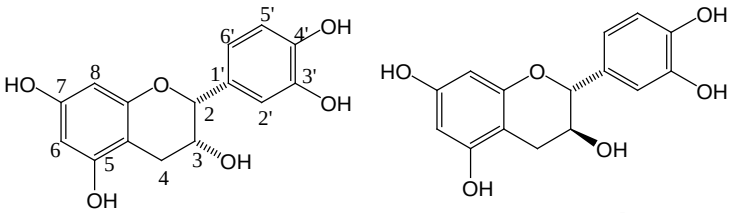
1.1 Polyphenolics and Tannins in Wine

Polyphenolic compounds are a diverse group of aromatic compounds that are ubiquitous in nature. Many of these compounds are found in red wine and play a significant role in colour, taste and mouth feel properties of red wines [1].

Polyphenolics are produced from many natural sources or processes, including grape skins, flesh, seeds, and vines; or from microbial directed breakdown products, from which they are extracted into wine. Winemakers may also add these compounds during or after fermentation. Oak barrel ageing can also contribute tannins to wine after fermentation[2].

There are several subclasses of the phenolics found in wine[1]. The condensed tannins, or proanthocyanidins, are one such structural class. These tannins are polymeric species predominantly derived from monomeric units known as catechin and epicatechin (figure 1). Other structural variations of these monomers include gallate esters of the 3-OH, such as (epi)catechin gallate and 3',4',5'-trihydroxylated analogues known as (epi)gallocatechins[3]. Condensed tannins have been associated with colour stabilisation, mouth feel and possibly some bitterness effects in wine [4].

Figure 1: Epicatechin (1) and Catechin (2) monomers of proanthocyanidins and the C-skeleton numbering system.



1- (-) Epicatechin **2**- (+)-Catechin

1.2 Benefits of tannins

Wine tannins are widely reported to have powerful health benefits, which are responsible for the phenomenon known as the "French Paradox"[1]. Regular consumption of red wine by the French has led to reduced occurrence of cardiovascular disease despite their diet

high in saturated fats. Proanthocyanidin health benefits are not limited to wine tannins. Many natural tannins have been shown by *in vitro* assays to have widespread potentially significant antiviral, enzyme-inhibiting, anti-oxidant and radical-scavenging properties[5,6]. There has also been increasing interest in theses compounds as possible anticancer agents[7,8]. Also, proanthocyanidin dimers and trimers have been shown to inhibit non-enzymatic browning reactions known as the Maillard Reaction[9,10]. The Maillard Reaction is important in foodstuffs as an indication of spoilage, as in fruits and beer, or as an ageing factor in increasing flavour complexity and mellowing, as in teas and aged wines[11,12].

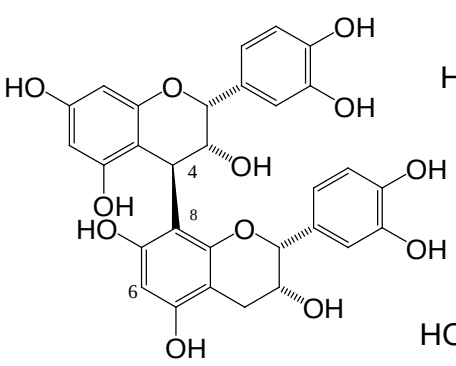
1.3 Challenges in Obtaining Tannin Material

Given that proanthocyanidin oligomers hold important health benefits, obtaining these compounds has gained importance. Many herbal medicines are used as mixtures containing polyphenolics as active ingredients. However, studies of biological activities of proanthocyanidins require pure samples so that activity can be traced to a particular compound. Traditionally, tannin material has been isolated from plant sources including, but not limited to, teas and cinnamon[13,14]. However, these isolations can be painstakingly time consuming and purity and reproducibility are often suspect. Also, yields of isolations are often small (Nonaka etal[14] isolated 1g of cinnamtannin C1 from 1kg of cinnamon, but yields are generally smaller) and information on biological activities is hard, sometimes impossible to assimilate on such small samples. Thus attention has turned towards synthetic methods for producing these compounds[7-10,15].

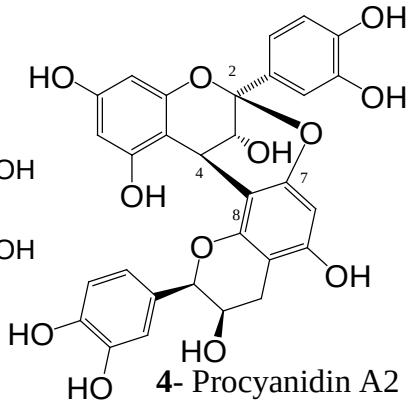
1.4 Oligomeric Proanthocyanidins

Oligomeric proanthocyanidins constituted of catechin and epicatechin are dominated by two groups that vary in their linkage between flavan (monomer) units. The B-type oligomers contain single C-C linkages from C4-C8 (Fig2-**3**) or C4-C6. The A-type oligomers have the B-type C-C linkage, but also have a second ether type linkage from C2-C7 (Fig 2-**4**) [16].

Figure 2: Dimers of the B-type and A-type Families of Proanthocyanidins.



3- Procyanidin B2



4- Procyanidin A2

B-type oligomers linked C4-C6 are rare in nature and have not been considered in this project to any extent.

1.5 Synthesis of B-type Oligomers

Synthetic methods in lower oligomeric (dimers, trimers) B-type proanthocyanidin have been undertaken in recent years[7-10,15]. These syntheses and subsequent modifications to reaction steps[17,18] have led to these compounds being available by efficient methods and in high yields. Biological assays using these synthetic compounds have been conducted and in some cases compared to natural procyanidins isolated from plant sources[9].

1.6 A-type oligomers

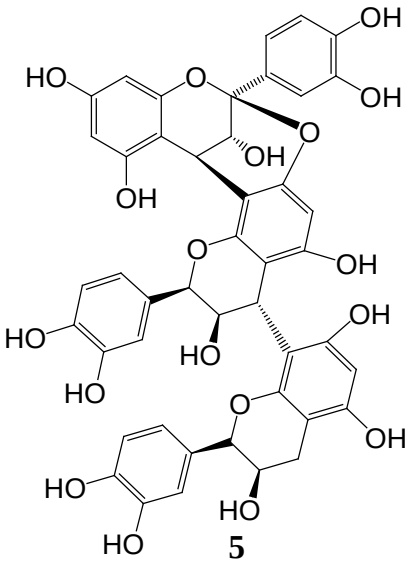
A-type proanthocyanidins have received much less attention than their B-type counterparts. The unusual second ether linkages lead to these compounds generally being much less abundant in nature. A number of the simpler dimers and trimers of the A-type series have been isolated from natural sources. Of these, a few have characterised by NMR and MS[19,20], but their properties have not been studied to the extent of B-types. From the limited studies on these compounds, evidence exists that the A-type conversion from B-type counterparts may occur under oxidative conditions [5,27], but no detailed research into synthetic pathways has been undertaken.

1.7 Research Aims

This study aims to take advantage of efficient methods developed for the synthesis of B-type dimers and trimers to synthesise B-type building blocks and then use these compounds in developing methods for efficient B-type to A-type conversion. Currently, only two low-yielding methods exist, DPPH radicals in ethanol/water solution[5], and H_2O_2 in $NaHCO_3$ solution[27]. Given these conversion conditions, this research aims to test existing and new reagents for suitability in A-type conversion. These tests will be conducted using the B2(**3**) to A2(**4**) conversion system as a model. Both **3** and **4** are dimers and there are no regiochemical issues in the addition of the A-type linkage as the dimer can form only one A-type between its monomers.

Also, the synthesis of a trimer containing both A-type and B-type linkages (Figure3-**5**) will be attempted. The regiochemical issue of A-type conversion is of great importance with this trimer. Only conversion between the top two flavan units is desired. This regiochemistry will be attempted by using differential protecting groups during synthesis of the B-type trimer precursor.

Figure 3: Cinnamtannin C1 (**5**).



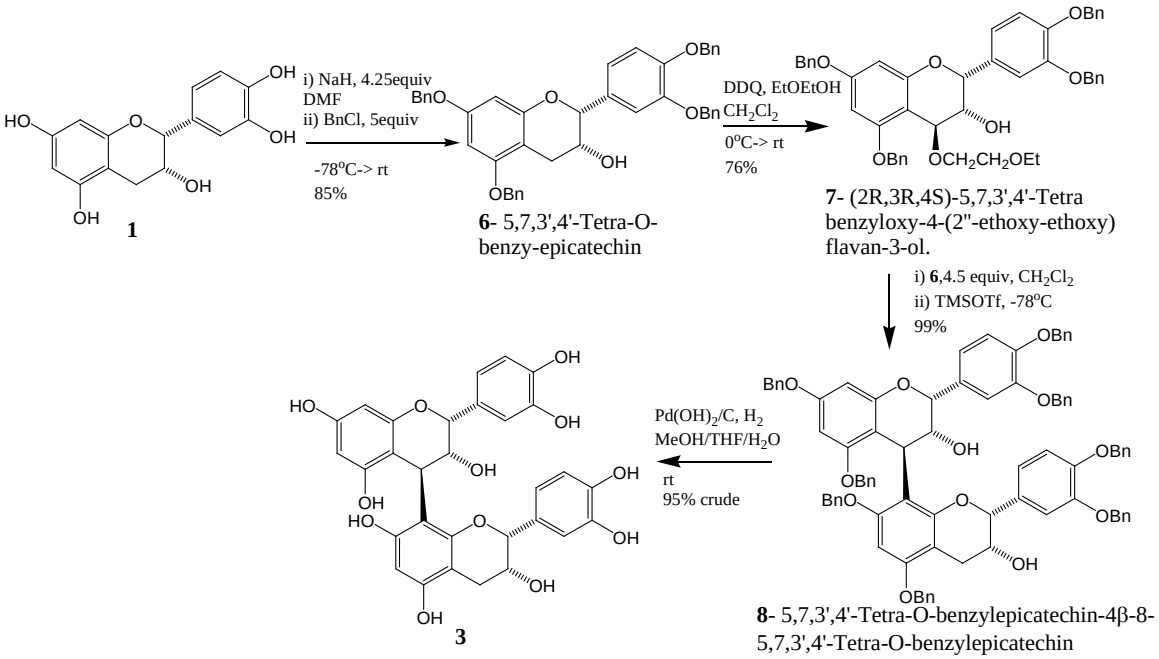
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2.1 Synthesis of B-type Analogues

2.1.1 B-type dimers and trimers

Synthetic studies in B-type proanthocyanidin dimers and trimers from catechin and epicatechin monomers have been conducted[7-10]. Two research groups, the Tuckmantel and Saito groups reported total syntheses of B2[7,9]. A combination of these syntheses with modifications was employed in the preparation of B2 to be used in the A2 conversion. Figure 4 below shows the synthetic pathway from epicatechin to the B2 dimer (**3**).

Figure 4: B2 synthetic pathway.



Overall, a monomer to dimer yield of 54% was achieved. Employing the O-benzylation technique, as reported in Mustafa *et al*[17], greatly increased this overall yield from 14% using the original Tuckmantel *et al*[7] O-benzylation (19% for benzylation reaction) to 54%. Tuckmantel O-benzylation involved addition of the benzylation species BnBr at -15°C and gave consistent yields of 15-20% of the all O-benzylated epicatechin. Mustafa *et al* benzylation employed the addition of BnCl as the benzylating agent at –78°C and gave the all O-benzylated species in 85% yield when conducted following the reported

procedure (reported yield was 91%). Adding the benzylating species at a lower temperature resulted in the vast increase in yield. The low yields reported by Tuckmantel *et al* resulted from multiple C and O benzylated products being produced in high yields. Reducing the temperature of solution (as in Mustafa synthesis) during addition of the benzylating agent altered C-benzylation and O-benzylation competition in favour of O-benzylation to such an extent that the all O-benzylated product is produced in far greater selectively.

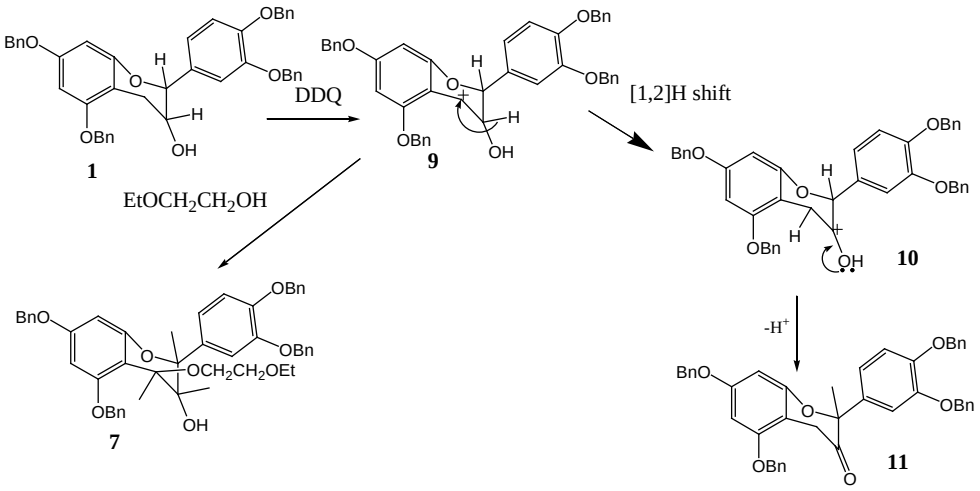
Large modifications to the workup and purification procedures for the C4-ethoxylation reaction (**4.2.2, 4.2.3**) resulted in an increased isolation yield (76%) of **7** compared to that reported by Saito *et al*[18] (64%). Removal of DDQ products was achieved in the Saito synthesis by addition of DMAP to complex and precipitate DDQ products, which required filtration. Filtration of this precipitate was difficult and it retained a large amount of solvent, which may have resulted in some product loss. In the modification, saturated $NaHCO_3$ solution was used to ionise the DDQ products, and subsequently removed by water washing. Thus, removal of the filtration step may have resulted in product being isolated from the reaction mixture in greater yield.

Only the 4S stereochemistry of this reaction was obtained in the reaction. Figure 5 over page shows the mechanism of formation of the carbocation **9**. The 2-ethoxy-ethanol present in large excess acts as a nucleophile to produce **7**. The 4S stereochemistry of C4 has the 2-ethoxy-ethanol in a pseudo-axial position, which should be expected to be less energetically favourable to the pseudo-equatorial position. The S_N1-type mechanism of formation favours the pseudo-axial substitution (4S) from steric interaction of the 3-OH, which is in a pseudo-axial position and thus blocks attack from below the planar carbocation **9** to form the 4R product.

This reaction was interesting when compared to the analogous reaction with catechin. Catechin C4-ethoxylation proceeded with 98% yield[21]. Variation in yield for the two isomers can be accounted for in part by the formation of (2R)-5,7,3',4'-Tetrakis(benzyloxy)flavan-3-one (**11**) as a by-product when an attempted recrystalisation

of **7** yielded the C3 oxidised material (7%) instead. This is proposed to have formed by the mechanism detailed in figure 5 below.

Figure 5: Proposed mechanism for the formation of the two DDQ oxidation products.



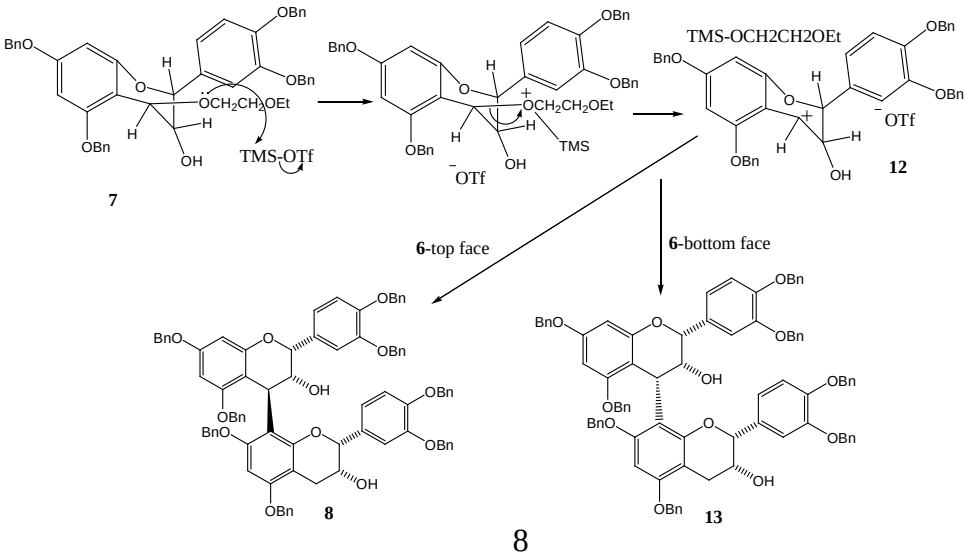
The ten-fold difference in the two products may be accounted for by thermodynamic stabilities of the two carbocations formed, (**9,10**). During formation of the C4-ethoxylated product, the intermediate carbocation **9** is formed. This carbocation is secondary and resonance stabilised by the ortho-para oxygenated aromatic ring. The C3-oxidised product involves an intermediate tertiary carbocation **10** resonance stabilised by the α-hydoxyl group. At room temperature, C4-ethoxylation predominated, suggesting carbocation **9** was far the more stable of the two intermediates. Another possible mechanism for formation of **11** is DDQ directly removing the C3-hydrogen to form carbocation **10**. The C3-hydrogen is α to a hydroxyl group and thus resonance stabilised by it. This hydrogen is far more sterically hindered compared to the two C4-hydrogens and thus if **11** formed by this mechanism the ten-fold difference in product formation could be accounted for by ease of removal of each hydrogen and statistical likelihood of hydrogen removal from each centre (C4 has two hydrogens, C3 only one). Chair conformations shown in figure 5 are not true representations of the actual conformations taken by the heterocycle. The heterocycle assumes a half chair conformation with the heteroatom and C4 taking arrangements planar to the fused aromatic ring, while C2 and C4 assume half chair arrangements with the C2 aryl group pseudo equatorial and C3-OH

pseudo axial[22]. These structures have been shown as chair conformations for ease of representation..

The analogous C4-ethoxylation of catechin produced no C3-oxidised by-product. This may be due to the stability of C3-hydroxyl in catechin versus epicatechin. Catechin has an equatorial-like C3-OH, whereas the analogous group is axial like in epicatechin and hence less stable. However, computational modelling at semi-empirical level (AM1) of the C4 carbocation of catechin and epicatechin model systems failed to show any significant energy differences and little influence of the C3-substiuents in the molecular orbitals of the C4-carbocation. Compound **11** was not isolated by recrystallisation in subsequent reactions as **7** was isolated directly using silica gel column chromatography (**4.2.3**).

Natural proanthocyanidin dimers generally show 3,4-*trans* stereochemistry[23,24]. This stereochemistry is exclusively obtained in the condensation reaction of **6** and **7**. This reaction proceeds via an S_N1-type mechanism shown in figure 6. No S_N2-type mechanism could occur as no 3,4-*cis* product is obtained. Given the 4S stereochemistry of **7**, if an S_N2 mechanism led to dimerisation, inversion of stereochemistry at C4 would result in exclusive formation of the 4R, or 3,4-*cis* product. Since this product is not obtained, an S_N2-type mechanism could not occur.

Figure 6: Dimerisation mechanism showing possible stereochemistry of products.



Stereochemistry at C4 is dominated by the relative ease of attack of nucleophile **6** on the top or bottom face of the planar carbocation **12**. Attack from below to form the 3,4-*cis* product, **13**, is hindered by the presence of the C3-OH. Given the large bulk of nucleophile **6**, a large steric interaction between it and the 3-OH prevented attack from below and led to exclusive formation of the 3,4-*trans* product (**8**) at –78^{o}C. This stereochemistry is not surprising given that formation of **7** produced only the 4S-ethoxylated product via an S_N1-type mechanism. The 2-ethoxy-ethanol was a far smaller nucleophile than **6** and the reaction was conducted at room temperature compared to -78^{o}C, where the lower temperature should favour the 3,4-*trans* product to a greater extent given the steric interactions between the incoming nucleophile and the 3-OH.

Stereochemistry at C4 can be controlled to give either 3,4-*cis* or 3,4-*trans* using intramolecular condensation[25,26] over the intermolecular conditions used. These intramolecular conditions involved the use of diesters to link electrophile and nucleophile via their 3-OH. Varying the length of carbon chain in this diester gave varying proportions of *cis* and *trans* products. Generally, small chains gave *cis* and long chains gave *trans*. Intermediate chains gave varying proportions of the two isomers. These methods gave reasonable yields of catechin-catechin dimers. However, when applied to epicatechin-epicatechin dimerisation, the method failed to produce any 3,4-*cis* product[26] and 8% of 3,4-*trans* product[25]. While exclusive stereoselection can be obtained by these methods, the low yields of these methods render them unsatisfactory for B2 synthesis. Given that stereoselection for the 3,4-*trans* product is very high for the intermolecular conditions and yields for this reaction are also excellent (99%), this method proved the far more preferable method of dimerisation in the B2 synthesis.

2.1.2 Oligomerisation studies

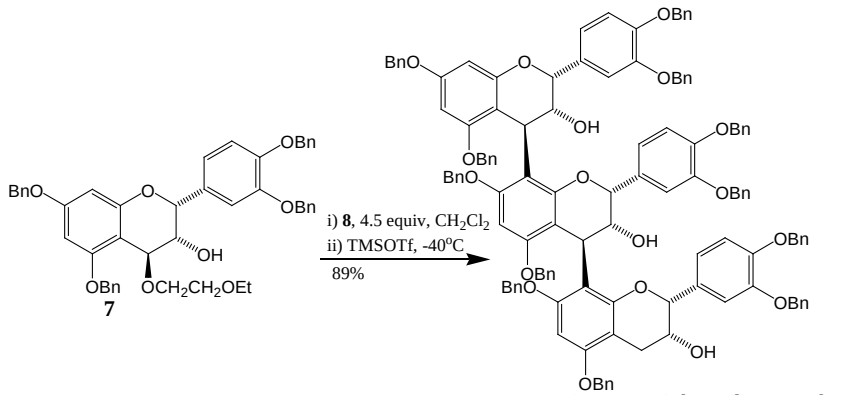
Selective B-type oligomerisation has been limited to dimers and trimers[7,9,15]. However, selective pathways to higher oligomers are desirable, as these higher oligomers have shown greater anticancer activity to smaller oligomers[8]. Three condensation reactions of varying ratios of nucleophile:electrophile (**6:7**) were conducted as per **4.2.**7. and analysed for oligomer composition using LC-MS. Products of condensations are shown in table 1.

Table 1: Product percentages from varying nucleophile:electrophile (**6:7**) ratios.

7:6 ratio	Monomer %	Dimer %	Higher oligomer%	Higher oligomer Identity	Unknowns/Artefacts (min)
1:4.5	69.2	24.4	-	-	14.87
1:1	66	<1%	33%	trimer	23.37
				MS not conclusive	
4.5:1	79.3	20.7	-	-	-

Table 1 shows that oligomers higher than trimers were not produced by any nucleophile:electrophile ratio. The 1:1 reaction showed a reasonable and selective yield for a peak that had a similar Rt to a trimer, but as this peak showed large overlap with the monomer peak it was not possible to gain resolved mass spectra of each peak. Therefore, this peak could not be concluded to be trimer. No method for selective higher oligomer synthesis was found by these results. Therefore, step-wise synthesis of proanthocyanidin oligomers remains the most selective method for obtaining unidisperse oligomers. Step-wise synthesis of oligomers (ie monomer to dimer, dimer to trimer) using electrophilic species **7** as a chain extension unit and a benzylated procyanidin as the nucleophilic chain-terminating unit has been used to stereoselectively produce dimers and trimers of both catechin and epicatechin monomers. The limits to this methodology are unknown. From figure 3, **8** was produced from monomer electrophile and nucleophile (**7,6**) in 99% yield at –78^{o}C. The analogous synthesis of the O-benzylated B-type trimer (**14**), shown in figure 7 below, produced **14** from electrophile **7** and nucleophile **8** in 89% yield at –40^{o}C.

Figure 7: Synthesis of Tri-5,7,3',4'-tetra-O-benzyl epicatechin (**14**).



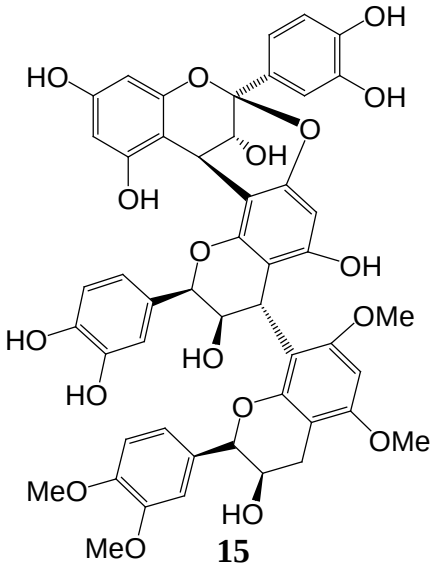
14- tri- 5,7,3',4' tetra-O-benzyl epicatechin

Saito *et al* conducted the trimerisation reaction at –78°C and reported **14** was not produced to any extent[16]. Therefore, temperature was increased to –40°C to give a good yield (89%) of **14**. Stereochemistry of the new interflavan bond was not examined, but it was reported increasing the reaction temperature may affect stereoselectivity and partial racemisation may occur. It is unknown if the trend of increased reaction temperatures may continue for production of tetramers and larger oligomers. If so, 3,4-trans stereochemistry obtained exclusively for dimers may be compromised and some racemisation at C4 may be seen in higher oligomers. If increased reaction temperatures are necessary for higher oligomers and partial racemisation occurs, it would suggest stepwise oligomer-building methods may not be suitable for larger oligomers.

2.1.3 B-type synthesis using varying protecting groups

The aimed synthesis of **5** required a varying approach to the all O-benzylated oligomers produced in **2.1.1** and **2.1.2** due to the regiochemistry challenges discussed in **1.7**. An alternative synthesis involving the use of O-methyl protected monomers in conjunction with O-benzyl protected monomers to produce the target model trimer **15** (figure 8) was proposed.

Figure 8: Proposed model trimer, **15**.

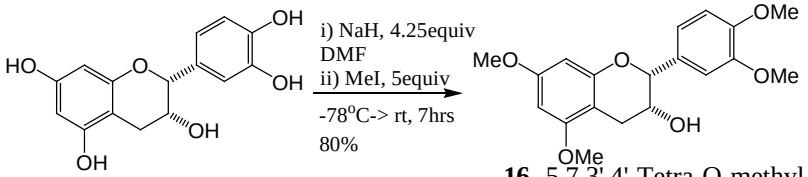


15

The proposed synthesis involved producing a B-type trimer with free phenolic groups for the top two monomer units and the bottom unit O-methyl protected.

Before commencing on a synthesis of **15**, an efficient method for gaining 5,7,3',4'-tetra-O-methyl-epicatechin was needed. Methylation of proanthocyanidins has been used extensively for derivatisation of isolated tannins from natural products[14,27] to assist in structural elucidation. These methylations mainly employed the use of diazomethane as the methylating agent[27]. Dangers of diazomethane are well known and an alternative synthesis was attempted using methyl iodide as the methylating agent. This methylation is shown in figure 9.

Figure 9: Methylation of epicatechin.



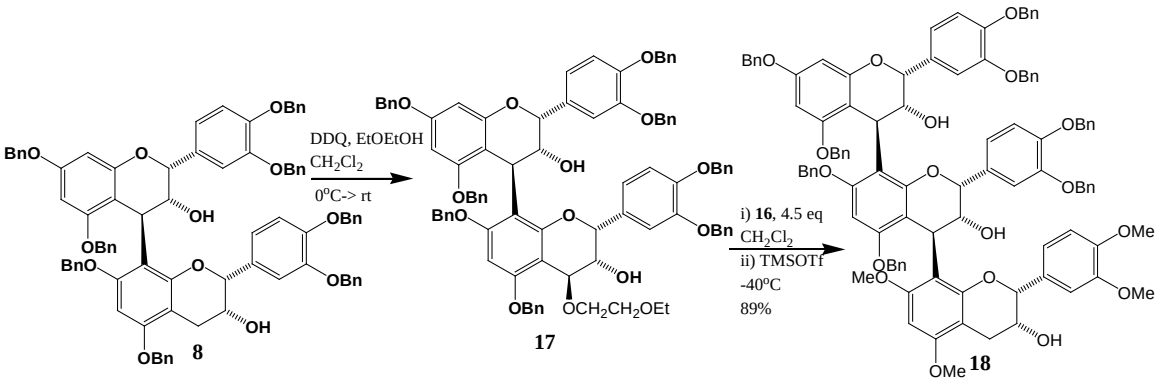
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16- 5,7,3',4'-Tetra-O-methyl epicatechin

The developed methylation procedure (**4.2.12**) used the same deprotonating and temperature conditions to the analogous O-benzylation method (**4.2.1**). This method produced **16** in good yield (80%). This compares well to yields obtained in proanthocyanidin methylations using diazomethane (generally 60-70% depending upon proanthocyanidin being methylated). This method was repeated at 0.5-1g (1.75-3.5mmol) scale of **1**. The all O-methyl product **16** was obtained in these subsequent reactions in gram-scale quantities at consistent 70-80% yields.

While the methyl-protecting group is easy to add to epicatechin in good yields, it is limited as a protecting group if targeting free phenolic proanthocyanidin oligomers as the harsh reaction conditions (strongly acidic) required to remove them can cause side-reactions such as polymerisation. Deprotection of the methyl groups was not attempted.
With **16** available, the first proposed synthesis of the B-type analogue (**18)** of **15** is described in figure 10.

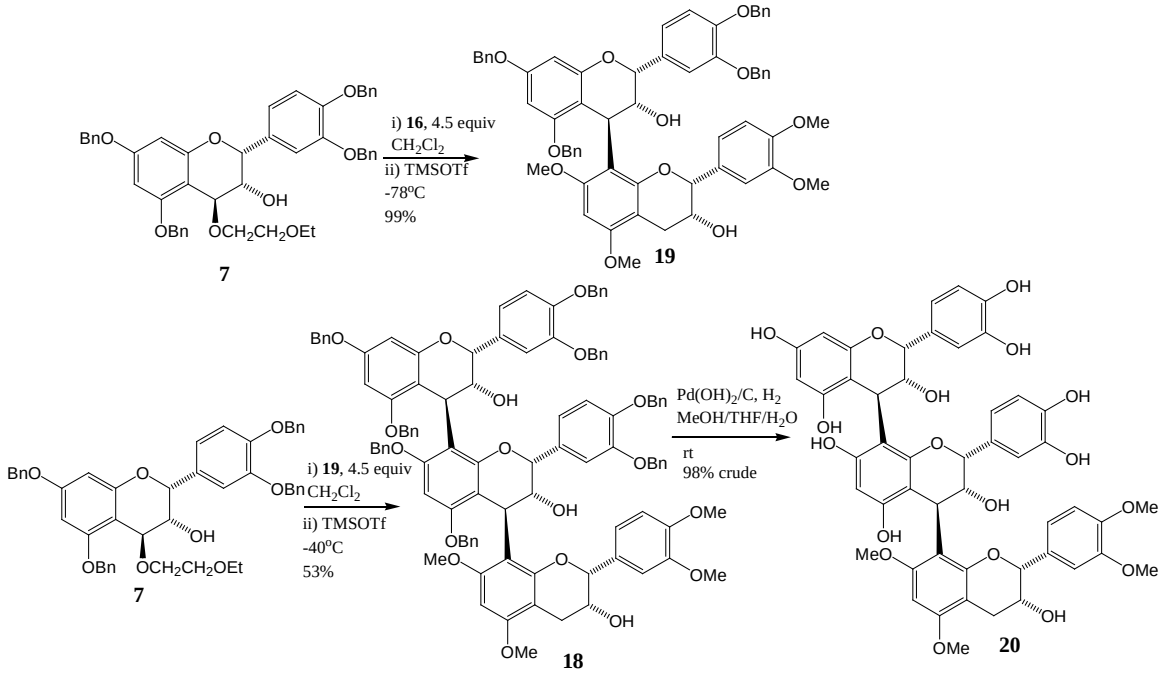
Figure 10: Proposed synthesis of **18**.



Attempts to synthesise **17** using the same conditions as **7** (**4.2.13**) yielded only starting materials. Rather than further explore this reaction, a synthesis was proposed using a step-wise building of oligomers constructing dimer **19** as an intermediate to the formation of trimer **18** (Figure 11 over page). This proposed synthesis proceeded smoothly through dimer **19** (99%) to trimer **18** (53%) and benzyl protecting groups removed by usual conditions to yield **20 (**98%) to give the B-type analogue of **15**. The syntheses of **19, 18, 20** show that B-type dimers and trimers can be constructed using varying protecting groups by straightforward methods developed for all benzyl-protected oligomers.

These oligomers with varying protecting groups could be used for a variety of purposes that all benzyl-protected dimers failed to fulfil. Ferreira *et al*[3] used **19** to prove the 3,4-*trans* stereochemistry of synthetic epicatechin-epicatechin dimer. The purpose for the construction of these oligomers was to control regiochemistry of A-type conversion. Using **20**, A-type regiochemistry must be confined to the top two monomer units to possibly give **15** as the methyl groups prevent A-type formation between the bottom two units. If the analogous all benzyl-protected trimer (**14**) was deprotected to the free phenolic trimer and A-type conversion attempted, regiochemistry could not be controlled. Conversion could occur between the top two units, the bottom two units or possibly both, giving a mixture of products.

Figure 11: Synthetic pathway to **20**.



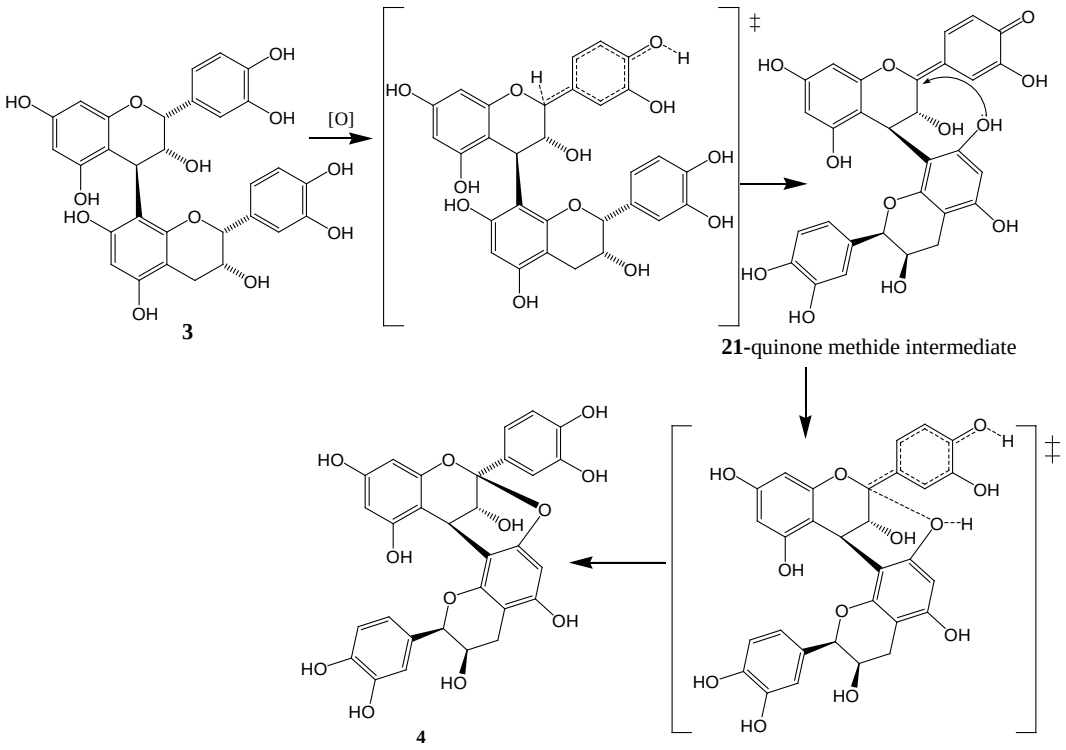
HPLC traces and mass spectra for **20** are shown in Appendix A-12 and Appendix B-4 respectively.

2.2 Conversion to A-type

2.2.1 Conversion Background

The B-type to A-type conversion has received little research focus, from either a mechanistic or synthetic point of view. The limited evidence available that A-type conversion occurs under oxidative conditions can be summed up by two key references showing B-type to A-type conversion using different oxidative reagents. Kondo *et al*[5] used DPPH radicals to convert B1 (epicatechin-catechin dimer) and B2 to A1 and A2 respectively. Nonaka *et al*[27] used H_2O_2/$NaHCO_3$ to convert B1 to A1 and then isolated the product as a heptamethyl ether. The Kondo reference[5] also studied the mechanism of B-type to A-type conversion using experimental evidence and molecular mechanics calculations. It was proposed that C2 and 4'-OH hydrogens are abstracted as radicals from the upper unit of B2 (**3)** to give the quinone methide intermediate (**21**). This transformation caused the C2 position to become electrophilic. If the lower epicatechin unit is in the correct conformational position, the 7-OH could then act as a nucleophile at C2 to give A2 (**4**). This conversion is shown below in figure 12.

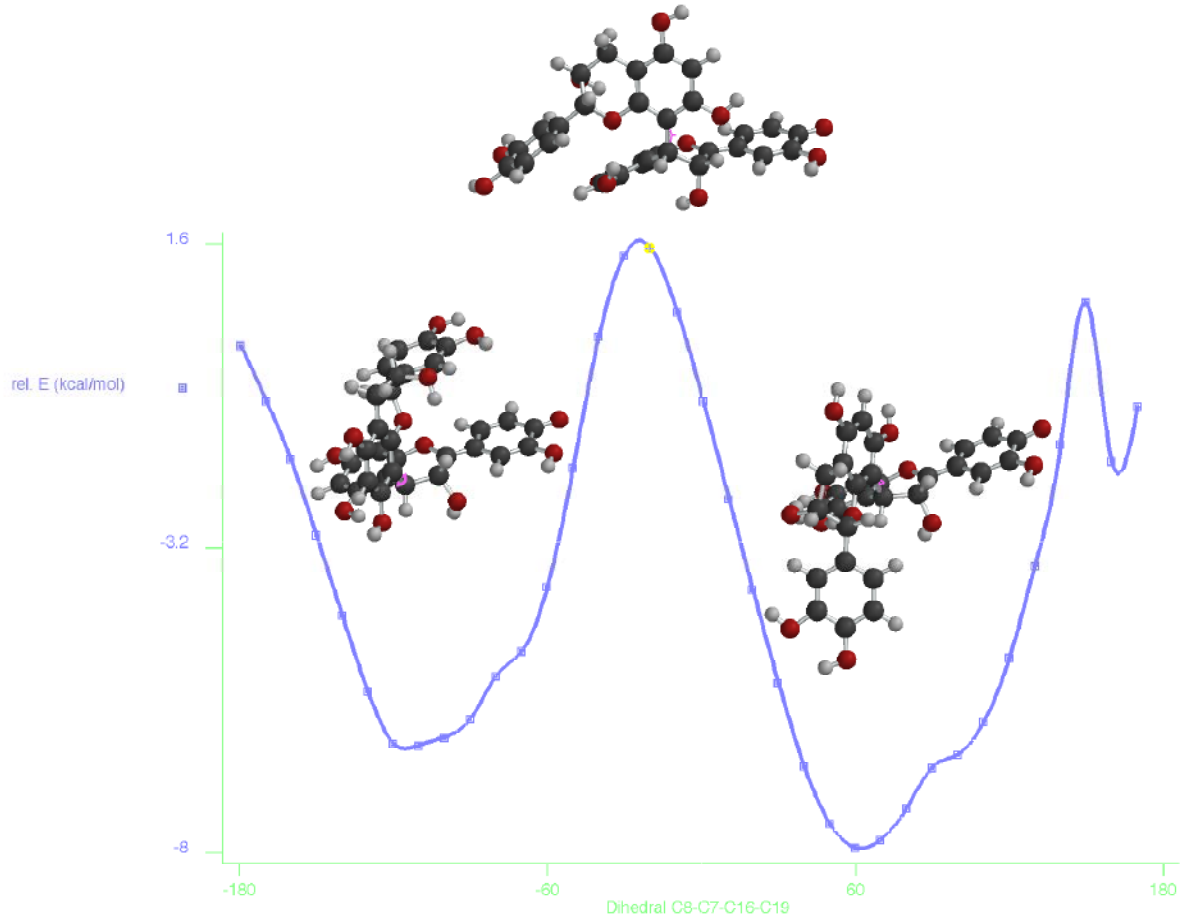
Figure 12: Proposed intermediate and possible mechanism for B2-A2 conversion.



The mechanism in figure 12 stands purely as a proposal. The transition states shown are pseudo transition states at best. Kondo *et al*[5] proposed that the 4'-OH hydrogen is abstracted first followed by the C2-hydrogen. These abstractions would certainly occur as a stepwise process and not as a concerted step as shown in the first pseudo transition state in figure 12. This would also be true for the proton loss and gain by 7-OH and 4'-O shown in the second pseudo transition state.

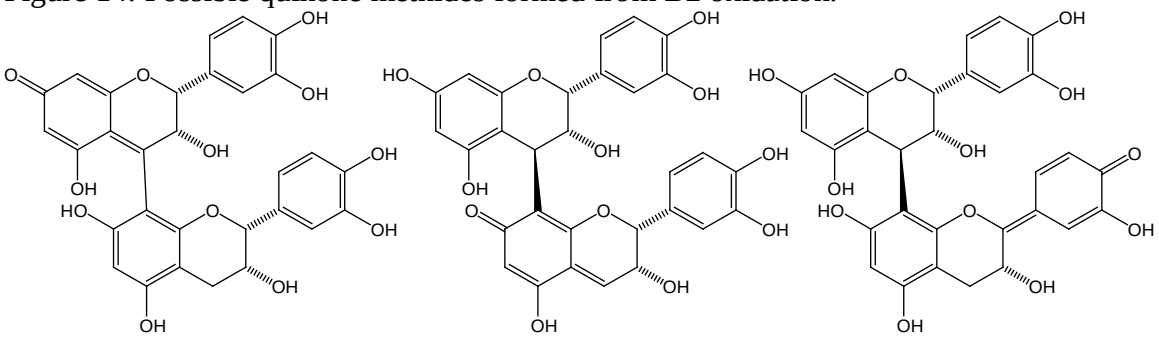
The attack of 7-OH on C2 would result in cyclisation of an acyclic precursor to a 6-membered heterocycle. Cyclisation of **21** to **4** was studied using molecular modelling. The key to A-type formation was the C4-C8 interflavan assuming the correct conformational position. The quinone methide must assume a conformation where the 7-OH of the lower unit is sufficiently close to C2 of the upper unit to allow cyclisation. Rotation about the interflavan bond for the quinone methide intermediate was modelled at semi-empirical (AM1) theory level. This rotation showed the low energy conformation of the intermediate to be the position taken when the 7-OH is close to C2 (2.7Å). Another low energy conformer about 2kcal/mol higher in energy to the lowest energy conformer was found when the heterocyclic oxygen of the lower flavan unit was close to C2 (3.2Å). The low energy conformation was about 10kcal/mol lower in energy to the maximum energy conformer taken when neither the 7-OH or heterocyclic oxygen was in close proximity to C2. These calculations show that there is a small driving force (~10kcal/mol) towards formation of the A-type linkage as the low energy conformation has the 7-OH in the correct position to attack the electrophilic C2. Figure 13 over page shows a plot of this rotation about the interflavan bond with energy plotted against angle of rotation with low and high energy conformers shown.

Figure 13: Plot of energy against angle for rotation about interflavan bond.



The C2-hydrogen is benzylic and α to an oxygen atom. Therefore, it is susceptible to homolytic abstraction by a radical reagent such as DPPH. Given the proposed mechanism for quinone methide formation, it is possible for **3** to form four different quinone methides from abstraction of benzylic hydrogens by radicals. The three other quinone methides are shown in figure 14 below.

Figure 14: Possible quinone methides formed from B2 oxidation.



The likelihood of each of these four intermediates forming would be dependent upon the ease that the radical is capable of accessing the hydrogen to be abstracted. Formation **22** and **24** requires removal of hydrogens sterically hindered by the other flavan unit and it would be expected **21** and **23** would form more readily. Formation of **23** requires the removal of the least sterically hindered hydrogen atom and perhaps the most likely to form using bulky radicals. Formation of this quinone methide results in C4 of the bottom flavan unit becoming electrophilic. Therefore, polymerisation can occur via this intermediate if C6 or C8 of a nearby oligomer acts a nucleophile in a similar manner to oligomerisation discussed in **2.1.1** and **2.1.2**. This mechanism may explain the polymerisation of samples that were seen for some oxidants in **2.2.2**.

2.2.2 A-type conversion

Given that evidence exists for the B-type to A-type conversion occurring via a quinone methide intermediate[5], several radical and transition metal reagents that could potentially oxidise B2 to the quinone methide **21** (figure 12) were trialed at different molar ratios as per **4.2.8**-**4.2.10**. Table 2 shows for each oxidant trialed: the major products of oxidation, any identification if possible and major Mass Spectral fragments.

Table 2: Oxidants and products of oxidants for B2-A2 conversion.

Oxidant	**Equiv.**	**Peak (min)**	**ID**	**% Area**	**Mass Spectra**
DPPH	100	6.47	Unknown	38.4	573.4
		9.3	unknown	17.5	573.2
		13.53	B2	38.3	289, 407, 425, 577
		23.17	A2	5.9	285, 423, 575
Ag_2O	2	6.5	unknown	38.1	573
		9.4	unknown	16.7	573.0, 232.4
		11.13	unknown	7.4	unidentifiable
		13.6	B2	16.4	289, 407, 425, 577
		19.63	unknown	8.7	575.4
		23.13	A2	12.7	285, 423, 575
H_2O_2/$NaHCO_3$	0.1	2.43	unknown	16.8	unidentifiable
		9	unknown	20	unidentifiable
		11.1	unknown	12	unidentifiable
		13.93	unknown	3.2	unidentifiable
		16.57	unknown	4.6	unidentifiable
		19.7	unknown	7.6	unidentifiable
		24.77	unknown	21.9	575
PIFA	11	2.57	unknown	78.6	508.2, 1155.8
		7.0-13.0	unknown	<10%	-
		25.37	unknown	<10%	738.2
		31.9	unknown	21.4	540.2, 861.6, 1202.0
ABTS	2	13.67	B2	12.3	289, 407, 425, 577
		21.13-25	ABTS?	86.3	512.4
Air	-	11.3	unknown	5.5	573.4
		13.9	B2	82.2	289, 407, 425, 577
		22.5	A2	6.1	285, 423, 575

From table 2, oxidants H_2O_2/$NaHCO_3$, PIFA and ABTS all gave complex mixtures of products with no product identifiable as A2. Possible reagents for B2-A2 conversion were DPPH, Ag_2O and air. These three reagents were trialed again on a larger scale and sampled at more frequent time intervals (**4.2.11**). Results of these oxidations for DPPH (7.5% A2 conversion) and air (6.2%) mirrored those of table y. However, silver oxide catalysed the polymerisation reaction and insoluble polymer resulted from this reaction

within 15 minutes. Given this result, silver oxide was ruled out as a possible A-type conversion reagent. HPLC traces of selected oxidations are shown in Appendix A.

After a failed B2 deprotection reaction, $Pd(OH)_2/C$ was considered as a possible conversion reagent. The reaction in question failed to go to completion. ^1H NMR of the product showed benzyl groups (δ7-7.5, m) still present. Therefore, the sample was placed under debenzylating conditions again and isolated. LC-MS studies of this sample showed a B2 peak (13.37min, 44.0%) and also A2 (23.03min, 19.7%) and an unknown oxidation product (10.87min, 14.8%). The HPLC trace for this failed deprotection is shown in Appendix A-1. It was inferred that the sample must have been oxidised during isolation of the first deprotection attempt and preparation of the second. Firstly, air was postulated to be the oxidising agent. However, air was shown (**4.2.11**) to convert B2 to A2 at 6% at best over one week. Therefore, the deprotection reagent, $Pd(OH)_2/C$, was considered for its possible involvement and trialed for the A-type conversion. This oxidation was carried out as per **4.2.17**. Results for this oxidation were very promising. At 24hrs, B2-A2 conversion had reached 16.7%. Beyond 24hrs, A-type conversion slowed or stopped completely and polymerisation had become evident from the hump forming at the baseline of the LC trace, which was characteristic of poly-disperse polymerisation using the described LC method (**4.1**). No reagent before $Pd(OH)_2/C$ had given more than 8% A-type conversion. Given these promising results, $Pd(OH)_2/C$ was trialed again three times at varying mass/mass ratios. However, for all three samples, at 24-48hr LC-MS analysis failed to show greater than 7% conversion to A2, generally less than 5%. While yields were significantly lower, it was postulated that $Pd(OH)_2/C$ had some involvement in the conversion process. For the second and subsequent trials yields were comparable those produced by air oxidation, but produced at an accelerated rate. For a comparable yield of A2 from a 24hr $Pd(OH)_2/C$ oxidation, air oxidation required 4 or more days.

Aside from the high yield, $Pd(OH)_2/C$ had the added advantage of being a “clean” oxidiser. Only one other unknown compound was produced at Rt=10-11min. No other reagent besides air oxidation gave so few by-products, which also produced this unknown at Rt=10-11min. DPPH showed two other by-products and other reagents produced large

numbers of by-products. Obviously, smaller number of by-products should make potential separations of products easier should it be attempted. The product at Rt=10-11min was interesting. It was produced by $Pd(OH)_2/C$, air and DPPH oxidations. The mass spectrum of this compound showed only a peak at 573, which was four mass units smaller than the B2 ($(M-H)^-$, 577) peak. It also absorbed UV at 280nm and 340nm, suggesting some quinone character. Polyphenolics such as A2 and B2 only absorb at 280nm. Presence of quinone oxidised phenolics increase conjugation and thus absorb at the longer wavelength of 340nm[29]. Perhaps the most interesting observation taken from this peak is that it has a lower Rt than B2. This means that the unknown must interact less with the C18 column used in LC, which suggests it would be more polar than B2 (hence less interaction with the non-polar column). However, the presence of a quinone suggests oxidation of a phenolic group to a less polar quinone. The origin and structure of this by-product remain a mystery.

There was also the phenomenon of polymerisation seen after about 24hrs. This was somewhat expected. Silver oxide, another transition metal Lewis acid, was shown to produce either A-type or polymerised products depending upon conditions. However, silver oxide favoured the polymerisation reaction quickly. With $Pd(OH)_2/C$, it appears that A-type conversion is favoured for short time periods before reaching a maximum and polymerisation became the favoured mode of reaction after this time period.

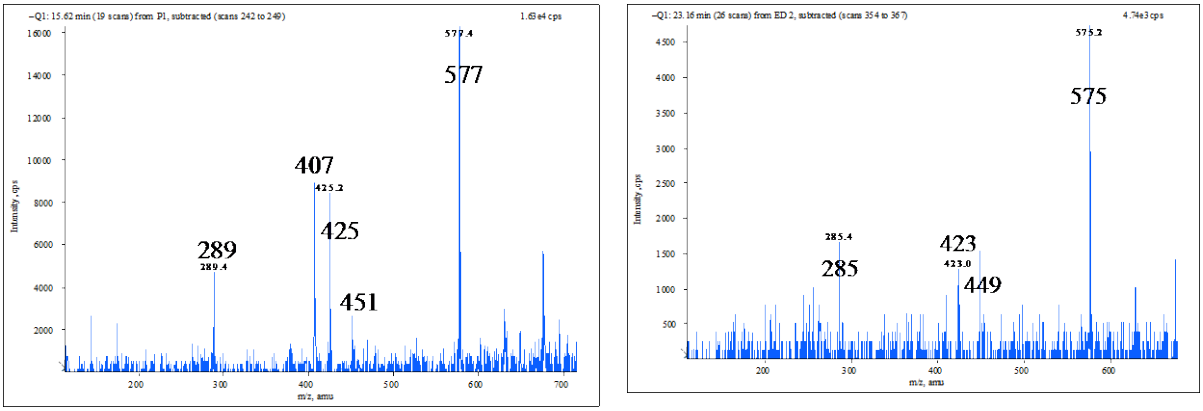
The mechanism of the $Pd(OH)_2/C$ oxidation was not studied, but given that the reagent is a strong absorber of hydrogen (its role in the debenzylation reaction), perhaps the 2-H and 4'-OH were able to be removed to form the quinone methide discussed in **2.2.1** in a mechanism similar to that of absorbing molecular hydrogen. Also, some involvement of an intermediate Palladium species (such as PdO) cannot be ruled out. A conversion of $Pd(OH)_2/C$ to another intermediate species under conditions present in the first trial that weren't reciprocated in the subsequent reactions (possibly increased UV intensity or increased heat) may have had some involvement in the conversion. Use of a poisoned catalyst may also have had some influence on the high conversion yield. The starting B2 material used in the initial oxidation that showed high conversion was from a different

batch to that used in subsequent oxidations that failed to gain large conversions. It is possible that an artefact of the debenzylation of the B2 batch used in the initial oxidation that was not present in the B2 batch used for subsequent oxidations may have poisoned the $Pd(OH)_2/C$ catalyst in some way and it was this poisoned catalyst (possibly a converted Palladium species) that was responsible for the conversion of B2 to A2.

2.2.3 A2 Confirmation

The peak corresponding to A2 was confirmed as 22-24min on the LC trace. This was done using a combination of MS and MSMS of the 22-24min peak. The B2 peak was located at 13-15min from existing reference compounds. The mass spectra of A2 and B2 show similar fragmentations varied by two mass units (A2 is two units smaller than B2). These two mass spectra are shown in figure 15 below.

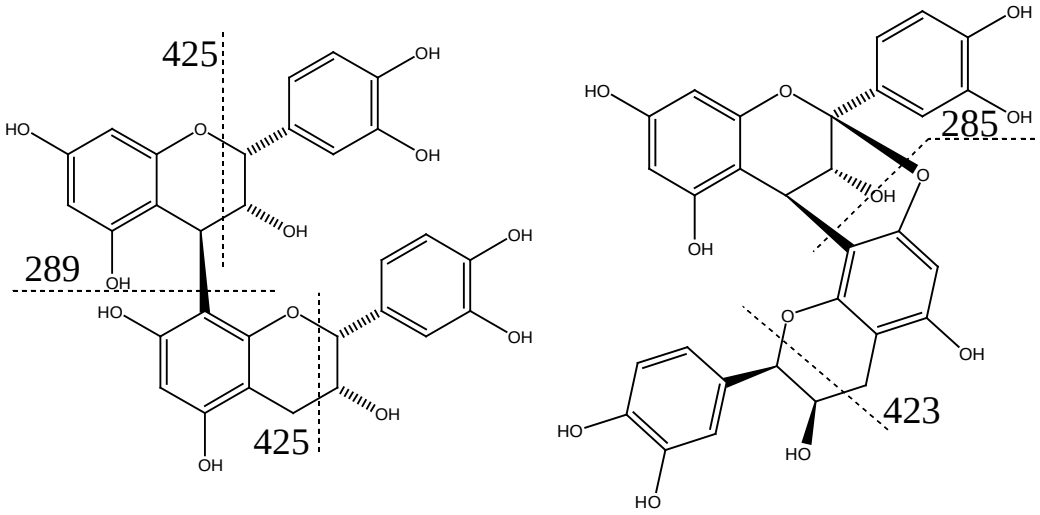
Figure 15: B2 (left) and A2 (right) mass spectra showing main fragments.



The mass spectra of the 22-24min peak matched that of A2. The major question to be answered was the peak indeed A2 or some oxidised B-type molecule, like the quinone methide intermediate **21**, with two mass units less than B2? The two spectra clearly show the similar pattern with variation of two mass units. The dimer-H peaks (577 and 575 respectively) are the most abundant in both spectra and "monomer" peaks (289 for B2, 285 for A2) show similar intensities. The peak at 423 is the key fragment for A2 confirmation. The intensity is greatly reduced in A2. Also no significant 405 peak is seen for A2. These observations result from the second inter-flavan ether linkage joining C2 of the upper unit to C7 of the lower unit. For B-type dimers, fragmentation to the monomer

occurs by direct fragmentation of the C4-C8 interflavan bond. For A-types, fragmentation to the monomer occurs by two paths due to the presence of the second interflavan bond. It can occur directly from the dimer in a retro-Diels-Alder fission or via the 423 intermediate formed from the retro-Diels-Alder fission of the lower heterocycle[30] shown in figure 16.

Figure 16: Key B2 and A2 fragments.



Using an MSMS parent ion scan of the 285 fragment of the 22-24min peak, final A2 confirmation was made. This fragment resulted directly from the 575 fragment and also from the intermediate 423 fragment as described by Karchesy *et al*[30]. The parent ion scan for 285 of the 22-24min peak is shown in Appendix B-3.

2.3 Regioselective A-type Trimer Synthesis

A synthesis of **15** was attempted by stirring the variably protected B-type trimer (**20**) with $Pd(OH)_2/C$ as described in **4.2.19**. LC-MS analysis at described intervals showed no A-type trimer was produced. The LC trace showed starting materials, the trimer **20** at 40min and an unknown artefact from trimer synthesis at 51-52min and also an unknown product peak at 26.8-27.0min that increased in area over time of the oxidation.

This unknown was interesting in that its mass spectra showed one peak that was four mass units less than the $(M-H)^-$ peak of the B-type trimer **20** (917 compared to 921) and absorbed UV at 280 and 340nm. The 340nm absorption suggested quinone character of the unknown. Both this unknown and the unknown at Rt=10-11min for the analogous oxidation of B2 (**2.2.2**) showed similar properties; four mass units smaller than the B-type starting material, absorbed at 340nm, eluted from the LC column earlier than the B-type starting material. It could be considered these unknowns have undergone the same change during $Pd(OH)_2/C$ oxidation and vary only by the presence of the methyl-protected epicatechin unit on the trimer.

Again the $Pd(OH)_2/C$ oxidation failed to give A-type conversion at reasonable yields as seen in the first oxidation using this oxidant (**4.2.17**). The failure to gain any A-type trimer where small amounts of A-type dimer were found may be due to the trimer being larger and thus more difficult to oxidise.

The trimer oxidation showed no polymerisation that occurred after around 24hrs with the analogous B2 oxidations. The B2 polymerisation was postulated to have formed via quinone methide **23** (**2.2.1**). With **20**, formation of the quinone methide at C4 of the terminal unit is precluded due to the presence of the methyl-protecting groups. Also, presence of the terminal unit blocks formation of the quinone methide at C4 of the central unit from attack by oxidising agents. Therefore, polymerisation was effectively precluded by the presence of the methyl-protected terminal unit.

3.1 B-type synthesis

B2 was successfully synthesised using a combination of referenced steps and modifications to give optimal yields. The overall yield from monomer to dimer was calculated at 54% over the four-step synthesis. This yield compared well to those obtained by the Tuckmantel *et al*[7] and Saito *et al*[9] syntheses (these were as low a 15-20%). The most significant modification made on literature precedents was the work-up and purification of **7**. These modifications discussed in **2.1.1** increased isolation yields from 64% as published[18] to 76%.

Trimers containing all benzyl-protected monomers (**14**) and varying benzyl-protected and methyl-protected monomers (**18**) were produced using steps set out in figures 7 and 11. Trimer **14** was produced as a reference compound and trimer **20** as a precursor to **15**, which was used as an A-type conversion model.

3.2 A-type conversion

A2 was successfully converted from B2 using:

1) DPPH radicals (100 equivalents) in ethanol/water solution (8%) over 4hrs.
2) Air in ethanol/water solution (6%) over one week.
3) $Pd(OH)_2/C$ (1.5-2 equivalents by mass) in ethanol/water solution (17%) over 24hrs.

The $Pd(OH)_2/C$ method for B2 to A2 conversion showed greatly improved yields from existing methods. However, conditions that produced that relatively large conversion (16%) were not reproducible (<7% on subsequent trials). DPPH and air oxidations produced low yields of A2 (~8% and ~6% respectively) to be considered as viable synthetic pathways in A-type oligomer production. $Pd(OH)_2/C$ is believed to play some role in the A-type role as accelerated rates compared to air oxidation are obtained. A poisoned catalyst or an intermediate Pd species being involved in the conversion were also considered as possibilities for the increased yields.

Given that air oxidation showed a slow conversion of B2 to A2, this may be the pathway of the conversion as it happens in nature.

3.3 A-type trimer

No A-type trimer **15** was isolated from oxidation of **20** using $Pd(OH)_2/C$ as the oxidising agent. Methods of conversion that were successful for the B2-A2 conversion may need to be modified for trimers given the extra monomer unit.

3.4 Future studies

3.4.1 B-type synthesis

Current selective B-type synthetic pathways are limited to step-wise building of dimers and trimers. There is an interest in selectively producing pure, larger oligomers such as tetramers and higher oligomers. Larger oligomers have been shown to have greater cell cycle arresting properties in breast cancers compared to lower oligomers[8]. True wine tannins as they are known are generally considered as tetramers and larger. Therefore, from a wine research point of view, these larger oligomers are of interest as model compounds and analysis standards. Currently, larger oligomers of up to 35 monomer units can be synthesised as a polydisperse mixture. Kozikowski *et al*[8] produced oligomers from 3 to 8 units as a mixture and Yoneda *et al*[28] methods produced polymers of 8 to 35 units with varying polydispersity. Neither method allows for selective synthesis particular oligomers. To gain pure, unidisperse oligomers requires tedious separations using silica gel for phenol-protected oligomers or Sephadex LH-20 or C18 for free phenolic oligomers. Preparative HPLC can be used, but is time consuming and yields are limited. For these reasons, developing methods for consistent, unidisperse, pure synthesis of higher oligomers become necessary.

3.4.2 A-type conversion

$Pd(OH)_2/C$ has potential as a viable conversion reagent. However, for this to become possible, further studies must be conducted to find optimal conditions for this conversion as subsequent studies after the initial success failed to gain high yields.

3.4.3 A-type oligomers

The possibility of regioselective addition of the A-type linkage in trimers using differential protecting groups has been shown to be plausible from the building of B-type precursors. Studies in the synthesis of B-type precursors to the A-type oligomers could be extended in two ways:

1) Using protecting groups other than methyl groups. Methyl protecting groups were an easy staring point, but for long-term syntheses of higher A-type oligomers, other protecting groups that can be removed under mild conditions should be considered so that the free-phenolic oligomers can be obtained at the end of syntheses.
2) Developing methods for larger B-type oligomers sets the scene for the production of large oligomers with varying protecting groups so that large oligomers with selectively positioned A-type linkages become possible. Building the B-type precursors require stepwise syntheses to allow each monomer to be added individually according to protecting group. Therefore the posed problems discussed in **2.1.2** become an issue.

If methods to build these B-type precursors can be found and the A-type conversion process optimised for trimers and larger oligomers, synthesis of higher A-type oligomers with selectively positioned A-type linkages could be constructed.

4.1 General Procedures

All chemicals were purchased from Sigma-Aldrich. ^{1}H NMR spectra were recorded at either 200MHz or 300MHz on a Varian Mercury or Varian Gemini spectrometer, while ^{13}C NMR spectra were recorded at either 50.3MHz or 75.5MHz on a Varian Mercury or Varian Gemini spectrometer. All spectra were recorded in either $CDCl_3$ as solvent and referenced to $CHCl_3$ (δ 7.26) for ^{1}H NMR and $CDCl_3$ (δ 77.0) for ^{13}C NMR or CD_3OD as solvent and referenced to CD_2HOD (δ 3.31) for ^{1}H NMR and CD_3OD (δ 39.0) for ^{13}C NMR. Signals of the proton-bearing aromatic carbons of the benzyl groups (region: δ128.6-127.0) have been omitted. All non-oxidative reactions were conducted under a nitrogen atmosphere using anhydrous solvents that were purified and dried using according to recommended methods, except for anhydrous DMF, which was purchased from Sigma-Aldrich. All oxidative reactions were conducted open to atmospheric conditions using solvents purified in the recommended procedures unless otherwise stated. All LC-MS data was obtained using a Synergi 4μHydro-RP 80A (2.1mm x 150mm: Phenomenex) LC column coupled to an API-300 triple quadruple mass spectrometer equipped with an electrospray (ES) ion source (MDS-Sciex, Concord, ON, Canada). Analysis was carried out by taking 20μL aliquot injected by an autosampler (HP1100, Agilent) into the above column. The method used a binary gradient with mobile phases containing formic acid:water:acetonitrile: (A) 5:95:0 and (B) 5:15:85 (v/v/v). The elution conditions were as follows: 180μL/min, linear gradient from 10%B to 35%B in 35min, from 35%B to 60%B in 25min and 60%B for 1min. The eluent from the LC was split using a T-piece and delivered at 22.5% of the total flow to the mass spectrometer (MS) and at 77.5% to a UV detector (HP1100, Aligent) monitoring wavelengths at 280 and 340nm. Negative ion mass spectra from m/z 150 to 2500 were recorded with a step size of 0.2u and dwell time of 0.25msec. The ES needle, orifice and ring potentials were set at –4500V, -30V, and –250V respectively. The curtain (nitrogen) and nebuliser (air) gases were set at 8 and 12 units respectively.

Molecular modelling calculations were conducted using Mac Spartan version 1.04e at semi-empirical theory level (AM1 basis set).

4.2 Experimental Procedures

4.2.1 5,7,3',4'-Tetra-O-benzylepicatechin (6)

To a stirred suspension of NaH (0.59g, 60% suspension in oil, 14.71mmol, 4.26 equiv) in anhydrous DMF (10ml) under nitrogen at –78^{o}C, a solution of **1** (1.00g, 3.46mmol) in anhydrous DMF (10ml) followed by neat BnCl (2.0ml, 17.38mmol, 5.04 equiv) were added. The mixture was stirred at –78^{o}C for 15 minutes and then the cold bath was removed and stirring was continued for 7hrs at rt. The reaction was quenched by adding HCl (1M, 6ml) and water (30ml). The aqueous phase was extracted with EtOAc (2x50ml) and the organic layer washed with water (2x20ml). The combined organic layers were dried (Na_2SO_4), filtered and concentrated to give a soft, brown solid. The solid was purified by SiO_2 column chromatography (Hexane/EtOAc 2:1) to yield 1.90g (85%) of a white crystalline solid, mpt 125-127^{0}C.

NMR: ^{1}H(200MHz, $CDCl_3$): 7.52-7.27(m,20H), 7.22(s,1H), 7.02-7.01(narrow ABq,2H), 6.31(s,2H), 5.22(s,2H), 5.20(s,2H), 5.04(narrow ABq,4H), 4.93(s,1H), 4.23(br s,1H), 3.10-2.90(ABq,2H,J=40Hz). ^{13}C(50MHz): 158.69, 158.24, 155.19, 148.94, 148.76, 137.17, 137.08, 136.93, 136.87, 131.40, 119.42, 114.95, 113.41, 100.91, 94.60, 93.97, 78.28, 71.28, 71.20, 70.04, 69.85, 66.24, 28.12.

4.2.2 (2R,3R,4S)-5,7,3',4'-tetrabenzyloxy-4-(2''-ethoxy-ethoxy)flavan-3-ol (7)

To 0.50g (0.77mmol) **6**, 2-ethoxyethanol (1.5ml) was added and dissolved in CH_2Cl_2 (20ml). The solution was stirred at 0^{o}C and DDQ (0.36g, 1.57mmol) in CH_2Cl_2 (25ml) was added dropwise, during which the solution turned from colourless to blue. The solution was stirred at rt for a further 2hrs and then cooled to 0^{o}C again. DMAP (360mg) in 25ml CH_2Cl_2 was added dropwise. A turbid purple solution resulted. The precipitate was filtered out and the purple filtrate was washed with H_20 (10ml) and brine (10ml). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2x10ml) and the combined organic phases were dried (Na_2SO_4), filtered and concentrated. The residue was filtered over a short SiO_2 column ($CHCl_3$: EtOAc 9:1) and the crude product was dissolved in EtOAc/Hexane (1:1) and crystallised in freezer. White crystals (30mg, 7%) precipitated, which were determined by NMR to be **11**. The remaining solution was

evaporated to give an orange oil. Further purification by SiO_2 (Hexane: $CHCl_3$:EtOAc 2:1:1) produced 0.35g (55%) of an orange oil.

NMR: ^{1}H(300MHz, $CDCl_3$): 7.47-7.27(m,20H), 7.16(s,1H), 7.08-7.02(ABq, 2H, J=18Hz), 6.16(s,2H), 5.24(s,1H), 5.21-5.19(ABq,4H,J=6Hz), 5.10(narrow ABq,4H), 4.65(d,1H,J=2Hz), 4.10(m,1H), 3.85(d,2H,J=1.5Hz), 3.63(d,2H,J=1.5Hz), 3.49(q,2H,J=3Hz), 2.21(d,1H), 1.24(t,3H,J=3Hz).

^{13}C(75MHz): 160.20, 159.48, 155.90, 148.68, 148.43, 136.99, 136.93, 136.51, 136.48, 131.10, 119.53, 114.66, 113.60, 101.82, 94.15, 93.70, 74.58, 71.01, 70.90, 70.13, 69.87, 69.53, 68.54, 67.92, 66.07, 14.94.

4.2.3 (2R,3R,4S)-5,7,3',4'-tetrabenzyloxy-4-(2''-ethoxy-ethoxy)flavan-3-ol (7) (alternative workup).

To 0.99g (1.52mmol) **6**, 2-ethoxyethanol (3.0ml) was added and dissolved in CH_2Cl_2 (30ml). The solution was stirred at 0^{o}C and DDQ (0.70g, 3.09mmol) in CH_2Cl_2 (30ml) was added dropwise, during which the solution turned from colourless to blue. The solution was stirred at rt for a further 2hrs. Sat. $NaHCO_3$ (50ml) was added to remove DDQ products and the phases were separated. The organic phase was washed with H_20 (25ml) and brine (25ml). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2x20ml) and the combined organic phases were dried (Na_2SO_4), filtered and concentrated to give purple oil. The residue was purified by SiO_2 column chromatography (Hexane: $CHCl_3$:EtOAc 2:1:1) to yield an orange oil (0.85g, 76%) of sufficient purity to use in dimerisation. NMR: Refer **4.2.2**.

4.2.4 5,7,3',4'-Tetra-O-benzylepicatechin-4β-8-5,7,3',4'-Tetra-O-benzylepicatechin(8)

To a stirring solution of **7** (0.21g, 0.28mmol) and **6** (0.83g, 1.28mmol, 4.5 equiv) in CH_2Cl_2 (20ml), TMSOTf (52.5μL) in CH_2Cl_2 (0.5mL) was added dropwise at –78^{o}C. After 10 min the reaction was quenched with sat. $NaHCO_3$ and the solution extracted with $CHCl_3$ and the phases separated. The organic phase was washed with H_2O (5ml) and brine (5ml) and the combined aqueous phase was extracted with $CHCl_3$ (2x5ml). The combined organics were dried (Na_2SO_4), filtered and concentrated. The red oil residue

was chromatographed over SiO_2 ($CHCl_3$:Hexane:EtOAc 13:13:1 for starting material, $CHCl_3$:Hexane:EtOAc 13:13:2 for product) to produce 0.36g (99%) of white/cream amorphous powder.

HRMS(ESI-TOF): Calc: 1299.5253, found: 1299.5232.

NMR: Complex mixture of rotational isomers, ^{1}H(300MHz,$CDCl_3$): 7.63-6.95(complex m,40H), 6.59-5.65(complex mixture of d,dd,ABq,8H), 5.15-4.95(complex m,16H), 4.80-3.96(complex mixture s,d,dd,6H), 3.09-2.97(m,2H), 1.43(s,1H), 1.23(s,1H). ^{13}C(75MHz): 158.20, 158.08, 157.94, 157.85, 157.78, 156.95, 156.80, 156.69, 156.56, 156.34, 155.74, 155.45, 155.14, 154.99, 154.32, 152.72, 149.03-148.04(8sig), 137.26-136.83(8sig), 132.54, 132.33, 131.07, 119.84, 119.71, 119.39, 118.71, 118.58, 114.91-111.07(9sig), 104.39, 102.22, 101.68, 100.90, 94.29-91.46(6sig), 78.72, 78.18, 77.94, 75.55, 72.30, 72.02, 71.32-68.93(9sig), 66.32, 64.99, 35.79, 35.68, 28.53, 28.25.

4.2.5 Epicatechin-4β-8-epicatechin (3)

A stirred solution of **8** (0.15g, 0.119mmol) in THF/MeOH/H_2O (12/12/1) (25ml) was hydrogenated over $Pd(OH)_2$/C (150mg) for 16hrs under a current of H_2 at rt. Filtration and concentration yielded black powder (0.07g,95%). NMR: ^{1}H(200MHz, CD_3OD): 7.15-6.60(m,6H), 6.11-5.91(m,3H), 2 signals under solvent OD peak(2H), 4.44(m,1H), 4.28-4.16(m,1H), 3.91(br s,1H), 2.99-2.86(m,2H). ^{13}C(50MHz): 158.08, 157.65, 156.22, 154.43, 145.67, 145.41, 132.49, 132.44, 131.99, 119.10, 115.77, 115.00, 107.18, 101.25, 100.32, 97.4-95.5(br), 79.55, 77.01, 73.37, 66.97, 37.01, 29.54.

4.2.6 5,7,3',4'-Tetra-O-benzylepicatechin-4β-8-5,7,3',4'-Tetra-O-benzylepicatechin-4β-8-5,7,3',4'-Tetra-O-benzylepicatechin (14)

To a stirring solution of **7** (19.5mg, 26.3μmol) and **8** (0.16g, 122.3μmol, 4.6 equiv) in CH_2Cl_2 (7ml), TMSOTf (4.8μL) in CH_2Cl_2 (1ml) was added dropwise at –78^{o}C. After 15 min the reaction was quenched with sat. $NaHCO_3$ and the solution extracted with $CHCl_3$ and the phases separated. The organic phase was washed with H_2O (5ml) and brine (5ml). The combined organics were dried (Na_2SO_4), filtered and concentrated. The red oil residue was chromatographed over SiO_2 ($CHCl_3$:Hexane:EtOAc 13:13:1 for starting

material, $CHCl_3$:Hexane:EtOAc 13:13:2 for product) to yield 45.8mg(89%) of a white amorphous solid.

4.2.7 Oligomerisation of Benzyl Epicatechin

Three separate stirring solution of **7** and **6** respectively (rxn1: 13.5mg, 18.3μmol; 55.4mg, 85.2μmol, 4.7equiv) (rxn2: 13.5mg, 18.3μmol; 13.8mg, 21.2μmol, 1.2equiv) (rxn3: 15.7mg, 21.2μmol; 3.1mg, 4.8μmol, 0.22equiv) each in CH_2Cl_2 (3ml) were made. TMSOTf (rxn1: 3.2μL) (rxn2: 3.2μL) (rxn3: 3.7μL) in CH_2Cl_2 (500μL) was added dropwise at -78^oC to each solution respectively. After 10 min the reactions were quenched with sat. $NaHCO_3$ and the solutions extracted with $CHCl_3$ and for each reaction the phases separated. The organic phase was washed with H_2O (5ml) and brine (5ml) and dried (Na_2SO_4). Filtration and concentration yielded for each reaction a colourless amorphous powder. To each reaction respectively, 71.7mg, 76.1mg, 22.2mg of $Pd(OH)_2/C$ and 10ml, 5ml, 3ml THF/MeOH/H_20(12:12:1) was added and the mixtures stirred overnight in a current of H_2 at rt. Each was then filtered and concentrated to yield an amorphous solid. These products were analysed by HPLC/MS to determine extent of oligomerisation.

4.2.8 Epicatechin-4β-8,2β-0-7-epicatechin (4)

3 (9.1mg, 15.6μmol) was dissolved in H_2O/EtOH (9:1, 9ml).

One ml of the **3** solution was added each to 9 eppendorf tubes (2ml) containing respectively 1.35mg(3.42μmol, 2 equiv), 13.84mg(35.13μmol, 20 equiv), 68.18mg(173.05μmol, 100 equiv), 0.34g(871.65μmol, 500 equiv) of DPPH, H_20_2 (0.5μL, 30%, 0.1 equiv) and $NaHCO_3$ (0.28mg), H_20_2 (5μL, 30%, 1 equiv) and $NaHCO_3$ (2.56mg), H_20_2 (50μL, 30%, 10 equiv) and $NaHCO_3$ (24.85mg), and 0.79mg (3.41μmol, 2 equiv) and 7.99mg (34.47μmol, 20 equiv) of Ag_2O. The tubes were vortexed to dissolve or disperse any solid and then shaken in the dark at 20 ^oC for 66hrs. LC-MS analysis of the resulting solutions showed following results:

DPPH 2equiv: 13.43min (72.6%), MS m/z 577$(M-H)^-$, 425$(M-H-152)^-$, 289$(M-H-288)^-$,

SM; 6.37min (5.4%), unknown; 9.23min (2.6%), unknown; 23.13min (3.6%), 575(M-H)$^-$, 423(M-H-152)$^-$, 285(M-H-290)$^-$, A2.

DPPH 20equiv: 13.53min (62.2%), MS m/z 577(M-H)$^-$, 425(M-H-152)$^-$, 289(M-H-288)$^-$, SM; 6.53min (14.1%), unknown; 9.33min (7.0%), unknown; 23.20min (4.5%), 575(M-H)$^-$, 423(M-H-152)$^-$, 285(M-H-290)$^-$, A2.

DPPH 100equiv: 13.53min (38.3%), MS m/z 577(M-H)$^-$, 425(M-H-152)$^-$, 289(M-H-288)$^-$ SM; 6.47min (38.4%), unknown; 9.30min (17.5%), unknown; 23.17min (5.9%), 575(M-H)$^-$, 423(M-H-152)$^-$, 285(M-H-290)$^-$, A2.

DPPH 500equiv: Showed no SM, flat TIC, suggested polymerisation beyond soluble level.

H_2O_2 0.1 equiv: >7 oxidation products, no A2.

H_2O_2 1 equiv: Showed broad hump in Chromatogram, suggested polymerisation.

H_2O_2 10 equiv: Showed flat TIC, suggested polymerisation beyond soluble level.

Ag_2O 2equiv: 13.60min (16.4%), MS m/z 577(M-H)$^-$, 425(M-H-152)$^-$, 289(M-H-288)$^-$; 23.13min (12.7%), MS m/z 575(M-H)$^-$, 423(M-H-152)$^-$, 285(M-H-290)$^-$, A2. Other unknown oxidation products (>7). Broad hump in chromatogram suggested some polymerisation.

Ag_2O 20equiv: Showed flat TIC, suggested polymerisation beyond soluble level.

4.2.9 Epicatechin-4β-8,2β-0-7-epicatechin (4)

3 (2.93mg, 5.07μmol) was dissolved in H_2O/EtOH (9:1, 3ml).

One ml of the **3** solution was added each to 3 eppendorf tubes (2ml) containing respectively 0.93mg(1.1 equiv), 8.61mg(11 equiv), and 81.32mg(110 equiv) of PIFA. The tubes were vortexed to dissolve or disperse any solid and then shaken in the dark at 20 ^oC for 66hrs. Analysis of these solutions by LC-MS showed:

1.1equiv: 13.53min(74.6%), 577(M-H)$^-$, 425(M-H-152)$^-$, 298(M-H-288)$^-$, SM; 9.30min (8.2%).

11equiv: Showed flat TIC, suggested polymerisation beyond soluble level.

110equiv: Showed flat TIC, suggested polymerisation beyond soluble level.

4.2.10 Epicatechin-4β-8,2β-0-7-epicatechin (4)

A stock ABTS solution was made by dissolving $(NH_4)_2$ABTS(0.29g) and AAPH(57.0mg) in EtOH/H_2O(1:9, 3ml) and heated at 70^oC for 15min.

To 3 eppendorf tubes (2ml) containing respectively 1.0mg, 1.1mg and 1.1mg of **3** were added 20μL ABTS stock and 980μL EtOH/H_2O(1:9), 200μL ABTS stock and 800μL EtOH/H_2O(1:9) and 1000μL ABTS stock. The tubes were vortexed and shaken at 20^oC in the dark for 72hrs. Analysis by LC-MS showed only starting materials.

4.2.11 Epicatechin-4β-8,2β-0-7-epicatechin (4)

To 3 vials containing respectively **3** (4.88mg, 8.82μmol) and DPPH (0.34g, 868.63μmol, 103 equiv), **3** (5.10mg, 8.82μmol) and Ag_2O (5.19mg, 22.40μmol, 2.54 equiv), and **3** (5.27mg, 9.03μmol) was added EtOH/H_2O (1:9, 5ml). The DPPH and Ag_2O solutions were sealed and covered from light and all were stirred at rt. After 5hrs and every 24hrs after that for 1 week, samples were taken for LC-MS analysis.

LC-MS:

DPPH 5hrs: 15.14min (54.2%), MS m/z 577$(M-H)^-$, 425$(M-H-152)^-$, 289$(M-H-288)^-$, SM; 8.53min (24.1%), MS m/z 573, unknown; 11.67min (13.9%), MS m/z 573, unknown; 23.30min (7.6%), MS m/z 575$(M-H)^-$, 423$(M-H-152)^-$, 285$(M-H-290)^-$, A2.

DPPH 24hrs+: Showed no SM, flat TIC, suggested polymerisation beyond soluble level.

Ag_2O 5hrs+: Showed no SM, flat TIC, suggested polymerisation beyond soluble level.

Air 5days+: 13.90min (82.2%) MS m/z 577$(M-H)^-$, 425$(M-H-152)^-$, 298$(M-H-288)^-$, SM; 11.30min (5.5%), unknown; 22.50min (6.1%), MS m/z 575$(M-H)^-$, 423$(M-H-152)^-$, 285$(M-H-290)^-$, A2.

4.2.12 5,7,3',4'-Tetra-O-methylepicatechin (16)

To a stirring suspension of NaH (60% dispersion in oil, 0.13g, 3.19mmol, 4.5 equiv) and anhydrous DMF (2ml) at –78^oC, epicatechin (**1**) (0.21g, 0.71mmol) in anhydrous DMF (3ml) was added all at once, followed by neat MeI (230μL, 3.7mmol, 5.2 equiv) added dropwise over 5min. The solution was stirred at –78^oC for a further 15min and warmed to rt, where stirring was continued for 48hrs. TLC control (Hexane:EtOAc, 1:1) showed

gradual elimination of starting material. The reaction was quenched with HCl (1M, 2ml) and water (10ml). The solution was then extracted with EtOAc(3x20ml) and the organic phase washed with water (3x20ml). The organic phase was dried (Na_2SO_4), filtered and concentrated. The product was purified by SiO_2 column chromatography (Hexane:EtOAc, 1:1) to yield a white crystalline solid (0.20g, 80%).

NMR: ^{1}H(200MHz, $CDCl_3$): 7.05(s,1H), 7.02,6.83((ABq,2H, J=38Hz, A part d with J=8Hz), 6.16,6.07(ABq,2H,J=18Hz, A part d with J=2Hz), 4.88 (s,1H), 4.19(s,1H), 3.87(s,3H), 3.85(s,3H), 3.75(s,3H), 3.72(s,3H), 2.85,2.84(narrow ABq,2H), 1.99(s,1H). ^{13}C: 159.33, 158.94, 154.99, 148.74, 148.48, 130.71, 118.43, 110.82, 109.44, 100.09, 93.04, 91.80, 78.19, 66.06, 55.64, 55.13, 55.04, 27.88.

4.2.13 5,7,3',4'-Tetra-O-benzylepicatechin-4β-8-(2R,3S,4S)-5,7,3',4'-tetrabenzyloxy-4-(2''-ethoxy-ethoxy)flavan-3-ol (17)

To a solution of **8** (0.20g, 0.16mmol) and 2-ethoxyethanol (0.3ml) in CH_2Cl_2 (2ml) at 0^{o}C, DDQ (0.08g, 0.33mmol) in CH_2Cl_2 (5ml) was added dropwise. The reaction was stirred at rt for 24hrs. TLC control (Hexane: $CHCl_3$:EtOAc, 3:1:1) showed only starting materials were present.

4.2.14 5,7,3',4'-Tetra-O-benzylepicatechin-4β-8-5,7,3',4'-Tetra-O-methylepicatechin(19)

To a stirring solution of **7** (0.03g, 40.9μmol) and **16** (0.07g, 191.7μmol, 4.7 equiv) in CH_2Cl_2 (7ml), TMSOTf (7.4μL, 40.9μmol) in CH_2Cl_2 (0.5mL) was added dropwise at –78^{o}C. After 10 min the reaction was quenched with sat. $NaHCO_3$ and the solution extracted with $CHCl_3$ and the phases separated. The organic phase was washed with H_2O (5ml) and brine (5ml) and the combined aqueous phase was extracted with $CHCl_3$ (2x5ml). The combined organics were dried (Na_2SO_4), filtered and concentrated. The red oil residue was chromatographed over SiO_2 (Hexane:EtOAc 1:2) to produce 0.040g (99%) of white amorphous powder.

HRMS(ESI-TOF): Calc: 995.4001 $(M+H)^+$, found: 995.3993.

NMR (complex mixture of rotational isomers): ^{1}H(300MHz, $CDCl_3$): 7.45-6.95(m,20H), 6.93-5.12(complex mixture s,d,dd,8H), 5.09-4.38(complex mixture s,d,dd,14H), 3.93-

3.61(complex mixture s,12H), 3.14(s,1H), 3.05-2.92(m,2H), 1.81(s,1H). ^{13}C(75MHz): 158.26-156.94(8sig), 155.59, 155.03, 154.22, 152.53, 148.99-148.27(7sig), 137.34-137.09(4sig), 132.46, 132.30, 130.49, 119.70, 118.35, 117.76, 115.05, 114.96, 113.81, 113.58, 111.56-109.52(6sig), 104.64, 104.56, 101.31, 94.32-93.10(4sig), 91.26, 88.41, 78.94, 77.90, 75.46, 72.11-69.02(8sig), 66.48, 65.06, 56.64-55.28(6sig), 35.47, 35.26, 28.53, 28.46.

4.2.15 5,7,3',4'-Tetra-O-benzylepicatechin-4β-8-5,7,3',4'-Tetra-O-benzylepicatechin-4β-8-5,7,3',4'-Tetra-O-methylepicatechin (18)

To a stirring solution of **7** (0.03g, 40.7μmol) and **19** (0.18g, 185.5μmol, 4.6 equiv) in CH_2Cl_2 (11ml), TMSOTf (8.0μL, 44.2μmol) in CH_2Cl_2 (1mL) was added dropwise at –40^{o}C. After 15 min the reaction was quenched with sat. $NaHCO_3$ and the solution extracted with $CHCl_3$ and the phases separated. The organic phase was washed with H_2O and brine and the combined aqueous phases were extracted with $CHCl_3$. The combined organics were dried (Na_2SO_4), filtered and concentrated. The residue was chromatographed over SiO_2 (Hexane:EtOAc: CH_2Cl_2 3:1:18) to yield 0.035g (53%) of white amorphous solid.

HRMS(ESI-TOF): Calc: 1643.6512 $(M+H)^+$, found: 1643.6508.

NMR (complex mixture of rotational isomers): ^{1}H(300MHz, $CDCl_3$): 7.50-6.72(m,40H), 6.53-5.22(complex mixture d,dd,12H), 5.20-4.78(m,16H), 4.76-4.00(complex mixture s,d,dd,9H), 3.92-3.76(complex mixture s,12H), 3.20(s,1H), 3.04-2.84(m,2H), 1.28(s,1H), 1.22(s,1H). ^{13}C(75MHz): 158.32-157.38(8sig), 156.61, 156.20-155.40(6sig), 154.70, 153.28, 152.96, 152.63, 152.38, 149.18-148.15(8sig), 142.71, 142.60, 137.35-137.20(overlapping signals unknown), 132.78, 132.62, 132.30, 132.23, 130.60, 130.54, 125.15, 120.04-117.84(7sig), 115.11-109.20(18sig), 105.99-104.49(7sig), 101.57, 101.28, 94.45-90.35(10sig), 78.00, 76.03, 75.64, 75.38, 75.20, 72.29-69.30(16sig), 65.29, 65.13, 64.94, 60.33, 56.82-55.31(6sig), 36.32-35.17(5sig), 29.65, 28.56.

4.2.16 Epicatechin-4β-8-epicatechin-4β-8-5,7,3',4'-Tetra-O-methylepicatechin (20)

A stirring solution of **18** (35.0mg, 21.3μmol) in THF/MeOH/H_2O (12/12/1, 25ml) was hydrogenated over $Pd(OH)_2$/C (150mg) overnight under a current of H_2 at rt. Filtration and concentration yielded 17.8mg of a brown powder (99%).

LC-MS: 39.18min (85%) MS m/z 633.6 $(M-H-288)^-$, 921.4$((M-H)^-$; 51.17min (15%) by MS m/z 723.4, unknown.

NMR (complex mixture of rotational isomers): ^{1}H(300MHz, CD_3OD): 7.40-6.60(m,8H), 6.38-5.90(complex mixture s,d,dd,12H), 5.85-3.70(complex mixture s,d,dd,9H), 3.37-3.29(complex mixture s,12H), 3.01-2.91(m,2H, overlapping s,1H), 1.82(s,1H), 1.21(s,1H). ^{13}C(75MHz): 159.86-153.99(13sig), 150.14, 149.65, 145.96, 145.86, 145.39, 133.38, 132.77, 129.97, 128.68, 126.12, 126.05, 119.99, 119.68, 119.40, 116.00-115.15(5sig), 112.72, 112.62, 111.95, 108.40, 103.94, 103.10, 100.08, 97.17-95.73(6sig), 92.75, 89.65, 80.51, 79.88, 79.74, 77.32, 76.82, 73.27, 72.76, 71.66, 68.85, 67.49, 66.93, 57.95-55.90(7sig), 37.46, 35.95, 30.89, 29.25, 27.56, 26.48, 22.96.

4.2.17 Epicatechin-4β-8,2β-0-7-epicatechin (4)

B2(6.5mg, 11.25μmol) and $Pd(OH)_2$/C (11.2g) were stirred in EtOH/H_2O (1:9, 6ml) and stirred continuously for a week open to the atmosphere. A sample was taken every 24hrs and analysed by LC-MS.

24hrs: 11.7min (11.7%), MS m/z 573.2, unknown; 15.6min (65.5%), MS m/z 577 $(M-H)^-$, 425 $(M-H-152)^-$, 289 $(M-H-288(monomer))^-$, SM; 23.5min (16.7%) MS m/z 575 $(M-H)^-$, 423$(M-H-152)^-$, 285$(M-H-290(monomer))^-$, A2.

48hrs: 11.7min (20.1%), MS m/z refer 24hrs; 15.6min (61.3%), MS m/z refer 24hrs; 23.5min (18.7%), MS m/z refer 24hrs

72hrs+: Polymerised material dominated.

4.2.18 Epicatechin-4β-8,2β-0-7-epicatechin (4)

B2(5.2mg, 9.00μmol) and $Pd(OH)_2$/C (7.8g) were stirred continuously in EtOH/H_2O (1:9, 5ml) for a week open to the atmosphere. A sample was taken every 24hrs and analysed by LC-MS.

72hrs: 16.60min (77.6%), MS m/z 577 $(M-H)^-$, 425 $(M-H-152)^-$, 289 $(M-H-288(monomer))^-$, SM; 24.23min (3.1%) MS m/z 575 $(M-H)^-$, 423$(M-H-152)^-$, 285$(M-H-290(monomer))^-$, A2.

4.2.19 Epicatechin-4β-8,2β-0-7-epicatechin-4β-8-5,7,3',4'-tetra-O-methylepicatechin(15)

20 (17.1mg) and $Pd(OH)_2/C$ (38.5mg) were stirred in $EtOH/H_2O$ (1:9, 20ml) for 50hrs, and samples taken at 0, 4, 6, 24, 29, 33 and 50hrs and analysed using LC-MS. LC-MS:

0hrs: 39.30min (76.2%), MS m/z 921.6 $(M-H)^-$, 633.6 $(M-H-288(monomer))^-$, SM; 51.56min (18.0%), MS m/z 729.6, unknown artefact.

4hrs: 39.87min (70.3%), MS refer 0hrs; 51.92min (17.8%), MS refer 0hrs; 26.77min (5.4%), MS m/z 917.6, unknown.

6hrs: 39.83min (68.5%), MS refer 0hrs; 51.83min (17.8%), MS refer 0hrs; 26.87min (6.7%), MS m/z refer 4hrs.

24hrs: 40.02min (67.6%), MS refer 0hrs; 52.00min (18.5%), MS refer 0hrs; 26.80min (7.9%), MS m/z refer 4hrs.

29hrs: 40.02min (67.3%), MS refer 0hrs; 51.92min (18.4%), MS refer 0hrs; 26.90min (8.2%), MS m/z refer 4hrs.

33hrs: 40.08min (67.6%), MS refer 0hrs; 51.87min (18.4%), MS refer 0hrs; 26.97min (7.8%), MS m/z refer 4hrs.

50hrs: 39.97min (67.4%), MS refer 0hrs, 51.87min (18.7%), MS refer 0hrs, 26.93min (7.9%), MS m/z refer 4hrs.

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