatechins. *Bioorganic & Medicinal Chemistry Letters* **2000,** 10, (15), 1673-1675.

53. Jin, G.; Yoshioka, H., Synthesis of lipophilic poly-lauroyl-(+)-catechins and radical-scavenging activity. *Bioscience, Biotechnology and Biochemistry* **2005,** 69, (3), 440-447.

54. Patti, A.; Mario, P.; Giovanni, N., Use of *Mucor miehei* lipase in the preparation of long chain 3-*O*-acylcatechins. *Journal of Molecular Catalysts B: Enzymatic* **2000,** 10, 577-582.

55. Sakai, M.; Suzuki, M.; Fumio, N.; Yukihiko, H. 3-*O*-Acylated catechins and method of producing same. E.P. Patent 0,618,203 A1, October 5, 2005.

56. Nkiliza, J. Process for esterifying a polyphenolic oligomeric extract of plant origin, composition thus obtained and use thereof. U.S. Patent 5,808,119, September 15, 1998.

57. Vercauteren, J.; Weber, J.-F.; Bisson, J.-L.; Bignon, J. Polyphenol derivative compositions and preparation thereof. U.S. Patent 5,844,061, December 1, 1998.

58. Constantinescu, G.; Cazacu, G.; Popa, V. I., Contributions to the modification of lignocellulosics by epoxidation and esterification. *Cellulose Chemistry and Technology* **2005,** 39, (3-4), 201-210.

59. Sroková, I.; Tomanová, V.; Ebringerová, A.; Malovíkova, A.; Heinze, T., Water-soluble amphiphilic *O*-(carboxymethyl)-cellulose derivatives - synthesis and properties. *Macromolecular Materials and Engineering* **2004,** 289, (1), 63-69.

60. Roelofsen, D. P.; Graaf, J. W. M. D.; Hagendoorn, J. A.; Verschoor, H. M.; Van Bekkum, H., Ester interchange using molecular sieves. *Recueil des Travaux Chimiques des Pays-Bas* **1970,** 89, 193-210.

61. Roelofsen, D. P.; Hagendoorn, J. A.; Van Bekkum, H., Ester interchange technique using molecular sieves. *Chemistry and Industry* **1966,** 39, 1622-1623.

62. Barry, J.; Bram, G.; Petit, A., Organic syntheses without solvent: Base-catalysed ester interchange. *Tetrahedron Letters* **1988,** 29, (36), 4567-4568.

63. Lin, S.-C.; Rei, M.-H. Process for the acetylation of a mono-substituted phenol or a mono-substituted naphthol. U.S. Patent 5,136,084, June 14, 1992.

64. Sroková, I.; Miníkova, S.; Ebringerová, A.; Sasinková, V.; Heinze, T., Novel *O*-(2-hydroxyethyl) cellulose-based biosurfactants. *Tenside, Surfactants, Detergents* **2003,** 40, (2), 73-76.

65. Ardhaoui, M.; Falcimaigne, A.; Ognier, S.; Engasser, J. M.; Moussou, P.; Pauly, G.; Ghoul, M., Effect of acyl donor chain length and substitutions pattern on the enzymatic acylation of flavonoids. *Journal of Biotechnology* **2004,** 110, (3), 265-271.

66. Ardhaoui, M.; Falcimaigne, A.; Engasser, J. M.; Moussou, P.; Pauly, G.; Ghoul, M., Acylation of natural flavonoids using lipase of *Candida antarctica* as biocatalyst. *Journal of Molecular Catalysis B-Enzymatic* **2004,** 29, (1-6), 63-67.

67. Passicos, E.; Santarelli, X.; Coulon, D., Regioselective acylation of flavonoids catalyzed by immobilized *Candida antarctica* lipase under reduced pressure. *Biotechnology Letters* **2004,** 26, (13), 1073-1076.

68. Todoroki, T.; Saito, A.; Tanaka, A., Lipase-catalysed kinetic resolution of (+)-*cis*-flavan-4-ol and its acetate: Synthesis of chiral 3-hydroxyflavanones. *Bioscience Biotechnology and Biochemistry* **2002,** 66, (8), 1772-1774.

69. Park, S. J.; Sin, Y. M.; Lee, S. O.; Lee, T. H., Enzymatic synthesis of an acylated phloroglucinol derivative with phloroglucinol and vinyl octanoate in acetonitrile. *Biotechnology Letters* **2002,** 24, (3), 181-184.

70. Freedman, B.; Butterfield, R. O.; Pryde, E. H., Transesterification kinetics of soybean oil. *Journal of the American Oil Chemists' Society* **1986,** 63, (10), 1375-1380.

71. Feuer, H.; Hooz, J., Methods of formation of the ether linkage. In *The Chemistry of the Ether Linkage*, Patai, S., Ed. Wiley: London, 1967; pp 445-498.

72. Barton, D.; Ollis, W. D., *Comprehensive Organic Chemistry: The Synthesis and Reactions of Organic Compounds*. Pergamon Press: Oxford, 1979; Vol. 1.

73. Glasser, W. G.; Wu, L. C. F.; Selin, J. F., Synthesis, structure, and some properties of hydroxypropyl lignins. In *Wood and Agriculture Residues: Research on Use for Feed, Fuels, and Chemicals*, Soltes, E. J., Ed. Academic Press: New York, 1983.

74. Cook, W. D.; Guise, G. B., *Polymer Update: Science and Engineering*. Royal Australian Chemical Institute: Victoria, 1989.

75. Summer, C. E., Jr.; Hitch, B. J.; Bernard, B. L. Process for the preparation of resorcinol bis(dihydroxyethyl)ether. W.O. Patent 9,116,292, October 31, 1991.

76. Johnstone, R. A. W.; Rose, M. E., A rapid, simple, and mild procedure for alkylation of phenols, alcohols, amides, and acids. *Tetrahedron* **1979,** 35, 2169-2173.

77. Badiger, M. V.; Kadam, V. S.; Lele, A. K.; Wadgaonkar, P. P.; Hourdet, D., Synthesis and characterization of novel hydrophobically end-capped poly(ethylene oxide)s [PEOs]. *Macromolecular Symposia* **2006,** 241, 9-13.

78. Wurm, G.; Rehn, D., Hydroxyalkoxyflavonoids, 1. Regioselective *O*-hydroxyethylation of quercetin. *Archiv der Pharmazie* **1985,** 318, (8), 747-753.

79. Zheng, X.; Meng, W. D.; Xu, Y. Y.; Cao, J. G.; Qing, F. L., Synthesis and anticancer effect of chrysin derivatives. *Bioorganic & Medicinal Chemistry Letters* **2003,** 13, (5), 881-884.

80. Wang, C. L.; Zheng, X.; Meng, W. D.; Li, H. Q.; Qing, F. L., Formation of the unexpected 3-alkylated flavonoids in the alkylation of B-ring substituted 5,7-dihydroxy flavones. *Tetrahedron Letters* **2005,** 46, (32), 5399-5402.

81. Babu, K. S.; Babu, T. H.; Srinivas, P. V.; Sastry, B. S.; Kishore, K. H.; Murty, U. S. N.; Rao, J. M., Synthesis and in vitro study of novel 7-*O*-acyl

derivatives of oroxylin A as antibacterial agents. *Bioorganic & Medicinal Chemistry Letters* **2005,** 15, (17), 3953-3956.

82. Miyase, T.; Sano, M. Preparation of catechin derivatives with increased stability. Japan Patent 2001253879, September 18, 2001.
83. Hoelldampf, S. Composition and characterisation of modified tannins. Diploma, Aalen University of Applied Science, Aalen, 2007.
84. Kessler, M.; Ubeaud, G.; Jung, L., Anti- and pro-oxidant activity of rutin and quercetin derivatives. *Journal of Pharmacy and Pharmacology* **2003,** 55, 131-142.
85. Rehn, D.; Wurm, G., Ethylene carbonate, a regioselective reactant for hydroxyethylation of flavonoids. In *Flavonoids and Bioflavonoids*, Farkas, L.; Gabor, M.; Kallay, F., Eds. Elsevier: New York, 1985.
86. Jurd, L., Plant polyphenols. V. Selective alkylation of the 7-hydroxyl group in polyhydroxyflavones. *Journal of the American Chemical Society* **1958,** 80, (20), 5531-5536.
87. Jurd, L.; Rolle, L. A., Plant polyphenols. IV. Migration of acetyl groups during alkylation of the partial acetates of flavonoid compounds. *Journal of the American Chemical Society* **1958,** 80, (20), 5527-5531.
88. Jurd, L., The selective alkylation of polyphenols. II. Methylation of 7-, 4'-, and 3'-hydroxyl groups in flavonoids. *Journal of Organic Chemistry* **1962,** 27, 1294-1297.
89. Szabo, V.; Litkei, G.; Farkas, E.; Bognar, R., Alkylation of rutin and quercetin with haloacetic acid derivatives. II. *Acta Universitatis Debreceniensis de Ludovico Kossuth Nominatae, Series Physica et Chemica* **1967,** 13, 167-179.
90. Gissot, A.; Wagner, A.; Mioskowski, C., Buffer-induced, selective mono-*C*-alkylation of phloroglucinol: application to the synthesis of an advanced intermediate of catechin. *Tetrahedron* **2004,** 60, 6807-6812.
91. Reabe, K. G.; Dressler, H. Method for the preparation of hydroxyalkyl ethers. U.S. Patent 3,644,534, February 22, 1972.
92. Dressler, H. Hydroxyalkylation of phenols or thiophenols with cyclic organic carbonates using thiorganophosphine catalysts. U.S. Patent 5,059,723, July 13, 1991.

93. Courbat, P.; Valenza, A., Contribution a l'etude de derives *O*-β-hydroxyethyles de la quercetine. Identification des derives par spectrophotometrie UV. *Helvetica Chimica Acta* **1973,** 56, (5), 1604-1614.

94. Su, Z. X.; Chang, X. J.; Zhan, G. Y.; Luo, X. Y.; Pu, Q. S., Synthesis and efficiency of an epoxy-tannin chelating resin for preconcentrating and separating various rare elements. *Analytica Chimica Acta* **1995,** 310, (3), 493-499.

95. Soto, R.; Freer, J.; Baeza, J., Evidence of chemical reactions between di- and poly-glycidyl ether resins and tannins isolated from *Pinus radiata* D. Don bark. *Bioresource Technology* **2005,** 96, (1), 95-101.

96. Simionescu, C. I.; Rusan, V.; Cazacu, G.; Macoveanu, M. M.; Popa, V. I.; Popa, M.; Bulacovschi, J., Polyphenolepoxy-As resins, based on alkaline extracts from the latex-bearing plant *Asclepias syriaca* L. 1. Influence of the synthesis conditions. *Cellulose Chemistry and Technology* **1987,** 21, 639-649.

97. Simionescu, C. I.; Rusan, V.; Cazacu, G.; Macoveanu, M. M.; Popa, V. I.; Popa, M.; Bulacovschi, J.; Ioanid, A., Polyphenolepoxy-As resins, based on alkaline extracts from the latex-bearing plant *Asclepias syriaca* L. 2. The physical and chemical characterisation. *Holzforschung und Holzverwertung* **1987,** 39, 62-65.

98. Kishi, H.; Fujita, A.; Miyazaki, H.; Matsuda, S.; Murakami, A., Synthesis of wood-based epoxy resins and their mechanical and adhesive properties. *Journal of Applied Polymer Science* **2006,** 102, (3), 2285-2292.

99. Lora, J. H.; Glasser, W. G., Recent industrial applications of lignin: A sustainable alternative to nonrenewable materials. *Journal of Polymers and the Environment* **2002,** 10, (1-2), 39-48.

100. Glasser, W. G.; Barnett, C.; Rials, T.; Kelley, S. S., Synthesis and characterization of several different hydroxyalkyl lignin derivatives. In *1983 International Symposium on Wood Pulping Chemistry*, TAPPI: Japan, 1983.

101. Glasser, W. G.; Selin, J. F.; Hall, P. L.; Drew, S. W., The upgrading of the raw material value of lignin by microbiological and chemical means. In *International Symposium on Wood and Pulping Chemistry (Ekman-Days 1981)*, Stockholm, Sweden, 1981; Vol. 4, pp 39-43.

102. Hofmann, K.; Glasser, W. G., Engineering plastics from lignin 21: Synthesis and properties of epoxidized lignin-poly(propylene oxide) copolymers. *Journal of Wood Chemistry and Technology* **1993,** 13, (1), 73-95.

103. Glasser, W. G.; Barnett, C.; Rials, T.; Sarad, V. P., Engineering plastics from lignin, 2. Characterization of hydroxyalkyl lignin derivatives. *Journal of Applied Polymer Science* **1984,** 29, 1815-1830.

104. Kelley, S. S.; Glasser, W. G., Engineering plastics from lignin XIV. Characterisation of chain-extended hydroxypropyl lignins. *Journal of Wood Chemistry and Technology* **1988,** 8, (3), 341-359.

105. Nonaka, Y.; Tomita, B.; Hatano, Y., Synthesis of lignin/epoxy resins in aqueous systems and their properties. *Holzforschung* **1997,** 51, (2), 183-187.

106. Zhao, B. Y.; Chen, G.; Liu, Y.; Hu, K.; Wu, R. J., Synthesis of lignin base epoxy resin and its characterization. *Journal of Materials Science Letters* **2001,** 20, (9), 859-862.

107. Miller, N. J.; Rice-Evans, C. A., Spectrophotometric determination of antioxidant activity. *Redox Report* **1996,** 2, (3), 161-171.

108. Miller, N. J.; Rice-Evans, C. A., Factors influencing the antioxidant activity determined by the $ABTS^{\cdot+}$ radical cation assay. *Free Radical Research* **1997,** 26, (3), 195-199.

109. Diffey, B. L.; Robson, J., A new substrate to measure sunscreen protection factors throughout the ultraviolet spectrum. *Journal of the Society of Cosmetic Chemists* **1989,** 40, (3), 127-133.

110. Diffey, B. L.; Jansen, C. T.; Urbach, F.; Wulf, H. C., A reference action spectrum for ultraviolet induced erythema in human skin. *International Commission on Illumination (CIE) Journal* **1987,** 6, (1), 17-22.

111. Herrero-Martínez, J. M.; Sanmartin, M.; Rosés, M.; Bosch, E.; Ràfols, C., Determination of dissociation constants of flavonoids by capillary electrophoresis. *Electrophoresis* **2005,** 26, (10), 1886-1895.

112. Slabbert, N. P., Ionisation of some flavanols and dihydroflavonols. *Tetrahedron* **1977,** 33, (7), 821-824.

113. Bloembergen, N.; Purcell, E. M.; Pound, R. V., Relaxation effects in nuclear magnetic resonance absorption. *Physical Review* **1948,** 73, (7), 679-712.

114. Sigma-Aldrich. Material safety data sheet: lauroyl chloride. http://www.sigmaaldrich.com (accessed 08/08/2007).

115. Sigma-Aldrich. Catalogue. http://www.sigmaaldrich.com/Area_of_Interest /Asia__Pacific_Rim/New_Zealand.html (accessed 16/10/2007).

116. Windholz, M., *The Merck Index*. Merck & Co: Rahway, 1976.

117. Sakai, S.; Sugiyama, T.; Ishii, Y., Steric effect in base-catalysed ring-opening reactions of epoxides. *Chemical Abstracts* **1963,** 59, 12605.

118. Ishizu, T.; Hirata, C.; Yamamoto, H.; Harano, K., Structure and intramolecular flexibility of β-cyclodextrin complex with (-)-epigallocatechin gallate in aqueous solvent. *Magnetic Resonance in Chemistry* **2006,** 44, (8), 776-783.

119. Martynov, I. V.; Zavel'skii, V. O.; Kovalenko, S. V.; Fetisov, V. I.; Yurtanov, A. I., Carbon acidity and mechanism of deuterium exchange of chloronitroacetic acid. *Doklady Akademii Nauk SSSR* **1983,** 269, (1), 152-156.

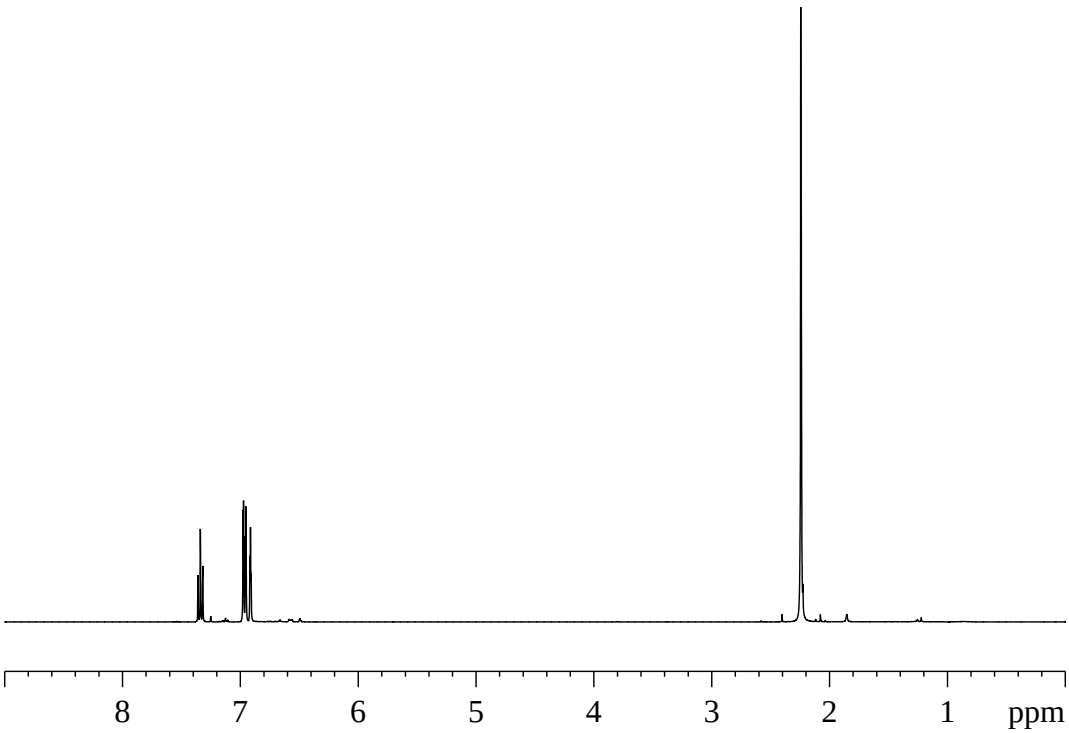
120. Cole, R. B.; Zhu, J., Chloride anion attachment in negative ion electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry* **1999,** 13, (7), 607-611.

121. Duer, M. J., *Introduction to Solid-State NMR Spectroscopy*. Blackwell: Oxford, 2004.

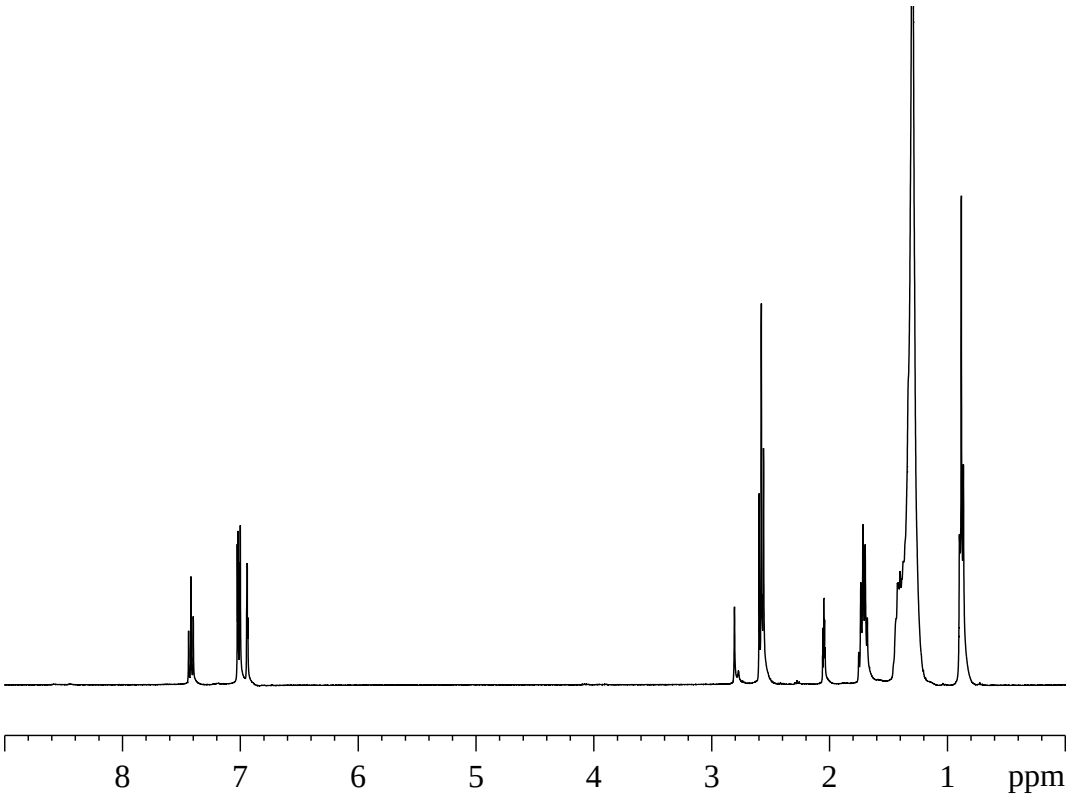
122. Levitt, M. H., *Spin Dynamics: Basics of Nuclear Magnetic Resonance*. Wiley: Chichester, 2001.

123. Friebolin, H., *Basic One- and Two-Dimensional NMR Spectroscopy*. 2nd ed.; VCH Publishers: Weinheim, 1993.

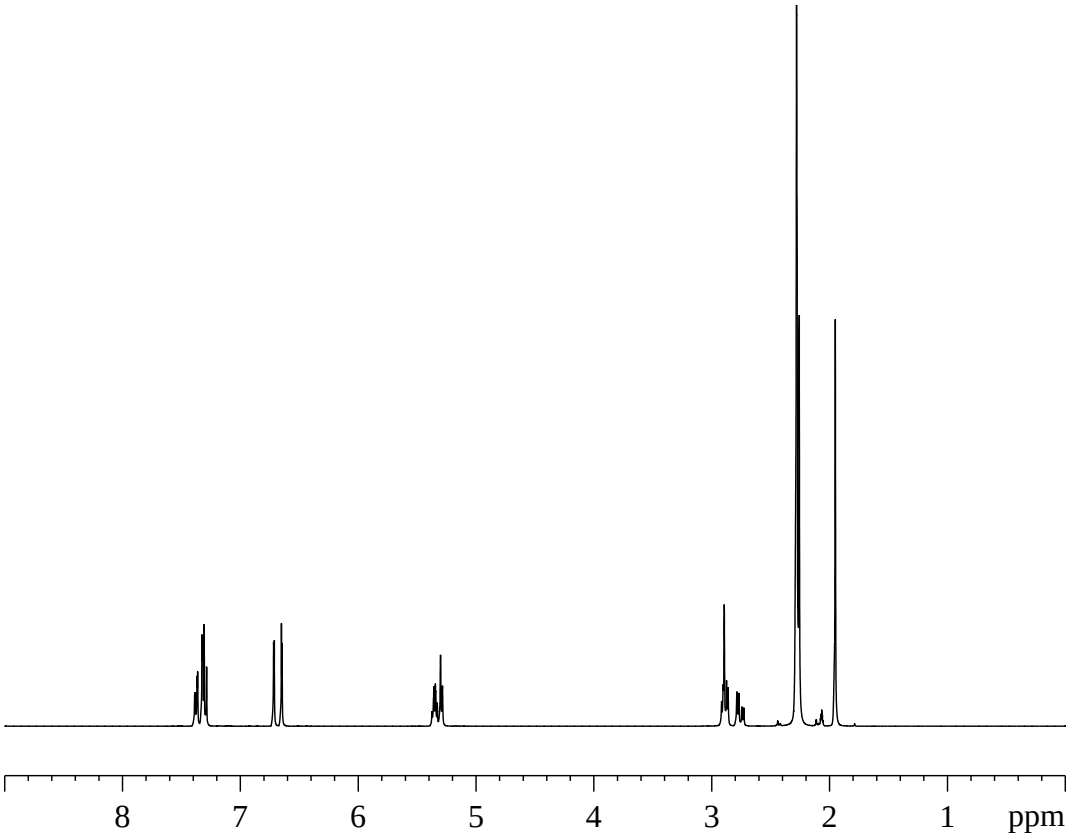
Appendices



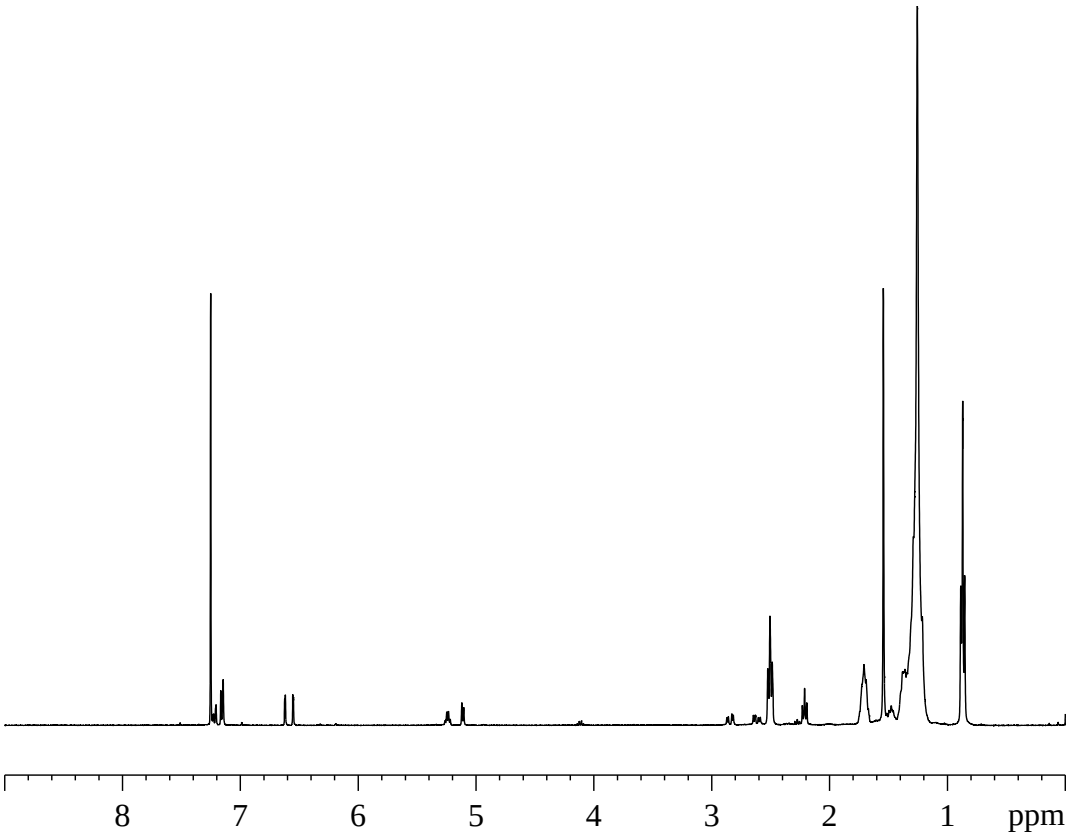
Appendix 1: ^{1}H NMR spectrum of **1**.



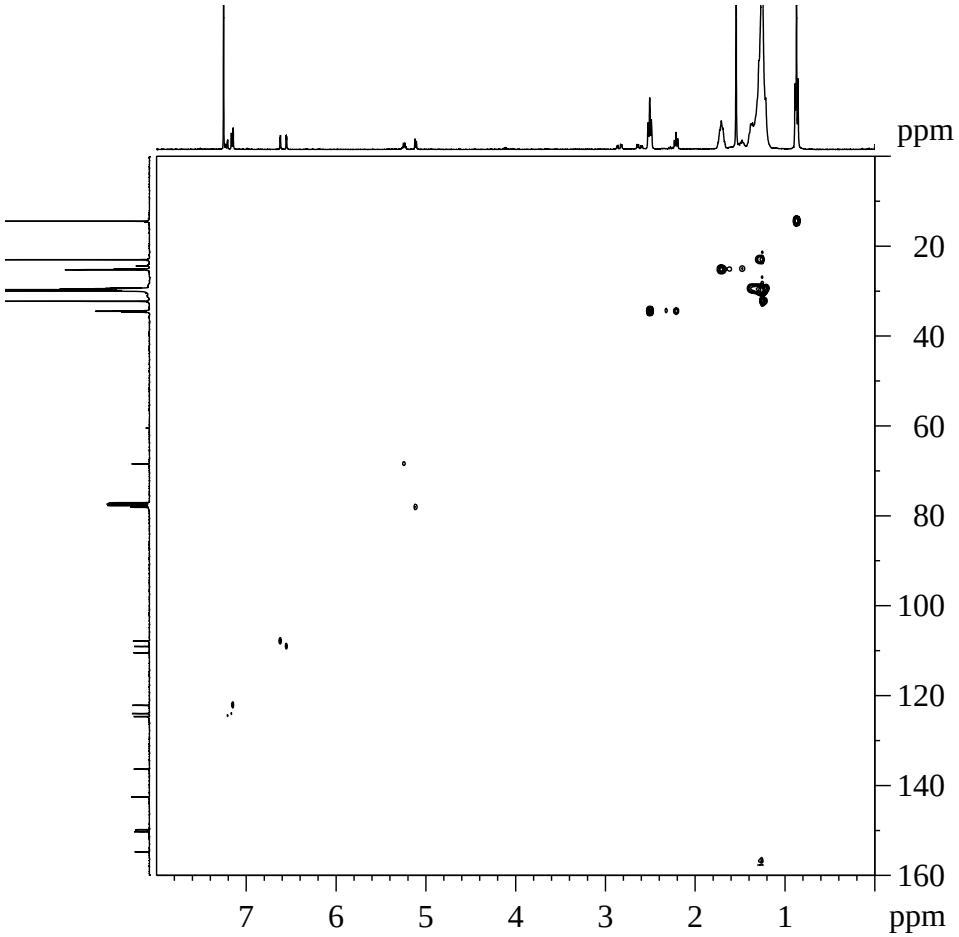
Appendix 2: ^{1}H NMR spectrum of **3**.



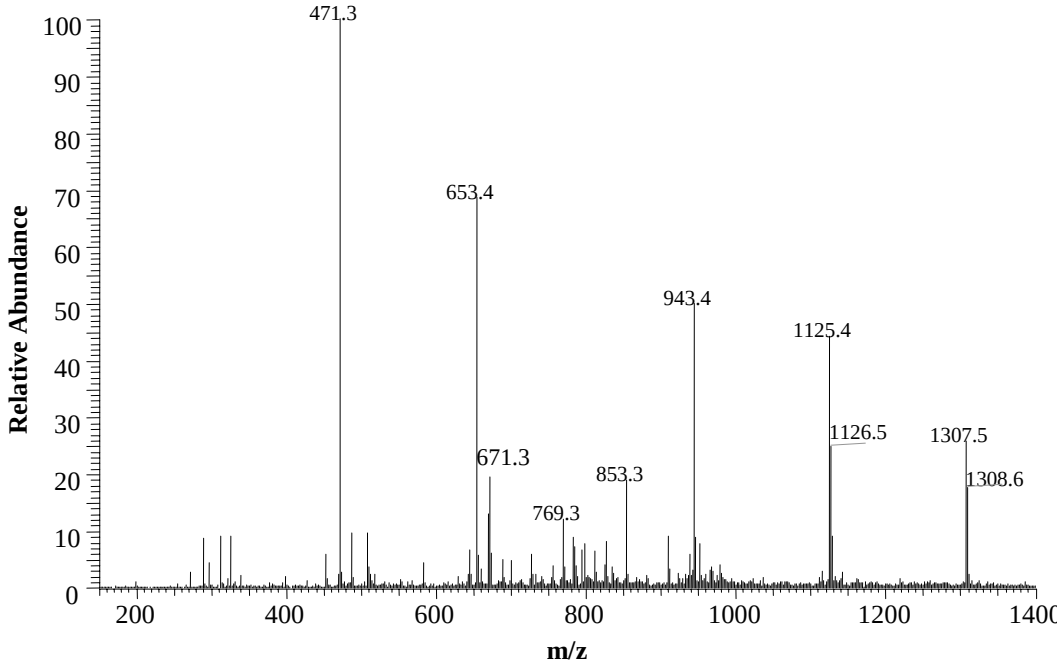
Appendix 3: ^{1}H NMR spectrum of **4**.



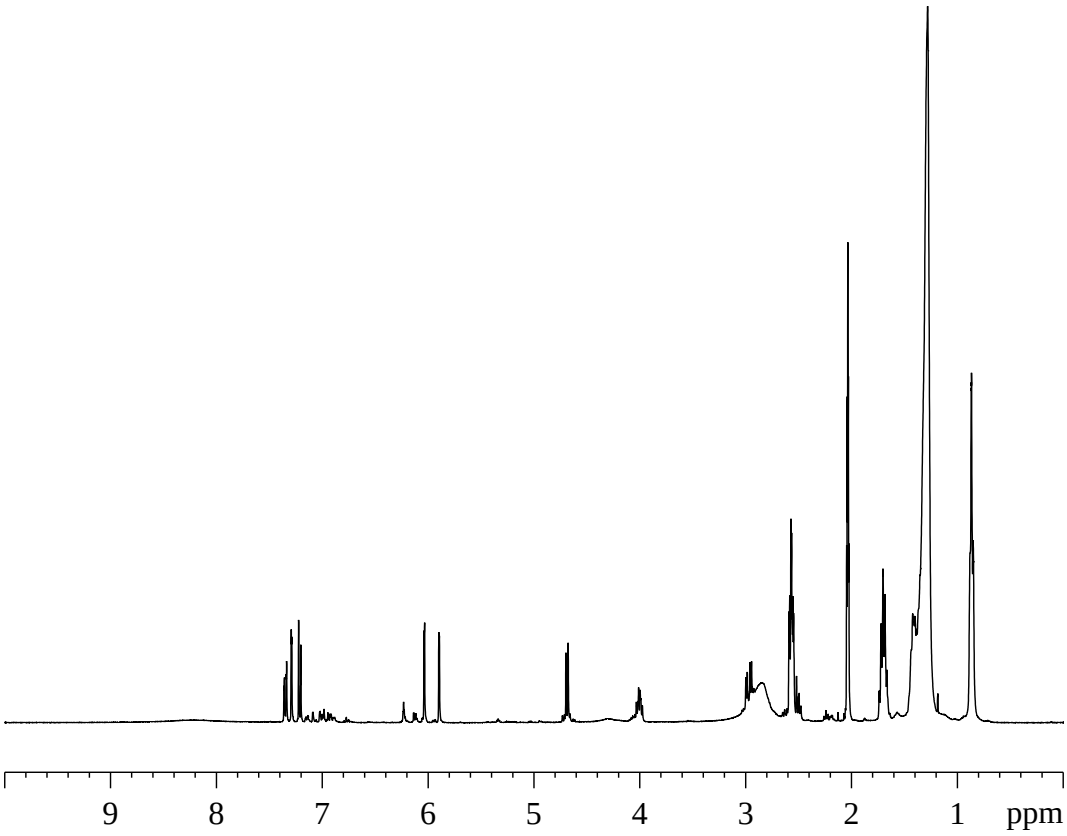
Appendix 4: ^{1}H NMR spectrum of **5**.



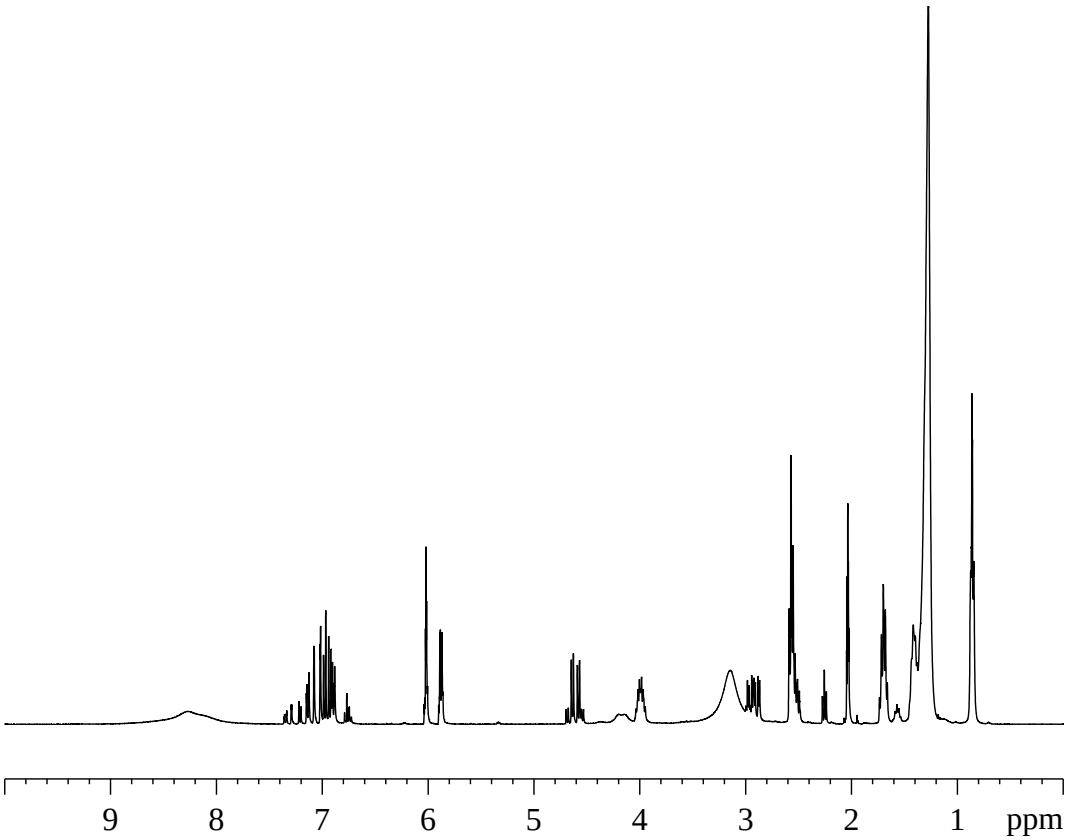
Appendix 5: HSQC NMR spectrum of **5**.



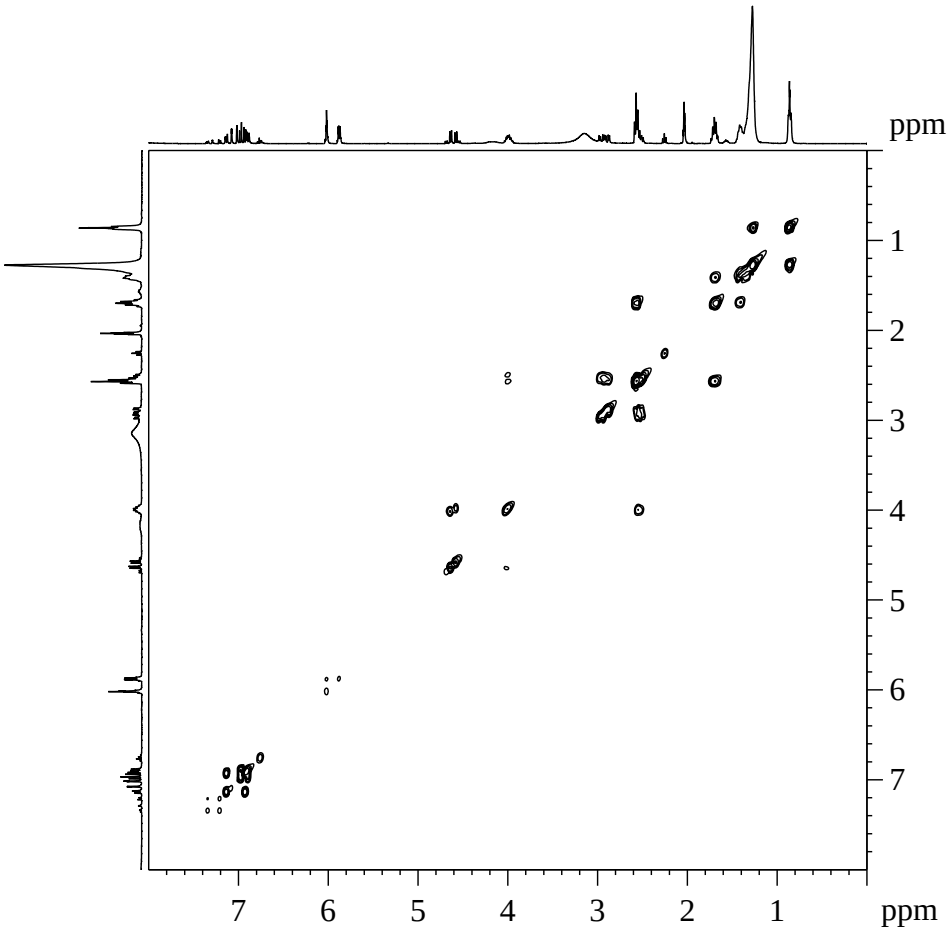
Appendix 6: ESI mass spectrum of **9**.



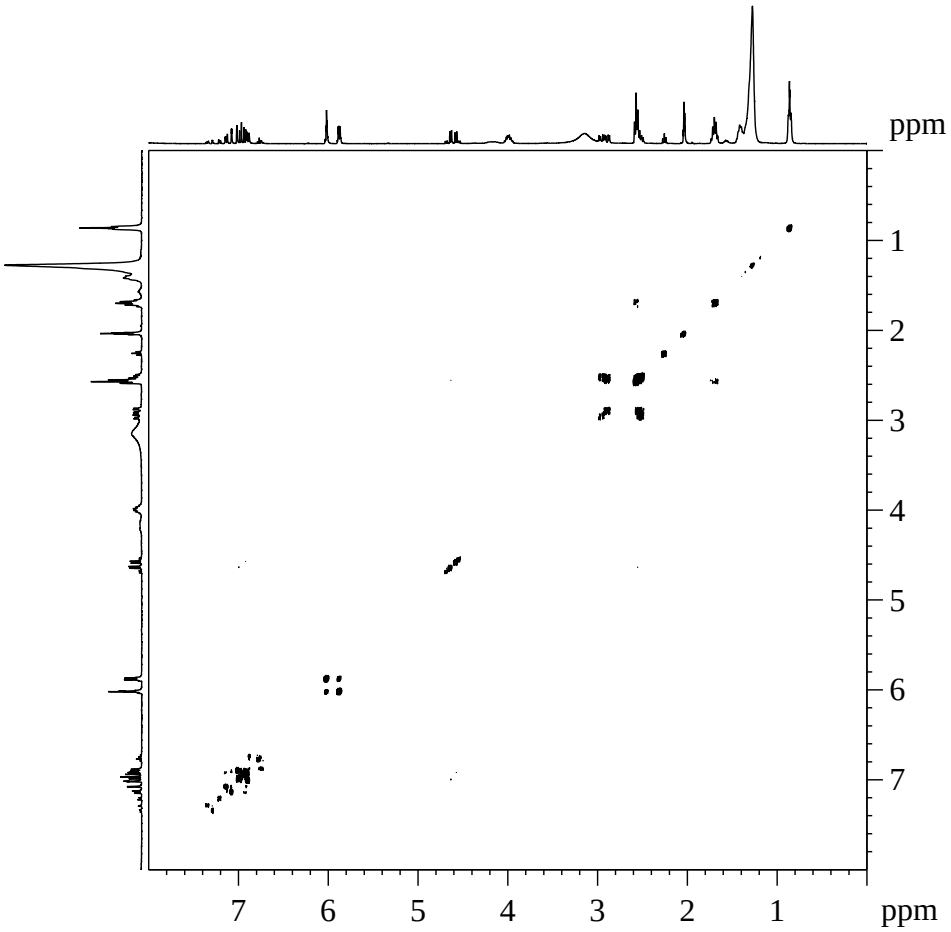
Appendix 7: ^{1}H NMR spectrum of **17**.



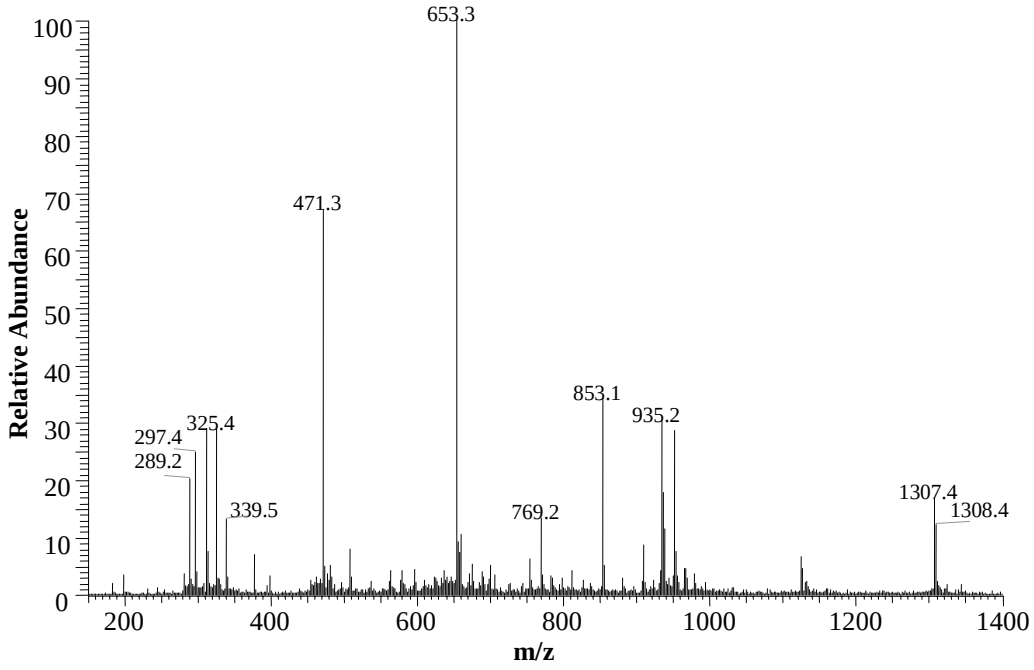
Appendix 8: ^{1}H NMR spectrum of **18**.



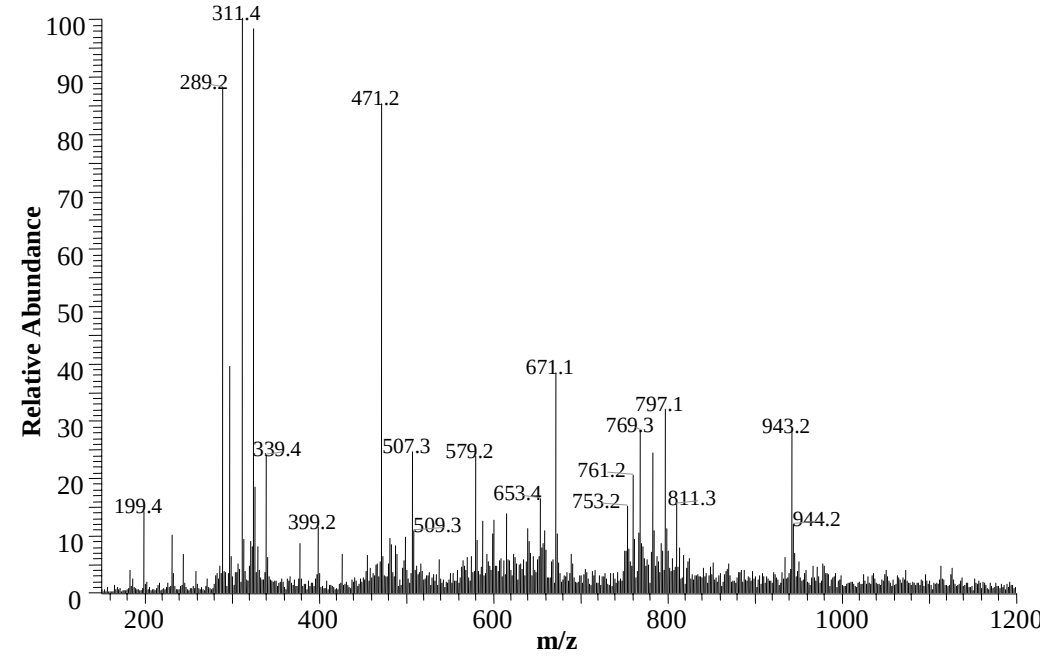
Appendix 9: COSY NMR spectrum of **18**.



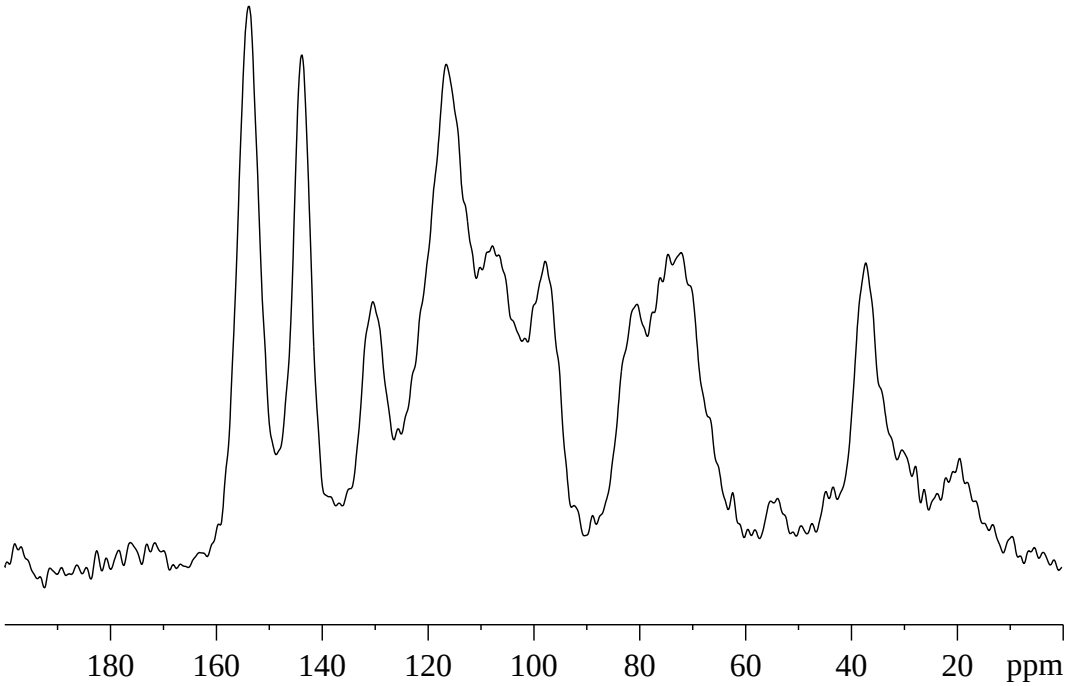
Appendix 10: LR-COSY NMR spectrum of **18**.



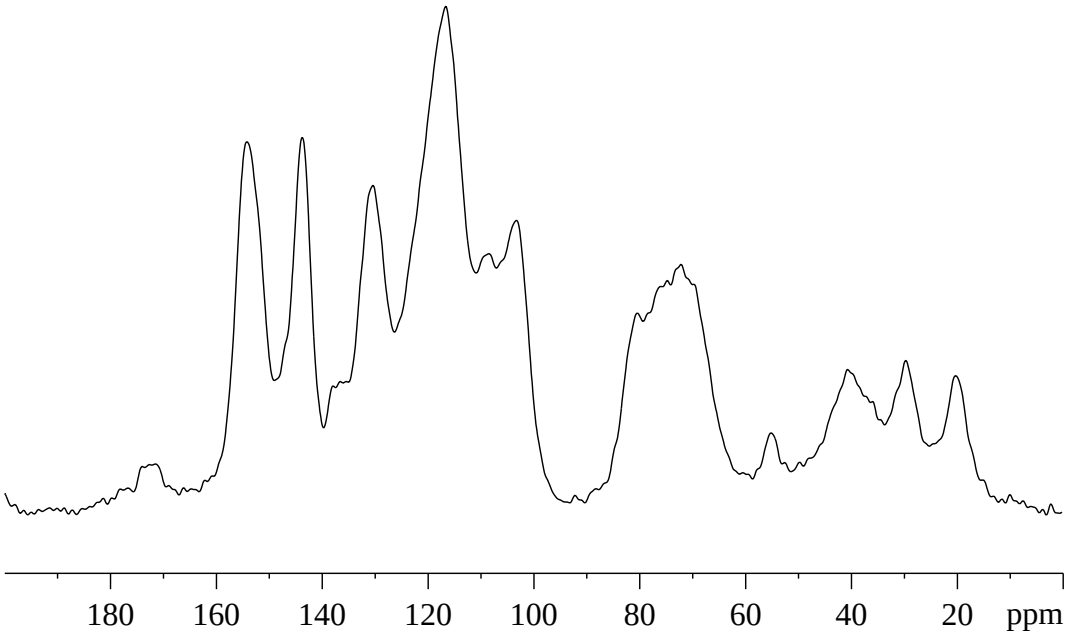
Appendix 11: ESI mass spectrum of **17**.



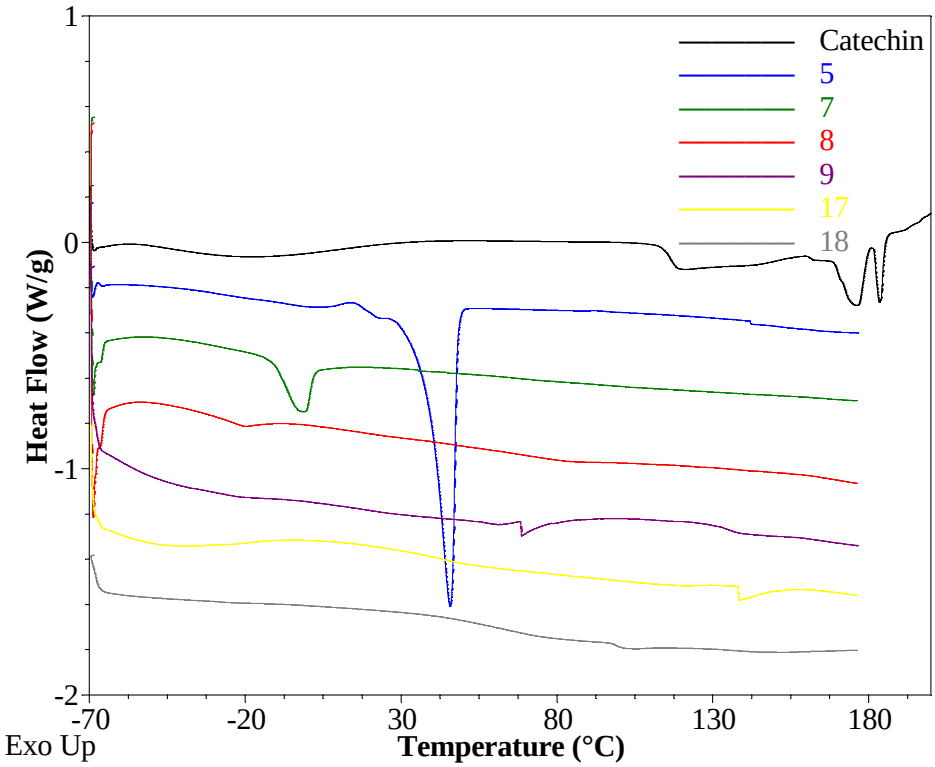
Appendix 12: ESI mass spectrum of **18**.



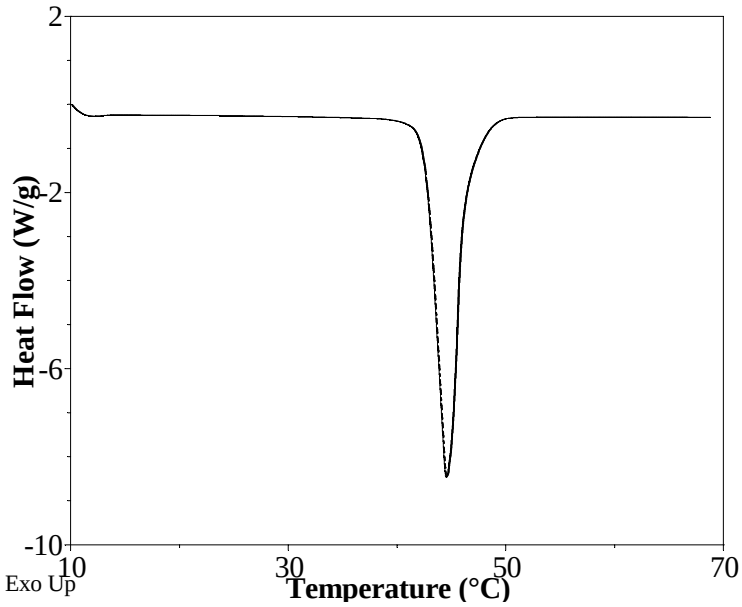
Appendix 13: ^{13}C CP-MAS NMR spectrum of **19**.



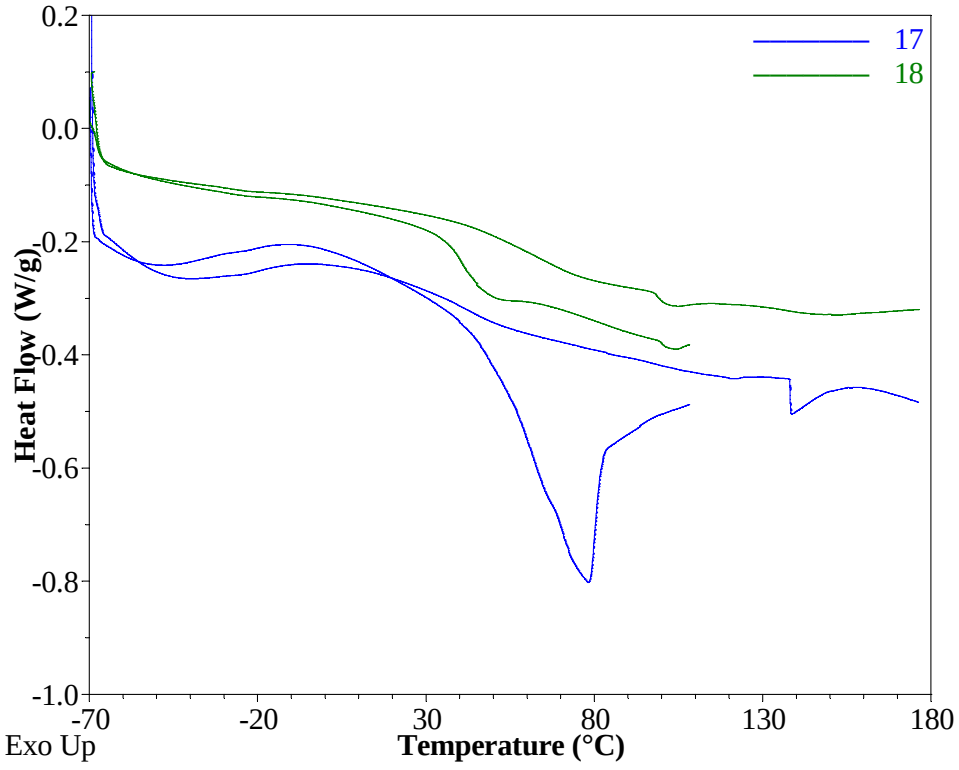
Appendix 14: ^{13}C CP-MAS NMR spectrum of **20**.



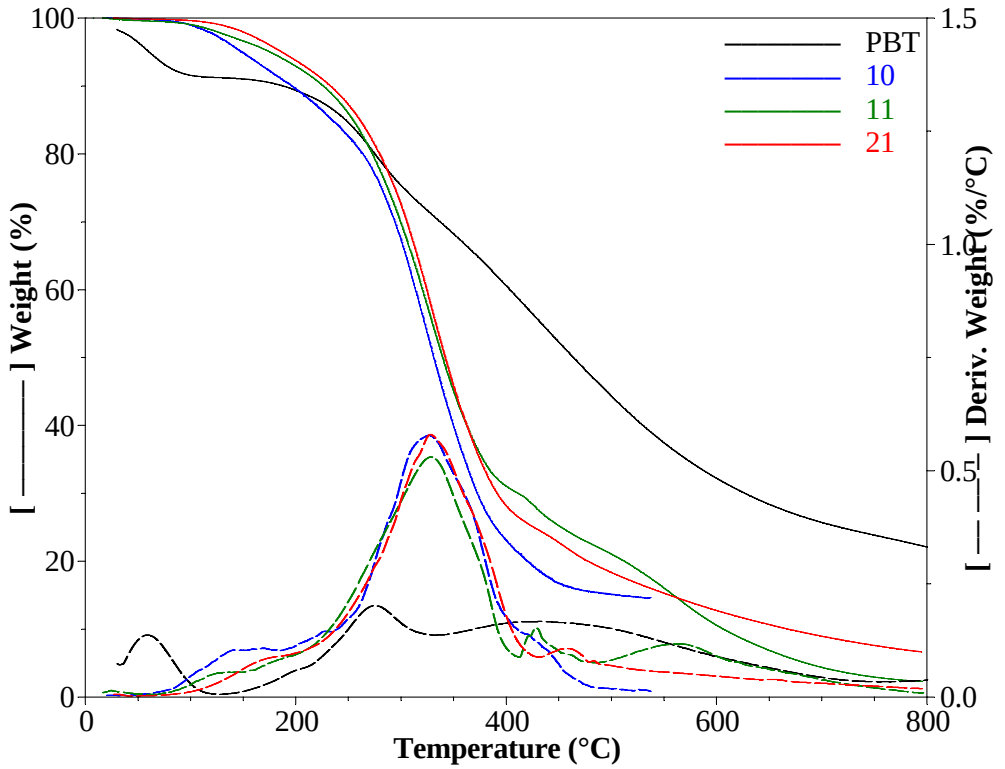
Appendix 15: DSC thermogram of catechin laurates (**5**, **7**, **8**, **9**, **17**, and **18**) compared with unreacted catechin (second heating cycle).



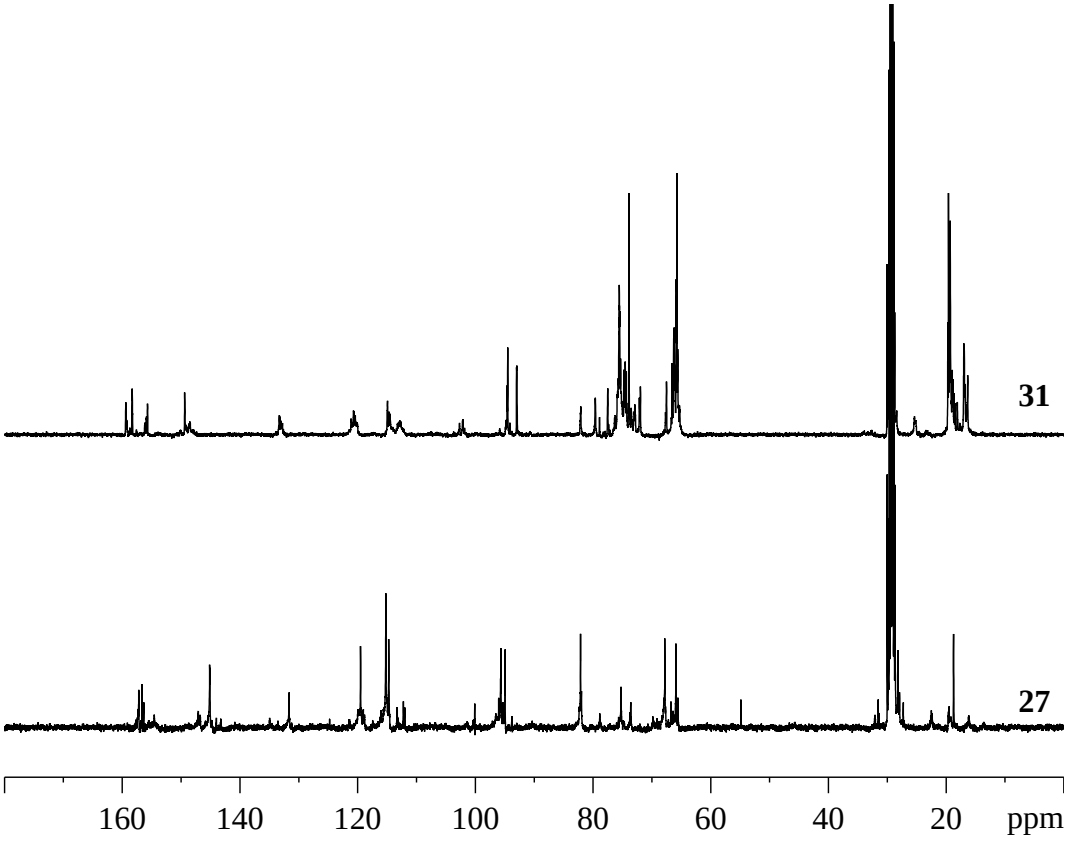
Appendix 16: DSC thermogram of lauric acid (second heating cycle).



Appendix 17: DSC thermogram of **17** and **18** showing both the first (–70 to 110 ^{o}C) and second heating cycles (–70 to 180 ^{o}C).



Appendix 18: TGA thermogram of PBT and PBT laurates (**10**, **11**, and **21**).



Appendix 19: ^{13}C NMR spectra of **27** and **31**.

Appendix 20: MS of PBT hydroxypropyl ethers (**32-36**) calculated by mass gains.

Product	Mole equivalents of PO	Initial mass of PBT (g)	Final mass (g)	% weight gain	MS
PBT hydroxypropyl ethers					
32	1	0.99	1.17	18.2	0.9
33	3	0.91	1.46	60.4	3.1
34	5	0.96	1.83	90.6	4.6
35	10	0.82	2.05	150.0	7.6
36	20	0.91	2.65	191.2	9.6

Appendix 21: MS for PBT hydroxypropyl ethers (**32-36**) calculated by integration of the ^{1}H NMR spectra.

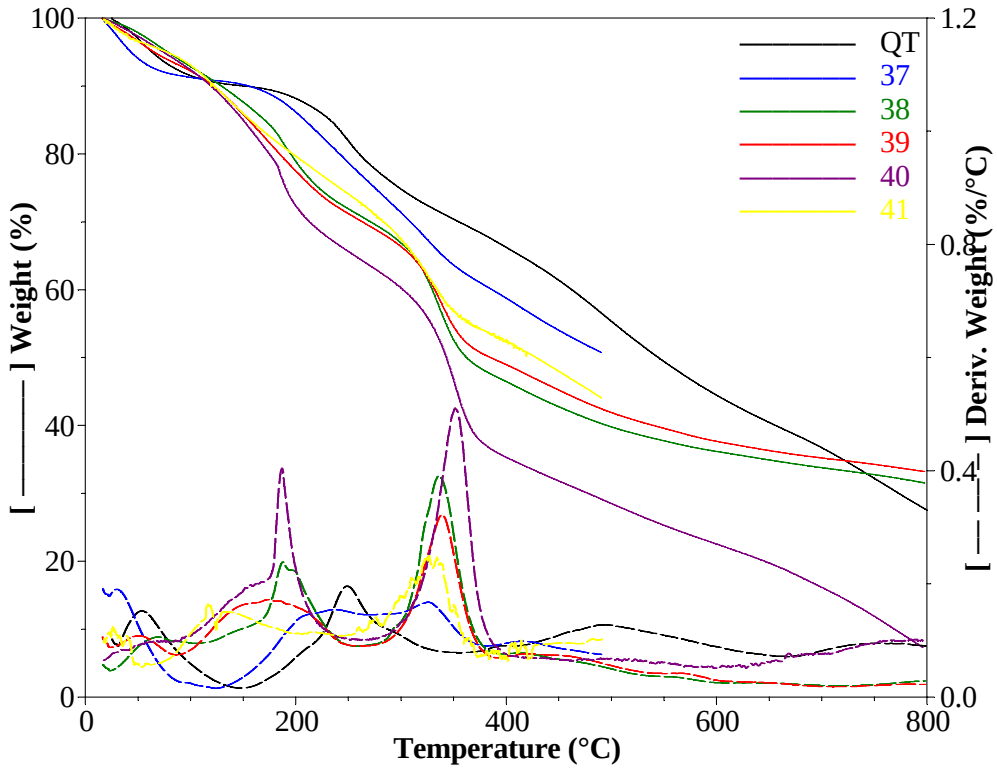
Product	Mole equivalents of PO	Integrals of ^{1}H NMR		Average MS
		Ar-H	CH_3	
PBT hydroxypropyl ethers				
32	1	3.9	4.6	1.5
33	3	3.9	17.3	5.8
34	5	3.9	21.1	7.0
35	10	3.9	40.2	13.4
36	20	3.9	41.8	14.0

Appendix 22: The MS of QT hydroxypropyl ethers (**37-41**) calculated by mass gains

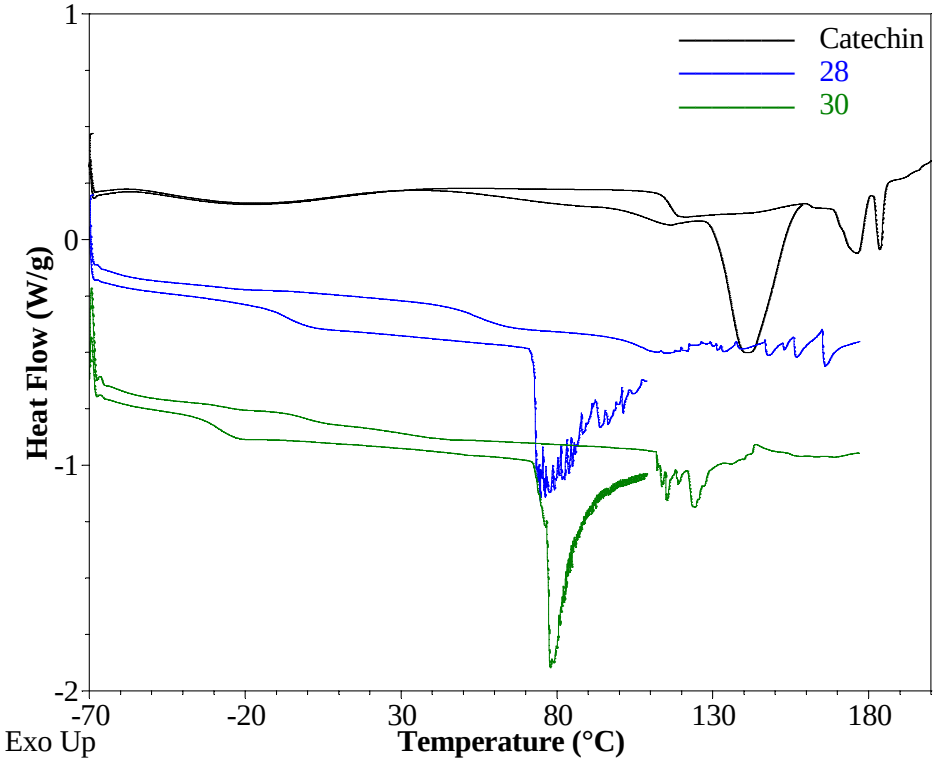
Product	Mole equivalents of PO	Initial mass of QT (g)	Final mass (g)	% weight gain	MS
QT hydroxypropyl ethers					
37	1	0.98	1.10	12.2	0.6
38	3	0.96	1.45	51.0	2.4
39	5	0.95	1.43	50.5	2.4
40	10	0.82	1.19	45.1	2.2
41	20	0.94	1.35	43.6	2.1

Appendix 23: The average MS for QT hydroxypropyl ethers (**37-41**) calculated by integration of the ^{1}H NMR spectra.

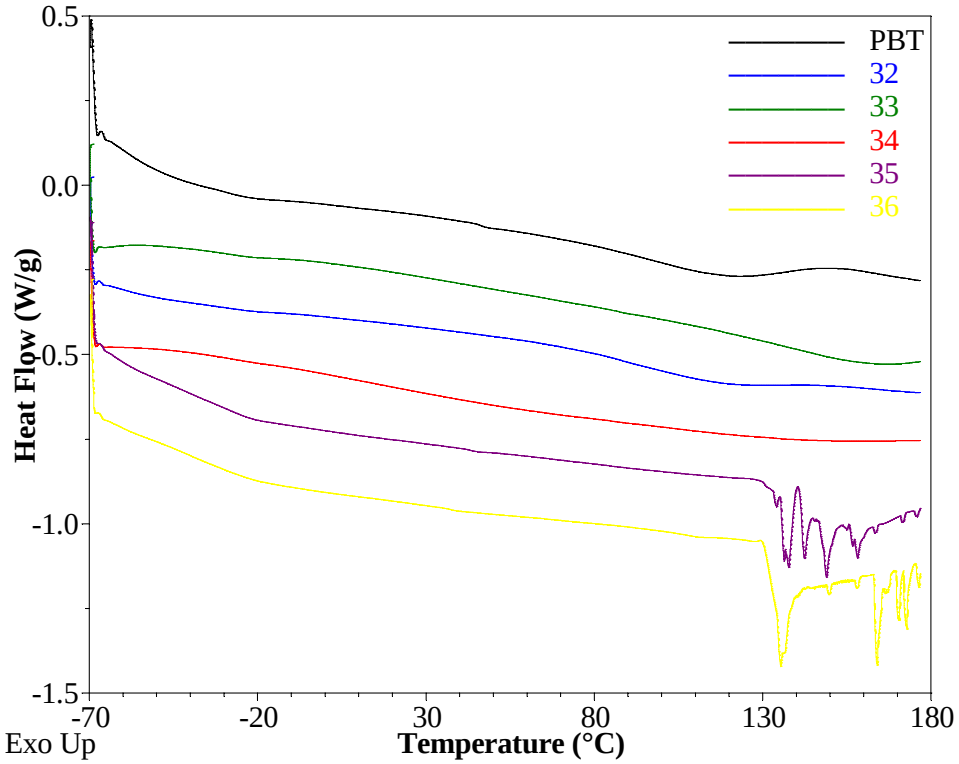
Product	Mole equivalents of PO	Integrals of ^{1}H NMR		Average MS
		Ar-H	CH_3	
QT hydroxypropyl ethers				
37	1	4.9	5.0	1.7
38	3	4.9	15.1	5.0
39	5	4.9	16.2	5.4
40	10	4.9	19.5	6.5
41	20	4.9	11.2	3.7



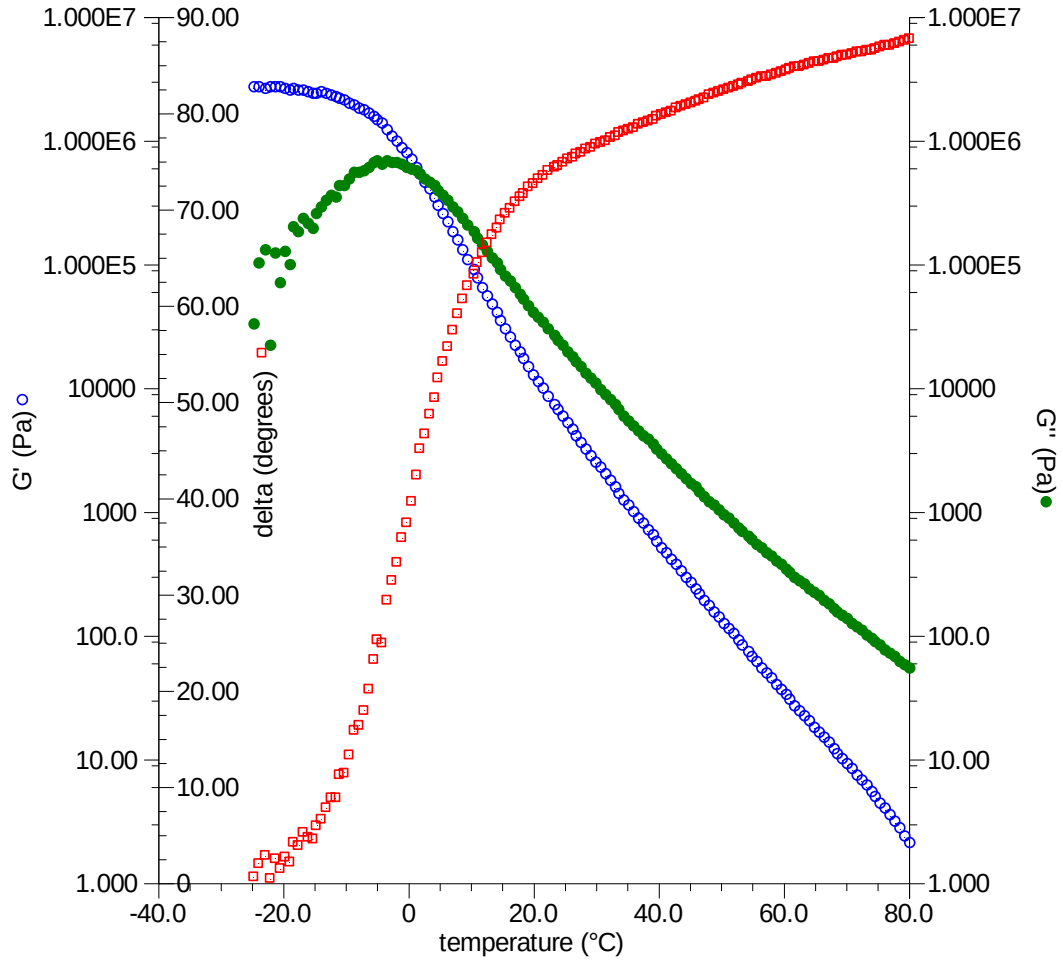
Appendix 24: TGA thermogram of QT hydroxypropyl ethers (**37**-**41**).



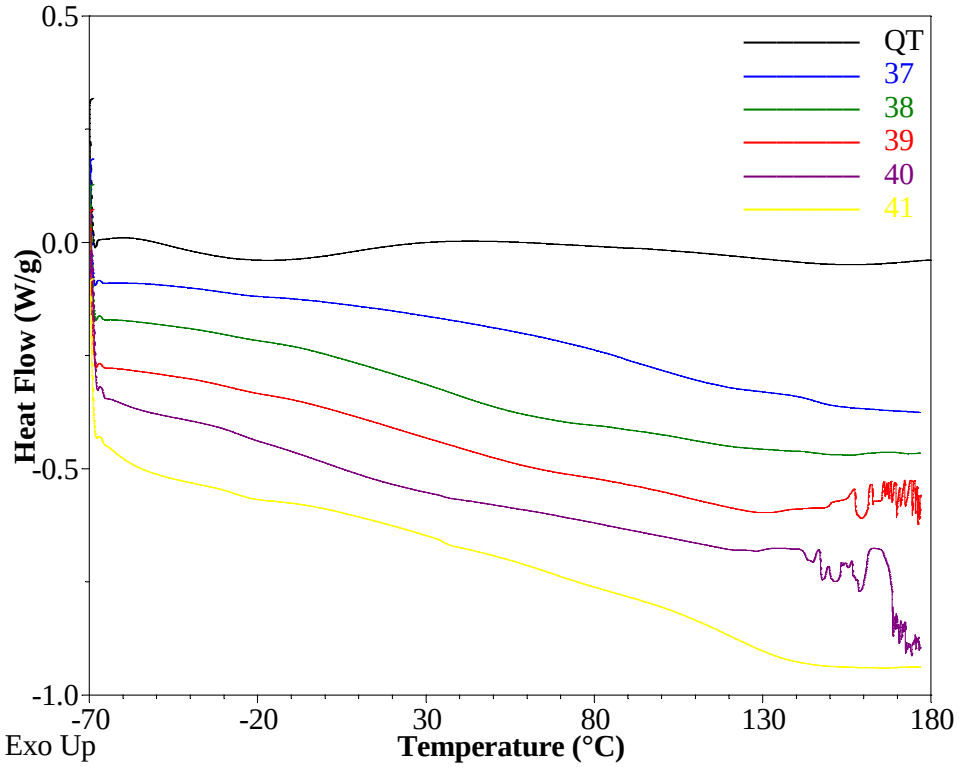
Appendix 25: DSC thermogram of catechin hydroxypropyl ethers (**28** and **30**) compared with unreacted catechin (first and second heating cycle).



Appendix 26: DSC thermogram of PBT hydroxypropyl ethers (**32-36**) compared with unreacted PBT (second heating cycle).

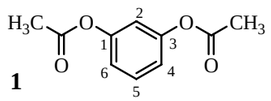


Appendix 27: Rheology measurements of **35** in an oscillatory mode. Where: G' is the elastic modulus, G'' is the loss modulus, and tan(delta) is a measure of the ratio of G' and G''.

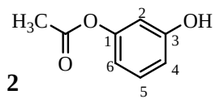


Appendix 28: DSC thermogram of QT hydroxypropyl ethers (**37**-**41**) compared with unreacted QT (second heating cycle).

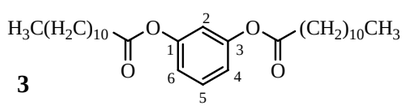
Structures



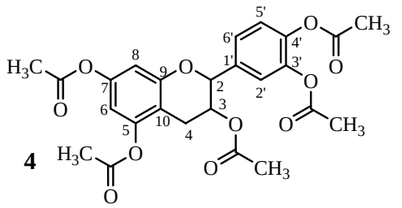
1



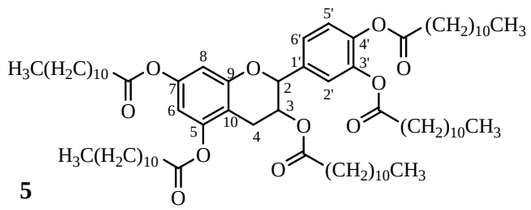
2



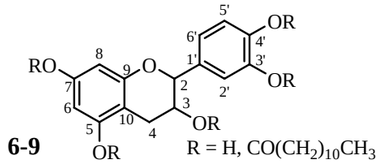
3



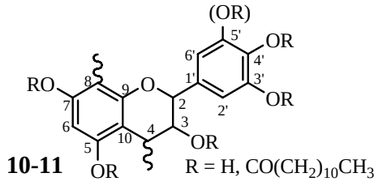
4



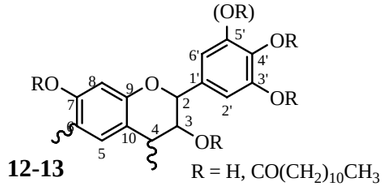
5



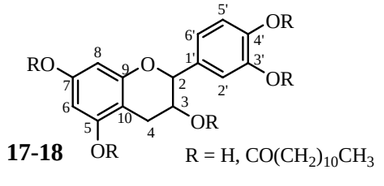
6-9 R = H, $CO(CH_2)_{10}CH_3$



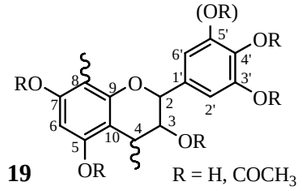
10-11 R = H, $CO(CH_2)_{10}CH_3$



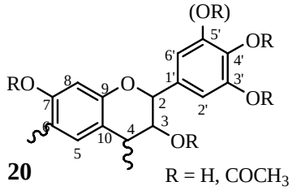
12-13 R = H, $CO(CH_2)_{10}CH_3$



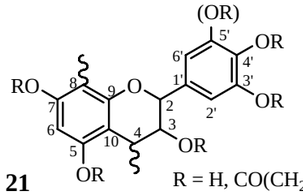
17-18 R = H, $CO(CH_2)_{10}CH_3$



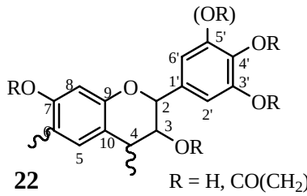
19 R = H, $COCH_3$



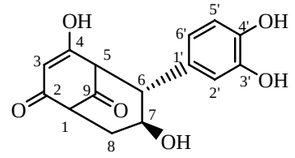
20 R = H, $COCH_3$



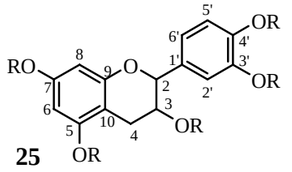
21 R = H, $CO(CH_2)_{10}CH_3$



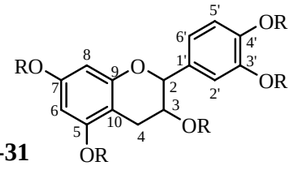
22 R = H, $CO(CH_2)_{10}CH_3$



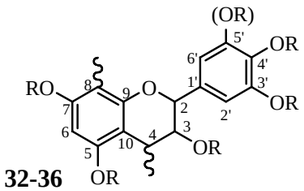
24



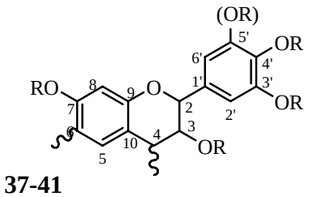
25 R = H, $(CH_2CH(CH_3)O)_nCH_2CHOHCH_3$



27-31 R = H, $(CH_2CH(CH_3)O)_nCH_2CHOHCH_3$



32-36 R = H, $(CH_2CH(CH_3)O)_nCH_2CHOHCH_3$



37-41 R = H, $(CH_2CH(CH_3)O)_nCH_2CHOHCH_3$