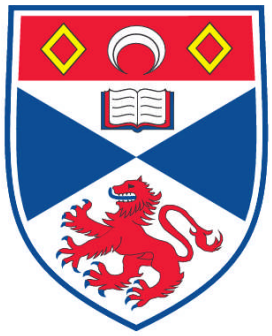
NOVEL METHODOLOGY FOR THE SYNTHESIS OF ^{13}C-LABELLED PHENOLS AND ITS APPLICATION TO THE TOTAL SYNTHESIS OF POLYPHENOLS

Laura Marshall

A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews



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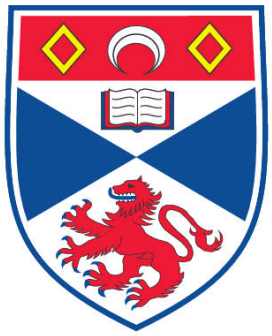

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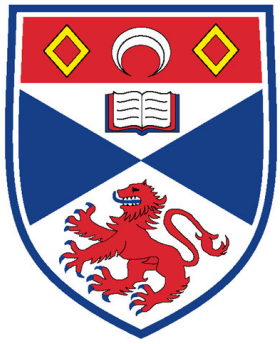

University of St Andrews

School of Chemistry

and

Centre for Biomolecular Sciences

Laura Marshall

PhD Thesis

March 2010

Supervisor: Dr Nigel P. Botting

Thesis Declaration

I, ……..…..……, hereby certify that this thesis, which is approximately 62,100 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in October 2006, and as a candidate for the degree of PhD in October 2006; the higher study for which this is a record was carried out in the University of St Andrews between 2006 and 2009.

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ABBREVIATIONS AND ACRONYMS

Å	Ångstrom
Ac	Acetyl group (-$COCH_3$)
AcOH	Acetic acid (CH_3CO_2H)
Ac_2O	Acetic anhydride ($(CH_3CO)_2O$)
br	Broad (spectral)
Bn	Benzyl group (-$CH_2C_6H_5$)
Boc	*tert*-Butyloxycarbonyl (-CO*t*C_4H_9)
Boc_2O	Boc anhydride ($(CO$*t*$C_4H_9)_2O$)
n-Bu	Primary butyl group
t-Bu	Tertiary butyl group
bpy	2,2'-Bipyridyl
*t*BuOH	*tert*-butanol
B_2Pin_2	Bis(pinacolato)diboron
°C	Degrees centigrade/celcius, temperature unit
calcd	Calculated
cf.	*Confer impe*r, (Latin), compared to
CI	Chemical ionisation, ionisation technique (mass spectrometry)
cm^{-1}	Wavenumber(s)
COD	Cyclooctadiene
Comp.	Compound
Conc.	Concentrated
COSY	Correlation spectroscopy (NMR)
d	Day(s); doublet (spectral)
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
Decomp.	Decomposition (melting point measurement)
δ	Chemical shift in ppm (NMR)
DEPT	Distortionless Enhancement by Polarisation Transfer (NMR)
DIBAL	Diisobutylaluminium hydride
DIPEA	Diisopropylethylamine
DMA	*N,N*-dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	Dimethyl sulfoxide
dppe	Diphos, 1,2-Bis(diphenylphosphino)ethane
d*t*bpy	Di-*tert*-butylbipyridyl
EI	Electron impact, ionisation technique (mass spectrometry)
Equiv./eq.	Equivalents
ES	Electrospray (mass spectrometry)
Et	Ethyl group (-CH_2CH_3)
et al.	*Et alia* (Latin), and others
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol

EWG	Electron withdrawing group
g	Gram(s)
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometry
GHz	GigaHertz
h	Hour(s)
HBPin	Pinacolborane
HMBC	Heteronuclear Multiple Bond Correlation (NMR)
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear Single Quantum Coherence (NMR)
Hz	Hertz
in situ	Latin, in the place
in vivo	Latin, within the living
*i*Pr	*iso*-Propyl group (-$CH(CH_3)_2$)
IR	Infrared
J	Coupling constant (NMR)
kg	Kilogram(s)
KO*t*Bu	Potassium *tert*-butoxide
L	Litre(s)
LC-MS	Liquid chromatography mass spectrometry
LDA	Lithium diisopropylamide
Lit.	Literature
m	Multiplet (spectral); metre(s); milli
M	Concentration, molar, mol l^{-1}; molecular ion (mass spectrometry)
*m*CPBA	*meta*-Chloroperbenzoic acid
MDAP	Mass directed auto prep (purification technique)
Me	Methyl group (-CH_3)
MeCN	Acetonitrile
MEM	2-Methoxyethoxymethyl (-$CH_2OCH_2CH_2OCH_3$)
MeOH	Methanol
mg	Milligram(s)
MHz	MegaHertz
min	Minutes
mL	Millilitres
mm	Millimetre(s)
mmol/mM	Concentration, millimolar, mmol l^{-1}
mol	Mole(s)
MOM	Methoxymethyl (-CH_2OCH_3)
mp	Melting point
MS	Mass spectrometry
m/z	Mass over charge ratio (mass spectrometry)
N	Normal solution, concentration
NFP	*N*-Formylpiperidine
nm	Nanometre(s)
NMP	*N*-Methylpyrrolidinone
NMR	Nuclear magnetic resonance
No.	Number

Oxone®	Potassium peroxomonosulfate salt, $2KHSO_5.KHSO_4.H_2SO_4$
pKa	Acid dissociation constant
PENDANT (NMR)	Polarisation enhancement nurtured during attached nucleus testing
Pet. ether	Petroleum ether
Pd/C	Palladium on carbon catalyst
Ph	Phenyl group ($-C_6H_5$)
PTC	Phase transfer catalyst
PTFE	Polytetrafluoroethylene
ppm	Parts per million
q	Quartet (spectral)
qt	Quintet (spectral)
R	Generic group
R_f	Retention factor (chromatography)
rt	Room temperature
s	Singlet (spectral); second(s)
t	Triplet (spectral)
$TBABr_3$	Tetrabutylammonium tribromide
TBAF	Tetrabutylammonium fluoride
TBDMSCl	*tert*-Butyldimethylsilyl chloride
TEA	Triethylamine
Tf	Triflate group, trifluoromethanesulfonate ($-OSO_2CF_3$)
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl group ($-Si(CH_3)_3$)
TOF	Time of flight (mass spectrometry)
µm	Micrometre(s)
U	Uniformly labelled (isotopic labelling)
UV	Ultraviolet
Vs	Versus
υ	Wavenumbers (IR spectroscopy)
W	Watt

ABSTRACT

The base-catalysed reaction of 4*H*-pyran-4-one with a range of nucleophiles, namely diethyl malonate, ethyl acetoacetate, nitromethane, acetylacetone and ethyl cyanoacetate, was developed as a reliable, high yielding method for the preparation of *para*-substituted phenols. The methodology was extended to include the use of the substituted pyranones, maltol, 2,6-dimethyl-4*H*-pyran-4-one and diethyl chelidonate. Reactions were studied using conventional heating methods and microwave irradiation. Microwave irradiation had definite beneficial effects, with improved yields, reduced reaction times and cleaner reaction profiles.

The potential of this methodology was examined for the regioselective placement of ^{13}C-atoms into benzene rings using ^{13}C-labelled nucleophiles or ^{13}C-labelled 4*H*-pyran-4-ones. [3,5-$^{13}C_2$]4*H*-pyran-4-one and [2,6-$^{13}C_2$]4*H*-pyran-4-one were prepared from various ^{13}C-labelled versions of triethyl orthoformate and acetone. This methodology was applied to the synthesis of [1,3,5-$^{13}C_3$]gallic acid, *via* the base-catalysed reaction of [3,5-$^{13}C_2$]4*H*-pyran-4-one with diethyl [2-^{13}C]malonate, followed by subsequent transformations to yield [1,3,5-$^{13}C_3$]gallic acid.

The preparation of [2-^{13}C]phloroglucinol was carried out *via* [2-^{13}C]resorcinol, with regioselective placement of a single ^{13}C-atom into the aromatic ring. This was accomplished from non-aromatic precursors, with the source of the ^{13}C-atom being [^{13}C]methyl iodide. The key step in this synthesis was the introduction of the third hydroxyl group, which was achieved using a modified iridium-catalysed C-H activation/borylation/oxidation procedure. The scope of an existing C-H activation/borylation reaction was modified and expanded to include a range of protected resorcinol derivatives. A catalyst system was developed which allowed high conversion to the intermediate arylboronic acids, followed by oxidation using aqueous Oxone® to yield the corresponding phenols.

Finally, to demonstrate the potential of these new methods for application in the synthesis of isotopically labelled natural products and polyphenols, the syntheses of ^{13}C-labelled anthocyanins were studied. A route was developed that could be applied to the synthesis of either cyanidin-3-glucoside or delphinidin-3-glucoside. Only the final coupling/cyclisation step to yield the desired anthocyanin targets remains to be carried out.

CONTENTS

1. INTRODUCTION

1.1 The Use of Stable Isotopes

During the drug discovery process, pharmacokinetic studies relating to the absorption, excretion and metabolism of the drug must be performed. Similar studies are also carried out on natural products, such as those found in foods, in order to determine their behaviour within a biological matrix.[1] LC-MS and GC-MS have become two of the most versatile and effective tools in bioanalytical chemistry for use in drug and metabolite studies, with the ability to separate and quantitate subnanomolar quantities of an analyte in complex matrices.[2] Other than degradation reactions, metabolic processes such as oxidation and alkylation must be studied, and mass spectrometry is an ideal analysis technique for this purpose due to its ability to monitor changes in the molecular weight of the analyte during the metabolic process. Although the mass spectrometer is extremely sensitive, the sensitivity is not constant, resulting in signal variation between compounds. Calibration is therefore required to compensate for this and for variations in sample preparation. This is best achieved by the use of a stable, pure internal standard, such as a stable isotope-labelled version of the analyte containing heavy isotopes such as ^{13}C, ^{15}N or ^{2}H. The introduction of isotopes into a compound will not change the chemistry of the process under investigation as the stable isotope version and analyte should have similar physical and chemical properties,[1] including, ideally, the same retention time by GC or LC. The use of a stable isotope standard results in the analyte and standard having different molecular ions and they can therefore be separately monitored by MS. In qualitative mass spectrometry the stable isotopes can also be used as a marker to distinguish drug-related material from endogenous compounds, to aid in the interpretation of complex mass spectra and also to help in the identification of metabolites.[2] If a metabolite contains the heavy isotope, this can be observed during MS analysis as its isotopic profile will differ from that of the unlabelled compound. The careful placement of heavy isotopes at metabolically inert positions throughout the analyte

allows a range of metabolites to be identified and studied, subsequently resulting in the deduction of potential metabolic pathways.[1]

One of the key characteristics of the internal standard is the mass difference between the analyte and standard. This must be large enough so that naturally occurring isotopes in the parent analyte do not interfere with the response of the internal standard and are therefore effectively cancelled out. The mass difference required will depend on the molecular weight of the analyte being studied and the presence of heteroatoms. For the majority of compounds examined here, a difference of +3 mass units is required. This makes for significant synthetic challenges, as outlined below.

Deuterated compounds are often used as internal standards in analysis due to their ease of synthesis by exchange reactions.[3] However, this process also has its disadvantages, as a range of species will be produced during deuteration. In the case of electron rich species such as phenols, the deuterium atoms can also exchange out of the compound in aqueous solution, producing a mixture of species containing varying numbers of deuterium atoms.[4] The resulting range of molecular ions significantly complicates the analysis procedure unnecessarily. For aromatic compounds – phenols in particular – back exchange may occur under sample preparation and analysis conditions, with deuterium atoms being replaced by hydrogen. This can result in a reduction in the amount of internal standard present and an apparent increase in the amount of analyte. The isotope effect must also be considered when deciding which atoms to substitute for their heavier isotopes.[5] This effect is directly related to the relative difference in mass between the two isotopic species and is most pronounced when the mass difference is greatest. For example, replacing a hydrogen (^{1}H) atom with a deuterium (^{2}H) atom gives a 100% increase in mass, however replacing a ^{12}C atom for a ^{13}C atom represents only an 8% mass increase. In terms of GC-MS and LC-MS analysis this can result in differing retention

times and chromatographic properties when compounds are multiply labelled using deuterium, giving carbon labelling yet another advantage over deuterium labelling.

In order to avoid the inherent disadvantages of deuterium labelled internal strandards, it is desirable to synthesise compounds containing ^{13}C atoms incorporated into the carbon framework of the desired compounds. This can be achieved using commercially available starting materials which have 99% incorporation of ^{13}C (*cf.* 1.1% natural abundance), and therefore it should be possible to obtain the final stable-labelled standard with 99% ^{13}C incorporation at the desired positions in the molecule. If, for example, a compound with four ^{13}C atoms incorporated is analysed, a major signal of four mass units higher than the unlabelled compound will be observed, avoiding the variable signals seen with deuterium incorporation. The isotopic purity is determined by monitoring an envelope of ions around the mass of interest. For example, for the [M+4] (labelled standard), MH^+ = 356 and [M_0] (unlabelled compound), MH^+ = 352. So the envelope being monitored will be around *m/z* 348, 352, 356 and 360.[2] *Diagram 1* below shows a schematic of a mass spectrum showing the labelled and unlabelled signals, and demonstrates the necessity of an appropriate mass increase of the labelled standard.

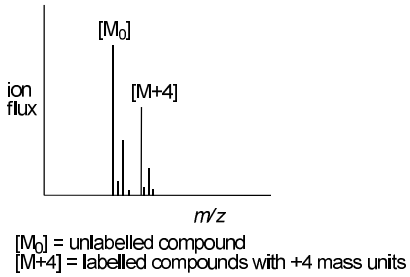


Diagram 1 – Mass spectrum schematic showing the mass difference between labelled and unlabelled compounds.

It is also important to note that the internal standards incorporating ^{13}C atoms will be chemically stable and the ^{13}C atoms do not have the potential to exchange out of the molecule, unlike deuterium atoms.[4] This will result in a more accurate analysis of an analyte and its metabolites,

as the presence of the ^{13}C atoms in the metabolic fragments can be observed by mass spectrometry and metabolites determined accordingly. These factors can be extremely important for ADME (absorption, distribution, metabolism and elimination) studies for potential new drugs.

1.2 Synthesis of Isotopically Labelled Compounds using Carbon-13

Due to the requirements outlined above for the use of isotopically labelled compounds as internal standards, it is essential to be able to synthesise these compounds as easily and efficiently as possible. This provides a significant synthetic challenge. Firstly, suitable ^{13}C-labelled building blocks must be synthesised. This task is made more challenging than standard organic synthesis, as the number of commercially available ^{13}C-labelled compounds is very limited. In particular, the number of commercially available ^{13}C-labelled benzene derivatives is very small, immediately limiting the routes available if choosing to start from a commercially available ^{13}C-labelled benzene derivative. Many syntheses of aromatic compounds must begin with either [$^{13}C_6$]phenol, aniline or bromobenzene. Therefore, the development of new efficient methods for the synthesis of substituted aromatic compounds containing ^{13}C-labelled atoms is necessary. Methods of these types would also be applicable to ^{14}C-labelling. The most frequently used method to obtain ^{14}C-ring-labelled aromatic compounds is *via* functionalisation of [U-^{14}C]benzene followed by substitutions or functional group interconversions. Alternative strategies would be particularly beneficial when considering the use of [U-^{14}C]benzene in synthetic procedures, due to the fact that [U-^{14}C]benzene is known to be a mixture of ^{14}C-labelled species, as demonstrated by the mass spectrum shown in *Diagram 2*.[6] The consequence is that when derivatisation of the ring is carried out, the ^{14}C-atoms will be observed in different positions within the ring, thus complicating analysis. The use of synthetic methods which construct the aromatic ring from acyclic and non-aromatic precursors will allow regioselective

placement of ^{14}C-atoms into the ring system, consequently removing the possibility of mixtures of species.

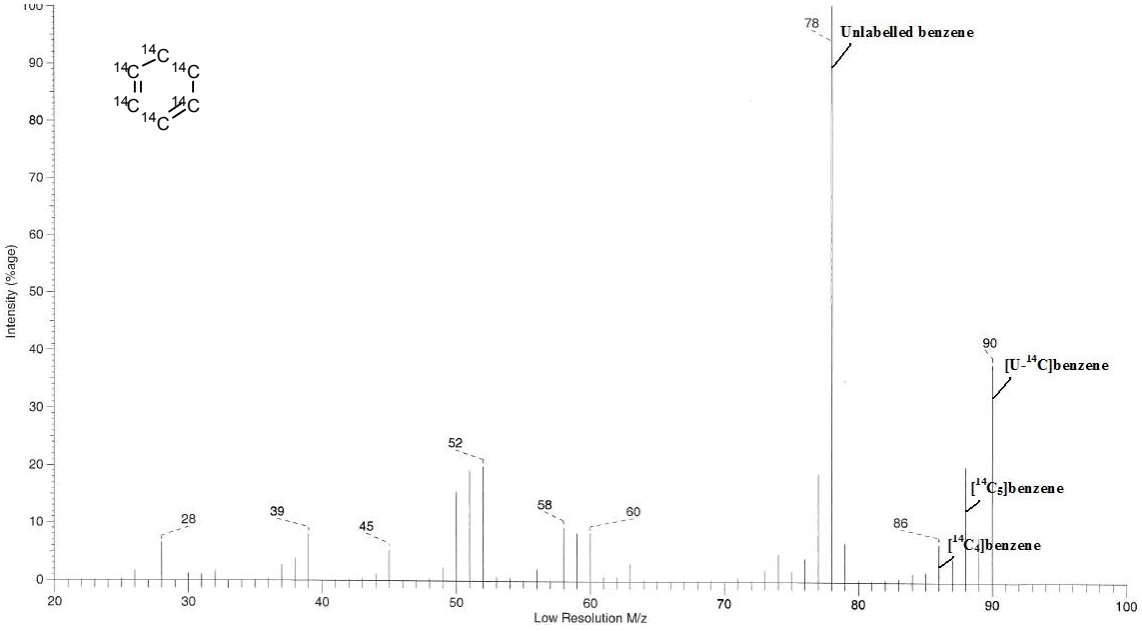


Diagram 2 – Mass Spectrum of [U-^{14}C]benzene.[6]

For these reasons our group is mainly interested in the ^{13}C-labelling of natural products. Examples of recently synthesised multiply ^{13}C-labelled polyphenol phytoestrogens in the Botting group include [3,4,8-$^{13}C_3$]daidzein **1**,[7] [3,4,1'-$^{13}C_3$]genistein **2**, [2,3,4-$^{13}C_3$]glycitein **3**,[8] and [6,6a,11a-$^{13}C_3$]coumestrol **4**,[9] as shown in *Diagram 3* below. All of these compounds have been successfully employed as internal standards in LC-MS analysis of the compounds in biological fluids.

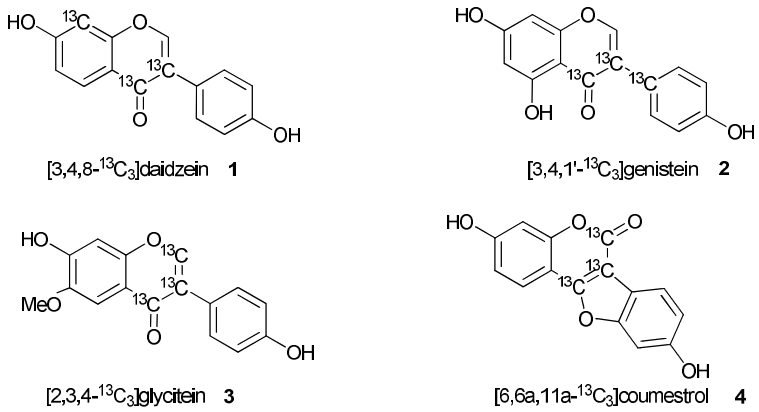


Diagram 3 – Recently synthesised multiply ^{13}C-labelled polyphenol phytoestrogens.[7-9]

1.3 Synthetic Routes to Phenols from Acyclic and Non-Aromatic Precursors

1.3.1 Background – Traditional Syntheses from Aromatic Compounds

Many natural products such as polyketides, coumarins and flavonoids contain mono-phenolic or polyphenolic moieties.[10] New methodology for the preparation of phenols is therefore potentially valuable in natural product synthesis. Traditional methods for the preparation of phenols[11] include hydrolysis of diazonium salts, Baeyer-Villiger oxidation, displacement of aromatic halides and oxidation of aryl boronates. More recent methods include the catalytic C-H activation/borylation/oxidation procedure developed by Maleczka and Smith (see **Section 2.4**)[12] and palladium-catalysed C-O bond formation.[13]

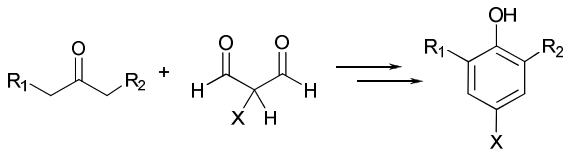
Traditionally, aromatic compounds were obtained from coal tar, which was a cheap and abundant supply of aromatic hydrocarbons such as benzene.[14] Substituted hydrocarbons were subsequently obtained by carrying out reactions such as nitration, sulfonation, reduction and oxidation on the hydrocarbon. At this time, ring functionalisation was one of the few methods available for the preparation of substituted benzenes.

An alternative synthetic strategy involves the construction of the benzene nucleus from acyclic, non-aromatic precursors with the substituents already in place.[14] The ring can then be functionalised further if required. One advantage of this route is that the substituted compound can frequently be prepared in fewer steps with the avoidance of "*ortho-meta-para*" mixtures which are common in traditional aromatic synthesis. This point also allows a way around the *ortho-*, *meta-* or *para*-directing problems which can limit the functionalisation and synthesis of many compounds.[15]

Leading on from this is the introduction of isotopic labels (e.g. ^{13}C, ^{14}C) into aromatic compounds, which is not an easy task by conventional synthesis, often due to the high cost or low availability of isotopically labelled benzene derivatives. This problem should therefore be overcome by synthesising the aromatic ring from isotopically labelled acyclic precursors, as they tend to be cheaper and are available in much greater variety then aromatic compounds.

1.3.2 Synthesis of Mono-Phenols

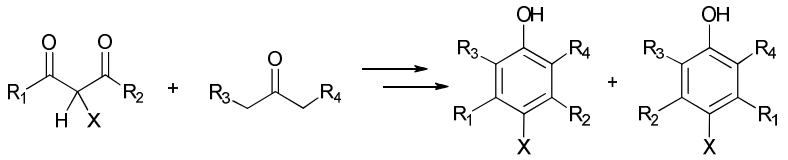
One of the most important aromatic compounds for use in natural product synthesis is phenol. Often in the synthesis of phenols, the hydroxyl group arises from either a ketone or an ester, giving a wide range of possibilities for their synthesis. Ketones usually react as three-carbon components *via* the two α-positions which are nucleophilic. The co-reactant must also be a three-carbon component in order to make the 6-membered ring. Two of the sites in the co-reactant must therefore be electrophilic. This is demonstrated in *Scheme 1*[14] below in the condensation of malondialdehydes (β-dicarbonyl compounds) with a variety of ketones. The significance of this type of reaction is increased by the fact that through the variation of the R_1, R_2 and X groups, a range of substituted phenols can be produced.[16]



X	R_1	R_2	%
H	CO_2R	CO_2R	41
NO_2	Ph	Ph	95
Br	CO_2R	CO_2R	53
NO_2	Me	Et	74

Scheme 1 – Formation of phenols from a ketone and a β-dicarbonyl compound.[14]

A similar route is shown in *Scheme 2* below for the synthesis of higher substituted phenols, where the aldehyde is replaced by a ketone which is reacted with either a β-ketoaldehyde or a β-diketone. One advantage of this method is that ketoaldehydes and diketones are generally more stable then malondialdehydes. Also, it is a useful alternative to conventional aromatic synthesis, as it allows the preparation of unsymmetrical substituted phenols, which may be difficult to prepare from other methods. However, it should be noted that when unsymmetrical β-diketones are used, there is the possibility of the generation of two isomers, as indicated in *Scheme 2*[14] below. This problem can be avoided by making use of preferential reactivity at one carbon atom in each of the reactants. This could be achieved by using an enolisable ketone which can be enolised preferentially in one direction and then react this with a β-dicarbonyl compound which possesses one carbonyl group which is more reactive than the other.[14]

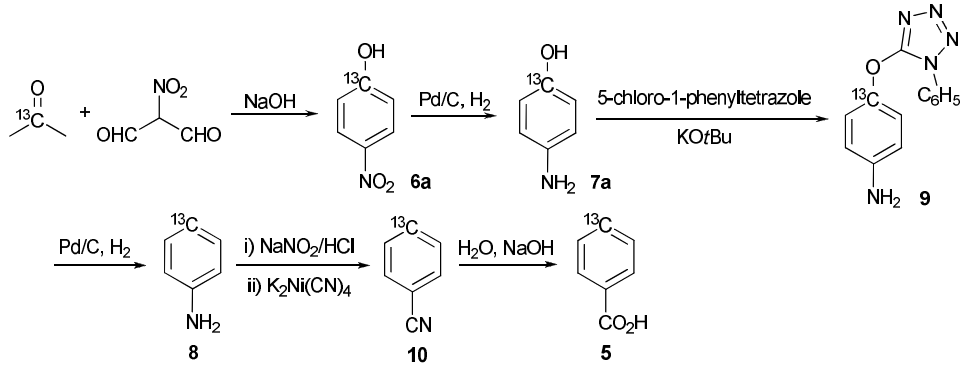


X	R_1	R_2	R_3	R_4	%
H	Me	CO_2H	CO_2H	CO_2H	76
H	Me	Me	H	COMe	64
Ph	$PhCH_2$	H	CO_2Et	CO_2Et	51

Scheme 2 – Formation of phenols from unsymmetrical β -ketones and β-dicarbonyl compounds.[14]

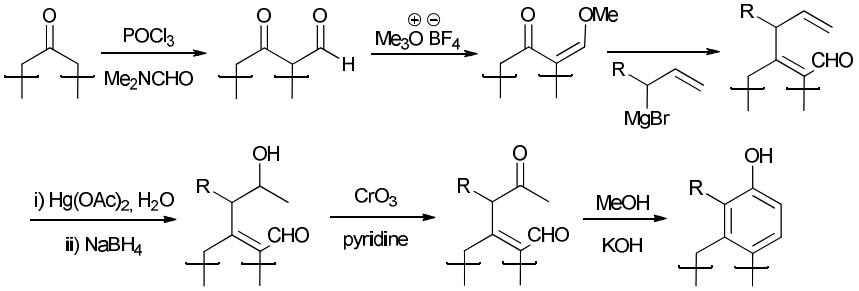
Benzoic acid is a food preservative which is also used in the treatment of hyperammonemia – a condition where elevated levels of ammonia in the blood are recorded. Baba *el al.*[17] reported the synthesis of [4-^{13}C]benzoic acid **5** by a seven-step synthetic route *via* 4-nitro[1-^{13}C]phenol **6a** and 4-amino[1-^{13}C]phenol **7a** using [2-^{13}C]acetone as the source of the isotopic label, as outlined in *Scheme 3* below. 4-Nitro[1-^{13}C]phenol **6a** was first prepared by the condensation of [2-^{13}C]acetone with nitromalonaldehyde. Reduction to 4-amino[1-^{13}C]phenol **7a** was carried

out by hydrogenation with a palladium-carbon catalyst. The aromatic hydroxyl group was then removed to give [4-^{13}C]aniline **8** *via* the formation of 1-phenyl-5-tetrazolyl ether **9** and subsequent hydrogenolytic cleavage. The aniline – which is a useful product itself – was easily converted to benzonitrile **10**, which was hydrolysed to give [4-^{13}C]benzoic acid **5**. It is important to note that each intermediate (with the exception of 4-nitrophenol) was used for the subsequent step without purification in order to increase the isotopic yield.



Scheme 3 – Route to [4-^{13}C]benzoic acid.[17]

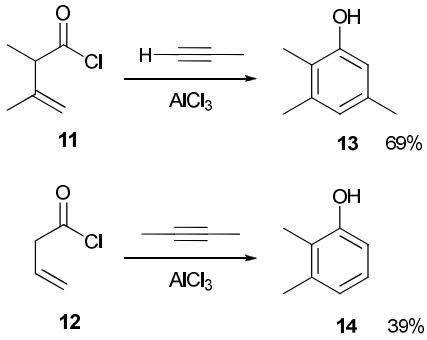
One other route to unsymmetrical substitution patterns in phenols is the use of the alkenyl Grignard reagent shown in *Scheme 4* below.[14,18] These can be reacted with a ketone, which can then ring-close *via* an intramolecular aldol reaction to the phenol after conversion to the corresponding aldehyde.



Scheme 4 – Use of Grignard reagents to yield unsymmetrical phenols.[14,18]

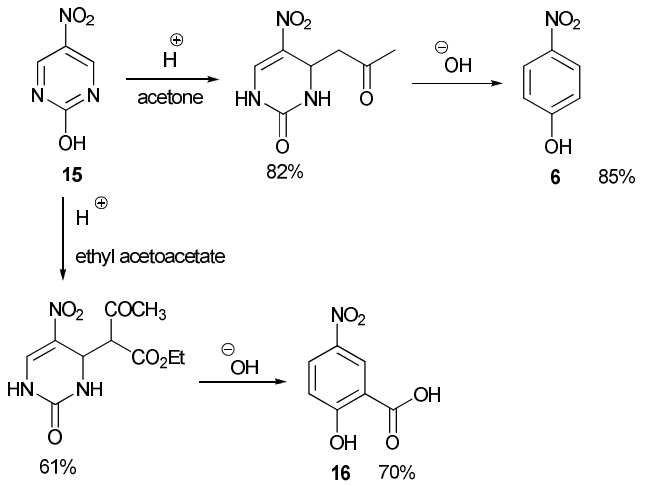
During their research into the synthesis of 5,5-disubstituted 2-cyclopentenones through the reaction of β,γ-unsaturated acid chlorides with trimethylsilyl derivatives of alkynes *via* an

intramolecular cyclisation-rearrangement process, Karpf *el al*[19] found that when the trimethylsilyl group is not present and the acid chlorides bear α-hydrogens, the ring contraction does not occur. This is demonstrated in *Scheme 5*, by the reaction of acid chlorides **11** and **12** with propyne and 2-butyne respectively, which led to polymethylphenols **13** and **14** after hydrolysis. This route is useful, as it provides a route to polyalkylated phenols from non-aromatic, acyclic starting materials.



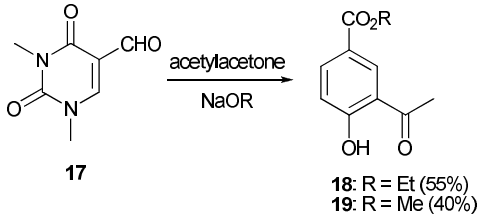
Scheme 5 – Synthesis of polyalkylated phenols from acid chlorides and alkynes.[19]

Another strategy in the synthesis of phenols is the use of heterocyclic precursors, with the synthetic route often involving ring cleavage of the heterocycle followed by ring-closure to yield the phenol. The pyrimidines have been shown to undergo acid-catalysed condensation with a variety of ketones to yield phenols (*via* pyrimidinones) as shown in *Schemes 6* and *7*.[20,21] In these examples, 2-hydroxy-5-nitropyrimidine **15** can be converted into 4-nitrophenol **6** through reaction with acetone, with the use of ethyl acetoacetate in place of acetone resulting in the formation of 5-nitrosalicyclic acid **16**.[20]



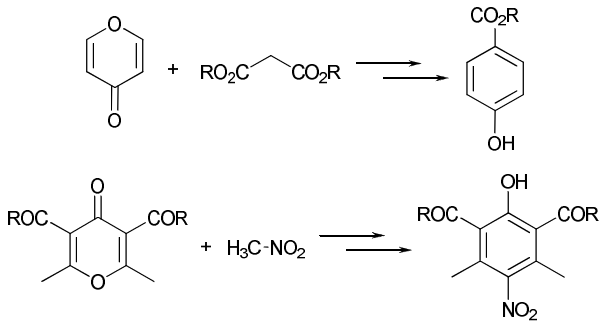
Scheme 6 – Use of pyrimidines in the synthesis of phenols.[20,21]

5-Formyl-1,3-dimethyluracil **17** can be used to produce 3-substituted 4-hydroxybenzoates **18, 19**, through reaction with acetylacetone, where the identity of the ester group in the 1-position depends upon the base used during the reaction. For example, when sodium ethoxide is used, the ethyl ester **18** is produced, whereas when sodium methoxide is used, the methyl ester **19** is produced (*Scheme 7*).[21]



Scheme 7 – Use of pyrimidines in the synthesis of phenols.[21]

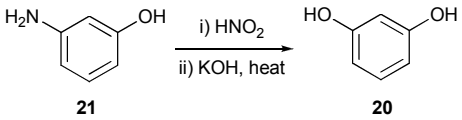
Another example of the use of heterocycles utilises the pyranones – oxygen heterocycles which can undergo either a rearrangement or an extrusion process. The pyranones are particularly susceptible to nucleophilic attack and hydrolysis as shown in *Scheme 8* below.[22] This gives potential routes to natural products which contain this moiety, as the ring-synthesis method can be used to build up the phenol ring from non-aromatic substrates. This will be discussed in more detail in **Section 2.1**.



Scheme 8 – Synthesis of phenols from pyranones.[22]

1.3.3 Synthesis of 1,3-Dihydroxybenzenes

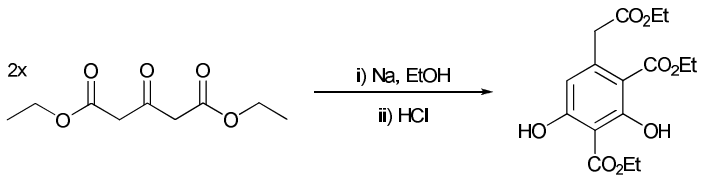
As phenols are important for their use in natural product synthesis, so too are the dihydroxybenzenes (catechols, resorcinols, etc). Resorcinol, or 1,3-dihydroxybenzene, **20** is found abundantly in nature, and can be obtained by the fusing of plant resins with potassium hydroxide, although it is often found in ether form or as the resorcinol moiety in larger plant polyphenols. Chemically it is traditionally synthesised by the treatment of 3-aminophenol **21** with nitrous acid as demonstrated in *Scheme 9*.[23]



Scheme 9 – Traditional preparation of resorcinol.[23]

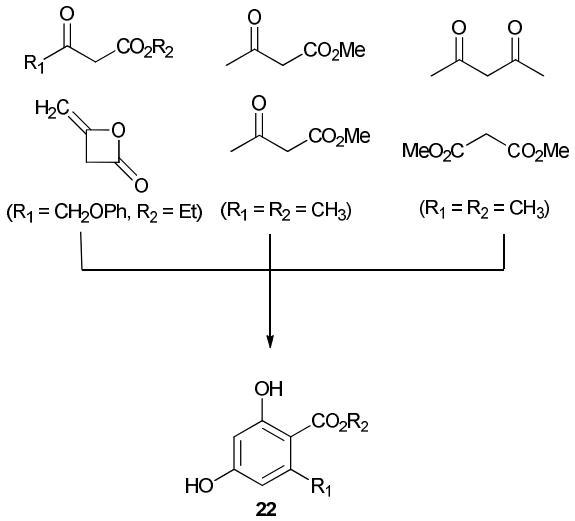
In general, the strategies for the preparation of dihydroxybenzenes are similar to those previously described for the phenols, as the hydroxyl groups tend to arise from carbonyl groups. One reaction which is more common in the preparation of dihydroxybenzenes is the Claisen condensation as it can be utilised in either the ring-closure step or in the assembly of the acyclic precursor.[14]

The synthesis of substituted 1,3-dihydroxybenzenes is the most commonly encountered procedure in the literature, giving a useful alternative to conventional methods. One example of this is the dimerisation of β-ketoesters, such as diethyl acetonedicarboxylate, as demonstrated in *Scheme 10* below in the synthesis of triethyl orcinoltricarboxylate.[24]



Scheme 10 – Synthesis of a highly substituted resorcinol from a β-ketoester.[24]

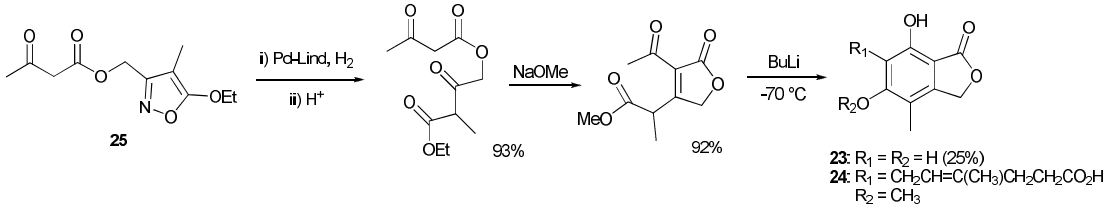
Bamfield *el al.*[14] recently reviewed the various routes towards the disubstituted 1,3-dihydroxybenzene (**22**, *Scheme 11*), which generally involve the use of dianions to give the desired product *via* regioselective condensations with the appropriate reagents.[25-28] Due to the simplicity and availability of these acyclic and non-aromatic precursors, the routes presented are very convenient for the synthesis of highly substituted compounds such as the substituted 1,3-dihydroxybenzenes.



Scheme 11 – Routes towards the disubstituted 1,3-dihydroxybenzene ***22.***[14, 25-28]

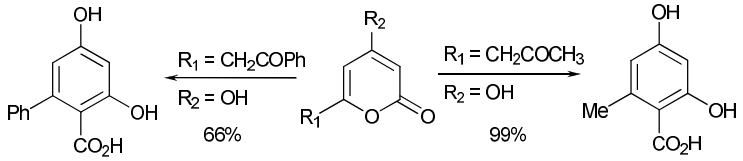
Isoxazolines can also be utilised in the synthesis of dihydroxyphthalides such as **23** (*Scheme 12*), which is a synthetic intermediate in the route towards mycophenolic acid **24** – a natural

phenol isolated from *Penicillium brevi-compactum* which possesses antibiotic and antiviral properties. The synthesis can be achieved in 3 steps from the isoxazoline **25** *via* a ring-opened intermediate as demonstrated in *Scheme 12* below.[14,29]



Scheme 12 – Synthesis of dihydroxy-phthalides from isoxazolines.[14,29]

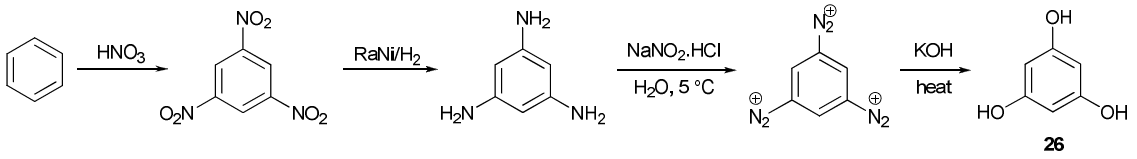
1,3-Dihydroxybenzenes can also be prepared from substituted pyran-2-ones as shown in *Scheme 13* below.[14,30,31]



Scheme 13 – Synthesis of resorcinols from lactones.[14,30,31]

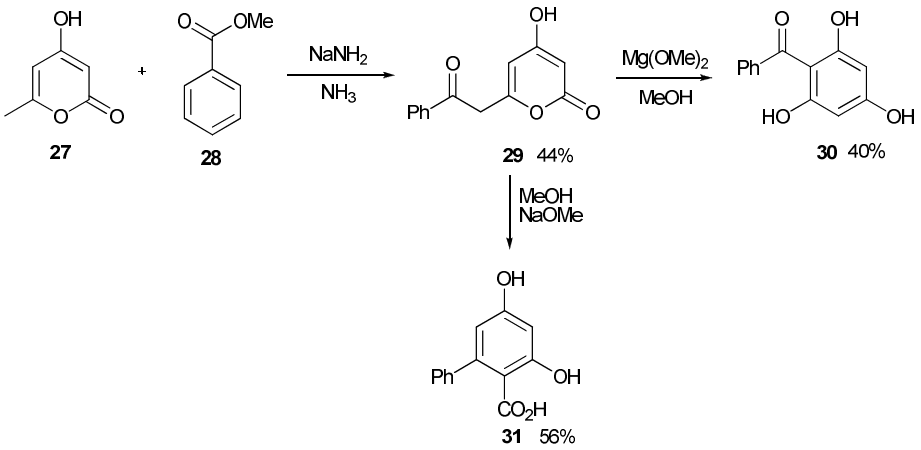
1.3.5 Synthesis of Polyhydroxybenzenes

Leading on from the 1,3-dihydroxybenzenes are the 1,3,5-trihydroxybenzenes, which are also extremely important moieties in plant polyphenols. Like the dihydroxybenzenes, they have also received a lot of attention in regards to their synthesis. One reason for this is due to the commercial importance of trihydroxybenzenes such as phloroglucinol **26** and its derivatives. Phloroglucinol can be isolated from tree bark and is often used in the industrial synthesis of pharmaceuticals and explosives. Commercially it is synthesised on a large scale from the following process, in which the final stages are similar to those shown previously for the synthesis of resorcinol (*Scheme 14*).



Scheme 14 – Synthesis of phloroglucinol ***26.***

An alternative route to substituted 1,3,5-trihydoxybenzenes uses substituted pyran-2-ones, as reported by Harris *et al*.[32] This route is analogous to the reactions previously examined for the synthesis of mono- and di-phenols. Acylation of **27** with methyl benzoate **28** (where the phenyl group can be replaced with other R groups as required) in the presence of sodium amide in liquid ammonia yielded the pyran-2-one **29**, which was subsequently treated with magnesium methoxide to yield the desired 2-substituted phloroglucinol **30** (*Scheme 15*).[32] The product of this reaction contrasts with that shown in *Scheme 13* above for the synthesis of resorcinols,[14,30,31] as in this case phloroglucinol **30** is the main product. It has been reported that aldol-type cyclisations are observed with the treatment of sodium methoxide to give products such as **31**, whereas Claisen-type cyclisations are observed with the use of methanolic magnesium methoxide.[32]



Scheme 15 – Synthesis of substituted 1,3,5-trihydroxybenzenes.[32]

The 1,2,3-trihydroxybenzene moiety is also present in several natural products, such as gallic acid **32**, which is a phenolic plant metabolite biosynthesised *via* the shikimic acid (or shikimate)

pathway.[33] It is normally encountered in plant tissues in the ester form, for example as the catechin esters in green tea (*Diagram 4*). Indeed the regular drinking of green tea has been associated with a reduced risk in several forms of cancer, which is assumed to be due to the antioxidant properties of the catechins. Epigallocatechin-3-gallate **33** is the most abundant catechin in green tea and has been shown to inhibit carcinogenesis.[34-36]

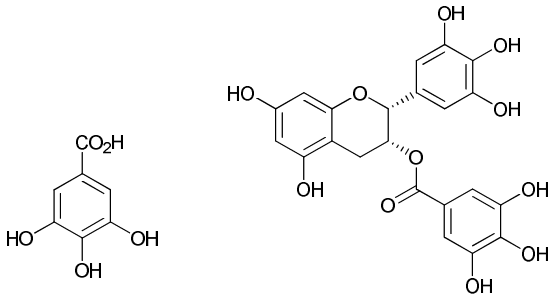
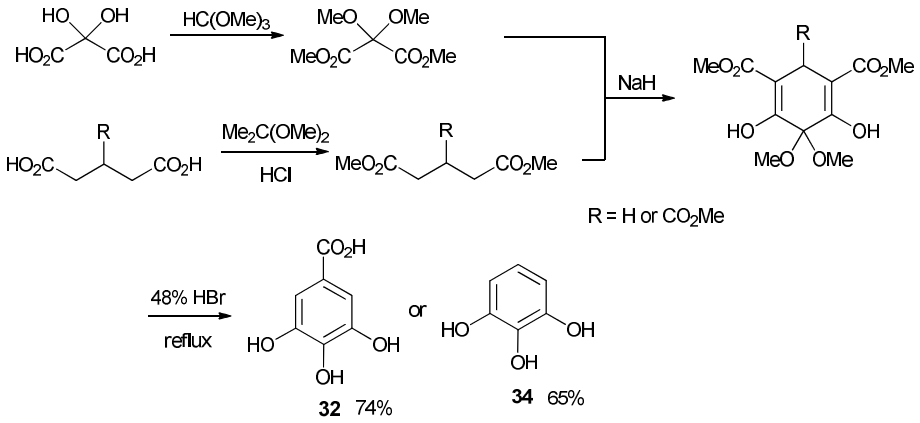


*Diagram 4 – Structures of gallic acid **32** and (-)-epigallocatechin-3-gallate **33**.*

One simple synthesis of gallic acid **32** (R = CO_2H) *via* the base-catalysed condensation of a ketal and a diester, followed by subsequent hydrolysis and decarboxylation is shown in *Scheme 16* below.[14,37] This route can also be applied to the synthesis of pyrogallol **34**, where R = H. Other routes to gallic acid will be examined later (see **Section 1.6**).

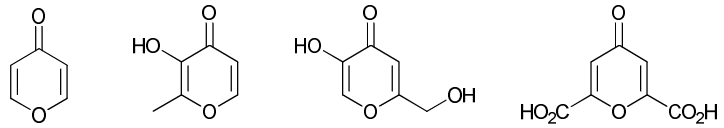


*Scheme 16 – Synthesis of gallic acid **32** and pyrogallol **34** from a ketal and diester.*[14,37]

1.4 Synthesis and Reactivity of 4*H*-Pyran-4-ones and their Derivatives

1.4.1 Synthesis of Pyran-4-ones

Six-membered oxygen heterocycles are a group of compounds which occur widely throughout the plant kingdom. They are also important constituents in pharmaceuticals due to their biological activity.[38] One group of these heterocycles which have already been touched upon are the pyranones – more specifically 4*H*-pyran-4-one **35**. A few of these pyranones do occur as natural products themselves, such as maltol **36** (present in pine needles), kojic acid **37** (isolated from fungus) and chelidonic acid **38** (from the herb *Chelidonium majus*), but their benzo-derivatives (e.g. the coumarins) are more common.[39] However, it seems that the majority of attention has centred around structural aspects of the pyranones rather than on the development of synthetic routes.



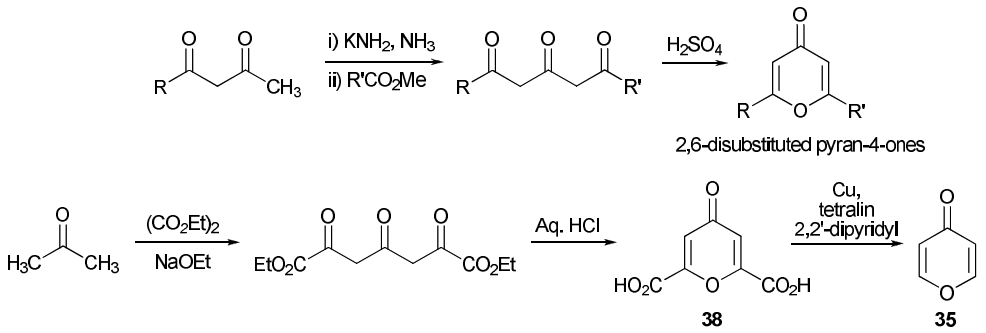
35 **36** **37** **38**

Diagram 5 – Naturally occurring pyran-4-ones.

Compounds of this type are frequently used as flavour enhancers in food. For example, maltol has the odor of caramel, and so is used to give a sweet scent to fragrances. It also has a taste similar to that of freshly baked bread, and is used as a flavour enhancer in breads and cakes.

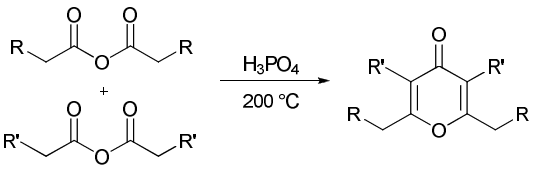
One simple general synthetic route to the ring system involves the initial preparation of a 1,3,5-triketone from the acylation of 1,3-diketones (*via* their anions) followed by acid-catalysed ring closure to yield 4*H*-pyran-4-ones (*Scheme 17*). It should however be noted that 1,3-diketones which are substituted on the central carbon atoms are less reactive under these conditions.[38]

This procedure can be applied to the synthesis of chelidonic acid *via* two Claisen condensations, followed by decarboxylation to produce 4*H*-pyran-4-one if desired (*Scheme 17*).[40]



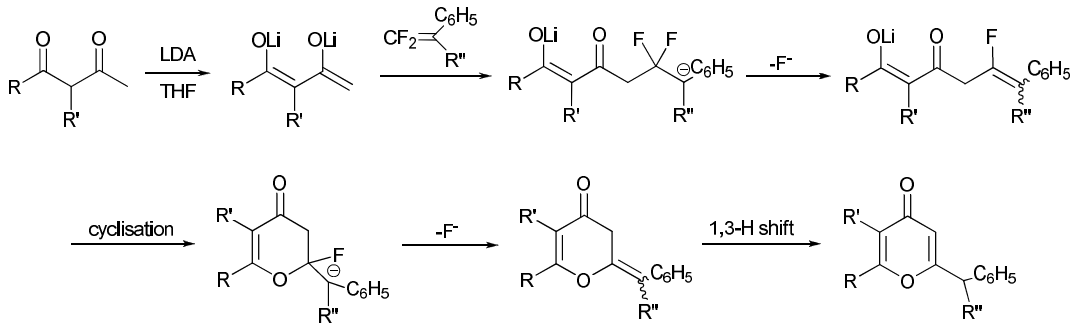
Scheme 17 – 4H-Pyran-4-ones from 1,3-diketones.[40]

Another route to alkyl-substituted pyran-4-ones is shown in *Scheme 18* below.[41] This protocol involves the reaction of aliphatic carboxylic anhydrides with polyphosphoric acid at high temperatures, to yield either 2,6-disubstituted or 2,3,5,6-tetrasubstituted pyran-4-ones depending on the nature of the R groups.



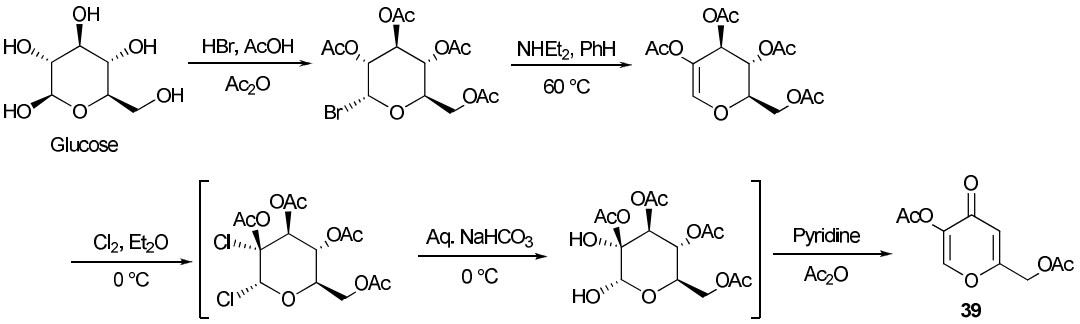
Scheme 18 – Route to alkyl-substituted pyran-4-ones.[41]

In 1997 Kim *el al.*[42] reported a novel synthesis of 4*H*-pyran-4-one derivatives by the treatment of 1-substituted 2,2-difluorostyrenes with dianions of 1,3-diketones, which are generated *via* the reaction of 1,3-diketones with LDA in tetrahydrofuran (THF) at -78 °C. This yields 4*H*-pyran-4-one derivatives in moderate yields after warming to room temperature (*Scheme 19* below).[42]



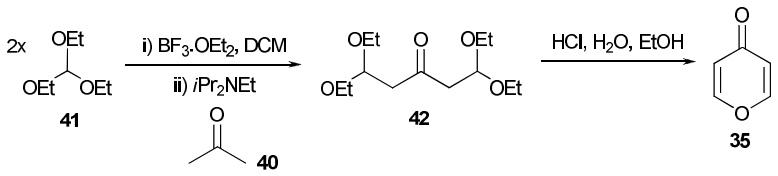
Scheme 19 – Synthesis of 4H-pyran-4-one derivatives.[42]

Another common pyran-4-one is kojic acid, which is produced by several species of fungi. It was first isolated from *Aspergillus Oryzae*, which is a mould used in the preparation of sake (Japanese rice wine) from the fermentation of malting rice. It is frequently used in food and cosmetics to preserve or change colours of substances, as it is a mild inhibitor of pigment formation in both plant and animal tissue. For example, it can prevent oxidative browning in fruits and has both antibacterial and antifungal properties. The synthesis of kojic acid diacetate **39** has been successfully carried out from glucose as shown in *Scheme 20* below.[40]



*Scheme 20 – Synthesis of kojic acid diacetate **39**.*[40]

However, the most efficient synthesis of 4*H*-pyran-4-one itself (**35**) appears to be the reaction between acetone **40** and triethyl orthoformate **41** *via* intermediate **42** as reported by Hobuss *el al.* (*Scheme 21*).[43] This procedure will be examined in more detail later (see **Section 2.2**).



Scheme 21 – Hobuss route to 4H-pyran-4one.[43]

1.4.2 Reactions of Pyran-4-ones

1.4.2.1 General Reactivity of 4*H*-Pyran-4-one

4*H*-Pyran-4-ones can undergo many types of reaction. For example, the pyran-4-one ring can undergo substitution at both the C-3 and C-5 positions when reacted with electrophilic reagents. The pyran-4-ones are more basic than their isomeric pyran-2-ones, due to their betaine structure (*Diagram 6*),[38] where the pyran-4-ones can be seen as 4-hydroxypyrylium salts. The structure possesses substantial π-electron delocalisation and, this can result in the formation of stable salts with acids such as perchloric acid.

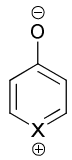
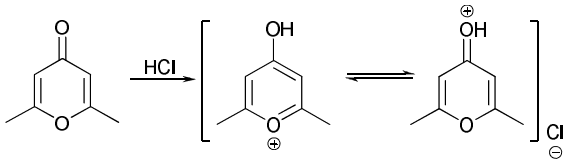


Diagram 6 – Betaine structure of 4H-pyran-4-one.

Many of the pyran-4-ones found in nature are acidic due to the presence of carboxylic acid or hydroxyl substituents.[41] Three such pyranones mentioned previously, maltol **36**, kojic acid **37** and chelidonic acid **38**, are examples of naturally occurring acidic pyran-4-ones.

1.4.2.2 Reactions with Electrophilic Reagents

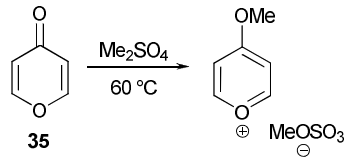
4*H*-Pyran-4-one can react with electrophilic reagents at either the carbonyl oxygen or at ring carbon atoms. It is a weak base with a pKa of 0.3, where protonation occurs at the carbonyl oxygen, as opposed to the ring oxygen. This allows the formation of salts with acids through participation of the betaine structure. *Scheme 22*[40] below shows the formation of a 4-hydroxypyrylium salt using hydrochloric acid. Indeed, the reaction of this 2,6-dimethyl substrate with *tert*-butyl bromide in hot chloroform yields the corresponding 4-hydroxy-2,6-dimethylpyrylium bromide.[44]



Scheme 22 – Pyran-4-one salt formation.[40]

Hydrogen-deuterium exchange can also occur in pyran-4-one. This is an acid-catalysed process and takes place at only the C-3 and C-5 positions, with no exchange occurring at the C-2 and C-6 positions. This process will be examined later (**Section 1.4.2.8**).

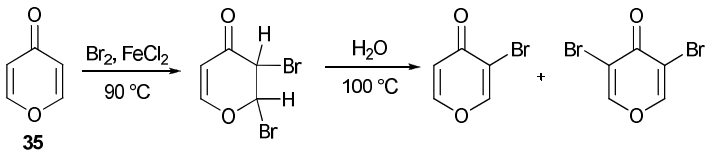
Alkylation of the carbonyl oxygen can occur with dimethyl sulfate[40] due to the polarisation of the pyranone, as previously discussed (*Scheme 23*).[45]



Scheme 23 – Alkylation of the pyranone carbonyl oxygen.[45]

Electrophilic substitution can also take place on ring carbon atoms in the C-3 and C-5 position (*Scheme 24*).[40] This includes the reaction with halogens such as bromine, which can

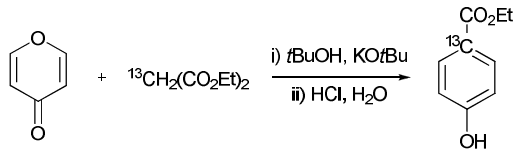
subsequently result in an addition-elimination process, as illustrated below. This is a very useful method for the synthesis of halogenated pyran-4-ones.



Scheme 24 – Electrophilic substitution.[40]

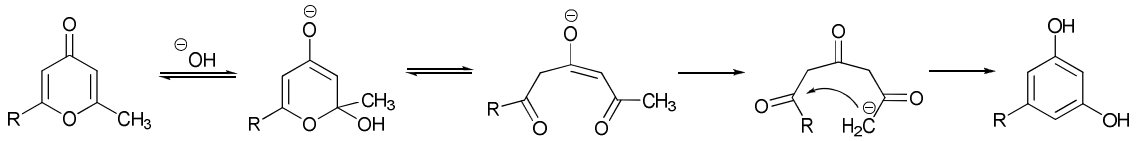
1.4.2.3 Reactions with Nucleophilic Reagents

The reaction of 4*H*-pyran-4-one with nucleophiles is one of the most important reactions in relation to our studies (**Section 2.1**), with pyranone being treated with pronucleophiles in the presence of base, as initially reported by Steglich *el al.* (*Scheme 25*).[22]



Scheme 25 – Steglich synthesis of ethyl 4-hydroxy-[^{13}C]benzoate.[22]

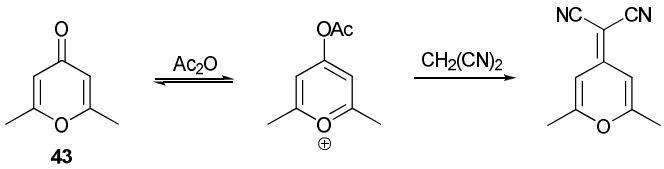
An interesting transformation of a pyran-4-one to a phenol is demonstrated in *Scheme 26* below.[41] This takes place through alkaline hydrolysis where attack takes place at the C-2 position adjacent to the methyl group. Ring opening, followed by an intramolecular aldol condensation yields the substituted phenol.



Scheme 26 – Conversion of pyran-4-ones to phenols.[41]

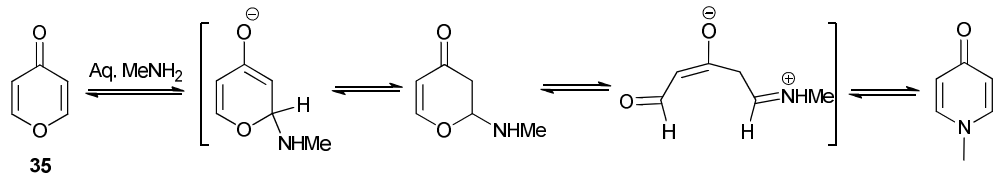
Pyran-4-ones also undergo reactions at the C-4 position in the presence of a Lewis acid. This is demonstrated in *Scheme 27* below where the addition of acetic anhydride to 2,6-dimethyl-pyran-

4-one **43** takes place at the carbonyl oxygen to give a pyrylium cation. Nucleophilic substitution with malononitrile can then occur at C-4.[40,41]



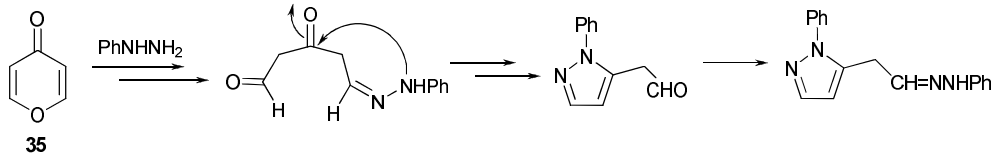
Scheme 27 – Nucleophilic substitution at C-4.[40,41]

Another important reaction of pyran-4-ones with nucleophilic reagents is the reaction with ammonia, or primary amines, to convert pyran-4-ones into pyrid-4-ones as shown in *Scheme 28* below.[40, 46] This occurs *via* attack at C-2, followed by ring opening and subsequent ring closure to yield the pyridone.



Scheme 28 – Pyrid-4-ones from pyran-4-ones.[40,46]

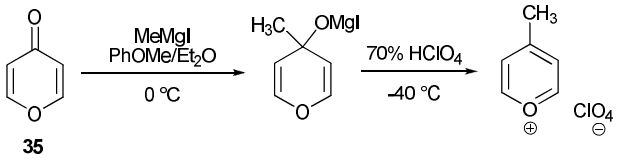
Pyran-4-ones also react with reagents such as phenylhydrazine, resulting in ring-opening. This is then followed by a further intramolecular reaction to give a substituted pyrazole, as shown below.[40]



Scheme 29 – Reaction of pyran-4-one with phenylhydrazine.[40]

As expected, the use of Grignard reagents and other 'hard' nucleophiles gives reaction at the carbonyl carbon. However, this does not allow for ring-opening to take place and therefore the reaction yields a 4-monosubstituted pyrylium salt after reaction with perchloric acid. This results in a simple synthesis of what could otherwise be a relatively inaccessible salt, as shown

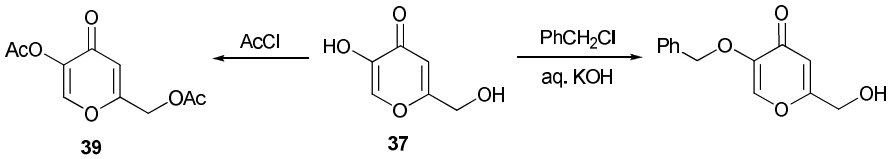
in *Scheme 30*.[40] It is also possible to produce 4,4-disubstituted pyran-4-ones under more vigorous conditions.[47]



Scheme 30 – Reaction of pyran-4-ones with Grignard reagents.[40]

1.4.2.4 4*H*-Pyran-4-one and *O*-Linked Substituents

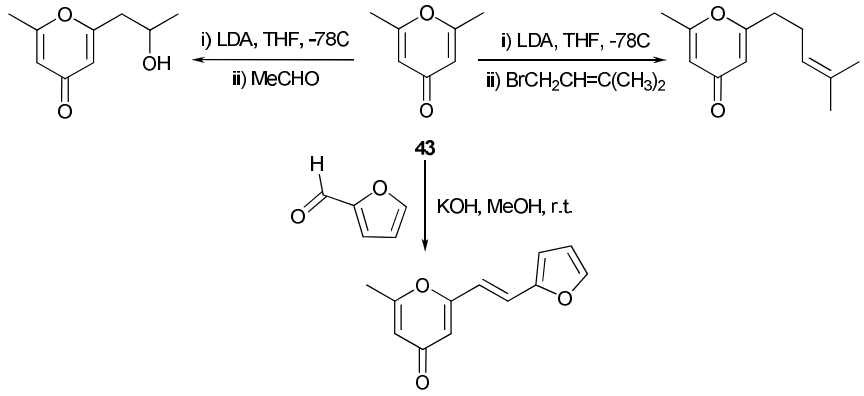
A large number of naturally occurring pyranones, chromones and flavonoids contain one or more oxygen-linked groups. For this reason, the alkylation and acylation of hydroxyl groups, along with the dealkylation of methoxy groups have been greatly studied. These reactions allow the scope of pyranone chemistry to be extended, as the synthesis of phenols from pyranones can be followed by substitutions on the hydroxyl groups. The protocols developed can then be applied to natural product synthesis. For example, kojic acid is one natural product previously discussed, and *Scheme 31*[38] below shows two examples of how the hydroxyl groups on kojic acid can be substituted. It should also be noted that due to the different reactivities of the hydroxyl groups, this can in some cases be utilised for regioselective substitution on one of the two groups.



Scheme 31 – Reactions of kojic acid hydroxyl groups.[38]

1.4.2.5 Side-Chain Reactions

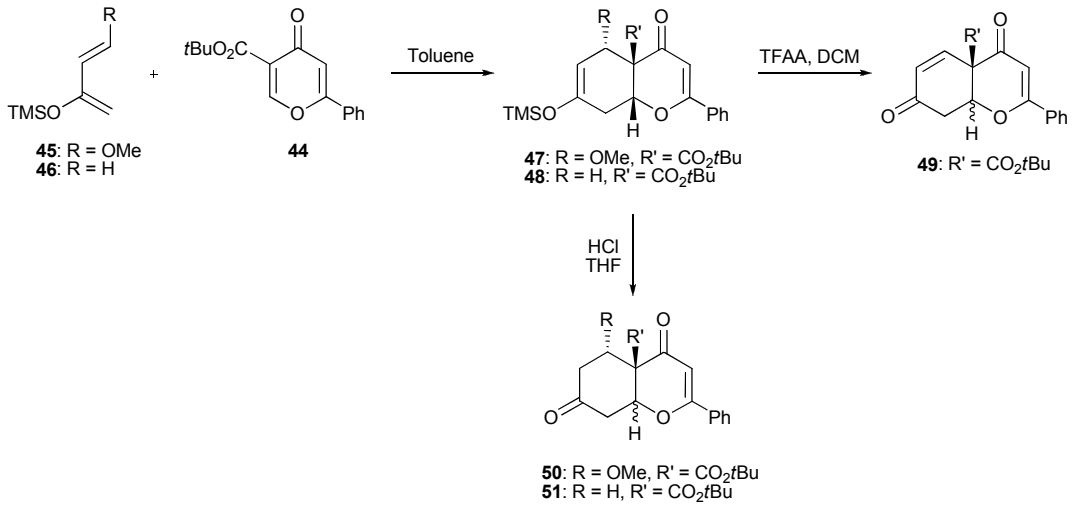
The presence of the carbonyl group has an effect on any ring substituents which may be present. It is known that pyran-4-ones with methyl groups in the 2- and 6-positions, such as 2,6-dimethylpyran-4-one **43**, can be deprotonated by bases such as LDA. Side-chain deprotonation followed by reaction with electrophiles and aromatic aldehydes has been exploited in the synthesis of more complex pyranones, such as those shown below.[48-50]



Scheme 32 – Side-chain reactions of 2,6-disubstituted pyran-4-ones.[48-50]

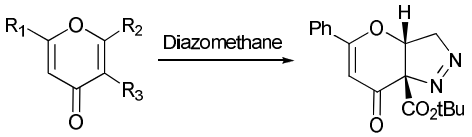
1.4.2.6 Reduced Flavones *via* Diels-Alder Addition to 4*H*-Pyran-4-one

Groundwater *el al.*[51] have reported the synthesis of reduced flavones from 4*H*-pyran-4-ones. This takes place *via* a Diels-Alder cycloaddition of the pyranone **44** with electron-rich dienes,[51] (*Scheme 33*), such as Danishefsky's diene **45**/**46**. The tetrahydroflavones **47** and **48** were then converted to their respective reduced flavone derivatives by reaction with either trifluoroacetic anhydride (**49**) or HCl (**50**) and (**51**).



Scheme 33 – Diels-Alder cycloaddition of pyran-4-ones and Danishefsky's diene.[51]

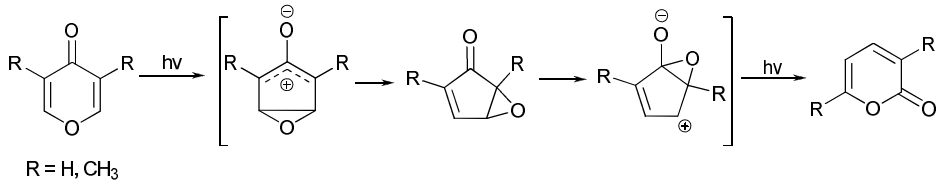
The Diels-Alder approach is an extremely useful route to higher substituted pyranones, as some substituted pyranones can also be used in the reaction, but it should be noted that there are limitations, as shown in *Scheme 34*[51] below, where the only successful reaction with diazomethane is that of pyranone **52** to give the product shown. This shows that the pyran-4-ones lacking electron-withdrawing groups (**53**) do not take part in the 1,3-dipolar cycloaddition. The reaction also fails when an electron-withdrawing ester group is placed in the position beta to the carbonyl carbon, as in pyranone **54**. However, when an ester group is placed in the position alpha to the carbonyl carbon, as in pyranone **52**, the cycloaddition does indeed take place, giving only one diastereoisomer as shown below.



Scheme 34 – Limitations of the 1,3-dipolar cycloaddition.[51]

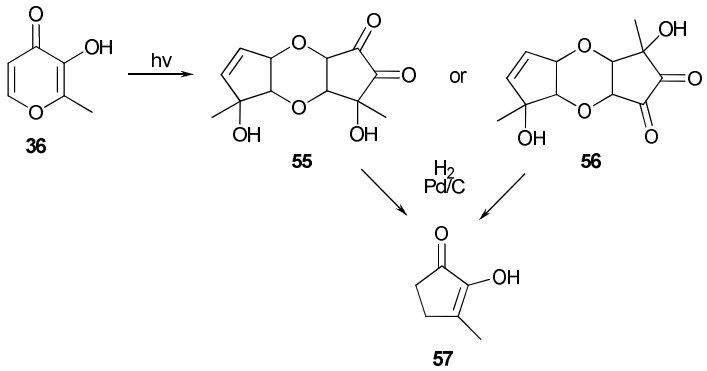
1.4.2.7 Photochemical Reactions

One interesting reaction of pyran-4-one is that it can be photoisomerised to pyran-2-one as shown in *Scheme 35*[38] below. The first stage in the conversion involves the formation of a bicyclic structure containing an epoxide moiety, which after rearrangement and further irradiation gives the final pyran-2-one. This could be potentially useful, and depending on the reactivity it may be possible to employ this procedure after the synthesis of substituted pyran-4-ones.



Scheme 35 – Photoisomerisation of pyran-4-one.[38]

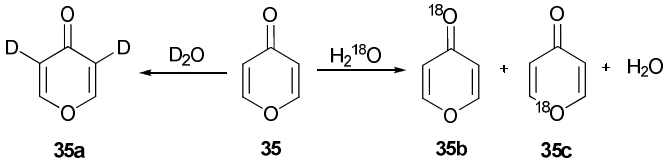
The photolysis of maltol **36** results in dimerisation, with the initial product being the dioxane **55** or **56**, which forms 2-hydroxy-3-methylcyclopent-2-en-1-one **57** upon hydrogenation, as demonstrated in *Scheme 36*[38] below. The final product in this route is one of the natural flavouring components in coffee.



*Scheme 36 – Photolysis of maltol **36.***[38]

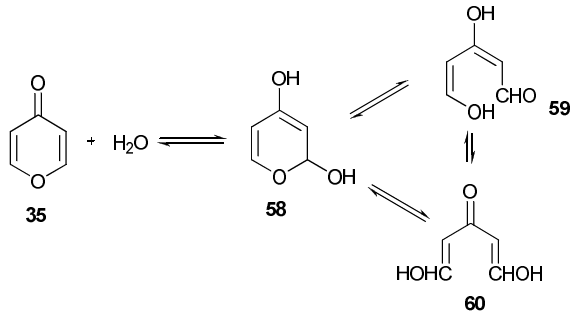
1.4.2.8 Isotopic Substitutions of 4*H*-Pyran-4-one and Derivatives

As previously mentioned in **Section 1.4.2.2**, substitution of protons with deuterium atoms in the C-3 and C-5 positions is possible. This is achieved by treatment with deuterium oxide (D_2O) under neutral or acidic conditions at 98 °C for 26 h, giving 4-[3,5-$^2H_2$]pyran-4-one **35a** as the major product.[52] In addition to this, upon treatment with oxygen-18 enriched water, oxygen-18 atoms can be incorporated into both the carbonyl group (**35b**) and the heterocyclic ring (**35c**), as shown in *Scheme 37* below.



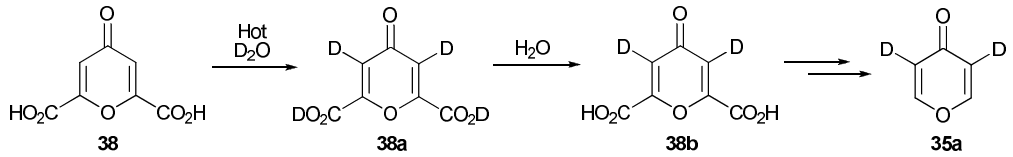
Scheme 37 – Incorporation of deuterium and ^{18}O into 4H-pyran-4-one.[52]

The proposed mechanism for the incorporation of ^{18}O into the pyranone is shown in *Scheme 38* below.[52] It involves the nucleophilic addition of water to the pyranone, which produces compound **58** which can subsequently undergo ring opening to give **59** and **60**. A hydration/dehydration route involving either the pyranone itself (**35**), **59** or **60** would result in the incorporation of ^{18}O into the carbonyl group. In order to incorporate ^{18}O into the ring, either tautomer **59** or **60** must be involved in the mechanism. It is likely that deuteration also occurs by the keto-enol equilibrium of **58**, **59** or **60**.



Scheme 38 – Proposed mechanism of isotope incorporation into 4H-pyran-4-one.[52]

It has been demonstrated that substitution of ring protons in the C-2 and C-6 positions with methyl groups deactivates the ring to proton-deuterium exchange,[53] as the methyl group exerts an inductive effect on the ring. Therefore, due to the presence of the electron-withdrawing 2- and 6-dicarboxyl groups, it was thought that chelidonic acid **38** would be as susceptible to the exchange mechanism as the unsubstituted system. Indeed, under the conditions of hot D_2O, chelidonic acid was converted to 4-[3,5-$^2H_2$]pyrone-2,6-dicarboxylic acid **38a**.[53] The difference in exchange rates between the hydrogen isotopes on the ring positions and the carboxyl groups allowed the preparation of 4-[3,5-$^2H_2$]pyron-2,6-dicarboxylic acid **38b**. This was achieved by rapid recrystallisation from H_2O. If desired, 4-[3,5-$^2H_2$]pyran-4-one **35a** could also be synthesised *via* this route by the hydrolysis of **38b**. The process is summarised in *Scheme 39* below.[53]



Scheme 39 – Exchange reactions of 4-pyrone derivatives.[53]

Although these exchange reactions and isotopic replacement of hydrogen atoms with deuterium are extremely interesting and straightforward, they are not particularly applicable to the chemistry of this project. As previously discussed in **Section 1.1**, deuterated standards are often not suitable for use in GC-MS or LC-MS analysis, as the deuterium atoms can easily exchange back out of the compound under analysis conditions. This is particularly apparent in both aromatic and phenolic compounds, and as the majority of compounds being examined are indeed phenolic, alternative labeling procedures (i.e. using ^{13}C) must be employed.

1.5 Naturally Occurring Polyphenols: Their Occurrence, Properties, Reactions, Syntheses and Importance as Targets for Isotopic Labelling

The flavonoid systems examined in this section are natural products which are widely distributed throughout the plant kingdom, with around 9000 structures identified to date.[54] Flavonoids are generally secondary metabolites and are found in all vascular plants, with many different flavonoids being found within the same species. They play a significant role in many areas of plant biology including protection from UV light, colouration of flowers and in plant defence.[54] Many also appear to have significant beneficial effects in humans, as will be discussed later in this section. The most important flavonoid systems are shown in *Diagram 7*.

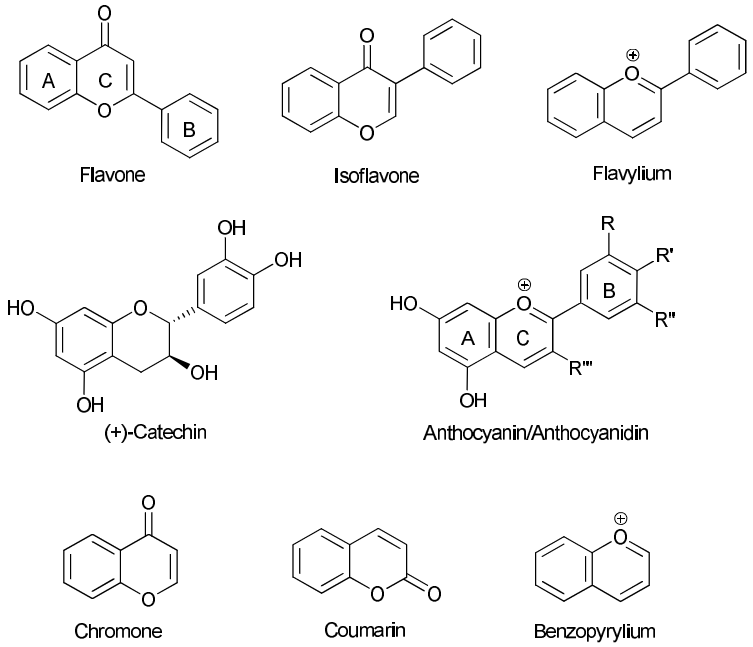


Diagram 7 – Parent structures.

1.5.1 Flavones

The flavones are an important class of naturally occurring flavonoids, based on the structure shown in *Diagram 7*. In plants they usually occur as glycosides with various sugars.[54] Due to the large amount of possible modifications – such as hydroxylation, *O*-methylation and glycosylation – the number of different flavones which can be formed is vast, with over 800

being isolated so far.[54] Naturally occurring flavones are found widely distributed within the plant kingdom, and can accumulate in almost any part of the plant, such as the flowers, fruit, stem, leaves and roots. For example, the bark of North American oak trees (*Quercus velutina*) contains the *O*-glycoside of quercetin **61**, known as quercetrin **62** (*Diagram 8*), and is used in the dye industry.

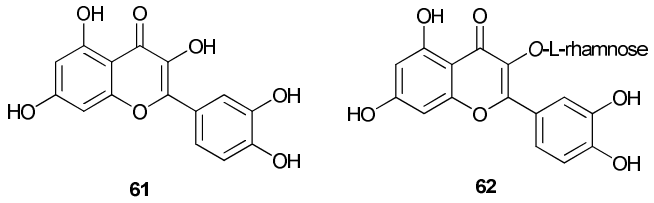


*Diagram 8 – Structures of quercetin **61** and quercetrin **62.***

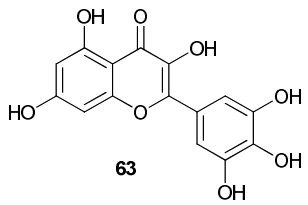
Flavones are currently becoming more commercially important due to their potential applications to various industries, including agriculture and pharmaceutical industries.[54] In terms of agriculture, there is a vast diversity of flavonoids present in the plant kingdom, and this diversity raises the question of the driving force behind the evolution of these compounds. One important benefit that the flavone glycosides have is that they are involved in the UV protection of the plants. They are also involved in various interactions between the plants and other organisms such as insects, microbes, and of course other plants.[54] For example, in blue-coloured flowers, the flavones are present as co-pigments with the anthocyanin delphinidin, producing an intense blue colour and therefore attracting pollinators, such as bees, to the flowers.[54]

Flavones are of specific interest to the dye industry at present due to the desire for "natural" dyes. Environmental concerns have forced the dye industry to reduce their amount of toxic discharges, therefore limiting the number of dyes and pigments which can be synthesised. Naturally-occurring flavones are yellow in colour, although with co-pigmentation with other

molecules they can produce a range of colours. Also they are relatively stable, so will not degrade quickly.[35]

Perhaps the most publicised potential application of flavones at present is that of nutrition and human health. The flavones are abundant in the human diet and can be consumed in the form of vegetables, fruits, juices and teas.[54] The mounting interest is due to increasing evidence that flavonoid compounds possess a wide range of biological activities and could therefore be used in the treatment of disease.[55] Medicinal properties documented include anti-tumour, anti-inflammatory, anti-atherosclerotic, antiviral, and antibacterial activity.[56] More specifically, further studies suggest that the consumption of flavones may be linked with a reduced risk of diseases such as osteoporosis, coronary heart disease and several cancers.[54]

Hydroxy-flavones such as quercetin **61** and myricetin **63** (*Diagrams 8* and *9*) have been shown to exhibit antioxidant activities, which are due to their ability to scavenge free radicals. This shows significant potential for the hydroxy-flavones to be used in the treatment of diseases caused by the action of free radicals.[57]

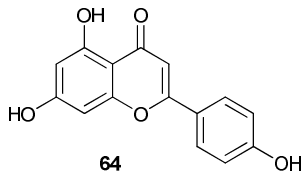


63

*Diagram 9 – Structure of myricetin **63**.*

This leads on to the anti-tumour activities of the flavones. The non-hydroxylated parent compound flavone itself has been proven to be a potent and selective inhibitor of cell proliferation was also found to be more effective towards inducing cell apoptosis than the well-established anti-tumour agent camptothecin.[54] Apigenin **64**, a common dietary flavone, (*Diagram 10*) has also shown to be a potent inhibitor of cell proliferation. This application is

supported by evidence that shows these compounds to be excellent scavengers of free radicals.[54] The use of such flavones to determine SARs (structure activity relationships) could also lead to new flavonoid-based anti-cancer, antiviral and antibacterial treatments.

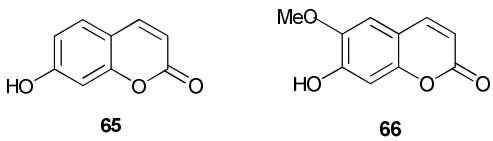


64

*Diagram 10 – Structure of apigenin **64.***

1.5.2 Coumarins

The coumarins (*Diagrams 7* and *11*) are oxygen-containing bicyclic systems which contain the benzo-2-pyrone nucleus. They are widely distributed throughout the plant kingdom as both the aglycone and glycoside derivatives, and can be found in plants such as vanilla grass, sweet grass, and in the highest concentrations in the Tonka bean. Coumarin itself can be found in sweet clover. The most widespread coumarins found in nature are umbelliferone and scopoletin, which are shown below. Umbelliferone **65**, found in carrots, has been used in sunscreens due to its ability to absorb UV radiation. Scopoletin **66**, isolated from passion fruit, is commonly used to regulate blood pressure.



65 **66**

*Diagram 11 – Umbelliferone **65** and scopoletin **66**.*

The coumarins contribute to the smell of newly cut hay and due to their sweet smell they can be used in fragrances.[35,58] During the fermentation of sweet clover, microorganisms produce 4-hydroxycoumarin which reacts with formaldehyde to give dicoumarol (*Diagram 12*). Dicoumarol possesses anticoagulant properties as discovered following the deaths of farm

animals, which could be attributed to the animals' consumption of fermented sweet clover. It interferes with the activity of vitamin K which is essential in the blood clotting mechanism. Therefore without the ability to clot, internal bleeding results. Dicoumarol was then developed for use as an anticoagulant in the treatment of thrombosis, but has more recently been replaced by the likes of warfarin. However, warfarin itself was originally developed for use as rat poison, causing death by internal haemorrhage. *Diagram 12* below shows the structures of 4-hydroxycoumarin, dicoumarol and warfarin.[59]

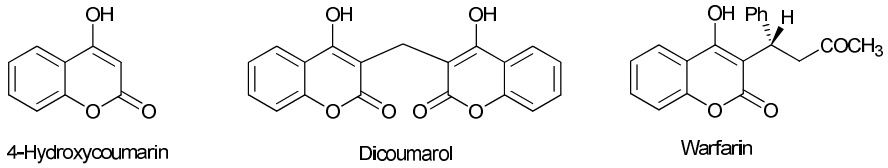


Diagram 12 – Structures of 4-hydroxycoumarin, dicoumarol and warfarin.[59]

The general core of the chromones differs very slightly from the coumarins in their structure, as the carbonyl group is at the 4-position as opposed to the 2-position (*Diagram 7* above). The chromones also possess a wide range of biological activities, including antiviral, anticancer, antifungal and antiallergenic properties. They have been shown to inhibit both tyrosine and protein kinase C, and are active at various receptors such as the benzodiazepine and cyclo-oxygenase receptors.[60] The chromones appear to have a lower toxicity towards humans than the coumarins and are present in large amounts in the human diet.[61,62]

The chromone khellin **67**, isolated from the plant *Ammi visnaga*, is known to cause vasodilation, but cannot be employed as a medicine due to its poor solubility and range of side-effects.[63] Disodium cromoglycate **68**, a derivative of chromone-2-carboxylic acid, is used in the treatment of asthma and has lower toxicity and better efficacy than khellin. The acid itself inhibits the formation of B lymphocytes and the release of inflammatory mediators, many of which are involved in the development of asthma.[64] In conjunction with the use of UV light, topical

khellin can be used in the treatment of the skin condition vitiligo.[65] *Diagram 13* below shows the structures of khellin and cromoglycate.

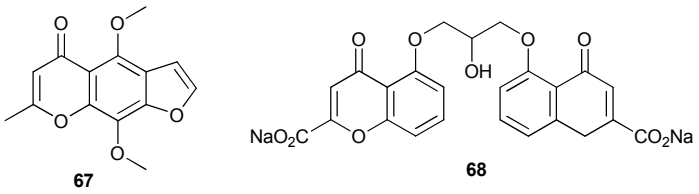


*Diagram 13 – Structures of khellin **67** and disodium cromoglycate **68**.*

1.5.3 Catechins

Diagram 14 below shows the structures of some catechins which have been discovered since catechin itself was first isolated from the Mimosa catechu plant.[66]

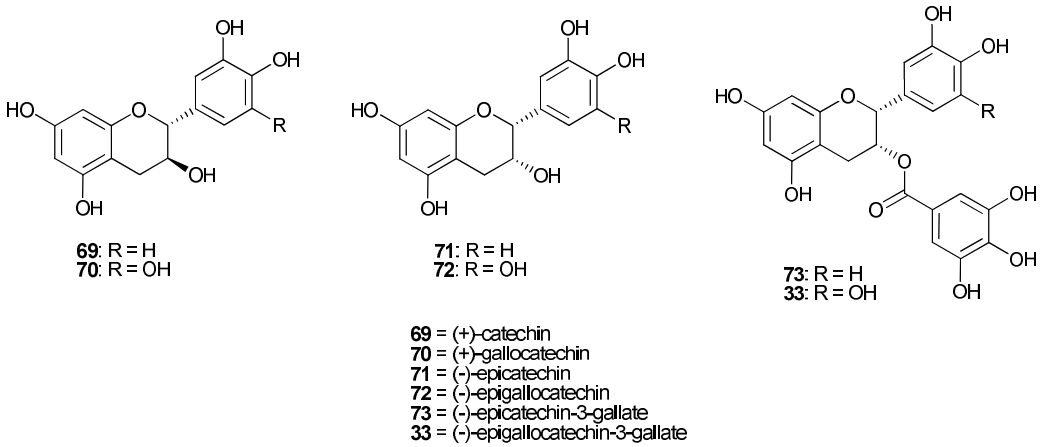


Diagram 14 – Catechins.[36]

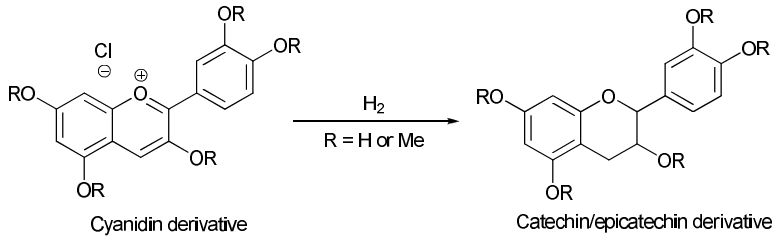
(+)-Catechin **69** and (-)-epicatechin **71** are epimers, whereas (+)-gallocatechin **70** and (-)-epigallocatechin **72** are also epimers but each contain an extra hydroxyl group on the B-ring. The 3-hydroxyl group can be esterified with gallic acid (**33** and **73**), with (-)-epigallocatechin-3-gallate **33** being the most abundant catechin in tea. Indeed, the largest source of catechins in the human diet is tea, which is probably one of the most widely consumed drinks worldwide.[35]

Catechins are also found in fruits, some legumes and wine, thus further increasing their dietary abundance. It was estimated that the average total intake of flavonoids in the United States was approximately 1 g per day, with catechins contributing to 20% of the total intake.[67] (-)-Epicatechin **71** is the most abundant catechin in apples, cherries and pears, whereas (+)-catechin **69** is present in higher concentrations in peaches and mangos. The amount of catechins in various foods varies from low (4.5 mg/kg) in kiwi fruit to very high (610 mg/kg) in dark chocolate.[68] Although the preparation of foods causes a decrease in catechin concentration, the majority of these foods are consumed without further preparation or cooking, and so this should not be an issue. In the case of cocoa and chocolate compared to roasted cocoa beans, different catechins can be isolated from each.[67] In dried, unroasted, unfermented cocoa beans (+)-catechin **69** and (-)-epicatechin **71** were found, whereas in roasted beans (-)-catechin was also found. This product is known to form during the manufacturing process through an epimerisation which is likely to be due to the high temperatures used in the process and also the alkalisation of the cocoa powder.[58] Dietary items such as chocolate and tea still constitute the best means of consuming catechins.

The potential benefits of catechins to humans are currently a topic of great interest and increasing research, as studies have shown decreases in atherosclerosis in animal models and a reduction in the growth of tumours during *in vitro* studies.[69] More specifically, (-)-epigallocatechin-3-gallate **33** has been shown to help in the protection of skin against damage caused by UV radiation, and therefore decreases the incidence of tumour formation.[70] Although the mode of action has not yet been fully established, these effects could be attributed to two factors. Firstly the polyphenolic structure of the catechins allows them to interact with proteins in biological systems, which could result in a vast range of biological activities such as increased or decreased production of hormones and enzymes, or the upregulation of genes. Secondly, like the other aromatic polyphenols, the catechins are strong antioxidants within biological systems, scavenging free radicals and therefore decreasing the amount of damage

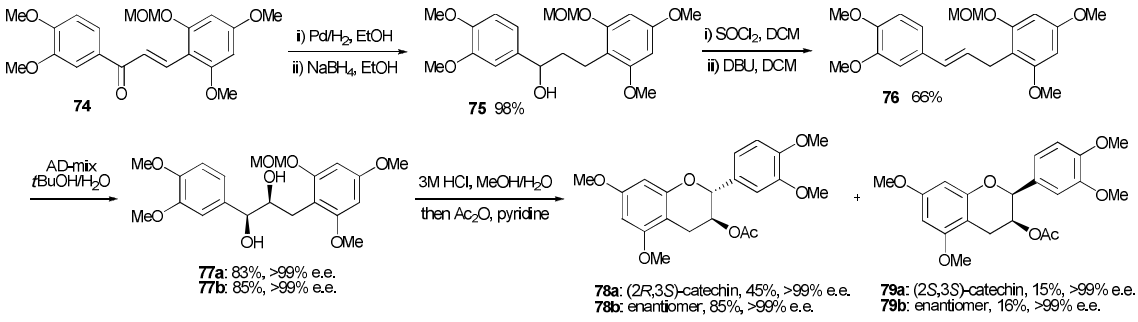
caused by these reactive species. As suggested, due to their antioxidant properties, the regular consumption of foods and drinks high in catechins can reduce the risk of various forms of cancer, along with heart failure, strokes and diabetes.[36] Indeed, one study carried out in Panama[71] showed that the incidence of these conditions was reduced in people who drank up to 40 cups of cocoa per week.

The first synthesis of catechin was reported by Freudenberg *el al.* and involved the hydrogenation of an anthocyanin derivative (*Scheme 40*).[72] The tetramethyl derivative of cyanidin was also successfully converted to the catechin/epicatechin derivative. However, there was no stereocontrol in this process and so a mixture of diastereoisomers was produced.



Scheme 40 – Hydrogenation of cyanidin derivatives to yield catechin/epicatechin derivatives.[72]

More recently van Rensburg *el al.*[73] developed a synthetic route based on the asymmetric dihydroxylation of an oxygenated 1,3-diarylpropene, as outlined in *Scheme 41* below.



Scheme 41 – Enantioselective synthesis of catechins.[73]

After reduction of the chalcone **74** to **75** using a combination of reagents (Pd/H_2 and $NaBH_4$) elimination of the alcohol was achieved by treatment with thionyl chloride and DBU. This

yielded the alkene **76** which was subjected to Sharpless asymmetric dihydroxylation[73] with AD-mix-α or AD-mix-β to give either (*S,S*)-*syn*-diol **77a** or (*R,R*)-*syn*-diol **77b** in good yields and high optical purity. Subsequent deprotection of the MOM groups followed by cyclisation and acetyl protection gave a mixture of **78a,b** and **79a,b** in good yields and high enantiomeric excess.

1.5.4 Anthocyanins

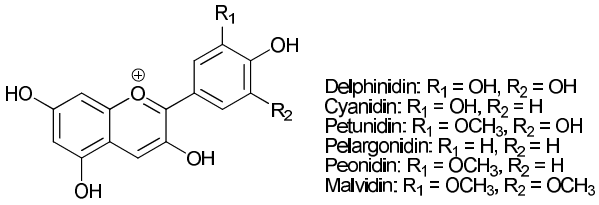
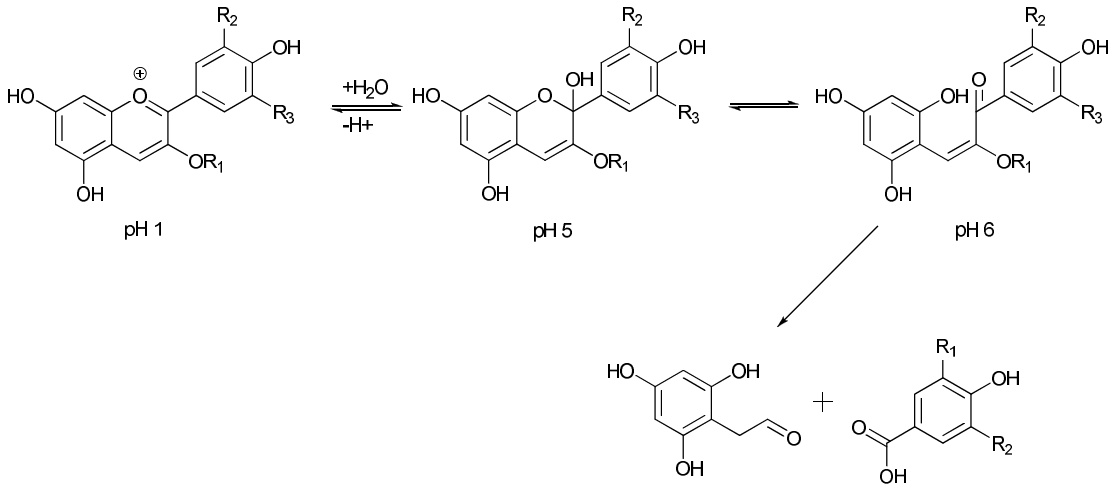


Diagram 15 – The six naturally-occurring anthocyanidins.

Anthocyanins are polyphenols based on the structure of the flavylium cation and were first isolated and characterised by Willstatter and Burdick.[74] They possess a positive charge, of which the counterion is usually Cl^-, and therefore differ from the other flavanoids, which are neutral. The anthocyanidins are sugar-free compounds, whereas the anthocyanins are water-soluble glycosides of the anthocyanidins, with the sugar moiety at the 3-position providing stability to the chromophore. The anthocyanidins are known to suffer irreversible degradation in weakly acidic to neutral conditions as found in nature, *via* the route shown in *Scheme 42* below.[75,76]



Scheme 42 – Degradation of anthocyanins in weakly acidic and neutral media.[75,76]

The extraction and purification of natural anthocyanins are now well-established procedures,[75] with extraction usually carried out using either acidified water or alcohol.[77] The extract is then concentrated to produce the commercial product. The anthocyanins are found in fruits and vegetables with red skin and/or tissue and provide the colour in leaves, stems, flowers, fruits etc. Generally, they are responsible for red, blue, pink and purple colours of these species. In terms of vegetables, cyanidin is the anthocyanidin which causes the colouring in red cabbage, and delphinidin is found in aubergines. These compounds occur in nature as either the glycosides, i.e. the anthocyanins, or the aglycones, i.e. the anthocyanidins. Only six different anthocyanidins occur naturally (*Diagram 15*), but due to the various glycosides which can be obtained a much larger number of anthocyanins can be formed, with many plant species containing a variety of different anthocyanins.[78] The anthocyanins are always glycosylated at the 3-position, but this can also occur at positions 5, 7, 3' and 4.' As well as glucose, other sugars such as galactose, rhamnose and arabinose can be found in anthocyanin structures. The main anthocyanins found in selected fruits are outlined in *Table 1* below,[78] with cyanidin being the most abundant of the anthocyanins.

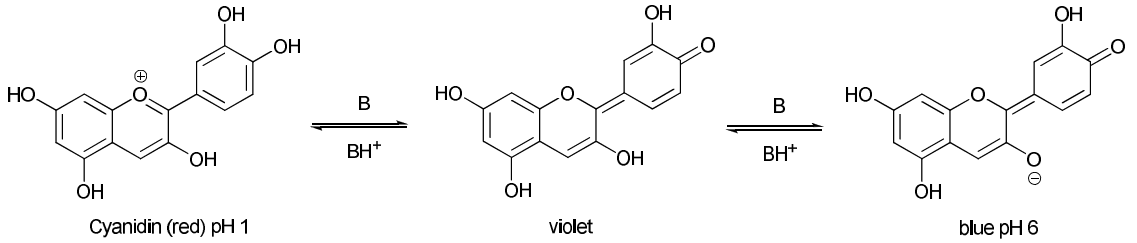
Table 1 – Anthocyanins found in selected fruits.[78]

Fruit	Principal Anthocyanins
Blackcurrant	3-glucosides and 3-rutinosides of delphinidin and cyanidin
Blackberry	3-glucosides and 3-rutinosides of cyanidin
Raspberry	3-glucosides, 3-sophorosides and 3-rutinosides of pelargonidin and cyanidin
Elderberry	3,5-diglucosides and 3-sambubiosides of cyanidin
Strawberry	3-glucosides of pelargonidin and cyanidin, 3-arabinoside of pelargonidin
European grape	3-glucosides, 3-acetylglucosides and 3-coumarylglucosides of malvidin, peonidin, delphinidin, petunidin and cyanidin

The grape anthocyanins in particular are noteworthy, as there is a greater amount of variety of anthocyanins in the grape than in most other plants. However, it is interesting to note that grapes do not contain pelargonidin, although they do contain the five other anthocyanidins – delphinidin, cyanidin, petunidin, peonidin and malvidin.[79] This is relevant in the wine-making process, as the anthocyanins are the pigments of the skins of red grapes and are responsible for the colour of red and rose wines. They are also responsible for the observed changes in colour during the ageing of red wines, with the sugar moiety often being conjugated with organic acids such as acetic, coumeric or caffeic acids.[79]

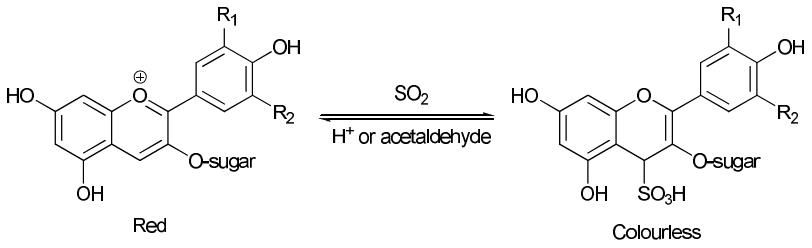
Recently, the anthocyanins have been the subject of a large amount of research with respect to their role in plants, their use in the colouration of foodstuffs and their biological effects on humans. As previously mentioned, the anthocyanins are highly coloured compounds. With increasing substitution on the B-ring the anthocyanins become more red in colour, however it is the combination of pH along with the presence of other substances which has the greatest effect on their colour.[78] For example, the anthocyanins are capable of forming hydrogen-bonded complexes through their hydroxyl groups with other flavonoid compounds. This can stabilise the blue quinoidal forms and enhance the intensity of the colour. As previously outlined in *Scheme 42*, at low pH values, the flavylium cation is the predominant form giving a red colour, but as the pH is raised, a proton is lost and the addition of water occurs to give a carbinol structure, which is colourless. As the pH rises above pH 3, the weakly purple quinoid structure is formed, followed by the quinoin anion at high pH values, giving a deep blue colour.[78] *Scheme*

43 below shows the anthocyanidin (R = H) structures present from pH 1 to pH 6 where the addition of water has not taken place. This pH-dependent colour allows the anthocyanins to be used as pH indicators, where the colour red indicates acidic conditions, and blue more basic conditions.



Scheme 43 – Anthocyanidin structures at pH 1-6.[78]

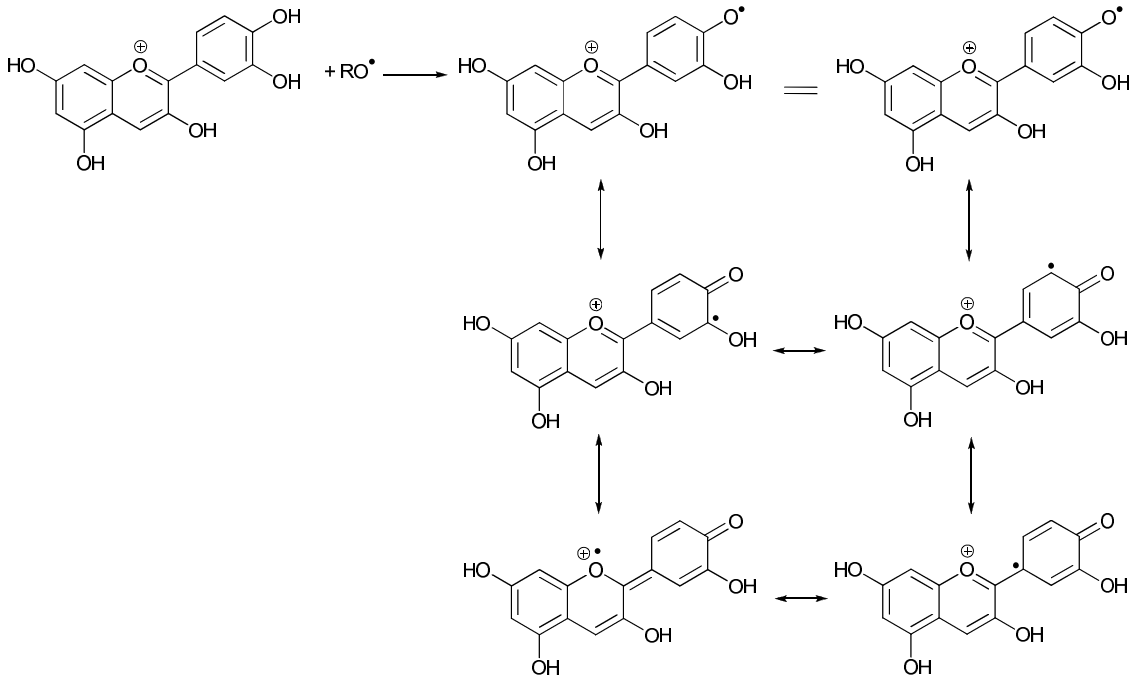
Due to the varied colours which can be obtained from anthocyanins, they are often used for colouring, such as in the confectionary industry and are increasingly replacing synthetic dyes in soft drinks and confectionary. However the pH dependent variation in stability and colour means that these compounds are not generally suitable for colouring foods with a pH above 4.5.[80] Due to a greater stability at low pH values, the anthocyanins are useful for colouring acidic products such as fruit sauces, jams, fruit drinks and sugar confectionary.[80] As the anthocyanins are relatively heat resistant, they can be used in the production of sugar confectionary as elevated temperatures are often reached during manufacture.[77] Some anthocyanins are sensitive to the presence of sulfur dioxide, which can cause problems in the food industry, but these limitations can be overcome by careful selection.[78] As the C-ring bears a cationic charge at the C-4 position, it reacts as an electrophile with the sulfur dioxide. Used at lower concentrations (500-2000 ppm) the SO_2 reacts with the anthocyanin in its flavylium form to give the colourless chroman-4-sulfonic acid, as shown in *Scheme 44* below. When it is present in relatively high concentrations (1-1.5%) bleaching of the anthocyanins occurs, which is not desirable in products where the anthocyanins are required to produce the colour. As white wine always contains sulfur dioxide, this is the basis of using white wine to remove red wine stains on fabrics.[78]



Scheme 44 – Decolourisation of anthocyanins by sulfur dioxide.[78]

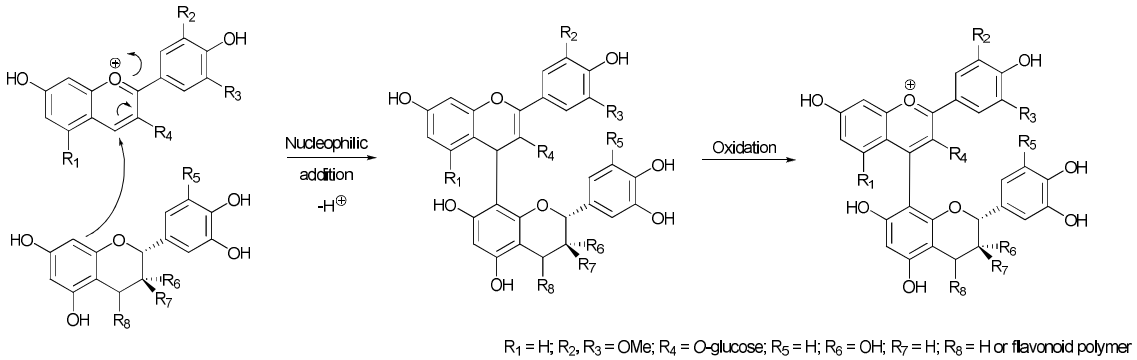
The anthocyanins are also powerful antioxidants, protecting the plant from radicals formed either by UV light or during metabolic processes. *Scheme 45* below[81] shows the route by which anthocyanidins scavenge and stabilise free radicals through multiple resonance structures, thus removing the amount of reactive free radicals present. Interest in anthocyanin pigments has intensified over recent years due to the possible health benefits to humans for a range of diseases.[82,83] Their antioxidant properties can result in reduced amount of oxidative damage to DNA. This in turn reduces the mutation rate of DNA, which is one of the main causes of cancer. Delphinidin and malvidin have been found to be potent inhibitors of enzymes which are responsible for the proliferation of tumours.[84,85] In terms of cardioprotection, antioxidant effects are also important, by preventing the peroxidation of LDLs (low-density lipoproteins), causing a decrease in the accumulation of atherosclerotic plaques, and subsequently reducing the risk of coronary heart disease.[82,86] This backs up a popular theory that the drinking of red wine can result in much lower rates of coronary heart disease. In countries such as France and Italy, where the consumption of red wine is higher than in North American and North European countries, there are significantly lower rates of coronary heart disease. One crucial factor for the anthocyanins is that this antioxidant property is retained after the plant has been consumed, and so fruits and vegetables containing anthocyanins are a good dietary source of antioxidants. Consumption of anthocyanin-containing foods may therefore help to prevent coronary heart disease, cancer and strokes. A small amount of research has also been carried out into the neuroprotective properties of the anthocyanins, and they have been found to enhance memory and contribute to preventing age-related declines in neural function through their inhibition of

oxidative stress on the brain.[86,87] The anthocyanins were also used in traditional medicine for the treatment of visual problems. They have been shown to increase blood circulation in the retina, improve night vision by enhanced generation of retinal pigments, and prevent conditions such as glaucoma and cataracts.[86]



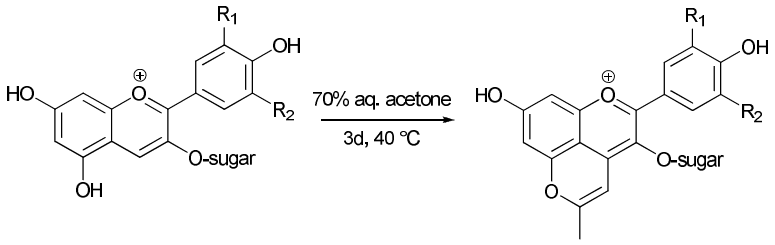
Scheme 45 – Anthocyanidin resonance forms after reaction with free radicals.[81]

The anthocyanins are also able to react with other flavonoids to produce procyanidins. This linkage takes place between the C-4-position of the anthocyanin and the C-8-position of the other flavonoid by nucleophilic addition to the anthocyanin. *Scheme 46* below shows a postulated mechanism[88] for the formation of anthocyanin-flavanol adducts involving nucleophilic addition followed by oxidation by a second molecule of anthocyanin to yield the adduct. The procyanidins formed from anthocyanins exhibit more intense colours and are more stable to both sulfur dioxide and to changes in pH.[78] The brown colour observed in old wines is due to these reactions between anthocyanins and other flavonoids.



Scheme 46 – Formation of anthocyanin-flavanol adducts.[88]

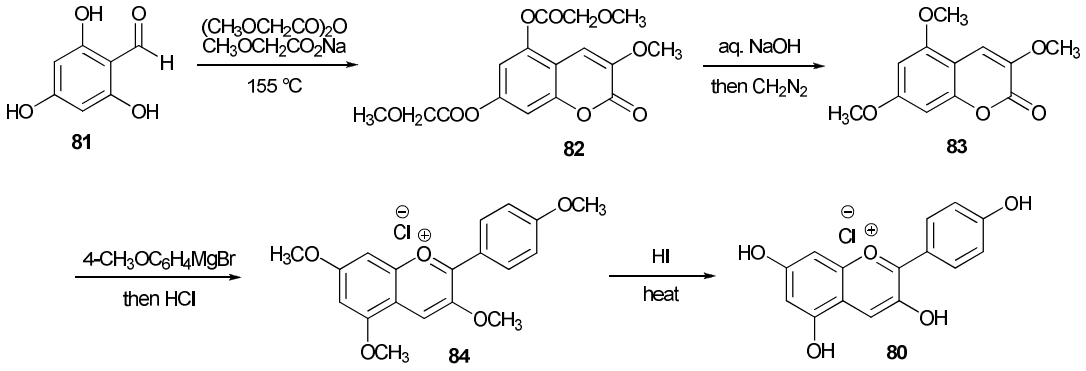
A relatively new reaction has been reported by Lu *el al.*[89] between the anthocyanins and acetone, yielding pyranoanthocyanins. Although previous reports of reactions involving flavylium compounds showed that they readily undergo addition at C-4 with a range of nucleophiles,[90] there were no reports of reactions with acetone. This reaction was discovered upon isolation of anthocyanins from blackcurrant seeds using acetone, until recently the solvent of choice, and alcohol as extraction solvents. It was found that the pyranoanthocyanin peaks were present in the acetone extracts but not in the alcohol extracts. Separate experiments were then carried out using isolated blackcurrant anthocyanins dissolved in 70% aqueous acetone at 40 °C (*Scheme 47*).[89] After 3 days HPLC analysis showed almost full conversion from the original anthocyanins to pyranoanthocyanins. Indeed it has also been shown that this extended conjugated system results in greater stability in relation to the anthocyanins and could potentially be used to stabilise colour in dye products.



Scheme 47 – Oxidative addition of acetone to anthocyanins to yield pyranoanthocyanins.[89]

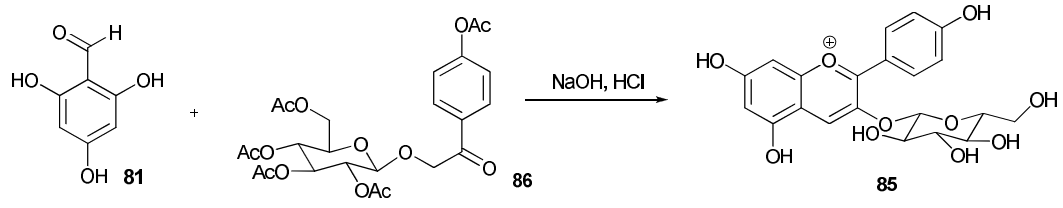
1.5.4.1 Synthesis of Anthocyanidins and Anthocyanins

The first reported synthesis of an anthocyanidin was in 1924 and was carried out by Willstaater *et al*.[91] They reported the synthesis of pelargonidin chloride **80** from 2-formylphloroglucinol **81**, as shown in *Scheme 48*. The resulting dimethoxyacetyloxy-substituted coumarin **82** was then concurrently deprotected and reprotected to give trimethoxycoumarin **83**. Treatment with the 4-methoxyphenyl Grignard reagent yielded the tetramethoxy-substituted anthocyanin **84**, which was subsequently deprotected using hydrogen iodide to give the desired pelargonidin chloride **80**.[91]



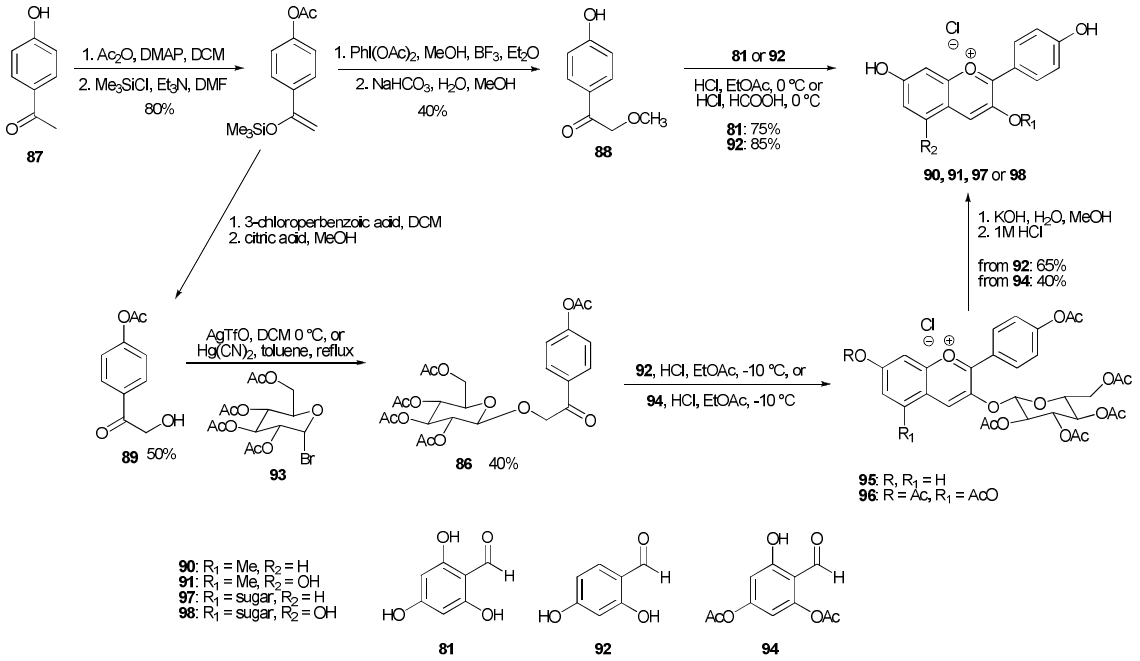
*Scheme 48 – Willstaatter synthesis of pelargonidin chloride **80**.*[91]

Shortly after this in 1928 was the first synthesis of an anthocyanin (**85**) by Robertson and Robinson[92] *via* the condensation and cyclisation of 2-formylphloroglucinol **81** and a protected glycosylated aromatic ketone **86** to give pelargonidin-3-glucoside **85** (*Scheme 40*).



*Scheme 49 – Robertson and Robinson synthesis of pelargonidin-3-glucoside **85**.*[92]

A similar route was utilised by Dangles *el al.*[75] in 1994 in the synthesis of a range of anthocyanins. In fact two routes (*Scheme 50*) were established which allowed the synthesis of glycosylated and non-glycosylated anthocyanins as desired.



Scheme 50 – Dangles route to anthocyanins.[75]

The first stage in both routes involved the acetylation of 4-hydroxyacetophenone **87** followed by treatment of the enol form with trimethylsilyl chloride. Treatment at this point was either with (diacetoxyiodo)benzene and boron trifluoride in methanol to yield the α-methoxy-substituted compound **88**, or with *m*CPBA followed by citric acid to yield the alcohol **89**. From the first route, the flavylium chlorides **90** and **91** were obtained upon condensation of **88** with either 2,4-dihydroxybenzaldehyde **92** or 2-formylphloroglucinol **81** in an atmosphere of hydrogen chloride to give the aglycones. *Via* the second route it was found that glycosylation of **89** could be achieved using 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide **93** with soft Lewis acids such as mercury cyanide or silver triflate. Condensation was once again carried out under a hydrogen chloride atmosphere in an aprotic solvent using either 2,4-dihydroxybenzaldehyde **92** or 2,4-diacetoxy-6-hydroxybenzaldehyde **94** to give **95** and **96** respectively. De-acetylation gave the target compounds **97** and **98**.

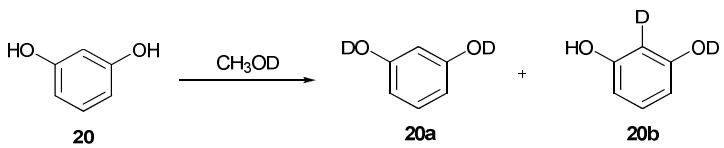
1.6 Previous Studies on the Isotopic Labelling of Resorcinol, Phloroglucinol, Gallic Acid and the Anthocyanins

1.6.1 Resorcinol

As previously discussed in **Section 1.3.3**, resorcinol **20** belongs to the family of dihydroxybenzenes found abundantly in nature, and in particular as a component of plant phenolic compounds. Often it is the resorcinol moiety which is present as part of a larger substructure, however resorcinol itself has also been isolated from the broad bean, tobacco leaves, and other plant species. The main use of resorcinol is in the rubber industry, but it also has medical applications as an antiseptic, a disinfectant, and a treatment for skin conditions such as eczema. In this section, the limited literature surrounding the synthesis of isotopically-labelled versions of resorcinol will be examined and discussed in relation to our strategy and desired routes.

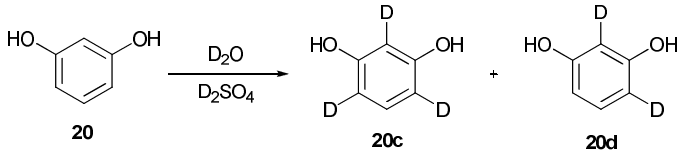
1.6.1.1 Hydrogen-Deuterium Exchange with Resorcinol

Few examples of deuterated versions of resorcinol were found, although there are examples of both the phenolic and aromatic protons being substituted. In the preparation of 1,3-dihydroxy-d_2-benzene **20a**, Schröder *el al.*[93] replaced the phenolic protons with deuterium by repetitive evaporation of a solution of resorcinol in deuterated methanol. Due to the presence of the labile aromatic proton at the C-2 position, an intermolecular self-exchange takes place between the ring and hydroxyl protons, resulting in a mixture of deuterated species **20b** (*Scheme 51*).[93]



Scheme 51 – Schröder method for preparation 1,3-dihydroxy-d_2-benzene.[93]

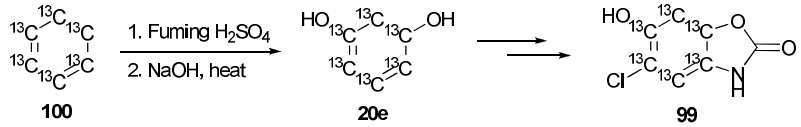
Methods for the deuteration of the aromatic protons include the use of D_2O and a deuterated acid catalyst such as D_2SO_4. In these cases the exchange takes place *via* an acid-catalysed proton-deuteron exchange. Calucci *el al.*[94] reported the preparation of resorcinol-d_3 **20c** (1,3-dihydroxy-[2,4,6-$^2H_3$]benzene) with deuterium replacing aromatic protons at the 2-, 4-, and 6-positions (*Scheme 52*). Nakashima *el al.*[95] also reported the synthesis of the 2,4,6-trideuterated compound **20c**, but with the 2,4-dideuterated compound **20d** also present. Neither route observed the formation of the 1,3-dideuterated compound as the hydroxyl proton exchanged with that of water and could not be independently observed on the NMR time scale.[95]



Scheme 52 – Calucci and Nakashima route for preparation of deuterated resorcinol.[94,95]

1.6.1.2 [$^{13}C_6$]Resorcinol[96]

Two routes towards ^{13}C-labelled versions of resorcinol have been reported, with the potential of application to the use of ^{14}C if desired. The first of these was reported by Jian *el al.*[96] as an intermediate in studies towards the synthesis of ^{13}C-labelled 6-hydroxychlorzoxazone **99** (*Scheme 53*). Their procedure yielded [$^{13}C_6$]resorcinol **20e** in 51% yield from [$^{13}C_6$]benzene **100** by treatment with fuming sulfuric acid, followed by fusion with sodium hydroxide at high temperatures. As [$^{14}C_6$]benzene is also commercially available, it would be possible to apply this route to the synthesis of [$^{14}C_6$]resorcinol.

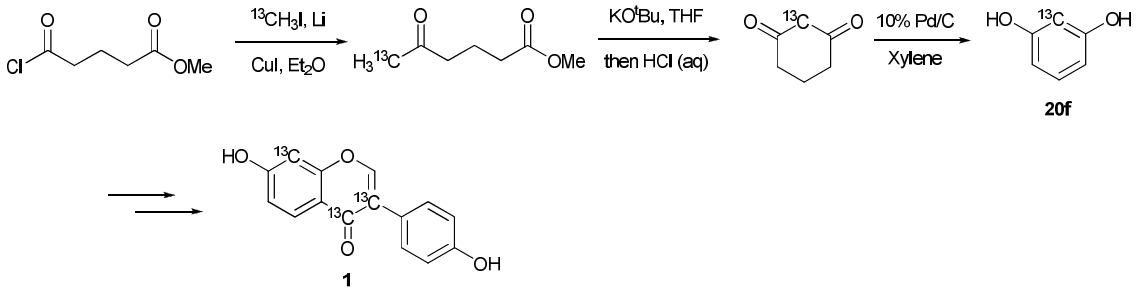


*Scheme 53 – Jian route to the preparation of [$^{13}C_6$]resorcinol **20e** in the synthesis of [$^{13}C_6$]6-hydroxychlorzoxazone **99**.*[96]

1.6.1.3 [2-^{13}C]Resorcinol[5]

The second route towards ^{13}C-labelled resorcinol was developed in the Botting group,[7] and involves the regioselective placement of one ^{13}C-atom into the aromatic ring. As previously discussed in **Sections 1.2** and **1.3.3** this approach is beneficial as it involves the use of ^{13}C-labelled small molecules, which are cheaper and more readily available than uniformly-labelled aromatic compounds.

This route to [2-^{13}C]resorcinol **20f** improved on a previous route to the compound,[97] and was developed further for use in the synthesis of the soy isoflavone [3,4,8-$^{13}C_3$]daidzein **1**.[7] Details of this route will be discussed later (see **Section 2.5**), as it will be the method used during the synthesis of phloroglucinol and the target anthocyanins.



*Scheme 54 – Oldfield route to [2-^{13}C]resorcinol **20f** in the synthesis of [3,4,8-$^{13}C_3$]daidzein **1**.*[7]

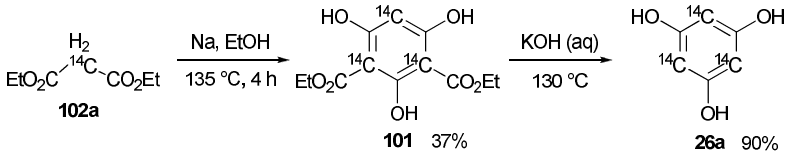
1.6.2 Phloroglucinol

Phloroglucinol itself was originally isolated from tree bark, but the 1,3,5-trihydroxybenzene moiety can be found in many natural products in either the protected or unprotected form. One route to phloroglucinol involves the functionalisation of benzene and therefore it can be synthesised in the $^{13}C_6$- or $^{14}C_6$-labelled forms *via* routes such as that outlined for resorcinol in *Scheme 53*. However, as our aim is to synthesise ring-labelled versions with regiospecific placement of the ^{13}C- or ^{14}C-atoms, this route is not applicable. Indeed very few examples

could be found in the literature relating to ^{14}C-labelled derivatives and none relating to ^{13}C-labelled derivatives. To the best of our knowledge, only two chemical routes have been published, and these are outlined below.[98,99]

1.6.2.1 [2,4,6-$^{14}C_3$]Phloroglucinol[98]

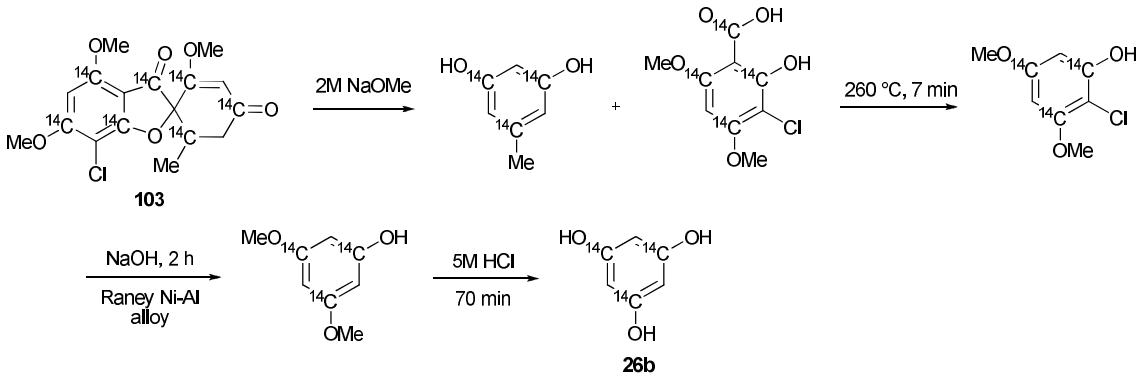
Pateschke *el al.*[98] developed a route to [2,4,6-$^{14}C_3$]phloroglucinol **26a** *via* the diester **101**, with the source of the isotope labels being diethyl [^{14}C]malonate **102a**. After reaction with sodium ethoxide (formed *in situ* from sodium and ethanol) at 135 °C for 4 h, phloroglucinol dicarboxylic diethyl ester **101** was hydrolysed in refluxing potassium hydroxide solution to yield the tri-labelled phloroglucinol **26a** in 33% over 2 steps.(*Scheme 55*).



*Scheme 55 – Patschke route to [2,4,6-$^{14}C_3$]phloroglucinol **26a.***[98]

1.6.2.2 [1,3,5-$^{14}C_3$]Phloroglucinol[99]

An alternative route to a tri-ring-labelled isotopomer of phloroglucinol **26b** was published by Birch *el al.*,[99] where the ^{14}C-atoms are adjacent to the hydroxyl groups. They were examining the incorporation of ^{14}C-labelled acetic acid ($CH_3{}^{14}CO_2H$) into griseofulvin **103**, which is a natural product isolated from mould and is used to treat fungal infections in humans and animals. Griseofulvin contains a phloroglucinol ring, and Birch *el al.* carried out the degradation of the griseofulvin ring system using chemical means to produce phloroglucinol (among other compounds).



*Scheme 56 – Chemical degradation of griseofulvin **103** to [1,3,5-$^{14}C_3$]phloroglucinol **26b**.*[99]

1.6.3 Gallic Acid

As previously discussed in **Section 1.3.5**, the 1,2,3-trihydroxybenzenes are present in several natural products, with gallic acid being given as one example of this group of compounds. In the context of our research, it is necessary to be able to synthesise gallic acid in isotopically labelled forms to allow incorporation into larger natural products if desired. This section will examine the few methods which have so far been used to synthesise labelled gallic acid, although it should be noted that previous to our work,[34] no ring-labelled versions of gallic acid had been prepared.

1.6.3.1 Deuteration of Gallic Acid[100,101]

One example of isotopically labelled gallic acid was developed by Zeng *el al.*[100] and involves the substitution of phenolic, acidic and aromatic protons with deuterium to give **32a** (*Diagram 16*). This was achieved by reaction of unlabelled gallic acid in a D_2O solution of trifluoroacetic anhydride, giving the desired compound in 92% yield. Although this is a fast and efficient procedure, it is not applicable to our synthetic route as the loss of deuterium atoms will be rapid, resulting in the presence of mixtures of deuterated species in solution.

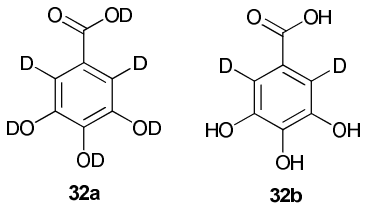
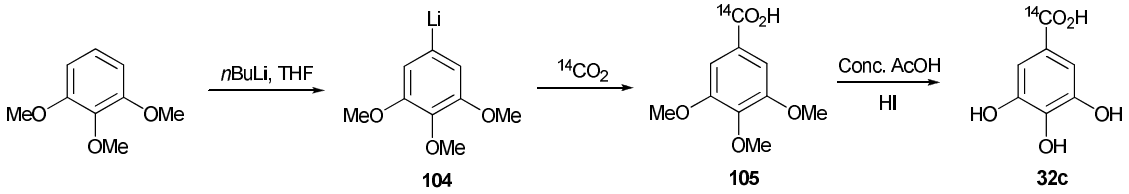


Diagram 16 – Deuterated gallic acid.[100,101]

Tuck *el al.* also studied the deuteration of gallic acid,[101] to give [2,6-$^2H_2$]gallic acid **32b** (*Diagram 16*). They developed a simple procedure for the deuteration of phenols using Amberlyst 15, which is a polymer-supported acid catalyst, and subsequently applied this to a number of phenols present in olive oil. However, as previously discussed, ring-deuterated phenols are not stable under GC-MS or LC-MS conditions and so this procedure will not be elaborated upon further.

1.6.3.2 [*carboxy*-^{14}C]Gallic Acid[102,103]

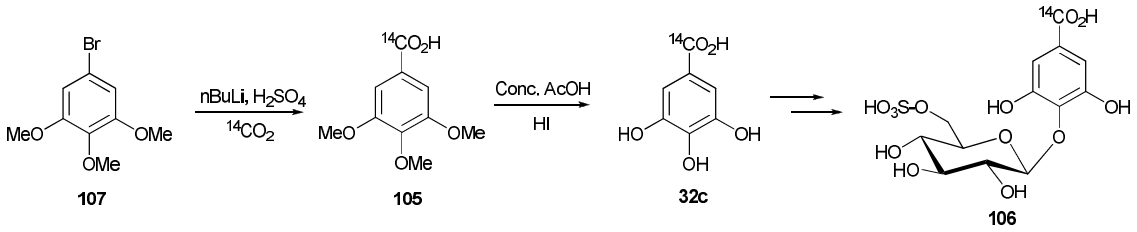
Two routes to [*carboxy*-^{14}C]gallic acid **32c** have been reported, by Zeng *el al.*[102] and Schildkeckt *et al*.[103] The first of these processes involves the reaction of $^{14}CO_2$ with lithiated 3,4,5-trimethoxybenzene **104**, followed by demethylation of **105** to give isotopically labelled gallic acid **32c** as demonstrated in *Scheme 57* below.



*Scheme 57 – Zeng route to [carboxy-^{14}C]gallic acid **32c.***[102]

The second route was developed during the synthesis of 4-*O*-(β-D-glucopyranosyl-6-sulfate)gallic acid **106**. This involved the reaction of the bromide **107** (obtained *via* lithiation)

with $^{14}CO_2$, once again followed by demethylation of **105** to yield [*carboxy*-^{14}C]gallic acid **32c**, as shown in *Scheme 58* below.[103]



*Scheme 58 – Schildkneckt route to [carboxy-^{14}C]gallic acid **32c**.*[103]

1.6.4 Anthocyanins, Anthocyanidins and Procyanidins

As outlined in **Section 1.5.4**, the anthocyanins are a group of polyphenols which are highly abundant in nature, with over 500 different anthocyanins being isolated from plant species. The anthocyanin group consists of the sugar-free anthocyanidins and the water-soluble anthocyanin glycosides – the form in which the majority of isolated anthocyanins are found (*Diagram 17*).

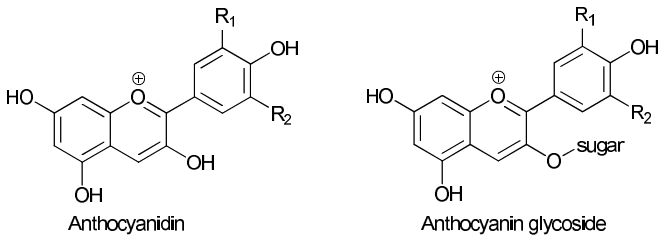


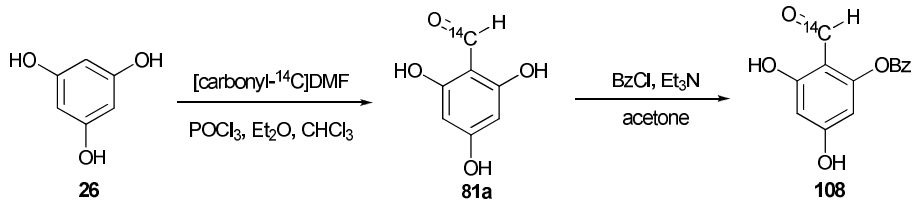
Diagram 17 – Structures of the anthocyanins and anthocyanidins.

Although the extraction and purification of anthocyanins from plant species are well-established, they only tend to yield small amounts of the desired compounds. In order for studies to be carried out on these compounds there is a requirement for the development of a simple and high yielding route to these compounds. Due to ongoing interest into the antioxidant properties of the anthocyanins, it is desirable to be able to synthesise anthocyanins in isotopically-labelled forms, with regioselective placement of labelled atoms into the molecule. This will allow LC-MS and GC-MS analysis to be carried out on biological samples, thus aiding

studies into the absorption, metabolism and excretion of the anthocyanins, as described in **Section 1.1**. To date only one example of the synthesis of carbon-labelled anthocyanidins has been published,[104] with no routes being found which use ^{13}C, and no examples of labelled anthocyanin glycosides. The presence of the glycosyl group at the 3-position is common to naturally occurring anthocyanins and is known to provide stability to the chromophore under weakly acidic to neutral conditions. It would therefore be desirable to obtain a synthetic route to these compounds in order for them to be studied further.

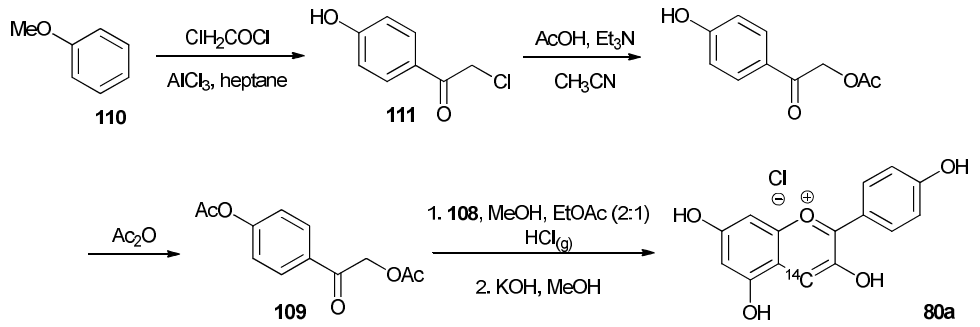
1.6.4.1 [4-^{14}C]Pelargonidin chloride[104]

The synthesis of [4-^{14}C]pelargonidin chloride **80a** as reported by Kraus *el al.*[104] was achieved *via* 2-(benzoyloxy)-4,6-[*formyl*-^{14}C]dihydroxybenzaldehyde **108** and ω,4-diacetoxyacetophenone **109**. The first step involved the introduction of a ^{14}C-labelled formyl group to phloroglucinol **26** by the Vilsmeyer reaction. Acylation of **81a** with benzoyl chloride followed to yield 2-(benzoyloxy)-4,6-dihydroxybenzaldehyde **108** which contained a mixture of mono- and di-benzoylated products (*Scheme 59*). In the final anthocyanidin **80a**, this moiety will represent the A-ring, with the ^{14}C-labelled carbon of the formyl group becoming the 4-position in the heterocyclic ring. This makes analysis of the ^{13}C-NMR of the final product more straightforward to interpret, as the shift of this carbon will be significantly different from that of the formyl group in component **108**, and will be easily observed in both spectra due to enhancement of the ^{13}C peak.



*Scheme 59 – Preparation of **108** in the synthesis of [4-^{14}C]pelargonidin chloride **80a.***[104]

The B-ring of the anthocyanidin originates from ω,4-diacetoxyacetophenone **109** (*Scheme 60*). The synthesis of this component began with the acylation of anisole **110** using chloroacetyl chloride and $AlCl_3$, which also resulted in deprotection of the methyl ether giving phenol **111**. Reaction with acetic acid in triethylamine followed by acetylation with acetic anhydride yielded **109**. The final coupling of **108** and **109** to yield [4-^{14}C]pelargonin chloride **80a** in an overall yield of 3.8% was achieved by treatment with methanol and ethyl acetate in an atmosphere of hydrogen chloride gas followed by deprotection using potassium hydroxide in aqueous methanol.

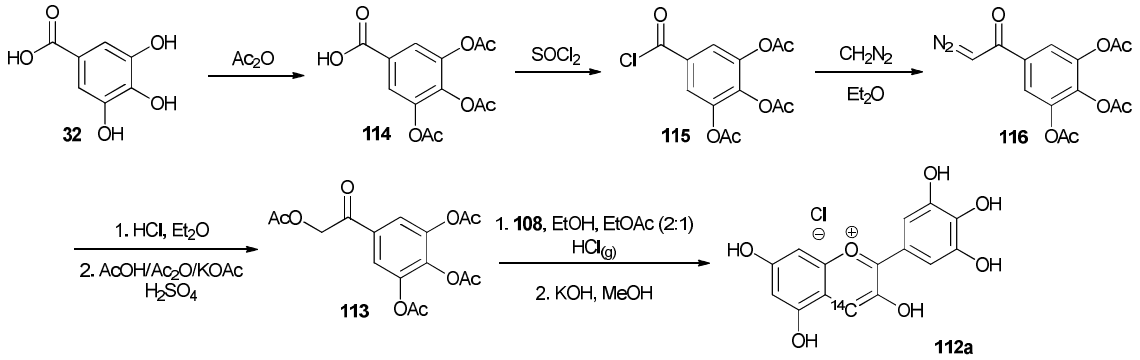


*Scheme 60 – Synthesis of **109** and final coupling to yield [4-^{14}C]pelargonidin chloride **80a**.*[104]

1.6.4.2 [4-^{14}C]Delphinidin chloride[104]

The second synthesis of a ^{14}C-labelled anthocyanin, [4-^{14}C]delphinidin chloride **112a**, was also reported by Kraus *el al.*,[104] with the preparation of the formylated component **108** being carried out using the same method as that shown in *Scheme 59* above. In this case the second component utilised in the final coupling was ω,3,4,5-triacetoxyacetophenone **113**. Preparation of this component began with the acetylation of gallic acid **32** to yield the 3,4,5-triacetoxybenzoic acid. Compound **114** was then converted into its acyl chloride (**115**) and converted to the diazoketone **116** through reaction with diazomethane. The final step in the preparation of **113** involved the reaction of **116** with hydrochloric acid in diethyl ether to yield the chloride, which was then reacted with a mixture of acetic acid, acetic anhydride and

potassium acetate. Coupling of **108** and **113** was once again carried out in an atmosphere of hydrogen chloride gas, but in this case in a solvent system of ethyl acetate and ethanol. Deprotection to yield [4-^{14}C]delphinidin chloride **112a** in an overall yield of 5.5% was again by the use of potassium hydroxide in aqueous methanol.

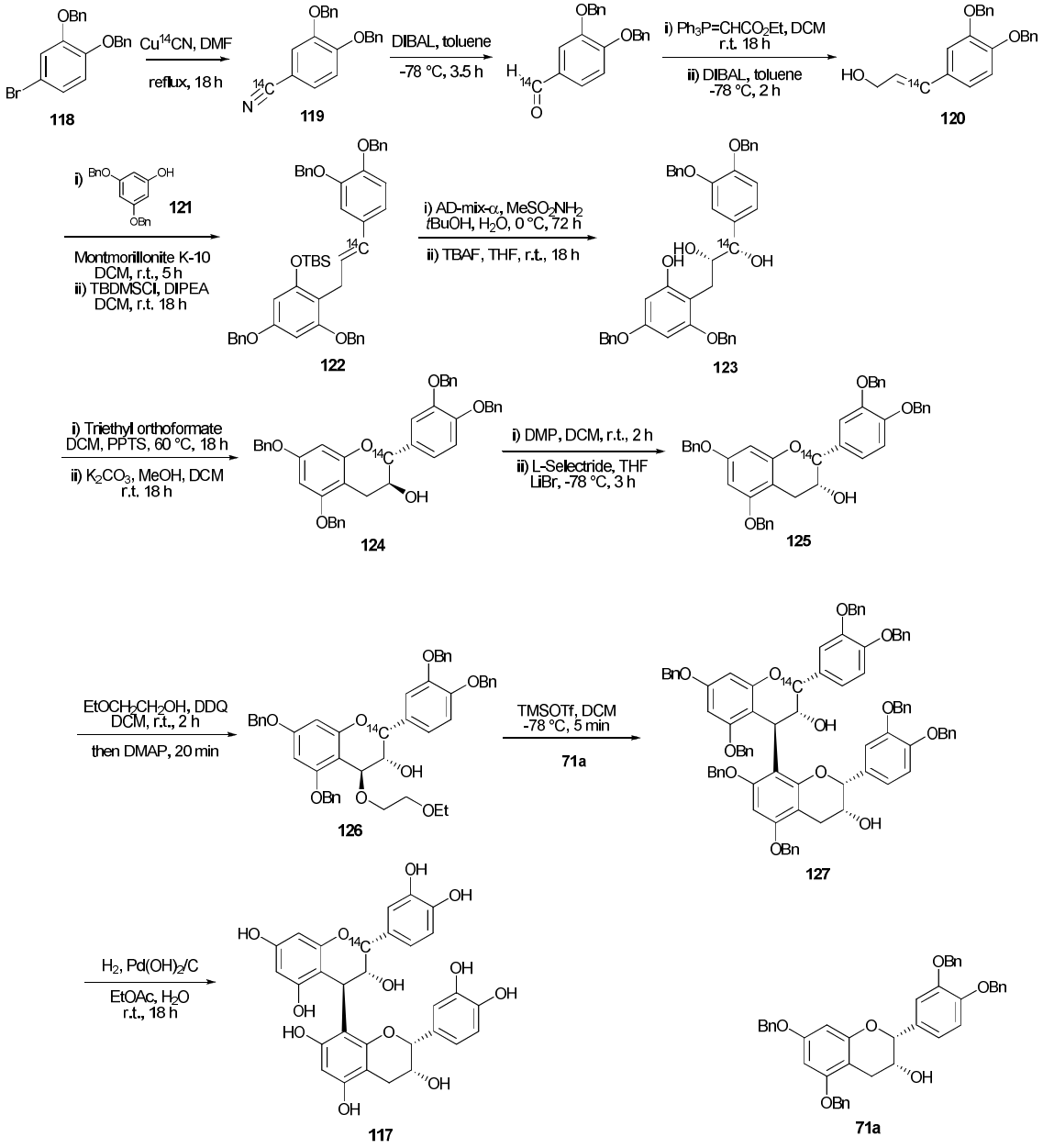


*Scheme 61 – Synthesis of **113** and final coupling to yield [4-^{14}C]delphinidin chloride **112a**.*[104]

1.6.4.3 [2-^{14}C]Procyanidin B2[105]

More recently, Barron *el al.*[105] published the first asymmetric total synthesis of ^{14}C-labelled procyanidin B2 **117** (*Scheme 62*), which is a major dietary polyphenol found in cocoa. In order to carry out the regioselective incorporation of a ^{14}C-label into the target molecule, a new route was required. Previous syntheses of procyanidins were not compatible as they were based on the coupling of flavanol monomers.[106] In the case of Barron *el al.*, the target was reached by the coupling of labelled and unlabelled (-)-epicatechin moieties, with an excess of the non-labelled form. The unlabelled form was prepared by benzylation of the phenolic hydroxyl groups of (-)-epicatechin **71**. Synthesis of the ^{14}C-labelled form began with aryl bromide **118**, with the ^{14}C-label being introduced in the first step using copper [^{14}C]cyanide (prepared from potassium [^{14}C]cyanide). Reduction of **119** using DIBAL followed by a stereoselective Wittig condensation and subsequent reduction gave the cinnamyl alcohol **120**. The alcohol was then coupled to 1,3-di-*O*-benzylphloroglucinol **121** in the presence of montmorillonite K-10, and silylated with TBDMSCl to yield **122**. In order to yield the desired diol **123**, a Sharpless

asymmetric dihydroxylation was carried out using AD-mix-α, followed deprotection of the silyl group using TBAF. Cyclisation to benzyl-protected (+)-catechin **124** proceeded with inversion of stereochemistry at C-2. However, for the preparation of procyanidin B2, the (-)-epicatechin derivative was required, and this was achieved by Dess-Martin oxidation to the ketone followed by reduction using L-Selectride® to yield **125**. Preparation of the activated monomer **126** was then required in order to carry out the subsequent coupling to an excess of the unlabelled benzylated (-)-epicatechin, and this was achieved through treatment of **125** with DDQ in the presence of 2-ethoxyethanol. Coupling of the two (-)-epicatechin moieties to yield the benzylated [2-^{14}C]procyanidin B2 **127** was successful in 8.5% overall yield from potassium [^{14}C]cyanide. The final step involved debenzylation, using palladium hydroxide catalysed hydrogenation, to give [2-^{14}C]procyanidin B2 **117**, however this was carried out on only small scale reactions prior to use in biological assays due to stability issues.



*Scheme 62 – Synthesis of [2-^{14}C]procyanidin B2 **117**.*[105]

1.7 The Use of Microwaves in Organic Synthesis

1.7.1 Background

Microwave radiation was first studied to a great extent during the second World War due to the ongoing development of radar, but it wasn't until the early 1970's that the first chemical applications were reported in the decomposition studies of organic compounds.[107,108] By the 1980's it was being recognised that reactions carried out in a microwave oven were showing considerable increases in rates, yields and product purity.[109] In fact, some success was also found in reactions which did not occur by conventional heating methods. This led to further research and the development of more advanced microwave ovens. Since that time, microwave irradiation has been successfully applied to many areas of chemistry, as will be discussed below.

Before the development of microwave technology, and even until relatively recently, the majority of organic reactions have been heated using only conventional methods such as the use of oil, water, or sand baths, or heating jackets.[110] Compared to microwave irradiation, these methods are slow as it takes time for the heat to penetrate throughout the entire sample. This results in the formation of a temperature gradient, which can lead to overheating in certain areas and decomposition of compounds in the reaction mixture. Microwave irradiation however is not introduced to the reaction mixture through direct contact with a heat source, and can penetrate the walls of the reaction vessel instantaneously resulting in uniform heating throughout the sample. This in turn can lead to the formation of fewer by-products or decomposition products. When the reaction is carried out in a sealed vessel, it is also possible for a higher pressure to be achieved with a rapid increase in temperature above the conventional boiling point of the solvent.[110]

The effects of microwave irradiation on a reaction are complex and involve many factors. Among those are thermal effects such as superheating, selective absorption of radiation by polar substances, and so called "hot spots," and non-thermal effects such as molecular mobility.[111]

Thermal effects are caused by the different temperature regime (dielectric heating) which is created due to the microwave heating, as microwave heating is immediate and volumetric – *cf.* conduction/convection in conventional heating methods. Non-thermal effects on the other hand are due to the radiation itself and not the temperature regime.[107] Research into these effects, and the changes they initiate during a reaction, is still at an early stage, but it is presumed that the thermal effect can be caused by either a faster heating rate compared to conventional methods, or the occurrence of "hot spots" within the reaction mixture.[107] In terms of rate acceleration, this will occur if energy from the microwave radiation is absorbed specifically by reactants – rather than the solvents – which can transform electromagnetic energy into heat. The activation energy for the desired transformation can therefore be reached more rapidly. In the case of conventional thermal heating methods, this radiation is not present and the transfer of heat relies purely on the conduction/convection of heat throughout the reaction mixture.[111] Therefore the same rate accelerations are not observed when this factor is examined independently.

However, the magnitude of heating during microwave irradiation depends on the dielectric properties of the compounds present in the reaction mixture – unlike conventional heating which is unspecific. This ensures that the absorption of radiation, and therefore the heating, may be performed selectively. Indeed this property can be exploited to heat polar substances in the presence of apolar ones, as the polar substances will absorb the radiation rapidly and undergo intense heating, whereas apolar substances will not. This in turn can result in the modification of selectivity (chemo-, regio- or stereoselectivity) of a given reaction through selective heating of solvents, catalysts and reagents, thus often improving processes which did not portray significant selectivity under conventional heating methods.[111]

The overheating observed in polar liquids under microwave irradiation could also be used to explain the acceleration of rates observed. Temperatures can range from 13-26 °C higher than the normal boiling point of the solvent being used. This can be explained by "inverse heat transfer," where heat travels from the interior of the reaction mixture towards the exterior.[111] Under conventional heating methods this is not observed, as the heat source directs the heat from the surroundings of the reaction vessel towards the inside of the reaction mixture.

A further thermal effect reported by several authors[111] when using dielectric heating is the presence of "hot spots" of up to 100 ppm diameter within the samples. These are most commonly found in solvent-free reactions where solids may be present, with temperatures in the range of ~100-200 °C higher than the bulk temperature of the sample. These conditions arise from the fact that in the presence of solids there is a relative heterogeneity of the applied radiation throughout the sample, therefore resulting in areas which are superheated. In fact, this can be particularly dangerous, as it can lead to the formation of holes in the reaction vessel, often resulting in explosion of the reaction due to the high pressures present. However, the presence of "hot spots" is postulated to be one of the causes of rate acceleration under microwave irradiation.[111]

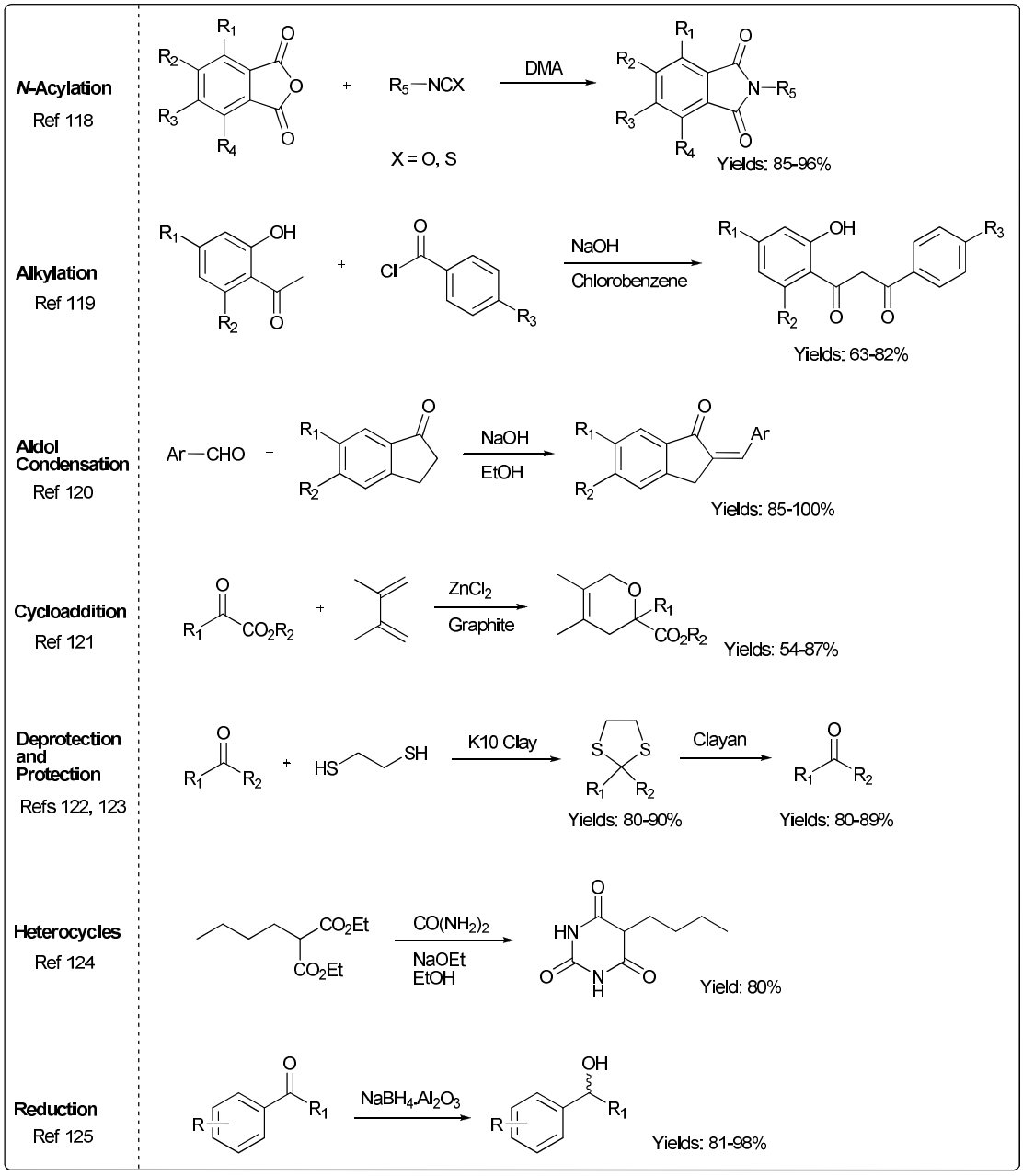
Although it was originally hypothesised that both thermal and non-thermal effects were involved in the process, it now seems to be accepted that the main cause is indeed the thermal factor due to the different heating method employed during the use of microwave irradiation on a sample. When reactions were carried out in a sealed tube under conventional heating methods, yields became comparable to those obtained when microwave irradiation was used. This suggests that the higher pressures achieved during microwave heating may be responsible for at least some of the effects observed in these reactions.[107]

Overall, the use of microwave dielectric heating can have significant beneficial effects on a reaction, with higher yields, shorter reaction times, and often milder reaction conditions. Due to the shorter reaction times, the decomposition of thermally unstable products or reagents can be avoided, as can the formation of unwanted side-products. As will be examined shortly, these factors can be particularly important in areas of chemistry where time is an important factor, such as in the preparation of radioisotopes which have a short half-life, in high throughput chemistry to produce a greater amount of novel chemical entities in a shorter period of time or in catalysis where shorter reaction times can prevent decomposition of the catalyst, thus making it a more economically viable process.[111]

1.7.2 Applications to Organic Synthesis

As previously discussed above, microwave heating provides an immediate source of heat, which can be very specific to the compounds present in the reaction vessel. This property can be of use in organic synthesis where specificity is preferred. Many reviews have been published in recent years detailing the application of microwaves in organic synthesis for the synthesis of radioisotopes,[112] solvent-free reactions,[113] cycloaddition reactions,[114] heterocyclic chemistry[115] and medicinal chemisty.[116] However, microwave chemistry is not restricted to organic chemistry alone and many publications relating to inorganic and polymer chemistry have also been investigated.[117] Due to this growing range of reactions which can exploit the use of microwave irradiation, the area is of great interest to the pharmaceutical industry, as the time required to produce a large number of novel chemical entities can be reduced significantly.[110]

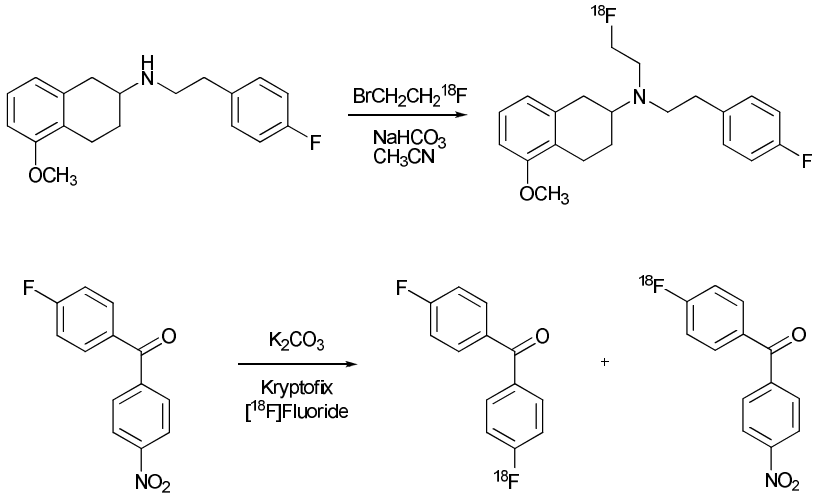
The wide range of reactions which have been previously examined under microwave irradiation is summarised in *Scheme 63* below.[110] It demonstrates the viability of many kinds of transformations, the wide scope of substrates and functional groups tolerated and in some cases the use of solvent-free conditions.



Scheme 63 – Examples of reactions previously examined under microwave conditions.[110]

It is also possible to carry out microwave reactions without the use of a solvent, as the dielectric heating used is a direct form of heating, with the presence of solvent not required for the conduction of heat.[107] One advantage of this is that there is no risk of solvent evaporation from the system. Pressure increases due to solvent evaporation are also avoided.

In the context of isotope labelling, the application of microwave technology to the synthesis of radiopharmaceuticals containing short-lived isotopes, such as ^{18}F, ^{11}C, ^{13}N and ^{15}O is of great interest. Commonly these isotopes are incorporated into structures for use in medical research such as positron emission tomography (PET) for imaging and diagnostics.[126] Due to their inherent radioactive properties and half-lives they often must be prepared immediately before use, and as quickly as possible in order to retain a high amount of radioactivity. Therefore any method which will shorten the reaction time when radioisotopes are in use will be beneficial.[107] Two examples of the use of microwave irradiation for the preparation of radioisotopes are outlined in *Scheme 64* below – firstly, the fluoroalkylation of secondary amines[127] and secondly aromatic substitution reaction with ^{18}F.[117]



Scheme 64 – Examples of the use of microwave irradiation during the synthesis of radioisotopes.[117,127]

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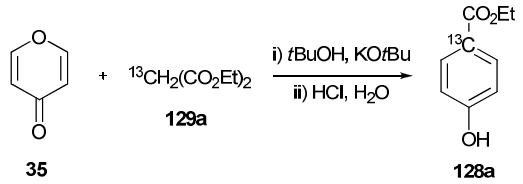
2. RESULTS AND DISCUSSION

2.1 Synthesis of Substituted Phenols from Pyran-4-one Precursors

2.1.1 Introduction

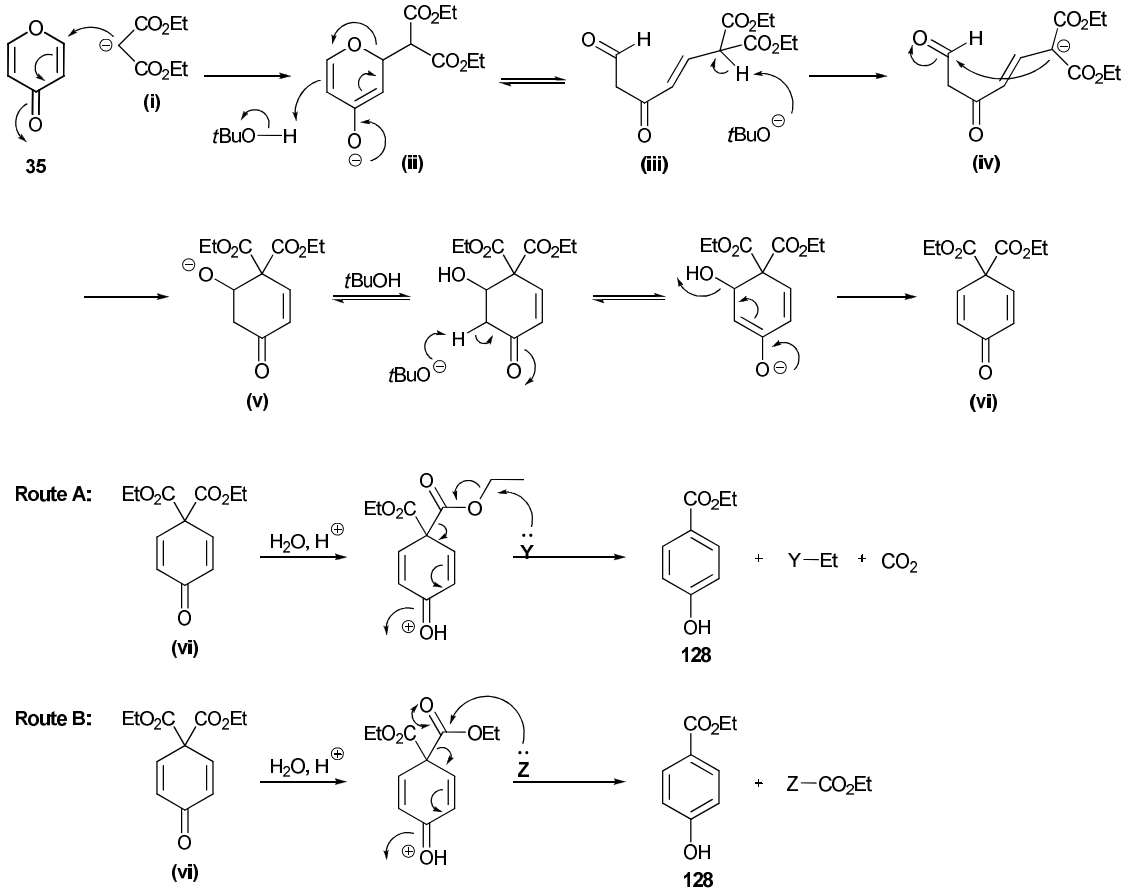
Whilst examining synthetic routes to phenols from acyclic or non-aromatic precursors (**Sections 1.3.2** and **1.4.2.3**), the reaction of 4*H*-pyran-4-one with diethyl malonate in the presence of base stood out as a potentially useful protocol from which a general methodology could be developed. The preparation of *para*-substituted phenol **128a** (*Scheme 65*) from the reaction of pyran-4-one **35** with diethyl [^{13}C]malonate **129a** in the presence of a suitable base, followed by acidic work-up, was originally reported by Steglich *el al.*[1,2] in 1998. We saw potential in this route for the incorporation of other nucleophiles in place of diethyl malonate, and Steglich's reaction did not appear to have been studied further since the time of their original publications.



Scheme 65 – Steglich´s synthesis of para-substituted phenols.[1]

A proposed mechanism for the reaction is outlined below (*Scheme 66*). The first stage involves deprotonation of the nucleophile by potassium *tert*-butoxide to form the anion of **129a (i)**, which subsequently attacks the pyranone **35**. Ring opening of the initial adduct **(ii)** then occurs to give an aldehyde **(iii)**. Deprotonation at the position alpha to the electron-withdrawing groups gives an anionic centre **(iv)** which can attack the aldehyde to form a 6-membered ring **(v)**. Subsequent elimination of water *via* an E1cB mechanism gives the conjugated intermediate **(vi)** with the two electron-withdrawing groups still present. Acidic work-up results in the loss of one group by one of two possible mechanisms, to yield the final product **128**. Route A involves the attack of a nucleophile **Y** (such as water) on the ethyl group of the ester, to give

loss of carbon dioxide and subsequent aromatisation, yielding product **128**. Route B differs in that attack (by **Z**) takes place at the carbonyl carbon, resulting in the formation of the desired product with no direct loss of carbon dioxide observed in this case. In either mechanism the driving force for the reaction is the formation of the final aromatic system.



*Scheme 66 – Proposed mechanism for the reaction between pyranone **35** and diethyl malonate **129**.*

We envisaged that if successful with the unsubstituted pyran-4-one **35** and a range of chosen nucleophiles, the scope of this reaction could be extended further to include the use of substituted pyranones, **36**, **38** and **43** in order to produce polysubstituted phenols (*Diagram 18*).[3] This could be particularly useful in the synthesis of natural products containing phenolic moieties as it presents an alternative route to phenols which does not require the direct functionalisation of the aromatic ring.[4]

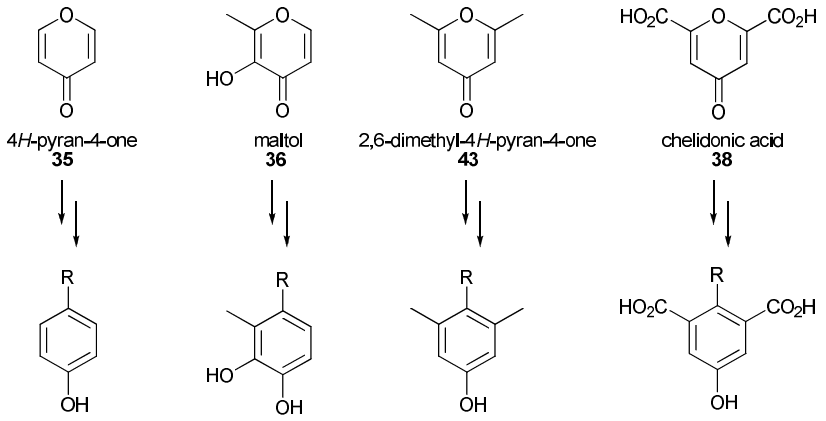


Diagram 18 – Pyran-4-one precursors for the synthesis of substituted phenols.[3]

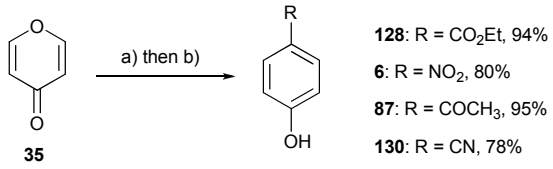
Following initial studies into these reactions we were also interested in the potential application of microwave irradiation to the methodology, as it has previously been demonstrated that the use of microwave heating techniques can accelerate a range of reactions (**Section 1.7**). Results for both conventional and microwave heating will therefore be presented and compared in the discussion which follows.

Applications to the synthesis of ^{13}C-labelled natural products are also feasible through the possible introduction of ^{13}C-atoms in the synthesis of the pyranone precursor or the use of suitably labelled commercially available nucleophiles, such as diethyl [2-^{13}C]malonate as demonstrated by Steglich.[1]

The aim was therefore to develop a methodology for the synthesis of phenols from pyranone precursors which would be high yielding and require the minimum amounts of purification in order to reduce losses of isotopically-labelled materials.

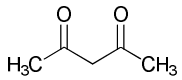
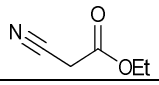
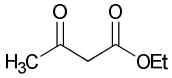
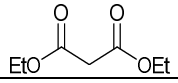
2.1.2 Reactions with 4*H*-Pyran-4-one (35)[3]

The reaction of a range of nucleophiles with the unsubstituted 4*H*-pyran-4-one **35** was studied to extend the methodology, as summarised in *Scheme 67* below. *Table 2* shows the nucleophiles studied and their respective pK_a values.



Scheme 67 – Reagents and conditions: a) *t*BuOH, KO*t*Bu, nucleophile, reflux; b) 1M HCl, reflux.[3]

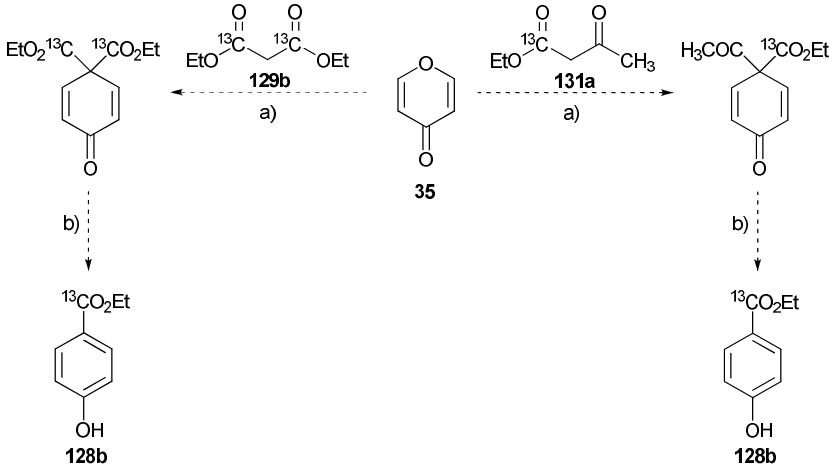
Table 2 –Nucleophiles and pK_a values relative to tert-butanol.

	Nucleophile		pK_a value
135		Acetylacetone	9
139		Ethyl cyanoacetate	9
133		Nitromethane	10
131		Ethyl acetoacetate	11
129		Diethyl malonate	13
-	-	*tert-butanol*	*18*

Initially we studied the reaction carried out by Steglich *el al.*[1] (*Scheme 65*) between 4*H*-pyran-4-one **35** and diethyl malonate **129**. They reported 85% yield by heating the pyranone (1.6 eq.) and diethyl malonate (1.0 eq.) with potassium *tert*-butoxide (1.3 eq.) in *tert*-butanol at reflux for 3 h, followed by a further 30 min reflux after the addition of dilute acid. Under these conditions we obtained a yield of only 49%. Repeating the reaction using 1.7 equivalents of the pyranone gave ethyl 4-hydroxybenzoate **128** in 94% crude yield, with further purification not being required. However, for our application of this procedure to the use of ^{13}C-labelled pyranones

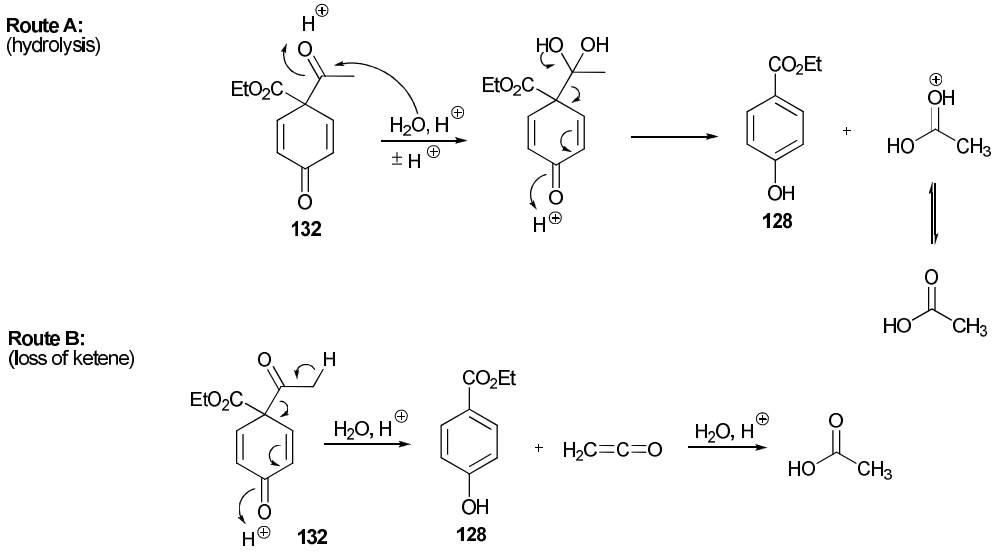
and nucleophiles it was desirable to find an alternative stoichiometric ratio where neither reagent was used in significant excess. This was achieved in 74% yield from 1 equivalent of the pyranone, 1.1 equivalents of diethyl malonate, and 1.1 equivalents of base. It was also found during the optimisation studies that a reflux time of 1 h (*cf.* 30 min) after addition of the acid was beneficial.[3] In the ^{1}H NMR spectrum of the product, a pair of doublets (*J* 8.0 Hz) was observed at 6.90 and 7.97 ppm corresponding to the aromatic protons, along with a triplet at 1.39 ppm and quartet at 4.36 ppm, further confirming the identity of the product, **128**.

Interestingly, ethyl 4-hydroxybenzoate **128** was also obtained when the reaction was carried out using ethyl acetoacetate **131** in place of diethyl malonate **129**. After optimisation, a 65% yield was achieved using 1 equivalent of the pyranone, 1.1 equivalents of ethyl acetoacetate, and 1.2 equivalents of base. The acetyl group appeared to be selectively removed in refluxing aqueous acid with no evidence for the formation of 4-hydroxyacetophenone **87** by loss of the ester group.[3] Although in our case this comparison was only carried out on unlabelled substrates, this selectivity would be of use when considering the use of ^{13}C-labelled nucleophiles to prepare **128b** (*Scheme 68*). Use of diethyl [1,3-$^{13}C_2$]malonate **129b** would ultimately result in loss of half of the isotopically labelled carbon atoms in the form of carbon dioxide during acid reflux. However, if ethyl [1-^{13}C]acetoacetate **131a** was used, 100% of the isotope label would be retained in the final product, thus reducing the potential financial impact.



Scheme 68 – Reagents and conditions: a) *t*BuOH, KO*t*Bu, reflux; b) 1M HCl, reflux.

The mechanism for this reaction (*Scheme 69*) is assumed to be similar to that outlined in *Scheme 66* above. In this case, the ester substituent is retained within the molecule and the acetyl moiety is lost during acid reflux, either as acetic acid *via* a hydrolysis mechanism or *via* loss of ketene.

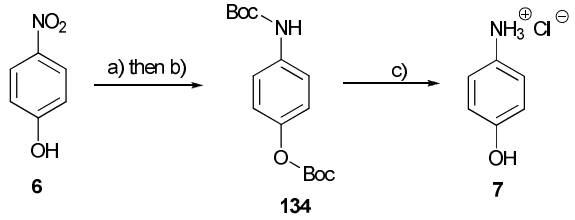


*Scheme 69 – Hydrolysis of pyranone-ethyl acetoacetate adduct **132** to give ethyl 4-hydroxybenzoate **128.***

With conditions established for the successful reaction of pyran-4-one **35** with both diethyl malonate **129** and ethyl acetoacetate **131**, these conditions were applied to the use of nitromethane **133** as the nucleophile. This yielded 4-nitrophenol **6** in 61% yield, with a

significant amount of the pyranone substrate remaining in the crude mixture. It was therefore concluded that a longer reaction time would be required. After heating for 5 h at reflux, reaction with nitromethane gave 4-nitrophenol **6** in 80% yield.[3] Due to the simplicity of the nitromethane nucleophile, aromatisation in the second stage of the reaction required only loss of a proton.

Derivatisation of 4-nitrophenol **6** to the corresponding amine **7** was carried out (*Scheme 70*), as the amine was considered to be a useful intermediate for applications in natural product synthesis.



Scheme 70 – Reagents and conditions: a) MeOH, Boc_2O, $NiCl_2.6H_2O$, $NaBH_4$; b) $(NH_2CH_2CH_2)_2NH$ (95%); c) DCM, 4M HCl in 1,3-dioxane (48%).[5]

An *in situ* reduction/protection procedure was used which involved sodium borohydride in presence of nickel(II) chloride as the reducing agent, and Boc-anhydride to protect the hydroxyl group and newly-formed amine functionality.[5] After the addition of sodium borohydride to the reaction mixture already containing Boc-anhydride and nickel chloride, a black precipitate of nickel(0) formed. Stirring overnight gave a green solution, which became purple after the addition of diethylenetriamine. After work-up, the Boc-protected product **134** was isolated in 95% yield. Deprotection using HCl in dioxane was carried out on the crude intermediate, with the hydrochloride salt of the product precipitating out of the reaction mixture. The desired product **7** was isolated pure in 48% yield (46% over two steps) with signals corresponding to the hydroxyl and amine protons visible in the ^1H NMR spectrum at 9.87 and 10.14 ppm respectively.

As previously discussed, the reaction of pyranone **35** with ethyl acetoacetate **131** yields the ester product **128** and not 4-hydroxyacetophenone **87**, as may have been expected through the loss of carbon dioxide during aromatisation. Therefore it was desirable to find an alternative route to the synthesis of 4-hydroxyacetophenone **87**. Application of the optimum conditions (1 equivalent pyranone, 1.1 equivalents nucleophile, 1.1 equivalents base, 4-5 h reaction time) to the reaction of 4*H*-pyran-4-one **35** and acetylacetone **135** was expected to yield the desired product, as it has already been established that the ketone group can be eliminated in the aromatisation by either a hydrolysis mechanism or loss of ketene (*Scheme 69*). However, the desired product **87** was only isolated in 22% yield. As was the case with nitromethane it was considered that a longer reaction time may be required, and indeed this was found to be beneficial. Optimisation studies led to the isolation of **87** in 95% yield after 20 h reflux using 2 equivalents of acetylacetone **135** and 2 equivalents of potassium *tert*-butoxide.[3] In consideration of the potential use of ^{13}C-labelled substrates, it was desirable to obtain conditions which required close to stoichiometric amounts of the two substrates. The use of 1.5 equivalents of acetylacetone and 1 equivalent of pyranone gave 4-hydroxyacetophenone **87** in 81% yield after 20 h reflux.[3]

Finally, we chose to introduce a *para*-nitrile group *via* the preparation of 4-hydroxybenzonitrile **130**. Following the use of nitromethane **133** in the synthesis of 4-nitrophenol the obvious nucleophile of choice would have been acetonitrile, due to the simplicity of its structure. However, as the pK_a value of acetonitrile is 25 (*cf.* 18 for *tert*-butanol) it did not meet the criteria for the application to our methodology as an alternative base would be required. The use of symmetrical malononitrile was subsequently examined (pK_a 11). The initial stages of the reaction appeared to proceed as expected, with a colour change to orange/red upon addition of the base. However, upon addition of hydrochloric acid, a deep red colour formed which had not been observed before. The recovered product mixture (mass recovery 25%) was found to be complex, with a minimum of 5 components present including a significant amount of the

pyranone substrate **35**, the non-aromatised intermediate **136** and only a small amount of the desired 4-hydroxybenzonitrile **130**. One pair of doublets observed within the aromatic region was found to have a large *J* value of 13 Hz, suggesting the presence of an alkene system, although it is not clear from the *J* value whether a *cis* or *trans* alkene is present, as this value is within the overlap region. It was considered likely that a conjugated dimer system **137** had formed which would be expected to be highly coloured and could therefore account for the deep red colour observed. A proposed structure for this conjugated system is shown below (*Scheme 71*), but could not be isolated successfully by column chromatography. The final product likely to be present is the Knovenagel product **138**, as malononitrile could react with the ketone at the 4-position of the pyranone in a Knovenagel condensation. Interestingly, such a side-product is not observed with the use of other nucleophiles.

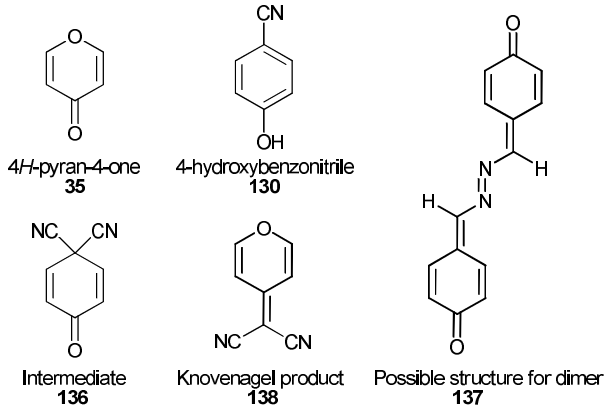


*Diagram 71 – Proposed structures of non-isolated compounds from the reaction of pyranone **35** with malononitrile.*

Due to the complexity of the crude reaction mixture above, it was decided to attempt the reaction using one final nucleophile, ethyl cyanoacetate **139** due to its similarity to both diethyl malonate **129** and ethyl acetoacetate **131** and low pK_a value of 9. Significant optimisation studies were carried out, with selected results outlined in *Table 3* below. Isolated yields are given where the conversions and reaction profiles were considered good enough for purification to be carried out.

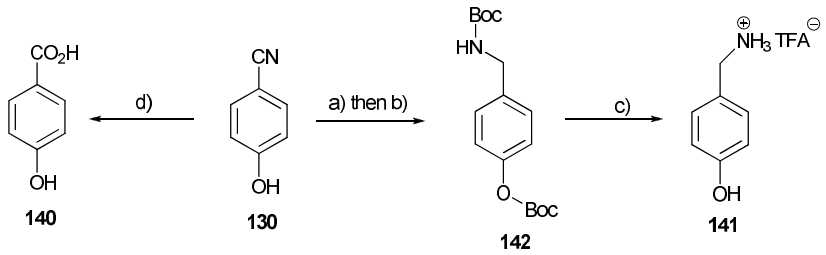
Table 3 – Reaction variables for the reaction of 4H-pyran-4-one and ethyl cyanoacetate.

Entry	Eq. nucleophile	Eq. KO*t*Bu	Reflux time (h)	Conversion (%)†	Isolated yield (%)
1	1.1	1.1	4	15	-
2	1.1	1.1	20	34	-
3	2.0	1.1	24	55	25
4	**1.6**	**3.0**	**24**	**90**	**78**

† Calculated from integration of ^{1}H NMR signals of starting material and product

In the first attempt, the conditions were kept similar to those previously optimised for use with other nucleophiles – 1.1 equivalents of base and nucleophile, 4 h reflux (entry 1). The results were analogous to those obtained using malononitrile, with a complex mixture being recovered. Integration of the ^{1}H NMR signals allowed a conversion of 15% to be calculated. The reaction was repeated with a reaction time of 20 h, this time giving 34% conversion to **130** (entry 2). Increasing the quantity of base to 2.0 equivalents was beneficial with a cleaner product being recovered (55% conversion) and purification by column chromatography giving **130** in 25% yield (entry 3). Finally, the quantities of both base and ethyl cyanoacetate were increased to 3.0 and 1.6 equivalents respectively (entry 4). Upon complete addition of base to the reaction mixture, a brown/orange precipitate formed which is similar to observations made in previous successful reactions of other nucleophiles. No red colour was observed upon addition of the acid. ^{1}H NMR analysis of the crude reaction mixture showed that conversion was high, and the desired 4-hydroxybenzonitrile **130** was recovered in 78% yield after purification by column chromatography.[3]

Two likely transformations of 4-hydroxybenzonitrile **130** (*Scheme 72*) which could subsequently be useful in applications of ^{13}C-labelling and natural product synthesis would be hydrolysis to the carboxylic acid **140**, or reduction to the corresponding amine **141** *via* **142**, using the reduction/protection procedure previously employed for the reduction of 4-nitrophenol **6**.



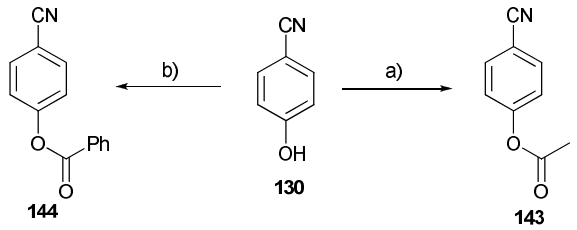
Scheme 72 – Reagents and conditions: a) MeOH, Boc_2O, $NiCl_2.6H_2O$, $NaBH_4$; b) $(NH_2CH_2CH_2)_2NH$ (78%); c) DCM, TFA (96%);[5] d) 2M NaOH, MeOH, reflux (76-82%).

The first stage of conversion to the TFA salt of 4-hydroxybenzylamine **130** once again involved simultaneous protection and reduction through the use of Boc anhydride, sodium borohydride and nickel(II) chloride.[5] The crude yield of **142** obtained was good (78%), although it could be seen from the signals in the ^{1}H NMR spectrum corresponding to the Boc methylene groups that a small amount of mono-protected product had formed. However, this is not an issue as full deprotection is carried out in the following stage. Trifluoroacetic acid (TFA) proved to be a more effective deprotecting agent yielding the desired salt **141** as a yellow oil which crystallised to give pale yellow crystals in an excellent yield for the deprotection (96%) and good yield over the two steps (75%).

The hydrolysis of 4-hydroxybenzonitrile **130** was carried out under basic conditions in order to yield the corresponding 4-hydroxybenzoic acid **140**. The reaction was successful using either pure or crude samples of 4-hydroxybenzonitrile **130**, although yields are slightly lower with the use of crude samples as would be expected (76% *cf.* 82%). However, this is an important result, due to the fact that if this procedure is applied to the use of ^{13}C-labelled substrates, purification is not required before the hydrolysis stage, thus decreasing potential losses of isotopically-labelled material. Hydrolysis under acidic conditions was less successful.

Finally, protection of the phenolic oxygen of 4-hydroxybenzonitrile **130** was examined using both acetate and benzoyl protecting groups (*Scheme 73*). It was hoped that these

transformations may also be useful for the isolation of a 4-hydroxybenzonitrile derivative, as protection of the hydroxyl group generates a less polar compound, thus potentially reducing losses during column chromatography.



Scheme 73 – Reagents and conditions: a) Pyridine, acetic anhydride, rt. 1 h (34-98%); b) Ethyl acetate, benzoyl chloride, NEt_3, rt. 1 h (67%).

Acetylation using sodium hydroxide and acetic anhydride was unsuccessful as the substrate was not soluble enough in the basic media to allow the reaction to take place. The reaction was then attempted using pyridine as base and solvent in the presence of an excess of acetic anhydride, with the reaction reaching completion after 1 h. Purification was not required when the reaction was carried out on a pure sample of 4-hydroxybenzonitrile, with the desired product **143** being obtained in 98% yield. However, purification by column chromatography was required for the use of crude 4-hydroxybenzonitrile, yielding the desired product in a much lower 34% yield.

It was expected that use of the benzoyl protecting group may produce more promising results in regard to the use of the crude substrate **130**, due to the greater increase in molecular weight imparted by the phenyl group simplifying the purification. Indeed this was the case with the desired product **144** being obtained in 85% crude yield and 67% after purification by column chromatography.

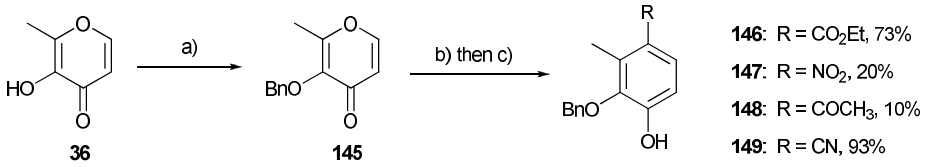
2.1.2.1 Summary of Reactions with 4*H*-Pyran-4-one

The successful syntheses of a number of *para*-substituted phenols have been developed using the principles of Steglich's method.[1-3] Synthesis of ethyl 4-hydroxybenzoate **128** from 4*H*-pyran-4-one **35** was achieved in excellent yields using both diethyl malonate **129** (94%) and ethyl acetoacetate **131** (65%) as nucleophiles. Nitromethane **133** was also successfully employed in the reaction to yield 4-nitrophenol **6** (80%) which was subsequently transformed to the HCl salt of 4-aminophenol **7** in 46% yield *via* a Boc-protected intermediate. 4-Hydroxyacetophenone **87** was isolated in 95% yield after optimisation of the number of equivalents of base and acetylacetone **135**. The preparation of 4-hydroxybenzonitrile **130** was the most troublesome of the reactions with considerable optimisation being required. The use of ethyl cyanoacetate **139** as nucleophile yielded the desired product **130** in 78% yield. Reduction of the crude product **130** to the TFA salt of 4-hydroxybenzylamine **141** was achieved in excellent yield (82%), thus further expanding the scope of the reaction.[3] Protection of the hydroxyl group to aid purification of the crude nitrile was also successful, with the use of the benzoyl protecting group giving the best results (67% after column chromatography).

2.1.3 Reactions with *O*-Benzyl-Maltol (145)[3]

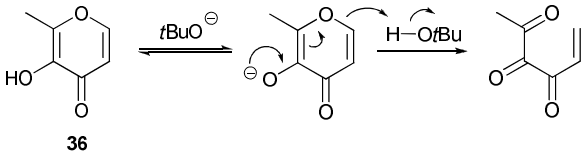
Encouraged by the results obtained using the unsubstituted 4*H*-pyran-4-one **35** we wished to extend the scope of this methodology to substituted pyran-4-ones, as this would allow the preparation of polysubstituted phenols. The substitution pattern would therefore be derived from the pyranone substrate, and could potentially remove the requirement for subsequent functionalisation of the aromatic ring. 3-Hydroxy-2-methyl-pyran-4-one (maltol) **36** is one of the few commercially available substituted pyran-4-ones as previously discussed in **Section 1.4**. In the case of maltol, the synthesis of polyphenols such as catechols could be achieved using this methodology due to the presence of an existing hydroxyl group on the pyranone substrate.

We therefore chose to investigate whether the methodology developed for the unsubstituted 4*H*-pyran-4-one **35** could also be applied to maltol **36** (*Scheme 74*).



Scheme 74 – Reagents and conditions: a) Benzyl bromide, aq. NaOH, MeOH, reflux, 20 h (89%); b) *t*BuOH, KO*t*Bu, nucleophile, reflux; c) 1M HCl, reflux.[3]

Like the nucleophiles being studied in these series of reactions, the pK_a of maltol (~8.5) is lower than that of *tert*-butanol (pK_a 18), and so deprotonation of the hydroxyl group would occur under our reaction conditions. In order to prevent unwanted side reactions, including ring opening (*Scheme 75*), the 3-hydroxy group was first protected as the benzyl ether **145** *via* treatment of maltol with benzyl bromide in a solution of methanol and aqueous sodium hydroxide (89%).



Scheme 75 – Maltol ring opening following deprotonation by KOtBu.

Initially, the reaction of *O*-benzyl-maltol **145** with diethyl malonate **129** failed to give any product when using similar conditions to those previously optimised for reactions using the unsubstituted pyranone **35** (*Table 4*, entry 1). An increase in the number of equivalents of base resulted in 14% conversion to **146** (entry 2), while increasing the reaction time to 47 h was beneficial (27% conversion, entry 3). However, by use of 3 equivalents of base, 1.6 equivalents of nucleophile and a reaction time of 47 h (entry 4), an excellent conversion of 85% and isolated yield of 73% were recorded. These optimised conditions were the same as those for the reaction between pyranone **35** and ethyl cyanoacetate **139**.[3]

Table 4 – Reaction variables for the reaction of O-benzyl-maltol and diethyl malonate.

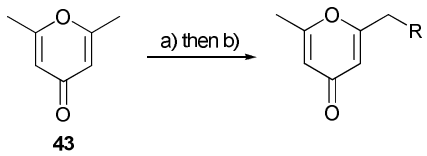
Entry	Eq. nucleophile	Eq. KO*t*Bu	Reflux time (h)	Conversion (%)†	Isolated yield (%)
1	1.6	1.3	18	0	-
2	1.6	2.3	23	14	-
3	1.6	2.3	47	27	-
4	**1.6**	**3**	**47**	**85**	**73**

† Calculated from integration of ^{1}H NMR signals of starting material and product

The reaction of **145** with nitromethane **133** and acetylacetone **135** resulted in low yields of **147** and **148** under all conditions studied. It was found in both cases that the use of 3 equivalents of base was detrimental to the reaction, with higher conversions being achieved after prolonged heating (>40 h) in the presence of 2.3 equivalents of base and 1.6 equivalents of nucleophile. Phenols **147** and **148** were isolated in 20% and 10% yield, respectively.[3]

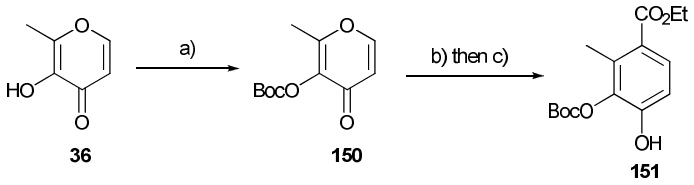
Interestingly, the reaction with ethyl cyanoacetate **139** was more successful than all other reactions using *O*-benzyl-maltol **145**. This is the opposite trend to that observed with the use of the unsubstituted pyranone **35**. In this case only two conditions were examined (2.3 equivalents of base and 3.0 equivalents of base), with the desired product **149** being obtained in 93% yield under conditions identical to those used in the reaction with diethyl malonate **129** to yield **146** (*Table 4*, entry 4).[3]

Compared to 4*H*-pyran-4-one **35**, *O*-benzyl-maltol **145** is relatively electron rich due to the presence of the oxygen atom at the 3-position. This may offer a possible explanation for the poor reactivity of **145** with some nucleophiles. However there was also evidence that the substituted pyranone starting material was being converted into other products by a competing pathway. As previously discussed (**Section 1.4.2**) the presence of the carbonyl group can have an effect on the ring substituents and it is known that pyranones with alkyl groups in the 2- and 6-positions, such as 2,6-dimethyl-4*H*-pyran-4-one **43**, can be deprotonated and reacted with electrophiles (*Scheme 76*).[6,7]



Scheme 76 – Reagents and conditions: a) LDA, THF, -78 °C; b) R-X (X = halide).[6,7]

Alternative protecting groups for the 3-hydroxy group were also examined, including the Boc group (*Scheme 77*). However, complex mixtures of products were obtained due to increased side reactions and cleavage of protecting groups during the acid hydrolysis step. Indeed, in the reaction of *O*-boc-maltol **150** with diethyl malonate **129**, only a 2% yield of **151** was achieved. After consideration of the vulnerability of many protecting groups to acidic conditions and their potential to undergo side-reactions under either the basic or acidic conditions utilised, it was concluded that the benzyl protecting group appeared to be the most effective for reactions under these conditions.



Scheme 77 – Reagents and conditions: a) MeOH, Boc_2O, DMAP, rt., O/N (88%); b) *t*BuOH, KO*t*Bu, diethyl malonate, reflux, 47 h; c) 1M HCl, reflux, 1 h (2%).

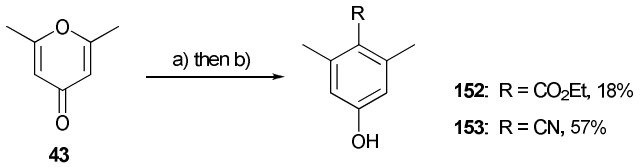
2.1.3.1 Summary of Reactions with *O*-Benzyl-Maltol

Conditions previously optimised for reactions with 4*H*-pyran-4-one **35** were subsequently applied to the use of the 2,3-disubstituted pyranone, maltol **36**, with the aim of synthesising a range of substituted phenols *via* the same methodology. Protection of the hydroxyl group was required in order to prevent unwanted side reactions. It was hypothesised that deprotonation of the methyl group occurred, and thus a greater amount of base was required in order for the desired reaction with nucleophiles to proceed. This could be confirmed by carrying out a

control reaction in deuterated *tert*-butanol (*t*BuOD), without addition of the nucleophile, as incorporation of deuterium into the methyl group would confirm that deprotonation does indeed take place under our reaction conditions. In some cases, side reactions were observed through the formation of complex product mixtures. After optimisation studies, reaction between *O*-benzyl-maltol **145** and diethyl malonate **129** gave phenol **146** in 73% yield. Reactions using nitromethane **133** and acetylacetone **135** gave the poorest results – 20% **147** and 10% **148**. The use of ethyl cyanoacetate **139** was most successful, with **149** being synthesised in an excellent yield of 93%.[3] Boc-protection of the 3-hydroxyl group was successful, giving **150** in 88% yield. However, reactions with nucleophiles were not successful using this substrate (2% yield of **151** using diethyl malonate **129**) due to the sensitivity of the protecting group to the conditions of the acid reflux.

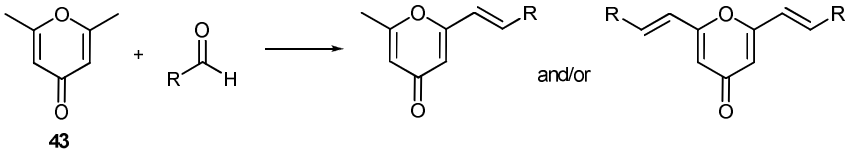
2.1.4 Reactions with 2,6-Dimethyl-4*H*-pyran-4-one (43)[3]

In the studies carried out thus far, the addition of more base generally appeared to improve conversion to the phenolic products, but in some cases also resulted in the generation of unwanted side-products. One likely explanation which would account for this observation involves the deprotonation of the methyl groups followed by reaction with other species in the reaction mixture (**Section 1.4**). In order to investigate whether it is the presence of the methyl group in *O*-benzyl-maltol **145** rather than the influence of the oxygen which is affecting the reaction, select reactions with 2,6-dimethyl-4*H*-pyran-4-one **43** were examined (*Scheme 78*).



Scheme 78 – Reagents and conditions: a) *t*BuOH, KO*t*Bu, nucleophile, reflux; b) 1M HCl, reflux.[3]

Over and above the side-chain reactions of **43** outlined previously with the use of LDA as base (*Scheme 76*), studies have shown that alkenes can be produced by the condensation of the dimethyl pyranone with an aldehyde using potassium *tert*-butoxide (*Scheme 79*).[8,9] This is highly relevant to our studies, as it suggests that under our reaction conditions (potassium *tert*-butoxide in *tert*-butanol) the methyl groups can be deprotonated, therefore accounting for the requirement of a greater number of equivalents of base, and also the possible formation of side-products depending on the nucleophiles utilised in the reaction.



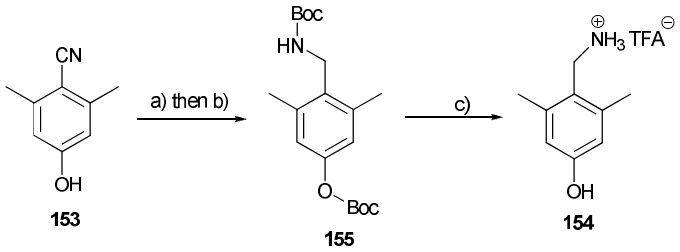
Scheme 79 – Reagents and conditions: a) KO*t*Bu, DMF or DMSO.[8,9]

Ethyl 4-hydroxy-2,6-dimethylbenzoate **152** was obtained in a very poor 2% conversion after heating the dimethylpyranone **43** at reflux for 47 h in the presence of 2.3 equivalents of base and 1.6 equivalents of diethyl malonate **129**. A similar pattern was observed to that of *O*-benzyl-maltol **145**, where an increase to 3 equivalents of base increased conversion. In this case a conversion of 25% and isolated yield of 18% was obtained. As with *O*-benzyl-maltol **145**, the reaction with ethyl cyanoacetate **139** was more successful than that with diethyl malonate **129**. Under similar conditions to those used in the preparation of **152**, 4-hydroxy-2,6-dimethylbenzonitrile **153** was obtained from ethyl cyanoacetate in 60% conversion and 57% isolated yield. Reactions of **43** with nitromethane **133** and acetylacetone **135** gave complex mixtures of products, with none of the desired phenols being observed.[3]

These yields were much lower than those obtained with both the unsubstituted 4*H*-pyran-4-one **35** and *O*-benzyl-maltol **145**. This supports the hypothesis that the pyranone starting material **43** was being consumed by the pathway described above, confirming that the methyl substituents are indeed having a significant effect on the reaction. With two methyl groups now

present in the substrate (*cf.* one methyl group in *O*-benzyl-maltol), it was thought that a higher number of equivalents of base would be required for the reaction to proceed further. However, an increase to 4 equivalents resulted in only 27% and 66% conversion for **152** and **153**, respectively, and so these observations do not merit further optimisation regarding the effects of the base.[3]

To complete the studies of 2,6-dimethyl-4*H*-pyran-4-one **43**, derivatisation of the nitrile **153** to the corresponding amine **154** (*Scheme 80*) was carried out using the procedure applied previously to the preparation of 4-hydroxybenzylamine **7**. The reduction/protection stage of the reaction was found to have gone to completion after a longer reaction time of 68 h (*cf.* 17 h), perhaps due to the increased steric influence of the methyl groups. The protected intermediate **155** was isolated in 44% crude yield with a small amount of only *N*-protected compound being present in the crude mixture. Deprotection yielded the TFA salt **154** in a yield of 65%, and 29% yield over the two steps.



Scheme 80 – Reagents and conditions: a) MeOH, Boc_2O, $NiCl_2.6H_2O$, $NaBH_4$; b) $(NH_2CH_2CH_2)_2NH$ (44%); c) DCM, TFA (65%).[5]

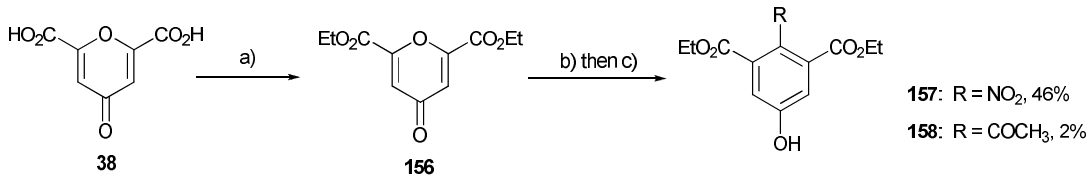
2.1.4.1 Summary of Reactions with 2,6-Dimethyl-4*H*-pyran-4-one

Due to the poor results of some reactions with *O*-benzyl-maltol **145** which were previously found to be successful with the unsubstituted pyran-4-one **35**, substituted pyranone **43** was employed in order to investigate the effect of the methyl groups on the reaction. Reaction with diethyl malonate **129** gave phenol **152** in low yield (18%). The use of ethyl cyanoacetate was

more successful with **153** being isolated in 57% yield and reduction of the nitrile to the corresponding amine **154** also achieved (29%). Reactions using nitromethane **133** and acetylacetone **135** gave poor results, with complex mixtures of products being observed. It could therefore be concluded that the presence of the methyl groups has a detrimental effect on the reaction due to deprotonation of the methyl groups by potassium *tert*-butoxide.

2.1.5 Reactions with Diethyl Chelidonate (156)[3]

Finally, we investigated the reactivity of an electron poor 2,6-disubstituted pyranone to determine how this would affect the reaction. Chelidonic acid **38** was converted into its diethyl ester **156** (*Scheme 81*) to avoid deprotonation of the acidic protons during the reaction.



Scheme 81 – Reagents and conditions: a) EtOH, conc. HCl, 24 h, reflux (99%); b) *t*BuOH, KO*t*Bu, nucleophile, reflux; c) 1M HCl, reflux.[3]

In contrast to the electron-rich substrates studied previously, reactions of **156** with diethyl malonate **129** and ethyl cyanoacetate **139** gave no trace of the desired phenols, with even more complex mixtures of products being observed. Initially the possibility was considered that the ester groups were being hydrolysed during the acid reflux. However, it was found that the reaction with nitromethane **133** could be carried out under milder conditions than those used for pyranones **43** and **145** with a reaction time of only 24 h being required. Phenol **157** was isolated in 46% yield (53% conversion) after heating at reflux in the presence of 3 equivalents of base and 1.1 equivalents of nitromethane. Reducing the reflux time to 5 h decreased the conversion slightly (37%), but still gave improved results from those obtained with *O*-benzyl-maltol **145** and 2,6-dimethyl-4*H*-pyran-4-one **43**. It was expected that due to the absence of acidic protons,

the amount of base required for substrate **156** would be lower. Reducing the amount of base to 1.1 equivalents did not affect the yield significantly (42%), thus supporting this hypothesis that the quantity of base would not affect the reaction outcome to any great extent.[3]

The reaction of **156** with acetylacetone **135** under the same conditions gave no conversion to the desired phenol **158** under conventional heating methods (*Table 5*, entry 1). Lowering the amount of base and decreasing the reaction time (entries 2 and 3) had no effect on the reaction. Finally, phenol **158** was isolated in a very poor 2% yield (5% conversion) with the use of 3 equivalents of base, 1.6 equivalents of acetylacetone and 48 h reflux.[3]

Table 5 – Reaction variables for the reaction of diethyl chelidonate and acetylacetone.

Entry	Eq. nucleophile	Eq. KO*t*Bu	Reflux time (h)	Conversion (%)†	Isolated yield (%)
1	1.1	3	24	0	-
2	1.1	1.1	24	0	-
3	1.1	3	5	0	-
4	**1.6**	**3**	**48**	**5**	**2**

† Calculated from integration of ^{1}H NMR signals of starting material and product

2.1.5.1 Summary of Reactions with Diethyl Chelidonate

To conclude our studies on the synthesis of polysubstituted phenols from pyranone precursors, the electron-poor 2,6-disubstituted diethyl chelidonate **156** was examined. The reaction of **156** with diethyl malonate **129** and ethyl cyanoacetate **139** gave complex product mixtures. Hydrolysis of the ester protecting groups during acid reflux could account for the formation of some side-products. Reaction conditions required for the reactions with nitromethane **133** and acetylacetone **135** to yield phenols **157** (46%) and **158** (2%) were milder than those previously required for *O*-benzyl-maltol **145** or 2,6-dimethyl-4*H*-pyran-4-one **43**, although yields were poor. An increase in the quantity of potassium *tert*-butoxide had a minimal effect on the conversion, which is assumed to be due to the absence of acidic methylene groups in diethyl chelidonate.[3]

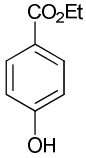
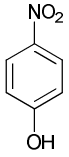
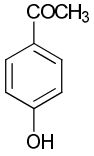
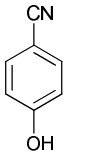
2.1.6 Effects of Microwave Irradiation on the Synthesis of Substituted Phenols from Pyran-4-one Precursors[3]

Following studies using conventional heating methods, microwave irradiation was examined in order to determine if the effects of microwave heating were beneficial to the reactions examined above. As previously discussed (**Section 1.7**), conventional heating methods are much slower than microwave irradiation due to their reliance on the convection/conduction of heat, resulting in the formation of a temperature gradient within the reaction mixture. The subsequent overheating observed in certain areas can lead to decomposition of compounds in the mixture. Microwave irradiation avoids this issue, as it penetrates the reaction vessel instantaneously, resulting in uniform heating of the reaction mixture, and the decreased formation of side-products or decomposition products. In our case, the use of conventional heating methods resulted in the formation of many side-products and in several cases reactions times were long and yields low. It is known that the use of microwave irradiation can accelerate the rate of reactions due to the energy from the microwaves being absorbed specifically by the reactants, and thus the activation energy for the reaction can be reached more rapidly. Therefore it was anticipated that shorter reaction times may suppress the formation of unwanted side-products and subsequently lead to higher yields of the desired phenolic compounds.

A wide range of conditions were studied in order to optimise each reaction where the desired phenol had previously been observed under conventional heating methods, and these are summarised in *Tables 6-9* below.

Firstly it was decided to study the reactions of 4*H*-pyran-4-one **35**, as this substrate had proven to be the fastest and highest yielding of the four pyranones. *Table 6* summarises the conditions investigated under microwave heating with each nucleophile (best results given in **bold type**), along with comparison of the results obtained using conventional heating methods (*italic type*).

Table 6 – Reaction variables for the reaction of 4H-pyran-4-one under microwave conditions, including comparison with yields obtained using conventional heating methods.[3]

Entry	Product	Eq. pyranone	Eq. nucleophile	Eq. KO*t*Bu	Time (min)	Temp. (°C)	Yield (%)
1		*1.7*	*1.1*	*1.3*	*3 h*	*110*	*94*†
2		1.0	1.1	1.1	15	120	58
3		**1.0**	**1.1**	**1.1**	**30**	**120**	**67**
4		1.0	1.1	2.0	15	120	47
5	**(128)**	1.0	1.1	2.0	30	120	63
6		*1.0*	*1.1*	*1.1*	*5 h*	*110*	*80*†
7		**1.0**	**1.1**	**1.1**	**15**	**120**	**80**
8		1.0	1.1	1.1	30	120	58
9		1.0	1.1	2.0	15	120	39
10	**(6)**	1.0	1.1	2.0	30	120	27
11		*1.0*	*2.0*	*2.0*	*20 h*	*110*	*95*†
12		1.0	1.1	2.0	15	120	12
13		**1.0**	**1.1**	**2.0**	**30**	**150**	**58**
14		1.0	1.1	3.0	15	150	14
15	**(87)**	1.0	1.1	3.0	30	150	13
16		*1.0*	*1.6*	*3.0*	*27 h*	*110*	*78*†
17		1.0	1.6	3.0	20	120	36
18		1.0	1.6	3.0	20	150	30
19		1.0	1.6	3.0	30	100	79
20	**(130)**	**1.0**	**1.6**	**3.0**	**30**	**150**	**86**

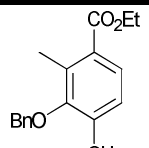
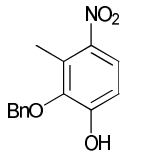
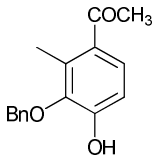
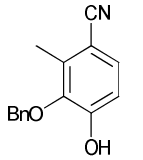
† Conventional yield

For the reaction of 4*H*-pyran-4-one **35** with diethyl malonate **129**, microwave reaction times of 15 min were initially examined with the quantity of base being varied (entries 2 and 4). In contrast to the results obtained using conventional heating methods, it was found that a lower quantity of base gave a higher yield. Increasing the reaction time to 30 min (entry 3, with 1.1 eq. base) give the best results (67%). These conditions were then repeated using nitromethane **133**. Once again, an increase in the quantity of base was detrimental to the reaction, and in this case a reaction time of 15 min was optimal (entry 7), giving 4-nitrophenol **6** in 80% yield. As the reaction with acetylacetone **135** was found to require harsher conditions under conventional heating methods, higher microwave temperatures were also investigated. At temperatures

suitable for the reactions of diethyl malonate and nitromethane (i.e. 120 °C) the yield was low (entry 12, 12%). Increasing both the quantity of base and the temperature gave similar results (entries 14 and 15). However, simply increasing the reaction time to 30 min and the temperature to 150 °C gave the best results (entry 13, 58%), with only 1.1 equivalents of nucleophile being required. Finally the reaction with ethyl cyanoacetate **139** was studied. This reaction was of particular interest due to the issues encountered during optimisation of the reaction under conventional heating methods. Keeping the quantities of nucleophile and base equal to those for the conventional heating methods, a range of temperatures and reaction times were investigated. Yields were low at both 120 °C and 150 °C with a reaction time of 20 min (entries 17 and 18). Increasing the reaction time to 30 min produced good results at 100 °C and 150 °C (entries 19 and 20) with the higher temperature being favoured and 4-hydroxybenzonitrile **130** being isolated in 86% yield – higher than that obtained using conventional heating methods and with a significantly shorter reaction time (30 min *cf.* 27 h).[3]

It could be concluded from these studies that although the yield increases for reactions of 4*H*-pyran-4-one **35** with nucleophiles were not significant, they were achieved through the use of shorter reaction times, and reaction profiles were generally cleaner than when conventional heating methods were utilised. Due to these promising results, application to the more troublesome reactions of the substituted pyranones was attempted. In experiments with *O*-benzyl-maltol **145** (*Table 7*), higher yields were obtained in all cases compared to conventional heating methods.

Table 7 – Reaction variables for the reaction of O-benzyl-maltol under microwave conditions, including comparison with yields obtained using conventional heating methods.[3]

Entry	Product	Eq. pyranone	Eq. nucleophile	Eq. KO*t*Bu	Time (min)	Temp. (°C)	Yield (%)
1	 **(146)**	*1.0*	*1.6*	*3.0*	*47 h*	*110*	*73*†
2		1.0	1.1	2.0	30	120	80
3		**1.0**	**1.6**	**2.3**	**30**	**120**	**99**
6	 **(147)**	*1.0*	*1.6*	*2.3*	*44 h*	*110*	*20*†
7		**1.0**	**1.6**	**2.0**	**30**	**120**	**53**
8		1.0	1.6	2.3	30	120	39
11	 **(148)**	*1.0*	*1.6*	*2.3*	*42 h*	*110*	*10*†
12		**1.0**	**1.6**	**2.3**	**30**	**150**	**86**
13		1.0	1.6	3.0	30	150	61
16	 **(149)**	*1.0*	*1.6*	*3.0*	*47 h*	*110*	*93*†
17		**1.0**	**1.6**	**2.3**	**30**	**120**	**99**
18		1.0	1.6	3.0	15	120	87

† Conventional yield

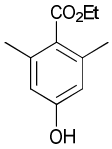
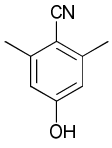
Reaction with diethyl malonate **129** required only 2.3 equivalents of base and 30 min reaction time (*cf.* 3.0 eq, 47 h) giving **146** in 99% yield. Decreasing the quantity of both nucleophile and base lowered the yield only slightly in this case (80%). Conditions for the reaction with nitromethane **133** (entry 7) differed in that only 2.0 equivalents of base were required to give a 53% yield of **147** (*cf.* 20%). For acetylacetone **135** a temperature increase to 150 °C was found to be beneficial to the reaction (entry 12), with a significant improvement in yield being observed (86% *cf.* 10%). As expected from studies using the unsubstituted pyranone **35**, increasing the quantity of base, in this case to 3 equivalents, lowered the yield (entry 13). Under conventional heating methods, reaction with ethyl cyanoacetate **139** was found to be the highest yielding of the reactions with *O*-benzyl-maltol **145** (93%). In this case, decreasing the

quantity of base and using 30 min reaction time gave the desired phenol **149** in 99% yield (entry 17).[3]

The application of microwave irradiation to reactions of *O*-benzyl-maltol **145** with nucleophiles was highly successful, with milder conditions and shorter reaction times being accompanied by increases in yields in all cases. Once again, fewer side-products were observed in the crude mixture, thus confirming that shorter reaction times can decrease the chance of side-reactions taking place.

As both *O*-benzyl-maltol **145** and 2,6-dimethyl-4*H*-pyran-4-one **43** contain active methylene groups, it was hoped that similar results would be obtained in the reactions of **43** (*Table 8*) due to the cleaner reaction profiles observed with *O*-benzyl-maltol, and lower quantities of base required compared to conventional heating methods.

Table 8 – Reaction variables for the reaction of 2,6-dimethyl-4H-pyran-4-one under microwave conditions, including comparison with yields obtained using conventional heating methods.[3]

Entry	Product	Eq. pyranone	Eq. nucleophile	Eq. KO*t*Bu	Time (min)	Temp. (°C)	Yield (%)
1		*1.0*	*1.6*	*3.0*	*44 h*	*110*	*18*†
2		1.0	1.6	2.0	15	150	46
3		**1.0**	**1.6**	**2.0**	**30**	**150**	**81**
4	**(152)**	1.0	1.6	3.0	30	150	78
16		*1.0*	*1.6*	*3.0*	*47 h*	*110*	*57*†
17		1.0	1.6	2.0	15	120	33
18		**1.0**	**1.6**	**2.0**	**30**	**120**	**47**
19	**(153)**	1.0	1.6	3.0	30	120	40

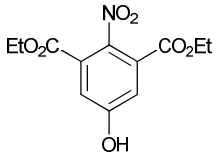
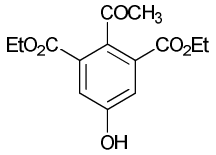
† Conventional yield

Reactions with diethyl malonate **129** and ethyl cyanoacetate **139** to give phenols **152** and **153**, respectively, were attempted under microwave irradiation. In both cases, under conventional heating methods long reaction times (44-47 h) and a high number of equivalents of base

(3.0 eq.) were required, giving **152** in low yield (18%), and **153** in good yield (57%). However, as trends have shown in the reactions of 4*H*-pyran-4-one **35** and *O*-benzyl-maltol **145**, shorter reaction times (30 min) and fewer equivalents of base (2.0 eq.) were beneficial to the reaction, with 3 equivalents being detrimental, presumably for reasons described earlier. Ethyl 4-hydroxy-2,6-dimethyl benzoate **152** was isolated in an excellent, improved yield of 81% when the temperature was increased to 150 °C. A good yield of 4-hydroxy-2,6-dimethylbenzonitrile **153** (47%) was also achieved. Although this was not an improvement from that obtained using conventional methods (57%), the shorter reaction time, milder conditions and cleaner reaction profile deemed both reactions a success.[3]

Reactions using diethyl chelidonate **156** were attempted using nitromethane **133** and acetylacetone **135** (*Table 9*). A small improvement in yield was achieved with the use of nitromethane, with the substituted phenol **157** being isolated in 48% yield (*cf.* 46%). In the case of acetylacetone, a very poor yield of 2% was obtained under conventional heating methods, with a long reaction time of 48 h. Using 2.3 equivalents of base and 30 min reaction time, an improved yield of 35% **158** was achieved. Once again, fewer side-products were observed in the crude reaction mixture.[3]

Table 9 – Reaction variables for the reaction of diethyl chelidonate under microwave conditions, including comparison with yields obtained using conventional heating methods.[3]

Entry	Product	Eq. pyranone	Eq. nucleophile	Eq. KO*t*Bu	Time (min)	Temp. (°C)	Yield (%)
1		*1.0*	*1.1*	*3.0*	*24 h*	*110*	*46*†
2		1.0	1.6	2.3	15	120	29
3		**1.0**	**1.6**	**2.3**	**30**	**120**	**48**
4	**(157)**	1.0	1.6	3.0	30	120	22
16		*1.0*	*1.6*	*3.0*	*48 h*	*110*	*2*†
17		1.0	1.6	2.3	15	120	34
18		**1.0**	**1.6**	**2.3**	**30**	**120**	**35**
19	**(158)**	1.0	1.6	3.0	30	120	12

† Conventional yield

2.1.6.1 Summary of Effects of Microwave Irradiation on the Synthesis of Substituted Phenols from Pyran-4-one Precursors

As expected, the use of microwave irradiation greatly accelerated the reactions with most giving optimum results after 30 min. Microwave experiments with 4*H*-pyran-4-one **35** gave yields similar to those obtained by conventional heating, but with shorter reaction times being required. Substantial improvements in yield were, however, observed with pyranones **43** and **145** when microwave heating was employed. In the case of *O*-benzyl-maltol **145**, 2-2.3 equivalents of base gave the cleanest reaction profiles. Addition of more base caused the formation of side-products and reduced yields. Reactions with 2,6-dimethyl-4*H*-pyran-4-one **43** gave varying results, with the best result being the reaction with diethyl malonate **129**. Microwave irradiation did not improve yields significantly in reactions with diethyl chelidonate **156**, with the best results being obtained in the reaction with acetylacetone **135**, which gave extremely poor results under conventional heating methods under microwave irradiation. In the majority of cases, it was found that the optimum quantities of nucleophile and base required in the microwave-assisted reactions were different from those required under conventional heating, often with a lower quantity of base being required. In several cases microwave heating at

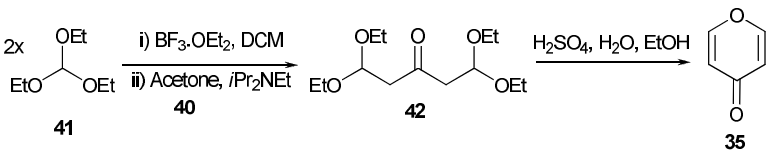
150 °C enhanced yields in reactions which gave poor yields by conventional heating. Overall, the use of microwave irradiation had beneficial effects on the reactions studied, with shorter reaction times accompanying higher yields, due to a decrease in compound decomposition and reduced formation of unwanted side-products.

2.1.7 Summary of the Synthesis of Substituted Phenols from Pyran-4-one Precursors

We have explored the scope of the base-catalysed reaction of substituted and unsubstituted pyran-4-ones with a range of carbon nucleophiles for the synthesis of phenols.[1,3,4] Under conventional heating, the most successful results were obtained with 4*H*-pyran-4-one **35** and 3-(benzyloxy)-2-methyl-4*H*-pyran-4-one (*O*-benzyl maltol) **145**. Limited success was achieved with 2,6-dimethyl-4*H*-pyran-4-one **43** and diethyl chelidonate **156**. The use of microwave irradiation accelerated the reactions with most being complete within 30 min. Milder reaction conditions and lower quantities of reagents were required in the majority of cases. When 4*H*-pyran-4-one **35** was used, there was little difference between the yields obtained using microwave and conventional heating. However substantial improvements in yield were observed with substituted pyran-4-ones when microwave heating was employed. This chemistry also has the potential application for the regiospecific placement of carbon isotopes into benzene derivatives through the use of suitably labelled pyranone precursors or nucleophiles.[4]

2.2 Synthesis of 4*H*-Pyran-4-one (35) and ^{13}C-Labelled Isotopomers (35d and 35e)

Having developed a methodology for the synthesis of phenols from pyranones, we wanted to exploit this route further for applications in isotopic labelling.[3] The introduction of isotope labels such as carbon-13 could be achieved by two main routes. Firstly, through the use of ^{13}C-labelled nucleophiles, as reported by Steglich *el al.*[1] in the synthesis of ethyl 4-hydroxy-[1-^{13}C]benzoate **128a** from the reaction of pyran-4-one **35** with diethyl [2-^{13}C]malonate **129a** (**Section 1.4**). This allows for the introduction of one ^{13}C-isotope label into the aromatic ring. The second method would be through the use of a ^{13}C-labelled pyranone, which could allow the introduction of multiple ^{13}C-labels into the aromatic ring.[4] As no previous routes to ^{13}C-labelled pyranones had been described, we chose to extend and modify an existing pyranone synthesis to incorporate ^{13}C-labelled starting materials. As previously mentioned (**Section 1.4.1**), the most efficient synthesis of the unsubstituted 4*H*-pyran-4-one appears to be the reaction between acetone **40** and triethyl orthoformate **41** (*Scheme 82*).[10] This route is particularly desirable to our studies as both triethyl orthoformate and acetone are available in ^{13}C-labelled forms, with the latter being available with either 1, 2, or 3 ^{13}C-labelled atoms.



Scheme 82 – Hobuss route to 4H-pyran-4-one.[10]

Hobuss *el al.*[10] originally reported the use of triethyl orthoformate and acetone in a ratio of 5:1. We chose to investigate this route further, beginning with optimisation of the preparation of the unlabelled intermediate **42**. Our studies showed that the use of a 2:1 ratio (i.e. the exact stoichiometry of the reaction) gave cleaner results, allowing the intermediate to be used *in situ* without further purification.[4] Interestingly it was found that with the use of 5 equivalents of

triethyl orthoformate to 1 equivalent of acetone, the bisacetal **42** (as reported by Hobuss) was the major product. However with a 2:1 ratio, we observed enone **159** as the major product. The formation of this unexpected product was discovered upon examination of the ^{1}H NMR spectrum, with the observation of a pair of doublets at 5.57 and 7.51 ppm with large *J* values of 12.3 Hz, suggesting the presence of an *E* (*trans*) alkene. The ethyl signals had also moved from 1.11 and 3.46 ppm to 1.27 and 3.87 ppm, and the characteristic CH_2-CH signals of the bisacetal at 2.71 and 4.84 ppm had disappeared. *Diagram 19* shows the ^{1}H NMR spectrum of a 0.4:1 mixture of bisacetal **42** and enone **159**. After further analysis by ^{13}C NMR spectroscopy and examination of the ^{1}H-^{13}C HSQC and HMBC spectra, it was clear that a symmetrical product had formed and this was determined to be the conjugated enone **159**.

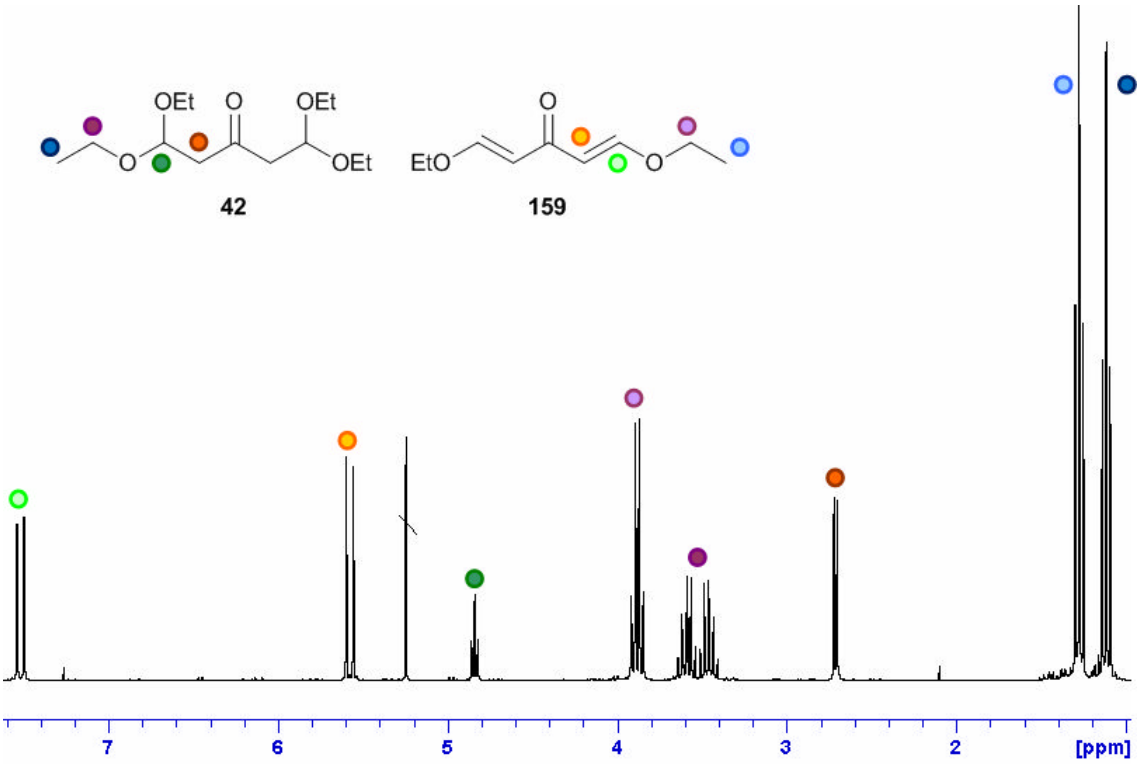
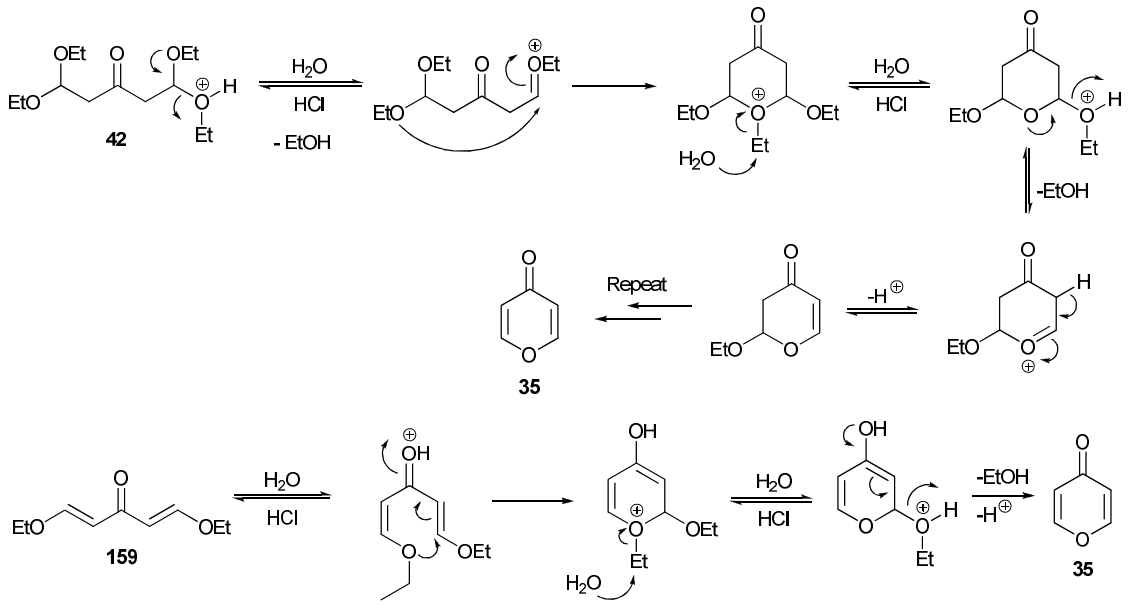


*Diagram 19 – ^{1}H NMR spectrum of 0.4:1 mixture of bisacetal **42** and enone **159** (400 MHz, $CDCl_3$).*

However, the presence of two possible intermediates is not an issue for the final cyclisation, as it has been shown to proceed from either intermediate (*Scheme 83*).

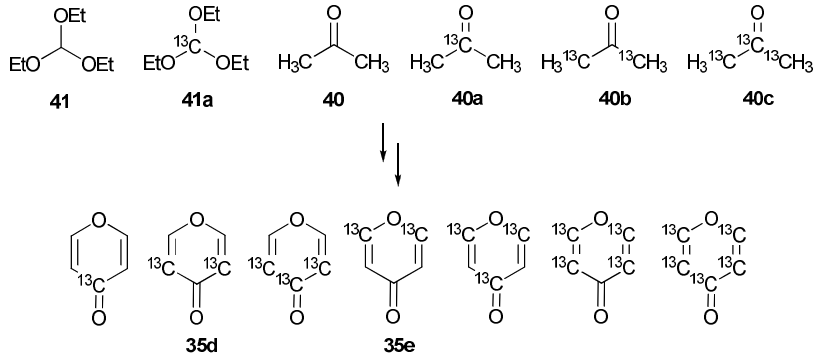


*Scheme 83 – Cyclisation to pyranone **35** from bisacetal **42** and enone **159**.*

Cyclisation to 4*H*-pyran-4-one **35** was achieved under acidic conditions with the use of hydrochloric acid in place of sulfuric acid. This change was required because during optimisation studies it was discovered that the pyranone was isolated from the aqueous phase and not the organic phase as implied in the original publication.[10] Therefore it was not possible to neutralise the aqueous reaction mixture with sodium hydrogen carbonate without contaminating the final product with large quantities of salt. It was also found that the pyranone was sensitive to light, heat and acid during the final work-up and so our procedure accounts for these factors.[4] The use of hydrochloric acid meant that solvents (including HCl) could be removed from the reaction mixture to give a residue which was not strongly acidic – this was not possible with the use of sulfuric acid. The residue was subsequently extracted with water to give the desired pyranone in excellent purity and yield (98%) after removal of the solvents at reduced pressure below 40 °C.

As previously mentioned, the Hobuss route is particularly desirable as both starting materials are available in ^{13}C-labelled forms. This then offers the potential for the synthesis of a range of isotopomers of the pyranone depending upon the labelled substrates employed. *Scheme 84*

below shows the 7 isotopomers which could be obtained from various combinations of triethyl orthoformate **41**, triethyl [^{13}C]orthoformate **41a**, acetone **40**, [2-^{13}C]acetone **40a**, [1,3-$^{13}C_2$]acetone **40b** and [1,2,3-$^{13}C_3$]acetone **40c**. Following subsequent reaction with pronucleophiles, this could yield *para*-substituted phenols containing between one and six ^{13}C-atoms, depending on the pyranone substitution pattern (*Scheme 84*) and whether a ^{13}C-labelled nucleophile is used in the subsequent reaction.[4]



Scheme 84 – Isotopomers of 4H-pyran-4-one.

In order to demonstrate the potential of our procedure for the regioselective placement of ^{13}C-atoms within the pyranone ring, the synthesis of [3,5-$^{13}C_2$]4*H*-pyran-4-one **35d** from [1,3-$^{13}C_2$]acetone **40b**, and [2,6-$^{13}C_2$]4*H*-pyran-4-one **35e** from triethyl [^{13}C]orthoformate **41a** were carried out using our optimised procedure.[4] Triethyl orthoformate (2 eq.) was treated with boron trifluoride diethyl etherate to give an orange slurry of diethoxycarbenium fluoroborate, to which *N,N*-diisopropylethylamine and acetone (1 eq.) were added. Vigorous stirring was required during the reaction (2.5 h at -78 °C) to ensure that solidification of the reaction mixture did not occur through the formation of a viscous mixture. The enone was isolated crude after work-up and used in the following cyclisation without further purification. Both [3,5-$^{13}C_2$]4*H*-pyran-4-one **35d** and [2,6-$^{13}C_2$]4*H*-pyran-4-one **35e** were synthesised in excellent yield (quantitative and 92%, respectively) and these yields were reproducible on scales ranging from 1-5 g acetone. The ^{1}H NMR spectra for both isotopomers are shown below (*Diagram 20*), where the large splitting due to ^{1}H-^{13}C coupling is visible. In the top spectrum, the ^{13}CH-3,5

protons of [3,5-$^{13}C_2$]4*H*-pyran-4-one **35d** are visible as a double multiplet at 6.29 ppm with a *J* value of 171.7 Hz, and the CH-2,6 protons as a multiplet at 8.13 ppm. The AB system observed in the unlabelled pyranone is not as clear in the spectra of the labelled pyranones due to the extra splitting observed. The bottom spectra shows the ^{13}CH-2,6 protons of [2,6-$^{13}C_2$]4*H*-pyran-4-one **35e** as a double multiplet this time at 8.20 ppm (*J* 202.0 Hz), and the unlabelled CH-3,5 protons as a multiplet at 6.33 ppm, thus reversing the splitting pattern observed for [3,5-$^{13}C_2$]4*H*-pyran-4-one.

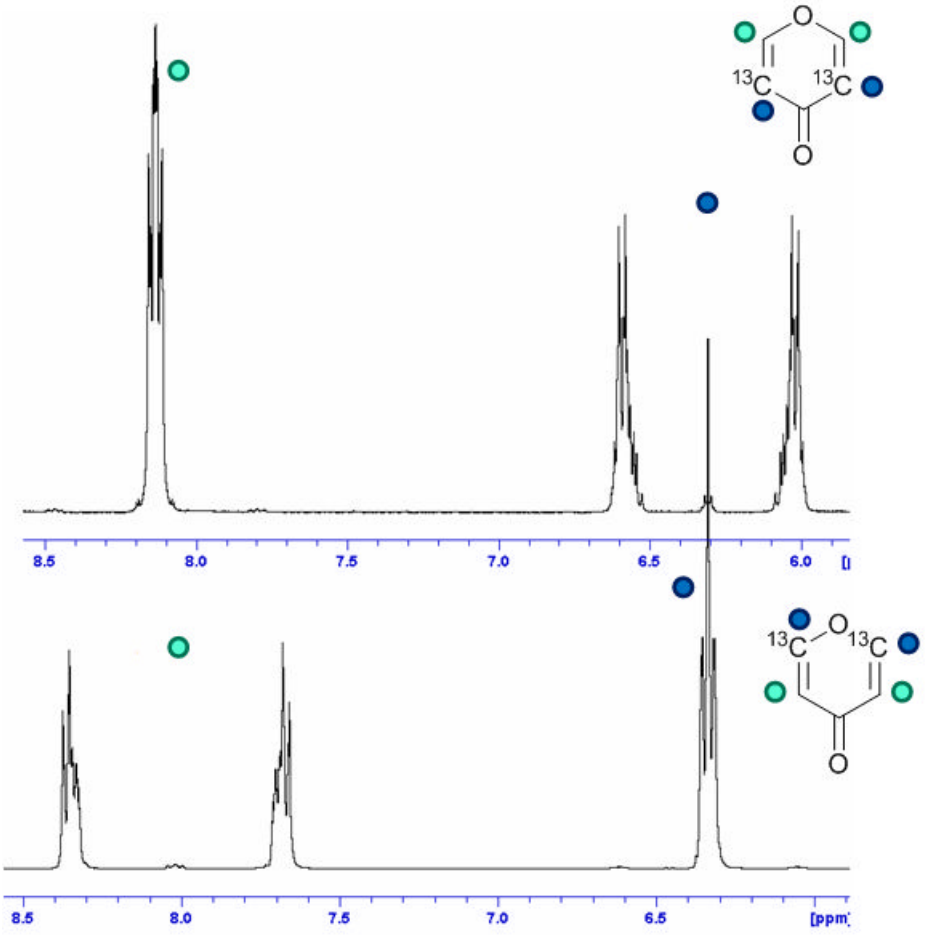


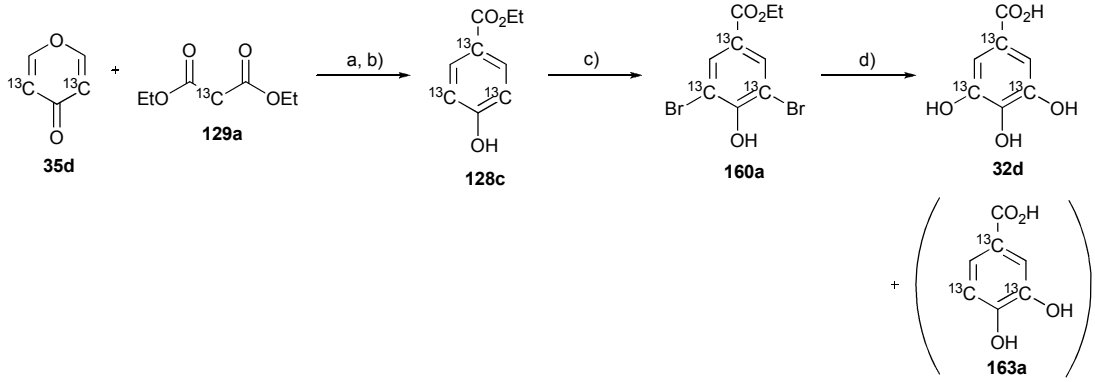
*Diagram 20 – ^{1}H NMR spectrum of [3,5-$^{13}C_2$]4H-pyran-4-one **35d (top)** (300 MHz, DMSO-d_6) and [2,6-$^{13}C_2$]4H-pyran-4-one **35e (bottom)** (300 MHz, MeOD).*

2.2.1 Summary of the Synthesis of 4*H*-Pyran-4-one and ^{13}C-Labelled Isotopomers

After optimisation and modification of the original literature procedure,[10] a high-yielding route to 4*H*-pyran-4-one **35** from triethyl orthoformate (2 eq.) and acetone (1 eq.) *via* the enone intermediate **159** was developed. This route was successfully applied to the synthesis of two of the seven possible ^{13}C-labelled isotopomers of the pyranone, namely [3,5-$^{13}C_2$]4*H*-pyran-4-one **35d** and [2,6-$^{13}C_2$]4H-pyran-4-one **35e**, through the use of ^{13}C-labelled substrates.[4] This demonstrates the potential of our route to the regioselective placement of ^{13}C-atoms into the pyranone ring, which can subsequently be applied to synthesis of ring-labelled phenols as discussed in **Sections 2.1**, **2.3** and **2.6**.[3]

2.3 Synthesis of [1,3,5-$^{13}C_3$]Gallic Acid (32d)

Gallic acid **32**, or 3,4,5-trihydroxybenzoic acid, is a naturally occurring polyphenol found in many plant species, often in the ester form – for example the gallate esters found in tea leaves, as previously examined (**Section 1.5.3**). As no ring-labelled versions of gallic acid had been reported, our aim was to synthesise gallic acid with the regioselective placement of three ^{13}C-atoms into the aromatic ring. It was also desirable to develop a synthesis which required the minimum amount of purification in order to minimise losses of expensive ^{13}C-labelled material. Following the successful synthesis of ^{13}C-labelled 4*H*-pyran-4-ones **35d** and **35e** (**Section 2.2**),[4] and development of Steglich's route for the preparation of *para*-substituted phenols from pyran-4-ones (**Section 2.1**),[1,3] the application of this procedure to the synthesis of a small natural product, gallic acid, was carried out (*Scheme 85*).[4]



*Scheme 85 – Route to [1,3,5-$^{13}C_3$]gallic acid **32d** from [3,5-$^{13}C_2$]4H-pyran-4-one **35d**; Reagents and conditions:* a) *t*BuOH, KO*t*Bu, reflux, 3 h; b) 1M HCl, reflux, 1 h (74%); c) AcOH, NaOAc, Br_2, rt, 1.5 h (99%); d) NaOH, H_2O, $CuSO_4.5H_2O$, 90 min, rt. then 18 h, reflux (48%).[4]

Optimisation studies of the reaction of pyranone with diethyl malonate showed that stoichiometric amounts of both substrates and 1.1 equivalents of base were the optimal conditions for the reaction. With [3,5-$^{13}C_2$]4*H*-pyran-4-one **35d** in hand, the reaction with diethyl [2-^{13}C]malonate **129a** in the presence of potassium *tert*-butoxide as base, followed by aromatisation and loss of carbon dioxide during the reflux in 1M hydrochloric acid, yielded

para-substituted phenol **128c** in good yield (74%) with ^{13}C-labels in three pre-determined positions in the ring. The signal corresponding to the ^{13}CH-3,5 protons were observed at 6.88 ppm as a doublet of multiplets (*J* 159.2 Hz), and that of the CH-2,6 protons at 7.97 ppm as a broad doublet (*J* 8.7 Hz) (*Diagram 21*). The ethyl signals were also clearly visible (4.36 and 1.39 ppm). Despite a large enhancement of the ^{13}C-3,5 and ^{13}C-1 signals in the ^{13}C NMR spectrum (115.1 and 123.0 ppm respectively), all carbons atoms were observed. The signal for the C-2,6 carbon atoms was visible as a doublet of triplets (131.8 ppm) due to splitting from the *ortho*- and *para*-^{13}C atoms in the ring (*J* 5.6 and 58.4 Hz). It should also be noted that if required, various isotopomers of 4*H*-pyran-4-one could be used at this stage, thus allowing a variety of ^{13}C-labelled *para*-substituted phenols to be synthesised with the required number of ^{13}C-atoms regioselectively placed within the aromatic ring.

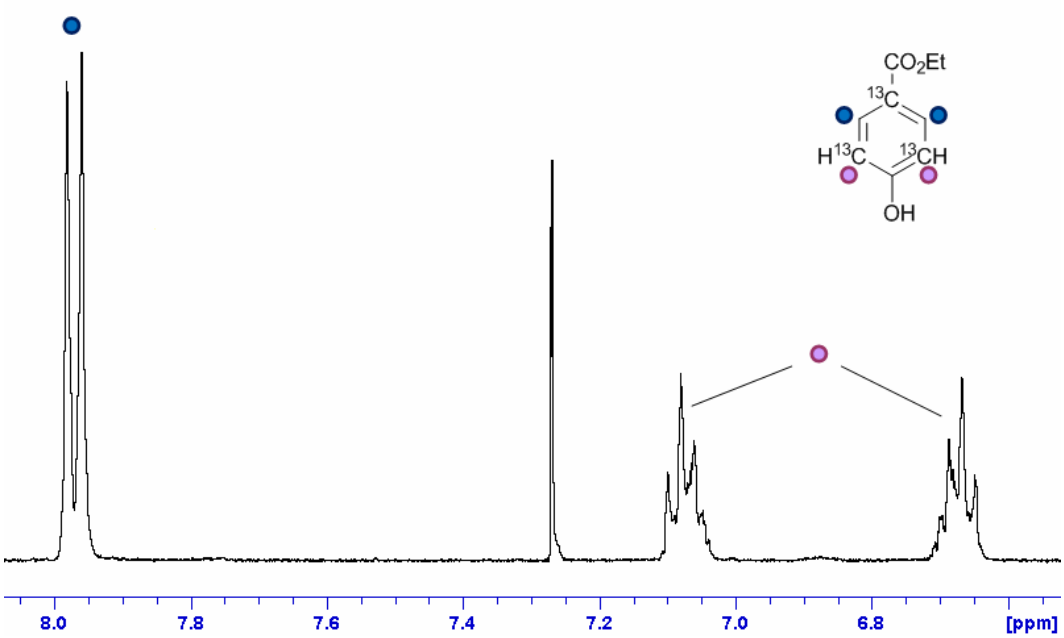


*Diagram 21 – Aromatic region of ^{1}H NMR spectrum of **128c** (400 MHz, $CDCl_3$).*

Para-substituted phenol **128c** was isolated in good purity, allowing the bromination to be carried out on the crude substrate. Bromination of **128c** to give ethyl 3,5-dibromo-4-hydroxy-[1,3,5-$^{13}C_3$]benzoate **160a** was first attempted on unlabelled substrates in order to optimise conditions. Adding a solution of the substrate **128c** in dichloromethane to an excess of bromine

(10 eq.) at 0 °C[1] was unsuccessful, giving only traces of the mono-bromo compound **161a** and none of the desired di-bromo compound **160a**, as may have been expected from using such a large excess of bromine. Solubility of the ester in dichloromethane at 0 °C was poor, and so the reaction was also attempted at room temperature over a range of reaction times. In this case solubility was greatly improved, however a mixture of products was recovered in each case which included starting material and multiply-brominated compounds. An alternative procedure was therefore sought. It was found that the use of an excess of bromine in acetic acid with sodium acetate at room temperature was an extremely fast and high yielding reaction (99%), with only the desired di-bromo compound **160a** being formed. Once again purification was not required. All carbon atoms were visible in the ^{13}C NMR spectrum with enhanced signals corresponding to ^{13}C-3,5 and ^{13}C-1 being observed at 109.6 and 125.1 ppm respectively. A double double doublet at 133.6 ppm (*J* 69.1, 59.4 and 4.4 Hz) was observed for C-2,6 due to coupling with the *ortho*- and *para*-^{13}C-atoms. The proton NMR spectrum also showed splitting of the CH-2,6 signal (8.07 ppm), with a doublet of triplets being observed (*J* 2.8 and 1.7 Hz) (*Diagram 22*).

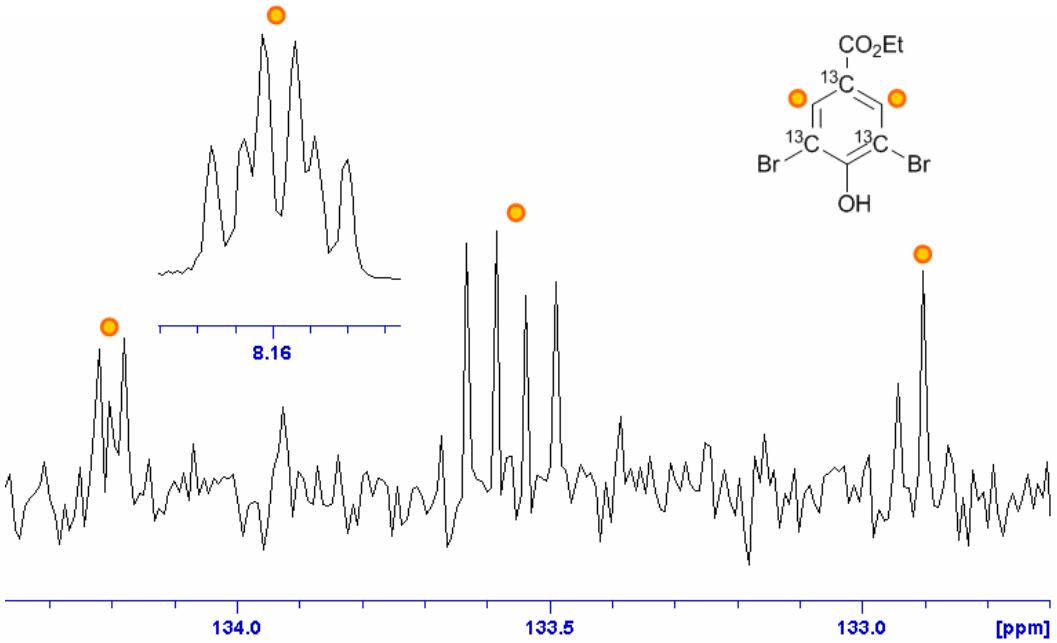
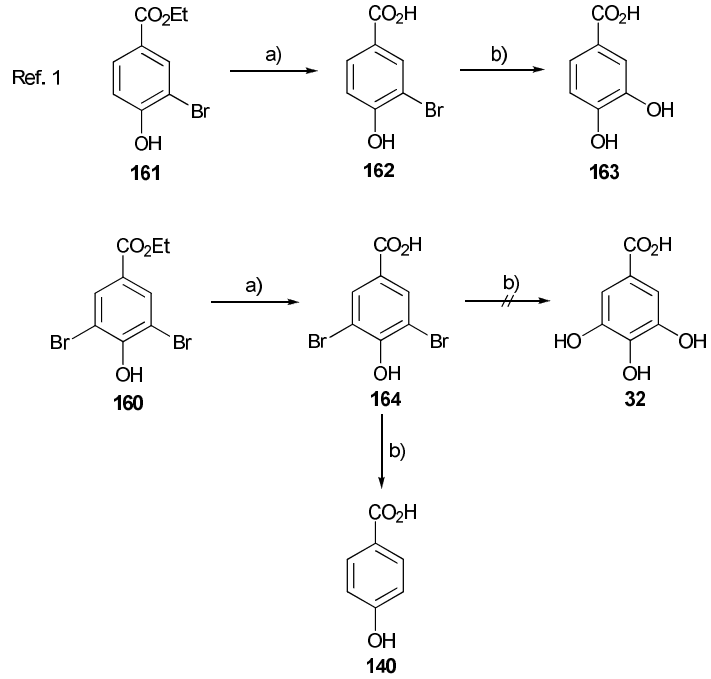


*Diagram 22 – Region of ^{13}C NMR spectrum of **160a** and aromatic region of ^{1}H NMR spectrum showing CH-2,6 resonances (400 MHz, $CDCl_3$).*

It had been shown that the mono-bromo derivative **161** could firstly be hydrolysed to the acid **162** using sodium hydroxide, followed by substitution of the bromine atoms with hydroxyl groups using activated copper and copper sulfate in aqueous potassium hydroxide to yield 3,4-dihydroxybenzoic acid **163** (*Scheme 86*).[1] This series of reactions was attempted on unlabelled substrates, and although the di-bromo ester was not soluble in aqueous sodium hydroxide, the acid product was, and so it could be seen after overnight stirring that the reaction had been successful. The hydrolysis product **164** was recovered in good yield (98%) as a white solid. However, when the subsequent reaction using activated copper was attempted using the di-bromo compound **160**, only 4-hydroxybenzoic acid **140** was recovered.



Scheme 86 –Reagents and conditions: a) NaOH, H_2O; b) KOH, H_2O, activated copper, $CuSO_4.5H_2O$.[1]

We then thought that it seemed possible to combine the hydrolysis step with the substitution step while optimising the substitution in order to prevent the formation of 4-hydroxybenzoic acid **140**. The failure of the reaction using the original procedure could be due to the conditions being too harsh or a requirement for a longer solvent degassing time. Therefore it was decided to remove the activated copper from the reaction, use only sodium hydroxide as base, and to

degass the sodium hydroxide over 4 h stirring under reduced pressure before transfer of the substrate to the reaction flask. Degassing the solution for shorter periods of time (<3 h) resulted in an increase in the amount of 3,4-dihydroxybenzoic acid **163** and 4-hydroxybenzoic acid **140** formed during the reaction. As the hydrolysis had previously been successful at room temperature, the reaction mixture was allowed to stir at room temperature for 90 min under reduced pressure after transfer of the substrate by cannula to the sodium hydroxide/$CuSO_4$ solution. Heating at reflux overnight followed by an acidic work-up yielded the desired gallic acid **32** in good yield, with a small amount of 3,4-dihydroxybenzoic acid **163** also present in the crude mixture. Using labelled substrates a ratio of 10:1 [1,3,5-$^{13}C_3$]gallic acid **32d** to 3,4-dihydroxy-[1,3,5-$^{13}C_3$]benzoic acid **163a** was isolated as a crude mixture. Purification was required at this stage to separate the two products and it was found that yields after purification were greatly increased by running the column under a positive pressure of argon. Purification therefore yielded **32d** in 48% yield, with a 6% yield of **163a** also being isolated, giving the [1,3,5-$^{13}C_3$]gallic acid **32d** in an overall yield of 35% over 5 steps from triethyl orthoformate **41** and [1,3-$^{13}C_2$]acetone **40b**.

The ^{1}H NMR spectrum of [1,3,5-$^{13}C_3$]gallic acid **32d** (*Diagram 23*) showed a double doublet at 7.02 ppm (*J* 3.8 and 1.9 Hz) corresponding to the aromatic protons which couple with the ^{13}C-labels in the ring. Broad singlets corresponding to the hydroxyl protons were also visible (7.90 and 8.10 ppm). All carbon atoms were visible in the ^{13}C NMR spectrum, with ^{13}C-1 and ^{13}C-3,5 signals enhanced at 122.0 and 146.0 ppm respectively, and that of ^{13}C-3,5 being observed as a doublet (*J* 2.4 Hz) due to splitting with ^{13}C-1.

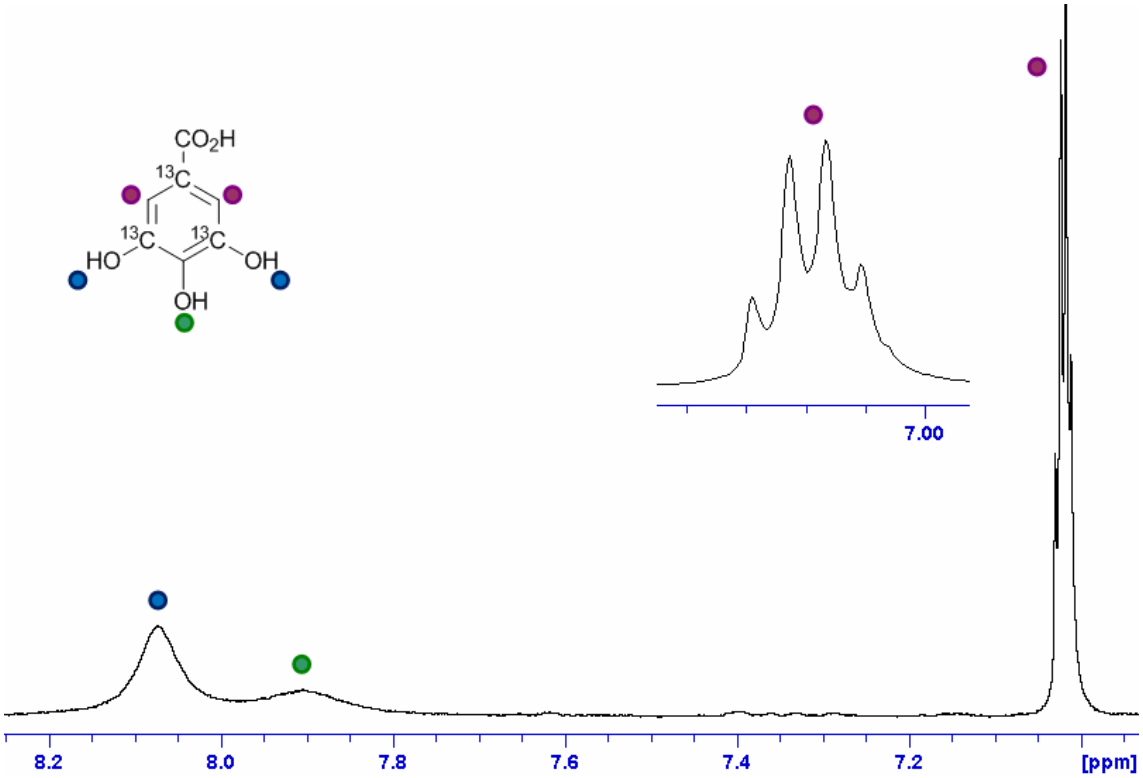


*Diagram 23 – ^{1}H NMR spectrum of [1,3,5-$^{13}C_3$]gallic acid **32d** with expansion of the aromatic region (300 MHz, acetone-d_6).*

The ^{1}H NMR spectrum of 3,4-dihydroxy-[1,3,5-$^{13}C_3$]benzoic acid **163a** (*Diagram 24*) was more interesting due to the lack of symmetry in the molecule compared to [1,3,5-$^{13}C_3$]gallic acid **32d**. A double double triplet was observed at 6.77 ppm with one large *J* value (159.6 Hz) corresponding to direct coupling of the proton attached to the ^{13}C-atom in position 5. Two smaller *J* values of 8.5 and 6.9 Hz were also measured due to coupling to H-6 and *meta*-^{13}C atoms. In the ^{13}C NMR spectrum, three signals were observed corresponding to the three ^{13}C-atoms. Both ^{13}C-5 and ^{13}C-3 signals were present as doublets (*J* 6.2 Hz), and ^{13}C-1 as a singlet.

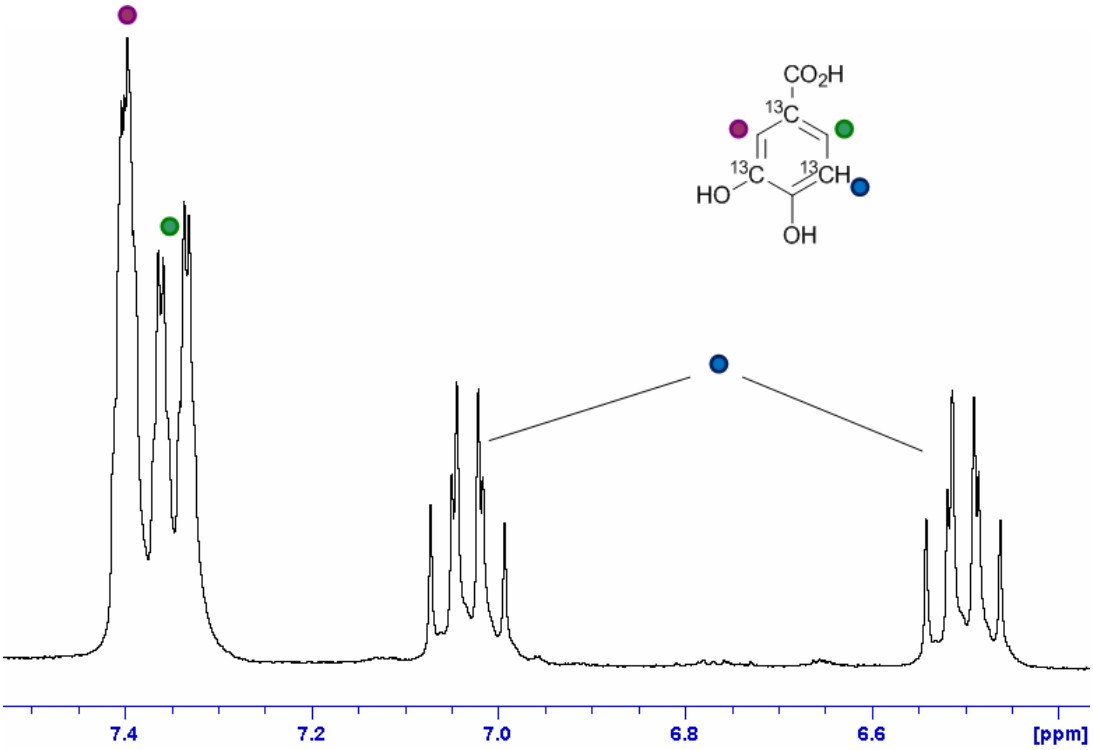


*Diagram 24 – ^{1}H NMR spectrum of 3,4-dihydroxy-[1,3,5-$^{13}C_3$]benzoic acid **163a** (400 MHz, acetone-d_6).*

If desired, this method therefore also has the potential to be applied to the synthesis of 3,4-dihydroxy-[1,3,5-$^{13}C_3$]benzoic acid **163a** directly. This could be achieved by carrying out a mono-bromination of **128c** using a similar method to that described by Steglich,[1] but where a solution of bromine in dichloromethane is added dropwise to a suspension of the substrate in dichloromethane. A similar procedure to this was developed for the bromination of 4-hydroxyacetophenone during studies towards the synthesis of the anthocyanin cyanidin-3-glucoside (see **Section 2.6**).

2.3.1 Summary of the Synthesis of [1,3,5-$^{13}C_3$]Gallic Acid

A fast, efficient and high yielding route to the synthesis of [1,3,5-$^{13}C_3$]gallic acid **32d** has been developed starting from commercially available ^{13}C-labelled acyclic building blocks. Our route

allows the regioselective placement of ^{13}C-atoms within the aromatic ring, demonstrated by the synthesis of both [2,6-$^{13}C_2$]4*H*-pyran-4-one **35e** and [3,5-$^{13}C_2$]4*H*-pyran-4-one **35d** (**Section 2.2**), followed by conversion of **35d** to *para*-substituted phenol **128c** and subsequent ring functionalisation. The final two transformations involving ester hydrolysis and conversion of bromine to hydroxyl groups were successfully modified to be carried out in one simple step, thus potentially minimising losses. The route has the potential to be applied to the synthesis of 3,4-dihydroxy-[1,3,5-$^{13}C_3$]benzoic acid **163a** if desired. Also the need for the minimum amount of purification has been met, with purification only being required in the final step, therefore minimising losses of ^{13}C-labelled material.[4]

2.4 Iridium-Catalysed C-H Activation/Borylation/Oxidation for the Generation of Phenols

2.4.1 Introduction

The formation of C-C and C-X (where X is a heteroatom) bonds can be achieved through the use of aryl boronate esters. Prior to 1995, these were generally prepared by the use of an arene (or aryl halide), a boron electrophile and a metallating agent such as lithium. Indeed lithiation reactions are still among the most common methods for functionalising C-H bonds in heterocycles.[11] Miyaura *el al.*[12] and Masuda *el al.*[13] have recently developed the synthesis of arylboronates *via* palladium-catalysed cross-coupling, with the use of only catalytic amounts of the metal reagent under mild reaction conditions, thus eliminating the stoichiometric quantities of metal reagent previously required. In 1993, Marder *el al.*[14] reported the borylation of toluene in the presence of catecholborane and an iridium catalyst, which was the beginning of iridium-catalysed C-H activation/borylation as we know it now. Examples of catalytic borylation of aryl, heteroaryl, and alkyl C-H bonds are now well established.[15]

Certain iridium(I) complexes (such as **165** and **166**) possessing a 4,4'-di-tert-butyl-2,2'-bipyridyl (d*t*bpy) ligand **167** are capable of exhibiting excellent activity and selectivity for aromatic C-H borylations, using either bis(pinacolato)diboron (B_2Pin_2) **168** or pinacolborane (HBPin) **169** (*Diagram 25*).[16] The use of d*t*bpy for these types of reaction over other commonly used ligands (such as dppe (bis(diphenylphosphino)-ethane) **170**) is beneficial as it has been shown to generate iridium complexes of greater solubility when solvents such as hexane and octane are used.[17] Due to the electron-donating properties and relatively small amount of steric hindrance from the bipyridyl ligand, the arene undergoes oxidative addition to the iridium(III) tris-boryl precursor resulting in the formation of an iridium(V) species (see mechanistic studies below, *Scheme 89*). The formation of this 7-coordinate iridium species is only possible due to

the small steric influence imparted by the aromatic ring and boron ligand.[16] It was discovered in 2002 by Miyaura *el al.*[17] that temperatures of between 150 °C and 200 °C were required for the process. It was also reported that both electron-rich and electron-poor monosubstituted arenes could undergo reaction to produce mixtures of *meta*- and *para*-borylated products. The *ortho* isomer was not observed due to the steric restraints put on the *ortho*-position by the arene ring substituents. In the case of 1,3-disubstituted arenes, regioselective *meta*-borylation was observed even when two different substituents were present on the substrate, with poly-borylation at either *ortho*- or *para*-positions rarely taking place.

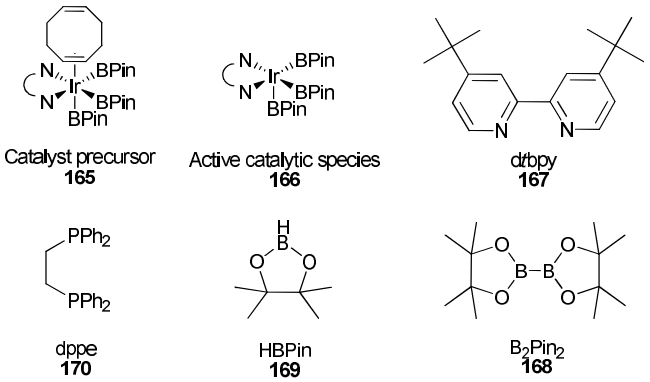
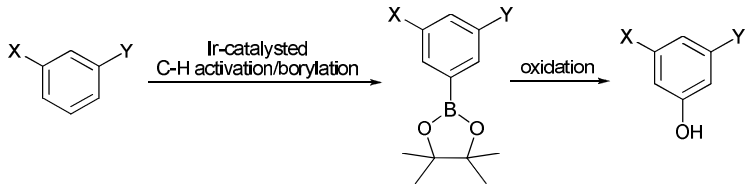


Diagram 25 – Structures of common reagents used in [Ir(OMe)(COD)]$_2$-catalysed C-H activation/borylation.[16-18]

In 2003, Maleczka *el al.*[18] published a noteworthy expansion of the C-H activation/borylation protocol, for the preparation of phenols. The procedure is of great significance as it is aimed at the introduction of a *meta*-arylboronic ester in arenes bearing *ortho*-/*para*-directing groups, followed by their conversion to the *meta*-substituted phenol *via* oxidation. The introduction of substituents at the *meta*-position is often difficult by traditional means (such as electrophilic aromatic substitution) when *ortho*-/*para*-directing groups are present. This is due to the electronic effects which govern regioselectivities in aromatic substitutions and therefore the 5-position in 1,3-disubstituted benzenes is particularly difficult to functionalise in the presence of *ortho*-/*para*-directors. However, in the case of transition metal catalysis, steric effects around the metal centre and substrate tend to dominate regioselectivities, and this is also true for

reactions involving the activation of aromatic C-H bonds. Maleczka *el al.* utilised iridium catalysis in their route due to the good functional group tolerance and selectivity of the iridium systems.[18] They proposed a one-pot two-step protocol involving conversion to the arylboronic ester, followed by oxidation to the phenol, as outlined in *Scheme 87* below, which would provide a direct route to a number of phenols.[18]



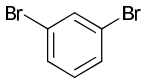
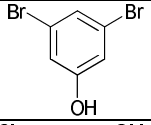
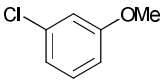
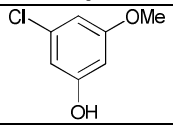
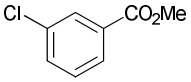
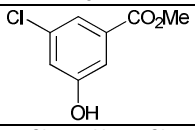
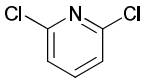
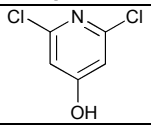
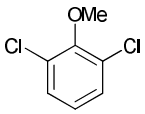
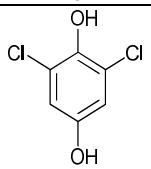
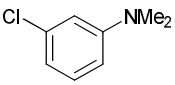
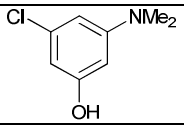
Scheme 87 – Maleczka protocol for synthesis of 3,5-disubstituted phenols.[18]

The first stage involves the transformation of the 1,3-disubstituted benzene into the arylboronic ester. The initial protocol developed involves heating the substrate in the presence of HBPin (1.5-2.5 eq.) with the iridium catalyst (Ind)-Ir(COD) (0.02 eq.) and either bis(dimethylphosphino)ethane (dmpe) at 150 °C or dppe at 100 °C until completion of the borylation. This stage could be carried out in solvent free conditions, but the use of inert solvents such as *iso*-hexane or octane is also possible. It was found that arenes presenting electron-donating substituents required longer reaction times for completion of the borylation, although subsequent oxidations were not affected.[18]

The second stage is conversion of the arylboronic ester to the phenol. After cooling the reaction mixture following the borylation, any solvents used in the first stage were removed. Acetone and an aqueous solution of oxone were then added sequentially. The oxidation could be performed in other water-miscible solvents, but it was found that a 1:1 mixture of water and acetone yielded the best results, with most oxidations going to completion after 10 minutes at room temperature, with no over-oxidation being observed.[18] *Table 10* outlines some examples of substrates used by Maleczka *el al.* (2 mol% catalyst and ligand, 100-150 °C).[18] Although not mentioned in this table, they discovered that while tolerance to bromide and chloride was good,

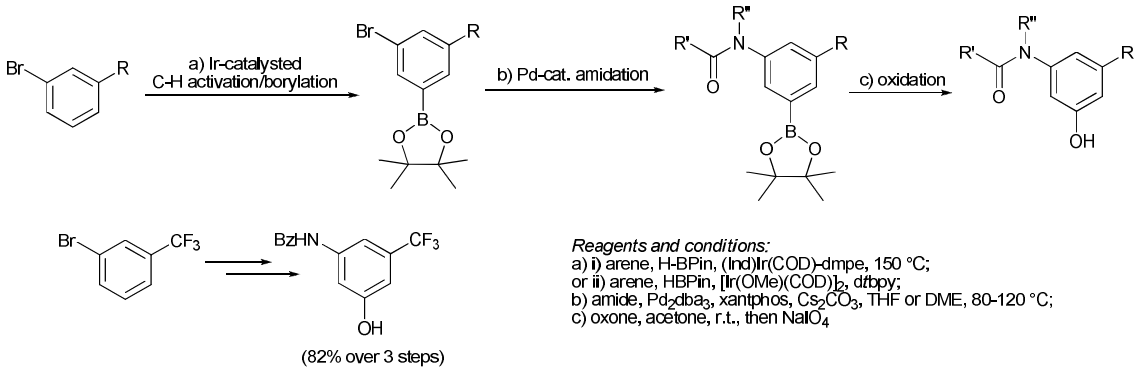
tolerance to iodide was not, although this could be avoided by the use of an alternative iridium catalyst-ligand system, namely $[Ir(OMe)(COD)]_2$ **171**, d*t*bpy **167**, and B_2Pin_2 **168**. This catalyst system was also reported by Miyaura *el al.*[9] to be the most effective system for these types of reaction, and in particular those reactions of substrates containing electron-rich substituents. Maleczka reported that both esters and ethers could survive the reaction conditions (entries 2 and 3), although partial demethylation was observed in the case of 2,6-dichloroanisole (entry 5). Where substrates contained nitrogen atoms susceptible to oxidation (entries 4 and 6), *N*-oxides were not observed.[18]

Table 10 – Summary of results obtained by Maleczka et al.[18]

Entry	Arene	H-BPin eq.	Borylation time (h)	Phenol	Yield (%)
1		2.0	18		87
2		2.0	18		79
3		1.5	3		70
4		1.5	3		64
5		2.5	16		68
6		2.0	18		79

They then went on to expand this chemistry to the synthesis of 5-substituted 3-amidophenols *via* the aromatic borylation/amidation oxidation of 3-substituted halobenzenes (*Scheme 88*).[19] Before this development, these types of compounds were routinely made *via* an amidation reaction between a functionalised aniline and a carboxylic acid derivative. However, often the required anilines were not commercially available, therefore imposing limits on this route. As

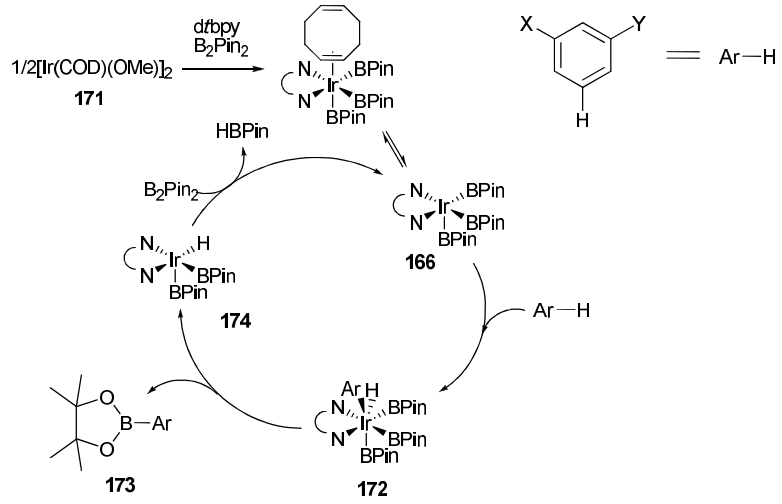
the borylation stage had already been shown to be successful with a range of substrates,[18] a three-step, one-pot route was developed for the synthesis of 5-subsituted 3-amidophenols in good yields over a range of substrates including 3-bromobenzotrifluoride (*Scheme 88*).[19]



Scheme 88 – Maleczka route to 5-substituted 3-amidophenols.[19]

At present, the mechanism of the C-H activation step has not been fully determined, although a plausible mechanism was proposed by Maleczka *el al.,*[18] which supports the mechanistic studies carried out by Sakaki *el al.* (*Scheme 89*).[20] During these studies it was found that the initial stages of the reaction involve coordination of the d*t*bpy ligand **167** and boron compound to the $[Ir(COD)(OMe)]_2$ complex **171**, followed by loss of the cyclooctadiene group to afford the active catalytic species – the iridium(III) tris(boryl)d*t*bpy complex **166**. The arene substrate subsequently undergoes oxidative addition to the active catalyst **166** to yield the 7-coordinate iridium(V) complex **172**. Reductive elimination of the arylboronate ester **173** from this species takes place in conjunction with the formation of an iridium(III) bis(boryl)hydride complex **174**, and occurs relatively easily due to the high oxidation state of the iridium(V) complex. When an excess of B_2Pin_2 is present in the reaction solution, further B_2Pin_2 can then react with **174** to afford the active catalytic species **166** and HBPin. It is also likely that this aids reductive elimination of the borylated product **173** from the metal centre. The high conversions to borylate esters are thought to be due to the strong σ-donor properties of boryl ligands when

attached to metal centres, thus favouring oxidative addition and stabilising the iridium(V) oxidation state.[15]



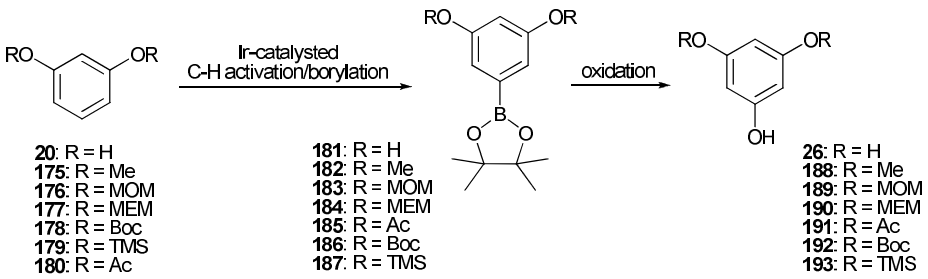
Scheme 89 – Proposed mechanism for the iridium-catalysed C-H activation/borylation.[18,20]

2.4.2 Results and Discussion

The use of C-H activation methods for the synthesis of phenols has the potential to be applied to the preparation of ^{13}C-labelled compounds. Previous work in our group had developed a successful synthesis of [2-^{13}C]resorcinol from [^{13}C]methyl iodide.[21] However, despite considerable work, a similar methodology could not be derived for phloroglucinol. This C-H activation chemistry offers the opportunity of introducing a third hydroxyl group into resorcinol to solve this problem. The use of a range of protecting groups on resorcinol would also increase the scope of the reaction beyond the use of halogen substituents and would provide a significant degree of flexibility in terms of the protecting groups which could be used in synthetic routes utilising this methodology.

After optimisation of conditions using the commercially available model substrate 1-bromo-3-iodobenzene, we therefore decided to investigate the reaction using seven different resorcinol

derivatives (for synthesis see **Section 2.5**) – resorcinol itself **20**, the methyl ether-protected **175**, MOM-protected **176**, MEM-protected **177**, Boc-protected **178**, TMS-protected **179** and acetyl-protected resorcinols **180** (*Scheme 20*) to yield the corresponding phloroglucinol derivatives. The use of protecting groups containing the benzene moiety or unsaturated hydrocarbon chains was not suitable as C-H activation and/or borylation would also take place on these protecting groups. As previously discussed, the first stage involves the C-H activation/borylation step with the conversion of a 1,3-disubstituted benzene into the arylboronic esters **181-187**. The second stage involves oxidation of the arylboronic ester to phenols **26** and **188-193** through the use of Oxone® in a 50:50 acetone/water solution.



Scheme 90 – Synthesis of 1,3,5-trisubstituted arenes from protected resorcinol.

Extensive optimisation studies on the C-H activation/borylation step were carried out, and selected results are given in *Tables 11-14* below (see **Appendix 1** for more conditions). Initially we attempted the conditions reported by Maleczka *el al.*[18] for the borylation of 1-bromo-3-iodobenzene using B_2Pin_2 **168**, $[Ir(OMe)(COD)]_2$ **171**, and d*t*bpy **167** in *iso*-hexane at room temperature for 18 h (*Table 11*, entry 1). However, the literature results were not reproducible, with no conversion to the arylboronic ester being achieved. Maleczka had also reported that often a lower loading of catalyst was beneficial to the reaction, so while decreasing the loading of catalyst and ligand accordingly, the temperature was increased to 40 °C and a conversion of 6% was observed (entry 2). Increasing the temperature further to 70 °C gave a conversion of 45% (entry 3), although this dropped once more upon a temperature increase to 110 °C (entry 4). It was therefore decided to raise the loading of both catalyst and ligand again,

along with a longer reaction time (entry 5), and this yielded the best results (66% conversion). To determine whether this increase in conversion was due to the higher loading of catalyst and ligand, or to the longer reaction time, the conditions of entry 3 were repeated with an extended reaction time of 60 h (entry 6), with a similar conversion of 63% being achieved. This demonstrated that indeed the longer reaction time was beneficial to the reaction. HPLC studies showed that although conversion increased significantly between 3 h and 18 h, only a marginal increase was observed after 18 h. Although it had been originally been hypothesised that these conditions may not be suitable for our substrates due to the fact that aromatic C-H activation/borylations are not as facile using electron-rich substrates,[16,22] the conditions from entry 5 (with 18 h reaction time) were attempted on our dimethoxy substrate **175**.

Table 11 – Reactions using 1-bromo-3-iodobenzene.

Entry	B_2Pin_2 (mmol)	$[Ir(OMe)(COD)]_2$ (mol%)	dtbpy (mol%)	Temp (°C)	Time (h)	Conversion (%)†
1	0.6	1.5	3	25	18	0*
2	0.6	1.0	2	40	18	6
3	0.6	1.0	2	70	18	45
4	0.6	1.0	2	110	18	39
5	**0.6**	**1.5**	**3**	**70**	**60**	**66**
6	0.6	1.0	2	70	60	63

† Calculated from integration of ^{1}H NMR signals of starting material and product; selected conversions confirmed by LCMS/HPLC analysis to confirm reliability
* Literature 76%[18]

Unfortunately, upon application of these conditions to 1,3-dimethoxybenzene **175**, a conversion of only 17% was achieved (*Table 12*, entry 1). As the 1,3-dimethoxy substituents are more electron-rich than the halides present on 1-bromo-3-iodobenzene, it was expected that the catalytic borylation would take place at a lower rate compared to the model substrate.[22] However, taking previous studies of C-H activation into account,[17] it was hoped that the increase in reaction temperature compared to the original literature would aid the reaction of our substrates. Significant optimisation studies were therefore undertaken to find conditions for the use of 1,3-dimethoxybenzene **175**. A decrease in catalyst/ligand loading was slightly beneficial in this case (entries 2 and 3), and an increase detrimental (entries 4 and 5) even with a longer reaction time (42 h *cf.* 18 h). It was thought that increasing the number of equivalents of the

boron reagent would increase conversion, and indeed, combined with a simultaneous increase in catalyst/ligand loading, conversions of 20-30% were achieved (entries 7-9). Increasing the temperature further to 110 °C also improved conversion, with similar results being obtained using 0.6 or 1.2 equivalents of B_2Pin_2 and catalyst/ligand mol% of 1.0 and 2.0 respectively (entries 10-13). Interestingly, the use of 1 mmol B_2Pin_2 (2 equivalents boron *cf.* arene) under these conditions (entry 14) produced the most successful results by far, with a conversion of 97% and isolated yield of 59%, due to the face that an increase in B_2Pin_2 excess may increase the rate of reductive elimination.[18] It had been suggested that the use of a less polar solvent such as octane may increase conversion, [22] and so using the conditions from entry 14, with temperatures of both 110 °C and 140 °C to account for the higher boiling point of octane, and a reaction time of only 3 h, good conversions of 57 and 65% (entries 15 and 16 respectively) were recorded. Disappointingly, allowing the reaction to proceed for 18 h (entry 17) gave no significant improvement in results, and so it was decided that the optimal conditions for the borylation of 1,3-dimethoxybenzene **175** were those outlined in entry 14.

*Table 12 – Reactions using 1,3-dimethoxybenzene **175.***

Entry	B_2Pin_2 (mmol)	$[Ir(OMe)(COD)]_2$ (mol%)	dtbpy (mol%)	Temp (°C)	Time (h)	Conversion (%)†	Isolated Yield (%)**
1	0.6	1.5	3.0	70	18	17	5
2	0.6	1.0	2.0	70	18	19	-
3	0.6	0.5	1.0	70	18	22	-
4	0.6	2.5	5.0	70	42	8	-
5	0.6	2.0	4.0	70	42	11	-
6	0.6	1.5	3.0	70	42	14	-
7	1.2	2.5	5.0	70	18	23	-
8	1.2	2.0	4.0	70	18	29	-
9	1.2	1.5	3.0	70	18	19	-
10	1.2	1.0	2.0	110	18	27	-
11	0.6	1.0	2.0	110	18	26	-
12	0.6	1.0	2.0	110	18	28	-
13	1.2	1.0	2.0	110	18	27	-
14	**1.0**	**1.0**	**2.0**	**110**	**18**	**97**	**59**
15	1.0	1.0	2.0	110*	3	57	18
16	1.0	1.0	2.0	140*	3	65	20
17	1.0	1.0	2.0	140*	18	69	21

† Calculated from integration of ^{1}H NMR signals of starting material and product; selected conversions confirmed by LCMS/HPLC analysis to confirm reliability

* Octane used as solvent (*cf. iso*-hexane)

** Isolated yields given where purification of the final product was carried out

On application of these conditions to the unprotected resorcinol **20**, no conversion to the arylboronic ester intermediate was achieved, with only starting material being recovered. The procedure was then carried out on various protected resorcinol derivatives. Interestingly, the optimised conditions from the reaction using 1,3-dimethoxybenzene were also found to be successful for the MOM- and MEM-protected substrates **176** and **177**, giving 95% conversion and 61% isolated yield **189** using the MOM-protected substrate, and 71% conversion (62% isolated yield **190**) using the MEM-protected substrate. Further optimisation using these protecting groups was not carried out.

An interesting result was obtained when applying the above conditions to the trimethylsilyl-protected substrate **179**. ^{1}H and ^{13}C NMR analysis showed 92% conversion to the desired arylboronic ester intermediate **187**. However, deprotection of the TMS groups was observed during the oxidation stage, with only phloroglucinol **26** being recovered in an excellent yield of 87%. This could be of importance for the synthesis of phloroglucinol or similar compounds, as a separate deprotection step would not be required following C-H activation/borylation/oxidation, and therefore potentially reducing the amount of losses of valuable material. Analysis of both the crude reaction mixture and pure product showed that the deprotection occurred during the oxidation and not during purification by column chromatography.

Although positive results were obtained for substrates **175**, **176**, **177** and **179**, it was desirable for us to also attempt the C-H activation/borylation on the acetyl-protected substrate **180**, as the acetyl protecting groups could be removed more easily than other protecting groups utilised after preparation of the final anthocyanin structure. As the acetyl-protected substrate **180** is less electron-rich than the methyl ether-protected **175**, MOM-protected **176** and MEM-protected **177** substrates, it was thought that our optimised conditions would be successful upon application to this substrate. The conditions from entry 14 (*Table 12*) were therefore attempted using both *iso*-

hexane (*Table 13*, entries 1 and 3) and octane (entries 2 and 4). Disappointingly, neither reaction was successful, with conversions of only 3 and 4% being achieved after 3 h, and only 7 and 9% after 18 h. Doubling the number of equivalents of B_2Pin_2 and loading of both catalyst and ligand, a conversion of 25% was achieved – the most promising result so far (entry 5). It was then decided to return to the initial conditions reported by Maleczka *el al.*[18] incorporating the use of the pinacolborane monomer (HBPin **169**) and dppe **170** ligand. It was hypothesised that the use of HBPin accelerates the reaction through faster coordination to the catalyst than the B_2Pin_2 dimer. This produced interesting results, as the conversion obtained from the conditions outlined in entry 6 where HBPin and dppe were used was similar to that from entry 5. During previous optimisation studies it was found that the optimal ratio of catalyst to ligand was 1:2. However, as can be seen from a comparison of entries 6 and 7, this is detrimental when dppe is used as the ligand in place of d*t*bpy, with dppe producing the best results when used in a 1:1 ratio with the catalyst.

Due to these results, it was thought that the use of B_2Pin_2 with a small amount of added HBPin may produce higher conversions, as B_2Pin_2 has been generally found to be the best borylating agent, and the presence of HBPin may result in acceleration of the rate of borylation. However, under these conditions (entries 8 and 9) with either dppe or d*t*bpy as ligand very poor conversions were achieved (10% and 16%, respectively), suggesting that an acceleration due the presence of HBPin is not taking place. To examine this more thoroughly, the quantity of HBPin was then increased to equal the number of mmol of B_2Pin_2, with a more favourable 1:1 catalyst to ligand ratio (entries 10 and 11). This gave the desired result, with a large increase in conversion in both reactions, and that of dppe yielding the highest conversion achieved so far (45%, isolated yield 44% **191**). Further increasing the quantity of HBPin to a higher concentration than B_2Pin_2 (entries 12 and 13) gave good results, but lower than that previously obtained in entry 10, which allowed the conclusion to be made that the number of mmol of HBPin should not exceed that of B_2Pin_2. This is supported by the theory previously discussed

that when an excess of B_2Pin_2 is present in the reaction solution, it can enter the catalytic cycle to regenerate the active catalytic species (and HBPin). Finally, using both d*t*bpy and dppe (entries 14 and 15) the loading of both B_2Pin_2 and HBPin were increased to 1.5 mmol in order to determine if this would lead to higher conversion to the arylborylate ester, however this was not the case, with the conditions of entry 10 still giving the best results.

It was decided at this stage not to continue with the optimisation of the reaction using the acetyl-protected substrate **180**, as the reaction continued to prove troublesome and excellent results had been achieved using the methyl ether-protected substrate **175**, MOM-protected substrate **176**, MEM-protected substrate **177** and TMS-protected substrate **179**.

*Table 13 – Reactions using 1,3-diacetoxybenzene **180.***

Entry	B_2Pin_2 (mmol)	HBPin (mmol)	$[Ir(OMe)(COD)]_2$ (mol%)	d*t*bpy (mol%)	Temp (°C)	Time (h)	Conversion (%)†	Isolated yield (%)*
1	1.0	-	1.0	2.0	110	3	3	-
2	1.0	-	1.0	2.0	140*	3	4	-
3	1.0	-	1.0	2.0	110	18	7	-
4	1.0	-	1.0	2.0	140*	18	9	-
5	2.0	-	2.0	4.0	110	18	25	10
6	-	1.5	2.0	2.0[L]	110	18	29	-
7	-	1.5	2.0	4.0[L]	110	18	11	-
8	1.0	0.2	2.0	4.0[L]	110	18	10	-
9	1.0	0.2	2.0	4.0	110	18	16	-
10	**1.0**	**1.0**	**2.0**	**2.0[L]**	**110**	**18**	**45**	**44**
11	1.0	1.0	2.0	2.0	110	18	26	-
12	1.0	1.5	2.0	2.0[L]	110	18	34	30
13	1.0	2.0	2.0	2.0[L]	110	18	37	29
14	1.5	1.5	2.0	2.0[L]	110	18	17	-
15	1.5	1.5	2.0	2.0	110	18	20	-

† Calculated from integration of ^{1}H NMR signals of starting material and product; selected conversions confirmed by LCMS/HPLC analysis to confirm reliability
* Octane used as solvent (*cf. iso*-hexane)
[L] dppe as ligand
* Isolated yields given where purification of the final product was carried out

Finally, we chose to investigate the reaction using the Boc-protected resorcinol **178**, as this was also a comparatively electron-poor substrate. *Table 14* outlines the conditions attempted. Optimal conditions for the electron-rich substrates (entry 1) were first attempted and gave an acceptable conversion of 51% and isolated yield of 22% **192**. Only starting material was recovered using the most successful conditions for the acetyl-protected substrate **180** (entry 2).

An increase in both conversion (73%) and isolated yield (36%) were observed upon returning to conditions similar to entry 1 but with an increase in the amount of B_2Pin_2 and catalyst loading (entry 3). It was anticipated that a further increase in B_2Pin_2, catalyst loading and ligand loading would increase conversion, and indeed this was observed (entry 4), with almost full conversion being and an isolated yield of 51% **192** being achieved.

*Table 14 – Reactions using the Boc-protected substrate **178.***

Entry	B_2Pin_2 (mmol)	HBPin (mmol)	$[Ir(OMe)(COD)]_2$ (mol%)	d*t*bpy (mol%)	Temp (°C)	Time (h)	Conversion (%)†	Isolated yield (%)*
1	1.0	-	1.0	2.0	110	18	51	22
2	1.0	1.0	1.0	2.0[L]	110	18	0	-
3	1.5	-	1.2	2.0	110	18	73	36
4	**1.6**	**-**	**1.3**	**2.1**	**110**	**18**	**99**	**51**

† Calculated from integration of ^{1}H NMR signals of starting material and product
[L] dppe as ligand
* Isolated yields given where purification of the final product was carried out

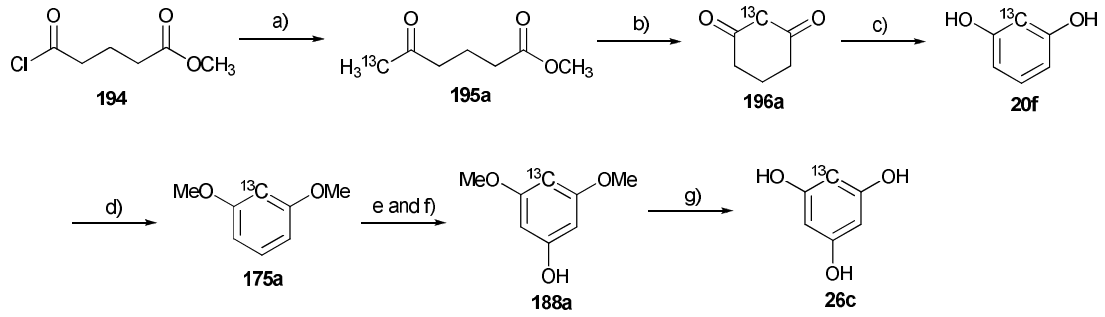
2.4.3 Summary

The two-step one-pot iridium-catalysed C-H activation/borylation/oxidation procedure originally reported by Marder *el al.*[14,15] and later developed by Maleczka[18,19] has been extensively studied and optimised in order to carry out the functionalisation of six di-protected resorcinols, **175-180**. Factors which we found to contribute to the success of the catalytic reactions include the use of a sealed reaction vessel under an argon atmosphere, a high reaction temperature (110 °C) in degassed *iso*-hexane, the iridium complex $[Ir(COD)(OMe)]_2$ **171** as a precursor to the active catalytic species, borylating reagent bis(pinacolato)diboron (B_2Pin_2 **168**), and a ligand containing the bipyridyl moiety (d*t*bpy **167**). This borylating agent is a strong electron-donator to the metal centre, therefore stabilising the iridium(V) species and facilitating oxidative addition. Likewise, the bipyridyl ligand also stabilises the high oxidation state through its electron-donating properties. The ligand and substrate structures are also relatively planar and this factor is favourable to the formation of the 7-coordinate species. The reaction was not successful using unprotected resorcinol **20**. The optimal conditions found for the

methyl ether-protected **175**, MOM-protected **176** and MEM-protected **177** resorcinols were as follows: 1 mmol B_2Pin_2, 1 mol% $[Ir(COD)(OMe)]_2$, 2 mol% d*t*bpy, 110 °C, 18 h, giving 97%, 95% and 99% conversion respectively (59% **188**, 61% **189** and 62% **190** isolated yields). A noteworthy result was obtained upon application of these conditions to the TMS-protected substrate **179**, with an excellent conversion of 92% to the desired intermediate being observed. Interestingly it was observed that deprotection occurred during the oxidation step to yield phloroglucinol **26** in 87% yield. Similar conditions were successful for the Boc-protected resorcinol **179**, giving 99% conversion and 51% isolated yield of Boc-protected phenol **192**. Limited success was achieved using the acetyl-protected substrate **180**, with 45% conversion (44% isolated yield **191**) being achieved using 1 mmol B_2Pin_2, 1 mmol HBPin, 2 mol% $[Ir(COD)(OMe)]_2$, 2 mol% dppe, 110 °C, 18 h. These successful results therefore gave us a range of substrates on which the subsequent deprotection to phloroglucinol could be attempted and significantly expand the scope of the C-H activation/borylation/oxidation procedure to include the use of electron-rich protected resorcinol derivatives.

2.5 Synthesis of [2-^{13}C]Phloroglucinol (26c)

As previously discussed in **Section 1.3.5**, the 1,3,5-trihydroxybenzenes, such as phloroglucinol **26**, are important and widely abundant moieties in plant metabolites. For this reason a route to the synthesis of isotopically-labelled [^{13}C] or [^{14}C]phloroglucinol is desirable and would give compounds useful for metabolism studies. Although a small number of routes to ^{14}C-labelled phloroglucinol have been reported,[23,24] to the best of our knowledge no synthesis involving ^{13}C derivatives has been documented as yet. In the context of our research, we therefore chose to develop a synthesis of [2-^{13}C]phloroglucinol **26c** with the regioselective placement of one ^{13}C-atom into the aromatic ring through the use of acyclic ^{13}C-labelled precursors – namely [^{13}C]methyl iodide (*Scheme 91*). This synthesis is also directly applicable to the preparation of ^{13}C-labelled anthocyanins (**Section 2.6**).



Scheme 91 – Reagents and conditions: a) Et_2O, Li, $^{13}CH_3I$, rt, 1 h, then CuI, 0 °C, 30 min, then -20 °C, 1 h, then rt, 18 h (43%); b) THF, KO*t*Bu, reflux, 7 h, then HCl (69%); c) Xylene, 10% Pd/C, reflux, 3 h, then HCl (88%); d) Acetonitrile, Cs_2CO_3, CH_3I, reflux, 6 h (70%); e) *iso*-Hexane, B_2Pin_2, d*t*bpy, $[Ir(OMe)(COD)]_2$, 110 °C, 18 h; f) Acetone, aqueous Oxone®, rt, 30 min (59%); g) DCM, BBr_3, -78 °C, 1 h, then rt, 18 h (81%).

For the initial stages of the synthesis we decided to incorporate an existing route to [2-^{13}C]resorcinol **20f** which was previously optimised in the Botting group[21] based on an earlier route to the compound.[25] This approach allows the regioselective placement of one ^{13}C-atom into the aromatic ring. The additional hydroxyl group would then be incorporated using the

C-H activation/borylation/oxidation developed previously (**Section 2.4**). As with the development of the route to [1,3,5-$^{13}C_3$]gallic acid **32d**, this approach is beneficial as it involves the use of ^{13}C-labelled small molecules, which are cheaper and more readily available than uniformly-labelled aromatic compounds.

The isotope label was introduced at the first stage of the synthesis (*Scheme 91*) through the treatment of [^{13}C]methyl iodide with lithium followed by the addition of copper iodide at low temperatures (0-4 °C) to give a lithium dimethylcuprate. This was subsequently treated *in situ* at -20 °C with the substrate, methyl 4-chloroformylbutyrate **194**. After stirring overnight at room temperature the crude product was isolated in good yield (86%), and it was hoped that purification would not be required at this stage. However, it was established during optimisation on unlabelled substrates that the crude product was not of suitable purity for the following step. Purification by column chromatography was therefore carried out and gave methyl 5-oxo-[6-^{13}C]hexanoate **195a** in 43% yield. The ^{13}C-atom was clearly observed in the ^{13}C NMR spectrum as an enhanced singlet at 29.9 ppm. In the ^{1}H NMR spectrum the signal for the $^{13}CH_3$ protons was visible as a doublet with a large *J* value of 127.1 Hz, corresponding to the ^{13}C-H coupling.

Cyclisation to [2-^{13}C]cyclohexane-1,3-dione **196a** was successful on the purified substrate **195a**. It was important at this stage to ensure careful drying of the potassium *tert*-butoxide and tetrahydrofuran, as yields were greatly reduced when traces of water were present. It was known from previous literature and optimisation studies[21] that the subsequent aromatisation was particularly low yielding (20-49%), and so purification was carried out after the cyclisation, giving the desired product **196a** in 69% yield. This was observed to be a mixture of keto and enol tautomers under NMR conditions in deuterated chloroform (*Diagram 26*). However, it was possible from ^{1}H-^{13}C HSQC and HMBC experiments to distinguish between the two species and therefore allow characterisation of both tautomers. Signals for the ^{13}C-atoms were visible as

enhanced singlets 58.4 and 104.3 ppm for the keto and enol tautomers respectively. As shown in *Diagram 26*, the CH_2-4,5,6 signals showed similar chemical shifts and *J* values. However, the signals corresponding to $^{13}CH_2$ (keto) and ^{13}CH (enol) were observed as doublets at 3.36 and 5.54 ppm respectively, with the enol form having the largest *J* value – 161.0 Hz *cf.* 130.1 Hz.

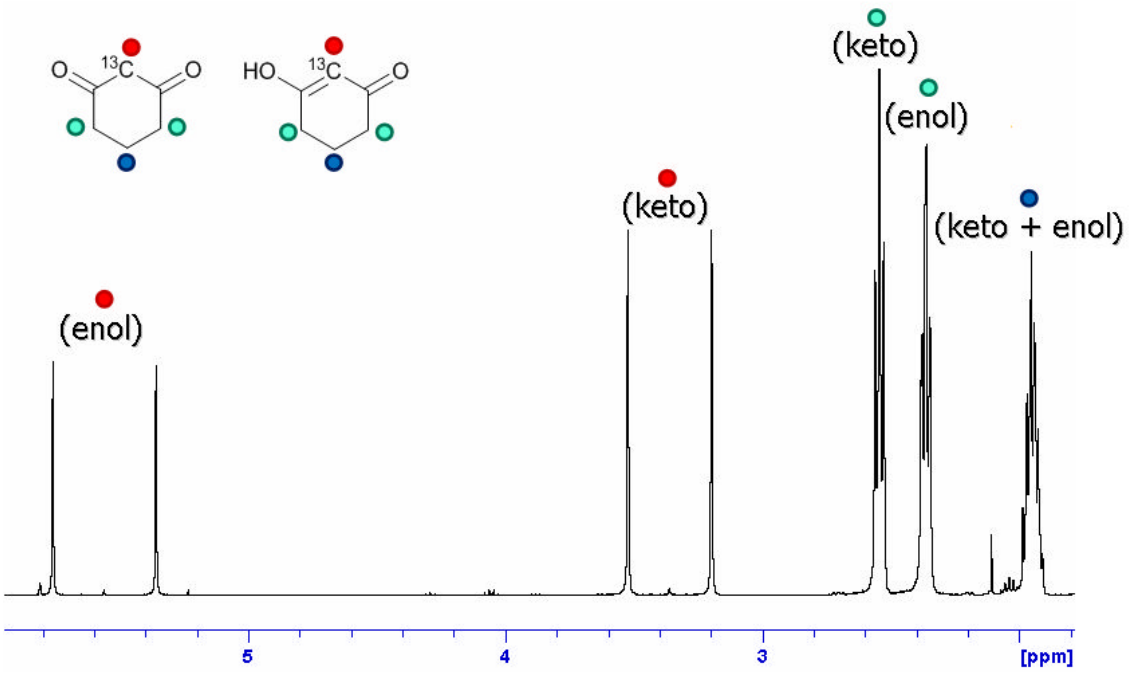
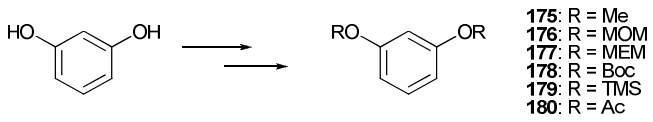


*Diagram 26 – ^{1}H NMR spectrum of [2-^{13}C]cyclohexane-1,3-dione **196a** (400 MHz, $CDCl_3$).*

The final stage in the original route to [2-^{13}C]resorcinol **20f** involved the aromatisation of [2-^{13}C]cyclohexane-1,3-dione **196a** by treatment with a palladium on charcoal catalyst in refluxing xylene at 138 °C for 3 h. Higher temperatures had previously been found to be detrimental to the reaction, with lower temperatures producing a cleaner product.[21] During optimisation studies, a range of reaction temperatures and times were examined, with no improvements being observed. The reaction was also attempted in both toluene and *o*-dichlorobenzene at reflux temperatures. However, in the case of *o*-dichlorobenzene only starting material was recovered, and toluene yielded a mixture of starting material and product even after prolonged reflux times (~48 h). It was concluded that the previous conditions (10% Pd/C, xylene) did indeed give the best conversion to product compared to the other conditions examined. It was later found that

extractions using ethyl acetate, rather than diethyl ether as before,[21] resulted in a much higher recovery of the desired compound **20f**, thus significantly increasing the overall yield compared to the original literature. After purification by column chromatography, [2-^{13}C]resorcinol **20f** was isolated in an improved 88% yield, giving a yield of 26% over three stages – this is comparable to the previously reported yield using this route (24%).[21] As expected through similarities of the aromatic ^{13}C-atom to the ^{13}C-environment in the enol form of **196a**, an enhanced singlet was observed at 103.5 ppm. In the ^{1}H NMR spectrum, the signal for H-2 was visible as a double triplet (6.36 ppm) with one large *J* value (157.0 Hz) corresponding to the ^{13}C-H splitting, and one smaller *J* value (2.3 Hz) corresponding to splitting to aromatic protons CH-4,6, which were themselves observed as a multiplet (6.29-6.37 ppm).

At this point in the optimisation using unlabelled substrates, protection of the hydroxyl groups was examined using the methyl ether **175**, MOM (methoxymethyl) **176**, MEM (methoxyethoxymethyl) **177**, Boc (*tert*-butyloxycarbonyl) **178**, TMS (trimethylsilyl) **179** and acetyl **180** protecting groups (*Scheme 92*).



Scheme 92 – Protection of resorcinol.

Protection using the MOM group was achieved through treatment of resorcinol **20** with chloromethyl methyl ether (MOMCl) in the presence of sodium hydride and dimethylformamide,[26] and gave good yields (67% **176**) after purification by column chromatography. Similarly, treatment of **20** with 2-methoxyethoxymethyl chloride (MEMCl) under identical conditions gave MEM-protected resorcinol **177** in 73% yield after purification. Boc protection was also successful in excellent yield (91% **178** after purification) using Boc anhydride with a catalytic amount of DMAP. Purification was not carried out after protection using trimethylsilyl chloride (pyridine, DMAP) due to deprotection occurring during the

chromatography on silica. However, purity was excellent, and the desired TMS-protected resorcinol **179** was isolated in 81% yield. Acetyl protection was also successful in excellent yield after purification (74% **180**) using pyridine and acetic anhydride. Although the acetyl protecting group would have been the protection of choice due to the ease of its removal, as previously discussed in **Section 2.4**, this substrate was not suitable for the subsequent catalysis step due to poor conversion. The use of iridium-catalysis to introduce the third hydroxyl group on the MOM-protected substrate gave excellent results (**Section 2.4**), with full conversion and good isolated yields. However, the later deprotection was troublesome, and often did not produce consistent results. The MEM-protected substrate **177** and Boc-protected substrate **178** also gave good results during the C-H activation/borylation/oxidation. However, as the conditions for these substrates had not been fully optimised at this point, and studies into their subsequent deprotection had not been carried, we chose to utilise the methyl ether protecting group due to the fact that it gave good conversion under catalysis, and good yields after purification by column chromatography. It was later found after the successful synthesis of [2-^{13}C]phloroglucinol **26c** that use of the TMS-protecting group at this stage would remove the requirement for a separate deprotection step (**Section 2.4**). It would therefore be desirable to use this alternative route in the future.

Methylation of the hydroxyl groups to give 1,3-dimethoxy-[2-^{13}C]benzene **175a** was therefore achieved by treatment of [2-^{13}C]resorcinol **20f** with methyl iodide in a suspension of cesium carbonate in acetonitrile.[27] The desired product **175a** was isolated in a good yield of 70% after purification. As in the NMR analysis of [2-^{13}C]resorcinol **20f**, the ^{13}C-atom was visible in the ^{13}C NMR spectrum as an enhanced singlet at 100.5 ppm, and H-2 as a double triplet (6.39 ppm) in the ^{1}H NMR spectrum with a large ^{13}C-^{1}H *J* value of 158.2 Hz and smaller ^{1}H-^{1}H *J* value of 2.4 Hz. All other ^{13}C-signals were observed as doublets with *J* values ranging from only 3.2 Hz (C-4,6) and 5.2 Hz (C-5) for *meta*- and *para*-^{13}C-^{13}C couplings, to 70.0 Hz (C-1,3) for *ortho*-^{13}C-^{13}C couplings.

For the introduction of the third hydroxyl group, an iridium-catalysed C-H activation/borylation/oxidation procedure as reported by Maleczka *el al.*[18] was utilised as outlined above. A range of reaction conditions, ligands and boron reagents were studied as previously examined in **Section 2.4**, and it was found that the optimum conditions for the dimethoxy-protected substrate involve the use of $[Ir(OMe)(COD)]_2$ catalyst, B_2Pin_2 as the borylating agent, d*t*bpy (di-*tert*-butyl-bipyridyl) as ligand and *iso*-hexane as solvent, with a reflux time of 18 h at 110 °C. 1,3-Dimethoxy-[2-^{13}C]benzene **175a** was treated accordingly and the boronic acid pinacol ester intermediate **182a** was recovered in 95% conversion. The subsequent oxidation using Oxone® in a 50:50 solution of acetone/water was carried out on the crude intermediate **182a**, with complete oxidation being achieved after 30 min. Formation of poly-hydroxylated species was not observed, although some starting material **175a** remained. Purification by column chromatography gave the desired 3,5-dimethoxy-[4-^{13}C]phenol **188a** in 59% yield. As with the ^{13}C NMR characterisation of **188a**, all carbon signals were observed as doublets (*J* 3.3, 4.1, and 71.0 Hz) except ^{13}C-4 which was present as an enhanced singlet (92.9 ppm). H-4 was once again observed as a double triplet (5.98 ppm, *J* 160.0 and 2.2 Hz) in the ^{1}H NMR spectrum and H-2,6 as a double doublet (5.97 ppm, *J* 4.7 and 2.2 Hz) due to coupling with the ^{13}C-atom and *meta*-protons (*Diagram 27*).

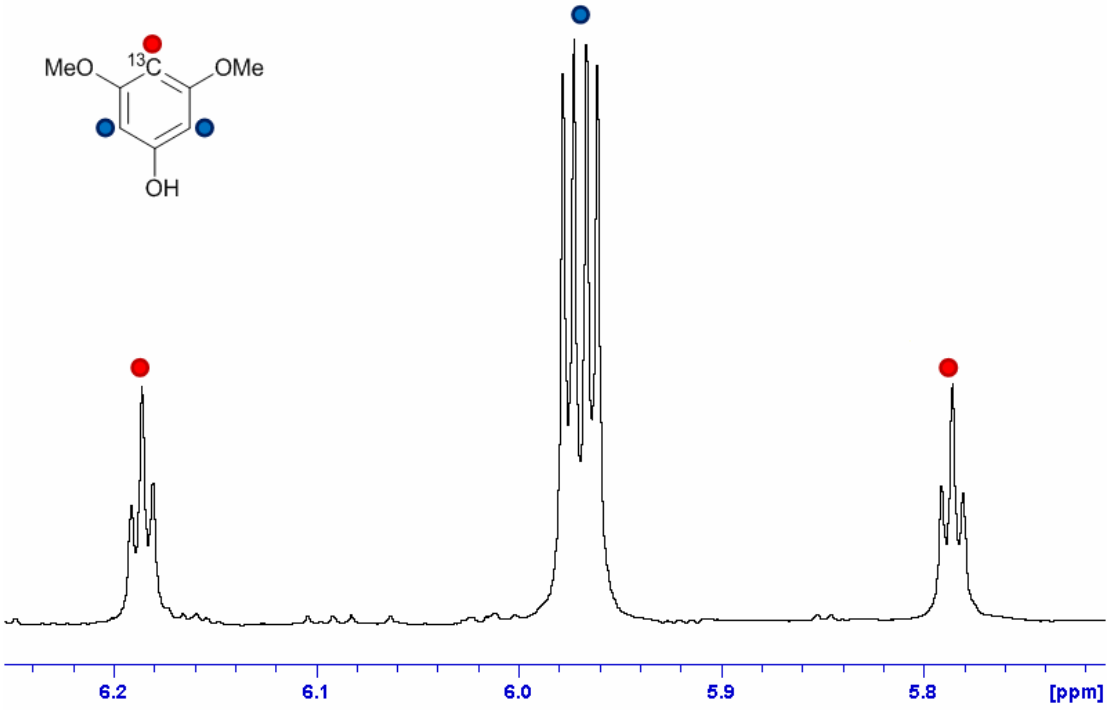


*Diagram 27 – Aromatic region of ^{1}H NMR spectrum of **188a** (400 MHz, $CDCl_3$).*

With the di-protected phloroglucinol **188a** in hand, the final demethylation to complete the synthesis of the desired [2-^{13}C]phloroglucinol **26c** could be carried out. A wide range of conditions were examined using unlabelled substrates as outlined (*Table 15*) and it was found that treatment with boron tribromide solution (10 eq.) in dichloromethane (-78 °C to rt) gave the cleanest results and highest yields (entries 1 and 2).[28]

*Table 15 – Reaction conditions for demethylation of 3,5-dimethoxyphenol **188.***

Entry	Solvent	Reagent	Time (h)	Temp. (°C)	Yield %
1	**DCM**	**BBr_3**	**1/10**	**-78/25**	**99**
2	**DCM**	**BBr_3**	**24**	**-78 to 25**	**99**
3	DCM	BCl_3	4 d	25	0
4	Chlorobenzene	$AlCl_3$	2	Reflux	0
5	Et_2O	$AlCl_3$	2	0 to 25, reflux	0
6	CH_3NO_2	$AlCl_3$	2	0 to 25, reflux	0
7	DCM	$AlCl_3$	2	0 to 25, reflux	0
8	NMP	Na_2S	3.5	Reflux	0
9	DMF	LiCl	27	Reflux	0
10	Pyridine	LiCl	27	25	0
11	Pyridine	LiI	48	25	0
12	-	Py.HCl	1-6	200	15

Bringmann *el al.*[29] reported that the demethylation of methoxyphenols could be achieved in excellent yields through the use of aluminium trichloride with chlorobenzene as solvent. We examined this procedure using four different solvents at temperatures ranging from 0-25 °C to reflux and recovered only starting material in all cases (entries 4-7). They also reported the use of boron trichloride, but once again this was unsuccessful (entry 3). Other reported methods for the demethylation in which we recovered only starting material included the use of sodium sulfide in *N*-methylpyrrolidinone (entry 8)[30] and lithium halides in either dimethylformamide or pyridine (entries 9-11).[31] An alternative solvent-free procedure was reported by Kelly *el al.*[32] where deprotection was achieved by heating the substrate at high temperatures in the presence of pyridinium hydrochloride (entry 12). Using this method we achieved 15% conversion, but it was decided to continue with the use of boron tribromide as it produced the most consistent results and highest yields.

Although introduction of the third hydroxyl group into the MOM-protected substrate was successful (**Section 2.4**), deprotection was problematic. A range of deprotection conditions were attempted, including treatment with hydrochloric acid in *iso*-propanol/tetrahydrofuran 50:50 (rt), hydrochloric acid in methanol (rt and reflux) and sulfuric acid in methanol (rt). Only partial deprotection was achieved under any of the above conditions, with the use of hydrochloric acid in *iso*-propanol/tetrahydrofuran producing the poorest results. An alternative route was therefore sought, and it was found that stirring a solution of the di-protected substrate in dichloromethane in the presence of a $NaHSO_4.SiO_2$ catalyst yielded the desired phloroglucinol in excellent yield and purity.[33] However, although in all cases the deprotection was successful, the results were not reproducible, as often the product could not be extracted from the catalyst without the use of DMSO. Therefore it was decided that this procedure was not suitable for use with labelled substrates due to the inherent risks relating to isolation of the final product, and thus methyl ether protection was chosen as the route of choice. Studies into

the deprotection of other disubstituted phenols obtained following C-H activation/borylation/oxidation has yet to be investigated.

After treatment of 3,5-dimethoxy-[4-^{13}C]phenol **188a** with boron tribromide in dichloromethane, purification was carried out in order to remove traces of impurities, and yielded [2-^{13}C]phloroglucinol **26c** in 81% yield, and in 9% over 6 steps. The ^{13}C-atom was clearly observed as an enhanced singlet at 93.9 ppm in the ^{13}C NMR spectrum. The quaternary carbon atoms were also visible (158.8 ppm), with C-1 and C-3 being observed as a doublet with a large *J* value of 66.4 Hz, due to coupling with the adjacent ^{13}C-atom. C-5 was observed as a doublet with a smaller *J* value of 3.4 Hz, due to coupling to the *para*-^{13}C-atom (*Diagram 28*).

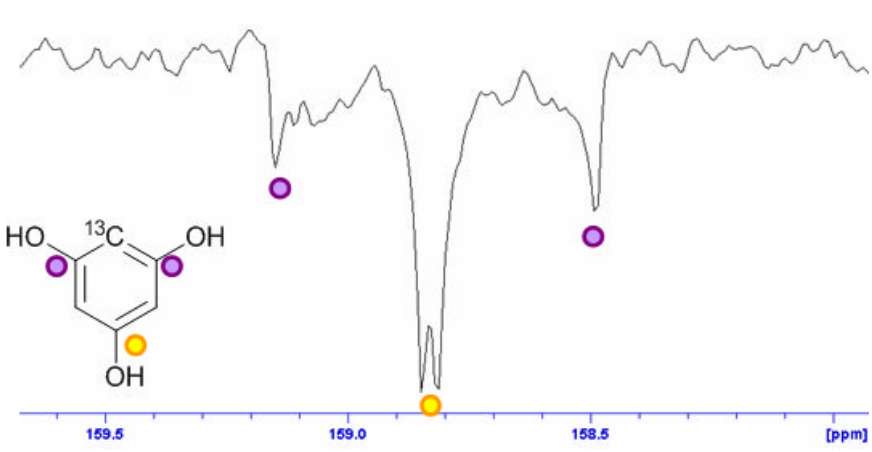


*Diagram 28 – C-1,3,5 section of ^{13}C DEPTQ NMR spectrum of [2-^{13}C]phloroglucinol **26c** (300 MHz, DMSO-d_6).*

The ^{1}H NMR spectrum was also interesting (*Diagram 29*), as the unlabelled phloroglucinol is symmetrical resulting in all three protons recording a chemical shift of 5.63 ppm. However, due to the presence of a single ^{13}C-atom and subsequent differences in the splitting, the two environments could be distinguished. The proton attached to the ^{13}C-atom (H-2) was observed as a double triplet with a large *J* value of 158.0 Hz due to direct coupling with the ^{13}C-atom, and a smaller *J* value of 1.8 Hz due to coupling with the protons at C-4 and C-6. These two protons

(H-4,6) themselves were observed as a double doublet with *J* values of 4.6 and 1.8 Hz due to coupling with the ^{13}C-atom and ^{13}C-H proton respectively.

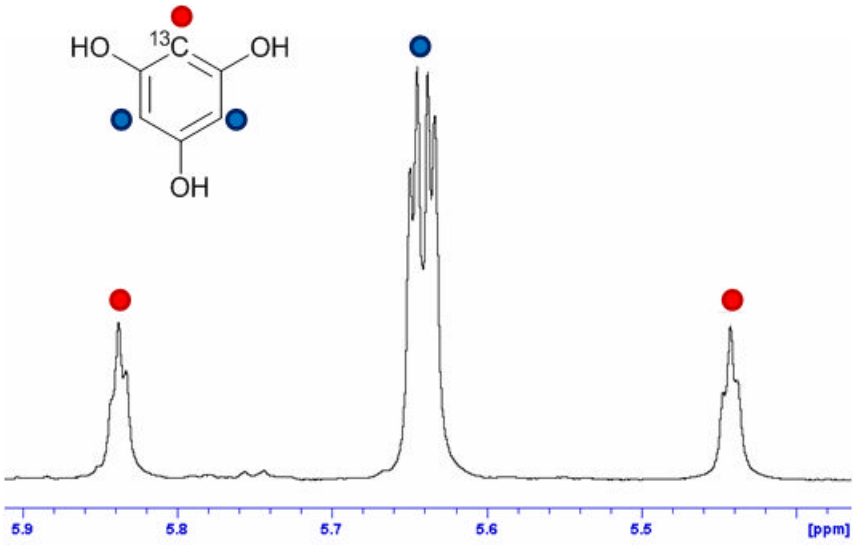


*Diagram 29 – ^{1}H NMR spectrum of [2-^{13}C]phloroglucinol **26c** (100 MHz, DMSO-d_6).*

2.5.1 Summary of the Synthesis of [2-^{13}C]Phloroglucinol

A fast and efficient route for the synthesis of [2-^{13}C]phloroglucinol **26c** in six steps from acyclic non-aromatic precursors has been developed, with the regioselective incorporation of a single ^{13}C-atom into the aromatic ring. The ^{13}C-label was introduced in the first step by the reaction of commercially available [^{13}C]methyl iodide, with methyl 4-chloroformyl butyrate **194**. Subsequent cyclisation *via* an intramolecular Claisen condensation, followed by aromatisation using a palladium on carbon catalyst gave [2-^{13}C]resorcinol **20f** in yields comparable to the original literature reported by the Botting group.[21] Following methylation of the hydroxyl groups, our new substrate was incorporated into a modified iridium-catalysed C-H activation/borylation/oxidation procedure[4] to introduce the third hydroxyl group to the aromatic ring. Demethylation using boron tribromide then yielded the desired [2-^{13}C]phloroglucinol **26c** in 9% yield over 6 steps.

2.6 Studies Towards the Synthesis of ^{13}C-Labelled Anthocyanins

2.6.1 Requirements for the Isotopic Labelling of Natural Polyphenols

As previously discussed in **Section 1.1**, pharmacological studies relating to the absorption, excretion and metabolism of natural products are carried out in order to determine their behaviour within a biological matrix,[34] and this is often achieved through the use of LC-MS and GC-MS analysis. Although many studies have been undertaken on the biological activity of the various classes of dietary polyphenols,[21,35,36] there are still questions to be answered concerning the absorption, metabolism and bioavailability of flavonoids such as the anthocyanins. The ability to synthesise stable isotopically labelled versions of these compounds is therefore an important goal. The availability of ^{13}C-labelled polyphenols as internal standards would greatly improve the accuracy and reproducibility of LC-MS and GC-MS based analytical methods. Accurate analysis is vitally important for establishing the exposure levels of the population to such dietary chemicals and also for epidemiological studies to investigate the associations between exposure and disease. Thus, new synthetic routes to isotopically labelled anthocyanins must be developed. To the best of our knowledge, only two synthetic routes to carbon-labelled anthocyanins have been reported to date (**Section 1.6.4**),[37] with both involving the use of carbon-14. No syntheses have been published of ^{13}C-labelled anthocyanidins or their glycosides.

Our objective was to develop a route to the synthesis of ^{13}C-labelled anthocyanins, namely delphinidin-3-glucoside **197** (*Diagram 30*). The route developed will also be applicable to the preparation of cyanidin-3-glucoside **198**, with the necessary modifications being outlined during the course of the following discussion. In order to aid metabolism studies and identification of the metabolites, it is desirable to incorporate ^{13}C-atoms into more than one of the aromatic rings. The aim was to introduce three ^{13}C-atoms through the use of acyclic, non-aromatic ^{13}C-labelled building blocks, utilising routes previously developed for the synthesis of [1,3,5-$^{13}C_3$]gallic acid

32d and [2-^{13}C]phloroglucinol **26c**.[3,4] This strategy would allow the regioselective placement of one ^{13}C-atom into the A ring and two ^{13}C-atoms into the B ring.

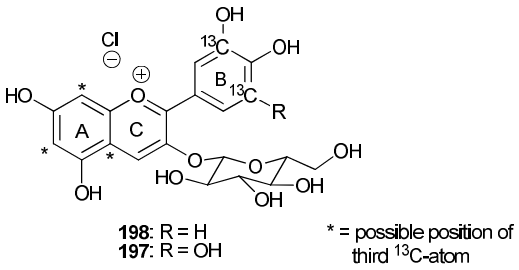
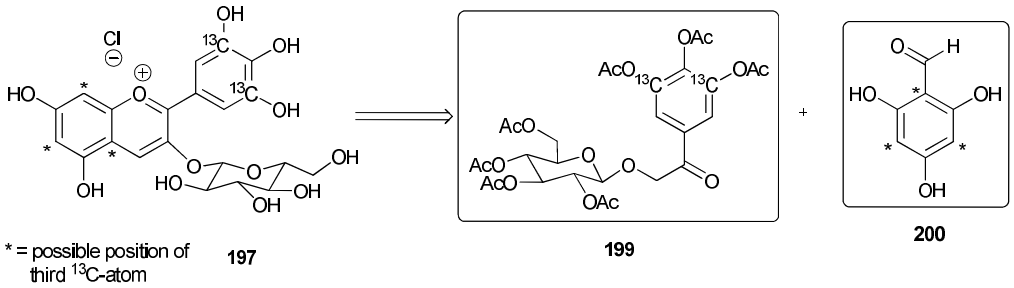


Diagram 30.

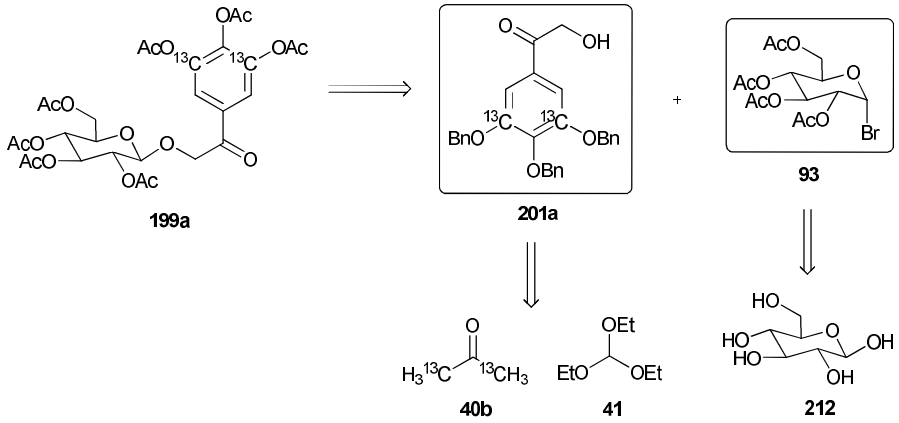
2.6.2 Retrosynthetic Analysis

Schemes 93-95 outline the retrosynthetic analysis for delphinidin-3-glucoside **197**. Initial retrosynthetic analysis (*Scheme 93*) gives the glycoside (**199a**) and aldehyde (**200**).



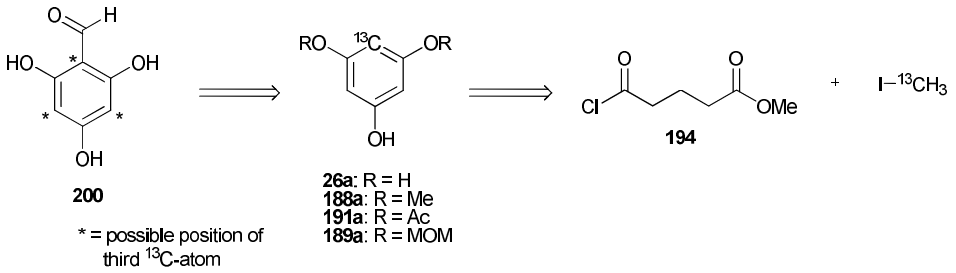
*Scheme 93 – Retrosynthetic analysis of delphinidin-3-glucoside **197**.*

The glycoside (**199**) can subsequently be broken down to the substituted α-hydroxyacetophenone **201a** and acetobromoglucose **93**. The first two ^{13}C-atoms would be introduced into the B ring by the use of [1,3-$^{13}C_2$]acetone **40b**[4] (*Scheme 94*)



*Scheme 94 – Retrosynthetic analysis of glycoside **199a.***

The aldehyde **200** can be synthesised by formylation of a ^{13}C-labelled phloroglucinol derivative which could be either phloroglucinol **26**, or one of three di-protected derivatives (as discussed previously in **Section 2.5**). The third and final ^{13}C-atom would be incorporated into the A ring using [^{13}C]methyl iodide[21] (*Scheme 95*).

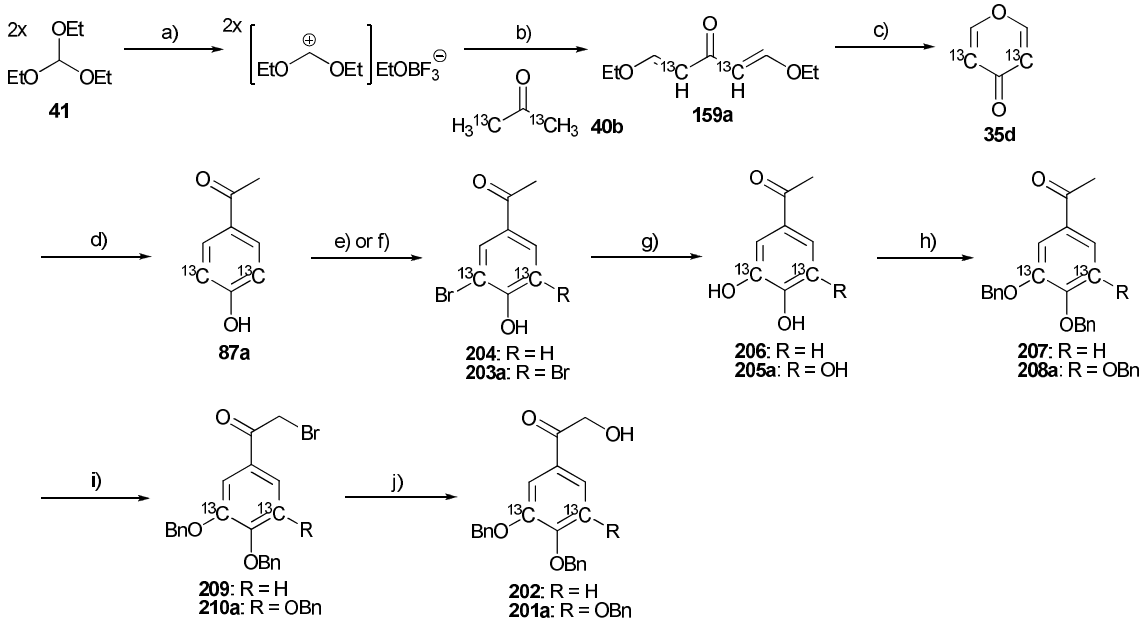


*Scheme 95 – Retrosynthetic analysis of aldehyde **200.***

2.6.3 Synthesis of α-Hydroxyacetophenones (201a and 202)

The synthesis of the α-hydroxyacetophenone (*Scheme 96*) was first optimised using unlabelled materials, to give both **201** and **202**, where R can be either hydrogen (for the synthesis of cyanidin-3-glucoside **198**) or *O*-benzyl (for the synthesis of delphinidin-3-glucoside **197**). The route to delphinidin-3-glucoside was then repeated using ^{13}C-labelled substrates. In order to demonstrate the potential application of the process to the synthesis of cyanidin-3-glucoside,

unlabelled studies in the route towards this product will be examined alongside studies towards the ^{13}C-labelled synthesis of delphinidin-3-glucoside. It should therefore be noted that in all Schemes, yields for compounds where R = H are quoted as unlabelled yields.



*Scheme 96 – Synthesis of α-hydroxyacetophenones **201a** and **202**; Reagents and conditions:* a) $BF_3.OEt_2$, DCM, -30 °C – 0 °C; b) *i*Pr_2NEt, -78 °C (quant.); c) Aq. HCl, EtOH, 80 °C, 24 h (quant.); d) Acetylacetone, KO*t*Bu, *t*BuOH, reflux, 20 h; then 1M HCl, reflux, 1 h (57%); e) DCM, Br_2, 0 °C – rt., 60 min (**204**, 99%); f) AcOH, NaOAc, Br_2, rt, 60 min (**203a**, quant.); g) $CuSO_4.5H_2O$, aq. NaOH, rt, 90 min, then 110 °C, 18 h (**206**, 82%; **205a**, quant.); h) Benzyl bromide, 18-crown-6, K_2CO_3, acetone, 69 °C, 6 h (**207**, 73%; **208a**, 48%); i) Tetrabutylammonium tribromide, DCM/MeOH, rt, 3 h (**209**, quant.; **210a**, quant.); j) Sodium formate, EtOH, 70 °C, 18 h (**202**, 66%; **201a**, 42%).

Preparation of the α-hydroxyacetophenone began with the previously optimised synthesis of [3,5-$^{13}C_2$]4*H*-pyran-4-one **35d** from triethyl orthoformate **41** and [1,3-$^{13}C_2$]acetone **40b** (**Section 2.2**).[4] Synthesis of *para*-substituted phenol **87a** was then achieved by reaction of **35d** with acetylacetone **135** in potassium *tert*-butoxide followed by aromatisation according to the conditions developed in **Section 2.1**.[3] The yield of 4-hydroxy-[3,5-$^{13}C_2$]acetophenone **87a** was lower at this point (57%) in comparison to the unlabelled synthesis (95%) as purification of the labelled compound was required before the subsequent bromination.[3,4]

The ^{1}H NMR spectrum of **87a** (*Diagram 31*) showed the ^{13}CH-3,5 protons as a double multiplet (6.93 ppm) with a large *J* value of 171 Hz due to ^{1}H-^{13}C coupling. The H-2,6 protons were present as a broad doublet at 7.91 ppm, with a smaller *J* value of 8.7 Hz due to coupling to the adjacent protons. An enhanced singlet was observed in the ^{13}C NMR spectrum (115.6 ppm) corresponding to the ^{13}C-3,5 atoms. Other resonances were weak, with that of C-2,6 being observed as a doublet (*J* 68.1 Hz) due to ^{13}C-^{13}C coupling.

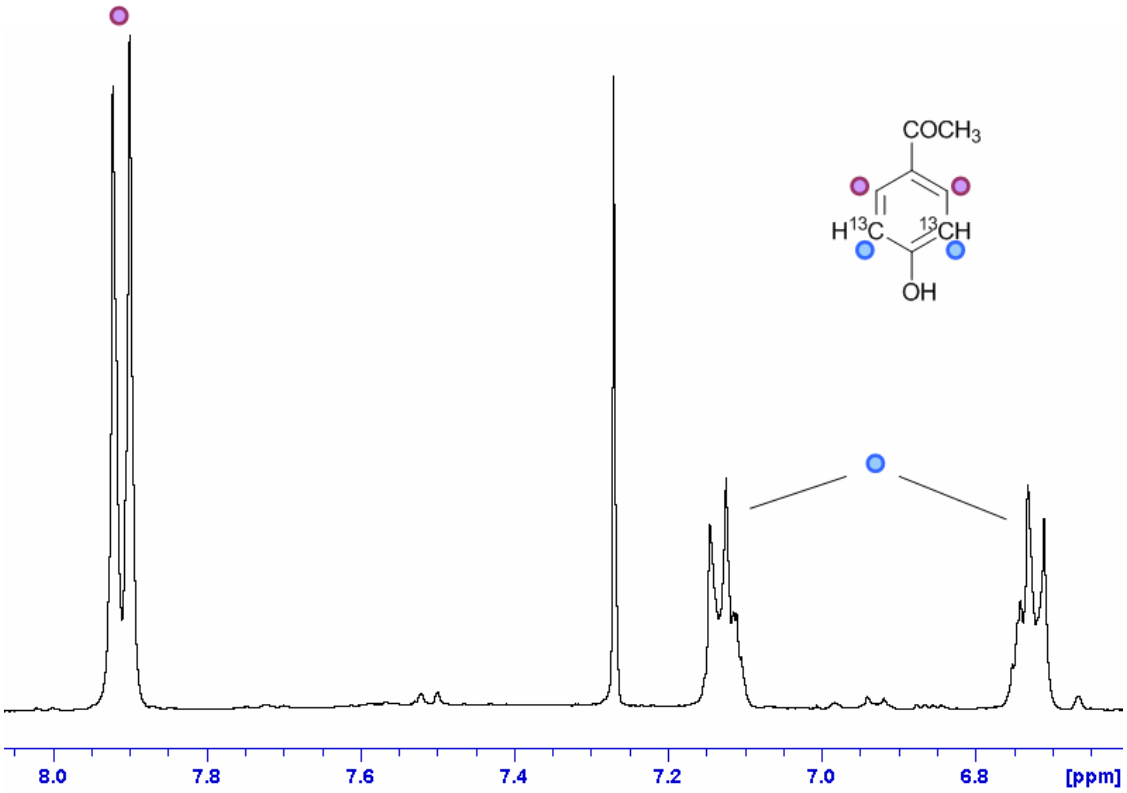


*Diagram 31 – Aromatic region of ^{1}H NMR spectrum of 4-hydroxy-[3,5-$^{13}C_2$]acetophenone **87a** (400 MHz, $CDCl_3$).*

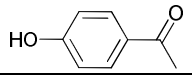
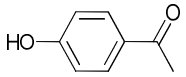
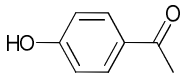
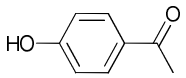
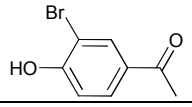
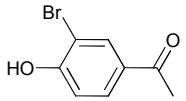
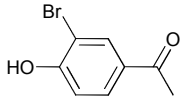
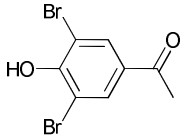
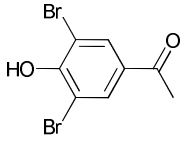
At this point the synthesis could be directed towards either delphinidin-3-glucoside **197** or cyanidin-3-glucoside **198** by the introduction of either one or two bromine atoms onto the aromatic ring. A method for the di-bromination of ethyl 4-hydroxybenzoate had already been optimised during the synthesis of [1,3,5-$^{13}C_3$]gallic acid **32d**, involving the use of bromine in the presence of sodium acetate and acetic anhydride.[4] This same method was employed with 4-hydroxy-[3,5-$^{13}C_2$]acetophenone **87a** and reached completion after 1 hour stirring at room

temperature, giving 3,5-dibromo-4-hydroxy-[3,5-$^{13}C_2$]acetophenone **203a** in excellent yield (>99%) and of sufficient purity that the following stage could be carried out on the crude material. The enhanced singlet in the ^{13}C NMR spectrum was observed at a lower chemical shift than that of 4-hydroxy-[3,5-$^{13}C_2$]acetophenone **87a** (111.5 ppm *cf.* 115.6 ppm) as expected following the introduction of two bromine atoms.[38]

For the introduction of a single bromine atom an alternative method was sought. In the synthesis of 3,4-dihydroxybenzoic acid **163**, Steglich *el al.* reported the use of bromine in dichloromethane at 0 °C for the bromination of ethyl 4-hydroxybenzoate **128**.[1] However, in our hands when this reaction was attempted on ethyl 4-hydroxybenzoate, only traces of the mono-bromo compound **161** were recovered (**Section 2.3**). This was found to be due to the poor solubility of the ester in dichloromethane. In the case of 4-hydroxyacetophenone **87**, this problem was not observed. Solubility was good, and so a range of conditions was attempted, with temperature, reaction time, and quantity of bromine being varied during optimisation. A solution of bromine in dichloromethane was thus added to a stirring solution of 4-hydroxyacetophenone in dichloromethane at 0 °C, with subsequent warming to room temperature, as indicated in *Table 16* below. Initial conditions attempted resulted in the recovery of mostly starting material **87** (entries 1 and 2). Doubling the quantity of bromine while using the same temperature profiles still gave a high recovery of starting material, but conversions to the mono-bromo product **204** and dibromo product **203** also increased (entries 3 and 4). Increasing the quantity of bromine further (entries 5 and 6) gave promising results, with the major product being the desired 3-bromo-4-hydroxyacetophenone **204**, although from results obtained thus far it appeared that allowing the reaction mixture to warm to room temperature was detrimental, as it resulted in the formation of the dibromo product. Allowing the reaction to stir at 0 °C for 90 min (1.6 eq. Br_2, entry 7) gave full conversion to the desired product, with no starting material or dibromo product being recovered. Entries 8 and 9 demonstrate that this route could also be applied to the synthesis of 3,5-dibromo-4-

hydroxyacetophenone **203** if desired, by simply increasing the quantity of bromine above the stoichiometric level and allowing the reaction to stir at room temperature for a longer period of time.

*Table 16 – Reaction variables for the bromination of 4-hydroxyacetophenone **87.***

Entry	Eq. Br_2	Time at 0 °C	Time at 25 °C	Major product	Ratio 87 : 204 : 203†
1	0.5	30	30		1 : 0.1 : 0
2	0.5	0	60		1 : 0.3 : 0.1
3	1	30	30		1 : 0.5 : 0.1
4	1	0	60		1 : 0.7 : 0.3
5	1.6	30	30		0.1 : 1 : 0.1
6	1.6	60	30		0 : 1 : 0.2
7	**1.6**	**90**	**0**		**0 : 1 : 0**
8	2	60	30		0 : 0.6 : 1
9	5	60	30		0 : 0.2 : 1

† Ratio of starting material **87**: mono-bromo **204**: dibromo **203** calculated by integration of ^{1}H NMR signals

Conversion of bromides **203a** and **204** to the di- and tri-hydroxybenzenes **205a** and **206** was achieved *via* the route previously optimised in the synthesis of [1,3,5-$^{13}C_3$]gallic acid (**Section 2.3**).[4] Although hydrolysis was not required in this case due to the absence of the ester group, the procedure was not altered. This was mainly due to the fact that this stage had previously been found to be very sensitive to the reaction conditions, especially in relation to the length of time that the aqueous mixture was stirred under reduced pressure. However, no adverse effects

were observed and further optimisation was not required. As with the synthesis of gallic acid, extractions with ethyl acetate significantly improved recoveries of the desired products. Unlabelled 3,4-dihydroxyacetophenone **206** was obtained in 82% yield, and 3,4,5-trihydroxy-[3,5-$^{13}C_2$]acetophenone **205a** in quantitative yield, with the shift of the ^{13}C NMR signal for C-3,5 from 111.5 ppm to 146.5 ppm confirming successful replacement of the bromine atoms with hydroxyl groups.[38] At this point in the synthesis of gallic acid, purification was carried out in order to obtain a pure sample of the final product. Despite the use of a positive pressure of argon during column choromatography, some oxidation was observed and small losses recorded due to the presence of 3 hydroxyl groups on the ring.[4] However, as crude samples of **205a** and **206** were found to be of good purity by ^1H NMR analysis, it was decided not to carry out purification at this point, but instead to continue with the following protection step.

Benzyl protection of the 3, 4 and 5-hydroxyl groups was chosen due to its inertness to subsequent reaction conditions, and also the ease of which it could be introduced. The acetyl protecting group was also examined for this reaction as will be discussed later (**Section 2.6.4**). Initially protection was attempted on unlabelled substrates using the procedure developed for the *O*-benzyl protection of maltol (**Section 2.1.3**).[3] However, the use of aqueous sodium hydroxide and methanol as solvent proved to be too harsh for our substrate. Alternative conditions[35] were found which were much milder, and used benzyl bromide in the presence of potassium carbonate, with acetone as solvent. Excess benzyl bromide was removed during purification by column chromatography, yielding 3,4-bis(benzyloxy)acetophenone **207** in 73% yield and 3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone **208a** in 48% yield after purification. Although ^1H-^{13}C HMBC analysis was required in order to visualise some quaternary carbon atoms, C-4 was visible at 148.6 ppm as a doublet (*J* 6.1 Hz) due to coupling with the adjacent ^{13}C-atoms. C-2,6 were also observed as a doublet (108.3 ppm), with a splitting of 70.4 Hz, along with an enhanced signal at 152.6 ppm corresponding to ^{13}C-3,5.

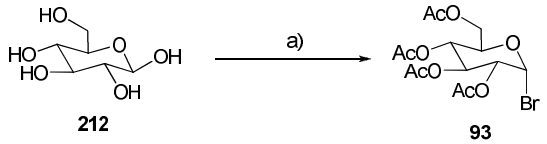
Two further transformations were required to complete the synthesis of **201a** and **202**. Firstly bromination of the methyl ketone was achieved by treatment with tetrabutylammonium tribromide in a mixture of dichloromethane and methanol.[26] The reaction proceeded smoothly to yield the desired bromides **209** and **210a** in excellent yield and of sufficient purity to be used crude in the next step. In the starting material (**208a**) the methyl group was visible in the ^{1}H NMR spectrum at 2.43 ppm and in the ^{13}C NMR spectrum at 26.3 ppm. After reaction with tetrabutylammonium tribromide, the new CH_2-Br group appeared at 4.23 ppm in the ^{1}H NMR spectrum and 30.5 ppm in the ^{13}C NMR spectrum as expected.[38] Analysis by mass spectrometry further confirmed the successful introduction of the bromine atom with signals for **210a** being observed at both 521 and 519 corresponding to $M^{81}Br+H$ and $M^{79}Br+H$ respectively, where M is the molecular ion.

The target alcohols α-hydroxy-3,4-bis(benzyloxy)acetophenone **202** and α-hydroxy-3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone **201a** were prepared in 66% and 42% yield respectively after reaction of the corresponding bromides with sodium formate in refluxing ethanol, followed by purification by column chromatography. A shift in the aliphatic CH_2 signal from 4.23 ppm to 4.64 ppm in the ^{1}H NMR spectrum and 30.5 ppm to 65.0 ppm in the ^{13}C NMR spectrum confirmed successful synthesis of the desired ^{13}C-labelled alcohol **201a**. An enhanced singlet corresponding to ^{13}C-3,5 was also observed (152.9 ppm). Mass spectrometry showed a molecular ion peak at 457 corresponding to M+H, further confirming the successful preparation of **201a**.

Over 8 steps, the ^{13}C-labelled α-hydroxyacetophenone **201a** was synthesised in 11.5% yield, and unlabelled α-hydroxyacetophenone **202** in 37% yield. It is possible that the lower yields for the ^{13}C-labelled reactions compared to the unlabelled substrates are due to the smaller scale for these reactions, which will result in greater losses during purification by column chromatography.

2.6.4 Synthesis of Glycosides (199a and 211) and Acetobromoglucose (93)

Before the synthesis of the glycosides (**199a** and **211**) could be carried out, preparation of the sugar moiety, acetobromoglucose **93** was required. This was achieved *via* treatment of a solution of *D*-glucose **212** in acetic anhydride with a 45% w/v solution of hydrogen bromide in acetic acid (*Scheme 97*).[39] Acetobromoglucose **93** was isolated as a white powder in 55% yield after recrystallisation from diethyl ether. Analytical data agreed with literature values and purity was confirmed by comparison of melting points. *Diagram 32* shows the ^{1}H NMR spectrum of 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide **93** (omitting the acetyl signals).



Scheme 97 – Reagents and conditions: a) Ac_2O, 45% w/v HBr in AcOH, 0 °C, 4 h, then rt. O/N (55%).

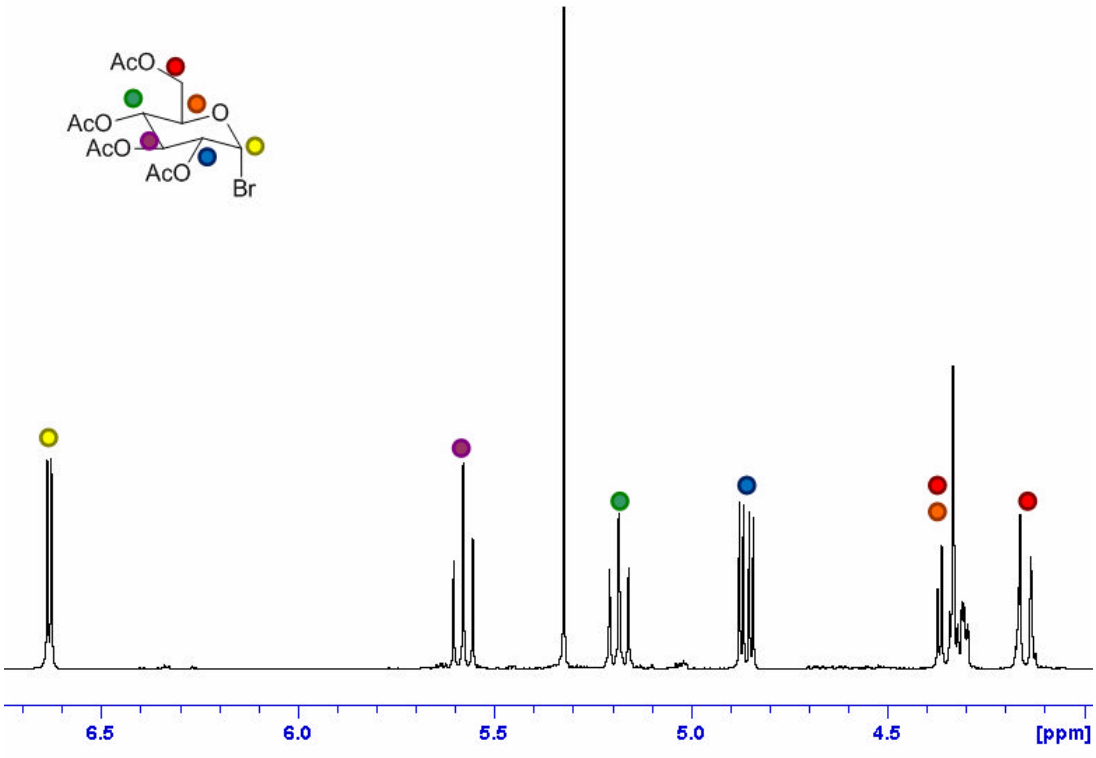
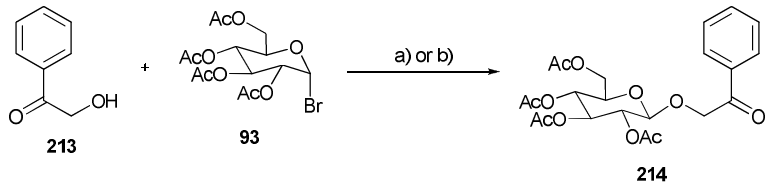


*Diagram 32 – ^{1}H NMR spectrum of **93** (4.00-6.70 ppm region) (400 MHz, $CDCl_3$).*

Coupling of α-hydroxyacetophenones **201a** and **202** with acetobromoglucose **93** was then examined. Two methods for the coupling of the alcohol and sugar moiety had been reported by Dangles *el al.* (*Scheme 98*),[40] and both were attempted using a commercially available model substrate **213** for optimisation studies.



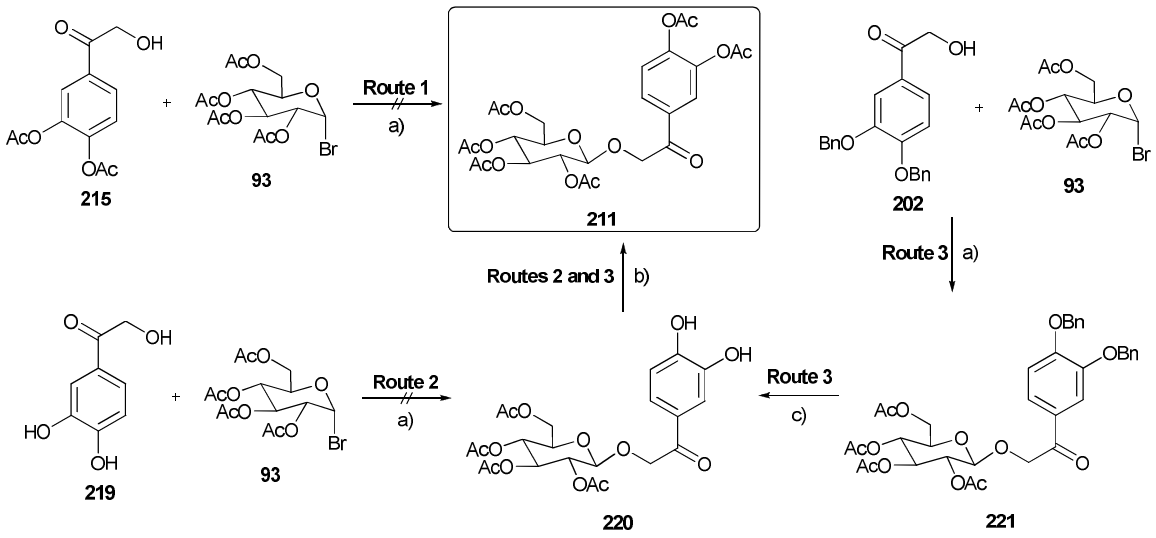
*Scheme 98 – Model conditions for the coupling of **213** and **93**:* a) AgOTf, DCM, $NaHCO_3$, 4Å mol. sieves, 0 °C; b) $Hg(CN)_2$, toluene, Na_2SO_4, 4Å mol. sieves, reflux.

The method which they appeared to favour involved the use of silver triflate as a Lewis acid at 0 °C. This reaction was attempted using 2 equivalents of the sugar (to 1 equivalent of alcohol) and 3 equivalents of silver triflate in dry dichloromethane in the presence of 4Å molecular sieves to ensure anhydrous conditions, and sodium bicarbonate to scavenge any triflic acid which may form during the reaction. Studies on the crude reaction mixture showed that a variety of components were present, including both starting materials, the hydrolysed sugar **212**, and the desired product **214**. Purification by column chromatography gave the coupled product **214** in 29% yield compared with the 40% reported by Dangles.[40]

Although it was reported that the use of mercuric cyanide gave similar yields to silver triflate,[40] we were eager to attempt the coupling using these alternative conditions in order to determine if the reaction proceeded with cleaner results. Indeed it was found that the use of 2 equivalents of mercuric cyanide and 2 equivalents of sugar **93** in refluxing toluene over 2 hours yielded the coupled species **214** as the major product. In order to ensure rigorously anhydrous conditions were maintained, 4Å molecular sieves and anhydrous sodium sulfate were also utilised in the reaction. Optimisation studies showed that the presence of molecular sieves and drying agents were indeed beneficial to the reaction, with increased yields when they were utilised. Reaction

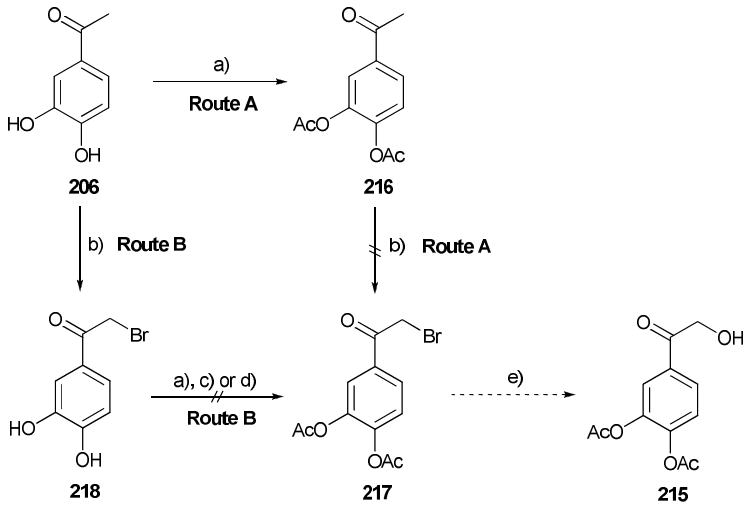
times were also varied during optimisation, but it was found that reactions of less than 2 hours gave a higher recovery of starting materials, and reaction times of greater than 2 hours resulted in the formation of unwanted degradation products. The desired coupled product **214** was isolated in 64% yield after purification by column chromatography, which is higher than the 40% yield quoted by Dangles *el al.*[40] for similar compounds. It is proposed that our incorporation of drying agents into the reaction accounts for this significant increase in isolated yield. Confirmation of the successful coupling was by ^{1}H NMR analysis, where the axial H-1 proton on the sugar moiety was observed as a doublet at 4.66 ppm (*cf.* 6.63 ppm for equatorial in **93**) with an appropriate *J* value of 7.9 Hz. The signal corresponding to C-1 in the ^{13}C NMR spectrum had also shifted from 86.6 ppm in **93** to 100.6 ppm in the coupled product **214**. In the ^{1}H NMR spectrum it could also be seen that the signal of the $COCH_2O$ protons were now visible as an AB system (*J* 16.4 Hz) compared to a doublet (*J* 3.7 Hz) in the alcohol substrate.

Following successful optimisation of the coupling conditions using the model substrate **213**, three potential routes to the glycoside **211** were envisaged (*Scheme 99*), and these were tested and developed, where appropriate, on unlabelled substrates before applying the most successful route to the preparation of ^{13}C-labelled glycoside **199a**.



*Scheme 99 – Proposed routes to glycoside **211**; Reagents and conditions:* a) $Hg(CN)_2$, toluene, Na_2SO_4, 4Å mol. sieves, reflux; b) Ac_2O, pyridine, rt.; c) Debenzylation *via* hydrogenation (Pd/H_2).

Route 1 was initially the route of choice, as it avoids the extra deprotection/protection stages in Routes 2 and 3. The synthesis of the α-hydroxyacetophenone with acetyl protecting groups (**215**) in place of benzyl protecting groups would therefore first be required, followed by coupling using the optimised conditions. It would not be possible to carry out a deprotection and acetyl protection after introduction of the aliphatic hydroxyl group on **202**, as this hydroxyl group would also be acetylated and thus could not undergo coupling to acetobromoglucose **93**. Therefore, two routes to **215** are plausible, both originating from 3,4-dihydroxyacetophenone **206** (*Scheme 100*).



*Scheme 100 – Proposed routes to **215**; Reagents and conditions:* a) Pyridine, Ac_2O, rt.; b) Tetrabutylammonium tribromide, DCM/MeOH or DCM, rt.; c) AcBr, acetone, 18-crown-6, K_2CO_3, reflux; d) Pyridine, acetyl bromide, rt.; e) Sodium formate, EtOH, reflux.

Route A was attempted first, with acetylation to give **216** in 95% yield after overnight stirring at room temperature. However, when subsequent bromination was attempted using tetrabutylammonium tribromide in methanol/dichloromethane a number of issues were encountered. Firstly, the presence of methanol in the reaction mixture was found to remove the acetyl protecting groups and so the reaction was then attempted using only dichloromethane. In the presence of the benzyl protecting groups, no side reactions were observed in the bromination, with mono-bromination taking place at the active methylene group. Conversely, in the presence of the acetyl groups, poly-bromination was observed on all three methyl groups in the compound. Lowering the reaction temperature had no significant effect on the reaction, with the preparation of **217** *via* this route being unsuccessful.

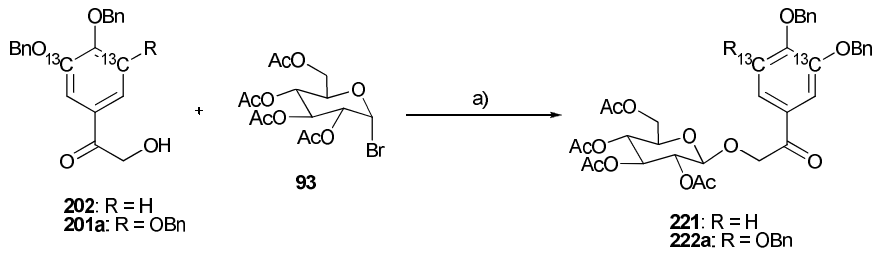
Direct bromination of the unprotected acetophenone **206** using $TBABr_3$ in methanol/dichloromethane was successful (Route B), with **218** being isolated in 99% yield. The conversion was confirmed by observing the shift of the aliphatic signals from 2.40 ppm (3H, CH_3) to 4.57 ppm (2H, CH_2-Br) as expected from the introduction of the bromine substituent.

Subsequent acetylation using acetic anhydride in pyridine gave a complex reaction mixture, which was presumed to be due to the presence of acetate anions in the reaction mixture causing unwanted side-reactions. It also appeared that the bromine atom had been lost during the attempted acetylation, as the CH_2 signal at 4.57 ppm was no longer present. An alternative procedure was sought which would not result in formation of the acetate anion. Acetic anhydride was replaced with acetyl bromide, as it was thought that formation of the bromide ion would cause fewer problems than the acetate anion. However, results were again disappointing, with no trace of the desired product **217** being isolated. Once again, the CH_2 signal at 4.57 ppm was not observed. A similar route to that used for the benzyl protection of 3,4-dihydroxyacetophenone **206** was adopted, replacing benzyl bromide with acetyl bromide. Unfortunately, a complex product mixture was recovered, with a large number of components observed by TLC and ^{1}H NMR analysis. Purification by column chromatography was not successful due to poor separation and streaking on the column. As all procedures developed would be applied to the use of ^{13}C-labelled substrates, it was desirable to obtain conditions which yielded the cleanest reaction profiles in order to avoid extensive purification and losses of expensive ^{13}C-labelled material. This route was therefore not examined further.

As the synthesis of α-hydroxy-3,4-bis(benzyloxy)acetophenone **202** had already been achieved, Route 2 (*Scheme 99*) was then investigated. Debenzylation *via* hydrogenation was successful using either 10% Pd/C or palladium black. α-Hydroxy-3,4-dihydroxyacetophenone **219** was isolated in quantitative yield and excellent purity after deprotection. Good purity was beneficial at this point, as similar products have previously exhibited oxidation during column chromatography and therefore purification was not desirable. Coupling of **219** with acetobromoglucose **93** using mercuric cyanide as the Lewis acid was attempted. Reaction times of 1-6 hours were investigated and all gave similar results. Complex mixtures were obtained which were found to consist mainly of the two starting materials. Comparison of the crude ^{1}H NMR spectrum with an authentic sample of 2-[3,4-dihydroxyphenyl]-2-oxoethyl 2,3,4,6-tetra-

O-acetylglucopyranoside **220** prepared previously (see **Scheme 99** and **101**) appeared to show no trace of the desired product. It could therefore be concluded that coupling of the unprotected substrate **219** with sugar **93** was unsuccessful using our optimised conditions, and this is likely due to possible side-reactions which could take place on the unprotected phenolic groups.

Following the failure of both Routes 1 and 2, the previously optimised conditions of the coupling using model substrate **213**, were applied to the use of both unlabelled α-hydroxy-3,4-bis(benzyloxy)acetophenone **202** (towards the synthesis of cyanidin-3-glucoside **198**) and ^{13}C-labelled α-hydroxy-3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone **201a** (towards the synthesis of delphinidin-3-glucoside **197**) *via* Route 3 (*Schemes 99* and *101*). It was anticipated that this procedure may be higher yielding due to the fact that a lesser number of side-reactions seemed possible using the fully protected benzylated substrates **201a** and **202**. Yields for compounds where R = H are quoted as unlabelled yields.



*Scheme 101 – Coupling of **201a** and **202** with **93**; Reagents and Conditions:* a) $Hg(CN)_2$, toluene, Na_2SO_4, 4Å mol. sieves, reflux (**221**, R = H, 28%; **222a**, R = OBn, 47%).

Optimisation was not carried out for the use of unlabelled substrates **201** and **202** as the initial yields recovered under mercuric cyanide conditions were comparable with the literature results for similar compounds.[40] Coupling of α-hydroxy-3,4-bis(benzyloxy)acetophenone **202** with sugar **93** was successful, giving **221** (R = H) in 47% yield after purification by column chromatography. It is hypothesised that partial hydrolysis of the glycoside takes place on silica during purification, therefore accounting for some losses in material. The coupling was confirmed by examination of the ^1H and ^{13}C NMR spectra of the starting materials and product.

Diagram 33 shows the ^{1}H NMR spectrum of the coupled product **221** with expansion of the 3.50-5.20 ppm region, where the signals of the sugar moiety are visible. The signal corresponding to the aliphatic CH_2 adjacent to the carbonyl group shifted from 4.69 ppm in the ^{1}H NMR spectrum of the starting material, where it was a doublet (*J* 3.7 Hz), to 4.69 and 4.80 ppm in the product, where an AB system was observed with a large *J* value of 16 Hz. A small shift was also observed in the ^{13}C NMR spectrum for the CH_2 from 65.0 ppm to 70.7 ppm. As with the model studies, the H-1 proton of the sugar moiety was in the equatorial position (6.63 ppm, *J* 4.0 Hz) in the starting material **93**, and in the axial position in product **221**. This was confirmed by the presence of a doublet at 4.59 ppm in the ^{1}H NMR spectrum of **221**, with a larger *J* value of 8.0 Hz. The ^{13}C spectrum showed a shift of the C-1 signal from 86.6 ppm in **93** to 100.6 ppm in **221**.

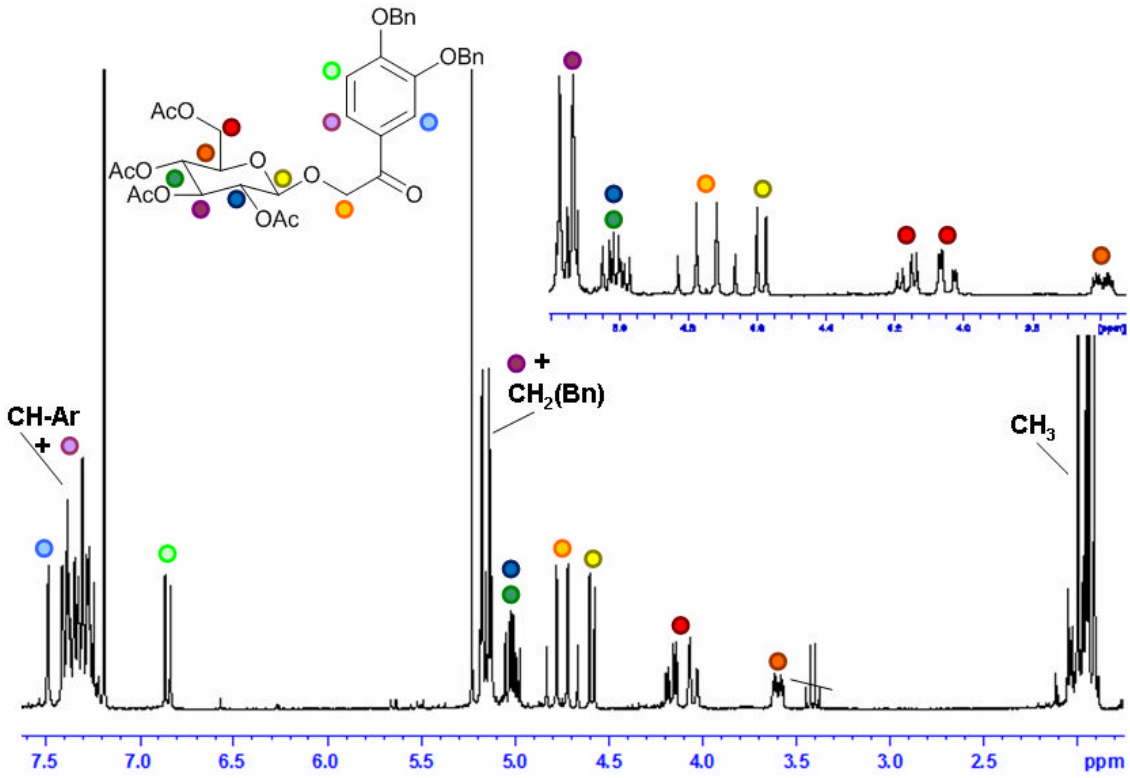


*Diagram 33 – ^{1}H NMR spectrum of **221** with expansion of region 3.50-5.20 ppm (300 MHz, $CDCl_3$).*

Preparation of the ^{13}C-labelled product **222a** from α-hydroxy-3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone **201a** was successful on a smaller scale, and yielded the desired product in 28% yield after purification by column chromatography. The lower yield is likely to be due to the smaller scale of the reaction, i.e. 41 mg ^{13}C-labelled substrate compared to 160 mg for the unlabelled substrate. The ^{13}C NMR spectrum showed the enhanced singlet for ^{13}C-3',5' at 152.7 ppm (*cf.* 152.9 ppm in **201a**). As this shift is not significantly large to confirm successful coupling, the regions of the aliphatic CH_2 signal and sugar H-1 signal were examined as before. As expected, the CH_2 signal shifted from 4.64 ppm to form an AB system at 4.55 and 4.83 ppm. The large *J* value (12.5 Hz) can be seen in the ^{1}H-^{13}C HSQC spectrum given in *Diagram 34* below, which shows the region corresponding to the signals of the sugar moiety and to the aliphatic CH_2. The signal corresponding to H-1 had also moved, in this case from 6.63 ppm (d, *J* 4.0 Hz) to 4.48 ppm, with the indicative coupling constant of 7.6 Hz due to the axial-axial coupling with H-2. The characteristic shift of the C-1 signal in the ^{13}C NMR spectrum from 86.6 ppm to 99.3 ppm was also observed, thus confirming successful synthesis of the desired coupled product **222a**. Although some impurities were still present in the ^{13}C-labelled sample after column chromatography (as can be seen in *Diagram 34*) the purity was considered high enough to continue with the following step in order to avoid further losses through additional purification. Until this point, analysis by high resolution mass spectrometry had been highly successful in providing accurate masses for both labelled and unlabelled compounds. However, the new coupling products **221** and **222a** appeared to be more difficult to ionise under electrospray methods, with fragmentation taking place easily under chemical ionisation methods. It was found that using a higher concentration of sample was sufficient to allow an accurate mass to be determined, and thus a mass corresponding to the molecular ion **222a** plus sodium (M+Na) (809.2679 ppm) was recorded within 1.7 ppm of the calculated value for the unlabelled product.

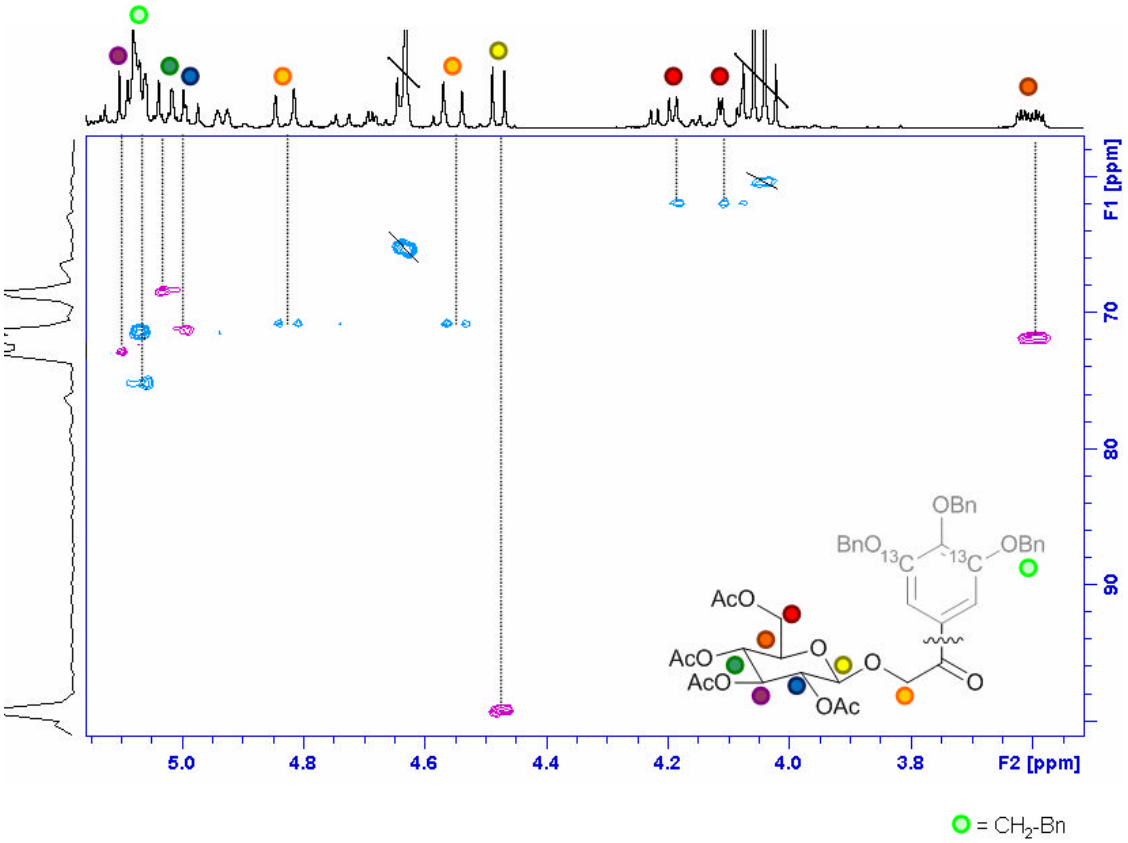
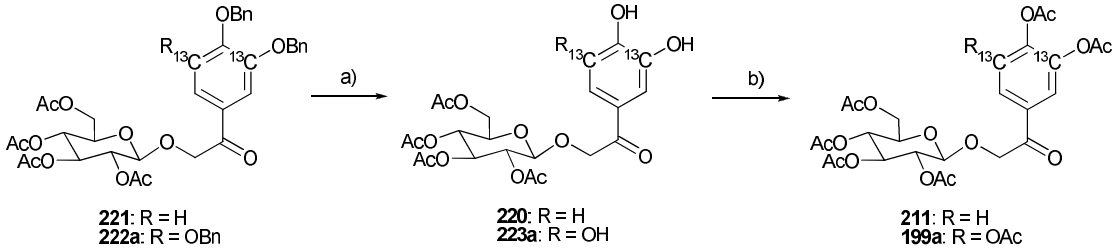


*Diagram 34 – 2D ^{1}H-^{13}C HSQC spectrum of **222a** showing the region 3.50-5.10 ppm (400 MHz, $CDCl_3$) (*residual solvent signals indicated by dash).*

Following successful coupling of α-hydroxyacetophenones **201a** and **202** with acetobromoglucose **93**, the final stages towards the synthesis of glycosides **199a** and **211** were examined. It was desirable to ensure that all hydroxyl protecting groups could be removed easily in a global deprotection after the final coupling with the phenolic aldehyde **200** to yield the desired anthocyanins (**Section 2.6.6**). Therefore it was chosen to deprotect the benzyl groups at this point, and subsequently re-protect with acetate protecting groups (*Scheme 102*). By this route, the protecting groups of the sugar hydroxyls and benzylic hydroxyls could both be removed under the same conditions.



*Scheme 102 – Completion of the synthesis of glycosides **199a** and **211**, Reagents and Conditions:* a) Palladium black, H_2, ethyl acetate, rt., 40 h (**220**, R = H, quant., **223a**, R = OBn, quant.); b) Ac_2O, pyridine, rt., 18 h (**211**, R = H, 10%, **199a**, R = OBn, 36%).

A range of deprotection conditions was attempted on the unlabelled substrate **221** (*Table 17*),[41] with the majority yielding only starting material. However, 10% palladium on carbon was found to give 100% conversion, and 47% isolated yield, after overnight stirring at 35 °C, but these conditions were found to be irreproducible depending on the batch of Pd/C used. In reactions using palladium hydroxide, deprotection was successful in two cases but, disappointingly, reduction of the ketone was also observed even at room temperature (entries 8 and 9). Raney nickel (entries 10 and 11) yielded only starting material. Palladium black (98% palladium on carbon) in ethyl acetate was found to be the most efficient and highest yielding of the catalysts (entries 12 and 13), with 100% conversion and quantitative yield of **220** being achieved after stirring at room temperature for 18 h. Reactions were carried out under a positive pressure of hydrogen *via* the use of a balloon filled with hydrogen gas. Purification was not desirable at this stage, due to reasons previously outlined for polyhydroxybenzenes. In all cases where the desired product was obtained, samples were of sufficient purity that purification was not required before the next step. Isolated yields are given only for those reactions where 90-100% conversion was achieved.

*Table 17 – Reaction variables for the debenzylation of **221.***

Entry	Catalyst	Solvent	Temp. (°C)	Time (h)	Conversion (%)[a]	Isolated Yield (%)
1	10% Pd/C	THF	25	18	0	-
2	10% Pd/C	THF	25	40	5	-
3	**10% Pd/C**	**THF**	**35**	**18**	**100**	**47**
4	10% Pd/C	EtOAc	25-40	40	0	-
5	10% Pd/C	Toluene	25-40	40	0	-
6	$Pd(OH)_2/C$	Toluene	25	18	0	-
7	$Pd(OH)_2/C$	EtOAc	25	18	0	-
8	$Pd(OH)_2/C$	EtOAc	40	40	Alcohol[b]	-
9	10% Pd/C, $Pd(OH)_2/C$	THF/Toluene	25-40	18	Alcohol[b]	-
10	Ra Ni	EtOAc	25-40	40	0	-
11	Ra Ni	THF/Toluene	25-40	40	0	-
12	**Pd black**	**EtOAc**	**25**	**18**	**97**	**99**
13	**Pd black**	**EtOAc**	**40**	**18**	**100**	**quant.**

[a] Conversion calculated by integration of ^{1}H NMR signals of starting material and product
[b] Reduction of the ketone functionality to an alcohol was also observed

Having confirmed the reproducibility of the results from the use of palladium black for the deprotection, conditions from entry 13 were then applied to the ^{13}C-labelled substrate **222a** to give **223a**. The reaction was found by TLC to have gone to completion after overnight stirring at 40 °C. ^{1}H NMR analysis of the product showed agreement with the unlabelled substrate, but also showed the presence of some additional signals in the aromatic region. It was apparent from overlaying the ^{1}H NMR spectra that these signals did not correspond to the benzyl signals from starting material **222a**, and it could be seen from the spectrum of the deprotected product that no signals corresponding to the benzylic CH_2 protons were present (~5.00-5.10 ppm). Also, correlation was not observed in the ^{1}H-^{13}C HMBC spectrum, thus confirming that the deprotection had been successful and that these signals must be due to a residual impurity in the sample. During optimisation of the reaction using unlabelled substrates, it was found that these signals were also observed in some cases, but following subsequent acetylation and purification by column chromatography the impurity was removed. Therefore, due to the comparatively small scale of this procedure (28 mg **222a**, 18 mg **223a**) and the use of ^{13}C-labelled substrates, it was decided that further purification would not be carried out until after the following step in order to avoid further losses of material. *Scheme 35* shows a segment of the ^{1}H-^{13}C HSQC spectrum of **223a** where signals corresponding to the sugar moiety can be seen. This should be compared to the ^{1}H-^{13}C HSQC spectrum of the benzylated substrate **222a**, where the aliphatic

CH_2 signals are visible. Their absence from the spectrum of **223a** demonstrates that full debenzylation was successful.

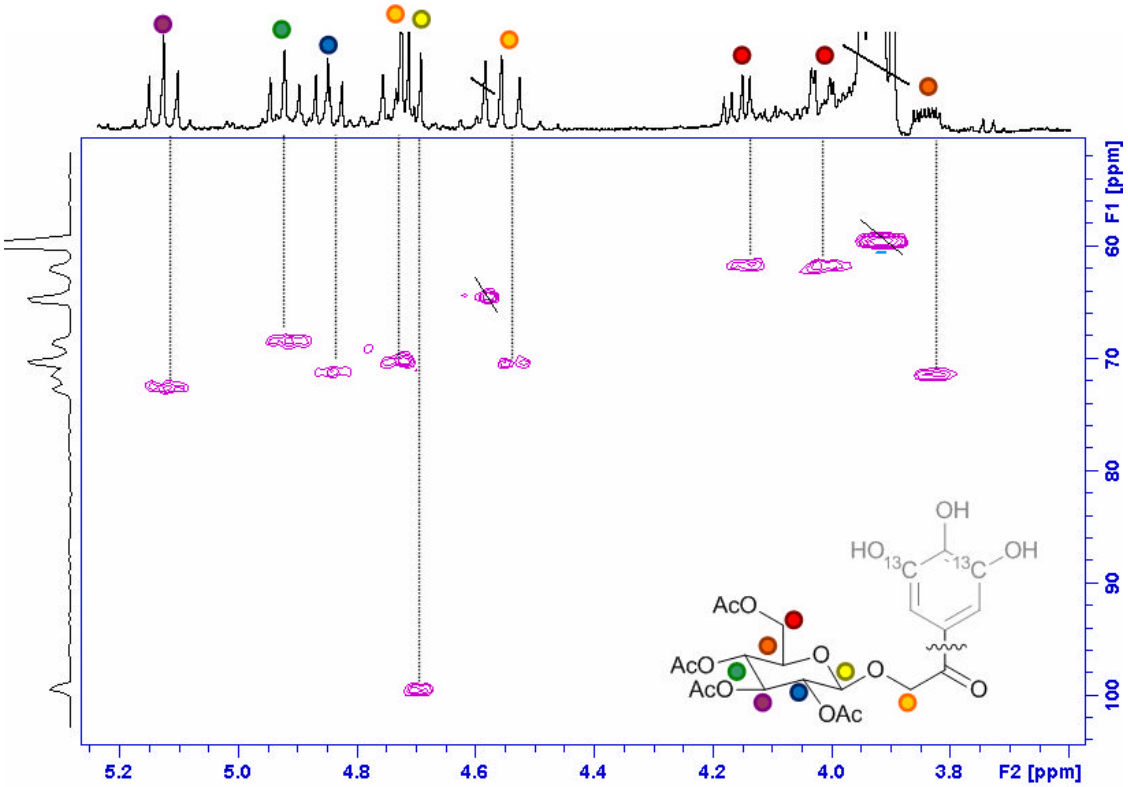


*Diagram 35 – 2D ^{1}H-^{13}C HSQC spectrum of **223a** showing the region 3.60-5.20 ppm (400 MHz, acetone-d_6) (*residual solvent signals indicated by dash).*

Further confirmation of the deprotection was by analysis of the ^{13}C NMR signals. It was expected that an upfield shift in the enhanced ^{13}C-3',5' signal would be observed following debenzylation.[38] Indeed this was observed, with a shift from 152.7 ppm in substrate **222a** to 146.2 ppm in **223a**. Taking into account our system of a multiply-substituted benzene, the magnitude of this shift is also consistent with theoretical values for mono-substituted benzenes, where OCH_3 was taken as being similar to *O*-benzyl (*Table 18*).[38]

*Table 18 – Predicted and observed shifts of ^{13}C-3',5' signals in the ^{13}C NMR spectrum of **223a**.*[38]

Substituent	Predicted shift (ppm)	Predicted change relative to OCH_3	Observed shift (ppm)	Observed change relative to OCH_3
OCH_3	128.5 +31.4	-	152.7	-
OH	128.5 +26.9	-4.5	146.2	-6.5
OAc	128.5 +22.4	-9.0	143.9	-8.8

Finally, the synthesis of glycosides **199a** and **211** could be completed by acetyl protection (*Scheme 102* above). This transformation was achieved by treatment of the deprotected compounds **220** and **223a** with acetic anhydride in pyridine. After overnight stirring at room temperature both reactions had reached completion. Purification of the unlabelled sample by column chromatography yielded 2-[3,4-bis(acetyloxy)phenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside **220** in 10% yield (2% over 3 steps, 0.74% overall from acetone **40** and triethyl orthoformate **41**). It would be desirable to attempt further optimisation of the purifcation in the future, as yields were poor. Two new signals in the ^{1}H NMR spectrum of **211** at 2.25 and 2.26 ppm (total integration = 6H) corresponding to the new acetyl protecting groups were observed. Analysis by high resolution mass spectrometry was carried out, and as with the initial coupling product **221**, and deprotected compound **220**, analysis required a high concentration of the sample to be used in order to obtain good ionisation. A signal corresponding to the molecular ion plus sodium was observed (M+Na = 605.1478), with the accurate mass being recorded to within 0.4 ppm of the theoretical value. The full ^{1}H NMR spectrum of **211** is shown in *Diagram 36* below, with expansion of the aromatic region.

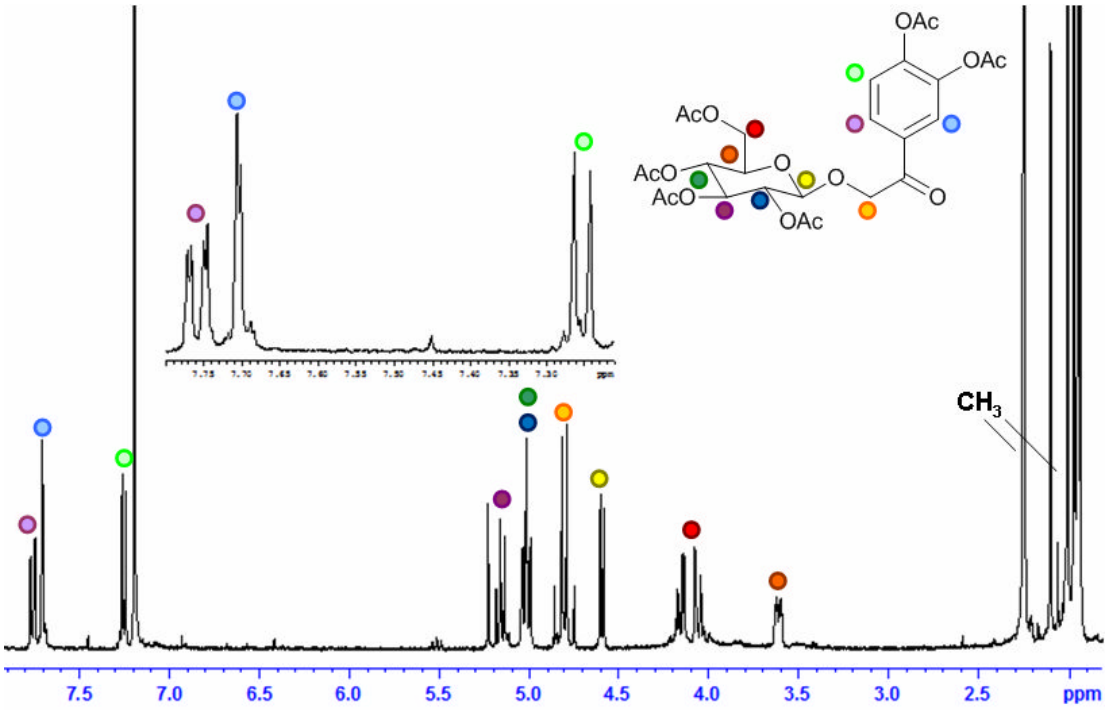


*Diagram 36 – ^{1}H NMR spectrum of **211** with expansion of the aromatic region 7.20-7.80 ppm (400 MHz, $CDCl_3$).*

The ^{1}H-^{13}C HSQC spectrum for the region 3.50-5.30 ppm is given in *Diagram 37*, which once again shows the area of the sugar and aliphatic CH_2 proton resonances.

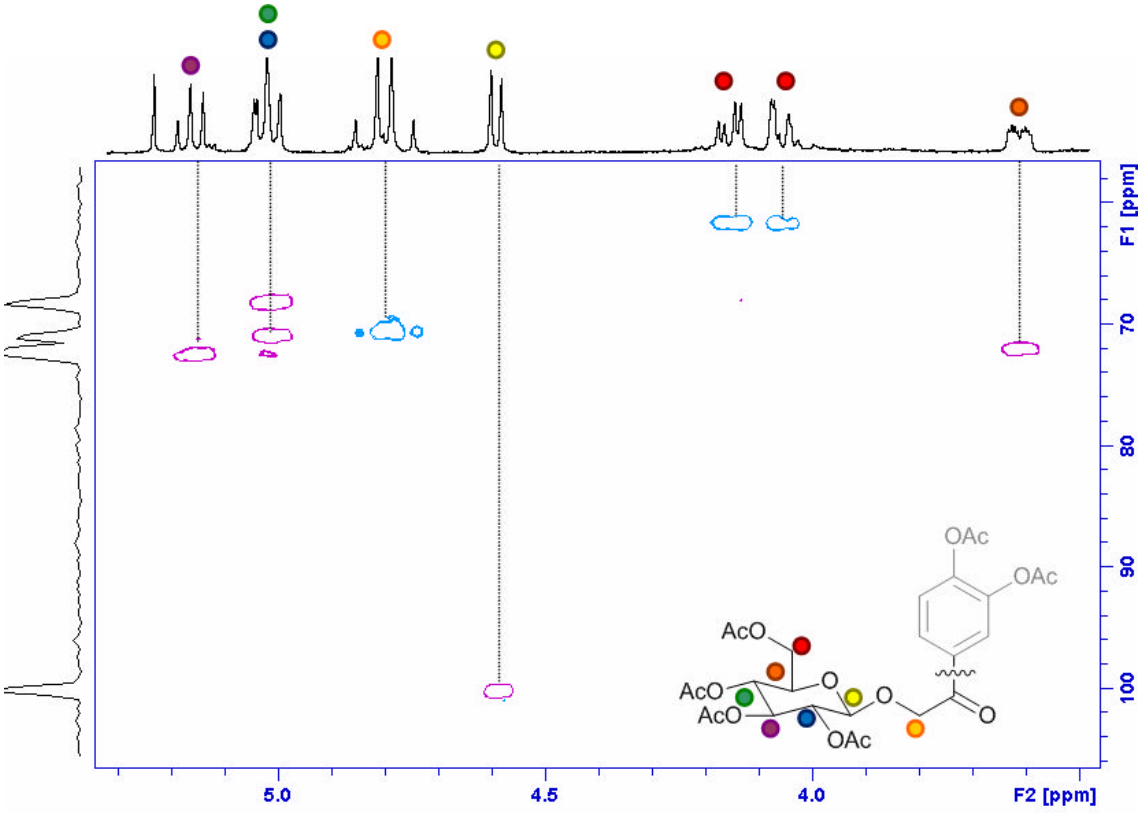


*Diagram 37 – 2D ^{1}H-^{13}C HSQC spectrum of **211** showing the region 3.50-5.30 ppm (400 MHz, $CDCl_3$).*

Acetyl protection of the ^{13}C-labelled trihydroxy substrate **223a** was carried out using identical conditions to those for **220**, with the reaction going to completion after stirring overnight. Purification by column chromatography yielded 2-[3,4,5-tris(acetyloxy)-[3,5-$^{13}C_2$]phenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside **199a** in 36% yield (10% over 3 steps, 2% overall from [1,3-$^{13}C_2$]acetone **40b** and triethyl orthoformate **41**). Optimisation of this step at a later date would be beneficial in order to increase yields. Due to the small quantity of **199a** isolated (10 mg), and the enhancement of the signals from the ^{13}C-atoms in the ^{13}C NMR spectrum, it was difficult to visualise other carbon atoms by that method alone. Therefore 2D ^{1}H-^{13}C HSQC and HMBC were required in order to observe the majority of the carbon signals. However, it should be noted that these signals recorded by the use of HSQC and HMBC

analysis did correlate with those observed in the unlabelled sample of **199** synthesised during route validation. Analysis by ^{1}H NMR spectroscopy showed that the additional signals in the aromatic region which were present after the debenzylation were still present at this point. However, it had been shown after the previous step that this appeared to be an impurity in the sample, and therefore it is likely that an alternative form of purification may be required in order to remove this impurity. At this point, no such method has been developed due to the small quantity of **199a** remaining after characterisation was carried out, and this remains an ongoing objective. This also caused issues for obtaining an accurate mass of the final product, as previously described, as there was not a sufficient quantity of **199a** isolated in order to provide a concentrated sample for analysis. An accurate mass correct to 0.2 ppm of the calculated value was however obtained for the unlabelled sample. Taking this factor into account along with the agreement of the ^{1}H and ^{13}C NMR spectra between the labelled and unlabelled compounds (see below) allows the conclusion to be made that the transformation was indeed successful.

Characterisation by both 1D and 2D ^{1}H and ^{13}C NMR was carried out on the ^{13}C-labelled product **199a**. In the ^{1}H NMR spectrum, a shift from 6.91-6.96 ppm (**222a**) to 7.61 ppm for the aromatic protons was observed, with this new signal present as a triplet (*J* 7.6 Hz) due to coupling with the *ortho*-^{13}C-atoms. Also, a singlet was observed at 2.25 ppm (integration = 9H), corresponding to the protons of the new acetyl protecting groups on the benzylic hydroxyl groups. In agreement with the data in *Table 18* relating to the predicted chemical shift of the ^{13}C-atoms in this series of transformation, an upfield shift was once again observed in the conversion of **223a** to acetyl-protected **199a**, with the new signal at 143.9 ppm corresponding to ^{13}C-3',5' on the aromatic ring. The ^{1}H NMR spectrum of **199a** is given in *Diagram 38* below, with the aromatic signals of the impurity clearly visible at chemical shift slightly higher than that of chloroform.

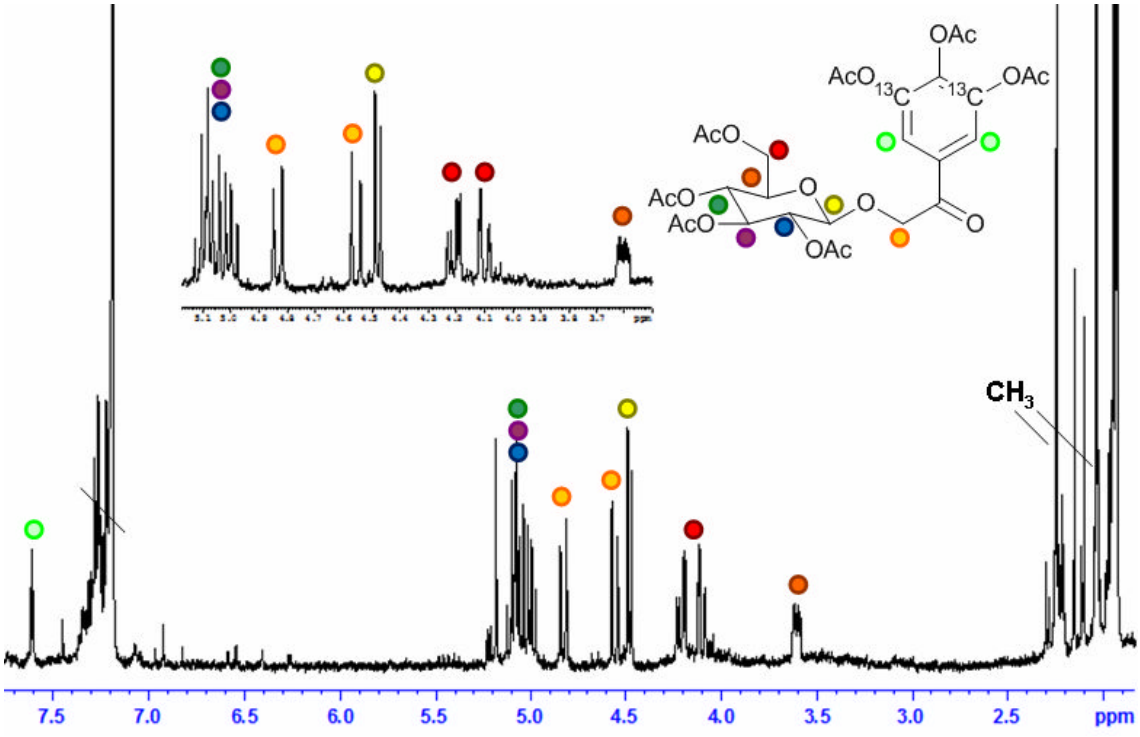


*Diagram 38 – ^{1}H NMRs pectrum of **199a** with expansion of the region 3.50-5.20 ppm (400 MHz, $CDCl_3$).*

Diagram 39 shows the 2D ^{1}H-^{13}C HSQC correlation for the region from 3.50-5.20 ppm, allowing the sugar moiety and aliphatic CH_2 signals to be clearly observed for comparison as before.

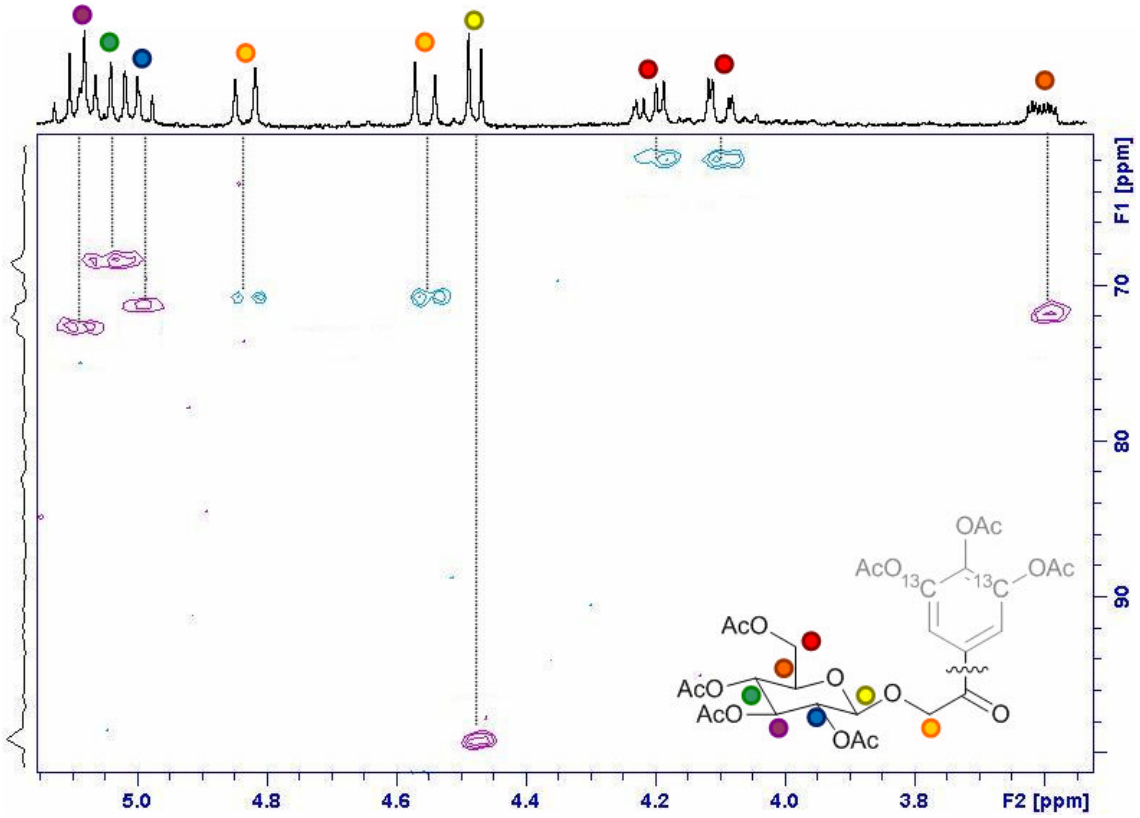
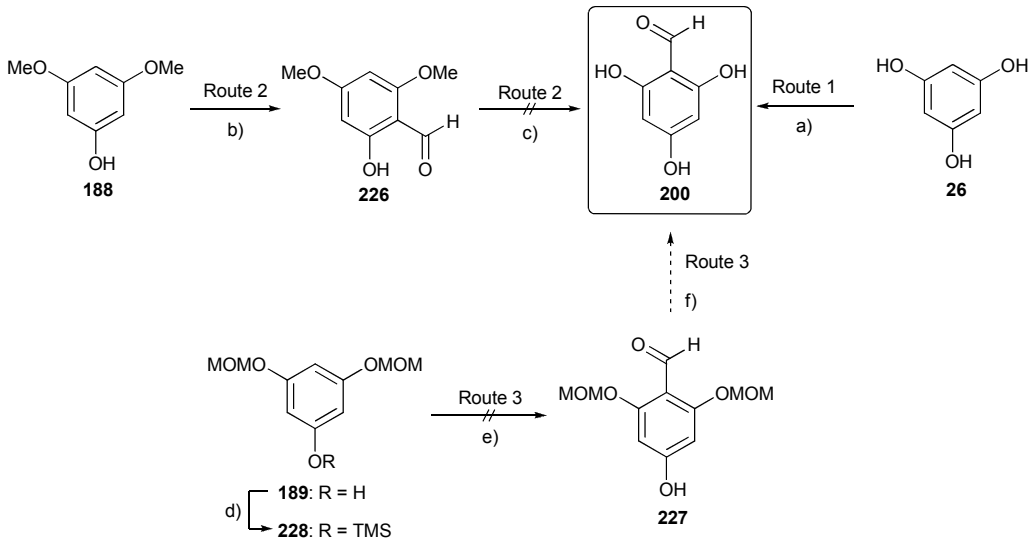


*Diagram 39 – 2D ^{1}H-^{13}C HSQC spectrum of **199a** showing the region 3.50-5.20 ppm (400 MHz, $CDCl_3$).*

It can therefore be concluded that the preparation of ^{13}C-labelled glycoside **199a** from α-hydroxy-3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone **201a** and 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide **93** was successful, although full purification and characterisation remains an ongoing objective after synthesis of a greater quantity of **199a**. Comparison and agreement with data from the unlabelled isomer allows this conclusion to be made. Glycosides **199a** and **211** can subsequently be used in the final cyclisation for the synthesis of anthocyanins **197** and **198**.

2.6.5 Synthesis of 2-Formylphloroglucinol (200)

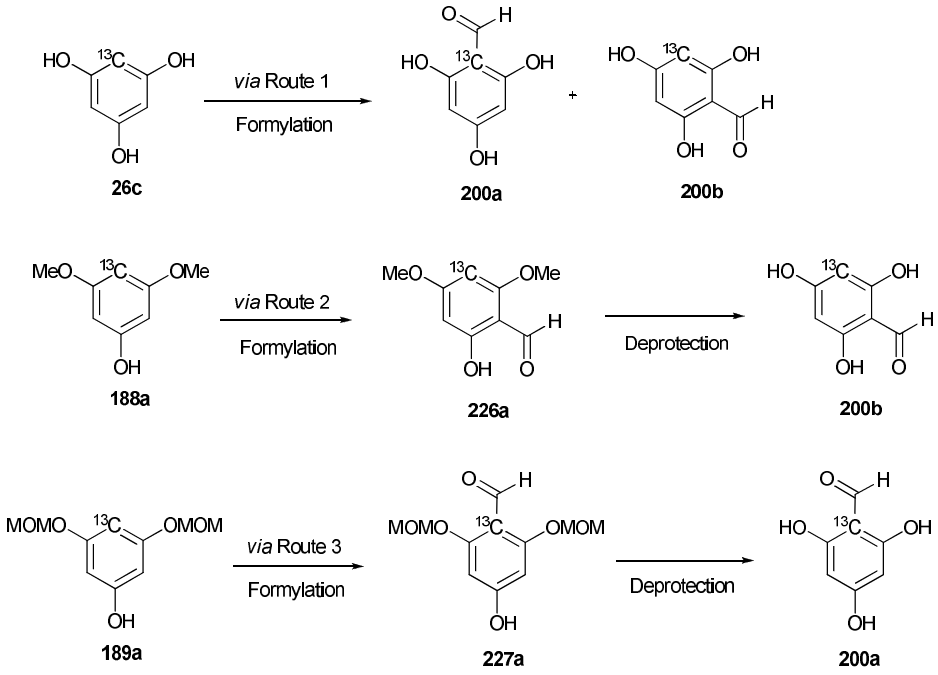
Three routes were proposed for the synthesis of 2-formylphloroglucinol **200** (*Scheme 103*), building upon the conditions employed for the synthesis of [2-^{13}C]phloroglucinol **26c** (**Section 2.5**), and utilising either phloroglucinol itself (**26**) or diprotected phloroglucinols **188** and **189** as substrates for the subsequent transformations. The use of MEM, Boc and acetyl protecting groups were not investigated at this stage as conditions for their preparation had not yet been optimised. As will be seen later, the route chosen for the preparation of **200** will determine the number of isotopomers present following the formylation step on either protected or unprotected [2-^{13}C]phloroglucinol **26c**. This in turn will have an effect on the position of the isotopic label in the final anthocyanin product (**Section 2.6.6** below). Ideally the synthesis of an isotopically-symmetrical formylphloroglucinol is desired, as will be discussed later.



*Scheme 103 – Proposed Routes to **200**; Reagents and conditions:* a) DMF, $POCl_3$, EtOAc; b) Dichloromethyl methyl ether, $TiCl_4$, DCM; c) BBr_3, DCM (see *Table 20*); d) Pyridine, TMSCl, DMAP; e) *n*BuLi, DMF, Et_2O; or *n*BuLi, THF, *N*-formylpiperidine; or Dichloromethyl methyl ether, $TiCl_4$, DCM; or DMF, $POCl_3$, EtOAc; f) DCM, $NaHSO_4.SiO_2$.

We previously synthesised [2-^{13}C]phloroglucinol **26c** in 6 steps from methyl 4-(chloroformyl)butyrate **194** and [^{13}C]methyl iodide (**Section 2.5**), *via* 3,5-dimethoxy-[4-^{13}C]phenol **188a**, with the potential to introduce various protecting groups following preparation of [2-^{13}C]resorcinol **20f**. Therefore at this stage, all three routes to **200** seemed plausible.

Scheme 104 below further demonstrates how the substrate utilised and the resulting position of formylation will have a significant effect on the number of isotopomers which will be obtained upon introduction of a ^{13}C-atom into the procedure. The implications for the use of the isotopically-symmetrical or unsymmetrical products in the final coupling to yield the anthocyanin products will be discussed in more detail in **Section 2.6.6**.

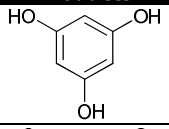
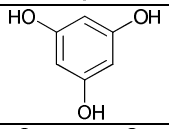
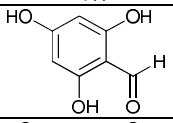
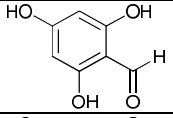
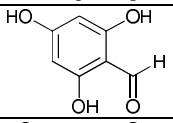
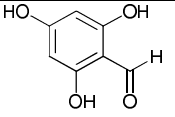
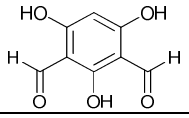
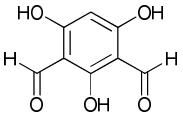
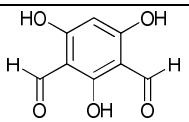


Scheme 104 – Preparation of different isotopomers of formyl-[2-^{13}C]phloroglucinol.

Formylation of phloroglucinol was first examined. Literature methods reported that the mono-formylation of phloroglucinol could be achieved in 70-90% yield through treatment with DMF in the presence of phosphorus oxychloride.[37] The reaction was also attempted in diethyl ether, dichloromethane, and ethyl acetate, with ethyl acetate giving the most promising results. A

range of conditions was examined varying the number of equivalents of phosphorus oxychloride and the reaction time (*Table 19*). One equivalent of DMF was used in all cases. After standard work-up, purification was carried out by column chromatography.

Table 19 – Results for the formylation of phloroglucinol ***26.***

Entry	$POCl_3$ eq.	Time (d)	Products	Isolated yield (%)
1	1.1	1		-
2	2.0	2		-
3	**3.0**	**1**		**42**
4	3.0	2		11
5	4.0	2		5
6	5.0	1	 	15 di, 6 mono
7	5.0	2		12
8	10.0	1		11

Initially the reaction was attempted using quantities of phosphorus oxychloride which had previously been reported to be successful.[37] However, in both cases (entries 1 and 2) only starting material was recovered. Increasing the number of equivalents of $POCl_3$ was beneficial, with 42% of the mono-formylated product **200** being isolated after purification (entry 3). An increase in reaction time from 1 to 2 days was detrimental, yielding only 11% of the desired

product (entry 4). Further increasing the quantity of $POCl_3$ to 4 equivalents reduced the yield further (entry 5). Interestingly, although stoichiometric amounts of phloroglucinol and DMF were used in the reaction, under conditions where a large excess of phosphorus oxychloride was used (entries 6-8) the di-formylated compound **224** was also isolated. *Diagram 40* shows the ^1H NMR spectra for both 2-formylphloroglucinol **200** (bottom spectrum) and 2,4-diformylphloroglucinol **224** (top spectrum). Integrations of the aromatic and formyl protons clearly show the mono-formylated and di-formylated products. The phenolic protons could also be observed in the case of 2-formylphloroglucinol **200**. Under the conditions outlined in entries 7 and 8, no mono-formylated product was isolated. Yields for 2,4-diformylphloroglucinol **224** were also very low (12% and 11%, entries 7 and 8) and phloroglucinol was also recovered during purification. As neither an increase in reaction time nor in the quantity of phosphorus oxychloride appeared to increase conversion and yields further, it was decided to attempt the preparation of **200** *via* Route 2 in order to try and improve yields.

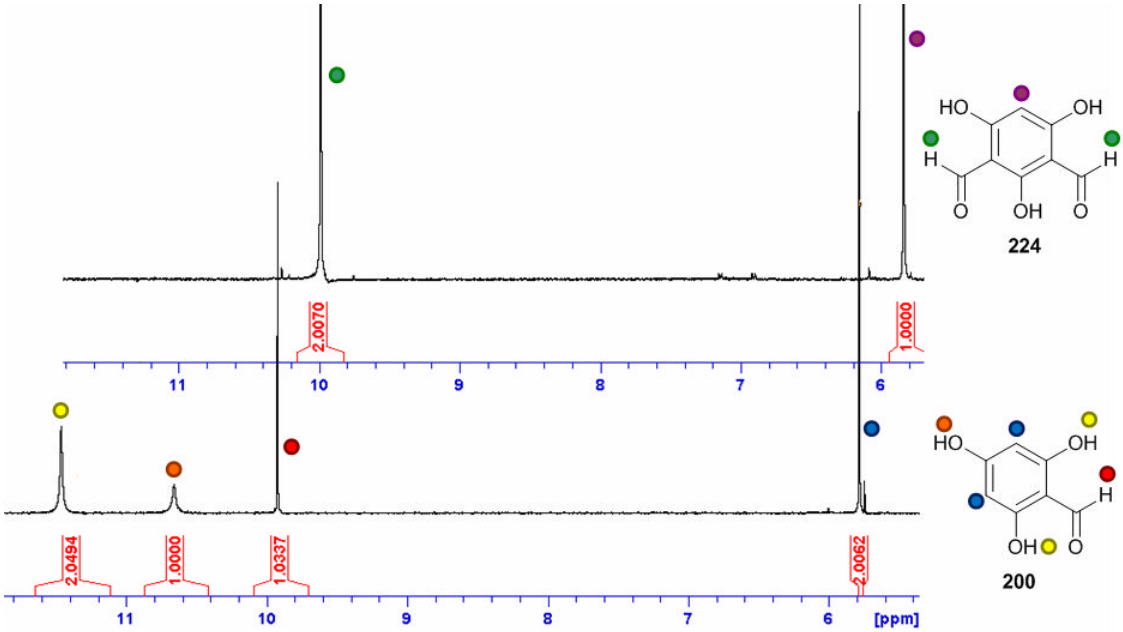
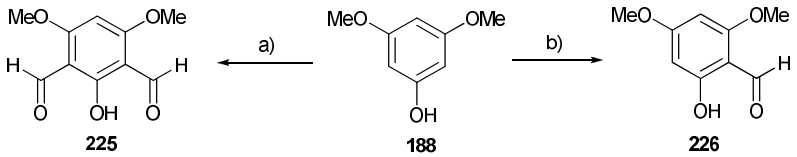


*Diagram 40 – ^1H NMR spectra for **200** and **224** (5.70-11.50 ppm) (400 MHz, DMSO-d_6).*

The formylation was also possible before the final demethylation (Route 2, *Scheme 103* above). Garcia *el al.*[42] employed dichloromethyl methyl ether in the presence of titanium tetrachloride for this reaction. Direct application of the literature procedure resulted in the formation of 2,6-diformyl-3,5-dimethoxyphenol (91%) **225** instead of the desired 2-formyl-3,5-dimethoxyphenol **226** (*Scheme 105*). However, halving the reaction time was successful, giving the desired mono-formylated product **226** in 93% yield after purification by column chromatography.



225 188 226

Scheme 105 –Reagents and conditions: a) Dichloromethyl methyl ether, $TiCl_4$, DCM ($TiCl_4$ added dropwise over 30 min, 1 h stirring after addition of $TiCl_4$, 2 hour stirring after addition of Cl_2CHOCH_3); b) Reagents as in (a) ($TiCl_4$ added dropwise over 15 min, 30 min stirring after addition of $TiCl_4$, 1 hour stirring after addition of Cl_2COCH_3).

At this point it seemed as if this route to 2-formylphloroglucinol **200** would be the most successful, as the formylation was significantly higher yielding than for phloroglucinol itself, and the demethylation of 3,5-dimethoxyphenol **188** to give phloroglucinol **26** had already proven to be successful and high yielding (**Section 2.5**). Demethylation of 2-formyl-3,5-dimethoxyphenol **226** was therefore attempted using the optimum conditions for 3,5-dimethoxyphenol (BBr_3, DCM). Unfortunately only starting material was recovered (*Table 20*, entry 1), and so a range of alternative conditions (*cf. Table 15*, **Section 2.5**) was investigated. Although the majority of these methods had previously failed for 1,3-dimethoxybenzene **188**, it was considered important to attempt them with the formylated substrate, as the optimum conditions for **188** were unsuitable for 2-formyl-3,5-dimethoxyphenol **226**, and therefore the demethylation appears to be affected by the presence of the formyl substituent.

Table 20 – Reaction conditions for demethylation of 2-formyl-3,5-dimethoxyphenol ***226.***

Entry	Solvent	Reagent	Time (d)	Temp. (°C)	Conversion %†
1	DCM	BBr_3	1	-78 to 25	0
2	DCM	BBr_3	5	-78 to 25	16
3	DCM	BBr_3	2	25	18
4	DCM	BCl_3	4	25	0
5	Et_2O	$AlCl_3$	2 h	25	0
6	Pyridine	LiI	6	25	0
7	Pyridine	LiI	2	25	0
8	-	Py.HCl	3 h	200	21
9	-	Py.HCl	8 h	200	3
10	**-**	**Aq. HCl**	**2 h**	**110**	**33**
11	-	Ac_2O, AcOH, HI	3 h	0 to 25	0
12	-	Aq. HCl	1	110	8

† Conversion calculated by integration of ^{1}H NMR signals

Demethylation was repeated using boron tribromide, but with longer reaction times and higher temperatures (entries 2 and 3). There was limited success, with conversions of 16-18%. Other conditions attempted including boron trichloride, aluminium trichloride and lithium iodide (entries 4, 5, 6 and 7) gave no conversion with only starting material being recovered. In line with the results obtained for **188** using pyridinium hydrochloride, partial conversion was achieved (21% after 3 h, entry 8).[43] Disappointingly, allowing the reaction to proceed for a longer period of time resulted in a lower conversion (3%, entry 9), with analysis suggesting the presence of degradation products most likely due to prolonged heating at 200 °C. A procedure employing the use of hydrogen iodide in acetic anhydride and acetic acid was then attempted, as originally reported by Delogu *el al.*[44] and utilised by Vilar *el al.*[45] in the demethylation of coumarins. However, application to our formylated substrate was unsuccessful with a complex mixture of products being observed in the crude mixture. Purification by column chromatography was unsuccessful and removal of iodine from the mixture was troublesome. The most promising results were achieved through the use of aqueous hydrochloric acid at reflux, with a conversion of 33% (entry 10). Disappointingly, increasing the reflux time was detrimental to the reaction. By following the reaction by TLC for one day (entry 12), it was clear that although demethylation was successful in the early stages of the reaction, decomposition of the compound appeared to be taking place gradually as the reaction progressed.

Following these poor results, the phloroglucinol protected with the methoxymethyl (MOM) group (**189**) was examined (Route 3, *Scheme 103*). Dichloromethyl methyl ether in the presence of titanium tetrachloride[42] was found to be efficient and high yielding for the formylation of 3,5-dimethoxyphenol **188**, although there was no literature precedent for its use in the formylation of MOM-protected substrates. Upon addition of $TiCl_4$ to the reaction mixture a dark red precipitate formed. This precipitate was found to be insoluble in all deuterated solvents attempted, and so analysis could not be carried out. As no starting material or product were recovered from the resulting filtrate it is probable that the precipitate was a complex of the substrate coordinated through the oxygen atoms on the MOM-groups to the titanium in the Lewis acid. These conditions were not pursued further.

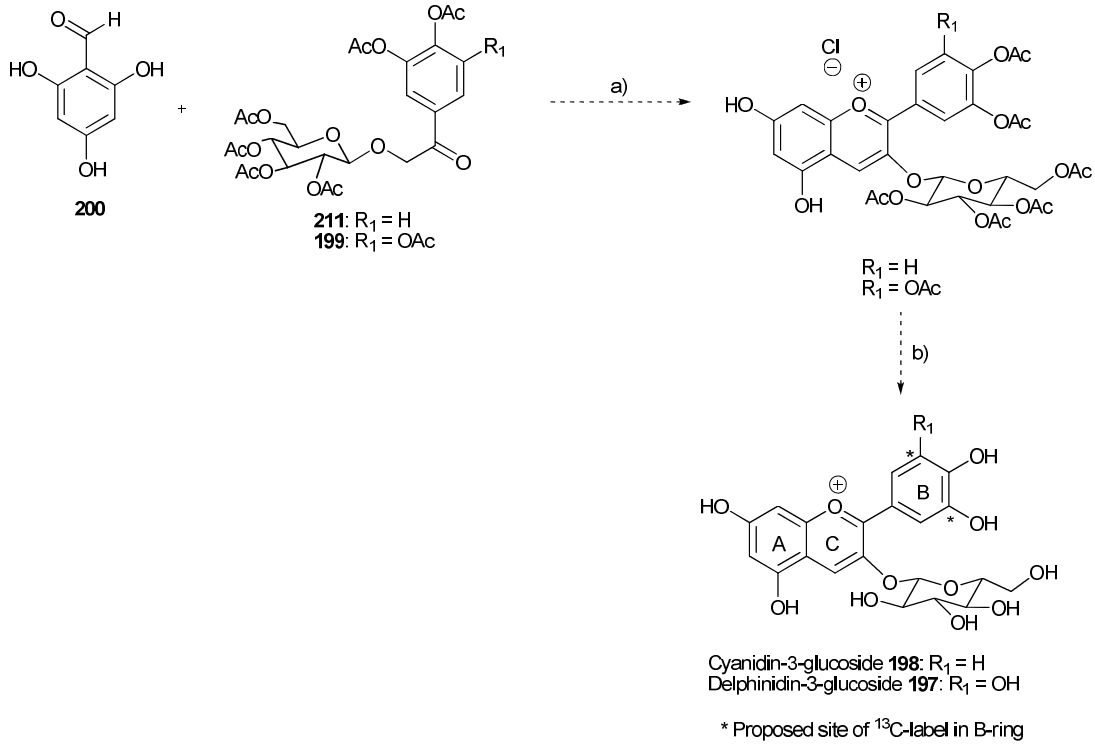
An alternative method for formylation involves ortho-lithiation of the aromatic ring, then reaction with DMF or a suitable equivalent to give **227**.[33,46] This requires protection of the remaining free hydroxyl group. TMS protection was successful giving **228** in an acceptable 54% yield, after purification by column chromatography. As some deprotection was observed during purification, it was expected that the TMS group may also be lost under the formylation conditions. However, this would not be an issue, as it would remove the requirement for a deprotection step later in the synthesis. Formylation was attempted using both DMF and *N*-formylpiperidine as the formylating agent, with *n*-buthyllithium as base. However, ^{1}H NMR analysis showed that the formylation was not successful, and that the TMS group had indeed been removed during the reaction. Subsequent formylations were attempted without the use of a protecting group. 3,5-Bis(methoxymethyl)phloroglucinol **189** was treated with *n*-butyllithium in either tetrahydrofuran or diethyl ether at 0 °C. After stirring for 2 h at room temperature, DMF or *N*-formylpiperidine was added. Reaction times examined ranged from 30 min to 5 days. The use of *N*-formylpiperidine gave only starting material in all cases. The most promising results were obtained using DMF in tetrahydrofuran, with a reaction time of 30 min. This gave a conversion of ~17% (from the integration of ^{1}H NMR signals). Increasing the

number of equivalents of *n*-butyllithium resulted in the formation of more complex reaction profiles. Longer reaction times and the use of an excess of DMF had no effect on the reaction.

It could be concluded from the above results that Routes 2 and 3 were at this point unsuitable for the preparation of 2-formylphloroglucinol **200** due to the poor results obtained in both the demethylation of 2-formyl-3,5-dimethoxyphenol **226** (Route 2) and the formylation of the MOM-protected susbstrate **189** (Route 3). As Route 3 is the only method which allows the synthesis of an isotopically-symmetrical product, it would be desirable in the future to investigate this route further. However, at this point the most successful results were obtained in the direct formylation of phloroglucinol itself (Route 1), giving **224** in 42% yield. However, this method has the drawback of giving a mixture of isotopomers. It would therefore be desirable to examine these routes further in the future, along with the use of alternative protecting groups.

2.6.6 Studies Towards the Construction of the Anthocyanin Targets

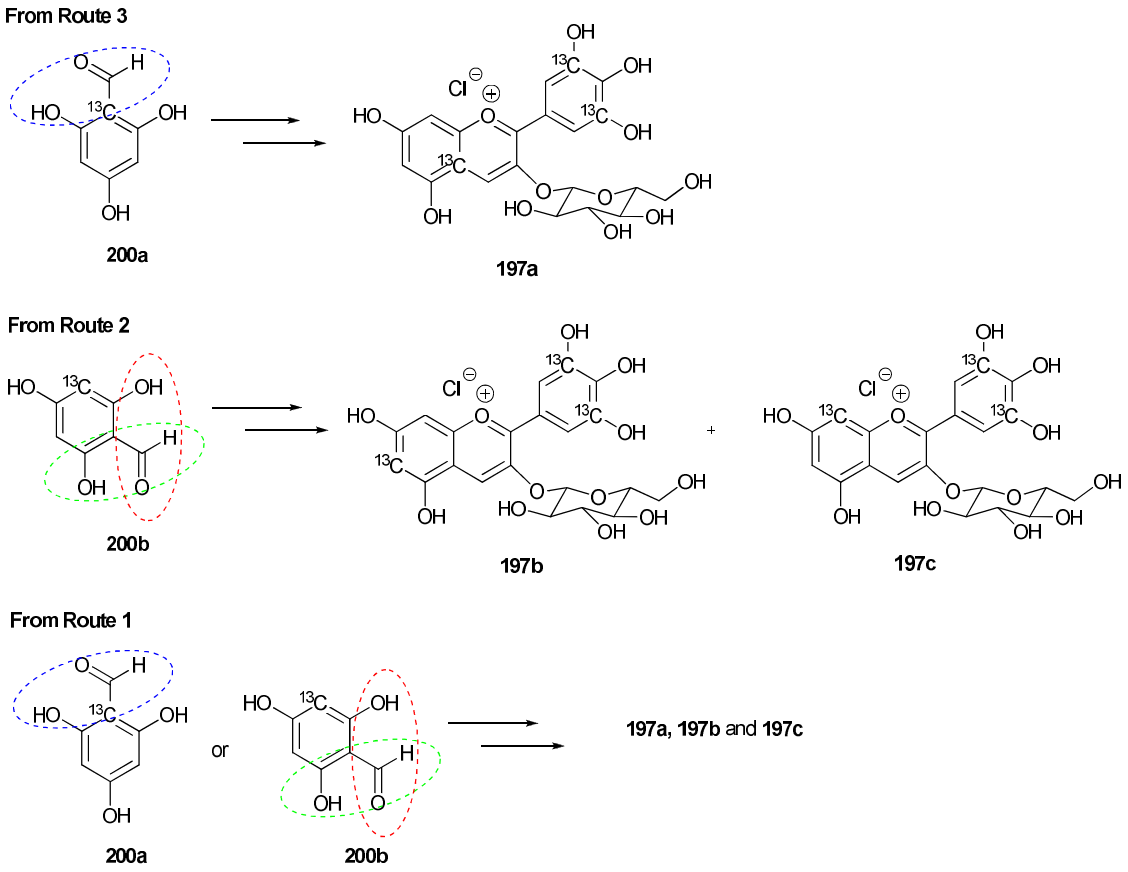
Coupling and cyclisation of glycosides, **199/199a** or **211**, with 2-formylphloroglucinol **200**, followed by subsequent deprotection were now the only stages remaining in the preparation of delphinidin-3-glucoside **197/197a** and cyanidin-3-glucoside **198**. *Scheme 106* outlines these steps and the proposed conditions to afford the anthocyanins in their unlabelled form.



*Scheme 106 – Proposed final stages in the synthesis of anthocyanins **197** and **198** (shown unlabelled); Proposed reagents and conditions:* a) Ethyl acetate, HCl gas, -10 °C, 1 h, then -20 °C, 48 h; b) KOH, water, methanol, rt., 3 h, then 1M HCl, 4 °C, O/N.

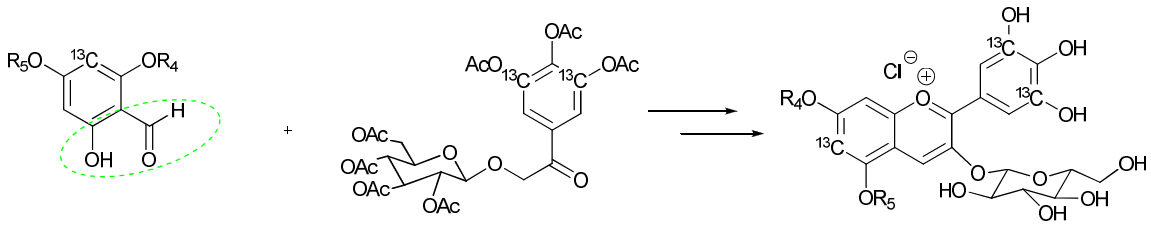
The labelling strategy derived will incorporate ^{13}C-atoms into the B-ring at the positions indicated in *Scheme 106* above. However, labelling in the A-ring may be complicated due to the symmetry of the intermediates, resulting in the mixture of isotopomers. This may not be a significant problem in terms of LC-MS and GC-MS metabolism studies of the anthocyanins as the breakdown products are likely to be symmetrical (e.g. formylphloroglucinol or derivatives thereof), but will complicate characterisation of the final labelled anthocyanin products. *Scheme 104* (**Section 2.6.5**) shows the various possible options for the preparation of 2-formylphloroglucinol **200** from [2-^{13}C]phloroglucinol **26c**, 3,5-dimethoxy-[2-^{13}C]phenol **188a**, or 3,5-bis(methoxymethyl)-[2-^{13}C]phloroglucinol **189a**. *Scheme 107* below outlines the potential coupling sites in substrates **200a**, and **200b** and demonstrates how this would impact upon the position of the ^{13}C-label in the A ring. Route 3 (*via* [2-^{13}C]phlorocglucinol **26c**) is the only method which results in the preparation of an isotopically symmetrical

formylphloroglucinol **200**, resulting in the formation of only one isotopomer of the anthocyanin (**197a**). Routes 1 and 2 would yield two further anthocyanin isotopomers, **197b** and **197c**.



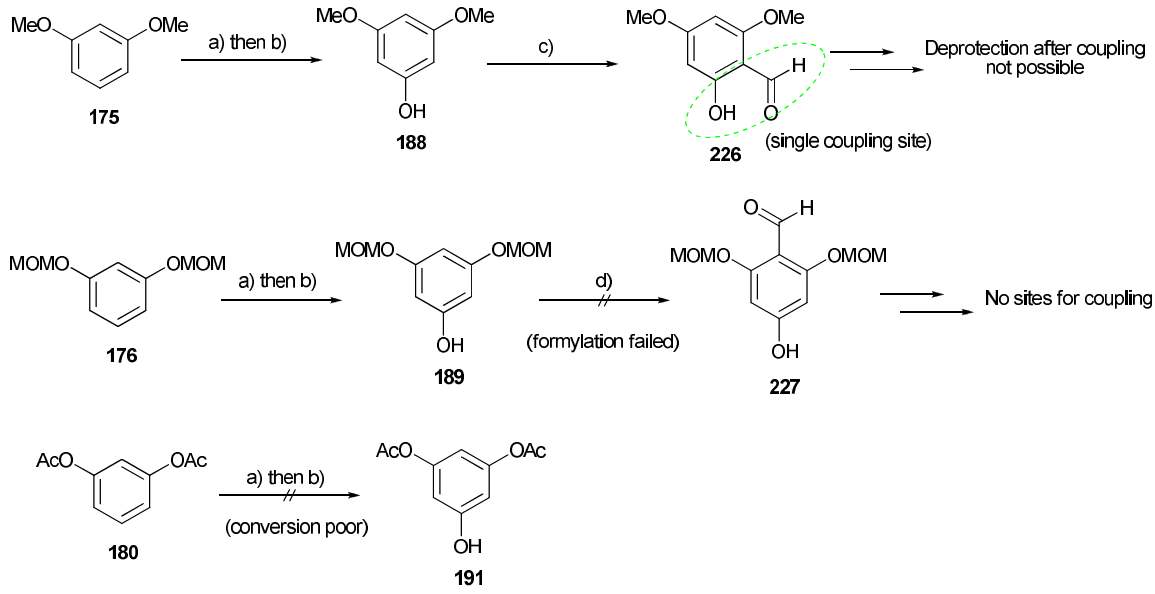
Scheme 107 – Possible isotopomers of anthocyanins via different preparations of formylphloroglucinol.

In order to avoid this potential complication, an alternative route *via* a di-protected formylphloroglucinol with the free hydroxyl group *ortho* to the formyl group would be preferred. This would result in the presence of only one coupling site and therefore the formation of only one isotopomer of the anthocyanin (*Scheme 108*), with the ^{13}C-atom situated between the two protected hydroxyl groups. The protecting groups utilised would therefore need to be easily removed under relatively mild conditions after the final coupling.



Scheme 108 – Use of a di-protected formylphloroglucinol.

However, as previously demonstrated in **Sections 2.4, 2.5** and **2.6.5**, the majority of these routes are not feasible (*Scheme 109* below). In summary, demethylation of 2-formyl-3,5-dimethoxyphenol **226** was not successful, and the conditions required for demethylation are considered too harsh to carry out after construction of the anthocyanin moiety. Therefore the use of 2-formyl-3,5-dimethoxyphenol **226** in the coupling stage is not possible under the conditions examined thus far. Formylation of the MOM-protected phloroglucinol **189** to give **227** was unsuccessful (*Scheme 109*), and deprotection was also problematic, thus rendering this route unfeasible. Also, in terms of the route outlined in *Scheme 109* with the use of the di-protected phloroglucinol **189**, this method would yield a formylated product with no *ortho*-hydroxyl groups, as the procedure employed would introduce the formyl group regioselectively between the two –OMOM substituents. The use of acetyl protecting groups would have been ideal, as they could be uniformly deprotected with the sugar and aromatic acetate groups after preparation of the anthocyanin ring system. However, as the introduction of the third phenolic hydroxyl group on 1,3-diacetoxybenzene **180** *via* iridium catalysis gave poor results, this route was also not an option (**Section 2.4**). *Scheme 109* summarises these issues.

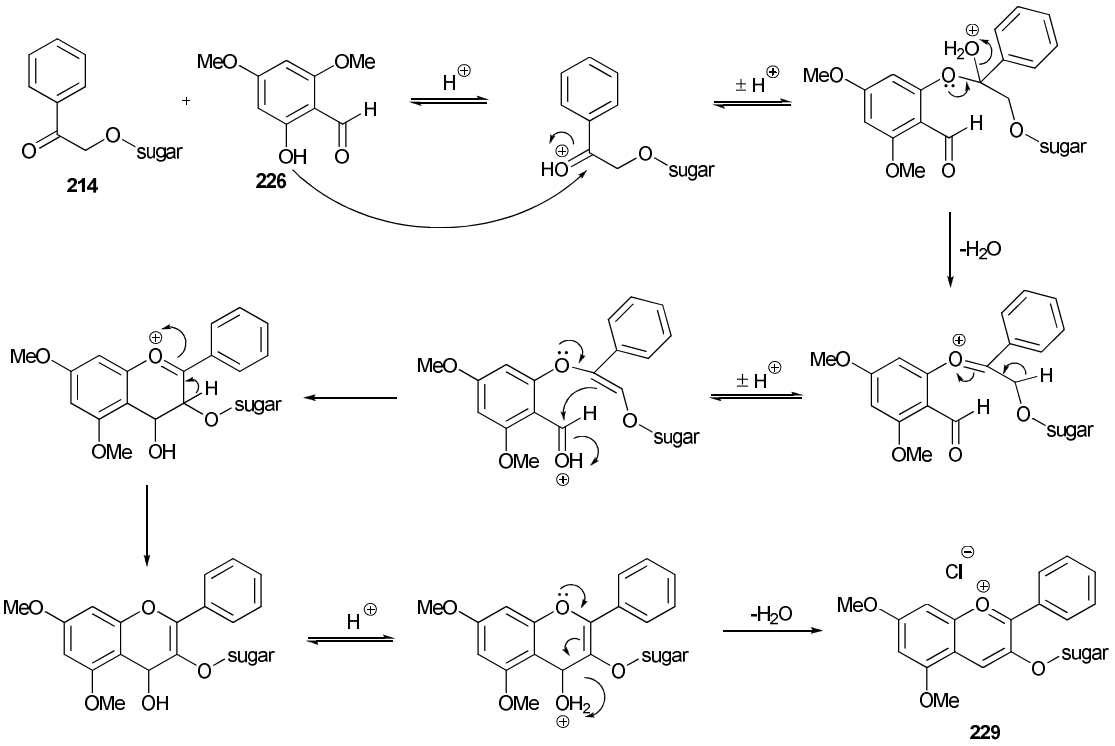


Scheme 109 – Possible routes to di-protected formylphloroglucinol; Reagents and conditions: a) *iso*-Hexane, B_2Pin_2, d*t*bpy, $[Ir(OMe)(COD)]_2$, 110 °C, 18 h; b) Acetone, aqueous Oxone®, rt., 30 min; c) Dichloromethyl methyl ether, $TiCl_4$, DCM; d) *n*-BuLi, DMF, EtOAc.

Therefore at this point, the only possible route to 2-formylphloroglucinol **200** is *via* the synthesis of [2-^{13}C]phloroglucinol **26c** (**Section 2.5**) followed by subsequent formylation to yield two isotopomers of formyl-[2-^{13}C]phloroglucinol **200a** and **200b**. As previously outlined (**Section 2.6.5** above), this was carried out using only unlabelled substrates as it would be desirable to obtain a higher yield during the formylation before application to labelled substrates. It would also be advantageous in the future to optimise Route 3 (*Scheme 103*) using the MOM-protecting groups to allow preparation of the isotopically symmetrical formylphloroglucinol.

Before cyclisation of glycosides **199** and **211** with 2-formylphloroglucinol **200** could be attempted using ^{13}C-labelled substrates, optimisation was attempted on unlabelled material. Due to the number of steps required in order to reach the precursors to delphinidin-3-glucoside (glycoside **199**) and cyanidin-3-glucoside (glycoside **211**), we chose to attempt the reaction using glycoside **214** (previously synthesised from the commercially available alcohol **213**) due

to its similarities to our substrates. 2-Formyl-3,5-dimethoxyphenol **226** was chosen as the other model substrate, as it had been prepared in good yield and showed good solubility in the reaction solvent. The coupling and cyclisation of the glycosides and formylphloroglucinol (either protected or unprotected) require the use of anhydrous acidic conditions, i.e. hydrogen chloride gas, and either ethyl acetate or an ethyl acetate/methanol/ethanol solvent mixture.[40,47,48] A range of reaction temperatures and times were reported varying from -20 °C to room temperature over 2 days.[40,47,48] *Scheme 110* outlines the proposed mechanism for the coupling and cyclisation to yield the model anthocyanin **229**, which can subsequently be diacetylated to give **230**.



Scheme 110 –Reagents and conditions: HCl, EtOAc/MeOH/EtOH, -20 °C-rt.

The reaction was initially attempted using ethyl acetate and dry hydrogen chloride gas as reported by Dangles *et al*.[40] The gas was introduced to a solution of **214** and **226** at -10 °C by the action of 98% sulfuric acid on solid sodium chloride, after first being passed through drying agents in order to maintain anhydrous conditions. Alternatively, concentrated

hydrochloric acid and calcium chloride was used to give dry hydrogen chloride gas. A deep red colour formed upon bubbling of the gas through the reaction mixture. After 30-60 min, the solution was allowed to stand at -20 °C for 48 h. Removal of the solvents at reduced pressure yielded a red powder to give what was anticipated to be **229**. This solid was subsequently treated with a basic methanol/water solution (aqueous potassium hydroxide in methanol) followed by acidification (1M HCl) in order to afford what was hoped to be the deacetylated product **230** as a brown solid. Analysis by ^{1}H NMR spectroscopy was inconclusive, with the sample showing poor solubility in the majority of deuterated solvents. Purification was therefore attempted, firstly using reverse phase column chromatography to remove traces of potassium chloride from the mixture. Secondly, column chromatography using cellulose microcrystalline powder was carried out and gave both a purple solid and a yellow solid. ^{1}H NMR spectra for both isolated solids were weak due to low recovery after purification and it could therefore not be concluded whether the coupling and cyclisation had been successful. However, the absence of acetate signals showed that deprotection of the acetylated species had gone to completion.

The reaction was repeated using identical conditions, with analysis by NMR spectroscopy following the initial coupling and cyclisation step. The red powder was found to be soluble in a mixture of 95% methanol and 5% trifluoroacetic acid. Disappointingly, the ^{1}H NMR spectrum showed only signals corresponding to both starting materials **214** and **226**, with the formyl proton of **226** observed at ~10.00 ppm and the CO(*C*H$_2$)OH of **214** at ~4.70-4.90 ppm, thus confirming the failure of the coupling and subsequent cyclisation. No trace of the desired product **229** was found. Analysis at this stage was important, as from this observation we could conclude that the de-acetylation using a basic methanol/water solution was not causing breakdown of the anthocyanin ring system.

The coupling and cyclisation was then attempted using a range of conditions (see *Table 22*,

Section 4, Experimental). Solvent mixtures of ethyl acetate/methanol and ethyl acetate/ethanol were unsuccessful. The use of dry HCl in diethyl ether was attempted as an alternative source of acid, with a large excess being added directly to the reaction solution. Temperatures ranged from -20 °C to room temperature, with all reactions being allowed to stand for 48 h before removal of the solvent at reduced pressure. The reaction was also attempted using the unlabelled glycoside **211**. In all cases only starting material was recovered. Further optimisation and determination of conditions is therefore required before the final stages can be applied to the coupling and cyclisation of both labelled and unlabelled substrates **199a**, **211** and **200/200abc**.

2.6.7 Summary of Studies Towards the Synthesis of ^{13}C-Labelled Anthocyanins

A route leading towards the synthesis of ^{13}C-labelled anthocyanins has been proposed, with only the final coupling/cyclisation step remaining to be carried out. During optimisation using unlabelled substrates, the stages requiring purification were determined, and purification kept to a minimum in order to maximise yields and reduce losses of ^{13}C-labelled material. For the synthesis of unlabelled cyanidin-3-glucoside **198**, α-hydroxyacetophenone **202** was synthesised in 36.8% yield in 8 steps from acetone and triethyl orthoformate, with the early synthetic stages being based upon routes previously developed in our group.[3,4] α-Hydroxyacetophenone **202** was then coupled successfully with 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide **93** through treatment with mercuric cyanide, followed by debenzylation and acetyl-protection to give the glycoside **211** in 2.4% yield over 3 steps and 0.74% from acetone and triethyl orthoformate.

Application of this route to the use of ^{13}C-labelled substrates was then carried out for the synthesis of delphinidin-3-glucoside **197**. Over 8 steps, α-hydroxy-3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone **201a** was prepared in 11.5% yield from [1,3-$^{13}C_2$]acetone **40b** and triethyl

orthoformate **41**, with di-bromination (*cf.* mono-bromination for **202**) introducing the required extra substituent for the preparation of delphinidin-3-glucoside. Subsequent coupling with acetobromoglucose **93** was successful and gave the ^{13}C-labelled glycoside **199a** in 10% yield (2% overall from [1,3-$^{13}C_2$]acetone **40b**) after debenzylation and acetylation.

Preparation of unlabelled 2-formylphloroglucinol **200** was successful (10% over 6 steps), and will subsequently be applied to the previously synthesised [2-^{13}C]phloroglucinol **26c** after further optimisation of the final formylation step. Further investigations into the use of alternative protecting groups may be carried out in the future in order to obtain an isotopically symmetrical product.

The coupling and cyclisation of glyclosides **199a** and **211** with 2-formylphloroglucinol **200** have yet to be achieved, so optimisation of conditions is required before these can be applied to ^{13}C-labelled substrates and the synthesis of anthocyanins **197**/**197a** and **198** can be completed.

2.7 References (Results and Discussion)

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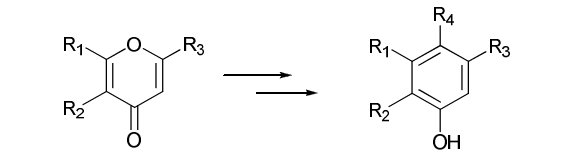
3. CONCLUSIONS AND FURTHER WORK

Our first aim was to explore the base-catalysed reaction of 4*H*-pyran-4-one with a range of common nucleophiles as a method for the preparation of phenol derivatives, and then exploit this chemistry for the synthesis of isotopically labelled of phenols (*Scheme 111*). After initially examining the reaction between 4*H*-pyran-4-one and diethyl malonate for the preparation of ethyl 4-hydroxybenzoate[1,2] we successfully expanded the scope of this reaction to generate a range of *para*-substituted phenols using both substituted and unsubstituted pyran-4-ones with a range of carbon nucleophiles, namely diethyl malonate, ethyl acetoacetate, nitromethane, acetylacetone and ethyl cyanoacetate.[3] Under conventional heating, the most successful results were obtained with 4*H*-pyran-4-one itself, with yields of the desired *para*-substituted phenols ranging from 65-94%. Further derivatisation of a selection of products was also carried out. The optimised conditions were then applied to the use of the 2,3-disubstituted pyranone *O*-benzyl maltol in order to generate a range of higher-substituted phenols *via* the same methodology. Success was achieved by increasing the number of equivalents of base to account for deprotonation of the methyl group. Reactions using diethyl malonate and ethyl cyanoacetate gave the best results for this substrate (73% and 93% yields respectively). Limited success was achieved with 2,6-disubstituted pyranone substrates. In the case of 2,6-dimethyl-4*H*-pyran-4-one only reactions with diethyl malonate and ethyl cyanoacetate were successful (18% and 57%). On the other hand, reactions using nitromethane and acetylacetone were successful for diethyl chelidonate (46% and 2%), with complex mixtures of products being observed for other nucleophiles.[3] In order to confirm if deprotonation of the methyl groups by potassium *tert*-butoxide is a competing reaction, a control reaction could be carried out in deuterated *tert*-butanol (*t*BuOD), without addition of the nucleophile, which would result in incorporation of deuterium into the methyl group.

The application of microwave irradiation to the above methodology greatly accelerated the reactions with most reaching completion within 30 min (*cf.* 24-48 h for conventional heating methods). In the majority of cases, it was found that the optimum quantities of nucleophile and base required in the microwave-assisted reactions were lower than those required under conventional heating. In several cases microwave heating at 150 °C enhanced yields in reactions which gave poor yields by conventional heating. Although little differences were observed between the yields obtained using microwave and conventional heating for 4*H*-pyran-4-one, reaction times were shortened. Substantial improvements in yield were observed with the substituted pyran-4-ones where microwave heating was employed, with the formation of fewer side products. Overall, we observed that the use of microwave irradiation had beneficial effects on the base-catalysed reaction of pyranones with nucleophiles, with shorter reaction times accompanying cleaner reaction profiles and higher yields.



35: $R_1 = R_2 = R_3 = H$ $R_4 = CO_2Et, COCH_3, NO_2, CN$

145: $R_1 = CH_3$, $R_2 = OBn$, $R_3 = H$

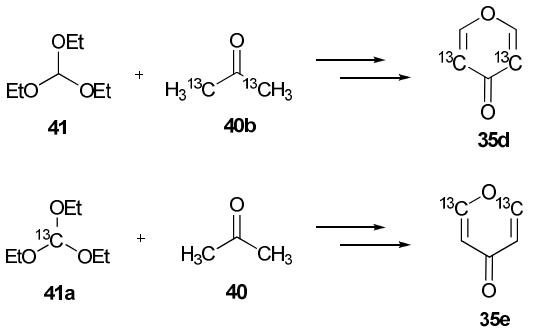
43: $R_1 = R_3 = CH_3$, $R_2 = H$

156: $R_1 = R_3 = CO_2Et$, $R_2 = H$

Scheme 111 – Synthesis of substituted phenols from pyran-4-one precursors.

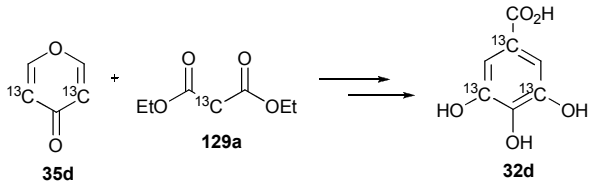
The developed methodology had significant potential for the regioselective placement of ^{13}C-atoms into benzene rings. This could either involve the use of ^{13}C-labelled nucleophiles, such as diethyl [2-^{13}C]malonate, or ^{13}C-labelled 4*H*-pyran-4-one. Therefore our next objective was the synthesis of ^{13}C-labelled 4*H*-pyran-4-one. After optimisation and modification of an existing literature procedure,[4] a high-yielding route to 4*H*-pyran-4-one from triethyl orthoformate and acetone was developed (*Scheme 112*). Through the use of either [1,3-$^{13}C_2$]acetone or triethyl [^{13}C]orthoformate we successfully prepared two ^{13}C-labelled isotopomers of the pyranone,

namely [3,5-$^{13}C_2$]4*H*-pyran-4-one and [2,6-$^{13}C_2$]4*H*-pyran-4-one.[5] Using this route with various combinations of ^{13}C-labelled acetone and triethyl orthoformate, the synthesis of all seven possible ^{13}C-labelled isotopomers of 4*H*-pyran-4-one could be achieved.



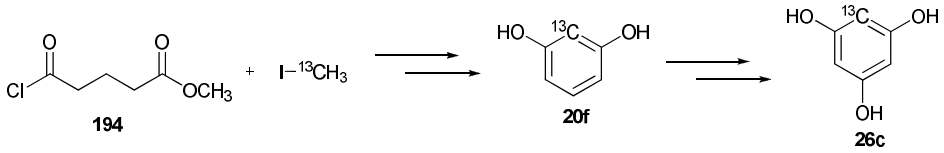
Scheme 112 – Synthesis of ^{13}C-labelled 4H-pyran-4-one.

Through the application of the above methodologies, a fast, efficient and high yielding route to the synthesis of [1,3,5-$^{13}C_3$]gallic acid was developed, giving the desired product in 35% yield over 5 steps (*Scheme 113*). Our route allowed the regioselective placement of three ^{13}C-atoms within the aromatic ring *via* the reaction of [3,5-$^{13}C_2$]4*H*-pyran-4-one with diethyl [^{13}C]malonate to yield ethyl 4-hydroxy-[1,3,5-$^{13}C_3$]benzoate (74%). Bromination followed to give ethyl 3,5-dibromo-4-hydroxy-[1,3,5-$^{13}C_3$]benzoate (99%). The final two transformations involving ester hydrolysis and conversion of bromine to hydroxyl groups were successfully telescoped into one simple step. Purification was only required after the final step, thus minimising losses of ^{13}C-labelled material. Our route also has the potential to be applied to the preparation of 3,4-dihydroxy-[1,3,5-$^{13}C_3$]benzoic acid if desired.[5]



Scheme 113 – Synthesis of [1,3,5-$^{13}C_3$]gallic acid.

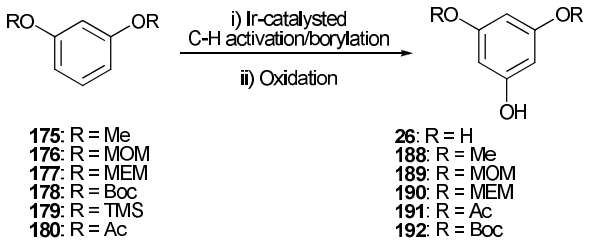
Our second target polyphenol for regioselective ^{13}C-labelling was phloroglucinol. This was achieved in six steps from non-aromatic precursors, with the source of the ^{13}C-atom being [^{13}C]methyl iodide (*Scheme 114*). Reaction of the corresponding ^{13}C-labelled lithium dimethylcuprate with methyl 4-chloroformyl butyrate followed by subsequent cyclisation and aromatisation gave [2-^{13}C]resorcinol in yields comparable to the literature.[6] Following methylation of the hydroxyl groups, a modified iridium-catalysed C-H activation/borylation/oxidation procedure[7,8] was employed to introduce the third hydroxyl group at the 5-position. Demethylation using boron tribromide then yielded the desired [2-^{13}C]phloroglucinol in 9% yield over 6 steps.



Scheme 114 – Synthesis of [2-^{13}C]phloroglucinol.

The C-H activation/borylation/oxidation procedure which was employed above, had previously only been used with halide-substituted arenes.[7,8] After our initial studies on the reaction of 1,3-dimethoxybenzene, we successfully extended the methodology to include other diprotected resorcinol substrates. A catalyst system was developed which allowed high conversion to the intermediate arylboronic acids, followed by oxidation using aqueous Oxone® to yield the corresponding phenols in yields ranging from 51-87% (*Scheme 115*). The optimal conditions found for the methyl-, MOM- and MEM-protected resorcinols were as follows: 1 mmol B_2Pin_2, 1 mol% $[Ir(COD)(OMe)]_2$, 2 mol% d*t*bpy, 110 °C, 18 h. Similar conditions were successful for the Boc-protected substrate. A noteworthy result was obtained upon application of these conditions to the TMS-protected substrate, with deprotection during the oxidation step yielding phloroglucinol as the only product. If the synthesis of [2-^{13}C]phloroglucinol is repeated in the future, it would be desirable to use the TMS-protected resorcinol for this step. Limited success was achieved using the acetyl-protected substrate, with 45% conversion (44% isolated yield)

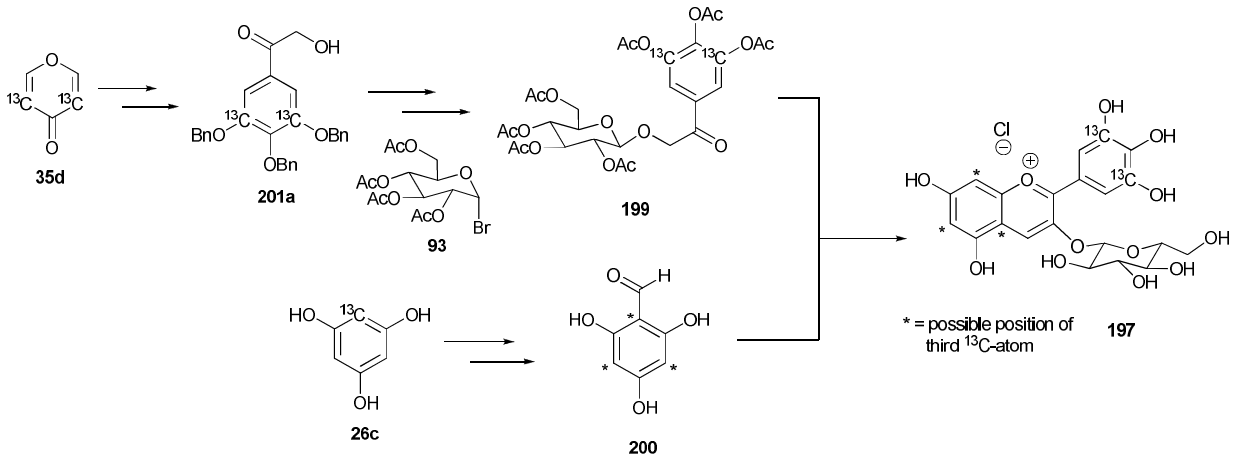
being achieved using 1 mmol B_2Pin_2, 1 mmol HBPin, 2 mol% $[Ir(COD)(OMe)]_2$, 2 mol% dppe, 110 °C, 18 h. These successful results significantly expand the scope of the C-H activation/borylation/oxidation procedure to include the use of electron-rich protected resorcinol derivatives. It may be interesting in the future to examine the use of other protecting groups for this reaction, although it should be noted that aromatic and unsaturated groups are not compatible with the methodology.



Scheme 115 – Synthesis of 1,3,5-trisubstituted arenes from protected resorcinol.

The final application of these newly developed methodologies was the preparation of ^{13}C-labelled anthocyanins (*Scheme 116*). The syntheses were not completed, however only the final coupling/cyclisation step remains to be carried out. Purification was kept to a minimum during the synthesis in order to maximise yields and reduce losses of ^{13}C-labelled material. For the preparation of unlabelled cyanidin-3-glucoside, α-hydroxy-3,4-bis(benzyloxy)acetophenone was prepared in 37% yield starting from acetone and triethyl orthoformate. Coupling of the resulting alcohol to acetobromoglucose was successful and the desired glycoside synthesised in 2.4% yield over 3 steps following debenzylation and acetyl-protection. For the preparation of ^{13}C-labelled delphinidin-3-glucoside, a similar route was utilised, with α-hydroxy-3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone being synthesised in 11.5% yield from [1,3-$^{13}C_2$]acetone and triethyl orthoformate. The corresponding ^{13}C-labelled glycoside was isolated in 10% yield. Preparation of unlabelled 2-formylphloroglucinol, the other aromatic component required for the final coupling and cyclisation, was successful (10% over 6 steps), and will subsequently be repeated with ^{13}C-labelled material. Further investigations into the use of alternative protecting groups may be carried out in the future in order to obtain an isotopically symmetrical product.

The coupling and cyclisation of the glycosides with 2-formylphloroglucinol has yet to be achieved, although studies are underway towards this final step in order to complete the synthesis of the desired anthocyanin targets.



Scheme 116 – Synthesis of ^{13}C-labelled delphinidin-3-glucoside.

In conclusion, we have successfully developed methodologies for the preparation of phenols from non-cyclic and non-aromatic ^{13}C-labelled precursors which allow the regioselective placement of ^{13}C-atoms into the aromatic ring. This has been demonstrated by the preparation of both [1,3,5-$^{13}C_3$]gallic acid[5] (*via* [3,5-$^{13}C_2$]4*H*-pyran-4-one) and [2-^{13}C]phloroglucinol. We have also applied the methodology to studies towards the synthesis of ^{13}C-labelled anthocyanins with only two steps remaining to be carried out to complete preparation of ^{13}C-labelled delphinidin-3-glucoside. These new methods also have potential for incorporation into the synthesis of other natural products and polyphenols, such as the flavonoids, for use as stable-labelled internal standards in LC-MS and GC-MS analysis and as tools for studies on pharmacokinetics and metabolism.

3.1 References (Conclusions and Further Work)

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[3] Marshall, L. J.; Cable, K. M.; Botting, N. P. *Tetrahedron*, **2009**, *65*, 8165-8170.

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4. EXPERIMENTAL

4.1 Instrumentation and General Techniques

NMR spectra were recorded on either a Bruker Avance 300 (^{1}H 300 MHz, ^{13}C 75.45 MHz) or a Bruker Avance II 400 (^{1}H 400 MHz, ^{13}C 100 MHz) spectrometer in the deuterated solvent stated. ^{13}C NMR spectra were recorded using the PENDANT or DEPTQ sequence and internal deuterium lock. Chemical shifts (δ) in ppm are given relative to tetramethylsilane, and are reported consecutively as positions (δ_H or δ_C), relative integral, multiplicity (s-singlet, d-doublet, t-triplet, q-quartet, qt-quintet, m-multiplet, dd-double of doublets, dt-doublet of triplets, and b-broad), coupling constants (*J*) given in Hz – recorded to the nearest 0.1 Hz – and assignment.

Low resolution (LR) and high resolution (HR) electrospray mass spectral analyses were acquired by chemical ionisation (CI) or electrospray (ES) within the School of Chemistry, University of St Andrews. ES-MS were recorded on a Micromass LC-T spectrometer and CI MS were recorded on a Micromass GC-T spectrometer (time-of-flight). Major fragments are given as percentages of the base peak intensity.

Melting points were recorded in open capillaries using an Electrothermal 9100 melting point apparatus and are uncorrected.

Elemental microanalyses were performed on a Carlo Erba CHNS analyser within the School of Chemistry and the University of St Andrews.

IR spectra were recorded on a Perkin-Elmer series 1420 FT IR spectrophotometer. The samples were prepared as Nujol mulls, thin films between sodium chloride discs, or thin films on PTFE IR cards. Absorption maxima are given in wavenumbers (cm^{-1}).

Analytical TLC was carried out on Merck 5785 Kieselgel $60F_{254}$ fluorescent plates (Aldrich). The components were visualised by ultraviolet fluorescence (254 nm) or using an aqueous potassium permanganate dip. Flash chromatography was performed according to the procedure of Still[1] using silica gel of 35-70 μ particle size or BakerBond C_{18} reverse phase silica gel. Additional purification was by Mass Directed Auto Prep (MDAP) when indicated. This system comprised:

- Hardware: Waters 600 gradient pump, Waters 2767 inject/collector, Waters reagent manager, MicroMass ZQ mass spectrometer, Gilson Aspec waste collector, Gilson 115 post-fraction UV detector;
- Software: MicroMass MassLynx v4.0;
- Column: Supelco LCABZ++, 20 mm internal diameter, 100 mm length, stationary phase particle size 5 μm;
- Solvents: Aqueous solvent = water + 0.1% formic acid; Organic solvent = MeCN : water 95:5 + 0.05% formic acid; Make up solvent = MeOH : water 80:20 + 50 mM ammonium acetate; Needle rinse solvent = MeOH : water : DMSO 80:10:10;
- Flow rate: 20 mL/min;
- Methods: 15 min runtime comprising of 10 min gradient followed by 5 min column flush and re-equilibration step, Method B = 5-30% B, Method C = 15-55% B.

tert-Butanol was dried by stirring with 3Å molecular sieves overnight, followed by filtration. Dichloromethane (DCM), diethyl ether (Et_2O), toluene, tetrahydrofuran (THF) and hexane were obtained dry using the MBRAUN solvent purification system MB SPS-800.

All chemicals and reagents were used as delivered from Sigma-Aldrich, Acros or Alfa-Aesar unless otherwise indicated. All reactions involving moisture sensitive reagents were performed in oven-dried glassware under a positive pressure of argon.

Microwave-assisted reactions were carried out on a Biotage Initiator-60, using a single mode resonator, with dynamic field tuning at a maximum power of 300 W at 2.45 GHz.

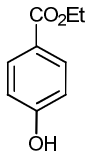
4.2 Reactions using 4*H*-Pyran-4-ones

4.2.1 General Procedure

A solution of the pyranone and pronucleophile in dry *tert*-butanol (20 mL) was heated to reflux under argon. Potassium *tert*-butoxide (1M solution in *tert*-butanol) in a further volume of dry *tert*-butanol (30 mL) was added dropwise. The resulting mixture was heated at reflux for the desired time after which 1M hydrochloric acid was added. The reaction solution was then heated at reflux for a further 1 h. The solvents were removed at reduced pressure, and water (40 mL) added to the residue. The aqueous phase was extracted with diethyl ether or ethyl acetate (3 x 30 mL). The combined organic layers were washed with water (2 x 30 mL) and brine (30 mL), dried ($MgSO_4$) and the solvent removed at reduced pressure to give the product. Purification by MDAP was carried out in some cases in order to confirm characterisation.

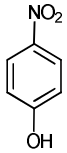
4.2.2 Reactions with 4*H*-Pyran-4-one (35)

Ethyl 4-hydroxybenzoate (128)



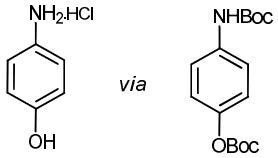
Using the general procedure with 4*H*-pyran-4-one **35** (1 g, 10.4 mmol) and diethyl malonate (1 g, 6.2 mmol) or ethyl acetoacetate (1.17 g, 9 mmol), potassium *tert*-butoxide (8.2 mL, 8.2 mmol) and a 3 h reflux time. Extractions were with diethyl ether. Product **128** was recovered as an orange solid (0.96 g, 94% from diethyl malonate, or 0.64 g, 65% from ethyl acetoacetate). mp 111-111.5 °C (lit[2] 112-115 °C); δ_H (400 MHz, $CDCl_3$) 1.39 (3H , t, *J* 7.0 Hz, CH_3), 4.36 (2H, q, *J* 7.0 Hz, CH_2), 6.90 (2H, d, *J* 8.0 Hz, CH-3,5) and 7.97 (2H, d, *J* 8.0 Hz, CH-2,6); δ_C (100 MHz, $CDCl_3$) 14.3 (CH_3), 61.0 (CH_2), 115.2 (CH-3,5), 122.5 (C-1), 131.9 (CH-2,6), 160.3 (C-4) and 167.1 (C=O). Data is in agreement with the literature.[2,3]

4-Nitrophenol (6)



Using the general procedure with 4*H*-pyran-4-one **35** (1 g, 10.4 mmol), nitromethane (0.7 g, 11.5 mmol), potassium *tert*-butoxide (11.5 mL, 11.5 mmol) and a 5 h reflux time. Extractions were with diethyl ether. Product **6** was recovered as an off-white solid (1.16 g, 80%). mp 109-110 °C (lit[4] 110-115 °C); δ_H (400 MHz, DMSO-d_6) 6.90 (2H, d, *J* 9.2 Hz, CH-2,6), 8.10 (2H, d, *J* 9.2 Hz, CH-3,5) and 8.26 (1H, s, OH); δ_C (100 MHz, DMSO-d_6) 115.9 (CH-2,6), 131.1 (CH-3,5), 139.1 (C-4) and 164.7 (C-1). Data is in agreement with the literature.[3,4]

4-Aminophenol HCl salt (7)

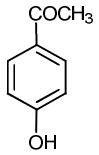


To a stirring solution of 4-nitrophenol **6** (0.546 g, 3.9 mmol) in dry methanol (30 mL) at 0 °C were added Boc_2O (1.7 g, 7.8 mmol) and $NiCl_2.6H_2O$ (92 mg, 0.39 mmol). $NaBH_4$ (1.032 g, 27.3 mmol) was then added in small portions over 60 min (the reaction was exothermic and effervescent). The resulting mixture, containing a finely divided black precipitate, was allowed to warm to room temperature and left to stir overnight. After this time, diethylenetriamine (0.53 mL, 5.56 mmol) was added, and the resulting mixture allowed to stir for 90 min. The solvents were then removed at reduced pressure. The resulting residue was dissolved in ethyl acetate (60 mL) and washed with saturated sodium bicarbonate solution (2 x 60 mL). The combined organic layers were dried ($MgSO_4$) and solvents removed at reduced pressure to give the Boc-protected amine **134** as an orange oil (1.64 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) 1.44 (9H, s, CH_3), 1.47 (9H, s, CH_3), 6.38 (1H, s, NH), 6.68 (2H, d, *J* 8.3 Hz, CH-2,6), and 7.09 (2H,

d, *J* 8.3 Hz, CH-3,5); ^{13}C NMR (75.45 MHz, $CDCl_3$) 27.7 (CH_3), 28.3 (CH_3), 83.5 ($C(CH_3)_3$), 115.7 (CH-2,6), 121.7 (CH-3,5), 136.5 (C-4), 147.8 (C-1), and 154.5 (C=O).

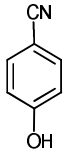
The crude protected amine **134** (1.64 g, 5.3 mmol) was dissolved in dry dichloromethane (12 mL). Dry hydrochloric acid in 1,4-dioxane (26.5 mmol, 6.6 mL of 4M solution) was added slowly. The resulting reaction mixture was allowed to stir overnight at room temperature. The product precipitated to give a latte-like appearance. The solid was filtered off and washed with diethyl ether to yield the HCl salt of **7** as an off-white solid (0.37 g, 48%). mp 180-181 °C (lit[5] 185-189 °C); ^{1}H NMR (300 MHz, DMSO-d_6) 6.85 (2H, d, *J* 8.8 Hz, CH-3,5), 7.19 (2H, d, *J* 8.8 Hz, CH-2,6), 9.87 (1H, s, OH) and 10.14 (3H, s, NH_3); ^{13}C NMR (75.45 MHz, DMSO-d_6) 116.5 (CH-3,5), 122.8 (C-4), 124.8 (CH-2,6) and 157.6 (C-1). Data is in agreement with the literature.[3,5]

4-Hydroxyacetophenone (87)



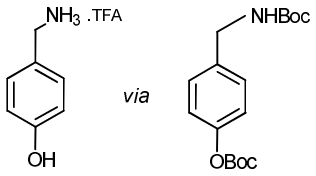
Using the general procedure with 4*H*-pyran-4-one **35** (0.95 g, 9.88 mmol), acetylacetone (2.69 g, 19.76 mmol), potassium *tert*-butoxide (19.8 mL, 19.8 mmol) and a 20 h reflux time. Extractions were with diethyl ether. Product **87** was recovered as a yellow solid (1.29 g, 95%). mp 105-105.5 °C (lit[6] 106-108 °C); δ_H (400 MHz, $CDCl_3$) 2.60 (3H, s, CH_3), 6.97 (2H, d, *J* 9.0 Hz, CH-3,5), 7.92 (2H, d, *J* 9.0 Hz, CH-2,6) and 8.17 (1H, s, OH); δ_C (100 MHz, $CDCl_3$) 26.3 (CH_3), 115.6 (CH-3,5), 129.4 (C-1), 131.2 (CH-2,6), 161.5 (C-4), and 198.8 (C=O). Data is in agreement with the literature.[6,34]

4-Hydroxybenzonitrile (130)



Using the general procedure with 4*H*-pyran-4-one **35** (0.96 g, 10.0 mmol), ethyl cyanoacetate (2.13 g, 16.0 mmol), potassium *tert*-butoxide (30 mL, 30.0 mmol), and a 27 h reflux time. Extractions were with ethyl acetate. Product **130** was recovered as a red solid (0.926 g, 78%). mp 109-109.5 °C (lit[6] 109-110 °C); δ_H (400 MHz, DMSO-d_6) 6.90 (2H, d, *J* 8.7 Hz, CH-3,5), 7.64 (2H, d, *J* 8.7 Hz, CH-2,6) and 10.62 (1H, s, OH); δ_C (100 MHz, DMSO-d_6) 101.0 (C-1), 116.4 (CH-3,5), 119.5 (CN), 134.2 (CH-2,6) and 161.6 (C-4); *m/z* (CI^+) 120 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 120.0449, C_7H_6NO requires 120.0450]. Data is in agreement with the literature.[3,6]

4-Hydroxybenzylamine TFA salt (141)

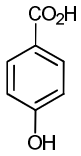


To a stirring solution of crude 4-hydroxybenzonitrile **130** (1.36 g, 11.4 mmol) in dry methanol (86 mL) at 0 °C, were added Boc_2O (4.98 g, 22.8 mmol) and $NiCl_2.6H_2O$ (0.27 g, 1.14 mmol). $NaBH_4$ (3.02 g, 79.8 mmol) was then added in small portions over 30 min (the reaction was exothermic and effervescent). The resulting mixture was allowed to warm to room temperature and left to stir overnight. After this time, diethylenetriamine (1.24 mL, 11.4 mmol) was added, and the resulting mixture allowed to stir for 90 min. The solvents were then removed at reduced pressure. The resulting residue was dissolved in ethyl acetate (60 mL) and washed with saturated sodium bicarbonate soluion (2 x 60 mL). The combined organic layers were dried ($MgSO_4$) and the solvents removed at reduced pressure to give the Boc-protected amine **142** as an orange oil (1.06 g, 78%). δ_H (300 MHz, $CDCl_3$) 1.46 (9H, s, CH_3), 1.56 (9H, s, CH_3), 4.23

(2H, d, *J* 5.3 Hz, CH_2), 6.79 (2H, d, *J* 8.6 Hz, CH-3,5), 7.13 (2H, d, *J* 8.6 Hz, CH-2,6) and 13.10 (1H, brs, NH); δ_C (75.45 MHz, $CDCl_3$) 27.7 (CH_3), 28.3 (CH_3), 60.2 (CH_2), 78.3 ($C(CH_3)_3$), 83.5 ($C(CH_3)_3$), 121.6 (CH-3,5), 128.4 (CH-2,6), 138.3 (C-1), 149.8 (C=O), 151.8 (C=O) and 156.2 (C-4).

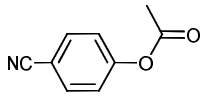
Trifluoroacetic acid (TFA) (6 mL) was added slowly to a stirring solution of the protected amine **142** (1.14 g, 3.5 mmol) in dry dichloromethane (6 mL) at 0 °C. The reaction was allowed to stir overnight at room temperature. The solvents were removed at reduced pressure to give a yellow oil which crystallised to give **141** as pale yellow crystals (0.83 g, 96%). mp 94-95 °C (lit[7] 94-95 °C); δ_H (300 MHz, DMSO-d_6) 3.90 (2H, q, *J* 5.5 Hz, CH_2), 6.82 (2H, d, *J* 8.5 Hz, CH-3,5), 7.25 (2H, d, *J* 8.5 Hz, CH-2,6), 8.12 (3H, s, NH_3) and 9.71 (1H, brs, OH); δ_C (75.45 MHz, DMSO-d_6) 40.3 (CH_2), 115.3 (CH-3,5), 124.0 (C-1), 130.4 (CH-2,6) and 157.7 (C-4). Data is in agreement with the literature.[3,7]

4-Hydroxybenzoic acid (140)



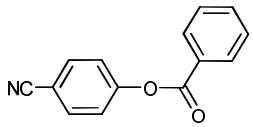
A mixture of 4-hydroxybenzonitrile **130** (1.2 g, 10 mmol), sodium hydroxide (2M, 93 mL) and methanol (9.3 mL) was heated at reflux for 18 h. The reaction mixture was then acidified using concentrated hydrochloric acid. The resulting suspension was extracted with a mixture of diethyl ether and methanol (6:1, 3 x 50 mL). The organic layers were combined, washed with water (2 x 30 mL), brine (30 mL) and dried ($MgSO_4$). The solvents were removed at reduced pressure to give **140** as a white solid (1.1 g, 82%). mp 220-222 °C (lit[8] 213-214 °C); δ_H (400 MHz, DMSO-d_6) 6.72 (2H, d, *J* 8.7 Hz, CH-3,5), 7.78 (2H, d, *J* 8.7 Hz, CH-2,6) and 9.82 (1H, s, OH); δ_C (75.45 MHz, DMSO-d_6) 116.2 (CH-3,5), 122.7 (C-1), 133.0 (CH-2,6), 163.4 (C-4) and 170.2 (C=O). Data is in agreement with the literature.[3,8]

4-Cyanophenylacetate (143)



To a solution of 4-hydroxybenzonitrile **130** (0.15 g, 1.26 mmol) in pyridine (5 mL) was added acetic anhydride (0.13 mL). The reaction mixture was stirred at room temperature for 1 h. After this time, solvents were removed at reduced pressure and the resulting residue dissolved in ethyl acetate (20 mL) and saturated ammonium chloride solution (20 mL). The aqueous phase was extracted with ethyl acetate (3 x 20 mL). The combined organic layers were washed with dilute ammonium chloride solution (20 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give **143** as a white solid (0.199 g, 98%). mp 51.5-52.5 °C (lit[9] 54.5-55.5 °C); δ_H (400 MHz, DMSO-d_6) 2.29 (3H, s, CH_3), 7.37 (2H, d, *J* 8.9 Hz, CH-2,6) and 7.91 (2H, d, *J* 8.9 Hz, CH-3,5); δ_C (75.45 MHz, DMSO-d_6) 20.8 (CH_3), 108.7 (C-4), 118.3 (CN), 123.3 (CH-2,6), 134.0 (CH-3,5), 153.9 (C-1) and 168.6 (C=O). Data is in agreement with the literature.[3,9]

4-Cyanophenyl benzoate (144)

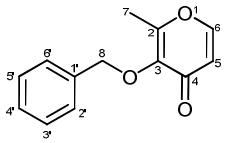


To a solution of 4-hydroxybenzonitrile **130** (0.25 g, 2.1 mmol) in ethyl acetate (5 mL) was added benzoyl chloride (0.32 g, 2.3 mmol), followed by triethylamine (0.23 g, 2.3 mmol). The resulting solution was stirred at room temperature for 1 h. The mixture was then filtered and the solvents removed at reduced pressure to give **144** as a white solid (0.46 g, 96%). mp 90-90.5 °C (lit[10] 91-91.5 °C); δ_H (400 MHz, DMSO-d_6) 7.56 (2H, d, *J* 8.9 Hz, CH-2,6), 7.59-7.65 (2H, m, CH-3',5'), 7.74-7.79 (1H, m, CH-4'), 7.98 (2H, *J* 8.9 Hz, CH-3,5) and 8.11-8.16 (2H, m, CH-2',6'); δ_C (75.45 MHz, DMSO-d_6) 108.9 (C-4), 118.3 (CN), 123.4 (CH-2,6), 128.4 (C-1'), 129.0

(CH-3',5'), 129.9 (CH-2',6'), 134.1 (CH-3,5), 134.3 (CH-4'), 154.1 (C-1) and 164.0 (C=O). Data is in agreement with the literature.[3,10]

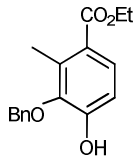
4.2.3 Reactions with Maltol

***O*-Benzyl-maltol (145)**



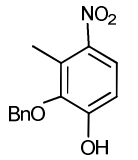
To a stirring solution of maltol **36** (6.55 g, 51.9 mmol) in methanol (57 mL) were added successively sodium hydroxide (2.28 g, 57.1 mmol) in water (5.2 mL) and benzyl bromide (10.21 g, 59.7 mmol). The mixture was heated at reflux for 20 h. The solvents were then removed at reduced pressure to give a yellow oil, which was dissolved in dichloromethane (50 mL) and washed with 5% aqueous sodium hydroxide solution (50 mL) and water (50 mL). The organic layers were combined, dried ($MgSO_4$), and the solvents removed at reduced pressure to give a pale yellow oil (10.96 g, 98%). Subsequent recrystallisation from diethyl ether gave **145** as colourless needles (9.94 g, 89%). mp 55.5-56 °C (lit[11] 53-55 °C); δ_H (300 MHz, $CDCl_3$) 2.09 (3H, s, CH_3), 5.16 (2H, s, CH_2), 6.37 (1H, d, *J* 5.7 Hz, CH-5), 7.25-7.36 (5H, m, CH-Ar) and 7.60 (1H, d, *J* 5.7 Hz, CH-6); δ_C (75.45 MHz, $CDCl_3$) 14.8 (CH_3), 73.5 (CH_2), 117.1 (CH-5), 128.3 (CH-4'), 128.4 (CH-2',6'), 129.0 (CH-3',5'), 136.9 (C-1'), 143.8 (C-3), 153.6 (CH-6), 159.8 (C-2) and 175.1 (C=O); *m/z* (CI^+) 217 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 217.0863, $C_{13}H_{13}O_3$ requires 217.0865]. Data is in agreement with the literature.[3,11]

Ethyl 3-(benzyloxy)-4-hydroxy-2-methyl benzoate (146)



Using the general procedure with *O*-benzyl-maltol **145** (1 g, 4.6 mmol), diethyl malonate (1.18 g, 7.4 mmol), potassium *tert*-butoxide (13.8 mL, 13.8 mmol) and a 47 h reflux time. Extractions were with diethyl ether. Product **146** was recovered as an orange oil (0.961 g, 73%). υ_{max}/cm^{-1} 3400 and 1277 (OH), 2981 (CH_3, CH_2), and 1731 (CO); δ_H (400 MHz, $CDCl_3$) 1.39 (3H, t, *J* 7.2 Hz, CH_3), 2.62 (3H, s, CH_3), 4.33 (2H, q, *J* 7.2 Hz, CH_2), 4.88 (2H, s, CH_2), 6.81 (1H, d, *J* 8.6 Hz, CH-5), 7.37-7.46 (5H, m, CH-Ar) and 7.72 (1H, d, *J* 8.6 Hz, CH-6); δ_C (100 MHz, $CDCl_3$) 14.2 (CH_3), 14.3 (CH_3), 60.5 (CH_2), 75.9 (CH_2-O), 112.4 (CH-5), 122.9 (CH-6), 128.3 (CH-2',6'), 128.4 (C-2), 128.8 (CH-3',5'), 134.4 (C-1), 136.4 (CH-4'), 144.6 (C-1'), 152.4 (C-3) and 167.1 (C=O); *m/z* (CI^+) 287 [$(M+H)^+$, 100%], 259 [$(M+H-Et)^+$, 23]; HRMS (CI^+) [Found: $(M+H)^+$, 287.1281, $C_{17}H_{19}O_4$ requires 287.1283]. Data is in agreement with the literature.[3]

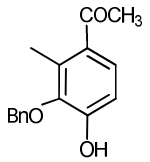
2-(Benzyloxy)-3-methyl-4-nitrophenol (147)



Using the general procedure with *O*-benzyl-maltol **145** (1 g, 4.6 mmol), nitromethane (0.45 g, 7.4 mmol), potassium *tert*-butoxide (10.6 mL, 10.6 mmol) and a 44 h reflux time. Extractions were with ethyl acetate. Product **147** was recovered as an orange oil (0.238 g, 20%). υ_{max}/cm^{-1} 2926 (CH_3, CH_2, OH), 1714 (NO_2), 1640 (ArH), and 1557 (CNO_2); δ_H (400 MHz, $CDCl_3$) 2.62 (3H, s, CH_3), 4.93 (2H, s, CH_2), 6.87 (1H, d, *J* 9.1 Hz, CH-6), 7.40-7.47 (5H, m, CH-Ar), 7.86 (1H, d, *J* 9.1 Hz, CH-5) and 8.00 (1H, s, OH); δ_C (100 MHz, $CDCl_3$) 13.8 (CH_3), 76.5 (CH_2), 112.8 (CH-5), 123.1 (CH-6), 128.3 (CH-2',6'), 129.1 (C-3) and 129.2 (CH-3',5'); *m/z* (CI^+)

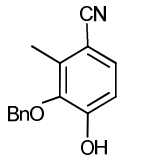
260 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 260.0970, $C_{14}H_{14}NO_4$ requires 260.0923]. Data is in agreement with the literature.[3]

1-(3-(Benzyloxy)-4-hydroxy-2-methylphenyl)ethanone (148)



Using the general procedure with *O*-benzyl-maltol **145** (1 g, 4.6 mmol), acetylacetone (1.01 g, 7.4 mmol), potassium *tert*-butoxide (10.6 mL, 10.6 mmol) and a 42 h reflux time. Extractions were with ethyl acetate. Product **148** was recovered as an orange oil (0.118 g, 10%). υ_{max}/cm^{-1} 3032 (CH_3, CH_2, OH), 1719 (C=O), 1648 (ArH), 1255 (OH), and 1187 (C-O); δ_H (400 MHz, $CDCl_3$) 2.01 (3H, s, CH_3), 2.57 (3H, s, CH_3), 4.88 (2H, s, CH_2), 6.83 (1H, d, *J* 8.6 Hz, CH-5), 7.39-7.45 (5H, m, CH-Ar), 7.54 (1H, d, *J* 8.6 Hz, CH-6) and 8.02 (1H, s, OH); δ_C (100 MHz, $CDCl_3$) 14.4 (CH_3), 29.3 (CH_3), 75.9 (CH_2), 112.1 (CH-5), 128.0 (CH-2',6'), 128.9 (C-2), 129.0 (CH-3',5'), 130.9 (CH-6), 133.4 (C-1), 136.3 (CH-4'), 145.0 (C-1'), 152.3 (C-3) and 200.0 (C=O); *m/z* (CI^+) 257 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 257.1176, $C_{16}H_{17}O_3$ requires 257.1178]. Data is in agreement with the literature.[3]

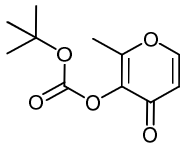
3-(Benzyloxy)-4-hydroxy-2-methylbenzonitrile (149)



Using the general procedure with *O*-benzyl-maltol **145** (1 g, 4.6 mmol), ethyl cyanoacetate (1.07 g, 7.4 mmol), potassium *tert*-butoxide (13.8 mL, 13.8 mmol) and a 47 h reflux time. Extractions were with ethyl acetate. Product **149** was recovered as an orange/red oil (1.13 g, 93%). υ_{max}/cm^{-1} 2929 (CH_3, CH_2, OH), 2216 (CN), 1456 (CH_3, CH_2) and 1376 (OH); δ_H (400 MHz, $CDCl_3$) 2.51 (3H, s, CH_3), 4.92 (2H, s, CH_2), 6.84 (1H, d, *J* 8.5 Hz, CH-5), 7.33 (1H,

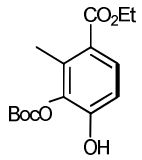
d, *J* 8.5 Hz, CH-6) and 7.39-7.47 (5H, m, CH-Ar); δ_C (100 MHz, $CDCl_3$) 14.9 (CH_3), 76.1 (CH_2), 105.3 (C-1), 114.2 (CN), 118.2 (CH-5), 128.3 (CH-2',6'), 129.1 (CH-3',5'), 130.1 (C-2), 131.6 (CH-6), 135.9 (C-4'), 144.5 (C-1') and 153.3 (C-3); *m/z* (CI^+) 240 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 240.1017, $C_{15}H_{14}NO_2$ requires 240.1025]. Data is in agreement with the literature.[3]

_O_-Boc-maltol (150)



To a stirring solution of maltol **36** (1 g, 7.9 mmol) in dry methanol (60 mL) at room temperature, were added Boc_2O (3.45 g, 15.8 mmol) and DMAP (0.1 g, 0.79 mmol) and the resulting mixture stirred overnight. After this time, the solvents were removed at reduced pressure and the resulting residue dissolved in ethyl acetate (30 mL). The organic phase was washed with saturated sodium bicarbonate solution (2 x 30 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give **150** as an off-white solid (1.58 g, 88%). mp 62-62.5 °C; Found: C, 58.2; H, 6.1, $C_{11}H_{14}O_5$ requires C, 58.4; H, 6.2%; υ_{max}/cm^{-1} 2896 and 1501 (CH_3), 1743 and 1620 (C=O), 1581 (C=C), 1326 (CH), 1211 and 1097 (C-O), 920 and 835 (CH); δ_H (300 MHz, $CDCl_3$) 1.48 (9H, s, CH_3), 2.24 (3H, s, CH_3), 6.33 (1H, d, *J* 5.7 Hz, CH-5) and 7.60 (1H, d, *J* 5.7 Hz, CH-6); δ_C (75.45 MHz, $CDCl_3$) 14.9 (CH_3), 27.5 (CH_3), 84.5 ($C(CH_3)_3$), 116.9 (CH-5), 139.0 (C-3), 150.1 (C=O), 154.2 (CH-6), 159.1 (C-2) and 172.1 (C=O); *m/z* (CI^+) 227 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 227.0921, $C_{11}H_{15}O_5$ requires 227.0919].

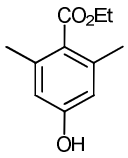
Ethyl 3-(*tert*-butoxycarbonyloxy)-4-hydroxy-2-methyl benzoate (151)



Using the general procedure with *O*-Boc-maltol **150** (1.5 g, 6.63 mmol), diethyl malonate (1.7 g, 10.6 mmol), potassium *tert*-butoxide (15.25 mL, 15.25 mmol) and a 47 h reflux time. Extractions were with diethyl ether. A mixture of starting material and product **151** was recovered as an orange oil (0.003 g, 2%). Data for **151**: υ_{max}/cm^{-1} 3409 and 1265 (OH), 2981 (CH_3, CH_2, OH), 1728 (C=O) and 1621 (ArH); δ_H (300 MHz, $CDCl_3$) 1.14 (3H, t, *J* 7.1 Hz, CH_3), 1.40 (9H, s, CH_3), 2.43 (3H, s, CH_3), 4.24 (2H, q, *J* 7.1 Hz, CH_2), 6.42 (1H, d, *J* 8.9 Hz, CH-5), 7.30 (1H, d, *J* 8.9 Hz, CH-6) and 11.00 (1H, s, OH); *m/z* (Cl^-) 296 [$(M-H)^-$, 2%], and 197 [$(M+H\text{-}boc)^-$, 99].

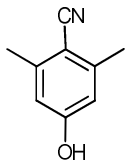
4.2.4 Reactions with 2,6-Dimethyl-4*H*-pyran-4-one (43)

Ethyl 4-hydroxy-2,6-dimethyl benzoate (152)



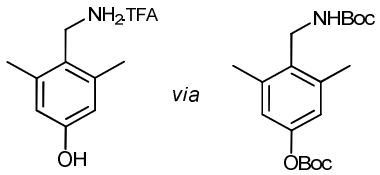
Using the general procedure with 2,6-dimethyl-4*H*-pyran-4-one **43** (1 g, 8.1 mmol), diethyl malonate (2.1 g, 12.9 mmol), potassium *tert*-butoxide (24.3 mL, 24.3 mmol) and a 44 h reflux time. Extractions were with ethyl acetate. Product **152** was recovered as an orange solid (0.283 g, 18%). mp 65-65.5 °C (lit[12] 62 °C); δ_H (400 MHz, $CDCl_3$) 1.38 (3H, t, *J* 7.1 Hz, CH_3), 2.29 (6H, s, CH_3), 4.37 (2H, q, *J* 7.1 Hz, CH_2) and 8.50 (2H, s, CH-3,5); δ_C (100 MHz, $CDCl_3$) 14.3 (CH_3), 20.1 (CH_3), 60.8 (CH_2), 114.5 (CH-3,5), 136.7 (C-2,6), 153.6 (C-4) and 169.9 (C=O). Data is in agreement with the literature.[3,12]

4-Hydroxy-2,6-dimethylbenzonitrile (153)



Using the general procedure with 2,6-dimethyl-4*H*-pyran-4-one **43** (0.67 g, 5.4 mmol), ethyl cyanoacetate (1.15 g, 8.6 mmol), potassium *tert*-butoxide (16.2 mL, 16.2 mmol) and a 47 h reflux time. Extractions were with ethyl acetate. Product **153** was recovered from the organic phase as an orange/red oil (0.449 g, 57%), and as an off-white solid after purification of a small portion of product by MDAP. mp 176-176.5 °C (lit[13] 177.5-177.7 °C); υ_{max}/cm^{-1} 3234 (OH), 2220 (CN), 1608, 1585 and 1457 (C=C), 1321 (OH), 1262, and 1147 (C-O), 845 (ArH), 717 and 705 (CH); δ_H (400 MHz, $CDCl_3$) 2.48 (6H, s, CH_3) and 6.69 (2H, s, CH-3,5); δ_C (100 MHz, $CDCl_3$), 20.8 (CH_3), 105.2 (C-1), 114.6 (CH-3,5), 117.7 (CN), 144.6 (C-2,6) and 158.8 (C-4); *m/z* (CI^-) 146 [$(M-H)^-$, 100%]; HRMS (CI^-) [Found: $(M-H)^-$, 146.0611, $C_9H_8O_6N$ requires 146.0606]. Data is in agreement with the literature.[3,13]

4-(Aminomethyl)-3,5-dimethylphenol TFA salt (154)



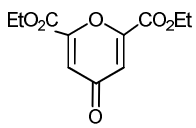
To a stirring solution at 0 °C of crude 4-hydroxy-2,6-dimethylbenzonitrile **153** (0.449 g, 3.05 mmol) in dry methanol (23 mL), was added Boc_2O (1.33 g, 6.1 mmol) and $NiCl_2.6H_2O$ (0.07 g, 0.31 mmol). $NaBH_4$ (0.81 g, 21.4 mmol) was then added in small portions over 30 min (the reaction was exothermic and effervescent). The resulting mixture (containing a finely divided black precipitate) was allowed to warm to room temperature and stirring continued for 68 h. After this time, diethylenetriamine (0.33 mL, 3.05 mmol) was added and the mixture stirred for 90 min. The solvents were then removed at reduced pressure. The resulting residue was dissolved in ethyl acetate (50 mL) and extracted with saturated sodium bicarbonate solution

(2 x 50 mL). The combined organic layers were dried ($MgSO_4$) and the solvents removed at reduced pressure to give the Boc-protected amine **155** as an orange oil (0.474 g, 44%). δ_H (300 MHz, DMSO-d_6) 1.47 (9H, s, CH_3), 1.48 (9H, s, CH_3), 2.30 (6H, s, CH_3), 4.20 (2H, d, *J* 5.4 Hz, CH_2) and 6.79 (2H, s, CH-2,6).

To a stirring solution at 0 °C of the crude protected amine **155** (0.474 g, 1.35 mmol) in dry dichloromethane (15 mL) was slowly added trifluoroacetic acid (15 mL). The resulting red/purple reaction mixture was allowed to stir for 23 h. The solvents were removed at reduced pressure and the resulting residue dissolved in water (30 mL) and diethyl ether (30 mL). The aqueous phase was extracted with diethyl ether (30 mL) and the combined organic layers washed with water (30 mL). The solvents were removed from aqueous phase at reduced pressure to give **154** as an orange solid (0.231 g, 65%). mp 64-70 °C; υ_{max}/cm^{-1} 3350 (NH_2), 1674 (C=C), 1316 (OH), 1202, 1146 and 1015 (C-O); δ_H (400 MHz, MeOD) 2.36 (6H, s, CH_3), 4.13 (2H, q, *J* 5.6 Hz, CH_2), 6.57 (2H, s, CH-2,6) and 7.78-8.20 (4H, brs, OH, NH_2); δ_C (75.45 MHz, $CDCl_3$) 19.8 (CH_3), 39.0 (CH_2), 115.4 (CH-2,6), 121.4 (C-4), 139.7 (C-3,5) and 157.8 (C-1); *m/z* (CI^+) 152 [$(M+H\text{-}TFA)^+$, 100%]; HRMS (CI^+) [Found: $(M+H\text{-}TFA)^+$, 152.1073, $C_9H_{14}ON$ requires 152.1076].

4.2.5 Reactions with Chelidonic Acid (38)

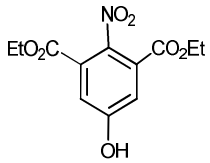
Diethyl chelidonate (156)



To a solution of chelidonic acid **38** (10 g, 54.3 mmol) in ethanol (500 mL) was added concentrated hydrochloric acid (50 mL). The reaction mixture was heated at reflux for 24 h, after which time the solvents were removed at reduced pressure. The resulting residue was dissolved in water (100 mL) and diethyl ether (100 mL). The aqueous layer was extracted with

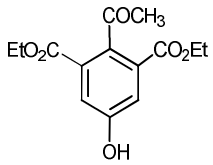
diethyl ether (2 x 100 mL). The combined organic layers were washed with water (100 mL), brine (100 mL) and dried ($MgSO_4$). The solvents were removed at reduced pressure to give **156** as a light yellow solid (12.85 g, 99%). mp 61-61.5 °C (lit[14] 62 °C); δ_H (400 MHz, $CDCl_3$) 1.42 (6H, t, *J* 7.1 Hz, CH_3), 4.46 (4H, q, *J* 7.1 Hz, CH_2) and 7.17 (2H, s, CH-2,6). Data is in agreement with the literature.[3,14]

Diethyl 2-nitro-5-hydroxyisophthalate (157)



Using the general procedure with diethyl chelidonate **156** (1 g, 4.2 mmol), nitromethane (0.29 g, 4.62 mmol), potassium *tert*-butoxide (12.6 mL, 12.6 mmol) and a 24 h reflux time. Extractions were with diethyl ether. Product **157** was recovered as a yellow solid (0.548 g, 46%). mp 84-84.5 °C; υ_{max}/cm^{-1} 3420 and 2989 (OH), 2902 (CH_3, CH_2), 1727 (C=O), 1655 (C=C), 1546, 1394 and 1376 (NO_2), 1250, 1051 and 1023 (C-O), 1002 (ArH), 823 and 760 (CH); δ_H (300 MHz, $CDCl_3$) 1.28 (6H, t, *J* 7.1 Hz, CH_3), 4.29 (4H, q, *J* 7.1 Hz, CH_2) and 7.46 (2H, s, CH-4,6); δ_C (100 MHz, $CDCl_3$), 13.6 (CH_3), 63.1 (CH_2), 120.6 (CH-4,6) 127.0 (C-1,3), 142.5 (C-2), 157.3 (C-5) and 163.6 (C=O); *m/z* (CI^+) 284 [$(M+H)^+$, 100%], 256 [$(M+H-Et)^+$, 19], 227 [$(M+H-Et_2)^+$, 5]; HRMS (CI^+) [Found: $(M+H)^+$, 284.0761, $C_{12}H_{14}O_7N$ requires 284.0770]. Data is in agreement with the literature.[3]

Diethyl 2-acetyl-5-hydroxyisophthalate (158)



Using the general procedure with diethyl chelidonate **156** (1 g, 4.2 mmol), acetylacetone (1.01 g, 7.4 mmol), potassium *tert*-butoxide (12.6 mL, 12.6 mmol) and a 48 h reflux time.

Extractions were with diethyl ether. The crude mixture contained product **158** in only trace amounts, which was recovered by purification using MDAP (0.023 g, 2%). mp 66-66.5 °C; υ_{max}/cm^{-1} 3512 (OH), 2986 (CH_3, CH_2), 1724 and 1688 (C=O), 1584 (C=C), 1283, 1243, 1223, 1203 and 1034 (C-O), 1009 (ArH) and 799 (CH); δ_H (400 MHz, $CDCl_3$) 1.37 (6H, t, *J* 7.2 Hz, CH_3), 2.64 (3H, s, CH_3), 4.34 (4H, q, *J* 7.2 Hz, CH_2) and 7.61 (2H, s, CH-4,6); δ_C (100 MHz, $CDCl_3$) 14.0 (CH_3), 32.0 (CH_3), 62.0 (CH_2), 121.2 (CH-4,6), 129.7 (C-1,3), 131.6 (C-2), 155.7 (C-5) and 165.0 (C=O); *m/z* (CI^-) 279 [$(M-H)^-$, 100%]; HRMS (CI^-) [Found: $(M-H)^-$, 279.0875, $C_{14}H_{15}O_6$ requires 279.0868]. Data is in agreement with the literature.[3]

4.2.6 Application of Microwave Irradiation to Reactions using 4*H*-Pyran-4-ones

4.2.6.1 General Procedure

A vial containing the pyranone, pronucleophile and potassium *tert*-butoxide in dry *tert*-butanol (7.5 mL) was treated with microwave irradiation for the desired time at the required temperature, as indicated in *Table 21*. After this time, 1M hydrochloric acid (7.5 mL) was added to the reaction mixture and microwave irradiation continued for a further 10 min at 120 °C. Solvents were then removed at reduced pressure and water (20 mL) added. The aqueous phase was extracted with diethyl ether (3 x 20 mL). The combined organic layers were washed with water (2 x 20 mL), brine (20 mL) and dried ($MgSO_4$). The solvents were removed at reduced pressure to give the product. Characterisations were as above.

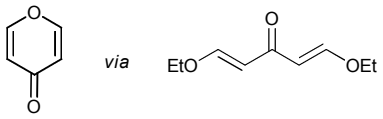
Table 21 – Reaction conditions and results for microwave-assisted reactions, including comparison with yields obtained using conventional heating methods.

Product	Pyranone	Eq. Pyranone	Eq. Nucleophile	Eq. KO*t*Bu	Time (min)	Temp. (°C)	Micro. yield (%)	Conv.l yield[a] (%)
128	**35**	1.0	1.1	1.1	30	120	67	94
6	**35**	1.0	1.1	1.1	15	120	80	80
87	**35**	1.0	1.1	2.0	30	150	57	95
130	**35**	1.0	1.6	3.0	30	150	86	78
146	**145**	1.0	1.6	2.3	30	120	99	73
147	**145**	1.0	1.6	2.0	30	120	53	20
148	**145**	1.0	1.6	2.3	30	150	86	10
149	**145**	1.0	1.6	2.3	30	120	99	93
152	**43**	1.0	1.6	2.0	30	150	81	18
153	**43**	1.0	1.6	2.0	30	120	47	57
157	**156**	1.0	1.6	2.3	30	120	48	46
158	**156**	1.0	1.6	2.3	30	120	35	2

[a] Using conditions previously discussed and given in **Section 2.3**.

4.3 Synthesis of 4*H*-Pyran-4-one (35) and ^{13}C-Labelled Isotopomers (35d and 35e)

4*H*-Pyran-4-one (35)

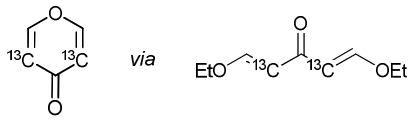


To triethyl orthoformate (17.78 g, 20 mL, 0.12 mol) at -30 °C (acetonitrile and dry ice) under argon, was added dropwise over 15 min, a solution of $BF_3.OEt_2$ (45.2 mL, 0.36 mol) in dichloromethane (20 mL). The reaction mixture was warmed to 0 °C with stirring and was stirred for 20 min to give a slurry of diethoxycarbenium fluoroborate. The mixture was cooled to -78 °C and acetone (4.41 mL, 0.06 mol) added. DIPEA (55.5 mL, 0.36 mol) was then added dropwise over 30 min with efficient stirring and the resulting reaction mixture stirred at -78 °C for 2.5 h. After that time, the reaction mixture was poured into conc. sodium bicarbonate solution (500 mL) and stirred vigorously for 10 min at room temperature. The resulting mixture was extracted with dichloromethane (3 x 100 mL). The combined organic layers were washed successively with ice cold 1M H_2SO_4 (75 mL), cold water (2 x 100 mL), and dried ($MgSO_4$). Removal of the solvents at reduced pressure gave the crude enone product **159** as a brown oil (10.33 g, quant.). δ_H (300 MHz, $CDCl_3$) 1.27 (6H, t, *J* 7.1 Hz, CH_3), 3.87 (4H, q, *J* 7.1 Hz,

CH_2), 5.57 (2H, d, *J* 12.3 Hz, CH) and 7.51 (2H, d, *J* 12.3 Hz, CH); δ_C (75.45 MHz, $CDCl_3$) 14.9 (CH_3), 67.4 (CH_2), 105.9 (CH), 161.6 (CH) and 189.3 (C=O).

A solution of the crude enone **159** (10.33 g, 0.06 mol) in ethanol (250 mL) and 10% aqueous hydrochloric acid (25 mL) was heated at 80 °C for 24 h. After this time, the solvents were removed at reduced pressure and the resulting residue dissolved in water (100 mL). The aqueous layer was extracted with toluene (3 x 100 mL) and the combined organic layers washed with water (2 x 100 mL). After filtering the aqueous phase to remove any solids, it was treated with decolourising charcoal and filtered through a bed of celite. Removal of the solvents from the aqueous phase at reduced pressure (at 40 °C) gave 4*H*-pyran-4-one **35** as an off-white solid (4.8 g, 98%). mp 31.5-32 °C (lit[15] 32.5 °C); δ_H (300 MHz, DMSO-d_6) 6.26 (2H, d, *J* 6.3 Hz, H-3,5) and 8.08 (2H, d, *J* 6.3 Hz, H-2,6); δ_C (75.45 MHz, DMSO-d_6) 117.3 (CH-3,5), 156.8 (CH-2,6) and 177.0 (C=O). Data is in agreement with the literature.[15]

[3,5-$^{13}C_2$]4*H*-Pyran-4-one (35d)

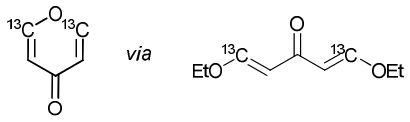


To triethyl orthoformate (4.74 g, 5.33 mL, 32 mmol) at -30 °C (acetonitrile and dry ice) under argon, was added dropwise over 15 min, a solution of $BF_3.OEt_2$ (12.14 mL, 96 mmol) in dichloromethane (13 mL). The reaction mixture was warmed to 0 °C with stirring, and stirred for 20 min to give a slurry of diethoxycarbenium fluoroborate. The mixture was cooled to -78 °C and [1,3-$^{13}C_2$]acetone (1.0 g, 14 mmol) added. DIPEA (16.7 mL, 96 mmol) was then added dropwise over 30 min with efficient stirring and the resulting reaction mixture stirred at -78 °C for 2.5 h. After that time, the reaction mixture was poured into conc. sodium bicarbonate solution (120 mL) and stirred vigorously for 10 min

at room temperature. The resulting mixture was extracted with dichloromethane (3 x 50 mL). The combined organic layers were washed successively with ice cold 1M H_2SO_4 (17.5 mL), cold water (2 x 50 mL) and dried ($MgSO_4$). Removal of the solvents at reduced pressure gave the crude enone product **159a** as a brown oil (2.40 g, quant.). δ_H (300 MHz, $CDCl_3$) 1.28 (6H, t, *J* 7.1 Hz, CH_3), 3.88 (4H, q, *J* 7.1 Hz, CH_2), 5.59 (2H, dd, *J* 159.3, 12.4 Hz, ^{13}CH) and 7.52 (2H, dd, *J* 12.4, 6.7 Hz, CH); δ_C (75.45 MHz, $CDCl_3$) 14.4 (CH_3), 62.5 (CH_2), 105.6 (enhanced, ^{13}CH) and 163.3 (t, *J* 41 Hz, CO).

A solution of the crude enone **159a** (2.75 g, 16 mmol) in ethanol (80 mL) and 10% aqueous hydrochloric acid (8 mL) was heated at 80 °C for 24 h. After this time, the solvents were removed at reduced pressure and the resulting residue dissolved in water (50 mL). The aqueous layer was extracted with toluene (3 x 30 mL), and the combined organic layers washed with water (2 x 30 mL). After filtering the aqueous phase to remove any solids, it was treated with decolourising charcoal and filtered through a bed of celite. Removal of the solvents from the aqueous phase at reduced pressure (at 40 °C) gave [3,5-$^{13}C_2$]4*H*-pyran-4-one **35d** as an off-white solid (1.37 g, quant.). mp 32-32.5 °C (lit[15] 32.5 °C for the unlablled); υ_{max}/cm^{-1} 1619 (C=O), 1590 (C=C), 1326 (CH), 1091 (C-O), 918 and 848 (CH); δ_H (300 MHz, DMSO-d_6) 6.29 (2H, dm, *J* 171.7 Hz, ^{13}CH-3,5) and 8.13 (2H, m, H-2,6); δ_C (75.45 MHz, DMSO-d_6) 117.3 (enhanced, ^{13}CH-3,5), 156.9 (d, *J* 72 Hz, CH-2, 6) and 177.1 (t, *J* 54 Hz, C=O); *m/z* (CI^+) 99 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 99.0357, $^{12}C_3{}^{13}C_2H_5O_2$ requires 99.0357]. Data is in agreement with the literature.[15,16]

[2,6-$^{13}C_2$]4*H*-Pyran-4-one (35e)



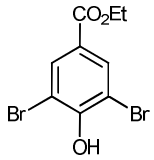
To triethyl [^{13}C]orthoformate (1 g, 1.12 mL, 6.7 mmol) at -30 °C (acetonitrile and dry ice) under argon, was added dropwise over 15 min, a solution of $BF_3.OEt_2$ (2.54 mL, 20.1 mmol) in dichloromethane (3 mL). The reaction mixture was warmed to 0 °C with stirring, and stirred for 20 min to give a slurry of diethoxycarbenium fluoroborate. The mixture was then cooled to -78 °C and acetone (0.24 mL, 3.35 mmol) added. DIPEA (3.5 mL, 20.1 mmol) was then added dropwise over 30 min with efficient stirring and the resulting reaction mixture stirred at -78 °C for 2.5 h. After that time, the reaction mixture was poured into conc. sodium bicarbonate solution (30 mL) and stirred vigorously for 10 min at room temperature. The resulting mixture was extracted with dichloromethane (3 x 15 mL). The combined organic layers were washed successively with ice cold 1M H_2SO_4 (4.2 mL), cold water (2 x 15 mL) and dried ($MgSO_4$). Removal of the solvents at reduced pressure gave the crude enone **159b** as a brown oil. (0.58 g, quant.). δ_H (300 MHz, $CDCl_3$) 1.28 (6H, t, *J* 7.0 Hz, CH_3), 3.88 (4H, dq, *J* 7.0, 2.9 Hz, CH_2), 5.57 (2H, dd, *J* 12.5, 3.2 Hz, CH) and 7.52 (2H, dd, *J* 181.4, 12.5 Hz, ^{13}CH); δ_C (75.45 MHz, $CDCl_3$) 14.6 (CH_3), 67.2 (CH_2), 105.6 (d, *J* 79 Hz, CH), 161.4 (enhanced, ^{13}CH) and 162.8 (C=O).

A solution of the crude enone **159b** (0.58 g, 3.35 mmol) in ethanol (20 mL) and 10% aqueous hydrochloric acid (2 mL) was heated at 80 °C for 24 h. After this time, the solvents were removed at reduced pressure and the resulting residue dissolved in water (15 mL). The aqueous layer was extracted with toluene (3 x 15 mL), and the combined organic layers washed with water (2 x 15 mL). After filtering the aqueous phase to remove any solids, it was treated with decolourising charcoal and filtered through a bed of celite. Removal of the solvents from the aqueous phase at reduced pressure (at 40 °C) gave [2,6-

$^{13}C_2$]4*H*-pyran-4-one **35e** as an off-white solid (0.304 g, 92%). mp 33-33.5 °C (lit[15] 32.5 °C for the unlabelled); υ_{max}/cm^{-1} 1623 (C=O), 1577 (C=C), 1325, 923 and 827 (CH); δ_H (300 MHz, CD_3OD) 6.33 (2H, m, CH-3,5) and 8.20 (2H, dm, *J* 202.0 Hz, ^{13}CH-2,6); δ_C (75.45 MHz, CD_3OD) 117.9 (d, *J* 66.8, 2.2 Hz, CH-3,5) and 156.8 (enhanced, ^{13}CH-2,6); *m/z* (CI^+) 99 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 99.0360, $^{12}C_3{}^{13}C_2H_5O_2$ requires 99.0357]. Data is in agreement with the literature.[15,16]

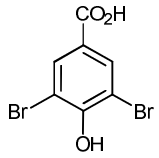
4.4 Synthesis of Gallic Acid (32)

Ethyl 3,5-dibromo-4-hydroxybenzoate (160)



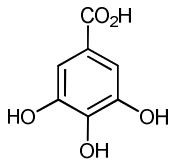
To a solution of ethyl 4-hydroxybenzoate **128** (0.6 g, 3.61 mmol) and sodium acetate (0.95 g, 11.6 mmol) in acetic acid (10 mL), was added dropwise over 20 min a solution of bromine (1.73 g, 10.83 mmol) in acetic acid (2.5 mL) at room temperature. The reaction mixture was stirred for 1.5 h at room temperature. After the addition of water (40 mL) to the reaction mixture, the aqueous phase was extracted with dichloromethane (3 x 40 mL). The combined organic layers were washed with saturated sodium metabisulfite solution (2 x 40 mL), water (40 mL), and brine (40 mL) and dried ($MgSO_4$). Removal of the solvents at reduced pressure gave the di-bromo product **160** as an orange/yellow solid (1.15 g, 98%). mp 147-148 °C (lit[17] 108-108.5 °C); δ_H (300 MHz, $CDCl_3$) 1.25 (3H, t, *J* 7.1 Hz, CH_3), 4.34 (2H, q, *J* 7.1 Hz, CH_2) and 8.09 (2H, s, CH-2,6); δ_C (75.5 MHz, $CDCl_3$) 14.3 (CH_3), 61.6 (CH_2), 109.7 (C-3,5), 125.1 (C-1), 133.6 (CH-2,6), 153.1 (C-4) and 164.0 (C=O); *m/z* (CI^+) 327 [$(M^{81}Br_2+H)^+$, 32%], 325 [$(M^{79}Br^{81}Br+H)^+$, 66], 323 [$(M^{79}Br_2+H)^+$, 33]; HRMS (CI^+) [Found: $(M)^+$, 324.8888, $C_9H_8O_3{}^{79}Br^{81}Br$ requires 324.8898]. Data is in agreement with the literature.[17]

3,5-Dibromo-4-hydroxybenzoic acid (164)



A solution of ethyl 3,5-dibromo-4-hydroxybenzoate **160** (2.1 g, 6.5 mmol) in 2M sodium hydroxide solution (9.1 mL) was stirred overnight at room temperature. The resulting solution was then acidified carefully with dilute hydrochloric acid and the aqueous phase extracted with *tert*-butyl methyl ether (3 x 30 mL). The combined organic layer were washed with brine (15 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the acid **164** as an off-white solid (1.9 g, 99%). mp 285 °C (lit[18] 275-276 °C); δ_H (300 MHz, acetone-d_6) 8.01 (2H, s, CH-2,6); δ_C (75.45 MHz, acetone-d_6) 110.3 (C-3,5), 124.6 (C-1), 133.8 (CH-2,6), 154.7 (C-4) and 164.2 (C=O); *m/z* (CI^+) 298, [$(M^{81}Br_2)^+$, 13%], 296 [$(M^{79}Br^{81}Br)^+$, 32] and 294 [$(M^{79}Br_2)^+$, 12]; HRMS (CI^+) [Found: $(M)^+$, 295.8508, $C_7H_4{}^{79}Br^{81}BrO_3$ requires 295.8507]. Data is in agreement with the literature.[18]

Gallic Acid or 3,4,5-trihydroxybenzoic acid (32)

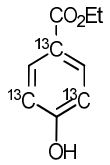


A solution of sodium hydroxide (4.5 g, 112.5 mol) in water (30 mL) was stirred at reduced pressure for 90 min. A solution of $CuSO_4.6H_2O$ (0.28 g, 1.78 mmol) was then added to the basic solution and stirring continued at reduced pressure for an additional 4 h. The aqueous solution was transferred by cannula to a flask containing ethyl 3,5-dibromo-4-hydroxybenzoate **160** (0.96 g, 2.96 mmol). After allowing the blue suspension to stir at room temperature under argon for 90 min, the mixture was heated at reflux at 110 °C for 18 h, cooled and acidified with hydrochloric acid. The aqueous mixture was extracted with

diethyl ether (3 x 30 mL). The combined organic layers were washed with brine (30 mL) and dried ($MgSO_4$). Removal of the solvents at reduced pressure gave gallic acid **32** as a yellow solid (0.344 g, 68%). mp 260-262 °C (lit[19] 258-260 °C); δ_H (300 MHz, DMSO-d_6) 6.92 (2H, s, CH-2,6), 9.26 (3H, brs, OH) and 12.37 (1H, brs, CO_2H). Data is in agreement with the literature.[19]

4.5 Synthesis of [1,3,5-$^{13}C_3$]Gallic Acid (32d)

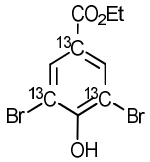
Ethyl 4-hydroxy-[1,3,5-$^{13}C_3$]benzoate (128c)



A solution of [3,5-$^{13}C_2$]4*H*-pyran-4-one **35d** (1.57 g, 16 mmol) and diethyl [2-^{13}C]malonate (2.34 g, 15 mmol) in dry *tert*-butanol (25 mL) was heated to reflux under argon. Potassium *tert*-butoxide (16 mmol, 16 mL of a 1M solution in *tert*-butanol) in dry *tert*-butanol (30 mL) was added dropwise. The resulting mixture was heated at reflux for 3 h, after which time hydrochloric acid (1M, 30 mL) was added. The reaction solution was heated at reflux for a further 1 h. The solvents were then removed at reduced pressure and water (40 mL) added. The aqueous phase was extracted with diethyl ether (3 x 30 mL). The combined organic layers were washed with water (2 x 30 mL), brine (30 mL), dried ($MgSO_4$) and the solvent removed at reduced pressure to give **128c** as an orange solid (1.88 g, 74%). mp 113-113.5 °C (lit[2] 112-115 °C for the unlabelled); υ_{max}/cm^{-1} 3207 (OH), 1714 (C=O), 1667 and 1569 (ArH), 1277 (OH), 1229, 1165, 1098 and 1016 (C-O), 840 and 762 (CH); δ_H (400 MHz, $CDCl_3$) 1.39 (3H, t, *J* 7.3 Hz, CH_3), 4.36 (2H, q, *J* 7.3 Hz, CH_2), 5.76 (1H, brs, OH), 6.88 (2H, dm, *J* 159.2 Hz, ^{13}CH-3,5) and 7.97 (2H, brd, *J* 8.7 Hz, CH-2,6); δ_C (100 MHz, $CDCl_3$) 14.4 (CH_3), 60.9 (CH_2), 115.1 (enhanced, d, *J* 1.6 Hz, ^{13}CH-3,5), 123.0 (enhanced, t,

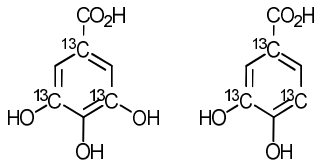
J 1.6 Hz, ^{13}C-1), 131.8 (dt, *J* 58.9, 5.6 Hz, CH-2,6), 161.4 (C-4) and 169.7 (C=O); *m/z* (CI^+) 170 [$(M+H)^+$, 100%], 142 [$(M-Et)^+$, 18]; HRMS (CI^+) [Found: $(M+H)^+$, 170.0808, $^{12}C_6{}^{13}C_3H_{10}O_3$ requires 170.0809]. Data is in agreement with the literature.[2,16]

Ethyl 3,5-dibromo-4-hydroxy-[1,3,5-$^{13}C_3$]benzoate (160a)



To a solution of ethyl [1,3,5-$^{13}C_3$]4-hydroxybenzoate **128c** (0.878 g, 5.2 mmol) and sodium acetate (1.94 g, 24 mmol) in acetic acid (20 mL), was added dropwise over 20 min a solution of bromine (3.54 g, 22 mmol) in acetic acid (5 mL) at room temperature. The reaction mixture was stirred for 1.5 h at room temperature. After the addition of water (40 mL) to the reaction mixture, the aqueous phase was extracted with dichloromethane (3 x 40 mL). The combined organic layers were washed with saturated sodium metabisulfite solution (2 x 30 mL), water (30 mL), brine (30 mL) and dried ($MgSO_4$). Removal of the solvents at reduced pressure gave the di-bromo product **160a** as an orange/yellow solid (1.68 g, 99%). mp 150-150.5 °C (lit[17] 108-108.5 °C for the unlabelled); υ_{max}/cm^{-1} 3313 and 2987 (OH), 1692 (C=O), 1458 (ArH), 1367 and 1281 (OH), 1240, 1212, 1112 and 1016 (C-O), 903 and 758 (CH) and 719 (C-Br); δ_H (400 MHz, $CDCl_3$) 1.40 (3H, t, *J* 7.1 Hz, CH_3), 4.37 (2H, q, *J* 7.1 Hz, CH_2), 6.28 (1H, brs, OH) and 8.16 (2H, dt, *J* 2.8, 1.7 Hz, CH-2,6); δ_C (100 MHz, $CDCl_3$) 14.3 (CH_3), 61.5 (CH_2), 109.6 (enhanced, ^{13}C-3,5), 125.1 (enhanced, ^{13}C-1), 133.6 (ddd, *J* 69.1, 59.4, 4.4 Hz, CH-2,6), 153.1 (C-4) and 164.7 (C=O); *m/z* (CI^+) 328 [$(M^{79}Br^{81}Br+H)^+$, 100%], 326 [$(M^{79}Br_2+H)^+$, 30], 330 [$(M^{81}Br_2+H)^+$, 27]; HRMS (CI^+) [Found: $(M+H)^+$, 327.8998, $^{12}C_6{}^{13}C_3H_8O_3{}^{79}Br^{81}Br$ requires 327.8999]. Data is in agreement with the literature.[16,17]

[1,3,5-$^{13}C_3$]Gallic Acid or 3,4,5-trihydroxy-[1,3,5-$^{13}C_3$]benzoic acid (32d) and 3,4-dihydroxy-[1,3,5-$^{13}C_3$]benzoic acid (163a)

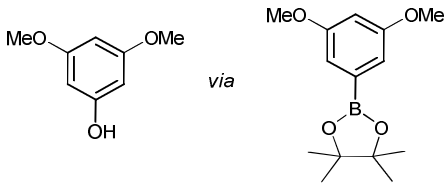


A solution of sodium hydroxide (7.0 g, 0.17 mol) in water (50 mL) was stirred at reduced pressure for 90 min. A solution of $CuSO_4.6H_2O$ (0.44 g, 2.75 mmol) was then added to the basic solution and stirring continued at reduced pressure for an additional 4 h. The aqueous solution was transferred by cannula to a flask containing ethyl 3,5-dibromo-4-hydroxy-[1,3,5-$^{13}C_3$]benzoate **160a** (1.5 g, 4.6 mmol). After allowing the blue suspension to stir at room temperature under argon for 90 min, the mixture was heated at reflux at 110 °C for 18 h, cooled and acidified with hydrochloric acid. The aqueous mixture was extracted with both diethyl ether (3 x 40 mL) and ethyl acetate (3 x 40 mL). The combined organic layers were washed with brine (2 x 40 mL) and dried ($MgSO_4$). Removal of the solvents at reduced pressure gave the crude product (0.6 g, 75%) which was a mixture of [1,3,5-$^{13}C_3$]gallic acid **32d** and 3,4-dihydroxy-[1,3,5-$^{13}C_3$]benzoic acid **163a** (10:1). Purification by column chromatography (silica gel, petroleum ether/ethanol, 10:1) gave pure **32d** as an off-white solid (0.38 g, 48%). mp 262-263 °C (lit[19] 258-260 °C for the unlabelled); υ_{max}/cm^{-1} 3272 and 2988 (OH), 1643 (C=O), 1574 (ArH), 1410, 1310 and 1262 (OH), 1219 and 1023 (C-O), 865, 760 and 731 (CH); δ_H (300 MHz, acetone-d_6) 7.02 (2H, dd, *J* 3.8, 1.9 Hz, CH-2,6), 7.90 (1H, s, OH), 8.10 (2H, s, (^{13}C)OH) and 10.60 (1H, brs, CO_2H); δ_C (75.45 MHz, acetone-d_6) 110.2 (CH-2,6), 122.0 (enhanced, ^{13}C-1), 140.5 (C-4), 146.0 (enhanced, d, *J* 2.4 Hz, ^{13}C-3,5) and 151.6 (C=O); *m/z* (CI^+) 174, [$(M+H)^+$, 100%], 156 [$(M-OH)^+$, 6]; HRMS (CI^+) [Found: $(M+H)^+$, 174.0397, $^{12}C_4{}^{13}C_3H_7O_5$ requires 174.0394]. Data is in agreement with the literature.[16,19] Data for 3,4,-dihydroxy-[1,3,5-$^{13}C_3$]benzoic acid **163a**: (41 mg, 6%). mp 183.5-184 °C (lit[20] 189 °C for the unlabelled); υ_{max}/cm^{-1} 3280 and 2990 (OH), 1656 (C=O), 1407 and 1275 (OH), 1231, 1113 and 1025 (C-O), 895 and 755 (CH); δ_H

(300 MHz, acetone-d_6) 6.77 (1H, ddt, *J* 159.6, 8.5, 6.9 Hz, ^{13}CH-5), 7.34 (1H, dm, *J* 8.5 Hz, CH-6) and 7.40 (1H, m, CH-2); δ_C (75.45 MHz, acetone-d_6) 115.7 (enhanced, d, *J* 6.2 Hz, ^{13}CH-5), 123.1 (enhanced, d, *J* 6.2 Hz, ^{13}C-1) and 145.5 (enhanced, d, *J* 6.2 Hz, ^{13}C-3); *m/z* (CI^+) 158 [$(MH)^+$, 100%], 157 [$(M)^+$, 7]; HRMS (CI^+) [Found: $(MH)^+$, 158.0445, $^{12}C_4{}^{13}C_3H_7O_4$ requires 158.0445]. Data is in agreement with the literature.[16,20]

4.6 Iridium Catalysed C-H Activation/Borylation/Oxidation for the Generation of Phenols

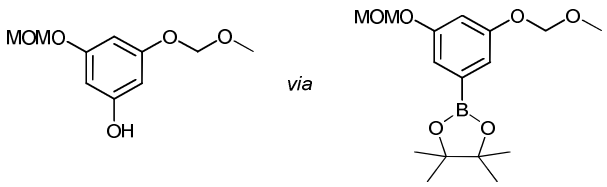
3,5-Dimethoxyphenol (188)



A solution of degassed 1,3-dimethoxybenzene **175** (138 mg, 1 mmol) in *iso*-hexane (5 mL) was transferred into an air-free flask containing B_2Pin_2 (254 mg, 1.0 mmol), $[Ir(OMe)(COD)]_2$ (6.6 mg, 1 mol%) and d*t*bpy (5.3 mg, 2 mol%). The flask was sealed and the reaction mixture stirred at 100 °C for 18 h. The *iso*-hexane was then removed at reduced pressure to give the intermediate borolane **182** (97% conversion) as an orange oil which was used without further purification. υ_{max}/cm^{-1} 1588 (ArH), 1421 (CH_3), 1370 (B-O), 1213 and 1151 (C-O), 780 (ArH) and 706 (B-C); δ_H (400 MHz, $CDCl_3$) 1.35 (12H, s, CH_3), 3.82 (6H, s, CH_3), 6.58 (1H, tm, *J* 2.4 Hz, CH-4) and 6.96 (2H, dm, *J* 2.4 Hz, CH-2,6); δ_C (100 MHz, $CDCl_3$) 24.8 (CH_3), 55.4 (CH_3), 83.8 ($CO(CH_3)_2$), 104.5 (CH-4), 116.3 (CH-2,6) and 160.3 (C-3,5); *m/z* (CI^+) 265 [$(M+H)^+$, 100%], 264 [$(M)^+$, 47]; HRMS (CI^+) [Found: $(M+H)^+$, 265.1616, $C_{14}H_{22}O_4B$ requires 265.1611].

Acetone (3.2 mL) was added to the crude borolane **182** and the mixture stirred to produce a homogeneous solution. An aqueous solution of Oxone® (738 mg, 1.2 mmol, in 3.2 mL H_2O) was then added dropwise over 2-4 min and the reaction mixture allowed to stir vigorously for 30 min. After this time, the reaction was quenched by the addition of saturated sodium sulfite solution (10 mL). The aqueous phase was extracted with diethyl ether. The organic solvents were removed at reduced pressure and the crude material dissolved in dichloromethane and passed through a plug of silica gel. The solvents were then removed at reduced pressure once more to give the crude product which contained traces of starting material (97% conversion). Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 3:1) to give the desired product **188** as a white solid (92 mg, 59%). mp 41-42 °C (lit[21] 40 °C); δ_H (400 MHz, $CDCl_3$) 3.76 (6H, s, CH_3), 6.04 (2H, d, *J* 2.2 Hz, CH-2,6) and 6.08 (1H, t, *J* 2.2 Hz, CH-4); δ_C (100 MHz, $CDCl_3$) 55.3 (CH_3), 93.1 (CH-4), 94.3 (CH-2,6), 157.4 (C-1) and 161.6 (C-3,5). Data is in agreement with the literature.[21]

3,5-Bis(methoxymethyl)phloroglucinol (189)

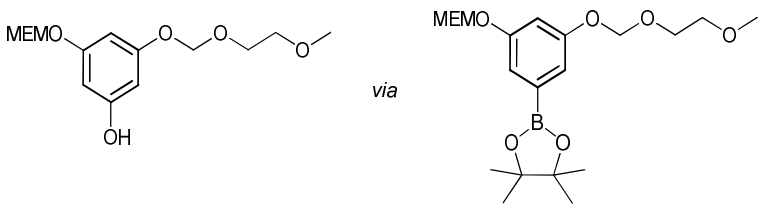


A solution of degassed 1,3-dimethoxymethylresorcinol **176** (495 mg, 2.5 mmol) in *iso*-hexane (10 mL) was transferred into an air-free flask containing B_2Pin_2 (635 mg, 2.5 mmol), $[Ir(OMe)(COD)]_2$ (16.6 mg, 1 mol%) and d*t*bpy (13.4 mg, 2 mol%). The flask was sealed and the reaction mixture stirred at 100 °C for 18 h. *Iso*-hexane was then removed at reduced pressure to give the intermediate borolane **183** as an orange oil which was used without further purification (810 mg, quant.). υ_{max}/cm^{-1} 2976 (CH_3, CH_2), 1586 (ArH), 1431 (CH_3, CH_2), 1355 (B-O), 1212, 1145, 1082 and 1027 (C-O), 989 and 850 (ArH) and 706 (B-C); δ_H (400 MHz, $CDCl_3$) 1.25 (12H, s, CH_3), 3.40 (6H, s, CH_3), 5.10 (4H,

s, CH_2), 6.75 (1H, t, *J* 2.3 Hz, CH-4) and 7.04 (2H, d, *J* 2.3 Hz, CH-2,6); δ_C (100 MHz, $CDCl_3$) 25.0 (CH_3), 25.3 (CH_3), 56.5 (CH_3), 84.3 ($CO(CH_3)_2$), 94.8 (CH_2), 108.8 (CH-4), 115.8 (CH-2,6) and 158.2 (C-3,5); *m/z* (CI^+) 325 [$(M+H)^+$, 30%]; 324 [$(M)^+$, 45]; 293 [$(M-(CH_3)_2)^+$, 100]; HRMS (CI^+) [Found: $(M+H)^+$, 325.1817, $C_{16}H_{26}O_6B$ requires 325.1822].

Acetone (6.4 mL) was added to the crude borolane **183** and the mixture stirred to produce a homogeneous solution. An aqueous solution of Oxone® (1475 mg, 2.4 mmol, in 6.4 mL H_2O) was then added dropwise over 2-4 min and the reaction mixture allowed to stir vigorously for 30 min. After this time, the reaction was quenched by the addition of saturated sodium sulfite solution (20 mL). The aqueous phase was extracted with diethyl ether. The organic solvents were removed at reduced pressure and the crude material dissolved in dichloromethane and passed through a plug of silica gel. The solvents were then removed at reduced pressure once more to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 3:1) to give the desired product **189** as a colourless oil (325 mg, 61%). υ_{max}/cm^{-1} 3365 (OH), 2958 (CH_3, CH_2, OH), 1601 (ArH), 1498 and 1467 (CH_3, CH_2), 1214, 1149 and 1031 (C-O), 922 and 828 (ArH); δ_H (400 MHz, $CDCl_3$) 3.39 (6H, s, CH_3), 5.03 (4H, s, CH_2), 6.14-6.17 (2H, m, CH-2,6), 6.20-6.23 (1H, m, CH-4) and 6.62 (1H, brs, OH); δ_C (100 MHz, $CDCl_3$) 56.0 (CH_3), 94.3 (CH_2), 97.2 (CH-4), 97.6 (CH-2,6), 157.8 (C-1) and 159.0 (C-3,5); *m/z* (CI^+) 325 [$(M+H)^+$, 30%], 215 [$(M+H)^+$, 60]; 183 [$(M-(CH_3)_2)^+$, 95]; HRMS (CI^+) [Found: $(M+H)^+$, 215.0919, $C_{10}H_{15}O_5$ requires 215.0919].

3,5-Bis(2-methoxyethoxymethoxy)phenol (190)

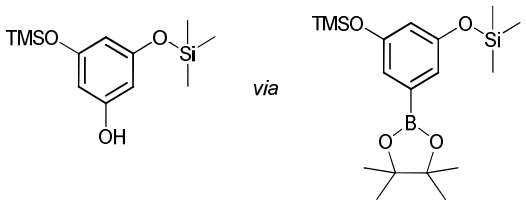


Degassed *iso*-hexane (10 mL) was transferred into an air-free flask containing 1,3-dimethoxyethoxymethyl resorcinol **177** (716 mg, 2.5 mmol) B_2Pin_2 (635 mg, 2.5 mmol), $[Ir(OMe)(COD)]_2$ (16.6 mg, 1 mol%) and d*t*bpy (13.4 mg, 2 mol%). The flask was sealed and the reaction mixture stirred at 100 °C for 18 h. The *iso*-hexane was then removed at reduced pressure to give the intermediate borolane **184** (99% conversion) as an orange oil which was used without further purification. υ_{max}/cm^{-1} 2955 and 1442 (CH_3, CH_2), 1579 (ArH), 1361 (B-O), 1210, 1131 and 1030 (C-O), 980 and 851 (ArH) and 713 (B-C); δ_H (400 MHz, $CDCl_3$) 1.25 (12H, s, CH_3), 3.31 (6H, s, CH_3), 3.46-3.51 (4H, m, CH_2OCH_3), 3.72-3.76 (4H, m, CH_2O), 5.19 (4H, s, OCH_2O), 6.76 (1H, t, *J* 2.4 Hz, CH-4) and 7.05 (2H, d, *J* 2.4 Hz, CH-2,6); δ_c (100 MHz, $CDCl_3$) 24.9 (CH_3), 59.0 (CH_3), 67.7 (CH_2OCH_3), 71.6 (CH_2O), 83.9 ($CO(CH_3)_2$), 93.4 (OCH_2O), 108.3 (CH-4), 115.5 (CH-2,6), 129.9 (C-1) and 157.8 (C-3,5); *m/z* (ES^+) 435 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 435.2156, $C_{20}H_{33}BO_8Na$ requires 435.2166].

Acetone (6.4 mL) was added to the crude borolane **184** and the mixture stirred to produce a homogeneous solution. An aqueous solution of Oxone® (1.48 g, 2.4 mmol, in 6.4 mL H_2O) was then added dropwise over 2-4 min and the reaction mixture allowed to stir vigorously for 30 min. After this time, the reaction was quenched by the addition of saturated sodium sulfite solution (20 mL). The aqueous phase was extracted with ethyl acetate. The organic solvents were removed at reduced pressure and the crude material dissolved in dichloromethane and passed through a plug of silica gel. The solvents were then removed at reduced pressure once more to give the crude product which contained a trace of starting material. Purification was by

column chromatography (silica gel, petroleum ether/ethyl acetate, 1:1) to give the desired product **190** as an off-white oil (468 mg, 62%). υ_{max}/cm^{-1} 3381 (OH), 2952 and 2899 (CH_3, CH_2, OH), 1598 (ArH), 1495 and 1469 (CH_3, CH_2), 1213, 1149 and 1029 (C-O), 918 and 830 (ArH); δ_H (400 MHz, $CDCl_3$) 3.31 (6H, s, CH_3), 3.48-3.52 (4H, m, CH_2OCH_3), 3.71-3.76 (4H, m, CH_2O), 5.12 (4H, s, OCH_2O), 6.19 (2H, d, *J* 2.1 Hz, CH-2,6) and 6.22 (1H, t, *J* 2.1 Hz, CH-4); δ_c (100 MHz, $CDCl_3$) 59.0 (CH_3), 67.6 (CH_2O), 71.6 (CH_2OCH_3), 93.5 (OCH_2O), 97.4 (CH-4), 97.6 (CH-2,6), 157.7 (C-1) and 159.0 (C-3,5); *m/z* (ES^+) 325 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 325.1262, $C_{14}H_{22}O_7Na$ requires 325.1263].

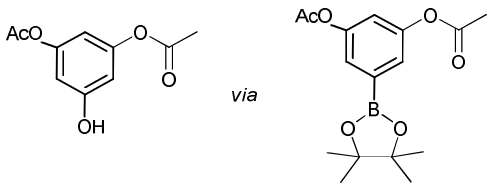
Attempted preparation of 3,5-bis[(trimethylsilyl)oxy]phenol (193)



Degassed *iso*-hexane (5 mL) was transferred into an air-free flask containing 1,3-di(trimethylsilyl) resorcinol **179** (318 mg, 1.25 mmol) B_2Pin_2 (318 mg, 1.25 mmol), $[Ir(OMe)(COD)]_2$ (8.3 mg, 1 mol%) and d*t*bpy (6.7 mg, 2 mol%). The flask was sealed and the reaction mixture stirred at 100 °C for 18 h. The *iso*-hexane was then removed at reduced pressure to give the intermediate borolane **187** (92% conversion) as an orange oil which was used without further purification. δ_H (500 MHz, $CDCl_3$) 0.08 (18H, s, CH_3), 1.15 (12H, s, CH_3), 6.27 (1H, t, *J* 2.3 Hz, CH-4) and 6.74 (2H, d, *J* 2.3 Hz, CH-2,6); δ_c (125 MHz, $CDCl_3$) 0.00 (CH_3), 24.6 (CH_3), 83.5 ($CO(CH_3)_2$), 114.8 (CH-4), 119.3 (CH-2,6) and 155.4 (C-3,5); *m/z* (ES^+) 403 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 403.1896, $C_{18}H_{33}BO_4NaSi_2$ requires 403.1908].

Acetone (3.2 mL) was added to the crude borolane **187** and the mixture stirred to produce a homogeneous solution. An aqueous solution of Oxone® (738 mg, 1.2 mmol, in 3.2 mL H_2O) was then added dropwise over 2-4 min and the reaction mixture allowed to stir vigorously for 30 min. After this time, the reaction was quenched by the addition of saturated sodium sulfite solution (10 mL). The aqueous phase was extracted with ethyl acetate. The organic solvents were removed at reduced pressure and the crude material dissolved in dichloromethane and passed through a plug of silica gel. The solvents were then removed at reduced pressure once more to give the crude product, which appeared to have undergone deprotection to phloroglucinol **26** during the oxidation. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 2:1) to give phloroglucinol **26** as a white solid (138 mg, 87%). mp 214-214.5 °C (lit[22] 216 °C); δ_H (300 MHz, $CDCl_3$-DMSO-d_6) 5.82 (3H, s, CH-2,4,6); δ_C (100 MHz, $CDCl_3$-DMSO-d_6) 94.8 (CH-2,4,6) and 158.8 (C-1,3,5). Data is in agreement with the literature.[22]

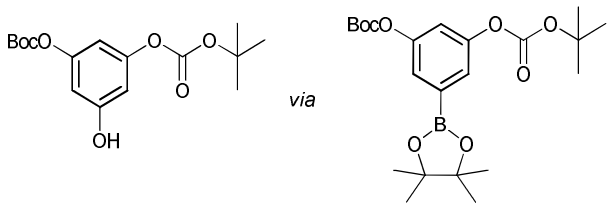
3,5-Diacetoxyphenol (191)



A solution of degassed HBPin (128 mg, 1.0 mmol) in *iso*-hexane (5 mL) was transferred into an air-free flask containing 1,3-diacetoxybenzene **180** (194 mg, 1.0 mmol) B_2Pin_2 (254 mg, 1.0 mmol), $[Ir(OMe)(COD)]_2$ (13.3 mg, 2 mol%) and dppe (8.0 mg, 2 mol%). The flask was sealed and the reaction mixture stirred at 100 °C for 18 h. The *iso*-hexane was then removed at reduced pressure to give the intermediate borolane **185** (45% conversion) as an orange oil which was used without further purification. δ_H (400 MHz, $CDCl_3$) 1.30 (12H, s, CH_3), 2.32 (6H, s, CH_3), 7.01 (1H, m, CH-4) and 7.41 (2H, d, *J* 2.2 Hz, CH-2,6).

Acetone (3.2 mL) was added to the crude borolane **185** and the mixture stirred to produce a homogeneous solution. An aqueous solution of Oxone® (738 mg, 1.2 mmol, in 3.2 mL H_2O) was then added dropwise over 2-4 min and the reaction mixture allowed to stir vigorously for 30 min. After this time, the reaction was quenched by the addition of saturated sodium sulfite solution (10 mL). The aqueous phase was extracted with diethyl ether. The organic solvents were removed at reduced pressure and the crude material dissolved in dichloromethane and passed through a plug of silica gel. The solvents were then removed at reduced pressure once more to give the crude product which contained a significant amount of starting material (45% conversion). Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 3:1) to give the desired product **191** as an off-white solid (68 mg, 44%). mp 165 °C (lit[23] 168 °C); δ_H (400 MHz, $CDCl_3$) 2.18 (6H, s, CH_3), 6.32 (1H, t, *J* 2.0 Hz, CH-4), 6.40 (2H, d, *J* 2.0 Hz, CH-2,6) and 8.40 (1H, brs, OH). Data is in agreement with the literature.[23]

Di-*tert*-butyl 5-hydroxy-1,3-phenylene biscarbonate (192)



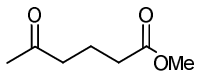
Degassed *iso*-hexane (5 mL) was transferred into an air-free flask containing di-*tert*-butyl 1,3-phenylenebiscarbonate **178** (388 mg, 1.25 mmol) B_2Pin_2 (476 mg, 1.88 mmol), $[Ir(OMe)(COD)]_2$ (9.9 mg, 1.2 mol%) and d*t*bpy (6.7 mg, 2 mol%). The flask was sealed and the reaction mixture stirred at 100 °C for 18 h. The *iso*-hexane was then removed at reduced pressure to give the intermediate borolane **186** (99% conversion) as an orange oil which was used without further purification. υ_{max}/cm^{-1} 2965 (CH_3), 1735 (C=O), 1377 (B-O), 1215 and 1126 (C-O), 856 and 775 (ArH) and 710 (B-C); δ_H (400 MHz, $CDCl_3$) 1.25 (12H, s, CH_3), 1.47 (18H, s, CH_3), 7.04 (1H, t, *J* 2.3 Hz, CH-2) and 7.40 (2H, d, *J* 2.3 Hz, CH-

4,6); δ_c (100 MHz, $CDCl_3$) 25.0 (CH_3), 27.9 (CH_3), 83.2 ($C(CH_3)_3$), 84.2 ($CO(CH_3)_2$), 117.8 (CH-2), 124.6 (CH-4,6), 151.5 (C-1,3) and 156.7 (C=O); *m/z* (ES^+) 459 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 459.2165, $C_{22}H_{33}BO_8Na$ requires 459.2166].

Acetone (4.0 mL) was added to the crude borolane **186** and the mixture stirred to produce a homogeneous solution. An aqueous solution of Oxone® (984 mg, 1.6 mmol, in 4.0 mL H_2O) was then added dropwise over 2-4 min and the reaction mixture allowed to stir vigorously for 30 min. After this time, the reaction was quenched by the addition of saturated sodium sulfite solution (10 mL). The aqueous phase was extracted with ethyl acetate. The organic solvents were removed at reduced pressure and the crude material dissolved in dichloromethane and passed through a plug of silica gel. The solvents were then removed at reduced pressure once more to give the crude product which contained a trace of starting material. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 3:1) to give the desired product **192** as a pale yellow oil (415 mg, 51%). υ_{max}/cm^{-1} 3373 (OH), 2950 and 2871 (CH_3, OH), 1597 (ArH), 1481 (CH_3), 1210 and 1124 (C-O), 915 (ArH); δ_H (400 MHz, $CDCl_3$) 1.48 (18H, s, CH_3), 6.45 (2H, d, *J* 2.1 Hz, CH-4,6) and 6.57 (1H, t, *J* 2.1 Hz, CH-2); δ_c (100 MHz, $CDCl_3$) 27.7 (CH_3), 84.0 ($C(CH_3)_3$), 106.5 (CH-2), 106.9 (CH-4,6), 151.4 (C=O), 151.9 (C-1,3) and 157.0 (C-5); *m/z* (ES^+) 349 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 349.1263, $C_{16}H_{22}O_7Na$ requires 349.1263].

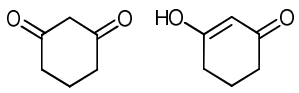
4.7 Synthesis of Phloroglucinol (26)

Methyl 5-oxo-hexanoate (195)



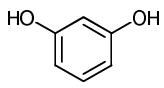
To dry diethyl ether (70 mL) under an argon atmosphere, were added lithium pieces (489 mg, 70.5 mmol). Methyl iodide (1.1 mL, 2.5 g, 17.6 mmol) was added and the reaction mixture stirred at room temperature for 1 h. After this time, the reaction mixture was cooled to 0 °C and copper iodide (3.35 g, 17.6 mmol) added. After stirring for 30 min at 0 °C, the resulting lithium dimethylcuprate was cooled to -20 °C. Pre-cooled methyl 4-chloroformylbutyrate **194** (2 mL, 2.39 g, 14.5 mmol) was then added dropwise with vigorous stirring. The temperature was maintained at -20 °C for 1 h and the reaction mixture stirred at room temperature overnight. The reaction was quenched with saturated ammonium chloride solution (45 mL) and filtered to remove the copper residue. The filtrate was extracted with diethyl ether (3 x 20 mL), washed with brine (20 mL) and dried ($MgSO_4$). The solvents were removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethanol, 15:1) to give **195** as a colourless oil (1.861 g, 89%). δ_H (400 MHz, $CDCl_3$) 1.90 (2H, quintet, *J* 7.2 Hz, CH_2-3), 2.15 (3H, s, CH_3), 2.35 (2H, t, *J* 7.2 Hz, CH_2-2), 2.51 (2H, t, *J* 7.2 Hz, CH_2-4) and 3.68 (3H, s, OCH_3); δ_C (100 MHz, $CDCl_3$) 18.8 (CH_2-3), 29.9 (CH_3), 33.0 (CH_2-2), 42.4 (CH_2-4), 51.6 (OCH_3), 173.6 (*C*O_2Me) and 208.0 (C=O); *m/z* (ES^+) 167 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 167.0678, $C_7H_{12}O_3Na$ requires 167.0684]. Data is in agreement with the literature.[24]

Cyclohexane-1,3-dione (196)



To a solution of methyl 5-oxo-hexanoate **195** (0.3 g, 2.1 mmol) in dry tetrahydrofuran (40 mL), was added dry potassium *tert*-butoxide (0.93 g, 8.3 mmol). The mixture was heated at reflux for 6 h, after which time the solvents were removed at reduced pressure. The residue was dissolved in water (10 mL) and acidified to pH 1 with concentrated hydrochloric acid. The aqueous layer was extracted with ethyl acetate (6 x 10 mL) and washed with 1M sodium hydroxide solution (10 mL). The organic layer was then dried ($MgSO_4$) and the solvents removed at reduced pressure to give an orange solid. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 1:4) to give **196** as a pale yellow solid (0.174 g, 74%). mp 103-104 °C (lit[25] 105 °C); Keto and enol tautomers were observed in the ^1H NMR spectrum. Data for keto form: δ_H (400 MHz, $CDCl_3$) 1.91-2.00 (2H, m, CH_2-5), 2.54 (4H, t, *J* 6.7 Hz, CH_2-4,6) and 3.37 (2H, s, CH_2-2); δ_C (100 MHz, $CDCl_3$) 17.6 (CH_2-5), 40.1 (CH_2-4,6), 57.9 (CH_2-2) and 204.4 (C-1,3); Data for enol form: δ_H (400 MHz, $CDCl_3$) 1.91-2.00 (2H, m, CH_2-5), 2.31 (4H, t, *J* 6.3 Hz, CH_2-4,6) and 5.30 (1H, s, CH-2); δ_C (100 MHz, $CDCl_3$) 20.7 (CH_2-5), 31.6 (CH_2-4,6), 103.8 (CH-2) and 188.1 (C-3); *m/z* (CI^+) 113 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 113.0601, $C_6H_9O_2$ requires 113.0603]. Data is in agreement with the literature.[25]

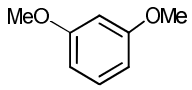
Resorcinol (20)



To a flask containing cyclohexane-1,3-dione **196** (0.25 g, 2.23 mmol) and xylene (40 mL), was added palladium on carbon (10%, 1.26 g). The mixture was heated at reflux for 3 h, then the palladium catalyst filtered off through a bed of celite. The reaction mixture was extracted with aqueous sodium hydroxide (20%, 4 x 30 mL). The combined aqueous layers

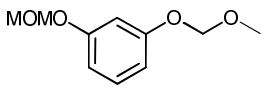
were cooled to 0 °C, acidified to pH 2 with concentrated hydrochloric acid and extracted with diethyl ether (3 x 30 mL). The solvents were then removed at reduced pressure to give an orange oil. Purification was by column chromatography (silica gel, diethyl ether/petroleum ether, 2:1) to give **20** as a white solid (76 mg, 29%). mp 109-109.5 °C (lit[26] 109-111 °C); δ_H (400 MHz, acetone-d_6) 6.34 (2H, dd, *J* 8.0, 2.3 Hz, CH-4,6), 6.37 (1H, t, *J* 2.3 Hz, CH-2) and 6.99 (1H, t, *J* 8.0 Hz, CH-5); δ_C (75.45 MHz, acetone-d_6) 103.9 (CH-2), 108.0 (CH-4,6), 131.2 (CH-5) and 159.9 (C-1,3). Data is in agreement with the literature.[26]

1,3-Dimethoxybenzene (175)



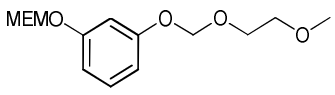
To a solution of resorcinol **20** (0.1 g, 9.1 mmol) and cesium carbonate (0.59 g, 1.82 mmol) in acetonitrile (40 mL) was added methyl iodide (0.64 g, 4.54 mmol). The mixture was heated at reflux for 5 h, after which time the solvents were removed at reduced pressure. The residue was dissolved in diethyl ether (2 x 100 mL), washed with water (3 x 30 mL) and the combined organic layers dried ($MgSO_4$). The solvents were removed at reduced pressure to give the product **175** as a pale yellow oil which could be used without further purification (0.12 g, 96%). δ_H (300 MHz, $CDCl_3$) 3.84 (6H, s, CH_3), 6.52-6.55 (1H, m, CH-2), 6.57 (2H, d, *J* 2.4 Hz, CH-4,6) and 7.24 (1H, t, *J* 8.3 Hz, CH-5); δ_C (75.45 MHz, $CDCl_3$) 55.7 (CH_3), 100.9 (CH-2), 106.6 (CH-4,6), 130.3 (CH-5) and 161.3 (C-1,3); *m/z* (CI^+) 139 [$(M+H)^+$, 100%], 138 [$(M)^+$, 21]; HRMS (CI^+) [Found: $(M+H)^+$, 139.0760, $C_8H_{11}O_2$ requires 139.0759]. Data is in agreement with the literature.[27]

1,3-Dimethoxymethylresorcinol (176)



Sodium hydride (4.8 g, 120 mmol) was washed with dry hexane and the residue suspended in dry diethyl ether (20 mL). Dry dimethylformamide (4.4 mL) was then added and the grey suspension cooled to 0 °C and resorcinol **20** (2.2 g, 20 mmol) added. The reaction mixture was allowed to stir for 30 min until gas evolution had ceased. Chloromethyl methyl ether (MOMCl) (3.1 mL, 40 mmol) was then added and the reaction mixture allowed to stir at room temperature overnight. After this time, excess sodium hydride was destroyed by the careful addition of water. The reaction mixture was diluted with diethyl ether (100 mL), washed with water (3 x 100 mL) and brine (2 x 100 mL). The organic layers were dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 3:1) to yield **176** as a colourless oil (3.25 g, 82%). δ_H (400 MHz, $CDCl_3$) 3.50 (6H, s, CH_3), 5.18 (4H, s, CH_2), 6.60-6.64 (2H, dd, *J* 8.1, 2.3 Hz, CH-4,6), 6.66 (1H, t, *J* 2.3 Hz, CH-2) and 7.21 (1H, t, *J* 8.1 Hz, CH-5); δ_C (100 MHz, $CDCl_3$) 56.0 (CH_3), 94.5 (CH_2), 105.0 (CH-2), 109.6 (CH-4,6), 130.0 (CH-5) and 158.4 (C-1,3). Data is in agreement with the literature.[28]

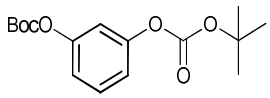
1,3-Dimethoxyethoxymethylresorcinol (177)



Sodium hydride (2.4 g, 60 mmol) was washed with dry hexane and the residue suspended in dry diethyl ether (10 mL). Dry dimethylformamide (2.2 mL) was then added and the grey suspension cooled to 0 °C and resorcinol **20** (1.1 g, 10 mmol) added. The reaction mixture was allowed to stir for 30 min until gas evolution had ceased. 2-Methoxyethoxymethyl chloride (MEMCl) (2.4 mL, 21 mmol) was then added and the reaction mixture allowed to stir at room temperature overnight. After this time, excess sodium hydride was destroyed

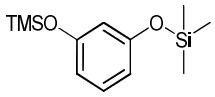
by the careful addition of water. The reaction mixture was diluted with diethyl ether (50 mL), washed with water (3 x 50 mL) and brine (2 x 50 mL). The organic layers were dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 2:1) to yield **177** as a colourless oil (2.08 g, 73%). δ_H (400 MHz, $CDCl_3$) 3.39 (6H, s, CH_3), 3.54-3.60 (4H, m, CH_2OCH_3), 3.79-3.87 (4H, m, CH_2O), 5.26 (4H, s, OCH_2O), 6.72 (2H, dd, *J* 8.2, 2.3 Hz, CH-4,6), 6.76 (1H, t, *J* 2.3 Hz, CH-2) and 7.19 (1H, t, *J* 8.2 Hz, CH-5); δ_C (100 MHz, $CDCl_3$) 59.0 (CH_3), 67.7 (CH_2O), 71.6 (CH_2OCH_3), 93.5 (OCH_2O), 105.1 (CH-2), 109.6 (CH-4,6), 129.9 (CH-5) and 158.3 (C-1,3); *m/z* (ES^+) 309 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 309.1325, $C_{14}H_{22}O_6Na$ requires 309.1314].

Di-*tert*-butyl 1,3-phenylenebiscarbonate (178)



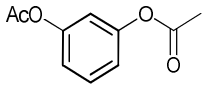
A solution of resorcinol **20** (1.1 g, 10 mmol), Boc_2O (4.8 g, 22 mmol) and DMAP (0.07 g, 0.5 mmol) in hexane was stirred at room temperature for 12 h. After this time, water (40 mL) was added and the aqueous phase extracted with ethyl acetate (3 x 40 mL). The combined organic layers were washed with water (2 x 40 mL), 1M hydrochloric acid (40 mL) and brine (40 mL). The organic layers were dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 2:1) to yield **178** as a colourless oil (3.01 g, 97%). mp 35-36 °C; δ_H (400 MHz, $CDCl_3$) 1.55 (18H, s, CH_3), 7.08 (2H, dd, *J* 8.2, 2.3 Hz, CH-4,6), 7.11 (1H, t, *J* 2.3 Hz, CH-2) and 7.35 (1H, t, *J* 8.2 Hz, CH-5); δ_C (100 MHz, $CDCl_3$) 27.7 (CH_3), 83.8 ($C(CH_3)_3$), 114.8 (CH-2), 118.5 (CH-4,6), 129.5 (CH-5) and 151.4 (C-1,3); *m/z* (ES^+) 333 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 333.1307, $C_{16}H_{22}O_6Na$ requires 333.1314].

1,3-Di[(trimethylsilyl)oxy]resorcinol (179)



To a solution of resorcinol **20** in pyridine (30 mL) was added a catalytic amount of DMAP and trimethylsilyl chloride (TMSCl, 4.3 mL, 40 mmol). The reaction mixture was stirred at 30 °C for 12 h, after which time water (40 mL) was added. The aqueous phase was extracted with ethyl acetate (3 x 30 mL), and the combined organic layers washed with dilute ammonium chloride solution (2 x 30 mL) and brine (30 mL). The organic layers were dried ($MgSO_4$) and the solvents removed at reduced pressure to give **179** as a colourless oil (2.06 g, 81%). δ_H (400 MHz, $CDCl_3$) 0.20 (18 H, s, CH_3), 6.30 (1H, t, *J* 2.3 Hz, CH-2), 6.42 (2H, dd, *J* 8.1, 2.3 Hz, CH-4,6) and 7.01 (1H, t, *J* 8.1 Hz, CH-5); δ_C (100 MHz, $CDCl_3$) -0.3 (CH_3), 112.1 (CH-2), 113.3 (CH-4,6), 129.4 (CH-5) and 155.9 (C-1,3); *m/z* (CI^-) 181 [$(M\text{-}Si(CH_3))^-$, 100%]. Data is in agreement with the literature.[29]

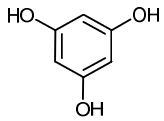
1,3-Diacetoxybenzene (180)



Resorcinol **20** (2 g, 18.2 mmol) was dissolved in pyridine (40 mL) and acetic anhydride (16 mL) added. The reaction mixture was stirred at room temperature overnight. After removal of the solvents at reduced pressure the residue was dissolved in ethyl acetate (60 mL) and dilute ammonium chloride solution (160 mL). The aqueous layer was extracted with ethyl acetate (2 x 60 mL), the combined organic layers washed with dilute ammonium chloride solution (2 x 60 mL), dried ($MgSO_4$) and solvents removed at reduced pressure to give the desired di-acetylated product **180** as a colourless oil. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 3:1) to yield **180** as a colourless oil (2.6 g, 74%). δ_H (300 MHz, $CDCl_3$) 2.21 (6H, s, CH_3), 6.85 (1H, t, *J* 2.2 Hz,

CH-2), 6.91 (2H, dd, *J* 8.2, 2.2 Hz, CH-4,6) and 7.29 (1H, t, *J* 8.2 Hz, CH-5); δ_C (100 MHz, $CDCl_3$) 21.1 (CH_3), 115.5 (CH-2), 119.0 (CH-4,6), 129.7 (CH-5), 151.1 (C-1,3) and 169.1 (C=O). Data is in agreement with the literature.[30]

Phloroglucinol (26)



Method 1: To a solution of 3,5-dimethoxyphenol **188** (0.154 g, 1 mmol) in dichloromethane (5.3 mL) at -78 °C under an argon atmosphere was added boron tribromide solution (1M in dichloromethane, 10 mL, 10 mmol). The reaction mixture was stirred at -78 °C for 1 h, then allowed to warm to room temperature. After stirring overnight at room temperature, the solution was cooled to 0 °C and water (3 mL) added. The solvents were then removed at reduced pressure and the aqueous phase extracted with ethyl acetate (3 x 25 mL). The organic layers were combined, dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as a light brown solid. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 1:3) to give **26** as an off-white solid (0.126 g, quant.). mp 213-214 °C (lit[22] 216 °C); δ_H (300 MHz, $CDCl_3$-DMSO-d_6) 5.82 (3H, s, CH-2,4,6); δ_C (100 MHz, $CDCl_3$-DMSO-d_6) 94.8 (CH-2,4,6) and 158.8 (C-1,3,5). Data is in agreement with the literature.[22]

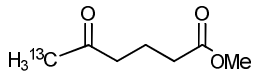
Method 2: To a solution of 1,3-dimethoxymethylresorcinol **189** (0.05 g, 0.234 mmol) in dichloromethane (4.5 mL) was added $NaHSO_4.SiO_2$ catalyst* (0.1 g). The resulting suspension was stirred at room temperature overnight. After this time the colourless supernatant containing an orange/red solid was poured into ice-cold water (10 mL). The aqueous layer was extracted with ethyl acetate (3 x 10 mL). The organic layers were

combined, dried ($MgSO_4$) and the solvents removed at reduced pressure to give **26** as a colourless oil (0.03 g, quant.). Spectroscopic data as before.

* $NaHSO_4.SiO_2$ catalyst was prepared by mixing $NaHSO_4.H_2O$ (4.14 g), silica gel (column chromatographic grade, 60 Å, 200-400 mesh) (10 g) and water (25 mL) and stirred for 15 min at 60 °C until a white powder was formed. The resulting powder was placed in an oven at 120 °C for 48 h to dry further before use.

4.8 Synthesis of [2-^{13}C]Phloroglucinol (26c)

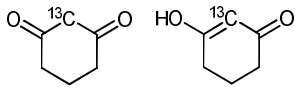
Methyl 5-oxo-[6-^{13}C]hexanoate (195a)



To dry diethyl ether (300 mL) under an argon atmosphere, were added lithium pieces (2.04 g, 294 mmol). [^{13}C]Methyl iodide (10 g, 70.5 mmol) was added and the reaction mixture stirred at room temperature for 1 h. After this time, the reaction mixture was cooled to 0 °C and copper iodide (13.4 g, 70.5 mmol) added. After stirring for 30 min at 0 °C, the resulting lithium dimethylcuprate was cooled to -20 °C. Pre-cooled methyl 4-chloroformylbutyrate **194** (7 mL, 8.33 g, 50.6 mmol) was then added dropwise with vigorous stirring. The temperature was maintained at -20 °C for 1 h and the reaction mixture stirred at room temperature overnight. The reaction was quenched with saturated ammonium chloride solution (90 mL) and filtered to remove the copper residue. The filtrate was extracted with diethyl ether (3 x 40 mL), washed with brine (40 mL) and dried ($MgSO_4$). The solvents were removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 9:1) to give **195a** as a colourless oil (3.17 g, 43%). δ_H (400 MHz, $CDCl_3$) 1.85 (2H, quintet, *J*

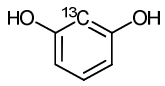
7.2 Hz, CH_2-3), 1.94 (3H, d, *J* 127.1 Hz, $^{13}CH_3$), 2.31 (2H, t, *J* 7.2 Hz, CH_2-2), 2.48 (2H, t, *J* 7.2 Hz, CH_2-4) and 3.63 (3H, s, OCH_3); δ_C (100 MHz, $CDCl_3$) 18.8 (CH_2-3), 29.9 (enhanced, $^{13}CH_3$), 32.9 (CH_2-2), 42.4 (d, *J* 14.8 Hz, CH_2-4), 51.5 (OCH_3), 173.5 (CO_2Me) and 207.9 (d, *J* 41.2 Hz, C=O); *m/z* (ES^+) 168 [$(M+Na)^+$, 45%]. Data is in agreement with the literature.[31]

[2-^{13}C]Cyclohexane-1,3-dione (196a)



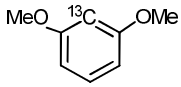
To a solution of methyl 5-oxo-[6-^{13}C]hexanoate **195a** (2.1 g, 14.5 mmol) in dry tetrahydrofuran (280 mL), was added dry potassium *tert*-butoxide (6.5 g, 57.9 mmol). The mixture was heated at reflux for 7 h, after which time the solvents were removed at reduced pressure. The residue was dissolved in water (120 mL) and acidified to pH 1 with concentrated hydrochloric acid. The aqueous layer was extracted with ethyl acetate (6 x 120 mL). The organic layer was then dried ($MgSO_4$) and the solvents removed at reduced pressure to give an orange solid. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 1:4) to give **196a** as a pale yellow solid (1.13 g, 69%). mp 106-106.5 °C (lit[25] 105 °C for the unlabelled); Keto and enol tautomers were observed in the ^{1}H NMR spectrum. Data for keto form: δ_H (400 MHz, $CDCl_3$) 1.88-2.00 (2H, m, CH_2-5), 2.55 (4H, t, *J* 6.5 Hz, CH_2-4,6) and 3.36 (2H, d, *J* 130.1 Hz, $^{13}CH_2$-2); δ_C (100 MHz, $CDCl_3$) 18.4 (CH_2-5), 39.9 (d, *J* 11.1 Hz, CH_2-4,6), 58.4 (enhanced, $^{13}CH_2$-2) and 204.0 (d, *J* 36.1 Hz, C=O); Data for enol form: δ_H (400 MHz, $CDCl_3$) 1.88-2.00 (2H, m, CH_2-5), 2.36 (4H, dt, *J* 6.5, 1.8 Hz, CH_2-4,6), 5.54 (1H, d, *J* 161.0 Hz, ^{13}CH-2) and 8.69 (1H, s, OH); δ_C (100 MHz, $CDCl_3$) 21.0 (CH_2-5), 32.1 (d, *J* 6.9 Hz, CH_2-4,6), 104.3 (enhanced, ^{13}CH-2) and 188.2 (C=O); *m/z* (CI^+) 114 [$(M+H)^+$, 100%]. Data is in agreement with the literature.[25,31]

[2-^{13}C]Resorcinol (20f)



To a flask containing [2-^{13}C]cyclohexane-1,3-dione **196a** (0.288 g, 2.55 mmol) and xylene (50 mL), was added palladium on carbon (10%, 1.44 g). The mixture was heated at reflux for 3 h, then the palladium catalyst filtered off through a bed of celite. The reaction mixture was extracted with aqueous sodium hydroxide (20%, 4 x 100 mL). The combined aqueous layers were cooled to 0 °C, acidified to pH 2 with concentrated hydrochloric acid and extracted with diethyl ether (3 x 100 mL) and ethyl acetate (3 x 100 mL). The solvents were then removed at reduced pressure to give an orange oil. Purification was by column chromatography (silica gel, diethyl ether/petroleum ether, 2:1) to give **20f** as a white solid (248 mg, 88%). mp 108-109 °C (lit[26] 109-111 °C for the unlabelled); δ_H (400 MHz, acetone-d_6) 6.29-6.37 (2H, m, CH-4,6), 6.36 (1H, dt, *J* 157.0, 2.3 Hz, ^{13}CH-2), 6.98 (1H, dt, *J* 8.1, 1.2 Hz, CH-5) and 8.21 (2H, d, *J* 5.1 Hz, OH); δ_C (75.45 MHz, acetone-d_6) 103.5 (enhanced, ^{13}CH-2), 109.0 (CH-4,6), 130.7 (CH-5) and 159.9 (d, *J* 67.2 Hz, C-1,3). *m/z* (CI^+) 112 [$(M+H)^+$, 100%]. Data is in agreement with the literature.[26,31]

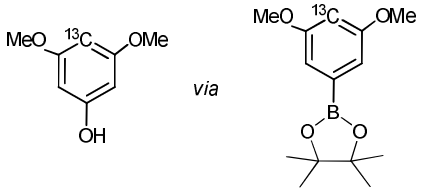
1,3-Dimethoxy-[2-^{13}C]benzene (175a)



To a solution of [2-^{13}C]resorcinol **20f** (0.24 g, 2.16 mmol) and cesium carbonate (1.41 g, 4.32 mmol) in acetonitrile (100 mL) was added methyl iodide (0.68 mL, 1.53 g, 10.8 mmol). The mixture was heated at reflux for 6 h, after which time the solvents were removed at reduced pressure. The residue was dissolved in diethyl ether (2 x 200 mL), washed with water (3 x 60 mL) and the combined organic layers dried ($MgSO_4$). The solvents were removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 4:1) to give **175a** as a

colourless oil (0.21 g, 70%). δ_H (400 MHz, $CDCl_3$) 3.70 (6H, s, CH_3), 6.39 (1H, dt, *J* 158.2, 2.4 Hz, ^{13}CH-2), 6.40-6.46 (2H, m, CH-4,6) and 7.11 (1H, dt, *J* 8.2, 1.3 Hz, CH-5); δ_C (100 MHz, $CDCl_3$) 55.3 (d, *J* 4.1 Hz, CH_3), 100.5 (enhanced, ^{13}CH-2), 106.1 (d, *J* 3.2 Hz, CH-4,6), 129.7 (d, *J* 5.2 Hz, CH-5) and 161.0 (d, *J* 70.0 Hz, C-1,3); *m/z* (CI^+) 140 [$(M+H)^+$, 100%], 139 [$(M)^+$, 15]; HRMS (CI^+) [Found: $(M+H)^+$, 140.0790, $^{13}C^{12}C_7H_{11}O_2$ requires 140.0793].

3,5-Dimethoxy-[4-^{13}C]phenol (188a)

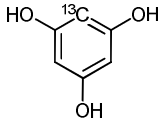


A solution of degassed 1,3-dimethoxy-[2-^{13}C]benzene **175a** (100 mg, 0.72 mmol) in *iso*-hexane (5 mL) was transferred into an air-free flask containing B_2Pin_2 (183 mg, 0.72 mmol), $[Ir(OMe)(COD)]_2$ (4.8 mg, 1 mol%) and d*t*bpy (3.9 mg, 2 mol%). The flask was sealed and the reaction mixture stirred at 100 °C for 18 h. The *iso*-hexane was then removed at reduced pressure to give the intermediate borolane **182a** (95% conversion) as an orange oil which was used without further purification. Analysis for crude intermediate: δ_H (300 MHz, $CDCl_3$) 1.35 (12H, s, CH_3), 3.82 (6H, s, CH_3), 6.58 (1H, dt, *J* 210.0, 3.2 Hz, ^{13}CH-4) and 6.96 (2H, dd, *J* 6.9, 3.2 Hz, CH-2,6); δ_C (100 MHz, $CDCl_3$)† 24.8 (CH_3), 55.5 (CH_3), 83.9 ($CO(CH_3)_2$), 104.4 (enhanced, ^{13}CH-4), 111.3 (CH-2,6) and 157.1 (C-3,5); *m/z* (CI^+) 266 [$(M+H)^+$, 100%], 265 [$(M)^+$, 30]; HRMS (CI^+) [Found: $(M+H)^+$, 266.1657, $^{13}C^{12}C_{13}H_{22}O_4B$ requires 266.1645]. † Observed by ^{1}H-^{13}C HSQC and/or ^{1}H-^{13}C HMBC, correlates with data from unlabelled compound.

Acetone (2.3 mL) was added to the crude borolane **182a** and the mixture stirred to produce a homogeneous solution. An aqueous solution of Oxone® (531 mg, 0.864 mmol, in 2.3 mL H_2O)

was then added dropwise over 2-4 min and the reaction mixture allowed to stir vigorously for 30 min. After this time, the reaction was quenched by the addition of saturated sodium sulfite solution (10 mL). The aqueous phase was extracted with diethyl ether. The organic solvents were removed at reduced pressure and the crude material dissolved in dichloromethane and passed through a plug of silica gel. The solvents were then removed at reduced pressure once more to give the crude product which contained traces of starting material. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 2:1) to give the desired 3,5-dimethoxy-[4-^{13}C]phenol **188a** as an off-white white solid (66 mg, 59%). mp 40-40.5 °C (lit[19] 40 °C for the unlabelled); δ_H (400 MHz, $CDCl_3$) 3.68 (6H, s, CH_3), 5.97 (2H, dd, *J* 4.7, 2.2 Hz, CH-2,6) and 5.98 (1H, dt, *J* 160.0, 2.2 Hz, ^{13}CH-4); δ_C (100 MHz, $CDCl_3$) 55.3 (d, *J* 4.1 Hz, CH_3), 92.9 (enhanced, ^{13}CH-4), 94.2 (d, *J* 3.3 Hz, CH-2,6), 157.7 (d, *J* 4.1 Hz, C-1) and 161.6 (d, *J* 71.0 Hz, C-3,5); *m/z* (CI^+) 156 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 156.0745, $^{13}C^{12}C_7H_{11}O_3$ requires 156.0742].

[2-^{13}C]Phloroglucinol (26c)

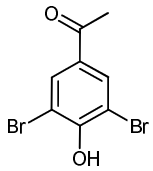


To a solution of 3,5-dimethoxy-[4-^{13}C]phenol **188a** (66 mg, 0.426 mmol) in dichloromethane (6 mL) at -78 °C under an argon atmosphere was added boron tribromide solution (1M in dichloromethane, 5 mL, 5 mmol). The reaction mixture was stirred at -78 °C for 1 h, then allowed to warm to room temperature. After stirring overnight at room temperature, the solution was cooled to 0 °C and water (3 mL) added. The solvents were then removed at reduced pressure and the aqueous phase extracted with ethyl acetate (3 x 15 mL). The organic layers were combined, dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as a light brown solid. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 1:1) to give **26c** as an off-

white solid (44 mg, 81%). mp 215-215.5 °C (lit[22] 216 °C for the unlabelled); δ_H (300 MHz, DMSO-d_6) 5.63 (1H, dt, *J* 158.0, 1.8 Hz, ^{13}CH-2), 5.63 (2H, dd, *J* 4.6, 1.8 Hz, CH-4,6) and 8.93 (3H, t, *J* 2.4 Hz, OH); δ_C (100 MHz, DMSO-d_6) 93.9 (enhanced, ^{13}CH-2 and CH-4,6), 158.8 (d, *J* 66.4 Hz, C-1,3) and 158.8 (d, *J* 3.4 Hz, C-5); *m/z* (CI^+) 128 [$(M+H)^+$, 100%], 127 [$(M)^+$, 25]; HRMS (CI^+) [Found: $(M+H)^+$, 128.0435, $^{13}C^{12}C_5H_7O_3$ requires 128.0429].

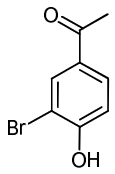
4.9 Studies Towards the Synthesis of Anthocyanins

3,5-Dibromo-4-hydroxyacetophenone (203)



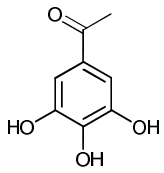
To a solution of 4-hydroxyacetophenone **87** (0.1 g, 0.73 mmol) and sodium acetate (0.12 g, 1.5 mmol) in acetic acid (3 mL), was added dropwise over 20 min a solution of bromine (5.6 g, 1.8 mL, 35 mmol) in acetic acid (0.7 mL) at room temperature. The reaction mixture was stirred for 1 h at room temperature after which time water (5 mL) was added. The mixture was then extracted with dichloromethane (3 x 5 mL), and the combined organic layers washed with saturated sodium metabisulfite solution (2 x 5 mL). The organic phase was then dried ($MgSO_4$) and the solvents removed at reduced pressure to give **203** as a pale yellow solid which was used without further purification (0.21 g, quant.). mp 185-185.5 °C (lit[32] 181 °C); δ_H (300 MHz, DMSO-d_6) 2.45 (3H, s, CH_3) and 8.00 (2H, s, CH-2,6); δ_C (75.45 MHz, DMSO-d_6) 26.4 (CH_3), 111.5 (CH-3,5), 130.9 (C-1), 132.5 (CH-2,6), 154.9 (C-4) and 194.7 (C=O); *m/z* (CI^+) 297 [$(M^{81}Br_2+H)^+$, 35%], 295 [$(M^{79}Br^{81}Br+H)^+$, 100], 293 [$(M^{79}Br_2+H)^+$, 40]; HRMS (CI^+) [Found: $(M+H)^+$, 294.8790, $C_8H_7O_2{}^{79}Br^{81}Br$ requires 294.8792]. Data is in agreement with the literature.[32]

3-Bromo-4-hydroxyacetophenone (204)



To a solution of 4-hydroxyacetophenone **87** (0.3 g, 2.2 mmol) in dichloromethane (5.5 mL), was added dropwise over 20 min a solution of bromine (0.56 g, 0.18 mL, 3.52 mmol) in dichloromethane (1.1 mL) at 0 °C. The reaction mixture was stirred for 90 min at 0 °C, after which time water (15 mL) was added. The mixture was then extracted with dichloromethane (3 x 15 mL) and the combined organic layers washed with saturated sodium metabisulfite solution (2 x 15 mL). The organic phase was then dried ($MgSO_4$) and the solvents removed at reduced pressure to give **204** as a pale yellow solid which was used without further purification (0.47 g, quant.). mp 110-111 °C (lit[33] 112 °C); δ_H (400 MHz, DMSO-d_6) 2.43 (3H, s, CH_3), 6.96 (1H, d, *J* 8.5 Hz, CH-5), 7.76 (1H, dd, *J* 8.5, 2.2 Hz, CH-6) and 8.00 (1H, d, *J* 2.2 Hz, CH-2); δ_C (100 MHz, DMSO-d_6) 26.28 (CH_3), 109.4 (C-3), 115.8 (CH-5), 129.6 (CH-6), 129.8 (C-1), 133.4 (CH-2), 158.5 (C-4) and 195.3 (C=O); *m/z* (CI^+) 217 [$(M^{81}Br+H)^+$, 95%], 215 [$(M^{79}Br+H)^+$, 100]; HRMS (CI^+) [Found: $(M+H)^+$, 214.9702, $C_8H_8O_2{}^{79}Br$ requires 214.9708], [Found: $(M+H)^+$, 216.9684, $C_8H_8O_2{}^{81}Br$ requires 216.9687]. Data is in agreement with the literature.[33]

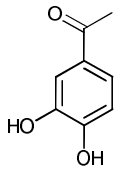
3,4,5-Trihydroxyacetophenone (205)



A solution of sodium hydroxide (2.58 g, 64.6 mmol) in water (17 mL) was stirred at reduced pressure for 90 min. $CuSO_4.6H_2O$ (0.16 g, 1.02 mmol, in 1 mL water) was then added to the solution and stirring continued at reduced pressure for an additional 4 h. The aqueous

solution was transferred by cannula to a flask containing substrate **203** (0.5 g, 1.7 mmol) and stirring continued at room temperature for 90 min. The resulting solution was heated at reflux at 110 °C for 18 h, cooled and acidified with concentrated hydrochloric acid. The aqueous mixture was extracted with both diethyl ether (3 x 20 mL) and ethyl acetate (3 x 20 mL), the combined organic layers washed with brine (20 mL), and dried ($MgSO_4$). The solvents were removed at reduced pressure to give **205** as a light brown solid, which contained a trace of the di-hydroxy product **206** (0.257 g, 90%). mp 176-178 °C; υ_{max}/cm^{-1} 3110 (br, OH), 1639 and 1593 (C=O), 1447 (CH_3), 1345 (OH), 1207, 1150 and 1014 (C-O), 862 and 799 (ArH); δ_H (400 MHz, MeOD) 2.37 (3H, s, CH_3) and 6.93 (2H, s, CH-2,6); δ_C (75.45 MHz, MeOD) 26.7 (CH_3), 109.7 (CH-2,6), 129.9 (C-1), 140.8 (C-4), 147.0 (C-3,5) and 200.3 (C=O); *m/z* (CI^+) 169 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 169.0498, $C_8H_9O_4$ requires 169.0501], [Found: $(M+H)^+$, 216.9684, $C_8H_8O_2{}^{81}Br$ requires 216.9687].

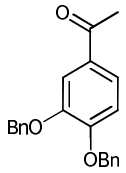
3,4-Dihydroxyacetophenone (206)



A solution of sodium hydroxide (7.1 g, 176.7 mmol) in water (50 mL) was stirred at reduced pressure for 90 min. $CuSO_4.6H_2O$ (0.45 g, 2.79 mmol, in 0.5 mL) was then added to the solution and stirring continued at reduced pressure for an additional 4 h. The aqueous solution was transferred by cannula to a flask containing substrate **204** (0.93 g, 4.32 mmol) and stirring continued at room temperature for 90 min. The resulting solution was heated at reflux at 110 °C for 18 h, cooled and acidified with concentrated hydrochloric acid. The aqueous mixture was extracted with both diethyl ether (3 x 40 mL) and ethyl acetate (3 x 40 mL), the combined organic layers washed with brine (40 mL), and dried ($MgSO_4$). The solvents were removed at reduced pressure to give **206** as a light brown solid (0.538 g,

82%). mp 119-120 °C (lit[34] 120 °C); δ_H (400 MHz, MeOD) 2.40 (3H, s, CH_3), 6.72 (1H, dm, *J* 8.1 Hz, CH-5), 7.30-7.33 (1H, m, CH-6) and 7.34 (1H, d, *J* 2.1 Hz, CH-2); δ_C (100 MHz, MeOD) 26.2 (CH_3), 115.8 (CH-5), 116.0 (CH-6), 123.5 (CH-2), 130.7 (C-1), 146.4 (C-3), 152.3 (C-4) and 199.8 (C=O); *m/z* (CI^+) 153 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 153.0550, $C_8H_9O_3$ requires 153.0552], [Found: $(M+H)^+$, 216.9684, $C_8H_8O_2{}^{81}Br$ requires 216.9687]. Data is in agreement with the literature.[34]

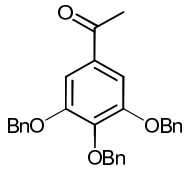
3,4-Bis(benzyloxy)acetophenone (207)



To a mixture of 3,4-dihydroxyacetophenone **206** (1 g, 5.95 mmol), 18-crown-6 (0.48 g, 1.8 mmol) and anhydrous potassium carbonate (5.95 g) in acetone (60 mL) was added benzyl bromide (2.2 mL, 3.06 g, 17.9 mmol). The reaction mixture was heated at 69 °C for 6 h, after which time the mixture was cooled and filtered to remove the solids. The solvents were removed at reduced pressure and the crude product dissolved in diethyl ether (20 mL). The organic layer was washed with water (3 x 20 mL), dried ($MgSO_4$) and the solvent removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 5:1 to 1:1; TLC 2:1) to give **207** as a white solid (2.07 g, 69%). mp 88-89 °C; υ_{max}/cm^{-1} 1668, 1588 and 1581 (C=O), 1512 and 1425 (CH_3, CH_2), 1216, 1125 and 1020 (C-O), 899, 812 and 730 (ArH); δ_H (400 MHz, $CDCl_3$) 2.54 (3H, s, CH_3), 5.24 (2H, s, CH_2), 5.27 (2H, s, CH_2), 6.96 (1H, d, *J* 8.4 Hz, CH-5), 7.32-7.52 (10H, m, CH-Ar), 7.56 (1H, dd, *J* 8.4, 2.1 Hz, CH-6) and 7.63 (1H, d, *J* 2.1 Hz, CH-2); δ_C (100 MHz, $CDCl_3$) 26.3 (CH_3), 70.8 (CH_2), 71.1 (CH_2), 112.9 (CH-5), 113.7 (CH-2), 123.5 (CH-6), 127.1 (CH-Ar), 127.4 (CH-Ar), 128.0 (CH-Ar), 128.5 (CH-Ar), 128.6 (CH-Ar), 130.8 (C-1), 136.5 (C-Ar), 136.8 (C-Ar), 148.6 (C-3), 153.1 (C-4) and 196.7 (C=O); *m/z* (CI^+)

333 [$(M+H)^+$, 100%] 91 [$(ArCH_2)^+$, 60]; HRMS (CI^+) [Found: $(M+H)^+$, 333.1486, $C_{22}H_{21}O_3$ requires 333.1491].

3,4,5-Tris(benzyloxy)acetophenone (208)

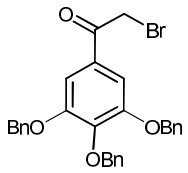


To a mixture of 3,4,5-trihydroxyacetophenone **205** (1.15 g, 6.84 mmol), 18-crown-6 (0.56 g, 2.1 mmol) and anhydrous potassium carbonate (4.65 g) in acetone (50 mL) was added benzyl bromide (3 mL, 4.1 g, 24 mmol). The reaction mixture was heated at 69 °C for 6 h, after which time the mixture was cooled and filtered to remove the solids. The solvents were removed at reduced pressure and the crude product dissolved in diethyl ether (30 mL). The organic layer was washed with water (3 x 30 mL), dried ($MgSO_4$) and the solvent removed at reduced pressure to give the crude product which contained traces of the dibenzyloxy product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 5:1 to 1:1; TLC 2:1) to give **208** as a white solid (2.07 g, 69%). mp 92-93 °C; υ_{max}/cm^{-1} 1670 and 1588 (C=O), 1423 (CH_3, CH_2), 1205 and 1120 (C-O), 845 and 731 (ArH); δ_H (400 MHz, $CDCl_3$) 2.43 (3H, s, CH_3), 5.07 (2H, s, CH_2), 5.08 (4H, s, CH_2), 7.21 (2H, s, CH-2,6) and 7.23-7.41 (15H, m, CH-Ar); δ_C (100 MHz, $CDCl_3$) 26.4 (CH_3), 71.4 (CH_2), 75.2 (CH_2), 108.4 (CH-2,6), 127.6 (CH-Ar), 128.0 (CH-Ar), 128.1 (CH-Ar), 128.3 (CH-Ar), 128.5 (CH-Ar), 128.6 (CH-Ar), 132.5 (C-1), 136.6 (C-Ar), 137.4 (C-Ar), 143.0 (C-4), 152.6 (C-3,5) and 196.9 (C=O); *m/z* (CI^+) 439 [$(M+H)^+$, 100%] 181 [$(M-COCH_3-(OBn)_2)^+$, 47], 91 [$(ArCH_2)^+$, 60]; HRMS (CI^+) [Found: $(M+H)^+$, 439.1915, $C_{29}H_{27}O_4$ requires 439.1909].

α-Bromo-3,4-bis(benzyloxy)acetophenone (209)

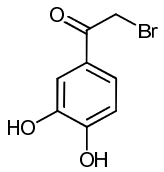
To a solution of 3,4-bis(benzyloxy)acetophenone **207** (1 g, 3 mmol) in dichloromethane/methanol (5:2, 7 mL) was added tetrabutylammonium tribromide (1.45 g, 3 mmol). The reaction mixture was stirred at room temperature for 3 h, after which time the reaction mixture was concentrated at reduced pressure and water (50 mL) added. The aqueous phase was extracted with a mixture of diethyl ether and ethyl acetate (50:50, 3 x 50 mL). The organic layers were washed with brine (50 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give **209** as an orange solid which was used without further purification (1.23 g, quant.). mp 85-86 °C (lit[35] 89-90 °C); δ_H (400 MHz, $CDCl_3$) 4.27 (2H, s, CH_2), 5.13 (2H, s, CH_2), 5.18 (2H, s, CH_2), 6.87 (1H, d, *J* 8.4 Hz, CH-5), 7.20-7.43 (10H, m, CH-Ar), 7.48 (1H, dd, *J* 8.4, 2.1 Hz, CH-6) and 7.54 (1H, d, *J* 2.1 Hz, CH-2); δ_C (100 MHz, $CDCl_3$) 31.0 (CH_2-Br), 71.2 (CH_2), 71.6 (CH_2), 113.2 (CH-5), 114.7 (CH-2), 124.5 (CH-6), 127.5 (CH-Ar), 127.6 (C-1), 127.8 (CH-Ar), 128.5 (CH-Ar), 128.6 (CH-Ar), 129.0 (CH-Ar), 129.1 (CH-Ar), 136.6 (C-Ar), 137.0 (C-Ar), 149.2 (C-3), 154.3 (C-4) and 190.3 (C=O); *m/z* (CI^+) 413 [$(M^{81}Br)^+$, 28%] 411 [$(M^{79}Br)^+$, 30] 181 [$(M\text{-}COCH_2Br\text{-}(OBn)_2)^+$, 51] 91 [$(ArCH_2)^+$, 55]; HRMS ($CI^+$) [Found: M^+, 411.0605, $C_{22}H_{20}O_3{}^{79}Br$ requires 411.0596]. Data is in agreement with the literature.[35]

α-Bromo-3,4,5-tris(benzyloxy)acetophenone (210)



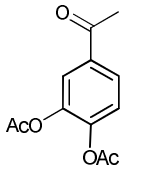
To a solution of 3,4,5-tris(benzyloxy)acetophenone **208** (1.15 g, 2.63 mmol) in dichloromethane/methanol (5:2, 7 mL) was added tetrabutylammonium tribromide (1.39 g, 2.89 mmol). The reaction mixture was stirred at room temperature for 3 h, after which time the reaction mixture was concentrated at reduced pressure and water (30 mL) added. The aqueous phase was extracted with a mixture of diethyl ether and ethyl acetate (50:50, 3 x 40 mL). The organic layers were washed with brine (30 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give **210** as an orange solid which was used without further purification (1.25 g, quant.). mp 92-93 °C; υ_{max}/cm^{-1} 1676 and 1581 (C=O), 1429 (CH_2), 1207, 1150, 1129 and 1029 (C-O), 873 and 732 (ArH), 695 and 625 (C-Br); δ_H (400 MHz, $CDCl_3$) 4.23 (2H, s, CH_2-Br), 5.08 (4H, s, CH_2), 5.09 (2H, s, CH_2), 7.22 (2H, s, CH-2,6) and 7.24-7.38 (15H, m, CH-Ar); δ_C (100 MHz, $CDCl_3$) 30.5 (CH_2-Br), 71.5 (CH_2), 75.3 (CH_2), 109.0 (CH-2,6), 127.6 (CH-Ar), 128.1 (CH-Ar), 128.2 (CH-Ar), 128.3 (CH-Ar), 128.5 (CH-Ar), 128.7 (CH-Ar), 129.0 (C-1), 136.4 (C-Ar), 137.2 (C-Ar), 143.0 (C-4), 152.7 (C-3,5) and 190.1 (C=O); *m/z* (CI^+) 519 [$(M^{81}Br+H)^+$, 30%] 517 [$(M^{79}Br+H)^+$, 35] 181 [$(M-COCH_2Br-(OBn)_2)^+$, 52] 91 [$(ArCH_2)^+$, 100]; HRMS (CI^+) [Found: $(M+H)^+$, 517.1017, $C_{29}H_{26}O_4{}^{79}Br$ requires 517.1017], [Found: $(M+H)^+$, 519.1004, $C_{29}H_{26}O_4{}^{81}Br$ requires 519.0994].

α-Bromo-3,4-dihydroxyacetophenone (218)



To a solution of 3,4-dihydroxyacetophenone **206** (0.2 g, 1.32 mmol) in dichloromethane/methanol (5:2, 3 mL) was added tetrabutylammonium tribromide (0.636 g, 1.32 mmol). The reaction mixture was stirred at room temperature for 3 h, after which time the reaction mixture was concentrated at reduced pressure and water (15 mL) added. The aqueous phase was extracted with a mixture of diethyl ether and ethyl acetate (50:50, 3 x 15 mL). The organic layers were washed with brine (15 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give **218** as an orange solid (0.305 g) which was used without further purification. mp 64-65 °C (lit[36] 61 °C); δ_H (400 MHz, $CDCl_3$) 4.57 (2H, s, CH_2-Br), 6.97 (1H, d, *J* 8.4 Hz, CH-5), 7.28-7.32 (1H, m, CH-6) and 7.50-7.53 (1H, m, CH-2); δ_C (100 MHz, $CDCl_3$) 24.1 (CH_2-Br), 116.3 (CH-5), 116.5 (CH-2), 122.2 (CH-6), 127.0 (C-1), 145.0 (C-3), 151.1 (C-4) and 190.0 (C=O); *m/z* (ES^+) 231 [$(M^{79}Br+H)^+$, 15%] 233 [$(M^{81}Br+H)^+$, 15]; HRMS (CI^+) [Found: $(M+H)^+$, 230.9665, $C_8H_8O_3{}^{79}Br$ requires 230.9657]. Data is in agreement with the literature.[36]

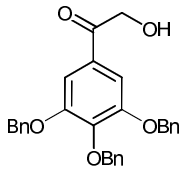
3,4-Diacetoxyacetophenone (216)



To a solution of 3,4-dihydroxyacetophenone **206** (0.5 g, 1.64 mmol) in pyridine (7 mL) was added acetic anhydride (1 mL). The reaction mixture was stirred at room temperature overnight. After this time the solvents were removed at reduced pressure and the residue dissolved in ethyl acetate (20 mL) and dilute ammonium chloride solution (20 mL). The

aqueous layer was extracted with ethyl acetate (2 x 20 mL). The combined organic layers washed with dilute ammonium chloride solution (20 mL), 2M hydrochloric acid (20 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give **216** as a white solid (0.776 g, quant.). mp 86-86.5 °C (lit[37] 86 °C); δ_H (300 MHz, $CDCl_3$) 2.33 (3H, s, CH_3), 2.34 (3H, s, CH_3), 2.61 (3H, s, CH_3), 7.33 (1H, d, *J* 8.4 Hz, CH-5), 7.81 (1H, d, *J* 2.1 Hz, CH-2) and 7.88 (1H, dd, *J* 8.4, 2.1 Hz, CH-6); δ_C (75.45 MHz, $CDCl_3$) 21.0 (CH_3), 21.1 (CH_3), 27.0 (CH_3), 124.0 (CH-2), 124.1 (CH-5), 127.3 (CH-6), 136.0 (C-1), 142.7 (C-3), 146.4 (C-4), 168.1 (C=O), 168.5 (C=O) and 196.4 (C=O); *m/z* (CI^+) 237 [$(M+H)^+$, 10%] 195 [$(M-COCH_3+H)^+$, 100]; HRMS (CI^+) [Found: $(M+H)^+$, 237.0763, $C_{12}H_{13}O_5$ requires 237.0763]. Data is in agreement with the literature.[37]

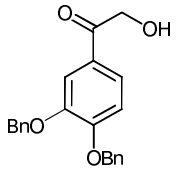
α-Hydroxy-3,4,5-tris(benzyloxy)acetophenone (201)



A solution of sodium formate (0.03 g, 0.406 mmol) in ethanol (2 mL) was stirred at room temperature for 15 min. After this time α-bromo-3,4,5-tri(benzyloxy)acetophenone **210** (0.07 g, 0.135 mmol) was added and the reaction mixture stirred overnight at 70 °C. The reaction mixture was then cooled to room temperature and water (2 mL) added. The mixture was extracted with ethyl acetate (3 x 2 mL), the combined organic layers dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as a yellow solid. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 4:1, TLC 2:1) to give **201** as a white solid (0.023 g, 52%). mp 99-100 °C; υ_{max}/cm^{-1} 3011 (br, OH), 1676 and 1589 (C=O), 1430 (CH_2), 1207, 1150, 1129 and 1054 (C-O), 895 and 731 (ArH); δ_H (400 MHz, $CDCl_3$) 3.37 (1H, t, *J* 4.3 Hz, OH), 4.65 (2H, d, *J* 4.3 Hz, CH_2), 5.08 (4H, s, CH_2), 5.09 (2H, s, CH_2), 7.13 (2H, s, CH-2,6) and 7.24-7.42 (15H, m, CH-Ar); δ_C

(100 MHz, $CDCl_3$) 65.2 (CH_2-OH), 71.5 (CH_2), 75.2 (CH_2), 107.6 (CH-2,6), 127.5 (CH-Ar), 128.1 (CH-Ar), 128.2 (CH-Ar), 128.3 (CH-Ar), 128.4 (C-1), 128.5 (CH-Ar), 128.7 (CH-Ar), 136.4 (C-Ar), 137.2 (C-Ar), 143.9 (C-4), 152.9 (C-3,5) and 197.1 (C=O); *m/z* (CI^+) 455 [$(M+H)^+$, 10%], 181 [$(M-COCH_2Br-(OBn)_2)^+$, 52] 91 [$(ArCH_2)^+$, 100]; HRMS (CI^+) [Found: $(M+H)^+$, 455.1964, $C_{29}H_{27}O_5$ requires 455.1858].

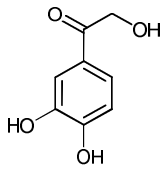
α-Hydroxy-3,4-bis(benzyloxy)acetophenone (202)



A solution of sodium formate (0.62 g, 9 mmol) in ethanol (50 mL) was stirred at room temperature for 15 min. After this time α-bromo-3,4-bis(benzyloxy)acetophenone **209** (1.23 g, 3 mmol) was added and the reaction mixture stirred overnight at 70 °C. The reaction mixture was then cooled to room temperature and water (30 mL) added. The mixture was extracted with ethyl acetate (3 x 30 mL), the combined organic layers dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as a yellow solid. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 4:1, TLC 2:1) to give **202** as a pale yellow solid (0.695 g, 67%). mp 105-106 °C; υ_{max}/cm^{-1} 3092 (br, OH), 1674 and 1582 (C=O), 1514 (ArH), 1426 (CH_2), 1208, 1150, 1095 and 1018 (C-O), 801 and 730 (ArH); δ_H (400 MHz, $CDCl_3$) 3.43 (1H, t, *J* 3.7 Hz, OH), 4.69 (2H, d, *J* 3.7 Hz, CH_2), 5.15 (2H, s, CH_2), 5.18 (2H, s, CH_2), 6.88 (1H, d, *J* 8.5 Hz, CH-5), 7.22-7.43 (11H, m, CH-6, CH-Ar) and 7.49 (1H, d, *J* 2.0 Hz, CH-2); δ_C (100 MHz, $CDCl_3$) 65.0 (CH_2-OH), 70.8 (CH_2), 71.2 (CH_2), 113.1 (CH-2), 113.3 (CH-5), 122.6 (CH-6), 126.7 (C-1), 127.1 (CH-Ar), 127.4 (CH-Ar), 128.0 (CH-Ar), 128.2 (CH-Ar), 128.6 (CH-Ar), 128.7 (CH-Ar), 136.2 (C-Ar), 136.6 (C-Ar) 148.9 (C-3), 154.1 (C-4) and 196.8 (C=O); *m/z* (CI^+) 349 [$(M+H)^+$,

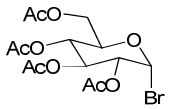
100%], 91 [$(ArCH_2)^+$, 40]; HRMS (CI^+) [Found: $(M+H)^+$, 349.1449, $C_{22}H_{21}O_4$ requires 349.1440].

α-Hydroxy-3,4-dihydroxyacetophenone (219)



A solution of α-hydroxy-3,4-bis(benzyloxy)acetophenone **202** (0.2 g, 0.57 mmol) and palladium black (40 mg, 0.34 mmol) in ethyl acetate (25 mL) under a positive pressure of hydrogen gas was stirred for 12 h at room temperature. After this time, the reaction mixture was filtered through celite to remove the palladium. The solvents were then removed at reduced pressure to give **219** as an off-white solid (0.1 g, quant.). mp 163-164 °C (lit[38] 161-162 °C); δ_H (400 MHz, acetone-d_6) 4.63 (2H, s, CH_2), 6.80 (1H, d, *J* 8.2 Hz, CH-5), 7.31 (1H, dd, *J* 8.2, 2.1 Hz, CH-6), 7.34 (1H, d, *J* 2.1 Hz, CH-2), 8.57 (1H, s, OH) and 8.81 (1H, s, OH); δ_C (100 MHz, $CDCl_3$) 65.5 (CH_2), 115.2 (CH-2), 115.9 (CH-5), 122.1 (CH-6), 128.5 (C-1), 145.1 (C-3),† 150.8 (C-4)† and 198.5 (C=O)†; *m/z* (CI^+) 169 [$(M+H)^+$, 100%], 151 [$(M-H_2O)^+$, 40]. † Observed by ^1H-^{13}C HMBC, correlates with literature data. Data is in agreement with the literature.[38]

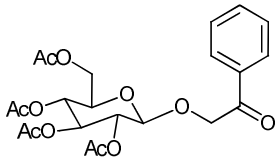
2,3,4,6-Tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide (93)



To *D*-glucose **212** (10 g, 55.5 mmol) in acetic anhydride (40 mL) at 0 °C under an argon atmosphere was added 45% w/v hydrogen bromide in acetic acid (16 mL). After 4 h, a further 45% w/v hydrogen bromide in acetic acid (44 mL) was added and the reaction mixture stirred at room temperature overnight. The reaction mixture was taken up in

dichloromethane (80 mL), poured into ice/water (120 mL) and the subsequent organic layer added to an ice/saturated sodium bicarbonate solution (150 mL) with stirring. Once gas evolution became less vigorous, the organic phase was added to a solution of saturated sodium bicarbonate (150 mL). The organic layer was dried ($MgSO_4$) and the solvents removed at reduced pressure to give a golden oil which solidified upon cooling to 0 °C. Recrystallisation from diethyl ether gave the pure product **93** as a white powder (6.29 g, 55%). mp 87.5-88 °C (lit[39] 88-89 °C); δ_H (400 MHz, $CDCl_3$) 2.06 (3H, s, CH_3-6), 2.07 (3H, s, CH_3-4), 2.12 (3H, s, CH_3-3), 2.13 (3H, s, CH_3-2), 4.11-4.19 (1H, m, CH-6a), 4.28-4.39 (2H, m, CH-6b, CH-5), 4.86 (1H, dd, *J* 10.0, 4.0 Hz, CH-2), 5.18 (1H, t, *J* 10.0 Hz, CH-4), 5.58 (1H, t, *J* 10.0 Hz, CH-3) and 6.63 (1H, d, *J* 4.0 Hz, CH-1); δ_C (100 MHz, $CDCl_3$) 20.6 (4 x CH_3), 60.9 (CH_2-6), 67.2 (CH-4), 70.1 (CH-3), 70.6 (CH-2), 72.1 (CH-5), 86.6 (CH-1), 169.5 (C=O), 169.8 (C=O), 169.9 (C=O) and 170.5 (C=O). Data is in agreement with the literature.[39]

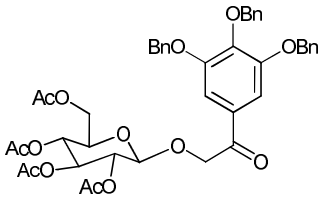
2-Oxo-2-phenylethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside (214)



To a solution of 2-hydroxyacetophenone **213** (0.1 g, 0.73 mmol), anhydrous sodium sulfate (0.2 g), 4Å molecular sieves (0.2 g), and $Hg(CN)_2$ (0.37 g, 1.46 mmol) in dry toluene (5 mL), was added dropwise a solution of 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide **93** (0.393 g, 1.46 mmol) in dry toluene (1.4 mL) under rigorously anhydrous conditions. The reaction mixture was heated at reflux for 2 h, after which time the reaction mixture was cooled and filtered through a bed of celite. The toluene was removed at reduced pressure and the residue dissolved in dichloromethane. The organic phase was washed with water (3 x 10 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as an orange syrup. Purification was by column chromatography (silica gel, dichloromethane/diethyl ether, 95:5) to give **214** as a pale

yellow oil (0.219 g, 64%). υ_{max}/cm^{-1} 1746, 1702 and 1598 (C=O), 1449 (CH_3, CH_2, CH), 1214, 1150 and 1037 (C-O), 906 and 780 (ArH); δ_H (400 MHz, $CDCl_3$) 1.95 (3H, s, CH_3-3), 1.96 (3H, s, CH_3-4), 1.97 (3H, s, CH_3-6), 2.01 (3H, s, CH_3-2), 3.58-3.65 (1H, m, CH-5), 4.08 (1H, dd, *J* 12.3, 2.3 Hz, CH_2-6a), 4.19 (1H, dd, *J* 12.3, 4.7 Hz, CH_2-6b), 4.66 (1H, d, *J* 7.9 Hz, CH-1), 4.83 (1H, AB, *J* 16.4 Hz, CO*CH*$_2$O), 4.92 (1H, AB, *J* 16.4 Hz, CO*CH*$_2$O), 5.00-5.07 (2H, m, CH-2,4), 5.19 (1H, t, *J* 9.5 Hz, CH-3), 7.39-7.47 (2H, m, CH-3',5'), 7.51-7.57 (1H, m, CH-4') and 7.82-7.88 (2H, m, CH-2',6'); δ_C (100 MHz, $CDCl_3$) 20.8 (CH_3-3), 20.9 (CH_3-4), 21.0 (CH_3-2), 21.1 (CH_3-6), 62.1 (CH_2-6), 68.6 (CH-4), 70.9 (CH_2-O), 71.3 (CH-2), 72.2 (CH-5), 72.9 (CH-3), 100.6 (CH-1), 128.5 (CH-2',6'), 129.1 (CH-3',5'), 134.1 (CH-4'), 135.0 (C-1'), 169.8 (C=O), 170.0 (C=O), 170.5 (C=O), 170.9 (C=O) and 195.2 (C=O); *m/z* (ES^+) 489 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 489.1382, $C_{22}H_{26}O_{11}Na$ requires 489.1373].

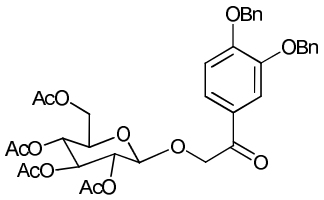
2-[3,4,5-Tris(benzyloxy)phenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside (222)



To a solution of α-hydroxy-3,4,5-tris(benzyloxy)acetophenone **201** (0.123 g, 0.27 mmol), anhydrous sodium sulfate (0.07 g), 4Å molecular sieves (0.07 g), and $Hg(CN)_2$ (0.136 g, 0.54 mmol) in dry toluene (1.5 mL), was added dropwise a solution of 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide **93** (0.145 g, 0.35 mmol) in dry toluene (1 mL) under rigorously anhydrous conditions. The reaction mixture was heated at reflux for 2 h, after which time the reaction mixture was cooled and filtered through a bed of celite. The toluene was removed at reduced pressure and the residue dissolved in dichloromethane. The organic phase was washed with water (3 x 5 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as an orange syrup. Purification was

by column chromatography (silica gel, dichloromethane/diethyl ether, 95:5 and petroleum ether/ethyl acetate, 3:1) to give **222** as an off-white oil (0.072 g, 34%). υ_{max}/cm^{-1} 1745 and 1593 (C=O), 1511 (CH_3, CH_2, CH), 1210, 1150 and 1037 (C-O), 799 and 739 (ArH); δ_H (400 MHz, $CDCl_3$) 1.91 (3H, s, CH_3-3), 1.93 (3H, s, CH_3-4), 1.95 (3H, s, CH_3-6), 1.99 (3H, s, CH_3-2), 3.55-3.65 (1H, m, CH-5), 4.05 (1H, dd, *J* 12.4, 2.3 Hz, CH_2-6a), 4.17 (1H, dd, *J* 12.4, 4.4 Hz, CH_2-6b), 4.57 (1H, d, *J* 7.8 Hz, CH-1), 4.65 (1H, AB, *J* 16.1 Hz, $COCH_2O$), 4.76 (1H, AB, *J* 16.1 Hz, $COCH_2O$), 4.98-5.05 (2H, m, CH-2,4), 5.07 (6H, s, CH_2-Bn), 5.15 (1H, t, *J* 9.4 Hz, CH-3), 7.17 (2H, s, CH-2',6'), 7.18-7.21 (3H, m, CH-Ar) and 7.23-7.39 (12H, m, CH-Ar); δ_C (100 MHz, $CDCl_3$) 20.6 (CH_3), 20.7 (CH_3), 61.7 (CH_2-6), 68.3 (CH-4), 70.6 (CH_2), 71.0 (CH-2), 71.3 (*C*H_2-Bn), 71.6 (*C*H_2-Bn), 72.0 (CH-5), 72.5 (CH-3), 75.2 (*C*H_2-Bn), 100.3 (CH-1), 108.0 (CH-2',6'), 127.6 (CH-Ar), 128.1 (CH-Ar), 128.2 (CH-Ar), 128.3 (CH-Ar), 128.5 (CH-Ar), 128.6 (CH-Ar), 129.7 (C-1'), 136.5 (C-Ar), 137.3 (C-Ar), 143.3 (C-4'), 152.8 (C-3',5'), 169.5 (C=O), 169.7 (C=O), 170.2 (C=O), 170.6 (C=O) and 193.6 (C=O); *m/z* (ES^+) 807 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 807.2617, $C_{43}H_{44}O_{14}Na$ requires 807.2629].

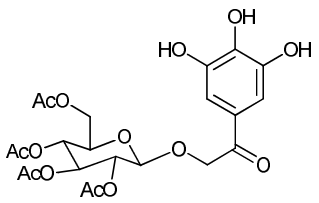
2-[3,4-Bis(benzyloxy)phenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside (221)



To a solution of α-hydroxy-3,4-bis(benzyloxy)acetophenone **202** (0.16 g, 0.48 mmol), anhydrous sodium sulfate (0.12 g), 4Å molecular sieves (0.12 g), and $Hg(CN)_2$ (0.2 g, 0.96 mmol) in dry toluene (4 mL), was added dropwise a solution of 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide **93** (0.26 g, 0.63 mmol) in dry toluene (1 mL) under rigorously anhydrous conditions. The reaction mixture was heated at reflux for 2 h, after which time the reaction mixture was cooled and filtered through a bed of celite. The toluene was

removed at reduced pressure and the residue dissolved in dichloromethane. The organic phase was washed with water (3 x 10 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as an orange syrup. Purification was by column chromatography (silica gel, dichloromethane/diethyl ether, 95:5) to give **221** as an off-white oil (0.153 g, 47%). υ_{max} /cm^{-1} 3032 and 2960 (CO-CH_3), 1750, 1695 and 1583 (C=O), 1499 and 1428 (CH_3, CH_2, CH), 1222, 1151 and 1038 (C-O), 908 and 738 (ArH); δ_H (400 MHz, $CDCl_3$) 1.91 (3H, s, CH_3-3), 1.94 (3H, s, CH_3-4), 1.95 (3H, s, CH_3-6), 1.99 (3H, s, CH_3-2), 3.55-3.64 (1H, m, CH-5), 4.05 (1H, dd, *J* 12.3, 2.3 Hz, CH_2-6a), 4.16 (1H, dd, *J* 12.3, 4.5 Hz, CH_2-6b), 4.59 (1H, d, *J* 8.0 Hz, CH-1), 4.69 (1H, AB, *J* 16.0 Hz, CO*CH_2*O), 4.80 (1H, AB, *J* 16.0 Hz, CO*CH_2*O), 4.96-5.06 (2H, m, CH-2,4), 5.14 (2H, s, CH_2-O), 5.15 (1H, t, *J* 9.3 Hz, CH-3), 5.17 (2H, s, CH_2-O), 6.85 (1H, d, *J* 8.4 Hz, CH-5'), 7.21-7.43 (11H, m, CH-6', CH-Ar) and 7.49 (1H, d, *J* 2.0 Hz, CH-2'); δ_C (100 MHz, $CDCl_3$) 21.0 (CH_3), 21.1 (CH_3), 62.1 (CH_2-6), 68.7 (CH-4), 70.7 (CH_2), 71.2 (*C*H_2-Bn), 71.4 (CH-2), 71.5 (*C*H_2-Bn), 72.3 (CH-5), 73.0 (CH-3), 100.6 (CH-1), 113.3 (CH-5'), 113.9 (CH-2'), 123.5 (CH-6'), 127.5 (CH-Ar), 127.8 (CH-Ar), 128.4 (CH-Ar), 128.5 (CH-Ar), 129.0 (CH-Ar), 129.1 (CH-Ar), 136.7 (C-Ar), 137.0 (C-Ar), 149.1 (C-3'), 169.9 (C=O), 170.1 (C=O), 170.6 (C=O), 171.1 (C=O) and 193.7 (C=O); *m/z* (ES^+) 701 [$(M+Na)^+$, 100%]; HRMS $(ES)^+$ [Found: ($M+Na^+$), 701.2199, $C_{36}H_{38}O_{13}Na$ requires 701.2210].

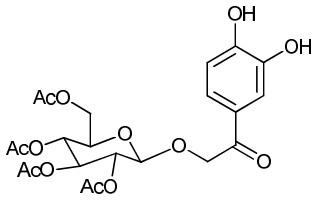
2-[3,4,5-Trihydroxyphenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside (223)



To a solution of 1-(3,4,5-tris(benzyloxy)phenyl)-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside **222** (0.17 g, 0.22 mmol) and 10% palladium on carbon (14 mg, 0.132 mmol) in dry tetrahydrofuran (3 mL) was attached a balloon containing hydrogen gas

at room temperature. The reaction mixture was stirred at 35 °C for 18 h, during which time the hydrogen in the system was periodically replaced. After this time, the mixture was filtered through a bed of celite and solvents removed at reduced pressure to give **223** as an off-white oil (0.107 g, 95%). υ_{max}/cm^{-1} 3012 (br, OH), 1750 and 1559 (C=O), 1436 (CH_3, CH_2), 1211, 1151 and 1021 (C-O) and 799 (ArH); δ_H (300 MHz, acetone-d_6) 1.82 (3H, s, CH_3-3), 1.86 (3H, s, CH_3-4), 1.87 (3H, s, CH_3-6), 1.89 (3H, s, CH_3-2), 3.77-3.87 (1H, m, CH-5), 3.99 (1H, dd, *J* 12.4, 2.4 Hz, CH_2-6a), 4.14 (1H, dd, *J* 12.4, 4.8 Hz, CH_2-6b), 4.68-4.75 (3H, m, CH_2, CH-1), 4.82 (1H, t, *J* 10.0 Hz, CH-2), 4.92 (1H, t, *J* 10.0 Hz, CH-4), 5.15 (1H, t, *J* 10.0 Hz, CH-3), 6.93 (2H, s, CH-2',6'), 8.09 (1H, s, OH) and 8.16 (2H, s, OH); δ_C (75.45 MHz, acetone-d_6) 21.0 (CH_3), 63.0 (CH_2-6), 69.8 (CH-4), 71.4 (CH_2), 72.4 (CH-2), 72.8 (CH-5), 73.8 (CH-3), 101.3 (CH-1), 109.0 (CH-2',6'), 127.7 (C-1'), 139.9 (C-4'), 146.6 (C-3',5'), 170.3 (C=O), 170.4 (C=O), 170.7 (C=O), 171.2 (C=O) and 194.2 (C=O); *m/z* $(ES)^-$ 513 $[(M-H)^-$, 100%]; *m/z* (ES^+) 537 $[(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 537.1229, $C_{22}H_{26}O_{14}Na$ requires 537.1220].

2-[3,4-Dihydroxyphenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside (220)

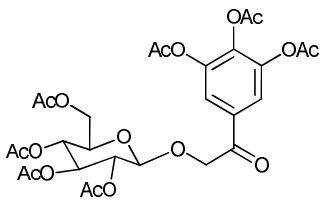


Method 1: To a solution of 1-(3,4-bis(benzyloxy)phenyl)-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside **221** (0.13 g, 0.19 mmol) and 10% palladium on carbon (10 mg, 0.1 mmol) in dry tetrahydrofuran (3 mL) was attached a balloon containing hydrogen gas at room temperature. The reaction mixture was stirred at 35 °C for 18 h, during which time the hydrogen in the system was periodically replaced. After this time, the mixture was filtered through a bed of celite and solvents removed at reduced pressure to give **220** as an off-white oil (45 mg, 47%). υ_{max}/cm^{-1} 3002 (br, OH), 1748 (C=O), 1440 (CH_3, CH_2), 1211,

1150 and 1019 (C-O), and 780 (ArH); δ_H (300 MHz, $CDCl_3$) 1.94 (3H, s, CH_3-3), 1.96 (3H, s, CH_3-4), 2.02 (3H, s, CH_3-6), 2.03 (3H, s, CH_3-2), 3.61-3.68 (1H, dm, *J* 10.0 Hz, CH-5), 4.12-4.18 (2H, m, CH_2-6), 4.62 (1H, d, *J* 7.7 Hz, CH-1), 4.68 (1H, AB, *J* 15.7 Hz, CO*CH*$_2$O), 4.87 (1H, AB, *J* 15.7 Hz, CO*CH*$_2$O), 4.99-5.09 (2H, m, CH-2,4), 5.17 (1H, t, *J* 9.4 Hz, CH-3), 6.86 (1H, d, *J* 8.3 Hz, CH-5'), 7.35 (1H, dd, *J* 8.3, 2.0 Hz, CH-6') and 7.50 (1H, d, *J* 2.0 Hz, CH-2'); δ_C (75.45 MHz, $CDCl_3$) 19.6 (CH_3), 60.9 (CH_2-6), 67.0 (CH_2), 68.4 (CH-4), 71.6 (CH-2), 72.9 (CH-5), 73.1 (CH-3), 100.1 (CH-1), 112.5 (CH-5'), 114.1 (CH-2'), 117.7 (CH-6'), 131.1 (C-1'), 142.9 (C-3'), 143.0 (C-4'), 168.5 (C=O), 168.9 (C=O), 169.1 (C=O) and 169.3 (C=O); *m/z* $(ES)^-$ 499 $[(M-H+D_2)^-$, 80%], 497 $[(M-H)^-$, 100]; *m/z* (ES^+) 523 $[(M+Na+D_2)^+$, 100%]; HRMS (ES^-) [Found: $(M-H)^-$, 497.1283, $C_{22}H_{25}O_{13}$ requires 497.1295].

Method 2: To a solution of 1-(3,4-bis(benzyloxy)phenyl)-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside **221** (17 mg, 0.025 mmol) and palladium black (2 mg, 0.017 mmol) in ethyl acetate (10 mL) was attached a balloon containing hydrogen gas at room temperature. The reaction mixture was stirred at room temperature for 40 h, during which time the hydrogen in the system was periodically replaced. After this time, the mixture was filtered through a bed of celite and solvents removed at reduced pressure to give **220** as an off-white oil (12.5 mg, quant.). Characterisation as above.

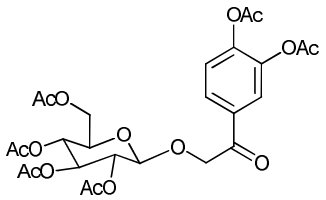
2-[3,4,5-Tris(acetyloxy)phenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside (199)



To a solution of 1-[3,4,5-trihydroxyphenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside **223** (0.09 g, 0.175 mmol) in pyridine (0.7 mL) was added acetic anhydride (0.08 mL). Further additions of acetic anhydride (0.08 mL) were made every

hour for 3 consecutive hours and the reaction mixture stirred at room temperature overnight. The solvents were then removed at reduced pressure and the resulting residue dissolved in ethyl acetate (3 mL) and dilute ammonium chloride solution (3 mL). The aqueous phase was extracted with ethyl acetate (3 x 3 mL). The combined organic layers were washed with dilute ammonium chloride solution (3 mL), saturated sodium bicarbonate solution (3 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as a yellow residue. The residue was washed with diethyl ether (1 mL) to give the crude product as a yellow solid (0.1095 g, 98%). Purification was by column chromatography (silica gel, dichloromethane/diethyl ether, 9:1) to give **199** as a colourless glass (62 mg, 55%). υ_{max}/cm^{-1} 1751 and 1748 (C=O), 1210, 1150 and 1040 (C-O), and 780 (ArH); δ_H (400 MHz, $CDCl_3$) 1.94 (3H, s, CH_3-3), 1.95 (3H, s, CH_3-4), 1.98 (3H, s, CH_3-6), 2.00 (3H, s, CH_3-2), 2.24 (9H, s CH_3), 3.58-3.64 (1H, m, CH-5), 4.05 (1H, dd, *J* 12.4, 2.2 Hz, CH_2-6a), 4.14 (1H, dd, *J* 12.4, 4.8 Hz, CH_2-6b), 4.57 (1H, d, *J* 7.8 Hz, CH-1), 4.76 (1H, AB, *J* 16.6 Hz, CO*CH*$_2$O), 4.81 (1H, AB, *J* 16.6 Hz, CO*CH*$_2$O), 4.98-5.04 (2H, m, CH-2,4), 5.16 (1H, t, *J* 9.5 Hz, CH-3) and 7.62 (2H, s, CH-2',6'); δ_C (100 MHz, $CDCl_3$) 20.2 (CH_3), 20.6 (CH_3), 20.7 (CH_3), 61.6 (CH_2-6), 68.2 (CH-4), 70.6 (CH_2), 70.9 (CH-2), 72.0 (CH-5), 72.5 (CH-3), 100.2 (CH-1), 120.8 (CH-2',6'), 132.3 (C-1'), 139.2 (C-4'), 143.8 (C-3',5'), 166.3 (C=O), 167.6 (C=O), 169.3 (C=O), 169.6 (C=O), 170.1 (C=O), 170.7 (C=O) and 192.2 (C=O); *m/z* (ES^+) 663 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 663.1539, $C_{28}H_{32}O_{17}Na$ requires 663.1537].

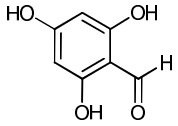
2-[3,4-Bis(acetyloxy)phenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside (211)



To a solution of 1-[3,4-dihydroxyphenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside **220** (0.04 g, 0.08 mmol) in pyridine (0.4 mL) was added acetic anhydride (0.03 mL). Further additions of acetic anhydride (0.03 mL) were made every hour for 3 consecutive hours and the reaction mixture stirred at room temperature overnight. Solvents were then removed at reduced pressure and the resulting residue dissolved in ethyl acetate (2 mL) and dilute ammonium chloride solution (2 mL). The aqueous phase was extracted with ethyl acetate (3 x 2 mL). The combined organic layers were washed with dilute ammonium chloride solution (2 mL), saturated sodium bicarbonate solution (2 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as a yellow residue. The residue was washed with diethyl ether (1 mL) to give the crude product as a yellow solid (0.1095 g, 98%). Purification was by column chromatography (silica gel, dichloromethane/diethyl ether, 9:1) to give **211** as a colourless glass (62 mg, 55%). υ_{max}/cm^{-1} 1748 (C=O), 1209, 1150 and 1037 (C-O), 799 (ArH); δ_H (400 MHz, $CDCl_3$) 1.94 (3H, s, CH_3-3), 1.95 (3H, s, CH_3-4), 1.97 (3H, s, CH_3-6), 2.00 (3H, s, CH_3-2), 2.25 (3H, s, CH_3), 2.26 (3H, s, CH_3), 3.58-3.64 (1H, dm, *J* 10.0 Hz, CH-5), 4.03-4.09 (1H, m, CH_2-6a), 4.12-4.19 (1H, m, CH_2-6b), 4.59 (1H, d, *J* 7.9 Hz, CH-1), 4.77 (1H, AB, *J* 16.3 Hz, CO*CH*$_2$O), 4.83 (1H, AB, *J* 16.3 Hz, CO*CH*$_2$O), 4.99-5.06 (2H, m, CH-2,4), 5.16 (1H, brt, *J* 9.5 Hz, CH-3), 7.25 (1H, d, *J* 8.5 Hz, CH-5'), 7.70 (1H, d, *J* 1.9 Hz, CH-2') and 7.76 (1H, dd, *J* 8.5, 1.9 Hz, CH-6'); δ_C (100 MHz, $CDCl_3$) 20.6 (CH_3), 20.7 (CH_3), 61.6 (CH_2-6), 68.0 (CH-4), 70.7 (CH_2), 70.9 (CH-2), 72.0 (CH-5), 72.6 (CH-3), 100.3 (CH-1), 123.7 (CH-2'), 123.9 (CH-5'), 126.7 (CH-6'), 133.0 (C-1'), 142.5 (C-3'),[†] 146.4 (C-4'),[†] 169.4 (C=O), 169.7 (C=O), 170.2 (C=O), 170.7

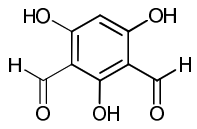
(C=O) and 192.9 (C=O); *m/z* (ES^+) 605 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 605.1478, $C_{26}H_{30}O_{15}Na$ requires 605.1482]. † Observed by ^1H-^{13}C HMBC.

2-Formylphloroglucinol (200)



To a solution of phloroglucinol **26** (0.2 g, 1.59 mmol) in ethyl acetate (25 mL) was added DMF (0.12 mL, 1.59 mmol) and $POCl_3$ (0.44 mL, 4.77 mmol) at room temperature. The reaction mixture was allowed to stir at room temperature for 2 days, after which time the reaction mixture was filtered and the solvent removed from the filtrate at reduced pressure. Water (20 mL) was added to the residue and the aqueous phase extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with brine (15 mL), dried ($MgSO_4$) and the solvent removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate 2:1) to give **200** as an off-white solid (104 mg, 42%). mp 291-291.5 °C (lit[40] 292-295 °C); δ_H (400 MHz, DMSO-d_6) 5.78 (2H, s, CH-4,6), 9.92 (1H, s, CHO), 10.66 (1H, s, OH) and 11.46 (2H, s, OH); δ_C (100 MHz, DMSO-d_6) 94.0 (CH-4,6), 104.5 (C-2), 164.0 (C-1,3), 167.2 (C-5) and 190.9 (CHO); *m/z* (CI^+) 155 [$(M+H)^+$, 100%], 156 [$(M+H_2)^+$, 75]. Data is in agreement with the literature.[40]

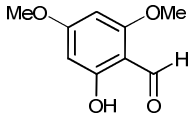
2,4-Diformylphloroglucinol (224)



To a solution of phloroglucinol **26** (0.1 g, 0.79 mmol) in ethyl acetate (12 mL) was added DMF (0.06 mL, 0.79 mmol) and $POCl_3$ (0.73 mL, 7.90 mmol) at room temperature. The reaction mixture was allowed to stir at room temperature for 24 h, after which time water

(10 mL) was added. The aqueous phase was extracted with ethyl acetate (3 x 15 mL), the combined organic layers washed with brine (15 mL), dried ($MgSO_4$) and the solvent removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate 2:1) to give **224** as an off-white solid (16 mg, 11%). mp 231 °C (decomp.) (lit[41] >220 °C (decomp.)); δ_H (400 MHz, acetone-d_6) 5.84 (1H, s, CH-6) and 9.98 (2H, s, CHO); *m/z* (CI^+) 183 [$(M+H)^+$, 100%], 184 [$(M+H_2)^+$, 70]. Data is in agreement with the literature.[41]

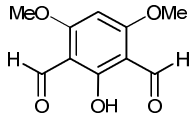
2-Formyl-3,5-dimethoxyphenol (226)



A solution of 3,5-dimethoxyphenol **188** (1 g, 6.5 mmol) in dichloromethane (1.5 mL, 1.5 mL/g) was cooled in an ice bath under an argon atmosphere. $TiCl_4$ (1M in DCM, 14.3 mL, 14.3 mmol) was then added dropwise over 15 min and the reaction mixture allowed to stir for 30 min. Dichloromethyl methyl ether (0.75 g, 6.5 mmol) was then added over 15 min and the reaction mixture allowed to stir for a further 1 h. The reaction was quenched by the addition of saturated ammonium chloride solution (30 mL) and the mixture allowed to stand for 1 h. The organic phase was then washed with 0.1M hydrochloric acid (30 mL), saturated sodium bicarbonate solution (30 mL), brine (30 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as a red solid. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 1:1) to give **226** as an off-white solid (1.1 g, 93%). mp 71-71.5 °C (lit[42] 72 °C); δ_H (400 MHz, $CDCl_3$) 3.75 (3H, s, CH_3), 3.76 (3H, s, CH_3), 5.82 (1H, d, *J* 2.2 Hz, CH-4), 5.92 (1H, d, *J* 2.2 Hz, CH-6), 10.00 (1H, s, CHO) and 12.44 (1H, s, OH); δ_C (100 MHz, $CDCl_3$) 55.8 (CH_3), 90.6 (CH-6), 92.9 (CH-4), 106.0 (C-2), 163.6 (C-3), 166.4 (C-1), 168.2 (C-5) and 191.9 (CHO);

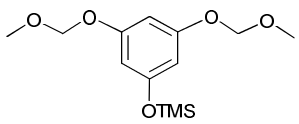
m/z (CI^+) 183 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 183.0651, $C_9H_{11}O_4$ requires 183.0657]. Data is in agreement with the literature.[42]

2,6-Diformyl-3,5-dimethoxyphenol (225)



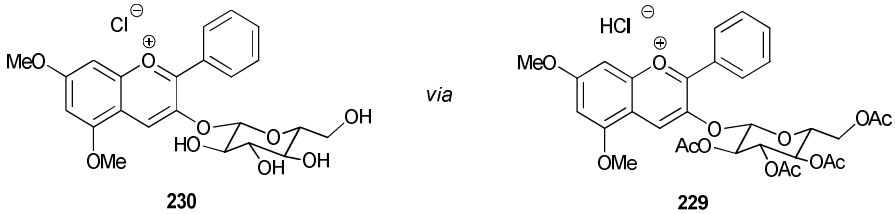
A solution of 3,5-dimethoxyphenol **188** (1 g, 6.5 mmol) in dichloromethane (1.5 mL, 1.5 mL/g) was cooled in an ice bath under an argon atmosphere. $TiCl_4$ (1M in DCM, 14.3 mL, 14.3 mmol) was then added dropwise over 30 min and the reaction mixture allowed to stir for 1 h. Dichloromethyl methyl ether (0.75 g, 6.5 mmol) was then added over 15 min and the reaction mixture allowed to stir for a further 2 h. The reaction was quenched by the addition of saturated ammonium chloride solution (30 mL) and the mixture allowed to stand for 1 h. The organic phase was then washed with 0.1M hydrochloric acid (30 mL), saturated sodium bicarbonate solution (30 mL), brine (30 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as a red solid. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 1:1) to give **225** as an off-white solid (1.24 g, 91%). mp 225-226 °C; υ_{max}/cm^{-1} 2983 (CH_3, OH), 1645 (C=O), 1426 (CH_3), 1305 (OH), 1221, 1158, 1112 and 1044 (C-O), 937, 803 and 728 (ArH); δ_H (300 MHz, $CDCl_3$) 3.92 (6H, s, CH_3), 5.23 (1H, s, OH), 5.87 (1H, s, CH-4) and 10.15 (2H, s, CHO); δ_C (100 MHz, $CDCl_3$) 56.4 (CH_3), 85.8 (CH-4), 106.2 (C-2,6), 168.4 (C-3,5), 168.7 (C-1) and 192.3 (CHO); *m/z* (CI^+) 211 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 211.0602, $C_{10}H_{11}O_5$ requires 211.0606].

1,3-Dimethoxymethyl-5-trimethylsilylphloroglucinol (228)



To a solution of 1,3-dimethoxymethylphloroglucinol **189** (0.128 g, 0.598 mmol) in pyridine (3 mL) under argon was added trimethylsilyl chloride (0.12 mL, 0.13 g 2.3 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred at 30-35 °C for 24 h. After this time, the reaction mixture was poured into water (15 mL) and the aqueous phase extracted with diethyl ether (3 x 15 mL). The combined organic layers were washed with water (3 x 15 mL), saturated ammonium chloride solution (15 mL), brine (15 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 2:1) to give **228** as a colourless oil (93 mg, 54%). υ_{max}/cm^{-1} 1211 and 1151 (C-O), 1008 (Si-O), 1021 (C-O), 837 (Si-C) and 780 (ArH); δ_H (300 MHz, $CDCl_3$) 0.11 (9H, s, CH_3), 3.31 (6H, s, CH_3), 4.96 (4H, s, CH_2), 6.07 (2H, d, *J* 2.2 Hz, CH-4,6), and 6.22 (1H, t, *J* 2.2 Hz, CH-2); δ_C (75.45 MHz, $CDCl_3$) 0.0 (Si(CH_3)$_3$), 55.8 (CH_3), 94.3 (CH_2), 98.0 (CH-2), 101.9 (CH-4,6), and 158.6 (C-1,3); *m/z* (CI^+) 309 [$(M+Na)^+$, 100%], 287 [$(M+H)^+$, 5]; HRMS (CI^+) [Found: $(M+Na)^+$, 309.1132, $C_{13}H_{22}O_5NaSi$ requires 309.1134].

Attempted Synthesis of Anthocyanin Model, 5,6-dimethoxy-2-phenylchromenium-3-yl-hexopyranoside HCl salt (230)



230 **229**

2-Oxo-2-phenylethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside **214** (0.05 g, 0.11 mmol) and 2-formyl-3,5-dimethoxyphenol **226** (0.02 g, 0.11 mmol) were dissolved in the desired dry

solvent (3 mL) (*Table 22*). Hydrogen chloride was introduced to the reaction mixture at the desired temperature by either the use of commercial HCl in diethyl ether or ethanol (2M solution), or by generation of dry HCl gas by the action of 98% H_2SO_4 on solid NaCl, or of concentrated HCl on solid $CaCl_2$. The reaction mixture was then stirred for 1 hour at the required temperature, and left for 48 h at either room temperature, 4 °C or -20 °C (*Table 22*). After this time, the solvents were removed at reduced pressure to give a red powder that was anticipated to be the crude acetylated product **229**.

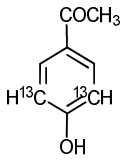
This red solid was then dissolved in a basic methanol/water solution (KOH, 0.16 g, 2.1 mL MeOH, 2.1 mL H_2O) and the resulting solution stirred at room temperature for 3 h. After this time, the reaction mixture was acidified to pH 1 with 1M HCl. The solution was left overnight at 4 °C and the solvents removed at reduced pressure. The resulting residue was triturated in methanol and the KCl filtered off. The solvents were removed once more at reduced pressure to give the crude powder. Purification was attempted by reverse phase column chromatography (C_{18} reverse phase silica gel, water/acetic acid, 9:1) and cellulose microcrystalline column chromatography (cellulose microcrystalline powder, water/acetic acid, 9:1). ^{1}H NMR analysis showed recovery of only 2-formyl-3,5-dimethoxyphenol **226** and non-acetylated 2-oxo-2-phenylethyl 2,3,4,6-tetra-*O*-acetylhexopyranoside **214**. No trace of the desired product **230** was observed.

Table 22 – Reaction conditions for attempted final coupling

Entry	Reaction Solvent	HCl source	Addition temp. (°C)	Stirring time (h)	Standing temp. (°C)
1	EtOAc	HCl gas	-10	0.5	-20
2	EtOAc	HCl gas	-10	1.0	-20
3	EtOAc/MeOH 2:1	HCl in ether	25	1.0	25
4	EtOAc/EtOH 1:1	HCl in ether	25	1.0	25
5	EtOAc	HCl in ether	25	1.0	25
6	EtOAc	HCl gas	-10	1.0	4
7	EtOAc	HCl in EtOH	-10	1.5	4

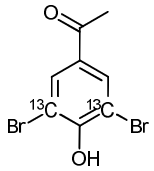
4.10 Studies Towards the Synthesis of ^{13}C-Labelled Delphinidin-3-Glucoside

4-Hydroxy-[3,5-$^{13}C_2$]acetophenone (87a)



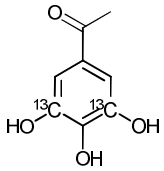
A solution of [3,5-$^{13}C_2$]4*H*-pyran-4-one **35d** (0.65 g, 6.6 mmol) and acetylacetone (1.33 g, 13.3 mmol) in dry *tert*-butanol (10 mL) was heated to reflux under argon. Potassium *tert*-butoxide (13 mL of a 1M solution in *tert*-butanol) in dry *tert*-butanol (17 mL) was added dropwise. The resulting mixture was heated at reflux for 20 h after which 1M hydrochloric acid (20 mL) was added. The reaction solution was then heated at reflux for a further 1 h. The solvents were removed at reduced pressure and water (30 mL) added to the residue. The aqueous phase was extracted with diethyl ether (3 x 20 mL). The combined organic layers were washed with water (2 x 20 mL), brine (20 mL), dried ($MgSO_4$) and the solvent removed at reduced pressure to give the crude product. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 3:1) to give **87a** as a yellow solid. (0.521 g, 57%). mp 104-105 °C (lit[6] 106-108 °C for the unlabelled); υ_{max}/cm^{-1} 3297 (br, OH), 1658 (C=O), 1565 (ArH), 1502 and 1462 (CH_3), 1275, 1210, 1150, 1103 and 1071 (C-O), 960, 844 and 812 (ArH); δ_H (400 MHz, $CDCl_3$) 2.58 (3H, s, CH_3), 6.93 (2H, dm, *J* 158.0 Hz, ^{13}CH-3,5) and 7.91 (2H, brd, *J* 8.7 Hz, CH-2,6); δ_C (75.45 MHz, $CDCl_3$) 26.3 (CH_3), 115.6 (enhanced, ^{13}CH-3,5), 131.2 (d, *J* 68.1 Hz, CH-2,6) and 198.3 (t, *J* 4.5 Hz, C=O); *m/z* (ES^-) 137 [$(M-H)^-$, 100%]; HRMS (ES^-) [Found: $(M-H)^-$, 137.0514, $^{13}C_2{}^{12}C_6H_7O_2$ requires 137.0513]. Data is in agreement with the unlabelled compound (**Section 4.9**).

3,5-Dibromo-4-hydroxy-[3,5-$^{13}C_2$]acetophenone (203a)



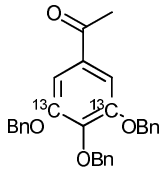
To a solution of 4-hydroxy-[3,5-$^{13}C_2$]acetophenone **87a** (320 mg, 2.32 mmol) and sodium acetate (0.38 g, 4.64 mmol) in acetic acid (3 mL), was added dropwise over 20 min a solution of bromine (0.57 mL, 1.78 g, 11.12 mmol) in acetic acid (10 mL) at room temperature. The reaction mixture was stirred for 1.5 h at room temperature after which time water (50 mL) was added. The mixture was then extracted with dichloromethane (3 x 50 mL) and the combined organic layers washed with saturated sodium metabisulfite solution (2 x 50 mL). The organic phase was then dried ($MgSO_4$) and the solvents removed at reduced pressure to give **203a** as a pale yellow solid which was used without further purification (0.688 g, quant.). mp 183-184 °C (lit[32] 181 °C for the unlabelled); δ_H (400 MHz, DMSO-d_6) 2.52 (3H, s, CH_3), 8.07 (2H, t, *J* 2.7 Hz, CH-2,6) and 10.92 (1H, brs, OH); δ_C (100 MHz, DMSO-d_6) 26.4 (CH_3), 111.5 (enhanced, ^{13}C-3,5), 130.9 (C-1), 132.5 (d, *J* 67.7 Hz, CH-2,6), 155.8 (C-4)† and 195.0 (C=O) †; *m/z* (ES$^-$) 297 [($M^{81}Br_2$-H)$^-$, 5%], 295 [($M^{79}Br^{81}$Br-H)$^-$, 100], 293 [($M^{79}Br_2$-H)$^-$, 6]; HRMS (ES$^-$) [Found: (M-H)$^-$, 294.8706, $^{12}C_6{}^{13}C_2H_5O_2{}^{79}Br^{81}$Br requires 294.8703]. † Observed by ^{1}H-^{13}C HMBC, correlates with data from unlabelled compound **203**. Data is in agreement with the unlabelled compound (**Section 4.9**).

3,4,5-Trihydroxy-[3,5-$^{13}C_2$]acetophenone (205a)



A solution of sodium hydroxide (3.53 g, 88.16 mmol) in water (23 mL) was stirred at reduced pressure for 90 min. $CuSO_4.6H_2O$ (223 mg, 1.4 mmol, in 1 mL water) was then added to the solution and stirring continued at reduced pressure for an additional 4 h. The aqueous solution was transferred by cannula to a flask containing 3,5-dibromo-4-hydroxy-[3,5-$^{13}C_2$]acetophenone **203a** (687 mg, 2.32 mmol) and stirring continued at room temperature for 90 min. The resulting solution was heated at reflux at 110 °C for 18 h, cooled, and acidified with concentrated hydrochloric acid. The aqueous mixture was extracted with both diethyl ether (3 x 30 mL) and ethyl acetate (3 x 30 mL), the organic layers combined, washed with brine (30 mL) and dried ($MgSO_4$). The solvents were removed at reduced pressure to give **205a** as a light brown solid which was used without further purification (394 mg, quant.). mp 174-174.5 °C; δ_H (400 MHz, MeOD) 2.37 (3H, s, CH_3) and 6.94 (2H, t, *J* 2.0 Hz, CH-2,6); δ_C (100 MHz, MeOD) 26.7 (CH_3), 108.8 (CH-2,6), 130.1 (C-1), 141.3 (C-4), 146.5 (enhanced, ^{13}C-3,5) and 201.8 (C=O); *m/z* (ES^-) 169 [$(M-H)^-$, 100%]; HRMS (ES^-) [Found: $(M-H)^-$, 169.0415, $^{13}C_2{}^{12}C_6H_7O_4$ requires 169.0411]. Data is in agreement with the unlabelled compound (**Section 4.9**).

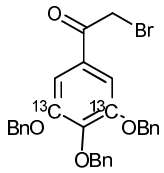
3,4,5-Tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone (208a)



To a mixture of 3,4,5-trihydroxy-[3,5-$^{13}C_2$]acetophenone **205a** (394 mg, 2.32 mmol), 18-crown-6 (180 mg, 0.7 mmol) and anhydrous potassium carbonate (1.58 g) in acetone

(16 mL) was added benzyl bromide (1.2 mL, 1.59 g, 9.3 mmol). The reaction mixture was heated at 69 °C overnight, after which time the mixture was cooled and filtered to remove the solids. The solvents were removed at reduced pressure and the crude product dissolved in diethyl ether (40 mL). The organic layer was washed with water (3 x 40 mL), dried ($MgSO_4$) and the solvent removed at reduced pressure to give the crude product as a yellow oil. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 5:1 to 1:1; TLC 2:1) to give **208a** as an off-white solid (494 mg, 48%). mp 93.5-94 °C; δ_H (400 MHz, $CDCl_3$) 2.43 (3H, s, CH_3), 5.07 (2H, s, CH_2), 5.08 (4H, s, CH_2), 7.21-7.22 (2H, m, CH-2,6) and 7.24-7.43 (15H, m, CH-Ar); δ_C (100 MHz, $CDCl_3$) 26.3 (CH_3), 71.4 (CH_2), 75.1 (CH_2), 108.4 (d, *J* 70.4 Hz, CH-2,6), 127.6 (CH-Ar), 128.0 (CH-Ar), 128.1 (CH-Ar), 128.2 (CH-Ar), 128.5 (CH-Ar), 128.6 (CH-Ar), 132.3 (C-1)†, 136.5 (C-Ar), 137.6 (C-Ar)†, 148.6 (d, *J* 6.1 Hz, C-4), 152.6 (enhanced, ^{13}C-3,5) and 196.8 (t, *J* 4.6 Hz, C=O); *m/z* (ES^+) 463 [$(M+Na)^+$, 100%]; HRMS (ES^+) [Found: $(M+Na)^+$, 463.1796, $^{13}C_2{}^{12}C_{27}H_{26}O_4Na$ requires 463.1796]. † Observed by ^{1}H-^{13}C HMBC, correlates with data from unlabelled compound **208**. Data is in agreement with the unlabelled compound (**Section 4.9**).

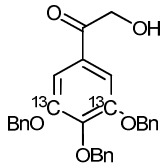
α-Bromo-3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone (210a)



To a solution of 3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone **208a** (494 mg, 1.12 mmol) in dichloromethane/methanol (5:2, 3 mL) was added tetrabutylammonium tribromide (595 mg, 1.23 mmol). The reaction mixture was stirred at room temperature for 6 h, after which time the reaction mixture was concentrated at reduced pressure and water (30 mL) added. The aqueous phase was extracted with a mixture of diethyl ether and ethyl acetate (50:50, 3 x 30 mL). The organic layers were washed with brine (30 mL), dried ($MgSO_4$) and the

solvents removed at reduced pressure to give **210a** as an orange oil which was used without further purification (580 mg, quant.). δ_H (400 MHz, $CDCl_3$) 4.23 (2H, s, CH_2-Br), 5.08 (4H, s, CH_2), 5.09 (2H, s, CH_2), 7.20-7.22 (2H, m, CH-2,6) and 7.23-7.39 (15H, m, CH-Ar); δ_C (100 MHz, $CDCl_3$) 30.5 (CH_2-Br), 71.5 (CH_2), 75.3 (CH_2), 109.0 (d, *J* 70.1 Hz, CH-2,6), 127.6 (CH-Ar), 128.0 (CH-Ar), 128.2 (CH-Ar), 128.3 (CH-Ar), 128.5 (CH-Ar), 128.7 (CH-Ar), 129.0 (C-1),† 136.4 (C-Ar), 137.2 (C-Ar),† 148.8 (d, *J* 6.2 Hz, C-4), 152.7 (enhanced, ^{13}C-3,5) and 190.1 (C=O);† *m/z* (CI^+) 521 [$(M^{81}Br+H)^+$, 85%], 519 [$(M^{79}Br+H)^+$, 95], 441 [$(M-^{81}Br+H)^+$, 100]; HRMS (CI^+) [Found: $(M+H)^+$, 519.1082, $^{13}C_2{}^{12}C_{27}H_{26}O_4{}^{79}Br$ requires 519.1082], [Found: $(M+H)^+$, 521.1063, $^{13}C_2{}^{12}C_{27}H_{26}O_4{}^{81}Br$ requires 521.1061]. † Observed by ^{1}H-^{13}C HMBC, correlates with data from unlabelled compound **210**. Data is in agreement with the unlabelled compound (**Section 4.9**).

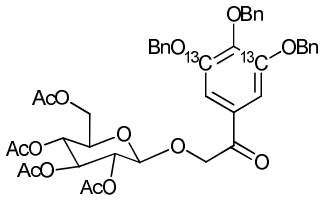
α-Hydroxy-3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone (201a)



A solution of sodium formate (229 mg, 3.36 mmol) in ethanol (16 mL) was stirred at room temperature for 15 min. After this time α-bromo-3,4,5-tri(benzyloxy)-[3,5-$^{13}C_2$]acetophenone **210a** (580 mg, 1.12 mmol) was added and the reaction mixture stirred overnight at 70 °C. The reaction mixture was then cooled to room temperature and water (50 mL) added. The mixture was extracted with ethyl acetate (3 x 50 mL), the combined organic layers dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as a yellow solid. Purification was by column chromatography (silica gel, petroleum ether/ethyl acetate, 4:1, TLC 2:1) to give **201a** as an off-white solid (219 mg, 42%). mp 97-97.5 °C; δ_H (400 MHz, $CDCl_3$) 4.64 (2H, s, CH_2), 5.07 (4H, s, CH_2), 5.09 (2H, s, CH_2), 7.12 (2H, t, *J* 2.1 Hz, CH-2,6) and 7.19-7.43 (15H, m, CH-Ar); δ_C (100 MHz, $CDCl_3$)

65.0 (CH_2-OH), 71.4 (CH_2), 75.2 (CH_2), 107.5 (d, *J* 70.9 Hz, CH-2,6), 127.5 (CH-Ar), 128.1 (CH-Ar), 128.2 (CH-Ar), 128.3 (CH-Ar), 128.3 (C-1),† 128.5 (CH-Ar), 128.7 (CH-Ar), 136.4 (C-Ar), 137.2 (C-Ar), 143.8 (C-4),† 152.9 (enhanced, ^{13}C-3,5) and 197.1 (t, *J* 5.9 Hz, C=O); *m/z* (CI^+) 457 [$(M+H)^+$, 100%]; HRMS (CI^+) [Found: $(M+H)^+$, 457.1923, $^{13}C_2{}^{12}C_{27}H_{27}O_5$ requires 457.1926]. † Observed by ^{1}H-^{13}C HMBC, correlates with data from unlabelled compound **201**. Data is in agreement with the unlabelled compound (**Section 4.9**).

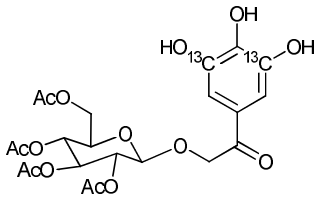
2-[3,4,5-Tris(benzyloxy)-[3,5-$^{13}C_2$]phenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside (222a)



To a solution of α-hydroxy-3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]acetophenone **201a** (41 mg, 0.09 mmol), anhydrous sodium sulfate (22 mg), 4Å molecular sieves (22 mg), and $Hg(CN)_2$ (46 mg, 0.18 mmol) in dry toluene (1 mL), was added dropwise a solution of 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide **93** (44.4 mg, 0.108 mmol) in dry toluene (1 mL) under rigorously anhydrous conditions. The reaction mixture was heated at reflux for 2 h, after which time the reaction mixture was cooled and filtered through a bed of celite. The toluene was removed at reduced pressure and the residue dissolved in dichloromethane. The organic phase was washed with water (3 x 5 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as an orange syrup. Purification was by column chromatography (silica gel, dichloromethane/diethyl ether, 95:5 and petroleum ether/ethyl acetate, 3:1) to give **222a** as an off-white oil (31 mg, 42%). δ_H (400 MHz, $CDCl_3$-acetone-d_6) 1.93 (3H, s, CH_3-3), 1.94 (3H, s, CH_3-4), 1.95 (3H, s, CH_3-6), 1.97 (3H, s, CH_3-2), 3.57-3.63 (1H, m, CH-5), 4.05 (1H, dd, *J* 16.4, 3.1 Hz, CH_2-6a), 4.17 (1H, dd, *J* 16.4, 6.0 Hz, CH_2-6b), 4.48 (1H, d, *J* 7.6 Hz, CH-1), 4.55 (1H, AB, *J* 12.5 Hz, CO*CH*$_2$O), 4.83 (1H,

AB, *J* 12.5 Hz, CO*CH*$_2$O), 4.98-5.04 (2H, m, CH-2,4), 5.05-5.09 (6H, m, CH$_2$-Bn), 5.17 (1H, t, *J* 9.6 Hz, CH-3), 7.15 (2H, dt, *J* 16.4, 2.2 Hz, CH-2',6') and 7.26-7.40 (15H, m, CH-Ar); δ_C (100 MHz, $CDCl_3$-acetone-d_6) 20.6 (CH_3), 20.7 (CH_3), 61.9 (CH_2-6), 68.4 (CH-4), 70.8 (CH_2), 71.3 (*C*H$_2$-Bn), 71.5 (*C*H$_2$-Bn), 71.8 (CH-2), 72.8 (CH-5), 72.9 (CH-3), 75.2 (*C*H$_2$-Bn), 99.3 (CH-1), 113.1 (d, *J* 6 Hz, CH-2',6'), 127.8 (CH-Ar), 128.1 (CH-Ar), 128.2 (CH-Ar), 128.3 (CH-Ar), 128.5 (CH-Ar), 128.6 (CH-Ar), 152.7 (enhanced, ^{13}C-3',5'), 169.4 (C=O), 169.5 (C=O), 170.3 (C=O) and 170.7 (C=O); *m/z* (ES$^+$) 809 [(M+Na)$^+$, 60%], 461 [(M-H$_2$O+Na)$^+$, 100]; HRMS (ES$^+$) [Found: (M+Na)$^+$, 809.2679, $^{13}C_2{}^{12}C_{41}H_{44}O_{14}Na$ requires 809.2696]. Data is in agreement with the unlabelled compound (**Section 4.9**).

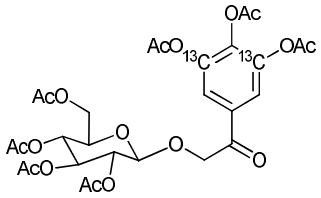
2-[3,4,5-Trihydroxy-[3,5-$^{13}C_2$]phenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside (223a)



To a solution of 1-(3,4,5-tris(benzyloxy)-[3,5-$^{13}C_2$]phenyl)-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside **222a** (28 mg, 0.035 mmol) and palladium black (4 mg, 0.035 mmol) in ethyl acetate (4 mL) was attached a balloon containing hydrogen gas. The reaction mixture stirred at room temperature for 18 h, during which time the hydrogen in the system was periodically replaced. After this time, the mixture was filtered through a bed of celite and solvents removed at reduced pressure to give **223a** as an off-white oil (18 mg, used crude). δ_H (400 MHz, acetone-d_6) 1.80 (3H, s, CH_3-3), 1.85 (3H, s, CH_3-4), 1.88 (3H, s, CH_3-6), 1.90 (3H, s, CH_3-2), 3.81-3.87 (1H, m, CH-5), 3.99 (1H, dd, *J* 15.5, 2.9 Hz, CH_2-6a), 4.14 (1H, dd, *J* 15.5, 5.1 Hz, CH_2-6b), 4.54 (1H, AB, *J* 12.4 Hz, CO*CH*$_2$O), 4.70 (1H, *J* 7.4 Hz, CH-1), 4.74 (1H, AB, *J* 12.4 Hz, CO*CH*$_2$O), 4.85 (1H, t, *J* 9.6 Hz, CH-2), 4.92 (1H, t, *J* 9.6 Hz, CH-4), 5.13 (1H, t, *J* 9.6 Hz, CH-3) and 6.91-6.96 (2H, m, CH-2',6'); δ_C (100 MHz, acetone-d_6)

20.6 (CH_3), 62.8 (CH_2-6), 69.5 (CH-4), 71.3 (CH_2), 72.4 (CH-2), 73.4 (CH-5), 73.5 (CH-3), 99.4 (CH-1),† 107.8 (CH-2',6'),† 126.6 (C-1'), 137.6 (C-4'),† 146.2 (enhanced, ^{13}C-3',5'), 170.9 (C=O) and 193.2 (C=O). † Observed by ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC, correlates with data from unlabelled compound **223**. Data is in agreement with the unlabelled compound (**Section 4.9**).

2-[3,4,5-Tris(acetyloxy)-[3,5-$^{13}C_2$]phenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside (199a)



To a solution of 1-[3,4,5-trihydroxy-[3,5-$^{13}C_2$]phenyl]-2-oxoethyl 2,3,4,6-tetra-*O*-acetylglucopyranoside **223a** (18 mg, 0.035 mmol) in pyridine (1 mL) was added acetic anhydride (0.2 mL). The reaction mixture was stirred at room temperature overnight. The solvents were then removed at reduced pressure and the resulting residue dissolved in ethyl acetate (2 mL) and dilute ammonium chloride solution (2 mL). The aqueous phase was extracted with ethyl acetate (3 x 2 mL). The combined organic layers were washed with dilute ammonium chloride solution (2 mL), saturated sodium bicarbonate solution (2 mL), dried ($MgSO_4$) and the solvents removed at reduced pressure to give the crude product as a yellow residue. Purification was by column chromatography (silica gel, dichloromethane/diethyl ether, 95:5) to give **199a** as colourless glass (10 mg, 44%). δ_H (400 MHz, $CDCl_3$) 1.93 (3H, s, CH_3-3), 1.94 (3H, s, CH_3-4), 1.95 (3H, s, CH_3-6), 2.04 (3H, s, CH_3-2), 2.25 (9H, s CH_3), 3.58-3.63 (1H, m, CH-5), 4.10 (1H, dd, *J* 12.3, 2.3 Hz, CH_2-6a), 4.20 (1H, dd, *J* 12.3, 4.7 Hz, CH_2-6b), 4.48 (1H, d, *J* 7.8 Hz, CH-1), 4.56 (1H, AB, *J* 12.3 Hz, COCH_2O), 4.83 (1H, AB, *J* 12.3 Hz, COCH_2O), 4.97-5.13 (3H, m, CH-2,3,4) and 7.61 (2H, t, *J* 7.6 Hz, CH-2',6'); δ_C (100 MHz, $CDCl_3$) 20.4 (CH_3), 20.6 (CH_3), 20.8 (CH_3), 61.9 (CH_2-6),

68.3 (CH-4),† 70.7 (CH_2),† 71.2 (CH-2),† 71.7 (CH-5),† 72.7 (CH-3),† 99.2 (CH-1),† 120.3 (CH-2',6'),† 127.7 (C-1'),† 139.8 (C-4'),† 143.9 (enhanced, ^{13}C-3',5'), 167.5 (C=O),† 169.6 (C=O)† and 170.7 (C=O).† Observed by ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC, correlates with data from unlabelled compound **199**. Data is in agreement with the unlabelled compound (**Section 4.9**).

4.11 References (Experimental)

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APPENDIX 1 – Selected Reaction Variables for Optimisation of C-H Activation/Borylation/Oxidation Reactions

*Table 23 – Optimisation of reaction variables for C-H activation/borylation/oxidation reactions (**Bold type** conditions given and described in **Section 2.4**)*

Entry	Substrate	B_2Pin_2 (mmol)	$[Ir(OMe)(COD)]_2$ (mol%)	dtbpy (mol%)	Temp. (°C)	Time (h)	Conversion (%)[†]	Isolated Yield (%)**
1	**a**	**0.6**	**1.5**	**3**	**25**	**18**	**0**	**-**
2	a	0.6	1.0	2	25	18	0	-
3	a	0.6	0.5	1	25	18	0	-
4	a	0.6	1.5	3	40	18	0	-
5	**a**	**0.6**	**1.0**	**2**	**40**	**18**	**6**	**-**
6	a	0.6	1.5	3	70	18	23	-
7	**a**	**0.6**	**1.0**	**2**	**70**	**18**	**45**	**-**
8	a	0.6	0.5	1	70	18	27	-
9	a	0.6	1.5	3	110	18	31	-
10	**a**	**0.6**	**1.0**	**2**	**110**	**18**	**39**	**-**
11	a	0.6	0.5	1	110	18	4	-
12	**a**	**0.6**	**1.5**	**3**	**70**	**60**	**66**	**51**
13	**a**	**0.6**	**1.0**	**2**	**70**	**60**	**63**	**44**
14	**175**	**0.6**	**1.5**	**3**	**70**	**18**	**17**	**5**
15	175	0.6	1.0	2	70	18	19	-
16	175	0.6	0.5	1	70	18	22	-
17	175	0.6	2.5	5	70	42	8	-
18	175	0.6	2.0	4	70	42	11	-
19	175	0.6	1.5	3	70	42	14	-
20	175	1.2	2.5	5	70	18	23	-
21	175	1.2	2.0	4	70	18	29	-
22	175	1.2	1.5	3	110	18	23	-
23	175	1.2	1.0	2	110	18	27	-
24	175	1.2	0.5	1	110	18	26	-
25	175	0.6	1.0	2	110	18	26	-
26	175	0.6	0.5	1	110	18	30	-
27	175	1.2	2.0	4	110	100	17	-
28	175	1.2	1.5	3	110	100	16	-
29	175	1.2	1.0	2	110	100	19	-
30	175	1.2	2.0	4	120	18	13	-
31	175	1.2	1.5	3	120	18	8	-
32	175	1.2	1.0	2	120	18	5	-
33	175	1.6	2.0	4	110	18	0	-
34	175	1.6	1.5	3	110	18	0	-
35	175	0.6	1.0	2	110	18	28	-
36	175	1.2	1.0	2	110	18	27	-
37	175	0.6	1.0	2	70	18	16	-

38	175	1.0	1.0	2	70	18	26	-
39	175	1.2	1.0	2	70	18	19	-
40	175	1.0	1.0	2	70	88	35	-
41	175	1.0	1.0	2	70	88	10	-
42	175	1.0	1.0	2	70	88	6	-
43	175	1.2	1.0	2	70	18	14	-
44	175	1.2	1.0	2	70	18	21	-
45	175	1.2	1.0	2	70	18	12	-
46	**175**	**1.0**	**1.0**	**2**	**110**	**18**	**97**	**59**
47	175	1.0	1.0	2	110	3	69	45
48	175	1.0	1.0	2	110	3	68	47
49	**175**	**1.0**	**1.0**	**2**	**110^{s}**	**3**	**57**	**18**
50	**175**	**1.0**	**1.0**	**2**	**140^{s}**	**3**	**65**	**20**
51	175	1.0	1.0	2	110	3	76	57
52	**175**	**1.0**	**1.0**	**2**	**140^{S}**	**18**	**69**	**21**
53	**180**	**1.0**	**1.0**	**2**	**110**	**3**	**3**	**-**
54	**180**	**1.0**	**1.0**	**2**	**140^{S}**	**3**	**4**	**-**
55	**180**	**1.0**	**1.0**	**2**	**110**	**18**	**7**	**-**
56	**180**	**1.0**	**1.0**	**2**	**140^{S}**	**18**	**9**	**-**
57	180	1.0	1.5	3	110	18	19	-
58	180	1.5	1.5	3	110	18	23	-
59	180	1.5	2.0	4	110	18	34	-
60	**180**	**2.0**	**2.0**	**4**	**110**	**18**	**25**	**10**
61	**180**	**HBPin (1.5)**	**2.0**	**dppe (2)**	**110**	**18**	**29**	**-**
62	**180**	**HBPin (1.5)**	**2.0**	**dppe (4)**	**110**	**18**	**11**	**-**
63	180	1.0	1.0	dppe (1)	110	18	8	-
64	180	1.0	1.0	dppe (2)	110	18	5	-
65	180	HBPin (1.5)	1.0	dppe (1)	110	18	21	-
66	180	1.0	2.0	dppe (2)	110	18	10	-
67	180	1.0	2.0	dppe (4)	100	18	0	-
68	180	1.0 + HBPin (0.2)	2.0	dppe (4)	50^{M}	18	0	-
69	180	1.0 + HBPin (0.2)	2.0	4	50^{M}	18	9	-
70	**180**	**1.0 + HBPin (0.2)**	**2.0**	**dppe (4)**	**110**	**18**	**10**	**-**
71	**180**	**1.0 + HBPin (0.2)**	**2.0**	**4**	**110**	**18**	**16**	**-**
72	180	1.0 + HBPin (0.2)	2.0	dppe (2)	50^{M}	18	0	-
73	180	1.0 + HBPin (0.2)	2.0	2	50^{M}	18	0	-
74	180	1.0 + HBPin (0.2)	2.0	dppe (2)	110	18	8	-

75	180	1.0 + HBPin (0.2)	2.0	2	110	18	13	-
76	**180**	**1.0 + HBPin (1.0)**	**2.0**	**dppe (2)**	**110**	**18**	**45**	**44**
77	**180**	**1.0 + HBPin (1.0)**	**2.0**	**2**	**110**	**18**	**26**	**-**
78	**180**	**1.0 + HBPin (1.5)**	**2.0**	**dppe (2)**	**110**	**18**	**34**	**30**
79	180	1.0 + HBPin (1.5)	2.0	2	110	18	25	-
80	**180**	**1.0 + HBPin (2.0)**	**2.0**	**dppe (2)**	**110**	**18**	**37**	**29**
81	180	1.0 + HBPin (2.0)	2.0	2	110	18	27	-
82	**180**	**1.5 + HBPin (1.5)**	**2.0**	**dppe (2)**	**110**	**18**	**17**	**-**
83	**180**	**1.5 + HBPin (1.5)**	**2.0**	**2**	**110**	**18**	**20**	**-**
84	180	HBPin (1.5)	2.0	dppe (2)	110	40	34	-
85	180	1.0 + HBPin (1.0)	2.0	dppe (2)	110	40	19	-
86	**176**	**1.0**	**1.0**	**2**	**110**	**18**	**95**	**61**
87	176	1.0 + HBPin (1.0)	2.0	dppe (2)	110	18	0	-
88	176	1.0	1.0	2	110	18	93	59
89	**177**	**1.0**	**1.0**	**2**	**110**	**18**	**71**	**62**
90	177	1.0 + HBPin (1.0)	2.0	dppe (2)	110	18	0	-
91	177	1.0	1.0	2	110	18	67	60
92	**179**	**1.0**	**1.0**	**2**	**110**	**18**	**92**	**87[P]**
93	**178**	**1.0**	**1.0**	**2**	**110**	**18**	**51**	**22**
94	**178**	**1.0 + HBPin (1.0)**	**1.0**	**dppe (2)**	**110**	**18**	**0**	**-**
95	**178**	**1.5**	**1.2**	**2**	**110**	**18**	**73**	**36**
96	**178**	**1.6**	**1.3**	**2.1**	**110**	**18**	**99**	**51**

* Substrate a = 1-bromo-3-iodobenzene
[†] Calculated from integration of ^{1}H NMR signals of starting material and product; selected conversions confirmed by LCMS/HPLC analysis to confirm reliability
** Isolated yields given where purification of the final product was carried out
[S] Octane as solvent (*cf. iso*-hexane)
[M] *Tert*-butyl methyl ether as solvent
[P] Phloroglucinol isolated as product

APPENDIX 2 – Publications

1. Laura J. Marshall, Karl M. Cable and Nigel P. Botting; First Synthesis of [1,3,5-$^{13}C_3$]Gallic Acid, *Org. Biomol. Chem.*, **2009**, *7*, 785-788.

2. Laura J. Marshall, Karl M. Cable and Nigel P. Botting; The Synthesis of Substituted Phenols from Pyranone Precursors, *Tetrahedron*, **2009**, *65*, 8165-8170.

In press:

3. Laura J. Marshall, Karl M. Cable and Nigel P. Botting; Abstracts of the 10th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, Chicago, June 2009: Studies Towards the Synthesis of [^{13}C]Anthocyanins, *J. Label. Compd. Radiopharm*., **2010**, in press.

4. Laura J. Marshall, Karl M. Cable and Nigel P. Botting; Abstracts of the 10th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, Chicago, June 2009: First Synthesis of [1,3,5-$^{13}C_3$]Gallic Acid, *J. Label. Compd. Radiopharm*., **2010**, in press.

5. Laura J. Marshall, Karl M. Cable and Nigel P. Botting; Abstracts of the 10th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, Chicago, June 2009: A General Methodology for the Synthesis of Substituted Phenols from Pyranone Precursors, *J. Label. Compd. Radiopharm*., **2010**, in press.

6. Laura J. Marshall, Karl M. Cable and Nigel P. Botting; Iridium-catalysed C-H Activation/Borylation/Oxidation for the Preparation of Diprotected Phloroglucinol Derivatives, *Tetrahedron Lett.*; **2010**, in press.

In preparation:

7. Laura J. Marshall, Karl M. Cable and Nigel P. Botting; First Synthesis of [2-^{13}C]Phloroglucinol, *J. Label. Compd. Radiopharm*., **2010**, in preparation.

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