



# **New synthetic applications of flavin dependent halogenases: Combining chemocatalysis with biological C–H functionalisation**

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# Abstract

The University of Manchester

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Doctor of Philosophy

## New synthetic applications of flavin dependent halogenases: Combining chemocatalysis with biological C–H functionalisation

Halogenases are nature's halogenating reagents. Flavin-dependent halogenases present an attractive alternative to current methods of halogenation as they can halogenate substrates in complementary positions. They show selectivity that is thus far not possible with transition metal catalysis. However, transition metal catalysis is incredibly important in organic synthesis.

The merging of chemocatalysis and biocatalysis into one pot processes is an emerging concept. This work describes a one pot reaction combining flavin-dependent halogenases with transition metal catalysis.

The palladium catalysed Suzuki reaction with 5-bromoanthranilamide was optimised and these conditions were integrated with the biohalogenation reactions to prepare a range of biaryls. The integration step required a filtration step post biohalogenation to remove deactivating enzymes.

HSP90 is a protein that is required for the stabilisation of cancerous cells. It is for this reason chemists are searching for new compounds that can bind to HSP90 and inhibit it. Radicicol is a known HSP90 inhibitor but displays low *in vivo* activity. RadH, a flavin-dependent halogenase, is believed to be involved in the chlorination of Monocillin I and/or II during the biosynthesis of radicicol. Herein is described the attempted synthesis of Monocillin I and the successful synthesis of Monocillin II and Monocillin III. The synthesis route is divergent and requires three components joined by an Mitsunobu esterification, benzylic acylation and a ring losing metathesis.

During the synthesis of these monocillins a one pot RadH and RCM biphasic protocol was optimised and progress towards substrates for these reactions is also presented.

## **Declaration**

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Parts of this work has been published in peer reviewed journals:

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*In the name of Allah, the Most Gracious, the Most Merciful*

This thesis is dedicated to my grandparents for their boundless love and affection,  
unwavering support and sacrifices.

## Abbreviations

2YT	yeast extract and tryptone media
Ac	Acetyl
ADH	alcohol dehydrogenase
Ar	Aryl
ATP	adenosine triphosphate
binap	2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene
br	Broad
CAN	cerium ammonium nitrate
cat.	catalyst/catalytic
CLEA	cross linked enzyme aggregate
COD	Cyclooctadiene
cp*	Pentamethylcyclopentadiene
CTAB	cetyltrimethylammonium bromide
d	Doublet
dba	Dibenzylideneacetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DEAD	diethyl azodicarboxylate
DET	diethyl tartrate
DIAD	diisopropyl azodicarboxylate
DIBAL	diisobutylaluminum hydride
DIPA	diisopropyl amine
DIPEA	<i>N,N</i> -diisopropylethylamine
DMAP	4-(dimethylamino)pyridine
DMF	Dimethylformamide
DMG	Dimethylguanidine
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPF	1,1'-ferrocenediyi-bis(diphenylphosphine)
EDC	<i>N</i> -(3-Dimethylaminopropyl)- <i>N'</i> -ethylcarbodiimide hydrochloride

EDTA	ethylenediaminetetraacetic acid
Eq	Equivalents
Et	Ethyl
EtOAc	ethyl acetate
FAD	flavin adenine dinucleotide
Fre	flavin reductase
GDH	glucose dehydrogenase
Gly	Glycine
Grubbs 2 <sup>nd</sup> generation	(1,3-Bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylenetricyclohexylphosphine)ruthenium
Grubbs Hoveyda 2 <sup>nd</sup> generation	(1,3-Bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro( <i>o</i> -isopropoxymethylmethylenetrifluoromethyl)benzyl)ruthenium
HATU	1-[Bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HBTU	<i>N,N,N',N'</i> -Tetramethyl- <i>O</i> -(1 <i>H</i> -benzotriazol-1-yl)uronium hexafluorophosphate
HMPA	Hexamethylphosphoramide
HOBr	1-hydroxybenzotriazole hydrate
HPIF	1,1,1,3,3-hexafluoro-2-propanol
HPLC	high-performance liquid chromatography
HSP90	heat shock protein 90
iPr	<i>iso</i> -propyl
IPTG	<i>isopropyl β-D-1-thiogalactopyranoside</i>
K	rate constant
KRED	Ketoreductase
LB	lysogeny broth
LCMS	liquid chromatography–mass spectrometry
LDA	lithium di <i>isopropylamide</i>
LED	light-emitting diode
M	Multiplet
MAO	monoamine oxidase

<i>m</i> CPBA	3-chloroperoxybenzoic acid
Me	Methyl
MeCN	Acetonitrile
mol	Moles
MOM	Methoxymethyl
MS	molecular sieves or mass spectrometry
Ms	Methanesulfonyl
MTBE	methyl <i>tert</i> -butyl ether
MWCO	molecular weight cutoff
NADH	$\beta$ -nicotinamide adenine dinucleotide
<i>n</i> Bu	Butyl
Ni-NTA	nickel -nitrilotriacetic acid
NMP	<i>N</i> -methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
OD <sub>x</sub>	optical density at x nm
PDB	protein data bank
PDMS	polydimethylsiloxane
PEG	polyethylene glycol
Ph	Phenyl
pH	potential of hydrogen
Pin	Pinacol
PMBM	<i>para</i> -methoxybenzyl chloromethyl ether
ppm	parts per million
PS	polymer supported
PVA	polyvinyl alcohol
q	Quartet
RCAM	ring closing alkyne metathesis
RCM	ring closing metathesis
rt	room temperature
s	Singlet
<i>s</i> Bu	<i>sec</i> -Butyl
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	2-(Trimethylsilyl)ethoxymethyl

SPhos	2-Dicyclohexylphosphino-2',6'-dimethoxybiphenyl
sSPhos	sodium 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl-3'-sulfonate
TB	terrific broth
TBAF	tetrabutylammonium fluoride
TBDPS	<i>tert</i> -Butyldiphenylsilyl
TBS	<i>tert</i> -butyldimethylsilyl
<i>t</i> Bu	<i>tert</i> -butyl
Tf	Trifluoromethanesulfonyl
TFA	trifluoroacetic acid
TFDO	methyl(trifluoromethyl)dioxirane
THF	Tetrahydrofuran
TLC	thin layer chromatography
TBDPS	<i>tert</i> -Butyldiphenylsilyl
TPPTS	triphenylphosphine-3,3',3''-trisulfonic acid trisodium salt
UV	Ultraviolet
XPhos	2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl

# 1. Introduction

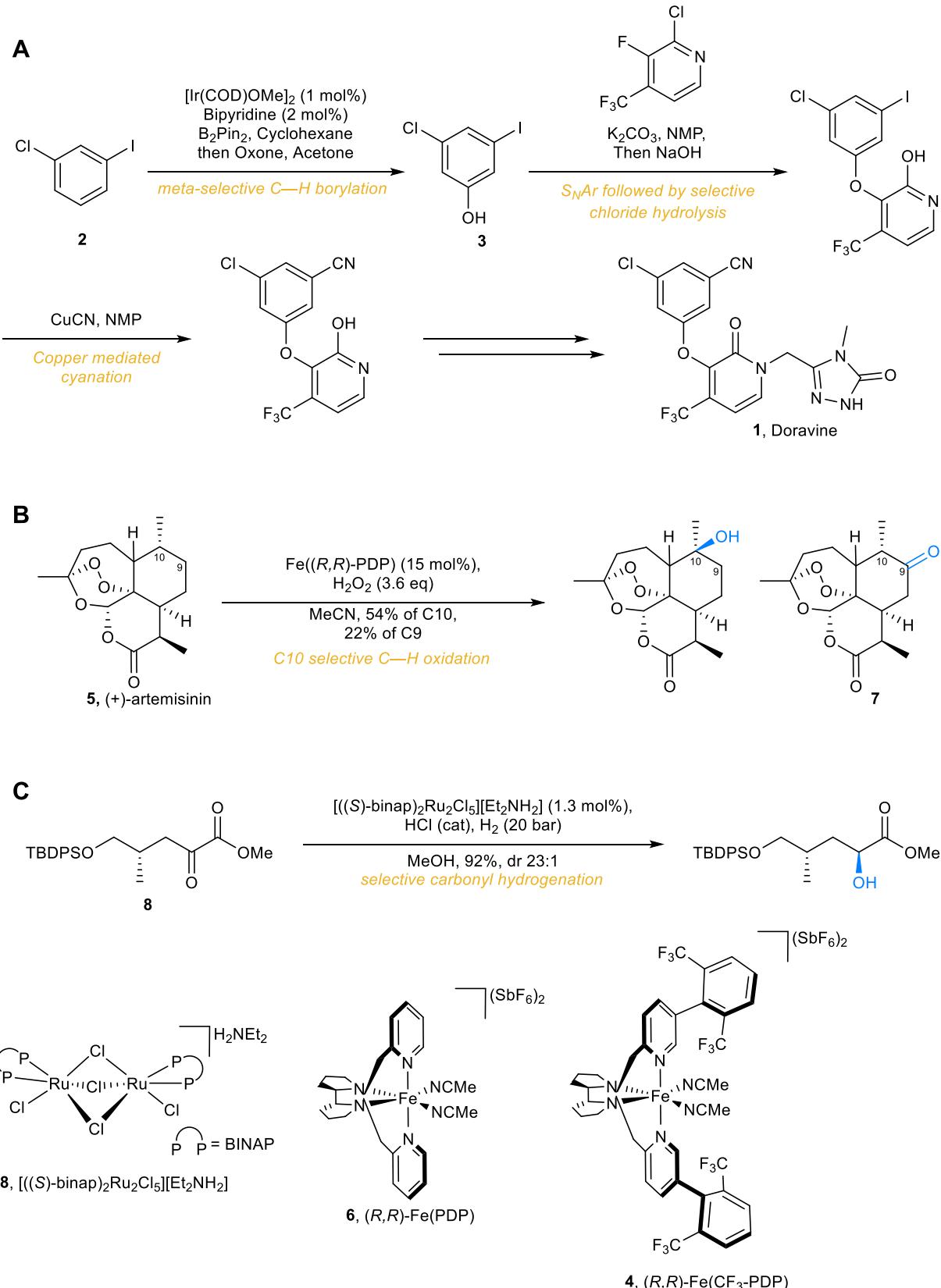
## 1.1. Selectivity in organic chemistry

Catalysis in chemistry has become a powerful tool in organic synthesis. One reason is because methods have now been developed which allow efficient discrimination between functional groups; thus streamlining syntheses. This functional group selectivity is important as it generates cleaner reaction profiles and as a result making synthesis more economical.<sup>1</sup>

An illustration of this functional group selectivity is in the synthesis of the pharmaceutical Doravirine, **1** (Scheme 1, A). The *meta*-selective C–H borylation–oxidation sequence of 1-chloro-3-iodobenzene **2** gives the desired phenol **3** in one pot. Significantly the iridium catalyst is selective for the C–H bond over the more reactive aryl halogen bonds present in the molecule. Gormisky and White was able to demonstrate the selectivity of the bulky chiral iron catalyst **4** developed by the White group. Oxidation with this catalyst occurs primarily at the tertiary C10 position of (+)-artemisinin, **5** over the multiple methylene sites and the ester group.<sup>2</sup> More interestingly switching the catalyst to one with more restrictive active site **6**, gave a switch in the ratio of the products to give predominantly the C9 oxidised product **7** (Scheme 1, B).

But in some cases the order of reactivity is determined by the intrinsic reactivity of the molecule. For example, the chemical reactivity, steric effects, chemical strain, electron density, bond strength and the acidity of a bond can be deciding factors for where the reaction can or will occur. This strategy was also used during the synthesis of **1**. The phenol **3**, containing two different aryl halide bonds partakes in a S<sub>N</sub>Ar on an electron poor pyridine.<sup>2,3</sup> After hydrolysis of the chloride the copper mediated cyanation is completely selective towards the weaker aryl iodide over the aryl chloride (Scheme 1, A).

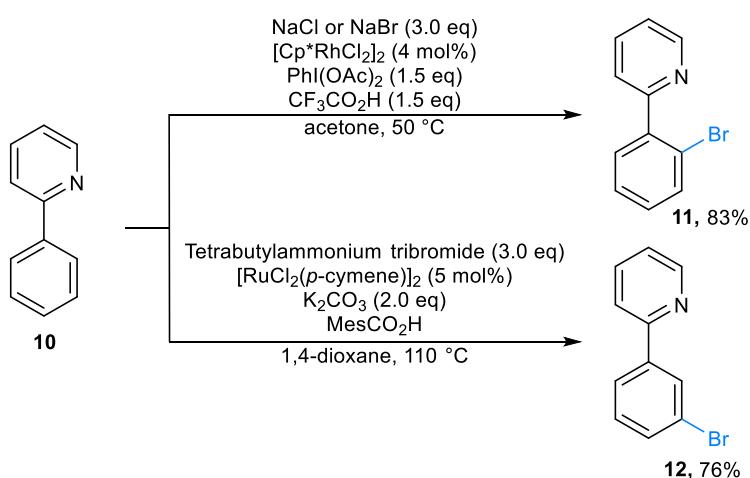
During the total synthesis of the polyketide natural products Amphidinolide X and Y asymmetric hydrogenation with ruthenium catalyst **8** was used to reduce the  $\alpha$ -ketoester **9**. The ketone is reduced over the ester carbonyl and the monoreduced product was isolated in 92% yield (Scheme 1, C).<sup>3</sup>



**Scheme 1.** Chemoselectivity in organic synthesis (A) Route to the synthesis of doravine an iridium catalysed *meta*-borylation shows functional group tolerance, the halides remain untouched during the reaction. The hydrolysis after the  $\text{S}_N\text{Ar}$  reaction occurs exclusively on the electron poor pyridine and the copper mediated cyanation occurs at the weaker C—I bond over the stronger C—Cl bond. (B) Using the catalysts developed by White *et al.* the C—H oxidation is selective for the tertiary C—H over other C—H bonds. (C) The selective monoreduction of an  $\alpha$ -ketoester, on route to the total synthesis of amphidinolide X the ketone is easier to reduce than the ester.

Being able to discriminate chemical bonds based on the environment that the catalyst creates, and not the molecule itself, is still the ‘holy grail’ of organic synthesis.<sup>4</sup> Significant advancements have taken place in many research groups, including within the Greaney group (Scheme 2). 2-Phenyl pyridine, **10** can be brominated in two complementary positions using a rhodium catalyst and NaBr the *ortho* position on the phenyl ring is brominated to **11**<sup>5</sup> but using a ruthenium catalyst and a different bromide source the *meta* position is brominated to give **12**.<sup>6</sup>

This discrimination is something that enzymes are already known to do. Enzymes or biocatalysts often show exquisite selectivity and this is due to the nature of the active site. The substrate usually binds specifically in the three-dimensional active site through a series of interactions, which include electrostatic, hydrogen bonding and  $\pi$ -stacking.

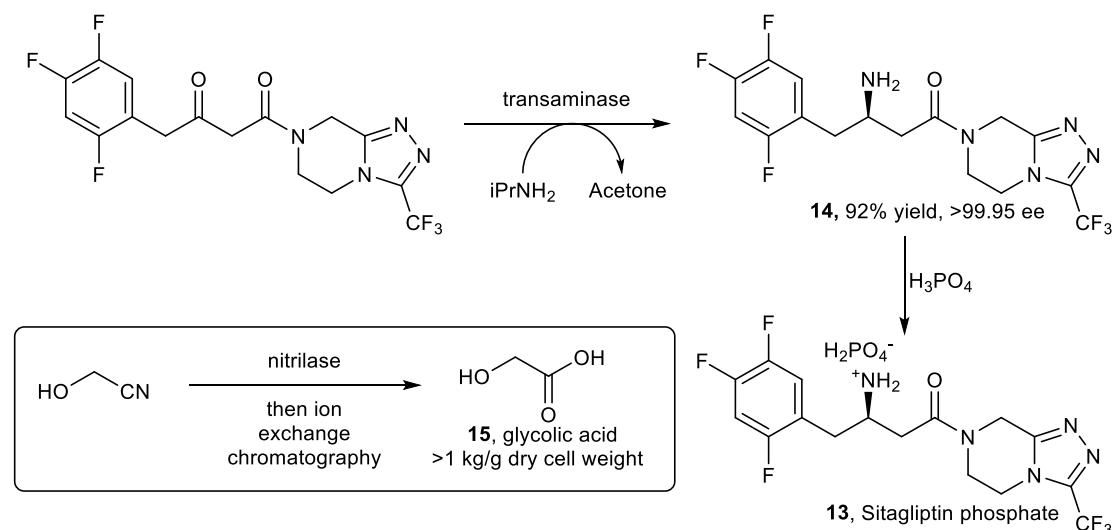


**Scheme 2.** Catalyst-controlled selectivity. Bromination of 2-phenylpyridine **10** can occur in the *ortho* or *meta* position depending on the reaction conditions.

Biocatalysis is the use of enzymes to conduct chemical transformations.<sup>7-9</sup> One of the main drawbacks to this selectivity of enzymes is the narrow substrate scope that biocatalysts can often have. Improvements in protein engineering have opened the door for the development of more suitable biocatalysts that can catalyse a wider range of reactions with a wider scope. With significant improvements over the years biocatalysis is now being used as a viable approach for accessing high value chemicals in industry.

Biocatalysts are already being used to synthesise fragments and key intermediates of important drug molecules such as Sitagliptin (**13**), Atorvastin and Lipitor on an industrial scale. A transaminase is used for the direct conversion of the ketone fragment to the chiral amine **14**. The excess of the amine source, isopropylamine drives the equilibrium to favour the product. Previously this chiral centre was installed by imine formation with ammonium acetate followed by a Noyori asymmetric hydrogenation. The non-biocatalytic route is unfavourable as it requires additional steps for the removal of the expensive rhodium from the hydrogenation catalyst.

Their use is extended beyond the fine chemicals and pharmaceutical industry: for example, they are used to prepare monomers for polymers on ton scale (Scheme 3, insert). Glycolic acid (**15**) is polymerised into the polymer poly(glycolic acid).<sup>10</sup>



**Scheme 3.** Using biocatalysts for important industrial applications. A transaminase is used to convert the  $\beta$ -ketoamide into the chiral amine **14** which is used to directly prepare the antidiabetic drug **13**. Insert; a nitrilase is able to convert 2-hydroxyacetonitrile into glycolic acid **15**, a monomer used for the synthesis of poly(glycolic acid).

### 1.1.1. C–H functionalisation

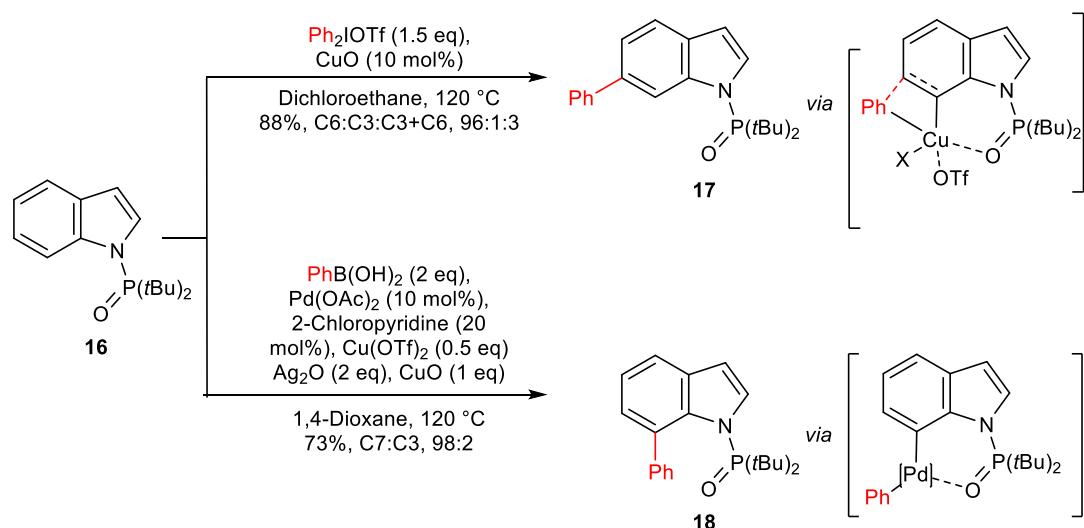
C–H functionalisation is where a C–H bond is cleaved to form a new carbon–carbon, or carbon–heteroatom bond. Direct functionalisation has had an impressive impact in organic synthesis and it is easy to see why, being able to install desired functionality without the need to prefunctionalise can lead to more streamlined synthesis.

#### *Regiocontrolled C–H arylation of indole*

To exemplify how the organic chemistry community is trying to address the issue of being able to selectively C–H functionalise at certain C–H bonds; some strategies for

the C–H bond functionalisation of the benzene core of indole will be described in this section.

Developing a collection of reaction conditions in which synthetic chemists can control the selectivity of a reaction is a coveted goal and so far, some progress has been made towards achieving this. The Shi group was able to selectively arylate the same *N*-phosphinoyl indole substrate **16**, either in the 6-position to **17** or the 7-position to **18** depending on the reaction conditions used. The phosphinoyl directing group directs C–H activation at the C7 position over the C2 position as it is sterically bulky and electron withdrawing. The combination of these two factors suppresses the formation of the more commonly seen 5 membered metallocycle and so blocking the C2 and/or C3 position when utilising this methodology is not necessary.<sup>11–13</sup>



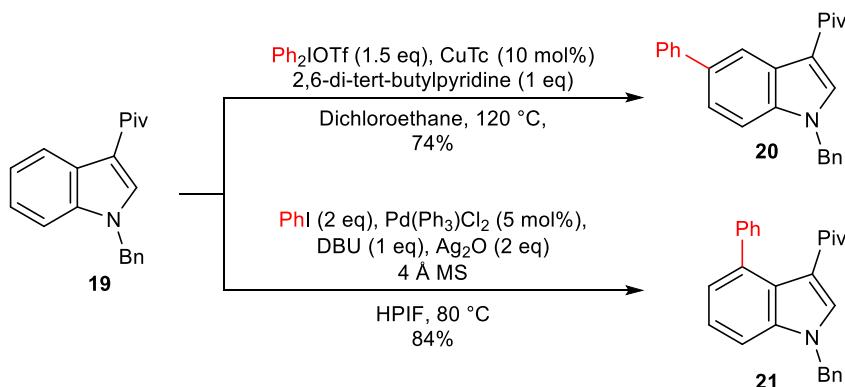
**Scheme 4.** Catalyst directed selectivity demonstrated in the indole ring.

Shi and coworkers were then able to further operate this strategy to arylate at C4 or C5.<sup>14</sup> By installing a pivaloyl group at C3 (**19**), this group could direct metalation at C4 (Scheme 5). Using a diaryliodonium and copper combination arylation occurs at C5 (**20**); using palladium and an aryl iodide, the aryl group is installed at C4 (**21**). Moreover, the pivaloyl directing group could be removed using a mild retro Friedel–Crafts reaction.

When metalation occurs with copper, oxidative addition with the diaryliodonium onto the copper is proposed. Then *via* a Heck type mechanism with the phenyl group on the copper, a four membered transition state (Scheme 4) is formed involving the C6 of

indole. A base-mediated E2 type elimination restores aromaticity affording the C6 arylated indole.<sup>12,13</sup>

When palladium is used as the catalyst, directed by the P=O bond, oxidative addition occurs at the 7-position giving the 6 membered palladacycle. It is likely that an aryl palladium species is formed with the phenylboronic acid (Scheme 4), reductive elimination then gives the corresponding 7-phenylindole. The directing group was found to be removable using LiAlH<sub>4</sub>.



**Scheme 5.** Catalyst directed selectivity demonstrated in the indole ring.

Regiodivergent synthesis is still an emerging research field and it is encouraging that some reliable methods have already been demonstrated, but as seen from the above examples, these methods generally require a complex ‘cocktail’ of metals and additives, extended reaction times and elevated temperatures.

### C6 functionalisation of indole

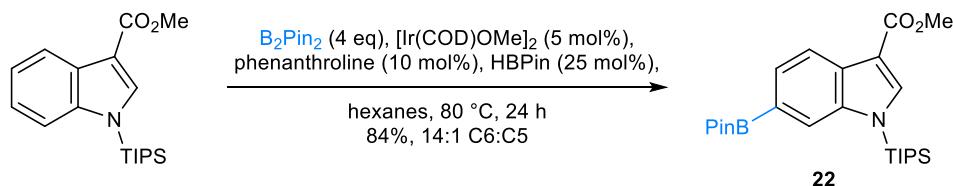
The C6 position of indole is the most challenging of the positions to functionalise and to date there only been a few approaches describing the access to this position.<sup>11,12,15–18</sup> They usually require the more reactive C2 and C3 to be protected (Scheme 6).

Feng *et al.* were able to demonstrate valuable methodology to preferentially borylate at the C6 position on N-TIPS indoles.<sup>15</sup> The bulky TIPS group was able to shield the more reactive C2 and C7 positions. Borylation also occurred at C5 (**22**), and the best regioselectivity obtained was 14:1, C6:C5. Although this methodology was used to complete the total synthesis of the alkaloids fumitremorgin A and verruculogen, substrates with more sensitive functionality were not tolerated.

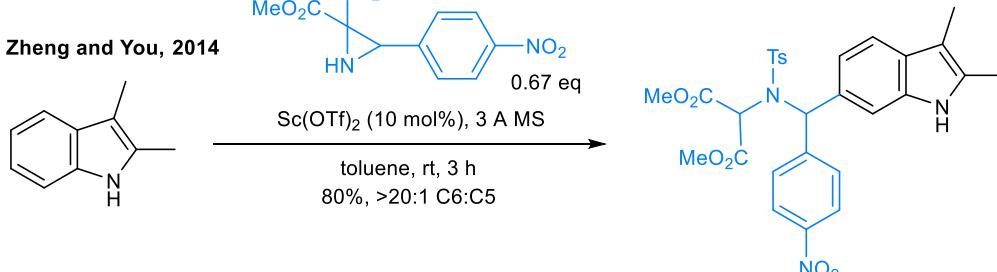
Yu and co-workers were able to alkenylated at the C6 position with very good selectivities using the specifically designed template. This U-shaped template contains

a nitrile group which directs palladation at the C6 position. The template is reported to be removable using magnesium turnings in methanol, these conditions also reduced the olefin.<sup>17</sup>

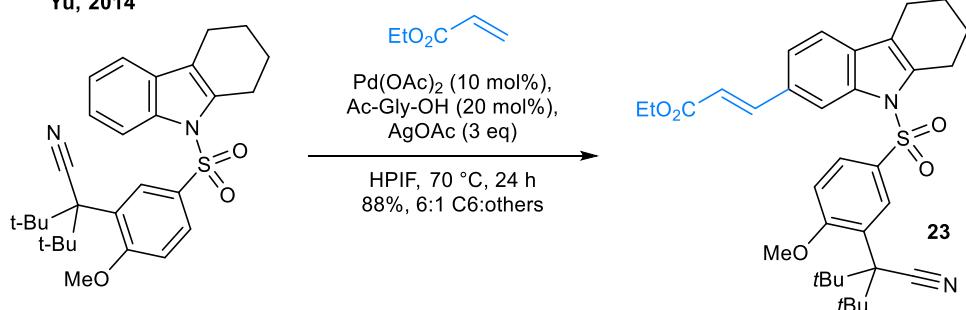
**Baran, 2015**



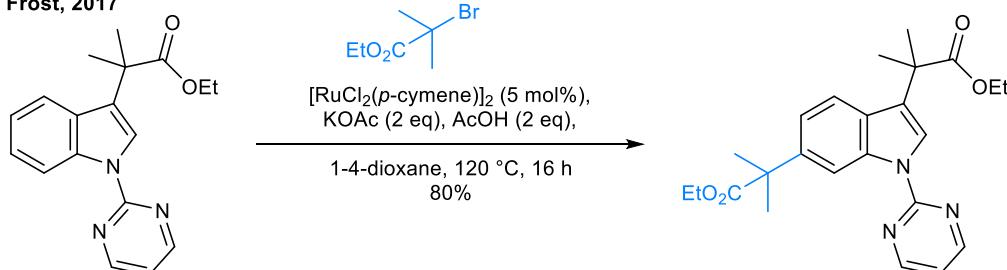
**Zheng and You, 2014**



**Yu, 2014**



**Frost, 2017**



**Scheme 6.** Direct C6 functionalisation of indole

## 1.2. Halogenation

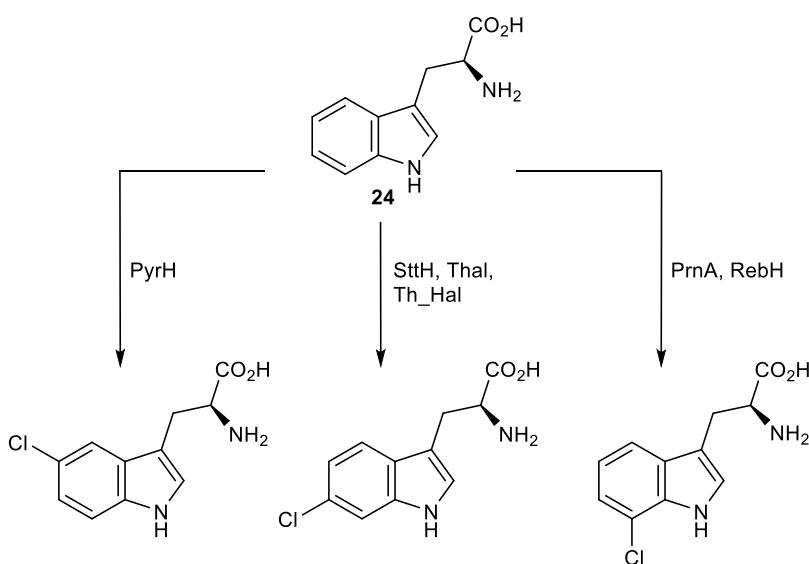
Halogens are present in many natural products<sup>19,20</sup> and are valuable building blocks in chemical synthesis. Many pharmaceuticals and agrochemicals also contain halogen atoms. The majority of the current methods of halogenation require stoichiometric halogenation reagents and atom economy is poor.<sup>21–27</sup> Halogenation of electron rich arenes are especially problematic as they can lead to a mixture of products.<sup>26</sup>

## 1.2.1. Biological Halogenation

Nature uses its own halogenating enzymes to selectively halogenate a range of compounds. Because these enzymes use innocuous, non-toxic halide ions as the halogen source they can be seen as an attractive alternative to traditional and current methods of halogenation.

### 1.2.1.1. Flavin-dependent halogenases

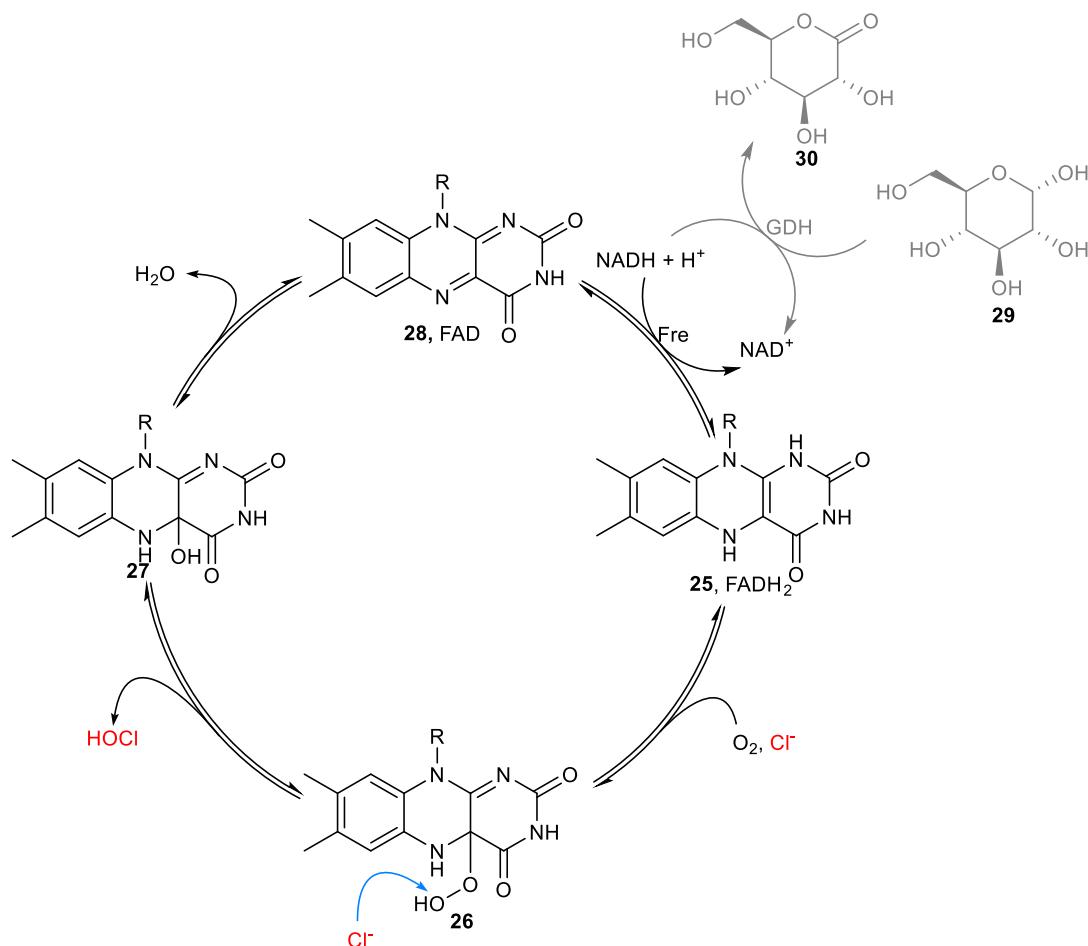
One of the most interesting class of halogenating enzymes synthetically are the flavin-dependent halogenases, this is because they show remarkable selectivity (Scheme 7). Different halogenases can halogenate in three complementary positions of tryptophan (**24**). They are grouped under the flavin-dependent monooxygenase protein superfamily and they work in conjunction with a flavin-reductase. The flavin-reductase is involved in generating hypohalous acid, which is the electrophilic halogenating agent. The flavin binding site and the substrate binding site of the halogenase is separated by only a 10 Å long tunnel, meaning that the hypohalous acid produced is not able to freely diffuse from the enzyme and halogenation is regioselective.



**Scheme 7.** Halogenation of tryptophan in nature. The tryptophan halogenases regioselectively halogenate tryptophan in nature at three different sites.

The mechanism for the formation of the electrophilic halogenating species hypohalous acid is drawn in scheme 8; reduced flavin adenine dinucleotide (**25**, FADH<sub>2</sub>) reacts with molecular oxygen to form a flavin-hydroperoxide intermediate (**26**). A chloride or bromide ion, present in solution then attacks this hydroperoxide to give the hypohalous acid and **27**, loss water from the flavin gives FADH<sub>2</sub>. Flavin reductase

(Fre) then converts FADH<sub>2</sub> (**25**) from FAD (**28**) to close the catalytic cycle; as this a reduction process it requires NADH. Halogenases are known to be compatible with a variety of NADH cofactor recycling enzymes.

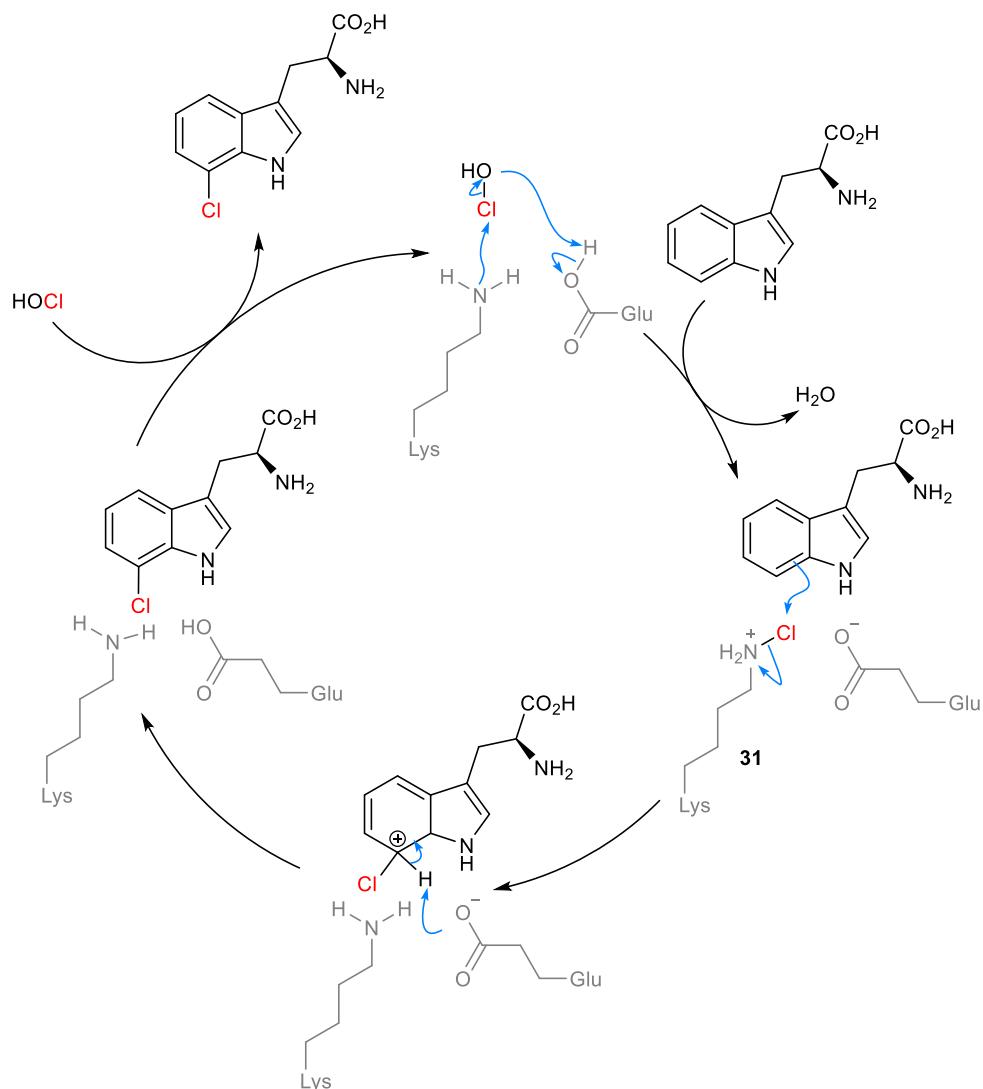


**Scheme 8.** The mechanism of the hypochlorous acid generation by a conserved bound flavin module. The NAD<sup>+</sup> regeneration system commonly used in the Micklefield lab is GDH, this uses a molecule of glucose **29** which is converted to gluconolactone **30**. Fre = Flavin reductase and GDH = glucose dehydrogenase.

The other major class of halogenases that chlorinate or brominate are haloperoxides. They use a vanadium or iron haeme complex to form the hypohalous acid which is liberated from the enzyme and thus they lack the regioselectivity of flavin-dependent halogenases. The metal complex is attacked by a hydrogen peroxide to give a metal-peroxy complex. This complex is then attacked by a halide ion to generate a hypohalous acid.

As mentioned earlier the protein trapped hypohalous acid, in flavin-dependent halogenases, then diffuses through a small tunnel into the active site. The origin of regioselectivity is believed to be from a lysine residue in the active site. The most

widely accepted mechanism (Scheme 9) is formation of chloramine species (**31**) in the active site by the reaction of the hypohalous acid with a lysine residue.



**Scheme 9.** The proposed mechanism of halogenation by the tryptophan-7-halogenase. The lysine residue is believed to react with the hypohalous acid in the active site to form a chloramine species close to a bound substrate; electrophilic aromatic substitution on the indole ring gives the Wheland intermediate which is stabilised and then deprotonated by a proximal glutamate residue. The 7-chlorotryptophan then diffuses out of the active site.

The halogenation process itself is seen to be atom economical as the only byproduct formed is water.

### Tryptophan halogenases

The most widely studied flavin dependent halogenases are the tryptophan halogenases and of these, the enzymes that are most widely described in literature are: PrnA, RebH, PyrH and SttH.

PrnA was the earliest halogenase to be reported in 2000 by the group of van Pée; it is part of the enzyme cluster involved in the biosynthesis of pyrrolnitrin and is a tryptophan 7-halogenase.<sup>28</sup> The crystal structure was subsequently published in 2005 by the same group. RebH is another tryptophan 7-halogenase and is part of the rebeccamycin biosynthesis.

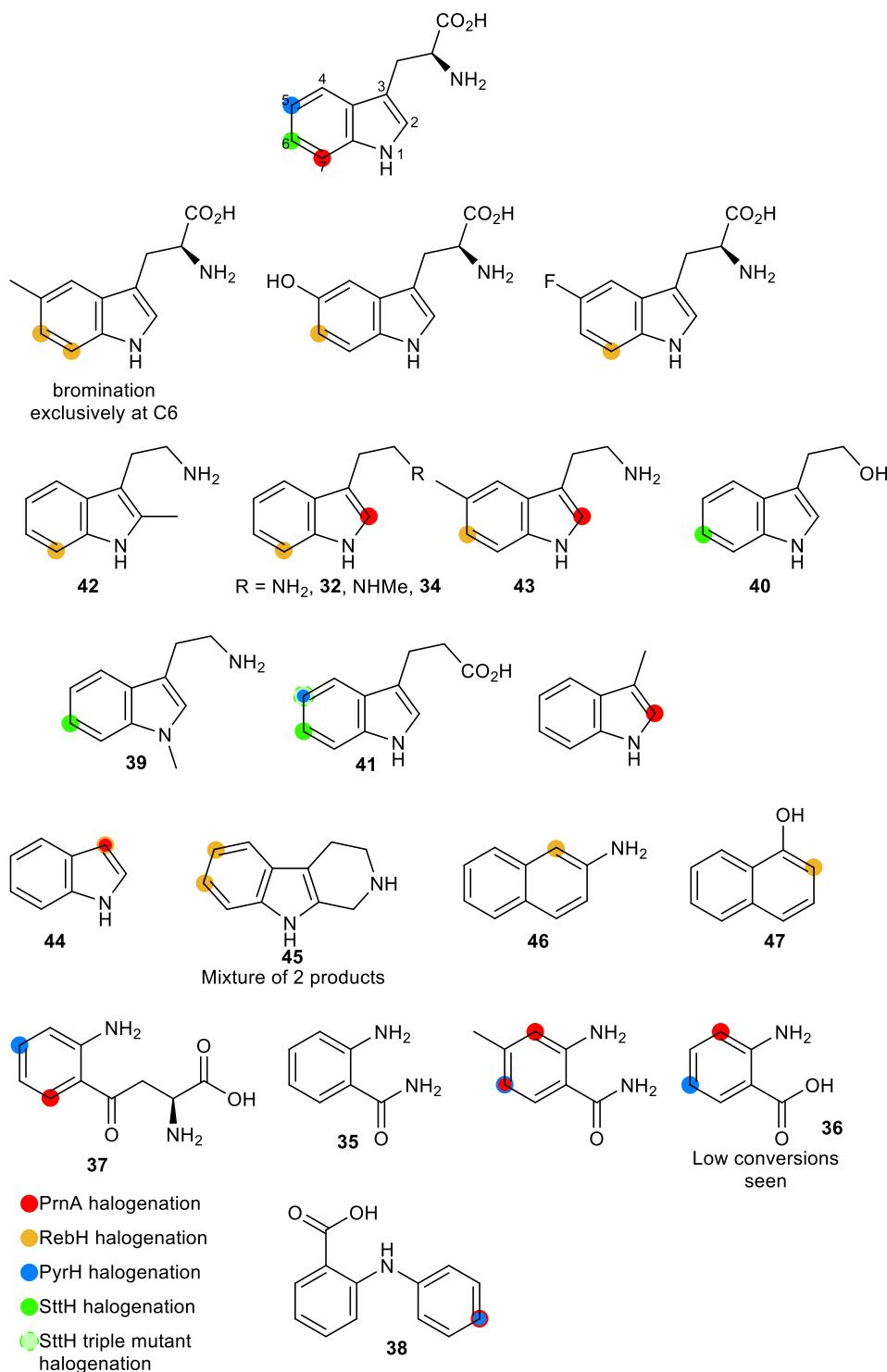
PyrH is a tryptophan 5-halogenase found in the pyrroindomycin B gene cluster and was first isolated and characterised in 2005.<sup>29</sup> This is the only tryptophan 5-halogenase within the literature that has been characterised. SttH is a tryptophan 6-halogenase found in *Streptomyces toxytricini* NRRL 15443 gene cluster.<sup>30,31</sup>

An overview of the substrates of these halogenases and their regioselectivity is shown in figure 1. Generally, the substrates require amino or primary amide groups to effect the regioselectivity.

The initial substrate scope of the wild type enzyme PrnA was demonstrated and shown to accept a range of indole containing compounds including tryptamines **32** and **33**; chlorination generally takes place at the 2 position of the indole on these substrates. Without substitution at C3 (**34**), chlorination takes place at C3.<sup>32</sup>

Later a mutagenesis study with PrnA was conducted in our group and a more diverse substrate scope was determined.<sup>33</sup> It was found that PrnA could accept a range of *ortho*-acyl anilines such as anthranilamide (**35**), anthranilic acid (**36**), kynurenone (**37**) and *N*-phenyl anthranilic acid (**38**); the activity towards these substrates were compared with PyrH and in some cases the selectivity was found to be complementary to PyrH.

Our group have also explored the substrate scope of the 6-halogenase SttH.<sup>34</sup> It was found that the C6 selectivity was retained on other indole substrates such as *N*-methyltryptamine (**39**), tryptophol (**40**) and indole-3-propanoic acid (**41**). The crystal structure of SttH was also obtained and compared to the previous published crystal structures. The chlorination selectivity of SttH on indole-3-propanoic acid, **41** could be switched from C6 to C5 by preparing a triple mutant of SttH (L460F/P461E/P462T).

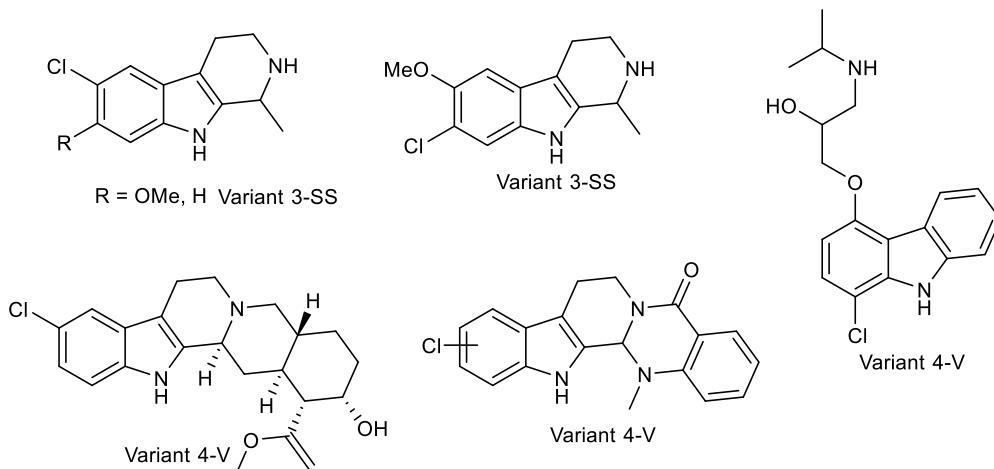


**Figure 1.** An overview of the substrates known to be halogenated by tryptophan halogenases.

RebH has been extensively studied by the group of Lewis. They found that they could improve the yields of protein by fusing RebH and RebF (a flavin reductase) to a maltose binding protein, this was then cleaved using proteases. They also discovered that using the chaperones GroEL and GroES the solubility of RebH was improved.<sup>35,36</sup>

Once they optimised the process for the overexpression of RebH, they explored the substrate scope. It was also proved that tryptophan could be brominated of using NaBr. The non-natural enantiomer D-tryptophan could also be chlorinated to complete conversion. It was found that a range of indoles (**32**, **34**, **42**) could be halogenated at the 7-position although with the indole containing the methyl group at C5 (**43**) chlorination occurs at C6. Indole (**44**) bearing no substituents is also chlorinated by RebH at C2. Other substrates are tryptoline (**45**), 1-substituted naphthalenes **46** and **47**.

Payne *et al.* have also developed a library of mutants using directed evolution to address the problem of stability attributed to this enzyme class.<sup>37</sup> They showed that by subjecting RebH through several rounds of mutagenesis, using directed evolution, they could expand the substrate scope to chlorination of various larger substrates including alkaloids (Figure 2).<sup>38</sup>



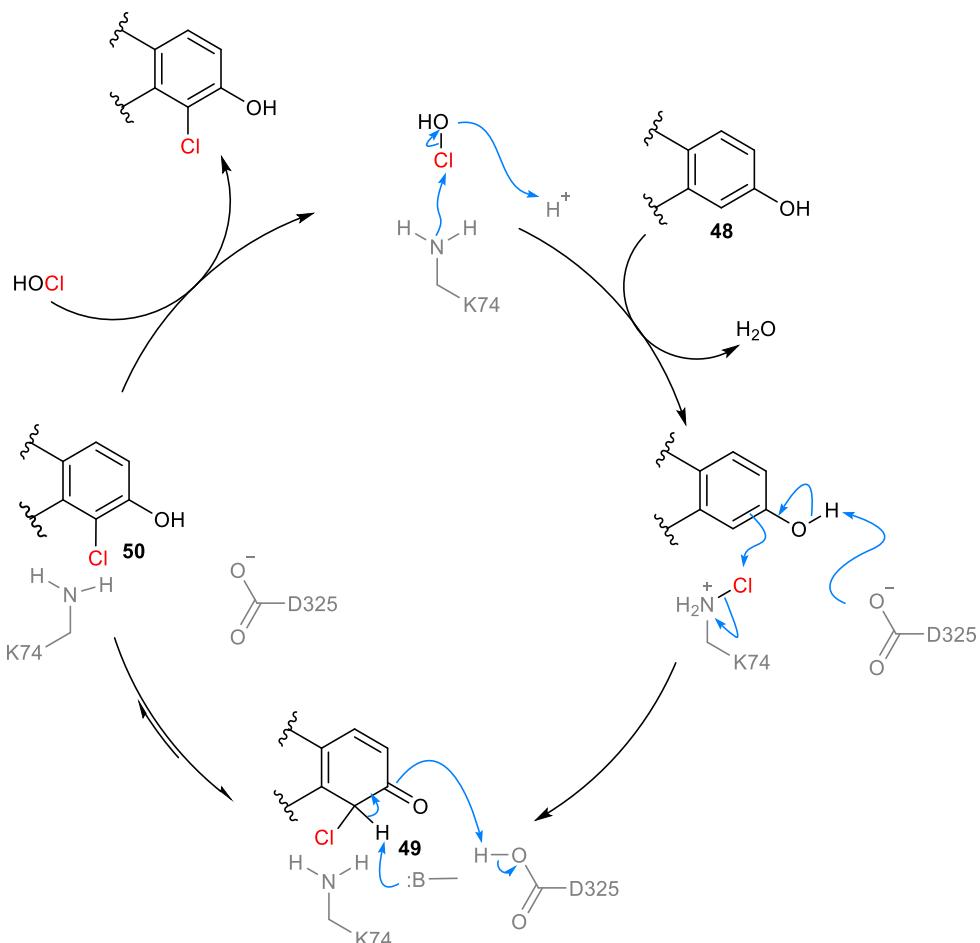
**Figure 2.** Halogenation of large indole containing substrates by RebH mutants obtained by directed evolution cycles.

### **Phenolic halogenases**

RadH is an enzyme responsible for chlorinating the phenolic ring in the natural product radicicol.<sup>39</sup> Rdc2 is the other known phenolic halogenase that has been characterised.<sup>39</sup> These are also flavin-dependent and require FAD to function. As a result of work conducted within our group, the mechanism is believed to differ to that of tryptophan halogenases: RadH is only able to chlorinate substrates bearing an *ortho*-phenol group.

Studies conducted by Eileen Brandenburger (E.B.) in our lab, have shown that an aspartic acid residue is crucial for the catalytic activity of RadH (Scheme 10) and it is postulated that this deprotonates the phenol (**48**) allowing it to act as a basic enolate

*via* this, halogenation can then occur at the  $\alpha$ -position (**49**). Rearomatisation by tautomerisation to its enol form would then deliver the 2-chlorophenol (**50**).<sup>40</sup>

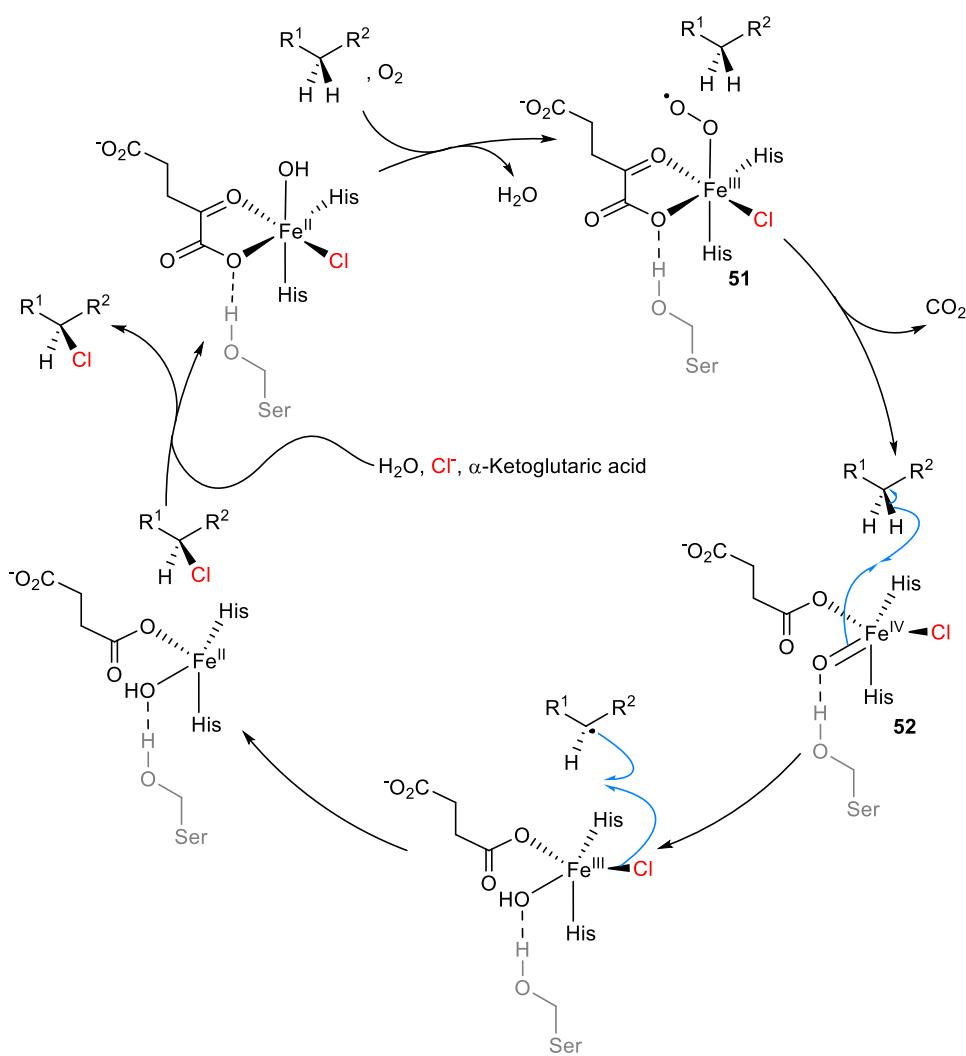


**Scheme 10.** The proposed mechanism of halogenation of a phenolic substrate (**48**) by RadH *via* a basic enolate formation, involving aspartate 325 and lysine 74.

### 1.2.1.2. Non-Haem Fe(II), $\alpha$ -keto glutarate dependent halogenases

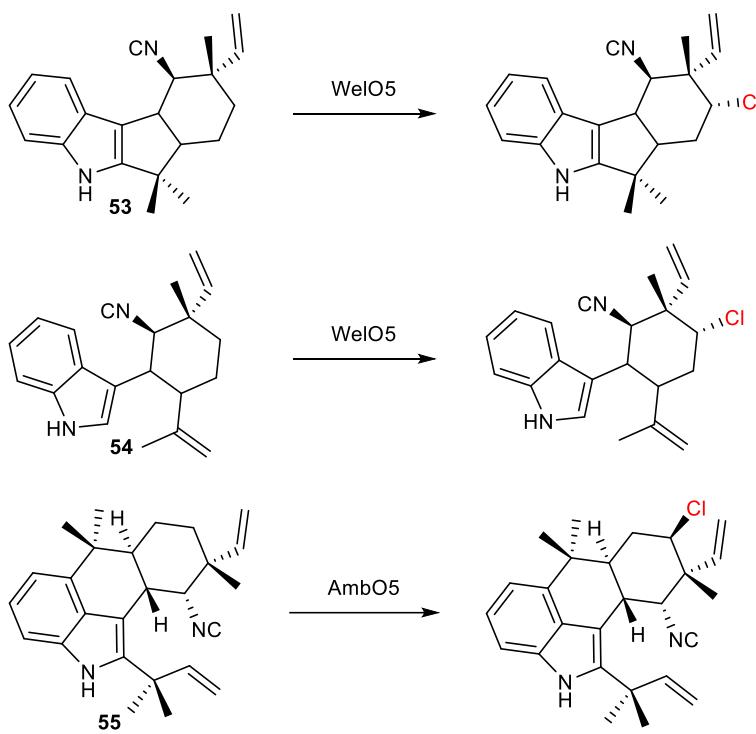
Another interesting class of halogenases that have shown selectivity is the  $\alpha$ -keto glutarate dependent halogenases. These halogenases regio- and stereoselectively chlorinate unactivated aliphatic substrates.

The mechanism (Scheme 11) is believed to be similar to hydroxylases and involves an  $\alpha$ -ketoglutarate chelated Fe(II) species with a chloride ligand (**51**). After ligand exchange with  $O_2$  and decarboxylation of the glutarate to form succinate, an Fe(IV) oxo species is formed (**52**), this is then capable of abstracting a H atom from the substrate forming a carbon centred radical. This carbon centred radical then reacts with the chloride substituent on the iron giving the new stereocentre.<sup>41–43</sup>



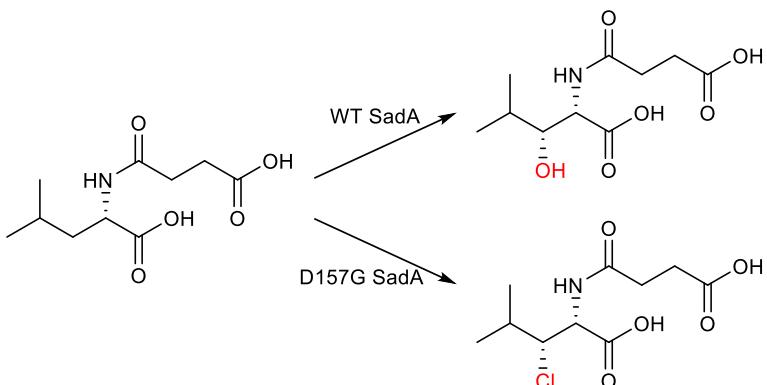
**Scheme 11.** The Mechanism of the Non-Haem Fe(II),  $\alpha$ -keto glutarate dependent halogenases, it involves the decarboxylation of  $\alpha$ -ketoglutaric acid to a succinate.

The majority of halogenases in this class usually act on substrates that are covalently attached to an acyl or peptidyl carrier protein (PCP). This secondary carrier protein can complicate the synthetic utility of these halogenases. However, there are two known exceptions in this class: WelO5 and AmbO5. The first characterised was WelO5 and is the halogenase responsible for the chlorine substituent on welwitindoline (**34**). WelO5 can chlorinate both 12-*epi* fischerindole U (**35**) and 12 *epi* hapalindole C (**36**); the chlorinated products were confirmed by mass spectrometry and  $^1\text{H}$  NMR spectroscopy.<sup>44</sup>



**Scheme 12.** The halogenation of welwitindoline (**53**), 12-epi fischerindole U (**54**) and 12-epi hapalindole C (**55**) by the  $\alpha$ -keto glutarate dependent halogenases.

The group of Liu and Boal was able to alter the functionality of the hydroxylase SadA to a chlorinase by comparing the gene sequences and the structural information between the halogenases and hydroxylases. It was found that a key difference was an Ala or Gly residue in halogenases, in place of a carboxylate. This change means that a chlorine atom coordinates to the Fe centre. It was found that by changing the ligands using site directed mutagenesis around the metal centre, the enzyme could be reprogrammed to give chlorinating ability (Scheme 13). However, the reaction was not completely selective and hydroxylase activity was still seen and the reaction with bromide was not as successful.<sup>45</sup>



**Scheme 13.** Converting the activity of SadA hydroxylase to a chlorinase by site specific mutagenesis.

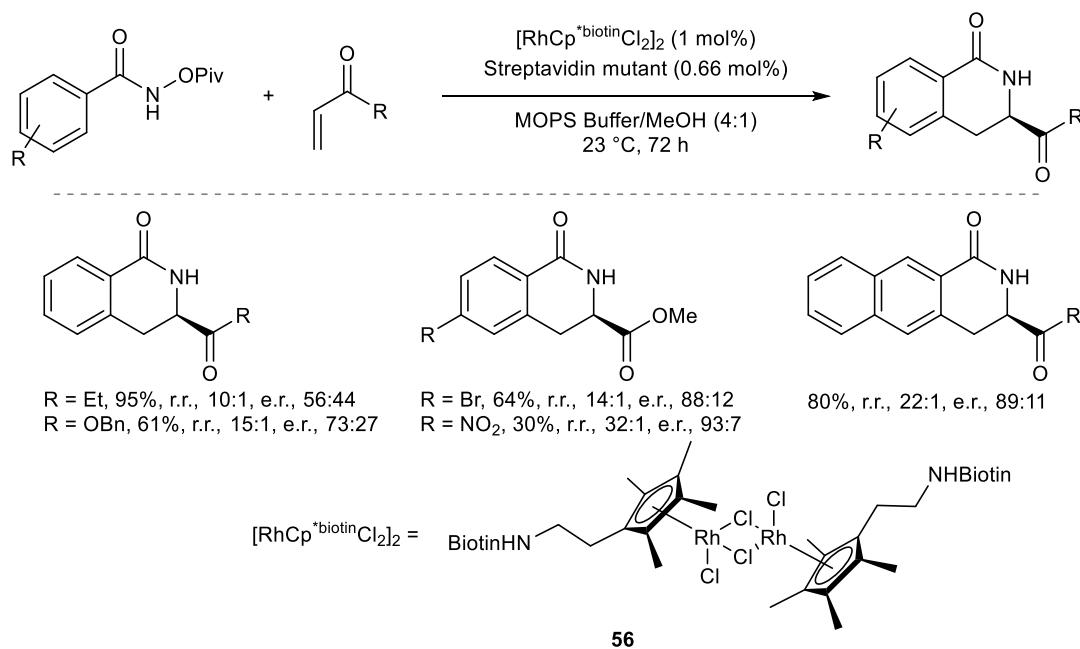
These halogenases, which work on freestanding substrates, not covalently attached to a PCP, are relatively new and show great promise for late stage halogenation strategies. This is because they accept relatively large substrates with sensitive functionalities.

### 1.3. Combining chemocatalysis with biocatalysis

Although enzymes have shown to be extremely valuable they are still limited in that they cannot incorporate a wide range of functional groups onto molecules. Attempts to address this has been attempted with artificial metalloenzymes or using enzymes containing the promiscuous haeme active site.<sup>46,47</sup>

#### 1.3.1. Metalloenzymes

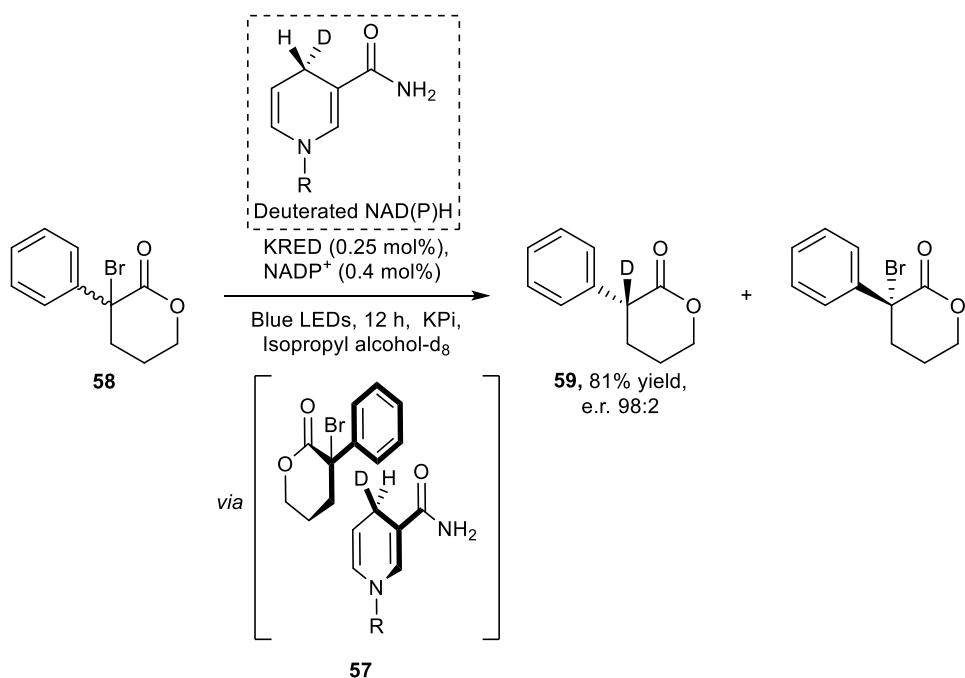
Pioneering work by the group of Ward used avidin-biotin binding to form artificial metalloenzymes.<sup>48</sup> These work by using the high affinity of avidin or streptavidin to biotin to affix a metal catalyst to a protein. The combined efforts by the groups of Ward and Rovis allowed for the anchoring of a rhodium(III) complex (**56**) to a protein (Scheme 14). This was to create a metalloenzyme capable of catalysing an asymmetric benzannulation reaction via C–H activation of protected benzhydroxamic acids.<sup>48</sup> Prior to this there were no known enantioselective benzannulation reactions utilising rhodium C–H activation.



**Scheme 14.** C–H activation of protected benzhydroxamic acids using a rhodium complex tethered to streptavidin enzyme.

Hartwig and co-workers were able to reconstitute a haem-porphrin containing enzyme by expressing the apo-protein lacking the haeme-unit and then treating it with stoichiometric amounts of different metal porphrin cofactors. It was found that the iridium containing metalloenzyme was able to form benzodihydrofurans or indanes by an intramolecular C–H carbene insertion reaction. Furthermore, it could also perform intramolecular C–H carbene insertion reactions onto phthalane or cyclopropanation reactions of olefins.<sup>49</sup>

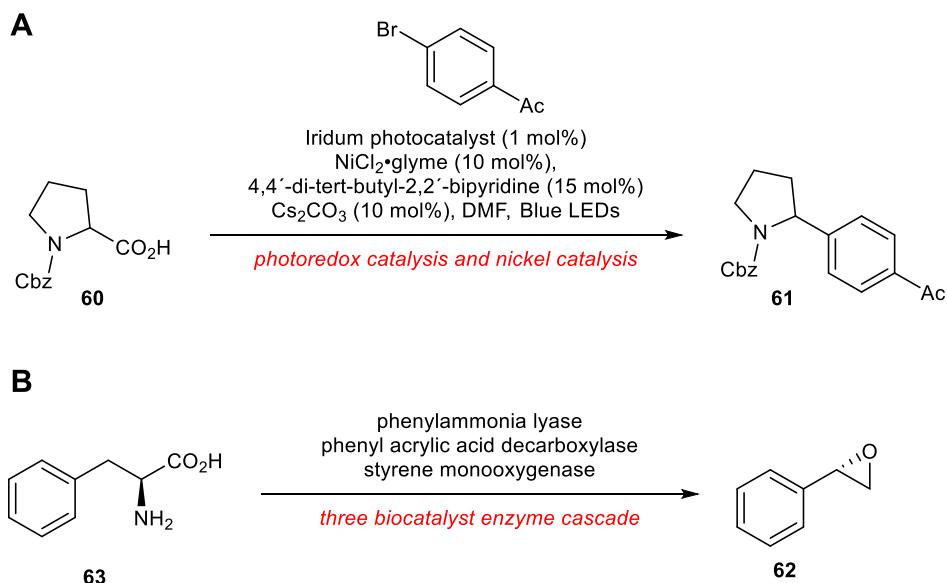
Another example of note is the switch of the reactivity of a nicotinamide-dependent ketoreductase (KRED) into a dehalogenase. This was achieved by irradiating a charge transfer complex (57) formed from nicotinamide and the halolactone substrate **58** (Scheme 15).<sup>50</sup>



**Scheme 15.** The debromination of lactone **37** using the combination of KRED and blue LED lights to give chiral lactone **59**.

Although these examples show remarkable reactivities they require specialist protein engineering techniques and thus making them difficult to optimise. In recent years, effort in particular by the groups of Hartwig, Zhao,<sup>51</sup> Hummel and Groger,<sup>52–55</sup> has also been in combining biocatalysts in one pot with chemocatalysts which can utilise both the selectivity of biocatalysts and the wide arena of chemocatalysts. (Scheme 15)

From the examples in literature it can be seen that it has been difficult to truly merge chemocatalysts and biocatalysts, as can often be done amongst its own class,<sup>56,57</sup> and typically needs some form of compartmentalisation. This is because there are compatibility issues; metal catalysed reactions can often require heating, bases or extended reaction times for catalytic turnover. Whereas enzymes require physiological conditions to prevent denaturation.

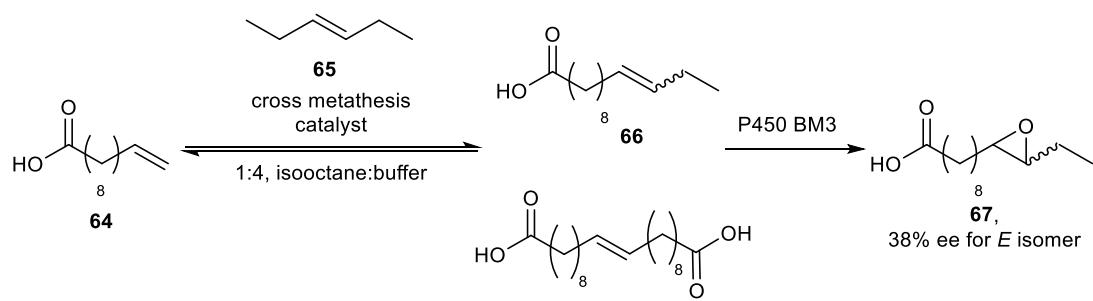


**Scheme 16.** Combining multiple reactions together can give rise to valuable compounds. **(A)** Coupling a photoredox reaction with a nickel catalysed cross coupling to give 2-arylpyrrolidines **61** from *N*-boc proline (**60**).<sup>58</sup> **(B)** The three enzyme cascade allows for the conversion of the enantiopure epoxide **62** from the amino acid phenylalanine (**63**).

### 1.3.2. Combining biocatalysis with olefin metathesis

The narrow selectivity of P450-BM3 was used as an advantage in a tandem chemocatalytic and biocatalytic reaction.<sup>51,59</sup> 10-undecenoic acid (**64**) could undergo a cross metathesis reaction with *trans*-3-hexene (**65**) to give internal alkene **66** which was selectively epoxidised using P450-BM3 enzyme to **67**. The P450 was able to discriminate the olefin based on size (Scheme 17).

Furthermore, this strategy benefited from the equilibration of the cross-metathesis step; the P450 allowed for the removal of the 10-tridecanoic acid from the equilibration. This meant that a higher yield of desired epoxide was formed than if the two steps were conducted separately. This system worked well and gave the epoxy-fatty acid in up to 90% yield.

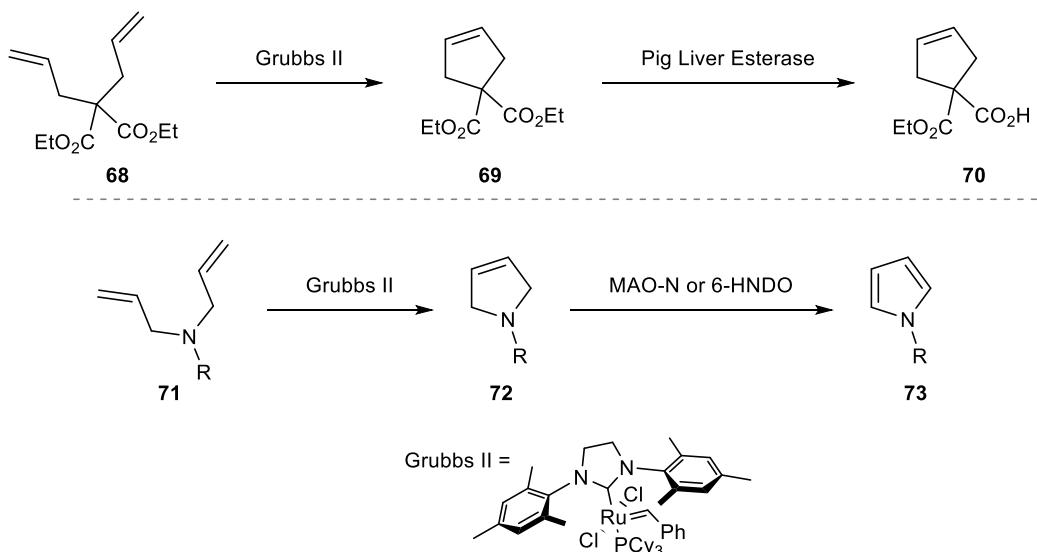


**Scheme 17.** The use of ring closing metathesis in combination with biocatalysis for the synthesis of 5-membered rings.

The compartmentalisation was achieved through the reaction occurring in biphasic, isooctane/buffer media; with the cross metathesis occurring in the organic phase and the P450 remaining in the aqueous phase.

Metathesis-biocatalysis tandem reactions have also been achieved to form cyclic structures. Diethyl malonates containing two terminal alkene groups (**68**) were also used as substrates in a two-step one pot reaction. The Grubbs II catalysed RCM reaction was conducted in water as the solvent before the adjustment of the ionic strength with NaCl and addition of pig liver-esterase and *tert*butanol which cleaved one of the gem diester groups of **69** to form **70** (Scheme 18).<sup>60</sup>

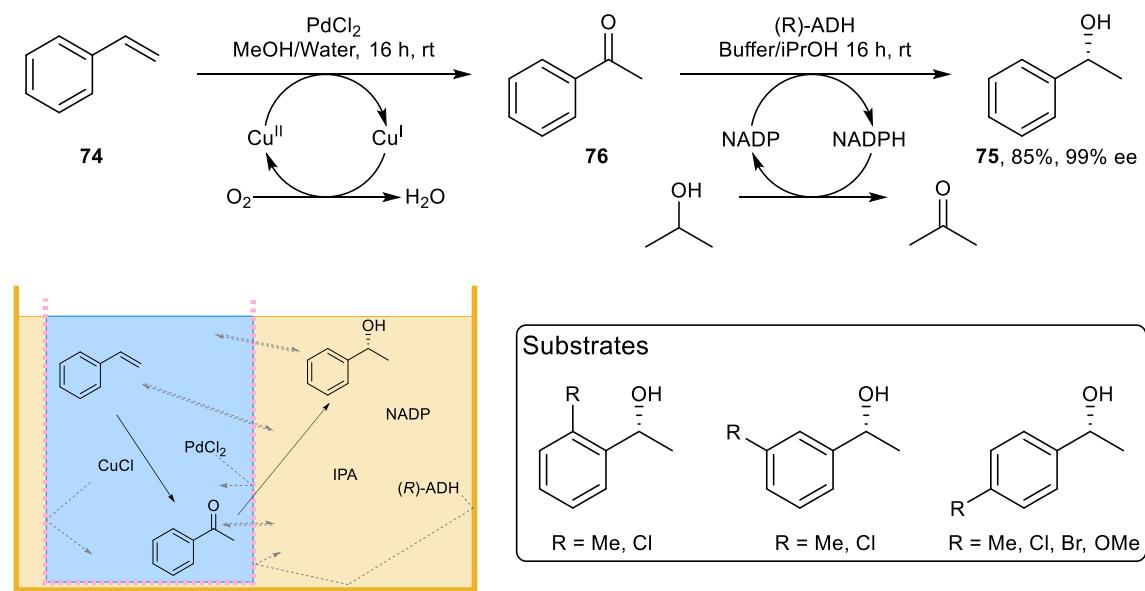
*N*-substituted pyrroles could be synthesised from a one pot two-step chemo-enzymatic approach from the simple to prepare diallylated alkyl amines or anilines (**71**) using biphasic reaction media. The diallyl amines were ring closed to the 3-pyrrolines (**72**) using Grubbs II catalyst. A whole cell monoamine oxygenase (MAO-N) biocatalyst could then be used to form the imine, which on spontaneous aromatisation forms the corresponding pyrrole (**73**). Whilst a similar iron catalysed process had been described previously, the use of MAO-N allows for a broader substrate scope, pyrroles containing an alkyl ester group at the 3 position or *N*-substituted could be prepared using this method (Scheme 18).<sup>61</sup>



**Scheme 18.** The use of ring closing metathesis in combination with biocatalysis for the synthesis of 5-membered rings.

### 1.3.3. Polymer compartmentalisation

Compartmentalisation using polymers has also been demonstrated. In 2015 Sato *et al.* showed that the polymer polydimethylsiloxane (PDMS) could be used to effectively separate mutually deactivating catalyst systems.<sup>54</sup> Using a PDMS ‘thimble’, a Wacker oxidation and alcohol reduction could both be run concurrently within the same vessel (Scheme 19).



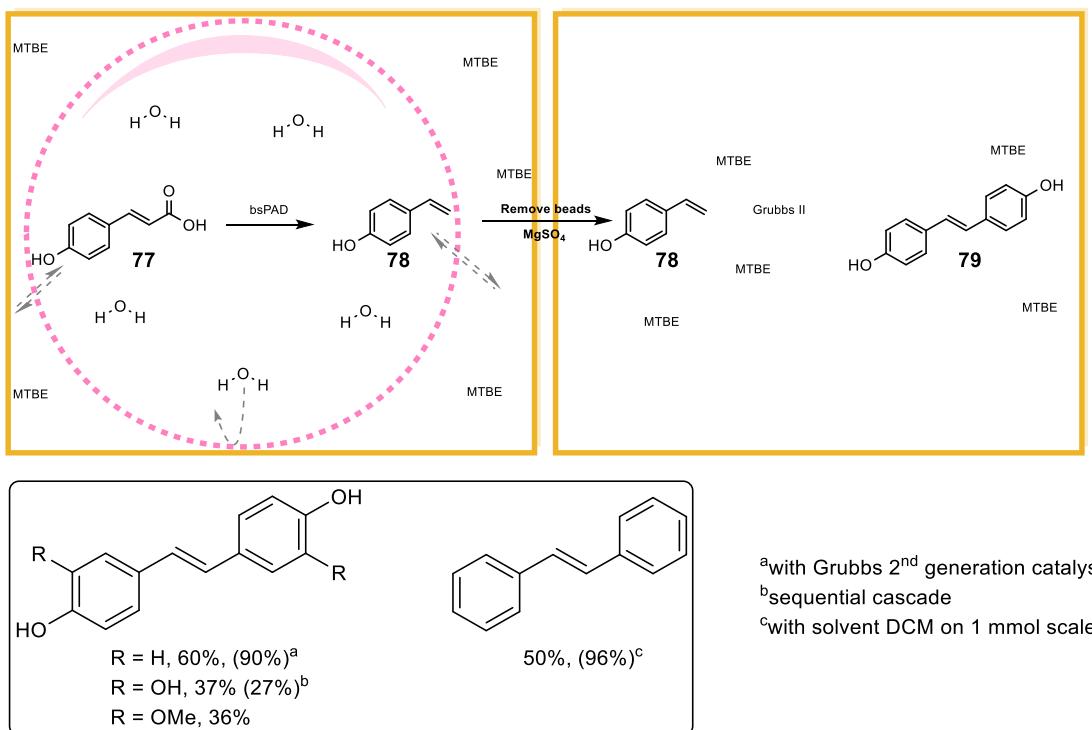
**Scheme 19.** Combining incompatible reactions. A Wacker oxidation can be combined with dehydrogenase into one pot through the use of PDMS membrane. Styrene (**74**) can be converted to enantiopure (*R*)-1-phenylethan-1-ol (**75**) via acetophenone (**76**)

The PDMS membrane is hydrophobic and so aqueous and polar components cannot traverse it. The need for the membrane arose when it was found unsurprisingly, that the presence of the copper catalyst required for the Wacker oxidation almost completely inhibited the alcohol dehydrogenase (ADH) catalysed reaction. The PDMS thimble containing the Wacker oxidation components was placed within a larger vessel with the ADH components and the substrate (**74**) and products (**75**, **76**) were lipophilic enough to move through the membrane. The catalysts were unable to move out of their respective vessels and thus could not inhibit each other.

After some optimisation it was found that the desired aryl ethanols could be produced with high enantiomeric purity and good yields. This procedure provides an elegant approach to separating two antagonistic systems.

Months later the groups of Gröger and Kourist reported the use of a PVA/PEG matrix to entrap the phenolic acid decarboxylase, *bsPAD*, in a chemoenzymatic route to stilbene derivatives.<sup>62</sup> The PVA/PEG cryogel in this case was prepared by adding cell lysate to an aqueous mixture of PVA/PEG, followed by dropping 40 µL of this mixture into silicone oil at -35 °C to form *bsPAD* cryogel beads. These porous PVA/PEG cryogels are able to hold the enzyme within their aqueous environment so that it can retain its required microenvironment (Figure 3).

The *bsPAD* encapsulated in PVA/PEG could catalyse the decarboxylation of **77** to **78** in the water immiscible MTBE. They could also be recycled and on the fifth reuse the beads retained 62% activity. Once the enzymatic reaction was complete the beads were removed from the medium, the reaction medium dried over MgSO<sub>4</sub> and then Grubbs II catalyst was added for the homodimerisation step. This process was demonstrated on a preparative scale; the reaction of 100 mg of hydroxycinnamic acid (**77**) with one gram of PVA/PEG beads and then 5 mol% of the metathesis catalyst gave the desired stilbene (**79**) in 60% overall yield.



**Figure 3.** The use of cryogel beads to conduct an enzymatic decarboxylation with cross metathesis reaction

Without the use of this compartmentalisation and using just a biphasic reaction mixture the conversions seen were unsatisfactory, this due to the instability of the metathesis catalyst towards water and air with this system. The electron rich hydroxystyrene **78** was prone to polymerisation and so isolating it was problematic. Being able to directly treat the product with the metathesis catalyst aided the efficiency of the reaction. This exemplifies the utility of one pot multi-step processes.

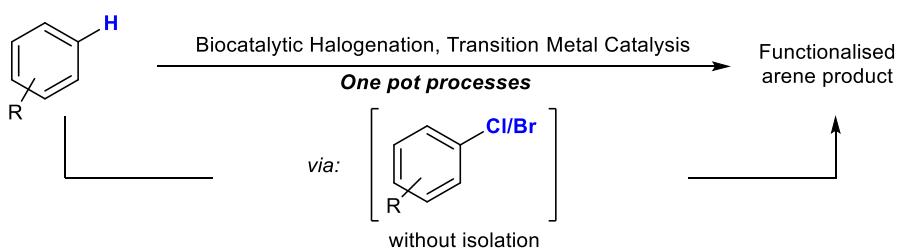
#### 1.4. Main Aims

Halogenated compounds are an extremely important class of compounds and currently have widespread use in organic synthesis. The flavin-dependent halogenases have been shown to be a favourable and more sustainable method to halogenating arenes.

Combining different catalytic cycles can give rise to novel products and can also streamline synthesis by eliminating workup and purification steps. In particular the combination of chemo and biocatalysts into tandem processes is a relatively

unexplored concept and so far, only a few elegant examples have been described in the literature.

The main aim of this thesis is to demonstrate the value of the flavin-dependent halogenases by employing them with more well established chemocatalysts in one pot reactions. The proposed strategy is shown in scheme 17. One pot reactions are where more than one reaction conducted in the same vessel without isolation of intermediates.



**Scheme 20.** Main aim of the project. Combining biohalogenation with chemocatalysis in a one pot fashion to give rise to functionalised arenes.

The combination of the two systems would require the two catalytic systems to occur at mutually compatible reaction conditions. Metal catalysis usually requires elevated temperatures for turnover and biological catalysis require ambient temperatures and pH to operate. The most challenging perception of this aim is that coordinating groups usually present in biological systems can deactivate metal catalysts and metals are frequently known to be deleterious to proteins.

To overcome this, we could initially scope transition metal catalysed reactions that have proven to be congruent with biological systems. It is supposed that these would be more likely to be functional in aqueous solvents, tolerate cofactors and other biological moieties present in the biocatalysed reaction and thus could possibly be combined one pot with halogenases.

## 2. C–H functionalisation using halogenases

Aryl halides are highly versatile compounds in organic chemistry and are capable of undergoing a large number of varied transformations. One such use is in palladium catalysed cross-coupling reactions. Since their development, palladium cross-coupling reactions have become an indispensable method of constructing C–C bonds and research is still being undertaken to develop these reactions.<sup>63</sup> Additionally palladium is known to be biocompatible as it has been used as a method of bioconjugation in living systems.<sup>64</sup>

### 2.1.1 Cross coupling in water

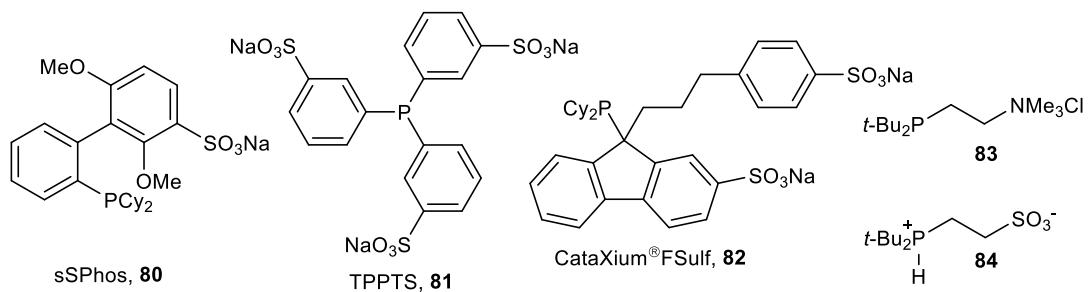
Biocatalysis usually only work in aqueous media, this is because enzymes require cofactors which are usually only soluble in water. It was for this reason only reactions that are known to be tolerant to water were explored.

Aryl halides can have different reactivities, in palladium catalysed cross coupling deactivated aryl halides are those into which oxidative insertion by palladium is difficult. Aryl halides bearing electron donating groups are considered to be electronically deactivated aryl halides.<sup>65,66</sup> Oxidative addition into aryl iodides is more facile than aryl bromides and chlorides.<sup>67</sup>

#### 2.1.1.1 Water soluble ligands

The ligand set used in metal catalysed reactions is usually important as they affect the reactivity of the metal centre. Of the most commonly used and studied are the phosphine-based ligands although other ligands are extensively used also. Electronically rich phosphines, though more active, tend to form more air sensitive catalysts. Electronically rich palladium catalysts are able to oxidatively insert into less reactive aryl halide bonds.<sup>68</sup> Bulky phosphines help with catalysis as they ensure that the palladium centre remains low coordinate; bulky phosphines would destabilise higher coordinate palladium species.

A range of hydrophilic ligands for palladium cross coupling reactions have been developed. Most of these are phosphines with solubilising groups such as **80-84** presented in figure 4

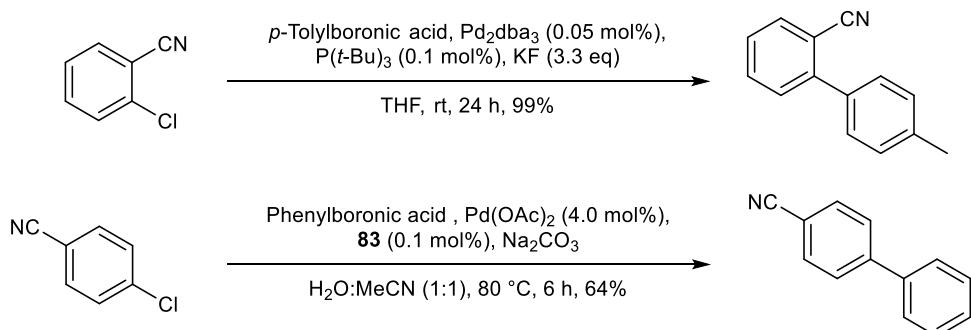


**Figure 4.** Some hydrophilic ligands employed in aqueous cross coupling reactions.

Triphenylphosphine-3,3',3''-trisulfonic acid trisodium salt (**81**, TPPTS), sulfonated triaryl phosphine, and was first synthesised by the group of Calabresse.<sup>69</sup> Later Shaughnessy showed that sulfonated trityl- and trixylyl- phosphines resulted in more efficient catalysts than TPPTS.<sup>70-72</sup> The TPPTS ligand has been used extensively and its uses have been shown not to be limited to palladium.

**82** developed by the group of Plenio, the 9-fluorenylphosphine ligand was doubly sulfonated to make it water soluble and was found to be compatible with heterocycles and unprotected amines.<sup>73-75</sup>

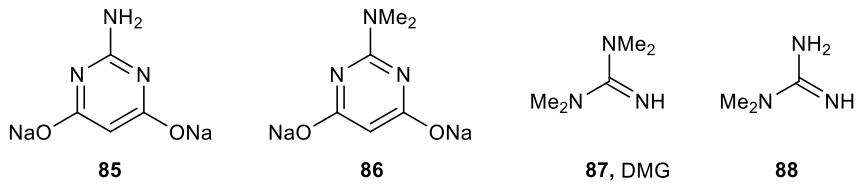
The trialkyl phosphines developed by Fu are known to give highly active catalysts which are effective with unactivated aryl chlorides but adding the cationic trimethylammonium portion (**83**) seems to markedly affect the catalytic ability (Scheme 21). The cross coupling reactions with **84** gave better results.<sup>76</sup>



**Scheme 21.** Relative catalyst activity comparison of L3 with P(t-Bu)<sub>3</sub>. Using L3 for the coupling of the aryl chloride requires higher temperatures and catalysts loadings than with P(t-Bu)<sub>3</sub> using a similar substrate.

Buchwald and coworkers were able to demonstrate that when the highly active biphenyl phosphine catalysts that he developed were sulfonated (**80**) that they retain their activity for a range of C–C bond forming reactions.<sup>77</sup>

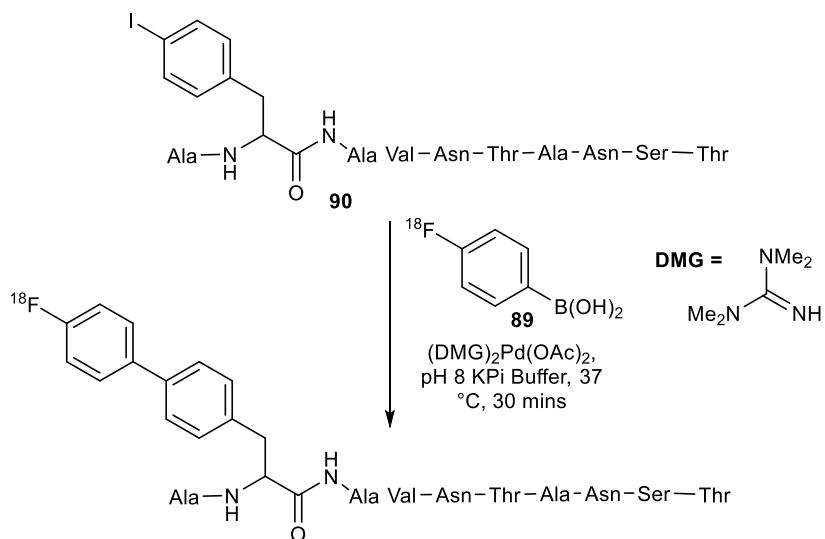
The group of Davis has developed guanidine and pyrimidine based ligands (Figure 5) and has shown them to be effective for the Suzuki cross coupling reaction. They are premixed in buffer with the Pd(II) source,  $\text{Pd}(\text{OAc})_2$  and are added to the substrate. These have the additional benefit of forming a palladium catalyst that is insensitive to oxygen and so reactions using this catalyst could be conducted under air.<sup>78</sup>



**Figure 5.** Guanidine and pyrimidine based ligands **85 – 88** developed by Davis.

### 2.1.1.2. Suzuki–Miyaura coupling reaction in aqueous medium

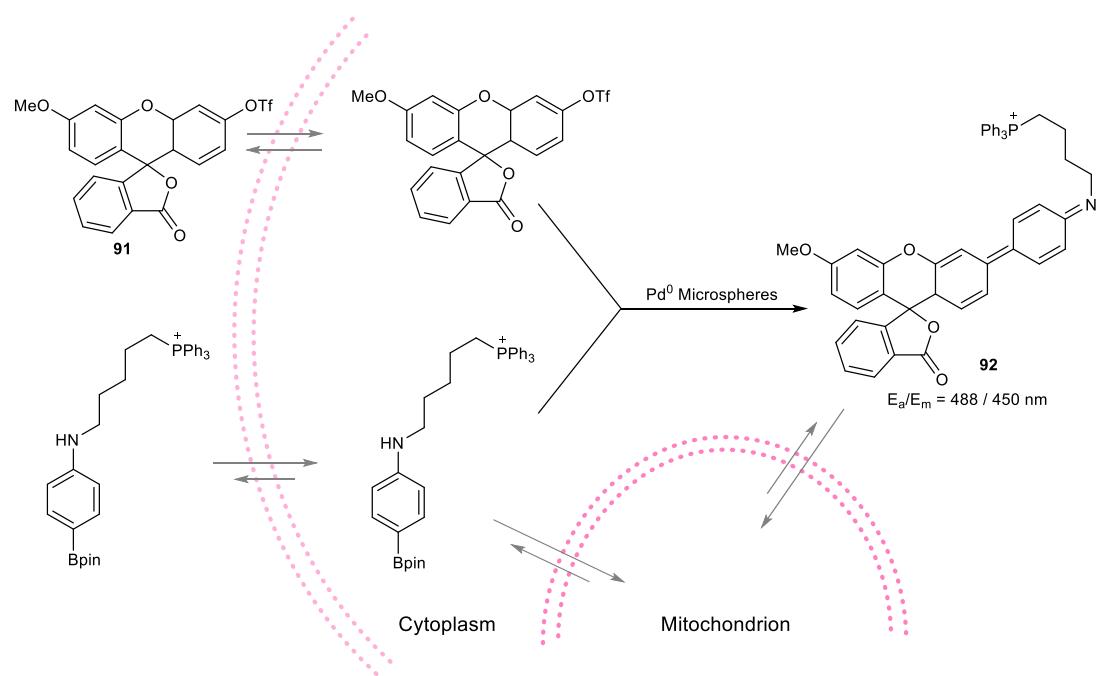
The Suzuki-Miyaura reaction is powerful method for the preparation of biaryls. There are several examples of Suzuki reactions being conducted in biological environments. In particular they have been used as a method of bioconjugation onto molecules of biological interest and proteins. Davis and others have used a pyrimidine ligand (**85**) to couple aryl iodides on the surface of proteins.<sup>79</sup> Using the methodology developed in their lab they could also attach radiolabelled boronic acid (**89**) onto peptides containing a 4-iodo phenylalanine (**90**), at ambient pH and temperature, within 30 minutes (Scheme 22).



**Scheme 22.** The cross coupling of a peptide containing an aryl iodide with a boronic acid radio labeled with  $^{18}\text{F}$  using the DMG ligand (**85**).

Bradley and co-workers were able to use the Suzuki reaction as a bioconjugation method (Figure 8). Nanoparticles functionalised with boronic acids were coupled with

a dye containing cargo moiety which also contained an aryl triflate, (**91**). After transport of the dye into the cell nucleus displayed fluorescence. This procedure also used **85** as the catalyst.<sup>80</sup>



**Scheme 23.** The use of a Suzuki reaction as a method of bioconjugation onto a fluorescent probe **91**. Once the Suzuki reaction occurs to form **92**. This is fluorescent accumulates in the mitochondria. So, fluorescence accumulates in the mitochondria.

Buchwald was able to show that by employing sSPhos (**80**) challenging aryl chloride substrates containing unprotected amino and hydroxyl groups could undergo the Suzuki reaction.<sup>77</sup> These examples remain the most difficult substrates coupled in water. The ligands **81** and **82** have been used in aqueous Suzuki reactions.<sup>75,81</sup>

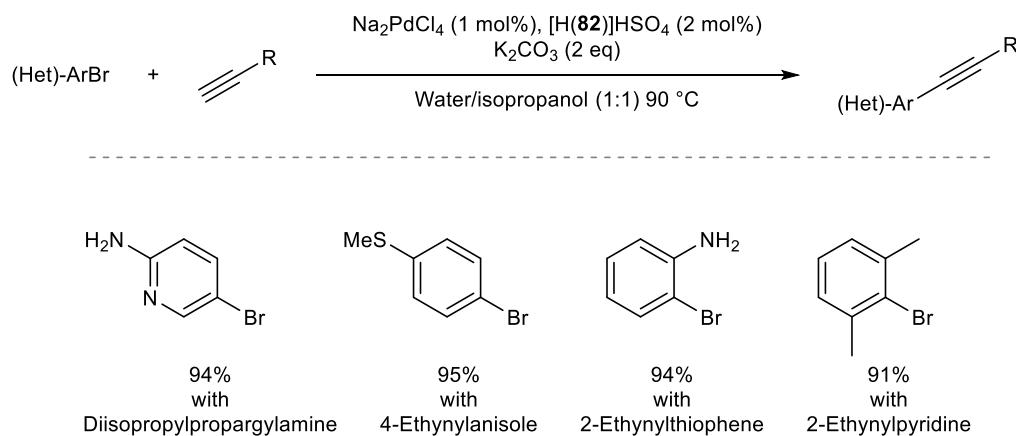
### 2.1.1.3. Alkenylation and alkynylation using palladium catalysis in aqueous media

The Mizoroki-Heck reaction is an invaluable palladium C–C bond forming reaction giving aryl alkenes or aryl alkynes. When copper is added as cocatalyst, to activate an alkyne, then the reaction is referred to as the Sonogashira cross-coupling reaction.<sup>63</sup> It is noteworthy that reactions commonly described as copper-free Sonogashira reactions, could in fact be referred to as a Heck alkynylation.<sup>82–84</sup>

The number of examples of alkenylations applied in biological systems are fewer than the Suzuki reaction, nevertheless there are examples of these reactions conducted in

aqueous media. Using the pyrimidine base catalyst used by Davis, there have been several examples of the Heck alkynylation demonstrated in biological systems.

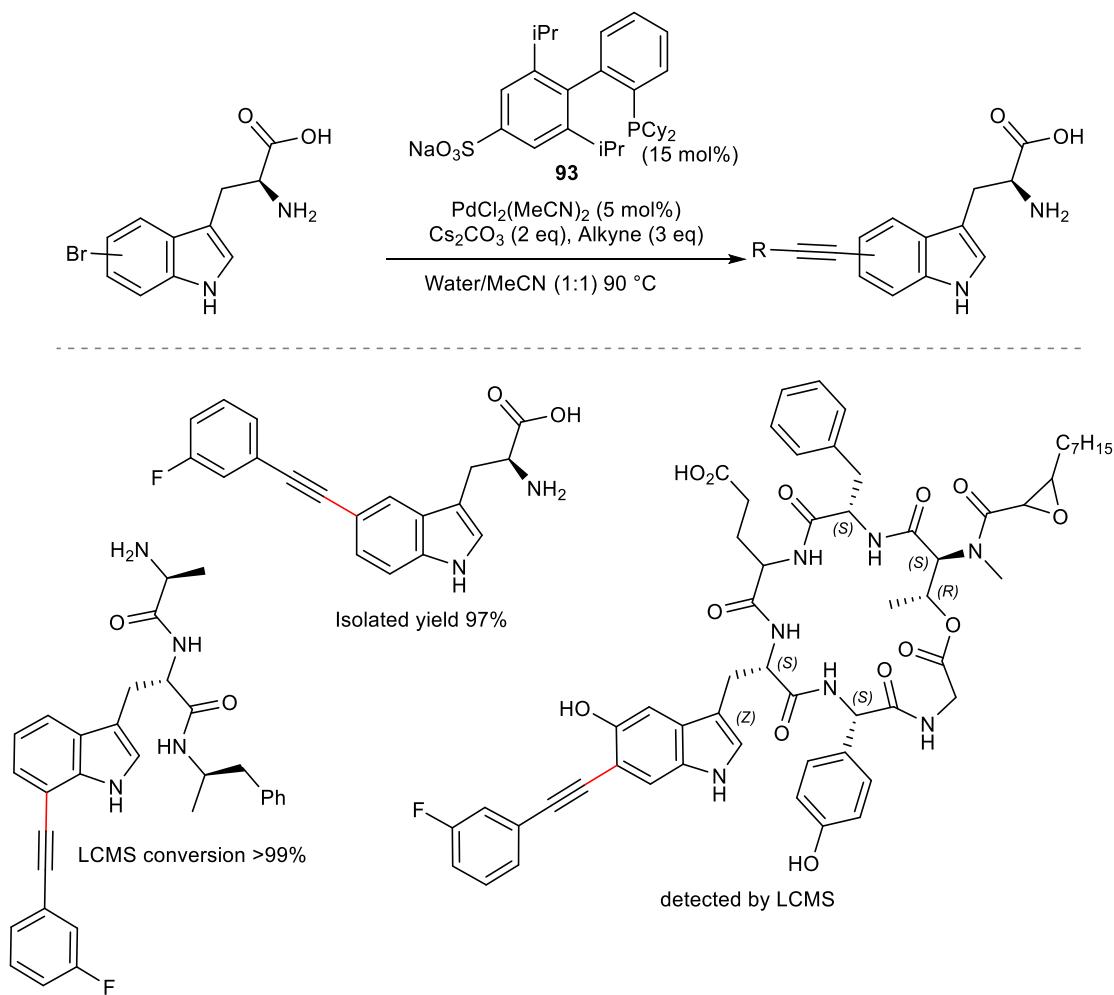
There have also been numerous reports of Heck reactions in water using aryl bromides. Heteroaryl chlorides and unactivated aryl bromides could be coupled with a range of terminal alkynes in the presence of ligand **82** in excellent yields (Scheme 20). However the active catalyst though had to be prepared before addition into the reaction; this was achieved by heating cataCXium®FSulf (**82**) with Na<sub>2</sub>PdCl<sub>4</sub> at 55 °C.<sup>74</sup>



**Scheme 24.** The coupling of a range of aryl bromides with alkynes using **82**.

Using a combination of palladium dichloride and PPh<sub>3</sub> in neat water with pyrrolidine as the base, a range of aryl bromides could be coupled with alkynes. However, higher temperatures of 120 °C were required.<sup>85</sup>

Furthermore, the group of Goss was able to couple bromo- and iodo-tryptophans using the sulfonated XPhos (**93**) catalyst system developed by Buchwald (Scheme 21).<sup>86</sup> The reaction using this ligand over sSPhos, TPPTS and the TXPTS gave better yields. This particular catalyst system was developed by Anderson *et al.* and it was found that in aqueous acetonitrile mixture unactivated aryl chlorides could be coupled with alkynes.<sup>77</sup> In the case of Goss and coworkers work it was found that the use of microwaves was beneficial and the reaction times could be shortened.



**Scheme 25.** The Heck alkynylation of various alkyne onto molecules containing a bromotryptophan moiety

Milder conditions can be employed when a copper cocatalyst is used, although the role of copper in the Sonogashira reaction is not fully understood.<sup>83,87</sup> Despite this, there are limited examples of the Sonogashira reaction in biological systems.<sup>88,89</sup> This is probably due to free amino groups having a strong affinity to copper, and so can denature proteins and DNA.<sup>90</sup> Nonetheless they are powerful reactions and there are many examples of Sonogashira reactions in water. The  $\text{Pd}(\text{OAc})_2/\text{TPPTS}$  ligand combination has been used to couple 2-iodophenols and 2-iodoanilines with a number of alkynes.

#### 2.1.1.4. Buchwald–Hartwig amination

The Buchwald–Hartwig amination is the reaction between an aryl halide and an amine to give an aniline. Amine coupling partners can vary in reactivity in comparison to the coupling partners in other coupling reactions and so the mechanism and/or the rate determining step can become different between different substrate classes. For

example heterocycles, anilines, amines, amides and hydrazones can all be used for amination reactions.<sup>91</sup>

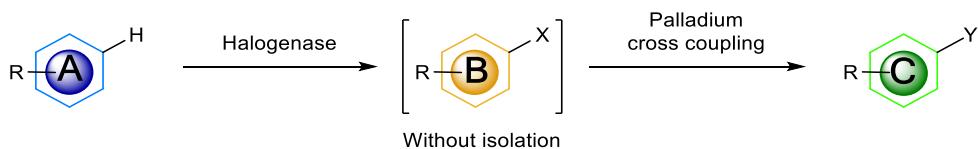
Generally, more nucleophilic amino groups are better coupling partners. Electronically rich anilines would generally be better coupling partners as they will not likely deactivate the palladium centre leading to the formation of catalytically non-active complexes. This reaction typically requires bulky ligands and it is found that bidentate ligands such as DPPF and BINAP give good yields. Mechanistic studies revealed that the use of bulky ligands with large bite angles increases yields as they prevent the competing dehydrohalogenation reaction. The dehydrohalogenation occurs through  $\beta$ -hydride elimination of the aryl group, thus the presence of the chelating ligand blocks the formation of an empty site on the palladium. To also suppress the competing dehalogenation reaction a strong base used is required. The base must be strong enough to deprotonate the amino group when complexed to the palladium.

To date in the literature there are only a few examples of Buchwald-Hartwig couplings conducted in aqueous conditions. Lipshutz and co-workers used the dicyclohexyl(2,2-diphenyl-1-methyl-1-cyclopropyl)phosphine (c-BRIDP) catalyst in the presence of TPGS-750m ((DL- $\alpha$ -tocopherol methoxypolyethylene glycol succinate) surfactant.<sup>92</sup> Stradiotto and Tardiff found that the mor-DalPhos ligand could be used to couple aryl chlorides including heteroaryl chlorides in water.<sup>93</sup>

### 2.1.2. Aims

Encouraged by the considerable literature reporting palladium cross-coupling methods with aryl-halides in aqueous media, we thought that it would be noteworthy to combine the regioselectivity of halogenases with the versatility of palladium catalysis. This would exemplify the utility of the halogenases whilst also developing a general approach to the C–H functionalisation of electron rich aromatic molecules (Scheme 22). Conducting the two reactions into one pot would be more enticing prospect.<sup>53,94</sup> With the halogenases being less established and less adaptable to change, a more reasonable effort would be to find a suitable palladium catalysed reaction for this purpose.

To be successful in our aims the palladium reaction would need to work in aerobic conditions as the halogenases require oxygen to operate. Additionally, the palladium would need to work at a neutral pH and at a temperature of 30 °C.



**Scheme 26.** Combining a halogenase reaction with palladium cross coupling reaction to form a C–C or C–N bond from a C–H bond.

Thus, the first objective is to identify a suitable substrate which would be halogenated most efficiently by the halogenases but would demonstrate the halogenases appealing reactivity.

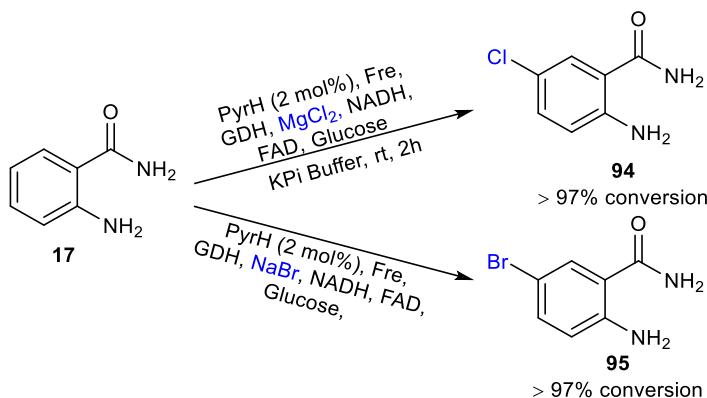
Using this substrate, a reaction screen would be conducted. Many of the reactions described in literature, discussed earlier in biological systems generally require the use of aryl iodides as coupling partners; this presents a problem as the halogenases used in the Micklefield lab are currently not known to iodinate substrates. Furthermore, the substrates halogenated by the halogenases usually contain a hydrogen bonding group to exert the selectivity. This can further complicate the process as coordinating groups can ligate to the metal catalyst and deactivate it.

Once suitable reactions are found then optimisation of these reactions would be required for them to operate in tandem with the halogenase reactions in one pot processes. It is envisaged that this would be challenging if the two systems interact overly with one another. Based on observations from previous literature, methods for compartmentalisation of the two reactions may need to be explored.

## 2.2. Results and discussion

Initially when we sought to develop a new reaction for the incorporation of the halogenases with a chemocatalysed reaction, we desired to identify a suitable substrate other to the natural substrate. The substrate scope of the enzymes being characterised within the Micklefield lab PyrH, PrnA and SttH was determined by Jonathan Latham (J.L.).<sup>33</sup> It was found that these enzymes could tolerate a range of substrates provided they had groups that were able to partake in hydrogen bonding and the substrate is smaller than the natural substrate. A more indepth discussion has been included earlier in the introduction. Also using a bromide source in place of a chloride salt gives rise to the same regioselectivity (scheme) and reactivity but using iodide sources was unsuccessful.

From his studies it was concluded that anthranilamide (**35**) was a good substrate for PyrH and could be converted quantitatively to 5-chloroanthranilamide (**94**) or 5-bromoanthranilamide (**95**) at a practical 2 mM substrate concentration depending on the halide source used (Scheme 27) and the halogenation efficiency was unaffected. Using higher substrate concentration lead to lower yields. We chose to initiate the reaction screening with 5-bromoanthranilamide as the oxidative addition into aryl bromides over aryl chlorides with palladium is more facile.<sup>63</sup>



**Scheme 27.** PyrH halogenation of anthranilamide **35**. Using a chloride source chlorination occurs but using a bromide salt the bromide **95** can be formed.

RadH the phenolic halogenase was more promiscuous as the natural substrate, radicicol is substantially larger than tryptophan. The substrates for RadH requires a phenolic group and halogenation occurs *ortho* to it. Substrate inhibition was not as problematic with this enzyme and could tolerate over 5 mM substrate concentration in most cases. The best non-natural substrate tested by Eileen Brandenburger was

6-hydroxy isoquinoline and halogenation occurred at the 5 position, at 5 mM substrate concentration and with 3 mol% catalyst loading the reaction only converted 11 to 22% after 18 hours. A lower 0.5 mM concentration of 6-hydroxy isoquinoline could be brominated or chlorinated using 3 mol% of RadH to full completion after 2 hours.

### 2.2.1. Suzuki-Miyaura Coupling

There are many reports in the literature of Suzuki reactions utilising aryl bromides conducted in aqueous solvent. With the intention to search for a reaction that could be used in one pot with the halogenases, we aimed to keep the conditions in the screening reactions as close as possible to the biocatalysed reaction. Initial screens (Table 1) involved testing the literature reported reactions but using 5-bromoanthranilamide (**95**) at 2 mM concentration in 10 mM phosphate buffer (table) at 50 °C.

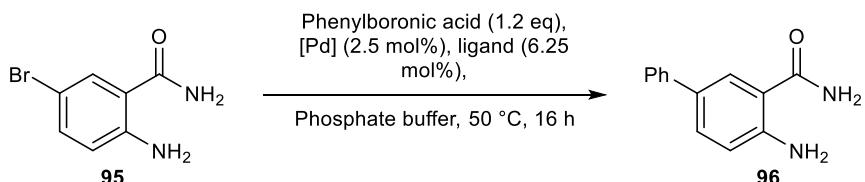
Pleasingly the two sulfonated ligands TPPTS (**81**) and the sulfonated Buchwald ligand (**80**), SPhos performed well in the reaction and quantitative conversions, to the biaryl **62**, were seen with both ligands (Table 1, entries 5-6).

The tetrakis(triphenylphosphine) palladium catalyst was found to be inactive (Table 1, entry 7), this could be attributed to its insolubility in water. TPPTS is expected to form a less active catalyst than triphenylphosphine as it bears electron withdrawing sodium sulfonate groups. Electron-rich ligands promote oxidative addition and are able to bind more tightly to palladium thus preventing catalyst deactivation.<sup>66</sup>

Two other water soluble ligands were tested and they both showed no activity on our substrate with the concentrations we tested (Table 1, entries 8-9). The *t*-Bu-Amphos ligand (**84**) is an aliphatic water-soluble phosphine bearing a trimethyl ammonium group and has been shown to work in a number of palladium catalysed cross-coupling reactions in aqueous solvents and Shaughnessy reported that this ligand is more active than TPPTS.<sup>95</sup> This ligand although was developed as water soluble form of the highly active Fu ligands, the reactivity *t*-Bu-Amphos (**84**) as described in literature is not comparable to the Fu catalysts.<sup>96</sup> This suggests that the inactivity of this ligand could be attributed to the trimethyl ammonium group; it could be involved in additional interactions with the palladium catalyst. The Suzuki reaction literature conditions utilising the 9-fluorenylphosphine cataCXium®FSulf ligand (**82**) required heating to 100 °C and it could be that this catalyst was not active at the lower temperature of

50 °C (Table 1, entry 9). Another plausible explanation of the reaction not proceeding in both cases is that the pH of the reaction conducted in the reaction screen would be dissimilar to the reactions reported in the literature. This is due the reactions being run at a higher dilution and in a buffer solution as opposed to neat water.

**Table 1** – Condition screening for the Suzuki reaction with 5-bromoanthranilamide (**95**)



Entry	Palladium source	Ligand	Conversion <sup>a</sup>
1*	Pd(OAc) <sub>2</sub>	2-Amino-4,6-dihydroxypyrimidine	32
2*	Pd(OAc) <sub>2</sub>	2-(Dimethylamino)-4,6-dihydroxypyrimidine	86
3*	Pd(OAc) <sub>2</sub>	1,1-Dimethylguanidine	56
4*	Pd(OAc) <sub>2</sub>	1,1,3,3-Tetramethylguanidine	32
5	Na <sub>2</sub> PdCl <sub>4</sub>	2'-Dicyclohexylphosphino-2,6-dimethoxy-1,1'-biphenyl-3-sulfonic acid sodium salt	Quantitative
6	Pd(OAc) <sub>2</sub>	Triphenylphosphine- <i>m</i> -trisulphonic acid trisodium salt	Quantitative
7	Pd(Ph <sub>3</sub> ) <sub>4</sub>	-	n.p.
8*	Pd(OAc) <sub>2</sub>	2-(Di- <i>tert</i> butylphosphino)-trimethylethanaminium chloride	n.p.
9*	Pd(OAc) <sub>2</sub>	CataCXium® FSulf	n.p.

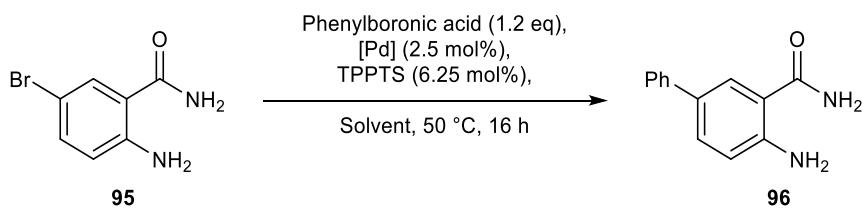
<sup>a</sup>LCMS conversion determined by integration of product and reactants. Conditions: 5-bromo anthranilamide (2.0 mM in 10 mM phosphate buffer), Pd salt (2.5 mol%), boronic acid (1.2 eq), base (2.0 eq), 10 mM potassium phosphate buffer to total volume of 10 mL. 50 °C, overnight. \* reactions conducted by JL, n.p. denotes no reaction occurs

The guanidine and pyrimidine based biocompatible ligands developed by Davis and coworkers<sup>79</sup>, which have been shown to work by other groups<sup>97</sup>, were able to convert 5-bromoanthranilamide to 5-phenylanthranilamide (**96**) in modest to good yields. Furthermore, these conversions were seen without the additional degassing step

required with the other phosphine based ligands. (Table 1, entry 3), this yield was obtained without deoxygenating the reaction solvent.

Pleased with the clean conversions at the initial stage we continued with our investigations to see if the reaction could be improved towards milder conditions. As TPPTS (**81**) was more readily available and cheaper than the sulfonated SPhos (**80**), this ligand was used in all subsequent optimisation reactions. The results of the optimisation reactions are shown in Table 2.

**Table 2** – Optimisation of the Suzuki reaction with 5-bromoanthranilamide (**95**) starting material



Entry	Base	Solvent	Under N <sub>2</sub>	Conversion <sup>a</sup>
1	K <sub>3</sub> PO <sub>4</sub>	Buffer	Yes	Quantitative
2	K <sub>3</sub> PO <sub>4</sub>	Buffer:MeCN, (1:1)	Yes	82%
3	DIPEA	Buffer	Yes	76%
4	DIPEA	Buffer:MeCN, (1:1)	Yes	64%
5	K <sub>2</sub> CO <sub>3</sub>	Buffer:MeCN, (1:1)	Yes	quantitative (80%) <sup>b</sup>
6	K <sub>2</sub> CO <sub>3</sub>	Buffer	No	n.p.
7	K <sub>2</sub> CO <sub>3</sub>	Buffer:MeCN, (1:1)	No	n.p.
8	None	Buffer	Yes	n.p.
9	None	Buffer:MeCN, (1:1)	Yes	n.p.
10	None	Buffer	No	n.p.
11 <sup>c</sup>	K <sub>2</sub> CO <sub>3</sub>	Buffer:MeCN, (1:1)	Yes	Quantitative

<sup>a</sup> LCMS conversion determined by integration of product and reactants, <sup>b</sup>isolated, <sup>c</sup>with Na<sub>2</sub>[PdCl<sub>4</sub>].

Conditions: 5-bromo anthranilamide (2.0 mM in 10 mM phosphate buffer), Pd salt (2.5 mol %), TPPTS (6.25 mol %), boronic acid (1.2 eq), base (2.0 eq), 10 mM potassium phosphate buffer to total volume of 10 mL. 50 °C, overnight n.p. denotes no reaction occurs

Moore *et al.* reported that the reaction using TPPTS required acetonitrile as co-solvent, but in our case it was found the reaction worked well without any co-solvent and no

drop in conversion was seen (Table 2, entry 1). Also, it can be seen that the TPPTS ligand works well using the weaker potassium carbonate base and with the organic base DIPEA (Table 2, entries 3-4). However, without adding base, the reaction yielded no product (Table 2, entries 9-10).

It would be desirable to add the cross coupling reagents to the halogenase reaction, without stringent removal of oxygen. The oxygen is required for the halogenase to function. To see if it was necessary to remove oxygen for the Suzuki to occur, the reaction was performed under air without degassing the solvents; this gave no reaction (Table 2, entries 8 and 10). This is unsurprising as the catalytic palladium(0) species bound to phosphines are known to be sensitive to oxygen. Working with water soluble catalysts allows for addition of the reagents from stock solutions in water thus minimising error when weighing out small quantities of the catalyst. Switching to a water soluble palladium source, does not affect the reaction adversely (Table 2, entry 11).

The reference conditions developed by Shaughnessy and coworkers using the water soluble triarylphosphines required the use of acetonitrile as a cosolvent<sup>70</sup> but it was found the reaction worked well without a significant drop in yield (Table 2, entry 1).

Also, it can be seen that the TPPTS ligand works well using most bases even with the organic base DIPEA (Table 2, entries 3-4). We aimed to find conditions closer to that of the halogenase reaction and using no base but the reaction yielded no product (Table 2, entries 9-10).

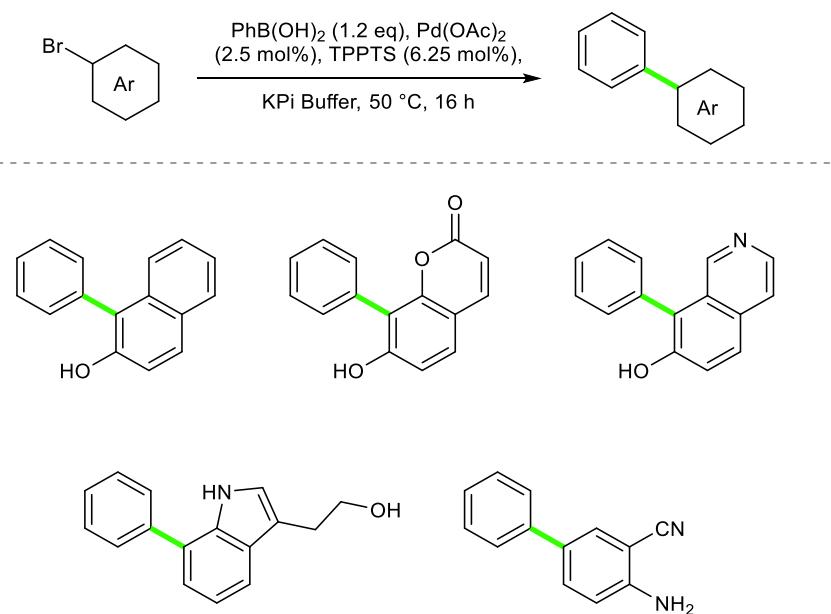
### 2.2.1.1. Suzuki substrate scope

Pleased with the conversion of 5-bromoanthranilamide (**95**) into 5-phenylanthranilamide (**96**), the scope of the reaction was next explored. A crude LCMS screen with a variety of boronic acid coupling partners was conducted and the reactivity followed a general trend. Electron deficient boronic acids were converted completely and no starting material was detected. When electron rich boronic acids were used unreacted starting material was observed. These observations were also corroborated by an independent screen by J.L. It was also noted that reaction with relatively unstable aliphatic boronic acids gave messy reaction profiles with trace or no desired product detected. This observation could be attributed to the stability of the

boronic acid to oxidation, aliphatic boronic acids are more susceptible to oxidation than aryl boronic acids.<sup>98</sup>

Once the generality with respect to the boronic acid was tested with XX, we wanted to see if these conditions could also be applied to other halogenase products as this would further expand the scope of the reaction. Pleasingly those arylhalides that were tested underwent the Suzuki reaction efficiently (Table 3). When reaction with bromotryptophol was tested a significant amount of side products including protodehalogenation was observed. This suggests that reductive elimination is inefficient on this substrate. Adding fluoride as an additive is known to aid the reductive elimination step and indeed adding an equivalent of CsF suppressed this side product and clean conversions were seen.<sup>99</sup>

**Table 3** - Substrate scope of the Suzuki reaction in water with respect to the aryl halide



N.B. These reactions were done on small scale so products were not isolated but greater than 90 % conversion, by LCMS, was seen in all cases. With 7-bromotryptophol CsF (1.0 eq) was added as an additive

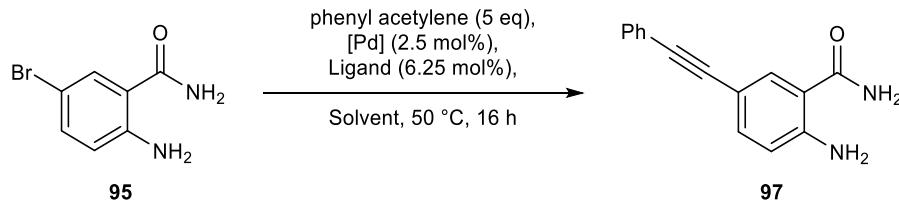
### 2.2.2. Alkynylation

With the optimised Suzuki reaction in hand, attention turned to a different class of C–C bond forming reactions. The alkynylation reaction would be an desirable reaction to optimise as alkynes are versatile functional groups and are also present in a range of biologically active compounds.<sup>100–106</sup>

5-Bromoanthranilamide (**95**) was subjected to the conditions set out in literature for the coupling of bromoarenes in aqueous conditions. The water-soluble electron

deficient propiolic acid was used as the coupling partner. As the halogenase reactions are dilute, the reactions were conducted on a 0.02 mmol scale. Phenylacetylene was later adopted as the coupling partner to ensure that no product was lost during the aqueous workup. Disappointingly, the yields for the Heck alkynylation were poor and starting material was recovered.

**Table 4** – Alkynylation reaction screen with 5-bromoanthranilamide (**95**)



Entry	Conditions	Conversion <sup>a</sup>
1	PdCl <sub>2</sub> (MeCN) <sub>2</sub> (2.5 mol%), sSPhos (7.5 mol%), CsCO <sub>3</sub> (1.5 eq), with acetonitrile cosolvent (1:1)	Trace
2	Pd(OAc) <sub>2</sub> (2.5 mol%), TPPTS (7.5 mol%), NEt <sub>3</sub> (1.5 eq)	Trace
3 <sup>b</sup>	PdCl <sub>2</sub> (2.5 mol%), pyrrolidine (1.5 eq)	n.p.
4	Pd(OAc) <sub>2</sub> (2.5 mol%), TPPTS (7.5 mol%), CsCO <sub>3</sub> (1.5 eq)	Trace
19	Pd(OAc) <sub>2</sub> (3 mol%), Davis ligand (3 mol%), Cs <sub>2</sub> CO <sub>3</sub> (1.5 eq), Buffer:MeCN (1:1)	n.p.

<sup>a</sup>NMR conversion, determined by integration of product and reactants Conditions: 5-bromoanthranilamide (2.0 mM in 10 mM phosphate buffer, with cosolvent the overall substrate concentration is 1.0 mM), phenyl acetylene (1.1 eq), CuI (1.0 eq), 50 °C, overnight. <sup>b</sup>with 5 equivalents of alkyne. n.p. denotes no reaction occurs

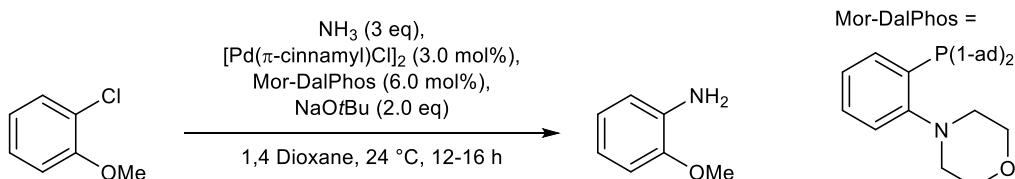
From these preliminary results, it was seen that optimisation was required for the efficient coupling of **95** with phenylacetylene. A short screen of the catalysts available commercially was conducted (Table 5) an equivalent of CuI was also added to activate the alkyne. Adding copper iodide made little difference and still only starting material was isolated.

**Table 5** – Catalyst screening for the alkynylation of 5-bromoanthranilamide (**95**)

Entry	Pd source	Ligand	Base	Solvent	Conversion <sup>a</sup>
1	Pd(OAc) <sub>2</sub> (3 mol%)	Davis ligand (3 mol%)	Cs <sub>2</sub> CO <sub>3</sub>	Buffer:MeCN (1:1)	n.p.
2	PdCl <sub>2</sub> (MeCN) <sub>2</sub> (2.5 mol%),	TPPTS	NEt <sub>3</sub>	Buffer	n.p.
3	Pd(dba) <sub>2</sub>	Sulf SPhos	Cs <sub>2</sub> CO <sub>3</sub>	Buffer:MeCN (1:1)	n.p.
4	Pd(dba) <sub>2</sub>	P( <i>o</i> -tol) <sub>3</sub>	K <sub>2</sub> CO <sub>3</sub>	Buffer:MeCN (1:1)	n.p.
5	Pd(dba) <sub>2</sub>	P( <i>o</i> -tol) <sub>3</sub>	K <sub>2</sub> CO <sub>3</sub>	Buffer:DMF (1:1)	n.p.

<sup>a</sup>NMR conversion determined by integration of product and reactants Conditions: 5-bromo anthranilamide (2.0 mM in 10 mM phosphate buffer, with cosolvent the overall substrate concentration is 1.0 mM), 80 °C, overnight. n.p. denotes no reaction occurs

Impressively, the catalyst system developed by Stradiotto is able to couple electronically rich aryl chlorides with ammonia at room temperature (Scheme 24) furthermore this ligand has been shown to work in solvent-free water.<sup>93,107</sup>



**Scheme 28.** A representative example of coupling an electron rich aryl chloride with ammonia using mor-DalPhos.

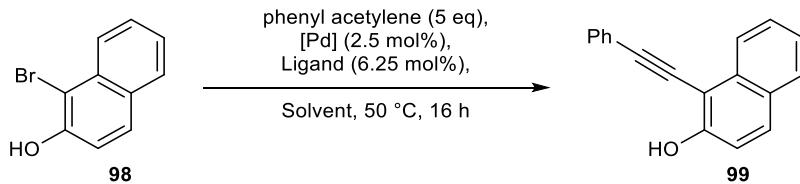
It was thought that by using this catalyst oxidative addition into 5-bromoanthranilamide should be facile. Treating 5-bromoanthranilade with the Pd( $\pi$ -cinnamyl) chloride dimer with Mor-DalPhos in neat water but also using dioxane, acetonitrile, DMF and THF as cosolvents gave no reaction (Table 6).

**Table 6-** Mor-DalPhos screening for the alkynylation of 5-bromanthranilamide (**95**)

Entry	Cosolvent	Conversion <sup>a</sup>
1	none	n.p.
2	MeCN	n.p.
3	DMF	n.p.
4	THF	n.p.
5	1,4-dioxane	n.p.

<sup>a</sup> NMR conversion determined by integration of product and reactants  
Conditions: 5-bromo anthranilamide (2.0 mM in 10 mM phosphate buffer, with cosolvent the overall substrate concentration is 1.0 mM), phenylacetylene (1.5 eq),  $[(\pi\text{-cinnamyl})\text{PdCl}]_2$  (10 mol%), MorDalPhos (20 mol%),  $\text{K}_2\text{CO}_3$  (1.5 eq), 90 °C, 16 h. n.p. denotes no reaction occurs

We rationalised the lack of coupling products as a result of two free amino groups which could bind to and deactivate the catalyst, sequestering it from the reaction. We then decided to move to anew substrate; Lewis showed that RebH could halogenate the 2 position of 1-naphthol (**47**).<sup>35</sup> **47** was tested with RebH by J.L. it was found that 2-naphthol was a better substrate for bromination. Using 1-bromo-2-naphthol (**98**) a ligand screen was conducted.

**Table 7 -** Catalyst screening for the alkynylation of 1-bromo2-naphthol (**98**)

Entry	Ligand	Conversion <sup>a</sup>
1	cyXPhos	n.p.
2	TPPTS	20
3	Sulf SPhos	9
3	P( <i>o</i> -tol) <sub>3</sub>	13
4	<i>t</i> Bu <sub>3</sub> PHBF <sub>4</sub>	n.p

<sup>a</sup> NMR conversion determined by integration of product and reactants  
Conditions: 1-bromonaphthol (2.0 mM in 10 mM phosphate buffer),  $\text{PdCl}_2(\text{MeCN})_2$  (10 mol%), ligand (20 mol%),  $\text{NEt}_3$  (2 eq.), 80 °C, 16 h. n.p. denotes no reaction occurs

Pleased with the result obtained with the TPPTS ligand (Table 8, entry 4), screening for bases was completed. It seems that the base used had an effect on the alkynylation

of bromonaphthol **98** with phenylacetylene and  $\text{Cs}_2\text{CO}_3$  (Table 8, entry 3) gave the best results.

**Table 8** - Base screening for the alkynylation of 1-bromo-2-naphthol (**98**)

Entry	Base	Conversion
1	$\text{K}_3\text{PO}_4$	22
2	Pyrrolidine	n.p.
3	$\text{Cs}_2\text{CO}_3$	30
3	$\text{K}_2\text{CO}_3$	13
4	$\text{NEt}_3$	20

<sup>a</sup> NMR conversion determined by integration of product and reactants Conditions: 1-bromonaphthol (2.0 mM in 10 mM phosphate buffer),  $\text{PdCl}_2(\text{MeCN})_2$  (10 mol%), TPPTS (20 mol%), base (2 eq) 80 °C, 16 h. n.p. denotes no reaction occurs

The choice of palladium source also seemed to be crucial, the palladium(II) salts bearing ligands with  $\pi$ -acceptor ability (Table 9, entries 1 and 2) were found to work better in the reaction over palladium salts containing only donor ligands (Table 9, entries 3 and 4). This may be due these palladium(II) sources being easier to reduce to the catalytically active palladium(0).

**Table 9** - Palladium precatalyst screening for the alkynylation of 1-bromo-2-naphthol (**98**)

Entry	Palladium (II) salt	Conversion <sup>a</sup>
1	$\text{PdCl}_2(\text{MeCN})_2$	21
2	$\text{PdCl}_2(\text{PPh}_3)_2$	28
3	$\text{Pd}(\text{OAc})_2$	Trace
4	$\text{Na}_2\text{PdCl}_4$	Trace

<sup>a</sup> NMR conversion determined by integration of product and reactants. Conditions: 1-bromonaphthol (2.0 mM in 10 mM phosphate buffer), Palladium salt (10 mol%), TPPTS (20 mol%),  $\text{NEt}_3$  (2 equiv.), 80 °C, 16 h.

A solvent screen revealed that adding DMF as a cosolvent improved the conversion (Table 10, entry 3). Conducting the reaction with other solvents, did not give an increase in yield.

**Table 10** - Further optimisation for the alkynylation of 1-bromo-2—naphthol (**98**)

Entry	Pd source	Solvent	Conversion <sup>a</sup>
1	PdCl <sub>2</sub> (MeCN) <sub>2</sub>	Buffer	30
3	PdCl <sub>2</sub> (PPh <sub>3</sub> ) <sub>2</sub>	Buffer:DMF (1:1)	44

<sup>a</sup> NMR conversion determined by integration of product and reactants Conditions: 1-bromonaphthol (2.0 mM in 10 mM phosphate buffer, with cosolvent the overall substrate concentration is 1.0 mM), Palladium salt (10 mol%), TPPTS (20 mol%), Cs<sub>2</sub>CO<sub>3</sub> (2 equiv.), 80 °C, 16 h.

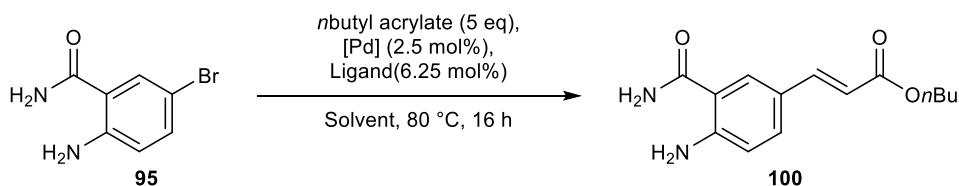
Shortly after conducting these optimisation reactions, work from the Goss group, reporting the Heck alkynylation on bromotryptophans using microwaves, showed that when the alkynylation reaction was conducted at a higher dilution a significant drop in conversion was seen.<sup>86</sup>

During the course of this study the yields could not be improved upon and so further efforts to optimise this reaction was not continued. It could be possible that the catalytically active palladium species may have not been forming and so oxidative insertion into the aryl bromide could not occur. If this is the case then to overcome this, the palladium salt and ligand could be premixed and heated before addition to the aryl halide and the alkyne. Alternatively, to improve the yields, a precatalyst such as those developed by Buchwald could be employed, these are air-stable palladium(0) species that have been precomplexed to the Buchwald ligand and only require deprotonation to become catalytically active.

### 2.2.3. Heck Alkenylation

Using the alkynes had shown some promise and so attention turned towards an alkene coupling partner. *n*Butyl acrylate was chosen as it is both electron poor and the boiling point is higher than methyl acrylate. The Heck alkenylation coupling with *n*butyl acrylate was also unsuccessful on 5-bromoanthranilamide using either TPPTS or the more electronically and sterically encumbered P(*o*-tol)<sub>3</sub> ligand (Table 11).

**Table 11** - Condition screening for the Heck alkenylation reaction with **95**.



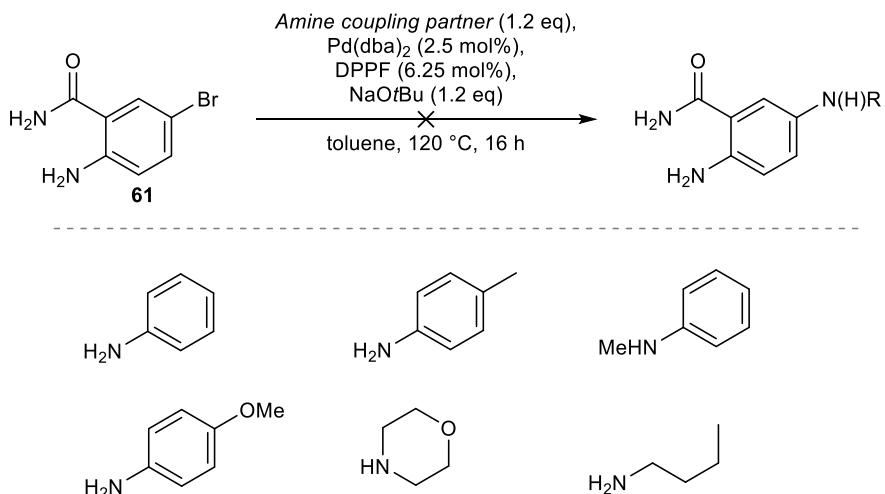
Entry	Pd source	Ligand	Base	Solvent	Conversion
50	Pd(OAc) <sub>2</sub>	TPPTS	K <sub>2</sub> CO <sub>3</sub>	Buffer:MeCN (1:1)	n.p.
52	Pd(OAc) <sub>2</sub>	TPPTS	K <sub>2</sub> CO <sub>3</sub>	Buffer	n.p.
53	Pd(dba) <sub>2</sub>	P( <i>o</i> -tol) <sub>3</sub>	NEt <sub>3</sub>	Buffer:MeCN (1:1)	n.p.
54	Pd(dba) <sub>2</sub>	P( <i>o</i> -tol) <sub>3</sub>	K <sub>2</sub> CO <sub>3</sub>	Buffer:MeCN (1:1)	n.p.
54	Pd(dba) <sub>2</sub>	P( <i>o</i> -tol) <sub>3</sub>	K <sub>2</sub> CO <sub>3</sub>	Buffer:DMF (1:1)	n.p.

Conditions: 5-bromo anthranilamide (2.0 mM in 10 mM phosphate buffer), n.p. denotes no reaction occurred

#### 2.2.4. Buchwald Hartwig-Amination

As described earlier, there are relatively fewer examples in the literature describing the Buchwald-Hartwig amination reaction in water. Using previously reported conditions on our substrate, which are HPtBu<sub>3</sub>BF<sub>4</sub> and a phase transfer agent CTAB in buffer solution and KOH as base,<sup>108</sup> the coupling of **95** with aniline or morpholine did not succeed. Using the XPhos catalyst as opposed to the trialkyl phosphine gave no coupling products.<sup>109</sup>

It was then thought to see if this reaction would proceed using conventional organic solvents as opposed to aqueous conditions. Using conditions developed by Hartwig using the ligand 1,1'-bis(diphenylphosphino)ferrocene (DPPF) and Pd(dba)<sub>2</sub> with sodium *tert*butoxide as the base and toluene solvent, trace amounts of product were detected by mass spectrometry. To further investigate if any product would form a variety of different amine coupling partners were used however, in all cases only starting material was seen by <sup>1</sup>H NMR spectroscopy (Scheme 29). Using 5 equivalents of amine also gave no reaction. When stoichiometric amounts of catalyst were used, starting material was consumed but the many products formed were not identifiable.

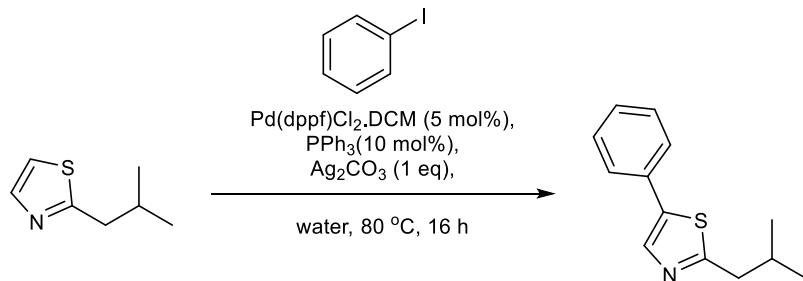


**Scheme 29.** The attempted Buchwald-Hartwig cross coupling reaction with a range of amine and aniline coupling partners.

The Ullmann coupling is another method for the construction of C–N bonds and uses copper rather than palladium and may work using 5-bromoanthranilimide (**95**) as a substrate. However, these reactions do not have any noteworthy advantage, apart from the cost of catalyst, over the Buchwald-Hartwig amination and are known to require significantly harsher reaction conditions such as higher temperatures and longer reaction times.

### 2.2.5. C–H activation

Previously in our group C–H activations of thiazole and indazoles with aryl halides were found to be accelerated in water (Scheme 30).<sup>110,111</sup>

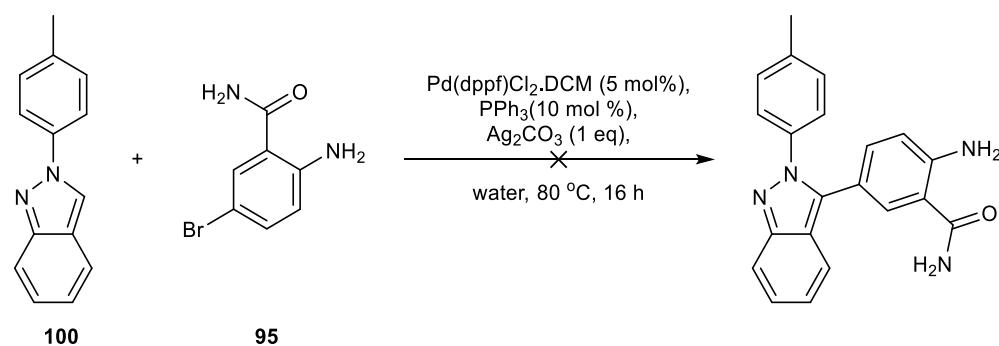


**Scheme 30.** The reported C–H activation of thiazole on water using iodobenzene.

This is an enticing prospect as if the C–H activation product is formed then in combination with the halogenase, then it would constitute as a formal dehydrogenative cross coupling reaction. To probe whether 5-bromoanthranilamide, (**95**) could participate in the C–H activation of thiazoles, the literature conditions were tested with

two different thiazoles. In both cases starting material was recovered and only trace product was formed, which was detected by mass spectrometry.

For this type of C–H activation aryl iodides were typically used, in the case of C–H activation of indazoles activated aryl bromides were used.<sup>110</sup> We thought that this reaction would be more likely to work with our substrates. Aryl bromide **95** was subjected to the reaction conditions with 2-tolyl indazole (**101**) as the coupling partner (Scheme 31). As with the thiazole, only trace product was detected by mass spectrometry. Similar to previous cross coupling attempts it could be rationalised that the bromoanthranilamide was not reactive enough for this coupling or that the substrate concentration is too low for the reaction to occur effectively.

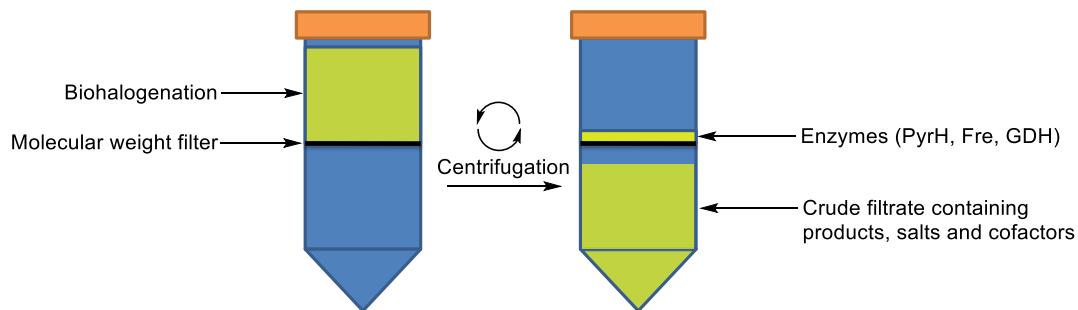
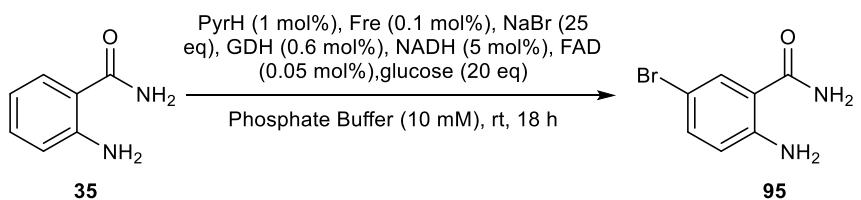


**Scheme 31.** The attempted C–H activation of 2-tolyl indazole with 5-bromoanthranilamide.

Ackermann has shown C–H activation of 2-phenyl pyridines with aryl chlorides: as ruthenium catalysis has shown to be active in aqueous media this could be an alternative avenue to explore.<sup>112</sup>

## 2.2.6. Combining the Suzuki reaction with halogenases

With the conditions for the aqueous Suzuki on **95** to **96** without protein now established, we desired to see what effects the components of the enzyme catalysed reaction would have on the conversions of the Suzuki reaction. It was found by J.L. that the enzyme components were particularly detrimental to the Suzuki reaction which was as we expected. Fortunately, J.L found that these could be removed using 10,000 kDa molecular weight filter Vivaspin 20. A schematic of the Vivaspin centrifugal filter has been depicted in figure 6. It is comprised of a molecular weight polyethersulfone membrane within a centrifuge tube. Those components, which are lighter than the molecular weight cutoff can pass the membrane whereas the larger components remain above it.



**Figure 6.** Compartmentalisation using the Vivavspin 20. Once the biohalogenation is complete it is filtered through a molecular weight filter by centrifugation.

We also explored the effect glycerol on the optimised reaction conditions (Table 12). It was thought that the glycerol in the protein could be problematic as it is known to reduce palladium to form palladium nanoparticles.<sup>113</sup>

**Table 12** – Determining the Suzuki tolerance to glycerol

Entry	Amount of glycerol added	Conversion <sup>a</sup>
1	5 mol%	35%
2	10 mol%	23%
3	20 mol%	47%
4	50 mol%	83%

<sup>a</sup>NMR conversion determined by integration of product and reactants,  
Conditions: 5-bromo anthranilamide (2.0 mM in 10 mM phosphate buffer), Pd(OAc) (2.5 mol %), TPPTS (6.25 mol%), boronic acid (1.2 eq), base (2.0 eq), 10 mM potassium phosphate buffer to total volume of 10 mL. 50 °C, overnight.

Glycerol is used as a protein cryoprotectant. Once protein is purified, it is often flash frozen as a mixture with a cryoprotectant and stored in the freezer so as to prevent the denaturation of the protein. Furthermore, owing to the molecular weight of glycerol, it would not be removed from the crude halogenase reaction during the filtration step. Adding glycerol in varying amounts to the optimised Suzuki reaction conditions

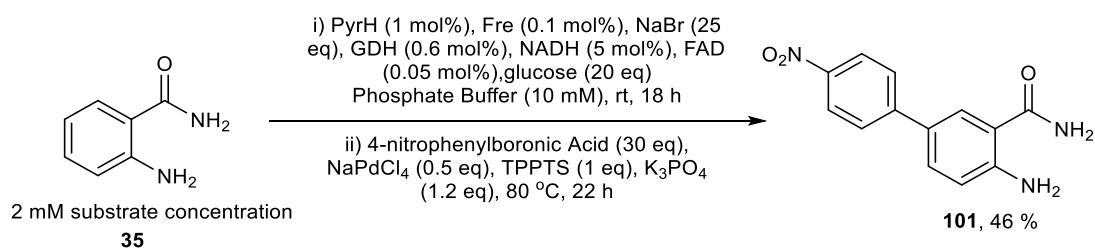
affected conversions. There was no trend on the amount of glycerol added and the conversion to the product.

With the knowledge that the palladium catalysts are inactive in aerobic conditions it was necessary to deoxygenate the filtrate by freeze-pump-thaw degassing. By eliminating these deactivating components to the Suzuki reaction, further optimisation combining these conditions with the PyrH halogenase was completed by J.L. He was able to isolate the desired biaryl **96** in an excellent 89% yield in the two-step process without isolation of intermediates or purification.

### 2.2.6. 1. Integrated halogenase-Suzuki reaction substrate scope

Confident that good yields could be obtained from the one pot reaction, work commenced on the substrate scope of the reaction. It was found that electron deficient boronic acid coupling partners gave the highest yields.<sup>114</sup> Work with J.L. and Jean-Marc Henry (J.-M.H.) then began on the scaling up of the tandem reactions.

The reaction with anthranilimide (**35**) required the use of purified PyrH enzyme. PyrH was overexpressed in *E. Coli* after transformation into *E. Coli* ArcticExpress (DE3)RP. The conversion of anthranilamide into 5-bromoanthranilamide with PyrH often gave high conversions ( $\geq 70\%$ ) if left overnight. Once conversion was determined complete by HPLC, the protein was removed by centrifugal filtration through a Vivaspin molecular weight filter (10 kDa MW cutoff). The filtrate was freeze-thaw degassed to remove oxygen which was required for halogenase but detrimental to the Suzuki reaction. The palladium catalyst, base and boronic acid was then added and the reaction was continued. The desired biaryl could be obtained after column chromatography (Scheme 28).

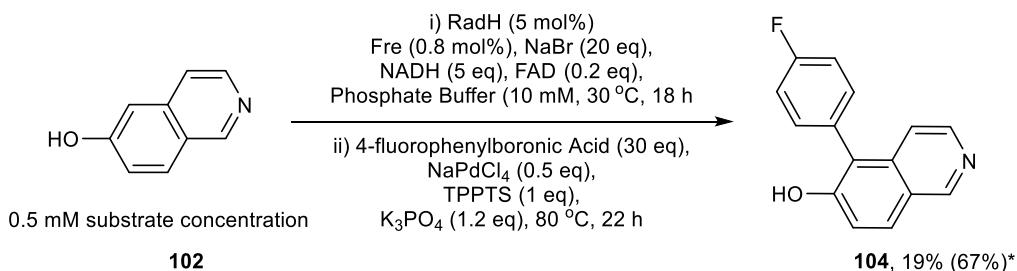


**Scheme 32.** The conversion of anthranilamide into 5-(*para*-nitrophenyl)anthranilamide by the one pot halogenase Suzuki reaction using RadH.

With the product of the PyrH in hand attention turned to the 6-hydroxy isoquinoline (**102**) as the substrate. This was halogenated using RadH. RadH was expressed in

*E.coli* Rosetta (DE3) cells. The process for the production of purified RadH protein was longer as it required longer incubation times, but RadH expressed well and protein yields could be increased by using 2YT as the growth media over LB media and using a larger bed of nickel-NTA. RadH was used to halogenate 6-hydroxyisoquinoline (**102**) 0.025 mmol scale. Cofactor recycling systems had been incompatible with RadH and so stoichiometric NADH was used in the reaction.

The second step procedure was conducted in a similar manner to that of the anthranilamide arylation. The Suzuki step worked well under the conditions and bromo-isoquinolinol (**103**) was fully converted to the arylated product **104** (Scheme 33). Isolation of the product was more challenging with this reaction as the solubility and the concentration of the product was lower. Purification of the product was achieved with semi-preparative reverse phase HPLC. The yield obtained was 19%, this was probably due to transfer losses as the conversion to product determined by HPLC was 97%. Using the same reaction conditions, the product could be isolated by J.L in 67% yield.

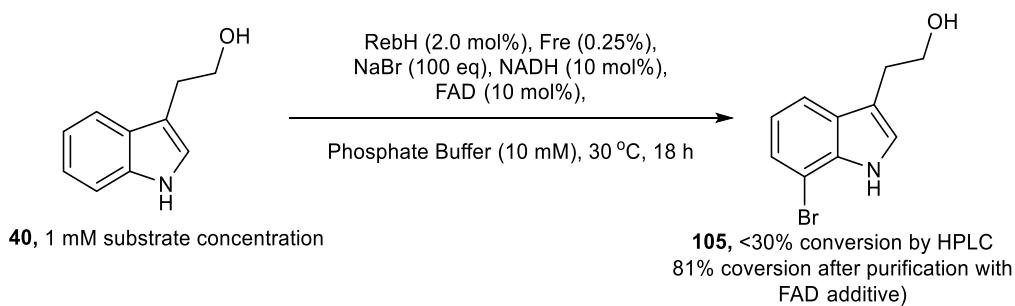


<sup>\*</sup>yield obtained by J.L.

**Scheme 33.** The conversion of 6-hydroxyisoquinoline (**102**) into 5-(4-fluorophenyl)isoquinolin-6-ol (**104**) by the one pot halogenase Suzuki reaction using RadH.

RebH was used to brominate tryptophol. RebH was transformed into ArcticExpress (DE3)RP and overexpressed in *E.Coli* using the protocols used in the Micklefield lab. Unfortunately, producing enough active RebH, using these conditions, to halogenate tryptophol (**40**) on preparative scale proved to be labour intensive and knowledge that glycerol was detrimental to the Suzuki reaction meant that RebH could not be frozen. Furthermore, doing the reaction with the fresh protein made was difficult as the amount of material that could be halogenated was less and isolation was arduous.

Using TB media instead of LB media for growing the *E. coli* cell containing over expressed RebH gave approximately twice as much cell mass. Even with this extra cell mass the protein obtained had little or no activity. On further investigation it was seen that protein seemed to be unstable to concentration after purification and that a significant proportion precipitated when buffering exchanging with the Vivaspin centrifugal concentrator; lowering the centrifuge speed had no effect.



**Scheme 34.** The RebH catalysed bromination of tryptophol (**40**) to 7-bromotryptophol (**105**).

Dialysing out the imidazole against phosphate buffer overnight gave less precipitation but the activities tested with the natural substrate tryptophan using this protein was determined to be always less than 30% by HPLC.

It was suggested that adding FAD during the purification steps may stabilise the protein and prevent it from degrading. Fortunately, this helped to an extent and the conversions improved. Pleased with the activity of the protein obtained through this procedure, the crude halogenation reaction was filtered through the Vivaspin and subjected to the Suzuki reaction. From LCMS the conversion to the biaryl was seen but also a significant amount of homocoupling product was noted. Separation of this product from homocoupled biaryl on such a small scale proved to be a challenging task. Loss from transfers and column chromatography meant that the desired product could not be isolated cleanly from the reaction for characterisation.

Whilst conducting the substrate scope, Sewald published a paper reporting the use of RebH combiCLEAs (cross-linked enzyme aggregate) for the halogenase.<sup>115</sup> Dr Sarah Shepherd (S.S.) was able to reproduce this work. Moreover, she found that the method for the preparation of the CLEAs with RebH worked just as well with the other enzymes, PrnA, SttH and PyrH.

This work proved to be a turning point, as these combiCLEAs were much easier to prepare than purified enzyme and the yields of active protein were significantly higher. The RebH construct was retransformed into BL21(DE3) containing the pGro7 chaperone by S.S. Using this the RebH was grown in *E. coli* overnight following the procedures set by Frese *et al.*<sup>36</sup>

The combiCLEAs were prepared by addition of Fre and ADH which were also cloned into BL21(DE3) containing the pGro7 chaperone. Next the crude protein was precipitated by increasing the ionic strength of the mixture with ammonium sulfate. Cross-linking was then achieved by incubating with glutaraldehyde.<sup>115</sup> This was a significant step change towards to production of 7-bromo-tryptophol (**105**).

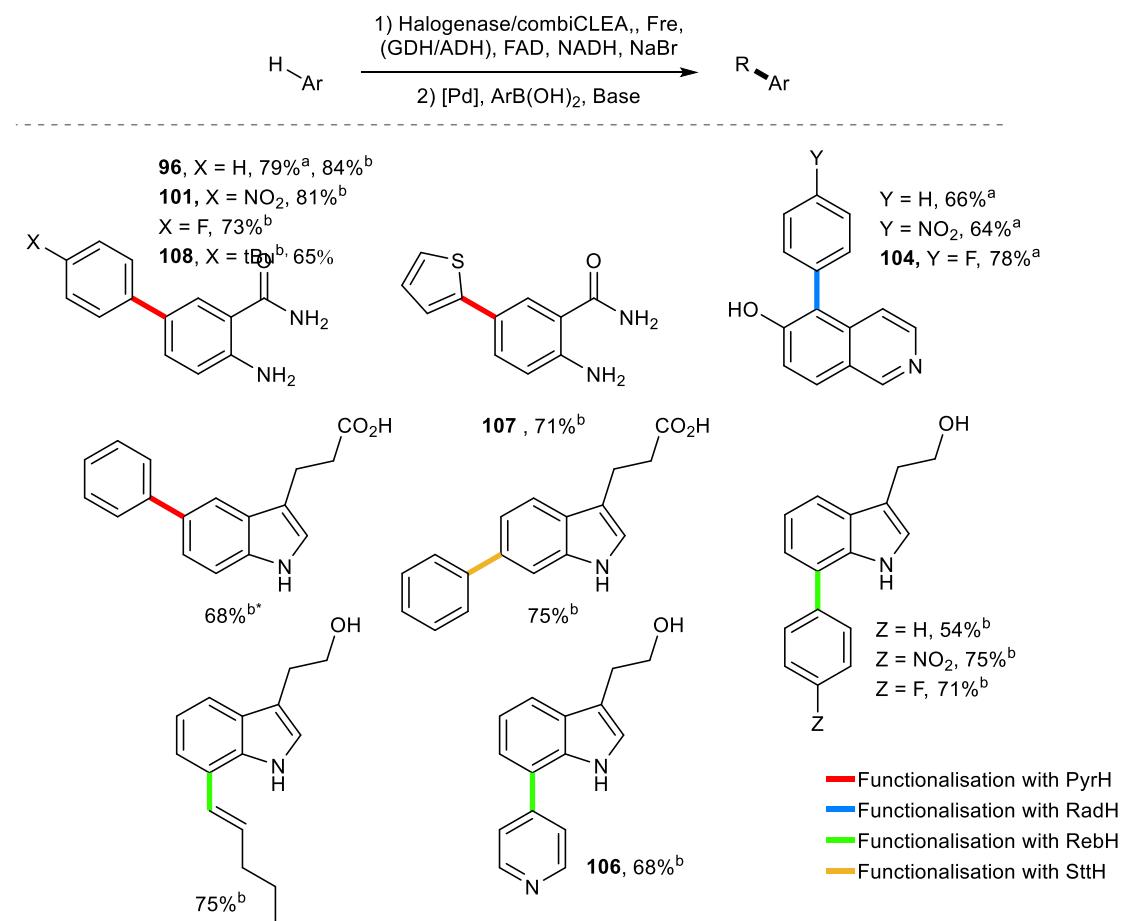
The combiCLEAs could also be stored in the fridge for several weeks whilst retaining activity. Furthermore, a good conversion could be seen with higher substrate concentrations (3.0 mM), meaning that more product could be formed from a reaction.

As well as being valuable in the biohalogenation step the CLEAs they also proved to be advantageous in the one pot reaction. Instead of using a Vivaspin the combiCLEA could be removed by a simpler centrifugation step before the addition of cross coupling reactants. Additionally, the palladium catalyst loadings could be lowered to a more amenable 10 mol% this is probably due to the cofactor recycling system switch from GDH to ADH. With the lower amount of palladium, the reaction profiles were cleaner and the stoichiometry of the boronic acid coupling partner could be lowered.

Using the RebH combiCLEAs a more comprehensive substrate scope was achieved by J.L and J.-M. H. The scope halogenase-Suzuki reaction is shown in Table 13 the reaction worked with other electron deficient boronic acid coupling partners including the heterocycles pyridine and thiophene to form biaryls **106** and **107** respectively. The moderately electron rich boronic acid containing the para *tert*butyl group was also coupled to anthranilamide and isolation of the product (**108**) was achieved in an excellent 65% yield over the two steps by J.L.

Attempts to form the RadH CLEA by S.S. and Eileen Brandenburger (E.B.) were unfruitful and transferring the conditions for preparing the CLEA had not been as straightforward as had been with the tryptophan halogenases.

**Table 13** – The substrate scope of the one pot halogenase Suzuki reaction



<sup>a</sup>obtained by filtrating through Vivaspin molecular weight filter prior to adding palladium components

<sup>b</sup>obtained by using halogenase combiCLEA and filtering prior to adding palladium components

\* Substrate scope was conducted by J.L. and J.-M.H.

## 2.2.7. Conclusions and Future work

Our goal to combine the halogenases into one pot with palladium catalysis was deconstructed into smaller objectives. Firstly, suitable substrates were chosen to conduct screening of chemocatalysed reactions in phosphate buffer. 5-Bromoanthranilamide (**35**) was chosen as a model substrate and tested with a variety of palladium catalysed reactions. The Suzuki reaction was found to work at 0.2 mM concentration with this substrate to form biaryl **36**. Optimisation was conducted so that milder reaction conditions could be used. It was found that the reaction required anaerobic conditions, base and elevated temperatures to proceed.

The Sonogashira and Mizoroki Heck reactions gave unsatisfactory yields and attempts to obtain near quantitative yields were unsuccessful. It was believed that the

5-bromoanthranilamide (**35**) was too unreactive due to the presence of the primary amide and anilino group. Efforts to optimise with other substrates, such as the naphthal **98**, was also unsuccessful. The optimisation reactions were conducted at the relatively low concentrations the halogenases work at and it is likely that at higher concentrations better yields could be obtained. Reports by Goss and coworkers have shown that at lower concentrations the Sonogashira reaction in biological conditions is greatly diminished.

Once the Suzuki reaction was optimised without enzymes, they were combined by J.L. with the biohalogenation reaction and further optimised. It was found that a protein removal step was required for the second step to work. The protein was removed by means of a molecular weight membrane filter before the components of the second step were added. Conducting the two reactions concurrently did not work due to the conditions and reagents required for each step being detrimental to the other.

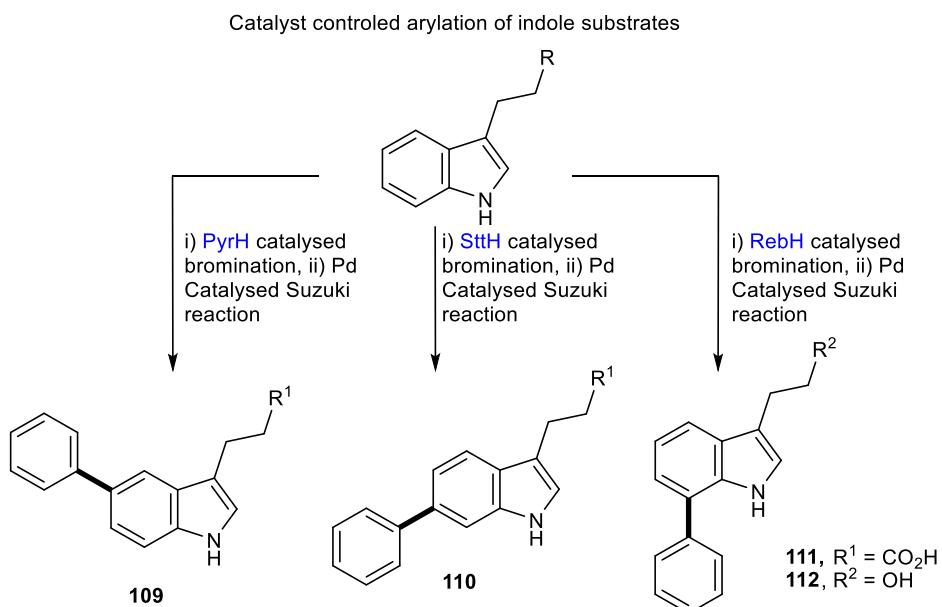
With both the reactions optimised and combined, a novel and general approach to the synthesis of biaryls using a biocatalysed/chemocatalysed sequence was developed. This reaction was demonstrated in conjunction with J. L and J.-M. H. that four different flavin dependent halogenase enzymes could be used. Furthermore, the Suzuki reaction was shown to be tolerant to unprotected functional groups widely believed to be problematic such as the hydroxyl and amino groups. The substrate scope with respect to the boronic acid was demonstrated.

The substrate scope of the combined reaction, with respect to the aryl halide, was limited by the halogenases available. The other drawback to this work was the stability of the halogenases, they were difficult to produce on preparative scale. This work was accomplished using wild type enzymes and although the substrate scope of these enzymes are quite broad, mutagenesis rounds such as those conducted within Lewis's group, could lead to more varied range of products and more robust biocatalysts.

The RebH combiCLEAs shown to halogenate tryptophan on gram scale by Sewald and co-workers were prepared in the labH and were shown by S.S that the combiCLEAs for the other tryptophan halogenases could also be prepared. The combiCLEAs were much easier to produce than purified protein and were significantly more robust. The combiCLEAs enabled higher substrate loadings. Moreover, the combiCLEAs could be removed by simple centrifugation and no

molecular weight filter was required. Hopefully as the halogenases are further developed and the substrate scope expanded, this procedure could be transformed into a more synthetically useful reaction making its way into syntheses of biologically active molecules.

The remarkable selectivity of the halogenases was utilised and using the protocol developed, three different positions of indole could be arylated, to give **109 – 112**, in one pot by merely altering the halogenase (Scheme 35). This is significant as previously, described earlier, selectively functionalising indoles required directing groups (Schemes 4 – 6). Prior to this work arylation of these substrates at these positions, required prefunctionalisation.<sup>116</sup>

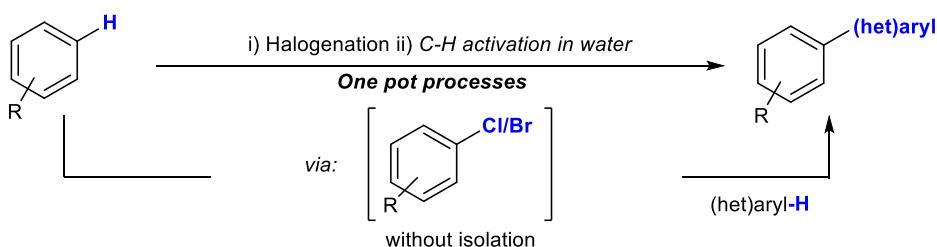


**Scheme 35.** Demonstration of the catalyst controlled selectivity using the developed methodology.

During the screening and the substrate scope it was found that this reaction required a high number of equivalents of boronic acid. After publishing this work, J.M.-H. was able to show that the boronic acid loading could be lowered when using ADH cofactor recycling enzyme as opposed to the GDH system. It seems that the glucose reacts with the boronic acid and it is for this reason a large excess was needed.

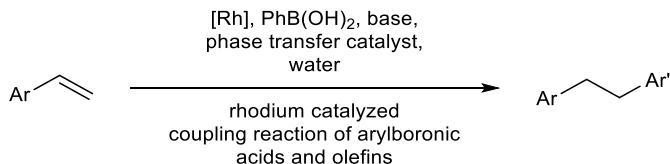
With the advantages of the CLEAs in hand, it could yet to be seen if the other coupling reactions previously found to be unsuccessful at 2.0 mM would work with higher substrate loadings thus forming more diverse products.

Efforts to find other reactions that could work with our substrates were unfruitful. The aryl halides tested did not undergo the C–H activation reactions or Buchwald-Hartwig amination reactions. Presently work within the group is ongoing to convert the bromide products into other potentially useful functional groups such as the cyano group. But as discussed earlier the C–H activation utilising the halogenase products, possibly with a metal other than palladium, could be an interesting reaction to develop, and with ongoing progress in the C–H activation field, this could be an achievable target (Scheme 36).



**Scheme 36.** A proposed scheme for the development of a novel formal cross dehydrogenative coupling reaction utilising halogenase.

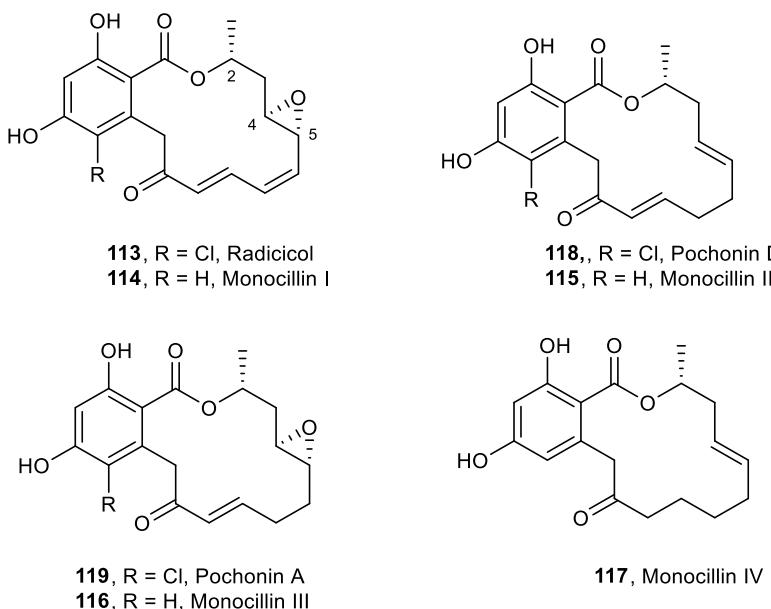
Another possible novel biocatalysed/chemocatalysed route to forming C–C bonds is possibly to utilise a rhodium catalysed hydroarylation of styrenes with boronic acids in water developed within the lab of Lautens (Scheme 37).<sup>117</sup> With the literature precedent for the formation of styrenes enzymatically; this could be combined with this reaction which has been shown to work in water.



**Scheme 37.** A proposed scheme for the development of a novel C–C bond forming reaction from enzymatically generated styrenes.

### 3. Synthesis of Radicicol

Radicicol (**113**, Figure 7) is a resorcylic acid lactone which is produced by various fungal species and was first reported in 1953.<sup>118</sup> Radicicol is a 14-membered benzofused macrolide containing an orsellinic acid portion with a chlorine substituent and an  $\alpha,\beta,\gamma,\delta$ -conjugated dienone at the benzylic position. It also contains a methyl substituent at the C2 position and an epoxide across C4 and C5. The compounds in this class not containing the chloro substituent are called the Monocillins (**114** – **117**).



**Figure 7.** Structures of selected monocillins and their related chlorinated compounds.

The compound which most resembles radicicol but does not contain the chloro- group is called monocillin I (**114**) and de-epoxy compounds (**118** and **119**, Figure 7) also are also isolated from the same fungal organisms as radicicol and are believed to be biosynthesised by the same molecular machinery.<sup>119</sup>

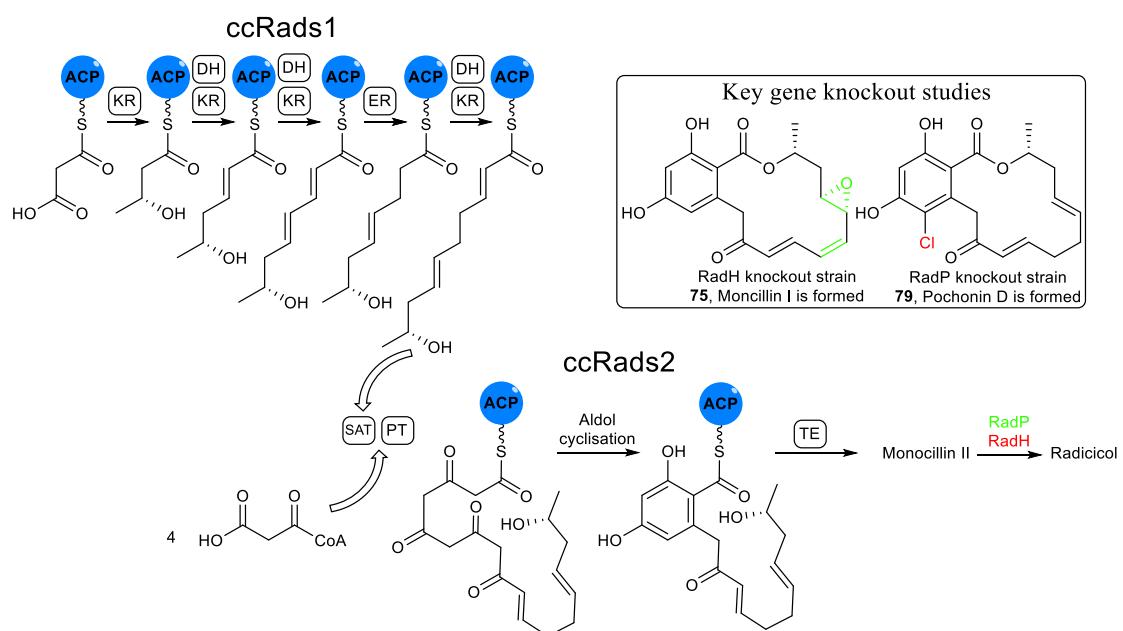
#### 3.1.1. Biosynthesis of radicicol

Studies on two radicicol producing fungal strains, *P. chlamydosporia*<sup>120</sup> and *C. chiversii* have been reported. These two genes have been found to have very similar sequence identities and contain homologous proteins.

The study of *C. chiversii* gene cluster conducted within the lab of Molnár define the function of two type I iterative polyketide synthases PKS, ccRads1 and ccRads2 and describe the presence of two post PKS proteins, RadH and RadP.<sup>121</sup> Figure 8 shows the sequence of events understood to occur in the RadH containing gene cluster.

ccRads1 is a highly reducing PKS (hrPKS); this cluster includes a single ketoacyl synthase domain (KS) which catalyses the decarboxylative condensation of malonyl-CoA onto the loaded ketide thioester, covalently attached to the acyl carrier protein (ACP). It also contains ketoreductase (KR) and dehydratase (DH).

The non-reducing PKS (nrPKS) ccRads2 is responsible for the resorcylate moiety present in radicicol and extends the polyketide by an additional four malonyl CoA units. It contains an N-terminal ACP acyltransferase domain (SAT), this transfers the pentaketide synthesised by ccRads1 to ccRads2. Once the nonaketide is formed, a product templating domain (PT) folds the nonaketide to aid the aldol cyclisation of the tri $\beta$ -keto intermediate giving the aromatic core. The thioesterase domain (TE) catalyses the lactonisation and liberates monocillin II.



**Figure 8.** The biosynthesis of Radicicol by the *C. chiversii* gene cluster. The biosynthesis involves two PKS systems and two post PKS proteins RadH and RadP. Gene knockout studies have shown that different products can be formed. ACP = acyl carrier protein, KR = keto reductase, DH = dehydratase, ER = ene reductase, PT = product templating domain, TE = thioesterase domain and SAT = acyl transferase domain.

The function of RadH was determined by gene knockout studies. It was found that when RadH was disrupted in the gene pathway the formation of the unchlorinated monocillin I (**75**) was observed.

An unusual feature in radicicol is the presence of the *trans*,*cis* dienone system. Although the *cis* isomer is more stable than the *trans* isomer, the mechanism by which it is formed is unclear. As there is only a single ER domain, it is unlikely that it is

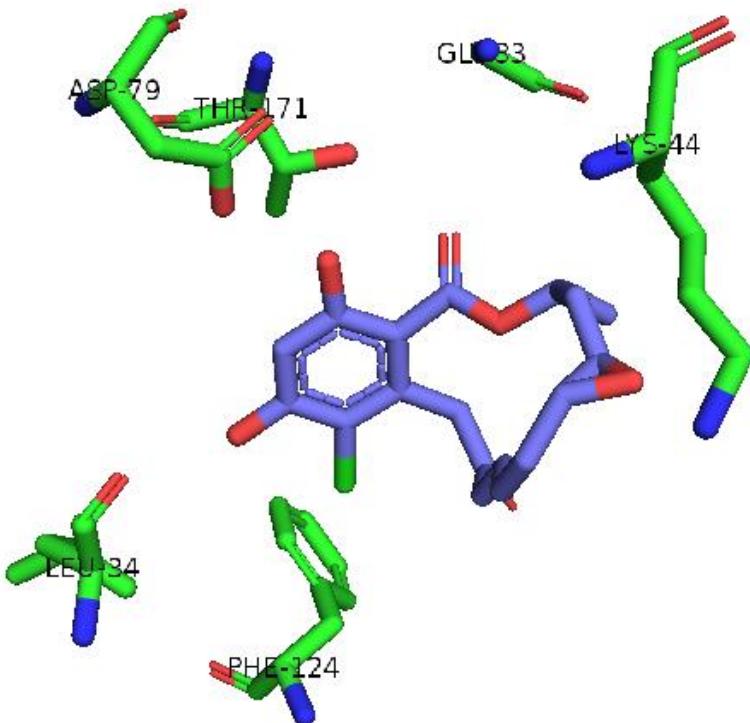
formed during the PKS step. It was thus proposed that the *cis* double bond is installed by the RadP, a putative cytochrome monooxygenase. This is confirmed by gene knockout studies; in the RadP knockout strain, the formation of the de-epoxy compound, pochonin D (**79**) was observed but no radicicol or triene (Figure 12). It is supposed that RadP hydroxylates and dehydration then yields the Z-configured double bond.

Tang and coworkers were able to reconstruct the radicicol biosynthesis *in vitro* but also in *S. cerevisiae* from the genes isolated from *P. chlamydosporia*.<sup>120</sup> Rdc5 and Rdc1 are the two PKS enzymes analogous to ccRads1 and ccRads2 respectively. They could be purified and incubated with malonyl CoA to give monocillin II (**76**). Monocillin II could then be chlorinated with Rcd2, the Flavin dependent halogenase with high sequence identity to RadH. Furthermore, feeding the pentaketide formed by Rdc5 to purified Rdc1 gave Monocillin II.

### 3.1.2. Biological activity of Radicicol

Heat shock protein 90 (HSP90) is a molecular chaperone which is believed to be essential for cancer cell survival.<sup>122</sup> Heat shock proteins protect against cellular stresses by protecting against protein misfolding and assisting in the stabilisation and/or activation of proteins. HSP90 is known to be involved in stabilising and activating over 200 different proteins. The HSP90 is also used by cancer cells to protect oncoproteins. Much work has been done to understand the mechanism and role of HSP90. It is found that HSP90 requires ATP to function and there are two binding sites which are referred to the *N*-terminal domain and the *C*-terminal domain. A crystal structure of radicicol bound in the *N*-terminal domain has been published and is shown in figure 9.

Radicicol has a binding affinity to HSP90 of 19 nM which is 50 fold stronger than the binding affinity of geldanamycin, the other naturally occurring molecule known to bind and inhibit HSP90.<sup>123</sup>



**Figure 9.** A crystal structure of radicicol bound in the *N*-terminal domain of HSP90. (PDB code 1BGQ)

Though radicicol and its compound class show high activities in cellular assays, they show little or no activity when tested in animal models. This is due to the metabolic instability of these compounds. Radicicol has a allylic epoxide which is strained, a conjugated dienone making it unstable to 1,6-Michael addition and a ketone that is readily enolisable, converting to unwanted isocoumarin products. Because of its metabolic instability radicicol and other natural compounds have not been used clinically.

It is because of this bioactivity, radicicol and its related class of resorcylic acid lactones, that much work has been done in preparing analogues related to these structures.<sup>123</sup>

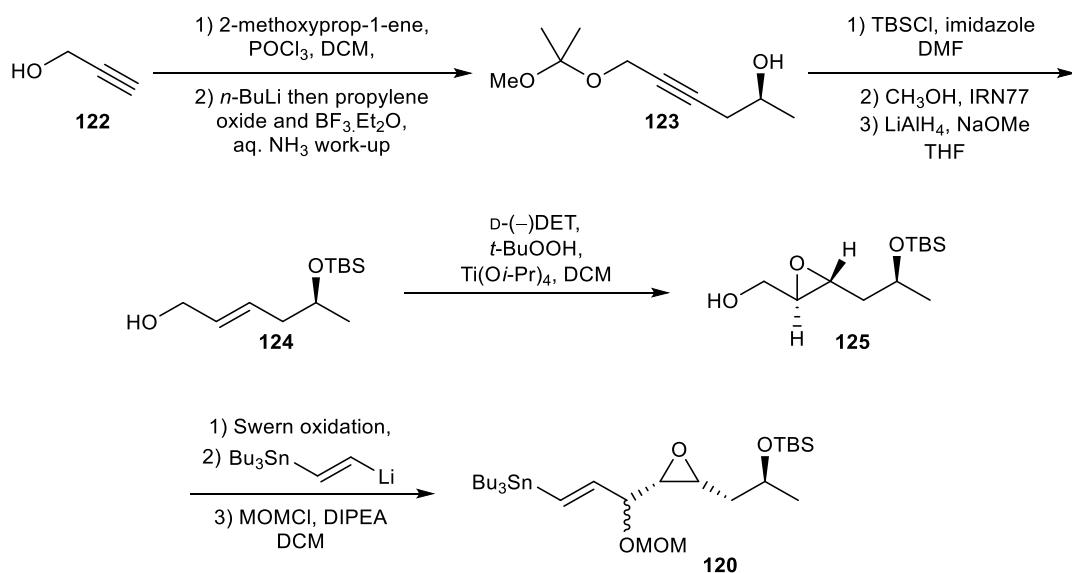
### 3.1.3. Synthesis of radicicol and related macrolides

The first total synthesis of radicicol dimethyl ether was completed by Lett and Lampilas in 1992<sup>124,125</sup> which was then modified and improved by the same group in 2002 to give radiciciol.<sup>125,126</sup> The synthesis of dimethyl radicicol and then radicicol was published by Danishefsky and coworkers later in 2000 and 2001 respectively.<sup>127,128</sup>

### 3.1.3.1. Lett's total synthesis of radicicol

The first total synthesis of monocillin I, radicicol and monocillin I dimethyl ether was reported in 1999 by the group of Lett.<sup>124</sup> The synthesis is convergent with two portions: an chiral alcohol fragment bearing the methyl substituent and the epoxide and an isocoumarin containing the orcinol moiety. They are both coupled together by a palladium catalysed cross coupling reaction followed by a Mitsunobu ring closing esterification.

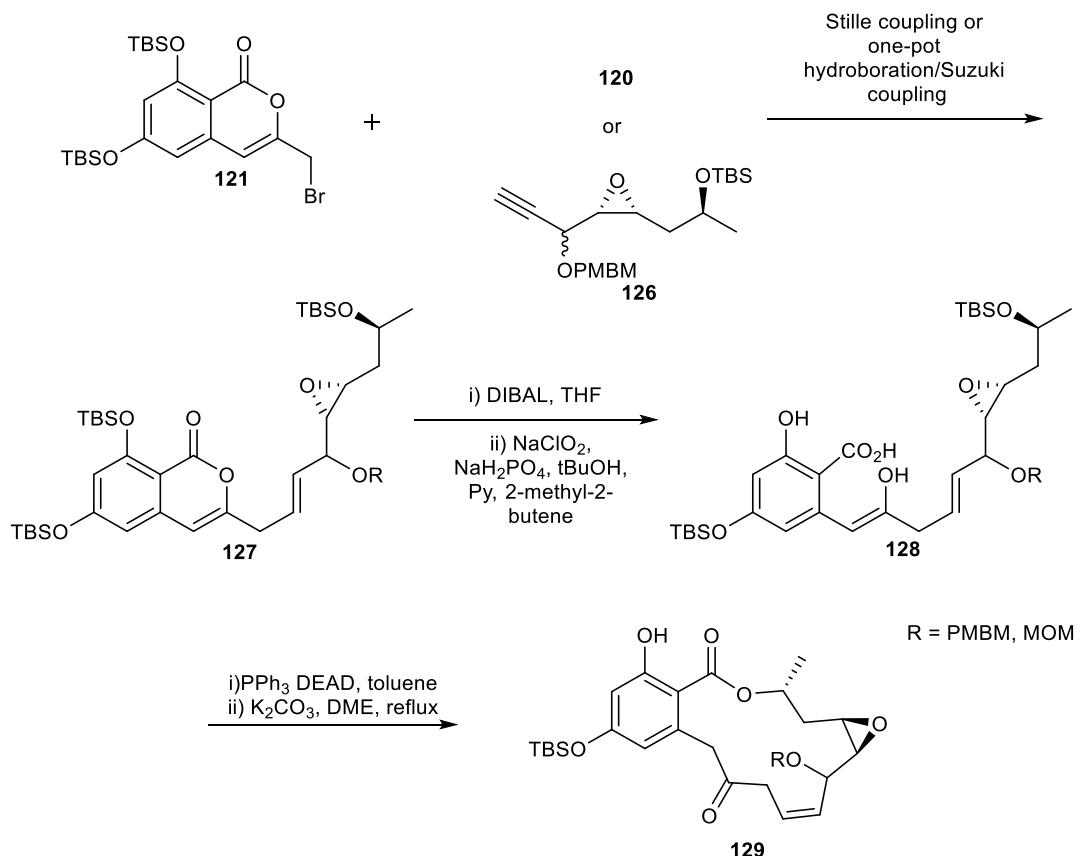
In the first generation synthesis (Scheme 38) tributylstannyl allyl epoxide **120** was used as the coupling partner in a Stille coupling with bromomethyl isocoumarin **121** (Scheme 39). This epoxide could be synthesised readily from propargyl alcohol (**122**, Scheme 38). The chiral centre (**123**) could be installed by treating the deprotonated *O*-protected propargyl alcohol with propylene oxide. The triple bond was reduced, to exclusively the *E* isomer (**124**) in 75-80% yield, using Corey conditions, LiAlH<sub>4</sub>, sodium methoxide in THF. Sharpless epoxidation was then used to get the desired diasteroisomer **125** in 95% yield.



**Scheme 38.** The synthesis of the epoxide containing stannane **120** for use in the coupling with isocoumarin **127**.

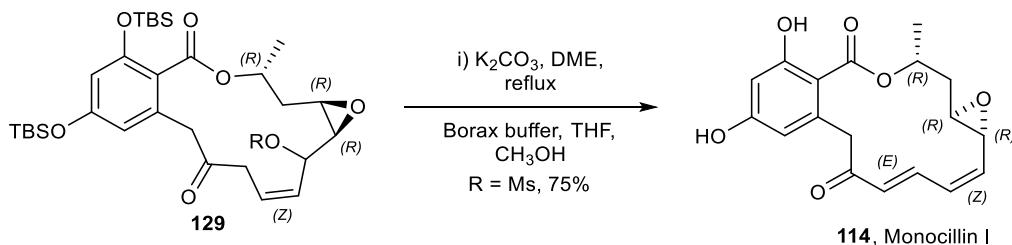
Swern oxidation then gave the aldehyde, which could then be treated with the lithiated vinyl stannane to access **120**. Later it was found that the homopropargyl epoxide **126** could undergo a one pot hydroboration and Suzuki cross coupling step with TBS protected isocoumarin to give intermediate **127**. Once these two parts were cross

coupled, the isocoumarin was cleaved with DIBAL and oxidised to the benzoic acid **128**. The TBS protecting group, proximal to the acid needs to removed before a Mitsunobu reaction closes the ring to give the 14 membered macrolide **129** (Scheme 34).



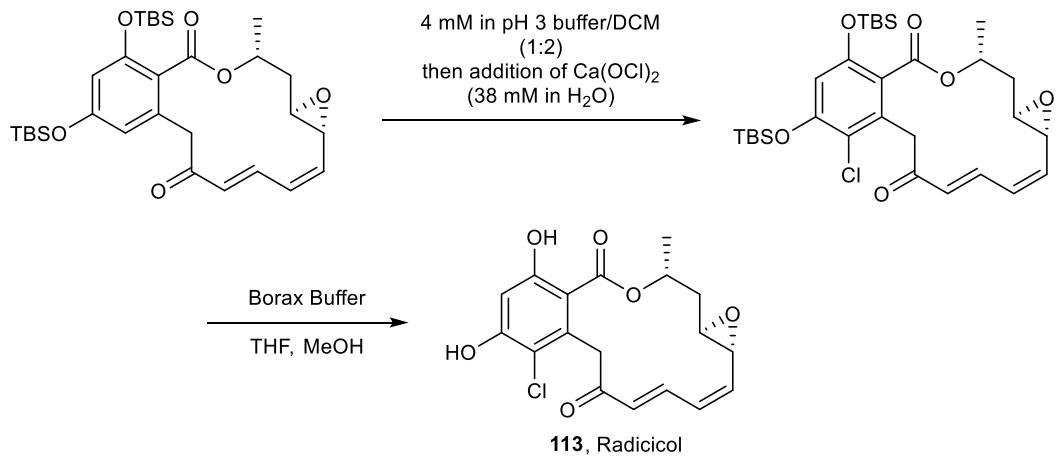
**Scheme 39.** The coupling of **120** or **126** to the isocoumarin fragment **121** using a cross coupling reaction, and the route to macrocycle **129**.

The 1,6-dienone feature was set-up by deprotonation  $\alpha$ - to the ketone with K<sub>2</sub>CO<sub>3</sub> and elimination of the methoxymethoxide from **129** in 25-30% yield (Scheme 36). In the second generation synthesis this step was addressed; the low yield was attributed to the equilibration of methoxymethoxide, with formaldehyde and the methoxide anion. This methoxide anion proves problematic as it further reacts with the sensitive conjugated dienone. This problem was solved by converting the protecting group to a mesylate before spontaneous elimination; this modification gave the desired macrolide in the more acceptable overall 75% yield.



**Scheme 40.** The end game approach in Lett's total synthesis of Monocillin I.

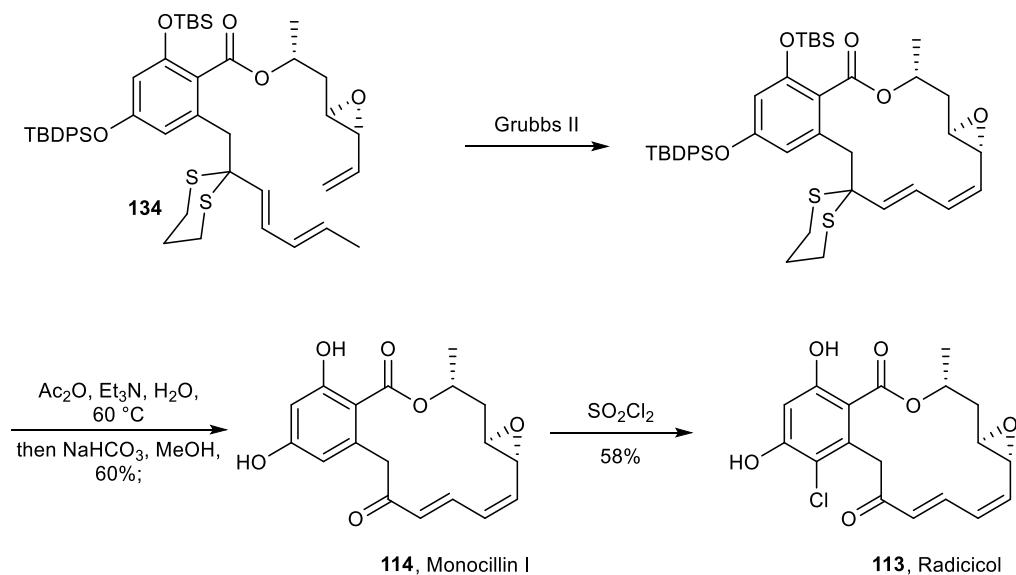
Chlorination of macrolide was achieved by calcium hypochlorite in dilute biphasic conditions (Scheme 41). It is of note that direct conversion of monocillin I to radicicol using these conditions was inefficient. The target radicicol (**113**) was then obtained by borax induced desilylation.



**Scheme 41.** Strategy used in the Lett synthesis for the installation of the chloride in Radicicol.

### 3.1.3.2. Danishefsky's total synthesis of radicicol

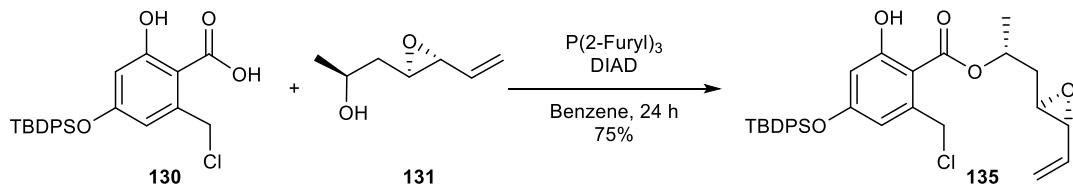
Danishefsky's synthetic route to radicicol (**113**) is also convergent and starts from three building blocks: orsellinic acid fragment **130** and two side arms (**131**, **132**, Scheme 42). Once the two side arm fragments are attached to the orsellinic acid core (**133**, Scheme 43 and 44) and protected (**134**), they were connected together by ring closing metathesis (RCM), as shown in Scheme 38.



**Scheme 42.** Ring closing metathesis of **134**, deprotection to give **114** and chlorination of monocillin I (**114**) to give radicicol (**113**).

The chiral secondary alcohol **131**, containing the methyl substituent and the allylic epoxide, is prepared in 8 steps by a series of chain extensions *via* oxidation reduction and olefination steps, from (S)-3 hydroxybutyric acid methyl ester.

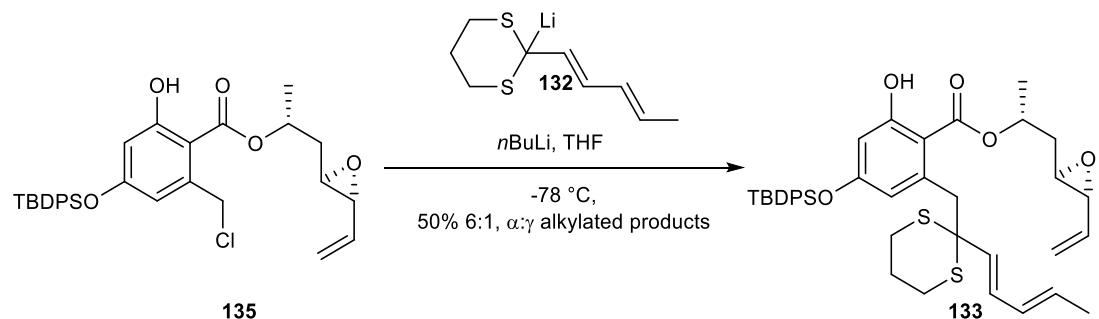
The secondary alcohol was attached to the monoprotected orsellinic acid by Mitsunobu esterification with P(2-furyl)<sub>3</sub> and DIAD in benzene to give **135** (Scheme 43). These unconventional conditions for Mitsunobu esterification over other esterification methods was chosen as, phthalide formation was seen when phenol protecting groups other than methyl ethers were used. Deprotection of the methyl ether was not successful and resulted in decomposition, undesired halogenation or ring opening of the epoxide.



**Scheme 43.** Synthesis of **135** by the Mitsunobu esterification of resorcylic acid **130** with alcohol **131**.

The other fragment **132** is the acyl anion equivalent, dithiane protected 1,6-dienone. It is prepared from commercially available hexadienal. This lithiated species is assembled onto the benzylic position by an S<sub>N</sub>2 reaction (Scheme 44). The macrocycle was then closed with Grubbs 2<sup>nd</sup> generation catalyst in DCM with 60% yield. For this

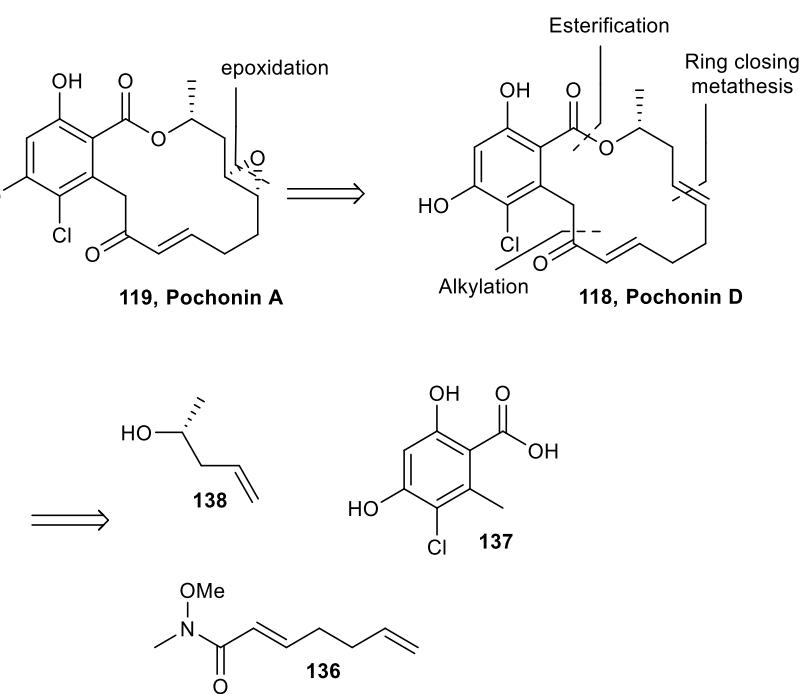
step the phenols had to be protected otherwise the reaction would not proceed (Scheme 42).



**Scheme 44.** Synthesis of **133** by addition of lithiated dithiane **132** to **135**.

### 3.1.3.3. Winssingers's total synthesis of radicicol and pochonin D

The retrosynthetic analysis taken by the lab of Winssinger was almost identical to Danishefsky's synthesis. The combinatorial synthesis was designed with the need to form analogues of radicicol for medicinal evaluation (Scheme 41).



**Scheme 45.** Retrosynthetic analysis used by Winssinger for the synthesis of Pochonin D (**118**) and Pochonin A (**119**).

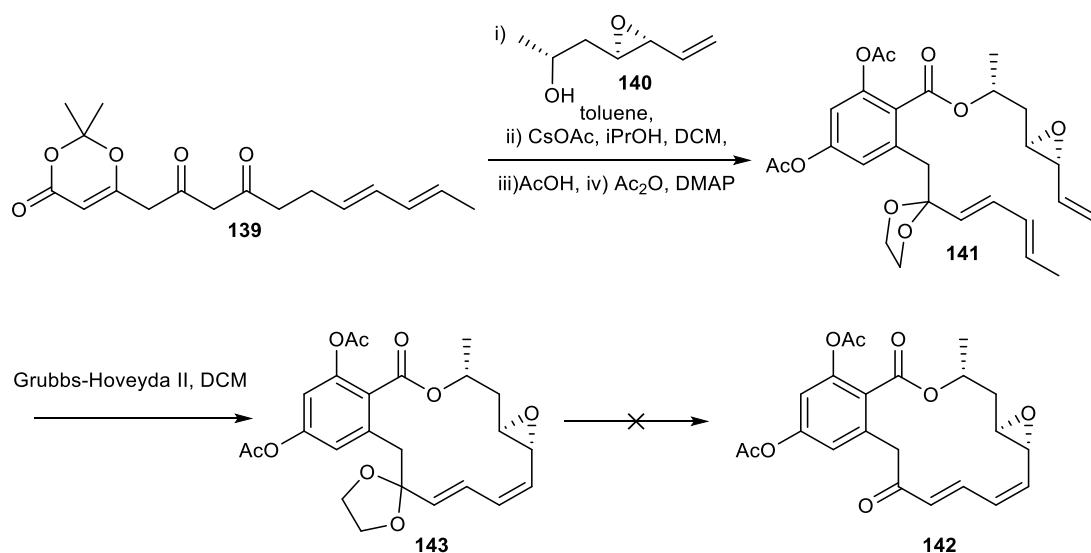
Winssinger's synthesis for pochonin D uses Weinreb amide **136** as opposed to an acyl anion: the benzylic anion was used over the benzylic chloride. This meant that

orsellinic acid could be used directly for the synthesis. The orsellinic acid **137** was coupled to the chiral homo allylic alcohol **138**.

For the synthesis of Radicicol the conjugated dienone was protected using a thioether. This thioether could be used to attach a solid phase resin in the synthesis.

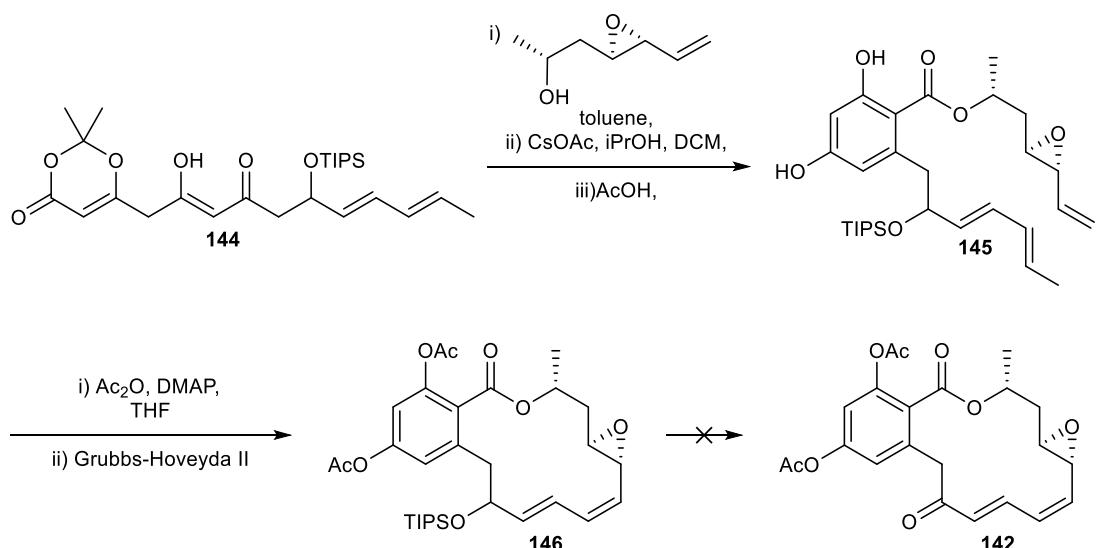
### 3.1.3.4. Barrett's biomimetic approach towards the total synthesis of radicicol

Barrett has attempted to approach the total synthesis of radiciol and monocillin I using a biomimetic strategy (Scheme 46).<sup>129</sup> The synthesis involved building a linear diketodioxinone **139**. The corresponding ketene could be formed from a retro-Claisen rearrangement and then trapped with an epoxide containing alcohol (**140**) giving the resorcylate **141**.



**Scheme 46.** Attempted formation of monocillin I diacetate **142** using a biomimetic strategy from **139** and alcohol **140**.

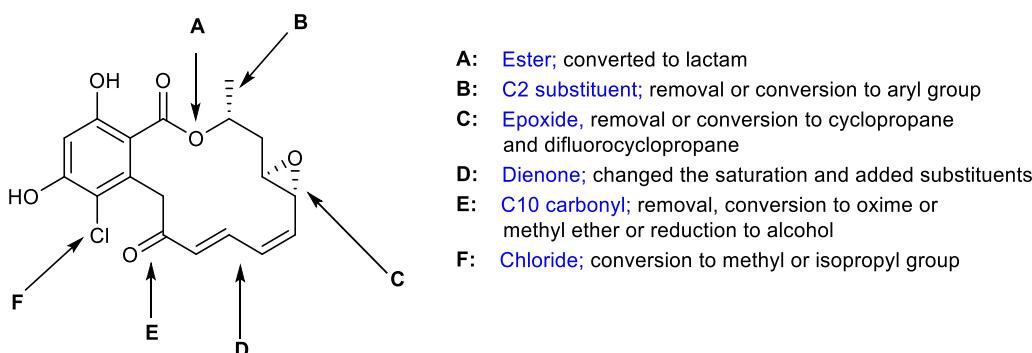
Unfortunately, after reaching the macrocycle **143** the deprotection of the ketal was problematic. Using acidic conditions resulted in opening of the epoxide ring. For this reason, the starting ketene precursor **144** was synthesised. This could be cyclised to give the resorcylate containing the TIPS protected alcohol **145** (Scheme 47). After reaching **146** using Grubbs-Hoveyda II catalyst the TIPS group could not be removed. When the TBS group over the TIPS group was used the RCM did not proceed.



**Scheme 47.** Attempted formation of monocillin I diacetate **142** using a biomimetic strategy from **144** and alcohol **140**.

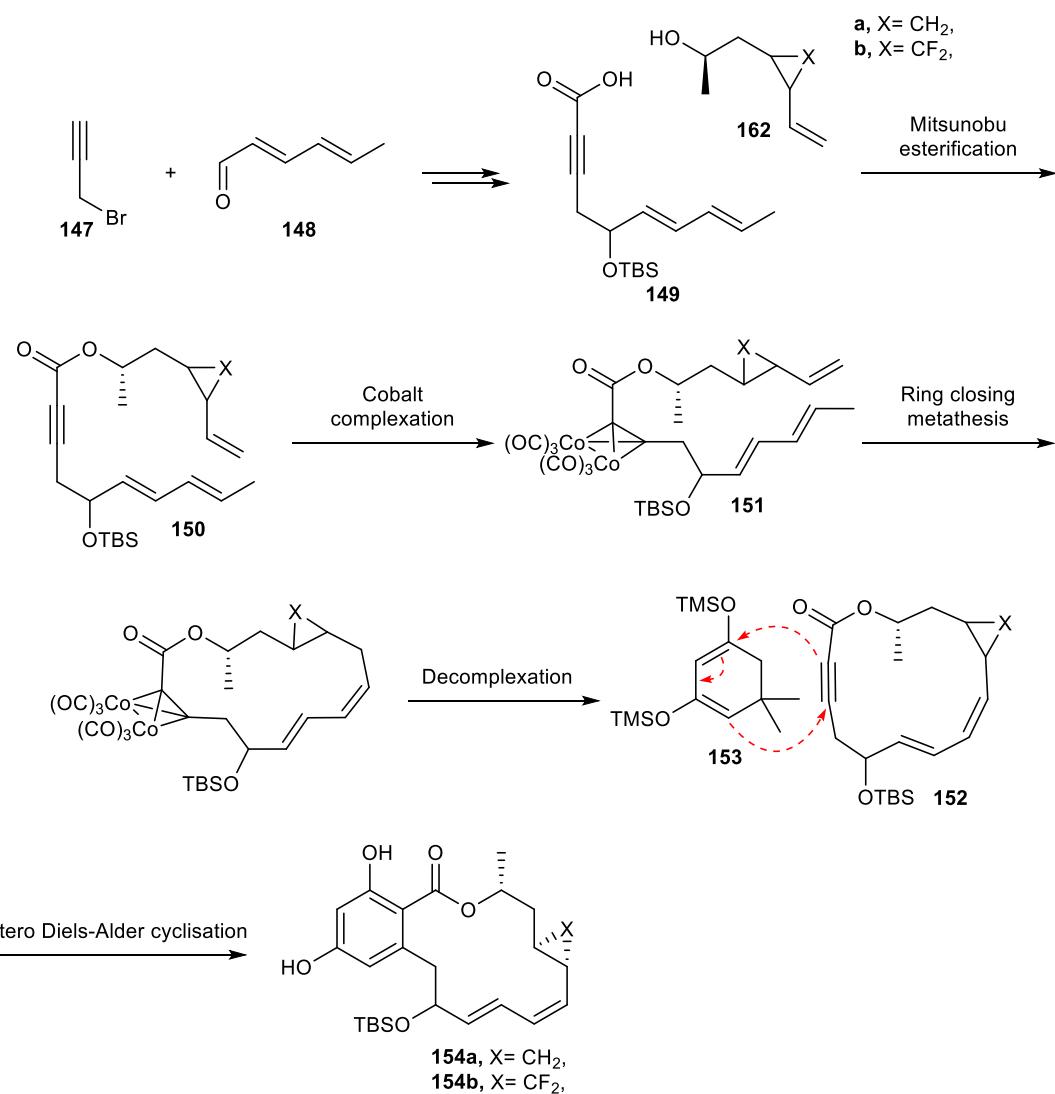
### 3.1.3.5. Synthesis of radicicol analogues

Due to the potency and instability of radicicol as described earlier, much effort has been made to prepare analogues in the hope that they would be more stable.<sup>123,130–135</sup> A summary of where variation has been made on the radicicol structure to generate a series of analogues to test their potency against HSP90 is shown in figure 10.



**Figure 10.** A summary of the points of variation that have been achieved to date to produce analogues of Radicicol

Danishefsky and co-workers were able to access the analogue cycloproparadicicol using an elegant Diels-Alder approach, in which a 14-membered lactone containing an acetylenic dienophile was reacted with a diene to construct the resorcinol moiety late in the synthesis (Scheme 48).<sup>135</sup>



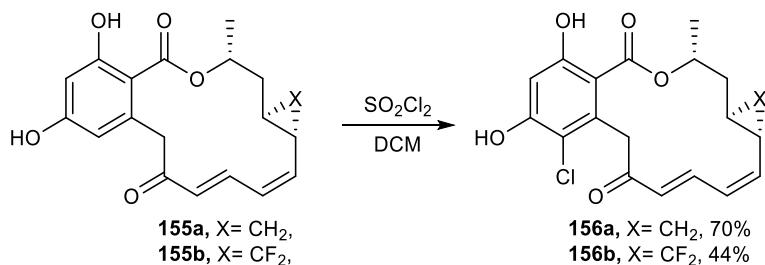
**Scheme 48.** Danishefsky's 'Ynolide' approach to cycloproparadicicol and Difluorocycloproparadicicol analogues.

Using this approach, zinc coupling of propargyl bromide (**147**) with hexadienal (**148**) provided the terminal alkyne which was then carboxylated to give acid **110**. The newly formed carboxylic acid then underwent Mitsunobu esterification to give the desired RCM precursor **150**. Attempts to close this cycle were unsuccessful, so the conformation of the ynolide was altered by complexing the alkyne with dicobalt carbonyl (**151**). Following the now fruitful RCM, the alkyne (**152**) was uncovered in 50% yield, using CAN in acetone buffered by 2,6-ditertbutylpyridine.

Once the 14 membered macrolide was achieved, it was then treated with a dimedone derived acyclic diene. The alkyne dieneophile proved to be underactivated for this reaction with acyclic dienes; the reaction had to be heated to 160 °C. At higher temperatures, it was necessary to use the more thermostable cyclic diene (**153**)

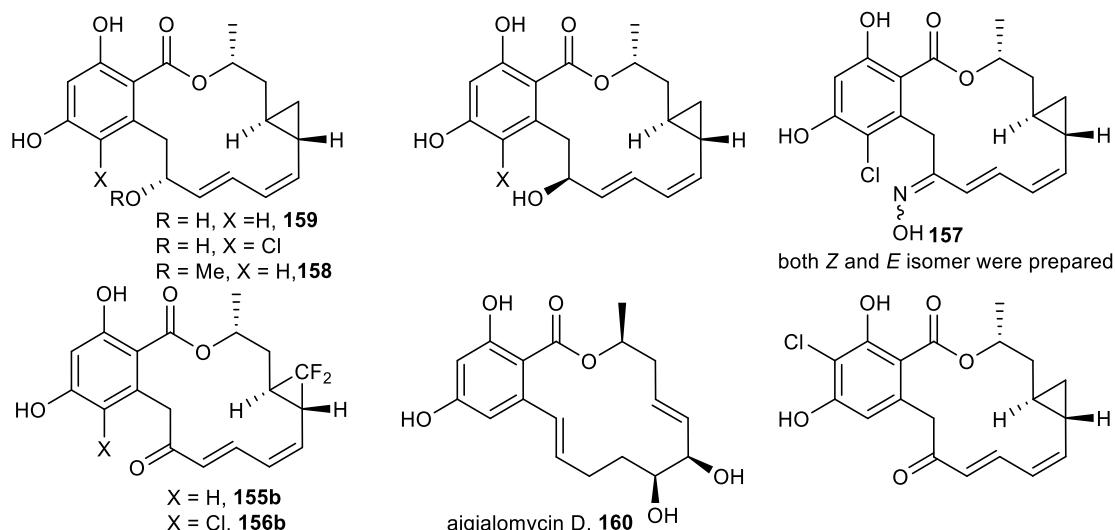
eliminating isobutylene after the Diels-Alder cyclisation to **154**. The Diels-Alder with the alkyne containing difluorocyclopropane proved to be challenging as it was not as thermostable as the cyclopropane compound and more decomposition was observed.

After the framework was in place the homobenzylic silyl ether was cleaved affording the alcohol. The oxidation of this  $2^\circ$  alcohol proved problematic. The free phenols were protected with acetate groups before oxidation with Dess-Martin periodinane to give the benzyl ketone in 68% yield. The chlorination with sulfonyl chloride gave the desired products (Scheme 49). It is important to note that this chlorination was not entirely regioselective and a mixture of two chlorinated isomers (13:5) was obtained.



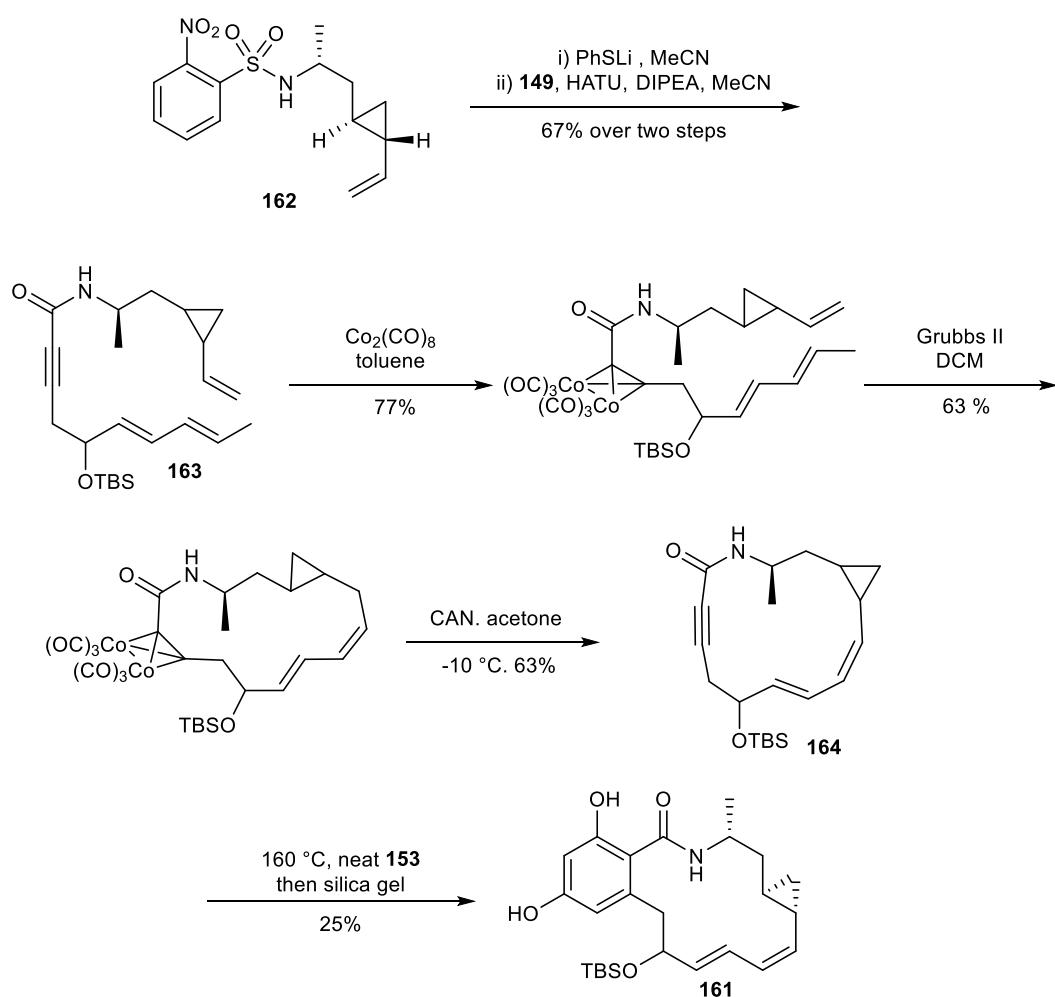
**Scheme 49.** The chlorination of macrocycles **155** to **156** using sulfonyl chloride.

This cycloproparadicicol analogue was then used to prepare further variants (Figure 11); the benzyl ketone **156a** was straightforwardly converted to the oxime **157**. The methyl ether **158** could also be installed at this position from the alcohol **159**, by treatment with methyl iodide and silver oxide in DMF. Using this protocol the group of Danishefsky was able to access another resorcylic acid lactone natural product, aigialomycin D **160**.



**Figure 11.** Development of resorcylic macrolides prepared by Yang *et al.* for the inhibition of HSP90

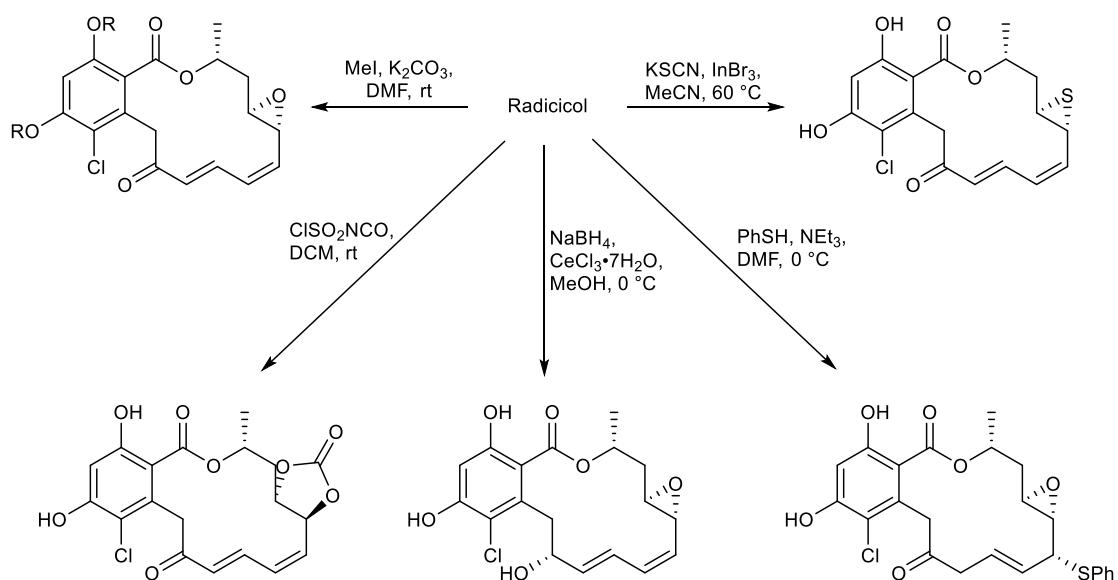
Cycloproparadicicol lactam **161** can be accessed through an HATU mediated amide coupling reaction of **162** to **163**. The amine was prepared by a Mitsunobu reaction of the cyclopropyl alcohol **162a** with nitrobenzenesulfonamide and then deprotection of the nosyl group. The Diels-Alder reaction to gave low yields as the ynamide **164** was too unreactive and so the synthesis to the desired product was suspended (Scheme 50). The group of Moody have also prepared various radicicol related lactams; it was thought that the lactam would be more metabolically stable.<sup>130,136</sup>



**Scheme 50.** Preparation of macrocyclic cycloproparadicicol analogue (**161**) using the Diels-Alder approach.

### 3.1.3.6. Semi-synthesis of radicicol analogues

Several analogues were prepared from monocillins I, II and III and radicicol isolated from *Pochonin chlamydosporia*. These included protecting the phenols, Luche reduction of the unsaturated ketone, Michael addition of various nucleophiles onto the dienone and conversion of the epoxide to a thiirane (Scheme 51).<sup>137</sup>



**Scheme 51.** Semi synthesis of radicicol analogues.

### 3.1.4. Objectives

The objective of this project is to design a flexible synthetic route to monocillin II. It is believed that monocillin II is the natural substrate for RadH. The RadH enzyme was being characterised within the Micklefield group. With this biologically important molecule and other molecules in this class in hand, it could prove to be valuable for the determination of the biosynthetic role of RadH and give us an insight into the biosynthesis of radicicol and related structure. Once the route has been established it is hopeful that the route can be adapted for the synthesis of several similar molecules.

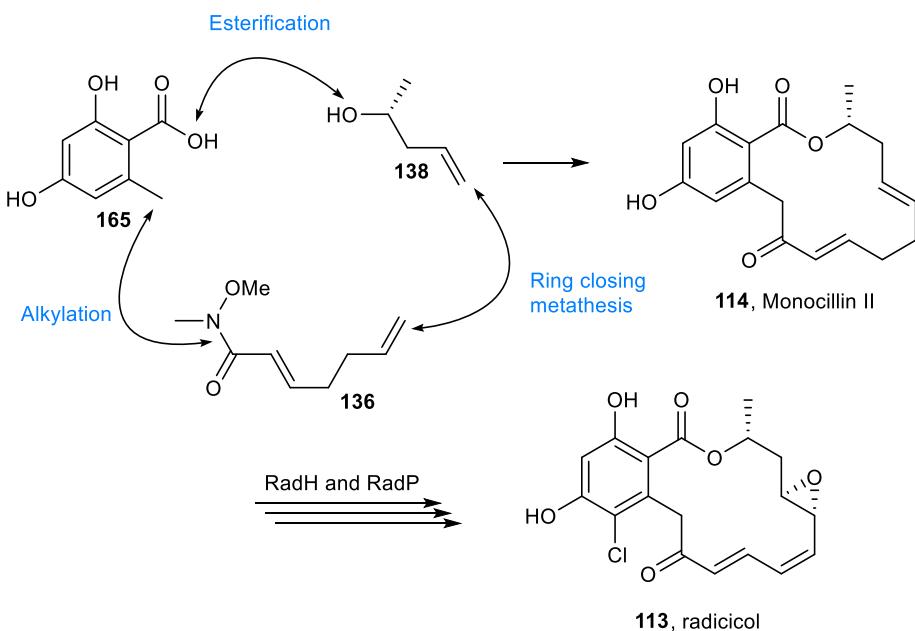
## 3.2. Results and discussion

RadH, the fungal derived halogenase is involved in the radicicol biosynthetic pathway.<sup>120,121</sup> The characterisation of this enzyme has not been achieved before in the literature and poses a significant interest, as it is fungal derived. To date the only other fungal halogenase characterised in the literature is rdc2.<sup>39</sup>

Work on the characterisation of RadH was being conducted by Dr Binuraj Menon (B.R.K.M.) and Eileen Brandenburger (E.B.) in the Micklefield Lab. To confirm the biosynthetic role of this enzyme the natural substrate needed to be tested. From the literature it was postulated that monocillin II was the natural substrate for RadH and then RadP furnished the chlorinated macrocycle with the *cis*-double bond and the epoxide. To test this hypothesis monocillin II was to be synthesised.

### 3.2.1. Monocillin II

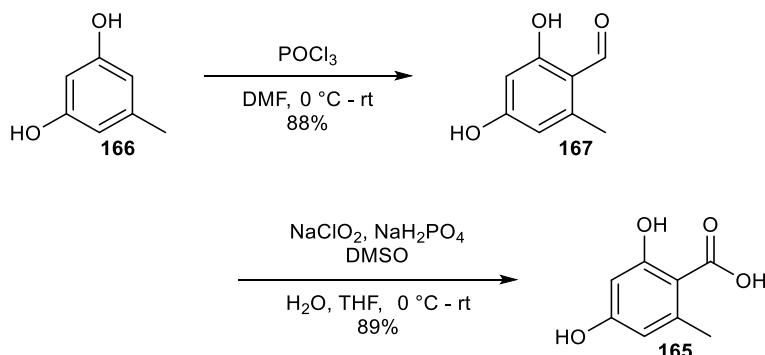
The route pursued was mapped out from literature and involves the coupling of three main components: orsellinic acid (**165**), (*S*)-pentenol (**138**) and the Weinreb amide of the skipped diene (**136**, Scheme 52). This convergent synthetic route taken by Danishefsky<sup>127,128,135</sup> then modified by Winssinger was desirable as it allows for diversification at later stage of the synthesis.



**Scheme 52.** The proposed convergent synthetic route towards the synthesis of Radicicol (**113**) using RadH and RadP to furnish the key intermediate Monocillin II (**114**).

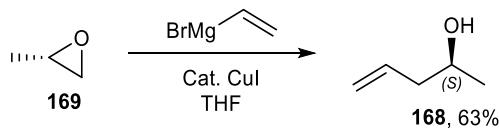
#### 3.2.1.1. Synthesis of fragments

The synthetic route towards orsellinic acid was taken from literature: Vilsmeier formylation of orcinol followed by Pinnick oxidation and this could be accessed on gram scales (Scheme 53).<sup>138</sup>



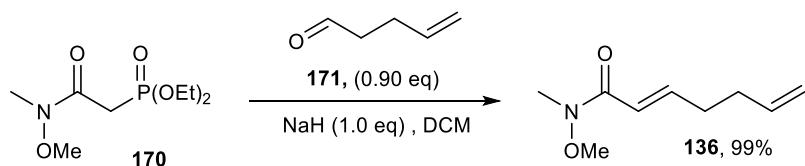
**Scheme 53.** The synthesis of orsellinic acid (**165**) from orcinol (**166**) via orcinaldehyde (**167**).

The desired enantiomer (*S*)-pentenol (**168**) could be synthesised on large scale in good yields by ring opening of optically pure propylene oxide (**169**) with vinylmagnesium bromide (Scheme 54) and it is also available commercially.



**Scheme 54.** The synthesis of the homoallylic alcohol **168** fragment.

Winssinger showed that the Weinreb amide could be made using a Julia-Kocienski olefination reaction. When this reaction was tried by Dr Thomas Williams (T.W.) in the Greaney group it was found that this reaction gave poor yields. It was thus decided that the Horner-Wadsworth-Emmons reaction would be a better route to the desired Weinreb amide. After deprotonation of the commercially available phosphonate ester **170** with NaH and treating it with skipped dienal **171**, the Weinreb amide **136** needed was obtained. This reaction proved to be extremely clean giving a high yield (Scheme 55).



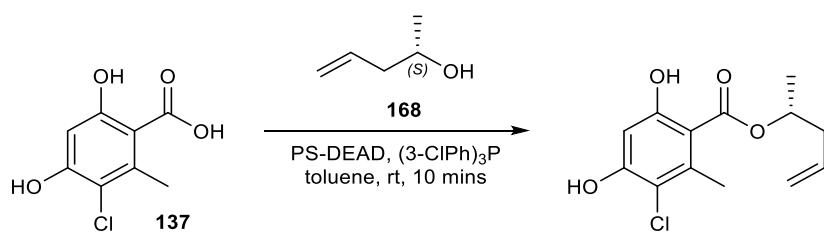
**Scheme 55.** Synthesis of **97** using a Horner-Wadsworth-Emmons reaction.

### 3.2.1.2. Esterification

The next stage in the synthesis required the esterification of the orsellinic acid (**165**) with the alcohol containing the terminal alkene (**168**).

During both Danishefsky's and Winssinger's approaches they divert from their initial route, *via* a more predictable  $(COCl)_2$  mediated esterification, to a Mitsunobu esterification reaction. Danishefsky found that when the phenolic protecting groups were not methyl ethers then acid chloride esterification gave an unwanted phthalide.<sup>128</sup> This phthalide was formed by the  $S_N2$  displacement of the acid onto the benzylic chloride. Using orsellinic acid (**168**) would not pose this problem as this benzylic chloromethyl group is absent.

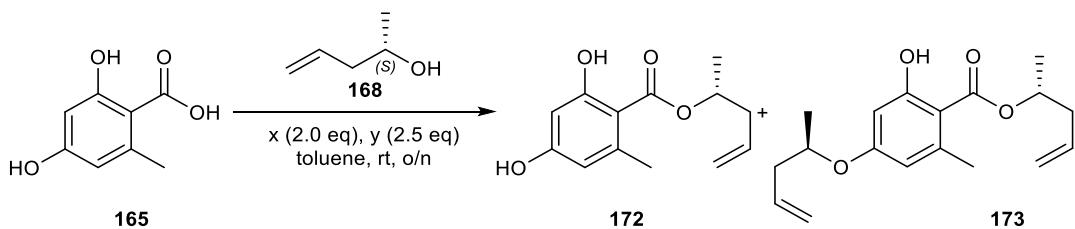
Moulin *et al.* were able to use the unprotected chlorinated orsellinic acid **137** and do a direct Mitsunobu esterification.<sup>139</sup> The conditions, used for this reaction, are shown in Scheme 56. Polymer-supported DEAD (PS-DEAD) and the deactivated tris *meta*-chloro substituted triphenylphosphine were the reagents.



**Scheme 56.** The conditions for the Mitsunobu reaction used in the Winsinger synthesis using chlorinated orsellinic acid (**137**).

The polymer supported reagent was a relatively costly for a step early on in the synthesis and so for this project neat DEAD was used instead. Using DEAD and tris-(3-chlorophenyl)phosphine ( $P(3-ClPh)_3$ ) gave the product (**172**) in just 21% yield which was much lower than that reported in the literature.<sup>139</sup> It was thought that using PS-DEAD could give a higher yield.

**Table 14** - Mitsunobu reaction of orsellinic acid (**165**), using P(3-ClPh)<sub>3</sub>



Entry	x	y	solvent	Yield	
				<b>172</b>	<b>173</b>
<b>1</b>	P(3-ClPh) <sub>3</sub>	DEAD	toluene	21 %	ni
<b>2</b>	P(3-ClPh) <sub>3</sub>	PS-DEAD	toluene	17 %	15 %

n.i means not isolated

From Table 14, it can be seen that when using a deactivated system, the conversions were low and using a more reactive phosphine competing alkylation would likely occur and give more of the unwanted dialkylated product **173**. It is also possible that the chlorine substituent on the benzene ring increases the nucleophilicity of the benzoic acid used in Winssinger's synthesis making the Mitsunobu reaction would be more facile, this rationalises, to an extent, the incomparable yields.

From these observations protecting the phenols to prevent the competing alkylation seemed to be required.

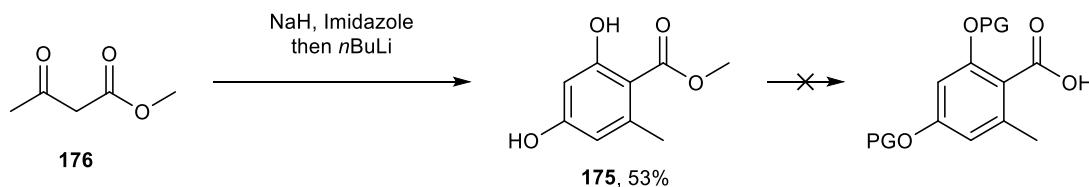
### 3.2.1.3. Protection strategy

Taking note from previous syntheses of radicicol (**113**) and pochonin D (**118**), it can be seen that the protecting group strategy seemed to be crucial.<sup>127,139</sup> Phenolic methyl ethers could not be cleaved and TBS ethers were found to be labile. If the epoxide functionality were to be installed later on in the synthetic route then it would not be stable to acidic deprotection conditions.

It was thought that using the SEM ether group over the TBDPS ether would be beneficial as it has been cleaved using the mild lewis acid MgBr<sub>2</sub>•Et<sub>2</sub>O.<sup>139</sup> Furthermore, it was noted that adding bulk *ortho* to the benzoic acid could affect the esterification.<sup>85,128</sup>

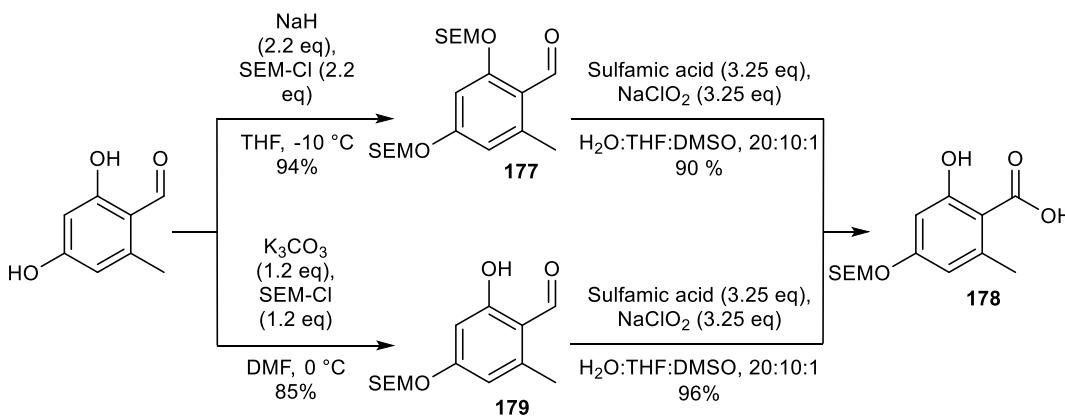
Work by T.W. showed that protecting directly the benzoic acid was challenging due to selectivity. It was found that methyl orsellinate (**175**) could be made by the self-condensation of methyl acetoacetate (**176**, Scheme 57).<sup>140</sup> Attempts on the hydrolysis

of the methyl ester group proved futile, probably due to the steric crowding around the ester.



**Scheme 57.** The synthesis of methyl orsellinate (**135**) deprotection of the methyl ester and the protection of phenol group was unsuccessful.

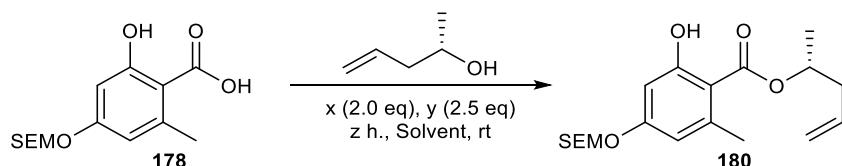
A route to the protected compound was proposed, involving the protection of the aldehyde before oxidation to the acid. Protection using NaH and SEM-Cl gave the desired diprotected benzaldehyde (**177**) in 94% yield. The subsequent oxidation conditions using NaClO<sub>2</sub> and sodium phosphate monobasic gave no reaction, but sulfamic acid and NaClO<sub>2</sub> worked well (Scheme 58). The *ortho*-SEM protecting group was found to be labile and was cleaved during column chromatography to **178** (Scheme 54). Initially mono-protection of the *para*-phenol to **179** was not straightforward, but after optimisation it could be accessed in 85% by treating orcinaldehyde (**167**) with potassium carbonate in DMF (Scheme 58).



**Scheme 58.** Synthesis of mono SEM protected orsellinic acid **178**.

It was seen that using an electron poor phosphine in a Mitsunobu reaction gave low conversions (Table 14, entry 1) and reaction with triphenylphosphine gave the desired ester **172** in decent yield with no competing alkylation (Table 15, entry 2). It was later found that conducting the reaction on a gram scale gave better yields; this was probably due to the reduction of adventitious water. It was also observed that this reaction to forming the ester was sensitive and gave irreproducible yields (34% to 72%).

**Table 15** - Mitsunobu reaction of protected orsellinic acid **178**



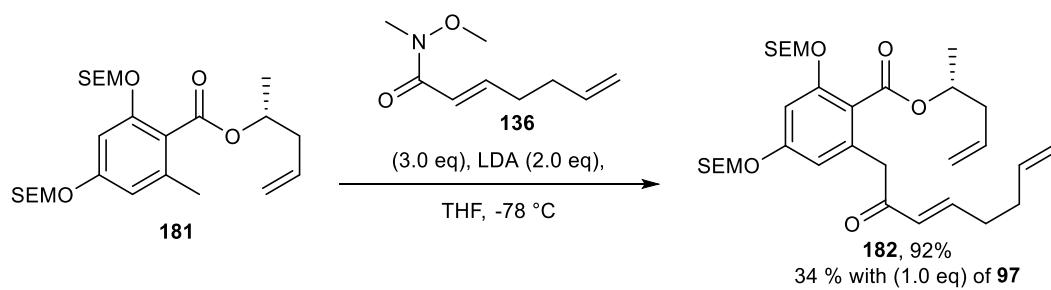
Entry	x	y	z	solvent	Yield
<b>1</b>	P(2-fur) <sub>3</sub>	DIAD	48	benzene	23%
<b>2</b>	PPh <sub>3</sub>	DEAD	18	THF	42%

Prior to the benzylic acylation step, the *ortho*-hydroxy group could then be protected (**181**) with sodium hydride and SEM-Cl in 97% yield. When using greater than one equivalent of SEM-Cl to NaH then extensive decomposition occurred. This is probably due to the presence of HCl generated on protection.

### 3.2.1.4. Installation of the benzyl ketone

Attention turned to acylating the benzylic position on ester **181**. This was achieved by Winssinger by using LDA to form the benzylic anion and treating it with hepta-2,6-diene Weinreb amide (**136**).

Gratifyingly the lateral lithiation protocol on **181** worked to a decent yield (34%) of **182** with no competing side reactions, as was observed by both Winssinger and Danishefsky.<sup>128,139</sup> When LDA was added to the substrate, a deep red colour was observed. At equimolar quantities of both the resorcylic ester **181** and the Weinreb amide **136**, it was noticed that the reaction was not complete, but **136** had been consumed. Increasing the equivalents of Weinreb amide to three gave full conversion of the ester to the desired product in good yield (Scheme 59).

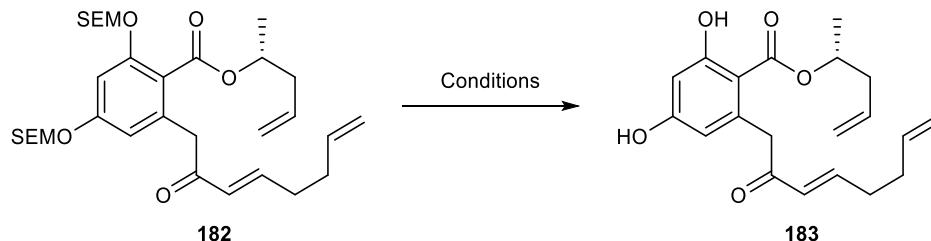


**Scheme 59.** The synthesis of the RCM precursor **182**.

### 3.2.1.5. Deprotection

The phenol ether of **182** needed to be removed to form **183**, it was envisaged that the removal would be straightforward. The various conditions tried for the deprotection of these SEM groups are shown in Table 16.

**Table 16** – Conditions used for the deprotection of **182**



Entry	Deprotection conditions	Conversion
1	MgBr <sub>2</sub> •Et <sub>2</sub> O, rt , 4 h	no reaction
2	MgBr <sub>2</sub> •Et <sub>2</sub> O, 40 °C , 24 h	decomposition
3	TFA, rt, 18 h	no reaction
4	PS-SO <sub>3</sub> H <sup>a</sup> , 18 h	no reaction
5	HCl, 3h	>99% <sup>b</sup>
6	Montmorillonite K10, 18 h	32%
7	TBAF, 1h	decomposition
8	HF•Pyridine, 3h	64%
9	HF•Pyridine, 18h	quantitative (82%)

<sup>a</sup>PS-SO<sub>3</sub>H is polymer supported sulfonic acid

<sup>b</sup>Addition of HCl was also observed, deprotection:addition product 65:45, 52%

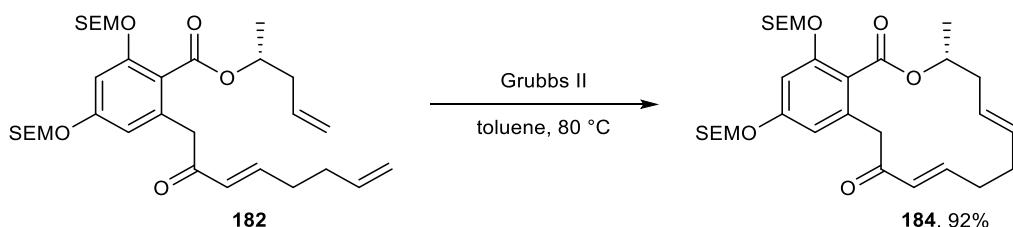
The deprotection of the SEM ethers on acyclic compound **182** using the mild Lewis acid MgBr<sub>2</sub>•EtO<sub>2</sub> as used in the Winssinger synthesis<sup>139</sup> did not yield the desired compound **183**, mild heat and a longer time gave extensive decomposition. Compound

**182** seemed to be more stable to weak Brønsted acids but deprotection with these proved to be sluggish (Table 16, entries 4 and 6). When HCl was used for deprotection, addition of HCl onto the double bond was also seen and separating this from the desired product proved to be difficult (Table 16, entry 5). Fluoride sources can promote desilylative deprotection, using TBAF gave decomposition, but HF•pyridine gave a clean reaction profile and leaving the substrate for longer gave complete conversion to the desired phenol **183** (Table 16, entry 9).

These conditions could also be applied to the deprotection of the macrocycle **184** to monocillin II (**115**) and comparative yields were also obtained (86%).

### 3.2.1.6. Closing the ring

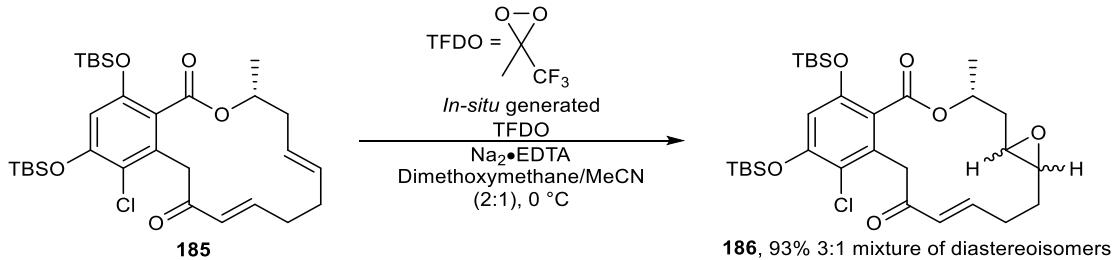
Pleasingly, the ring closing metathesis of the protected RCM precursor (**182**) with Grubbs II gave clean conversion and **184** could be isolated in 92% yield (Scheme 60).



Scheme 60. Grubbs catalysed RCM of **182** to macrocycle **184**.

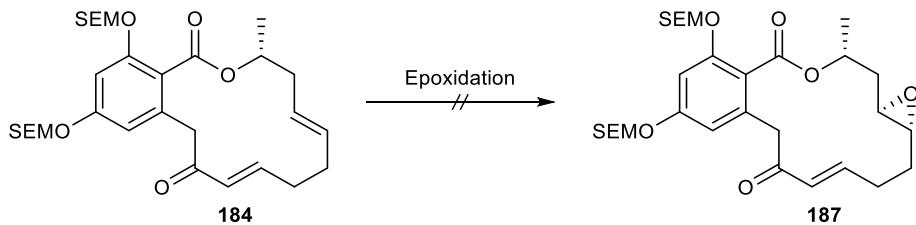
### 3.2.2. Total synthesis of Monocillin III

With monocillin II (**115**) in hand it attention turned to an epoxidation across the double bond of **184** towards monocillin III (**116**). Winssinger was able to selectively form the epoxide using TFDO generated *in situ* from the TBS protected chlorinated macrocycle **185** (Scheme 61). The corresponding SEM protected macrocycle could undergo an epoxidation with TFDO, but no selectivity was observed.



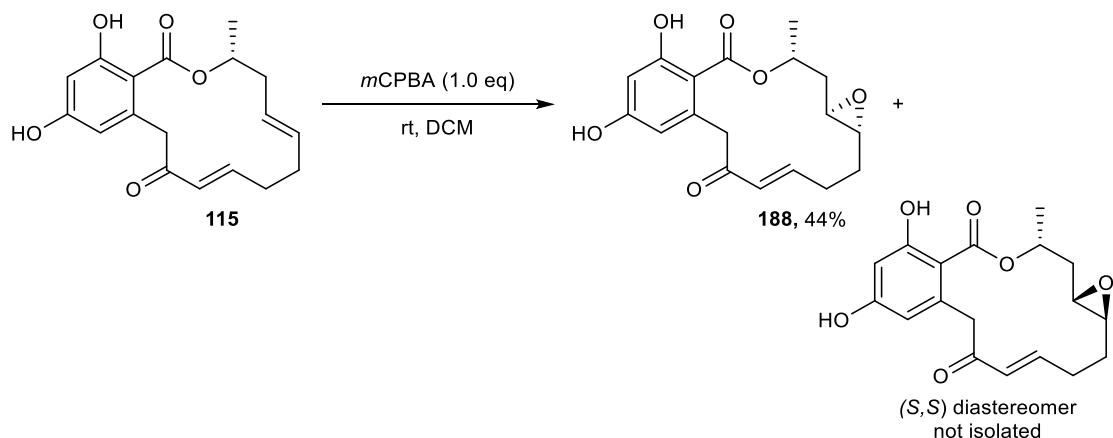
**Scheme 61.** The conditions for the epoxidation of **185** to **186** from the Winssinger synthesis of Pochonin D.

Using *m*CPBA is synthetically more straightforward to use over TFDO and it was stated in the literature that *m*CPBA can be used to form the epoxide of **185**.<sup>139</sup> Treating macrocycle **184** with *m*CPBA at 0 °C gave no reaction. It was then decided that Winssingers conditions should be used; although Winssinger could form the epoxide using *in situ* formed TFDO, it was decided that it would be beneficial to presynthesise TFDO and determine the concentration before adding to the substrate at 0 °C.<sup>139</sup> The product formed in 32% conversion. When treating **144** with 3 equivalents of TFDO decomposition of the compound occurred (Scheme 62).



**Scheme 62.** Attempted epoxidation of **184** to form **187**.

Gratifyingly treating the deprotected macrocycle **76** with *m*CPBA at room temperature gave a 1:1 mixture of diastereomers which could be separated by preparative TLC (Scheme 63). The correct diastereomer was confirmed by comparing the proton NMR with literature data.<sup>119</sup>

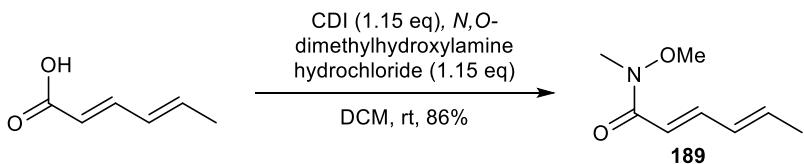


**Scheme 63.** Synthesis of monocillin III (**188**) from monocillin II (**115**)

### 3.2.3. Total synthesis of Monocillin I

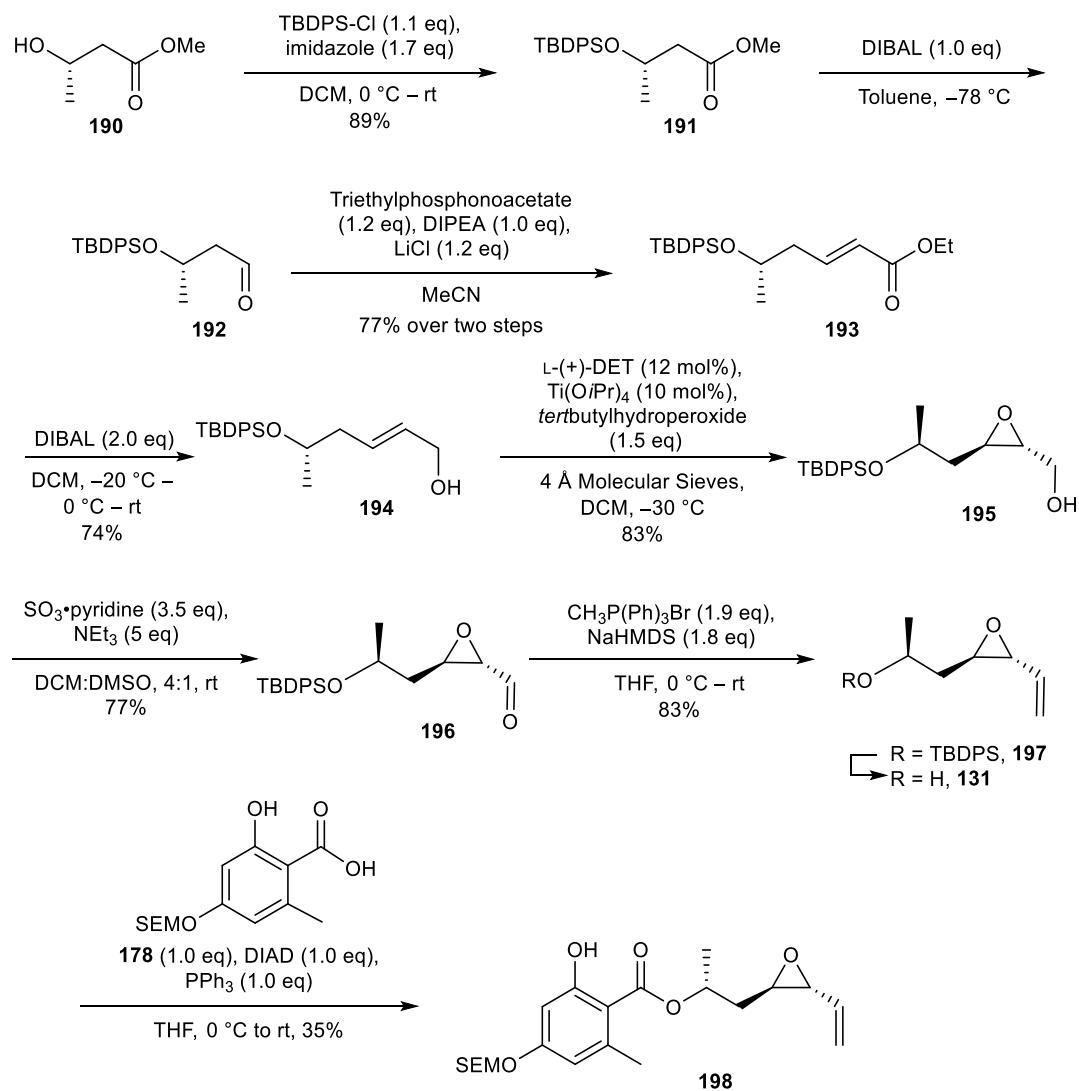
The most complex of the monocillins is monocillin I (**114**). Although Danishefsky<sup>128</sup> had used the orsellinic core containing a benzylic chloride (**135**) and treated it with an acyl anion equivalent (**132**), it was decided that following the approach embarked upon for monocillin II (**115**) would lead a more concise route to monocillin I (**114**).

Weinreb amide **189** could be prepared by an HBTU mediated amide coupling reaction in 86% yield (Scheme 64).



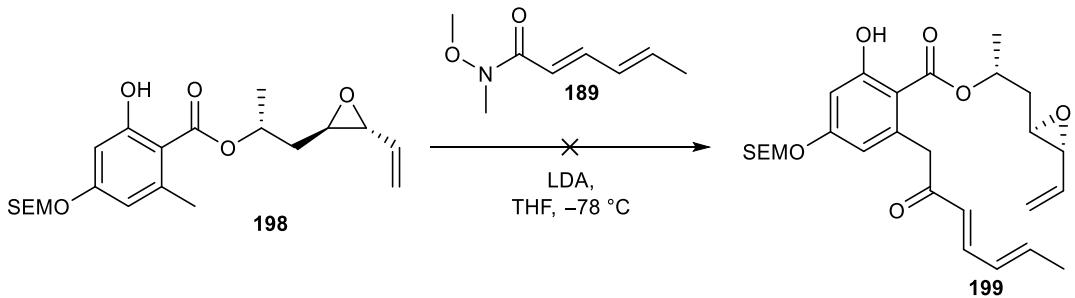
**Scheme 64.** Synthesis of Weinreb amide **189** via a CDI mediated cross coupling.

The epoxide containing alcohol coupling partner **131** for the Mitsunobu esterification reaction was prepared by the route established by Danishefsky (Scheme 65). The hydroxyl-methylbutyrate **190** was taken from the chiral pool for the synthesis of the desired enantiomer. Methyl (*R*)-3-hydroxybutanoate (**150**) was protected using a TBDPS group to **151**, followed by a reduction of the ester **151** to the aldehyde **152** with DIBAL. The allylic alcohol **154** then underwent Sharpless asymmetric epoxidation conditions, with L-(+)-DET to give the desired diastereoisomer (**155**). Parikh-Doering oxidation afforded the desired aldehyde **156** in 77% yield. Wittig methenylation gave **157** followed by fluoride induced silyl deprotection of the TDBPS group led to the desired allylic-epoxy-alcohol **92**. Pleasingly the Mitsunobu reaction gave the desired ester **158** in 35% yield.



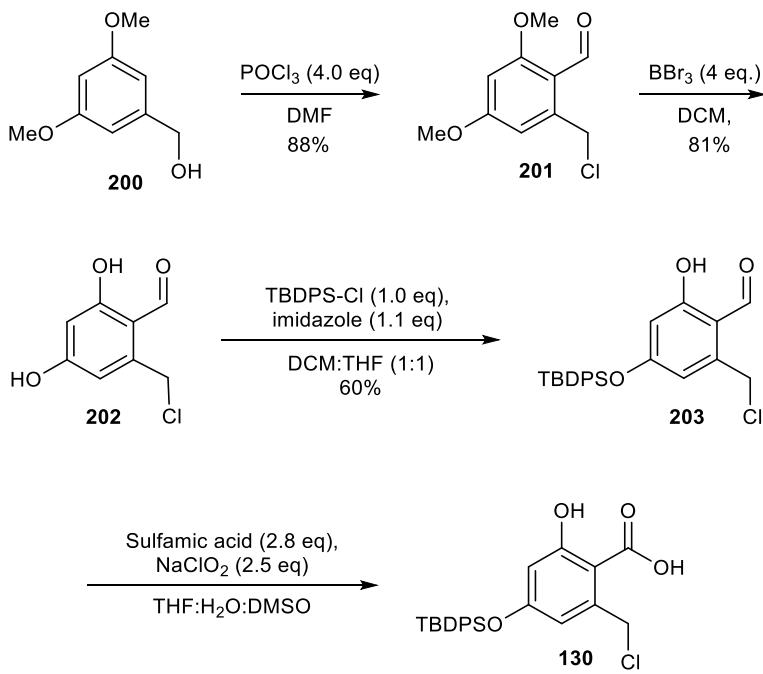
**Scheme 65.** Synthesis of the substrate **198** for the lateral lithiation reaction with **189**.

Next the unprecedented lateral lithiation reaction was attempted on this ester using the conditions optimised earlier with monocillin II. To our dismay, the lateral lithiation was unsuccessful. When **198** was treated with LDA the same deep red colour observed earlier when ester **181** was deprotonated, was also seen in this case (Scheme 66). This indicates that the deprotonation did occur but the steric bulk around the benzylic anion, coupled with the rigidity of the Weinreb amide **189** probably meant that the alkylation was unable to occur. This hypothesis could be validated by quenching the reaction with a smaller electrophile. Remarkably both starting materials **189** and **198** could be recovered.



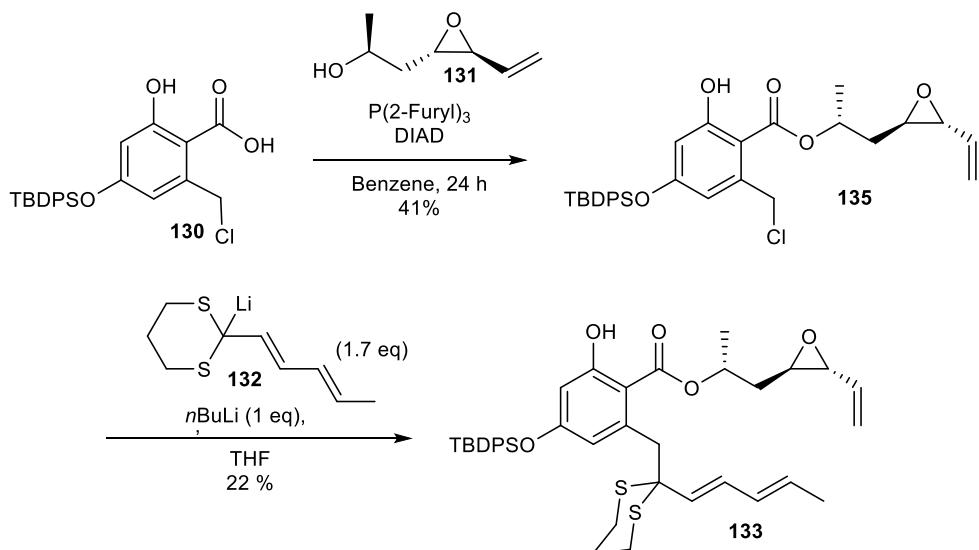
**Scheme 66.** The attempted synthesis of the RCM precursor **199** using the tolyl substrate **198**.

Undeterred by this, the orsellinic core **130** used during the Danishefsky synthesis was then prepared (Scheme 67).<sup>128</sup> The starting material for this was the 3,5-dimethoxybenzyl alcohol (**200**) and treating it with Vilsmeier-Haack reaction conditions gave the aldehyde containing the benzylic chloride (**201**). The dimethoxy aldehyde **201** was then deprotected to **202** using  $\text{BBr}_3$  in DCM in 81% yield. **201** could then be mono protected using TBDPS-Cl and imidazole in a DCM-THF solvent. Pinnick oxidation of **203** afforded benzoic acid **130**.



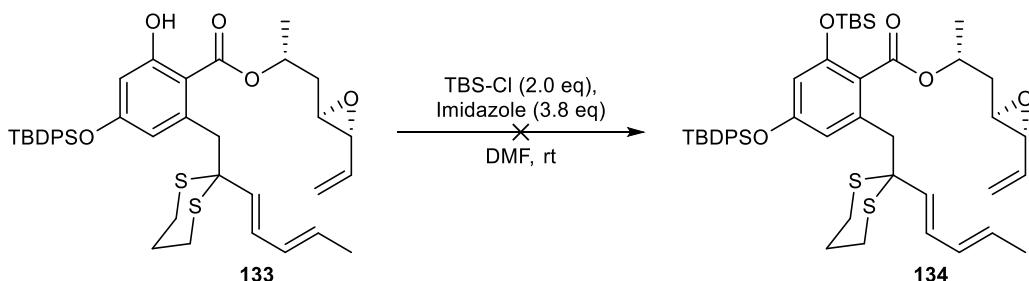
**Scheme 67.** Synthesis of the Mitsunobu precursor **130**.

Mitsunobu using the core **130** worked well and gave 41% yield of **135** (Scheme 68). Pleasingly treating the lithium-phenolate of **135** with lithiated dithiane gave in 63% yield, the  $\gamma$ -alkylation observed by Danishefsky was not observed in this case.<sup>127,128</sup>



**Scheme 68.** The synthetic route to **133** via a Mitsunobu reaction and reaction with acyl anion equivalent **132**.

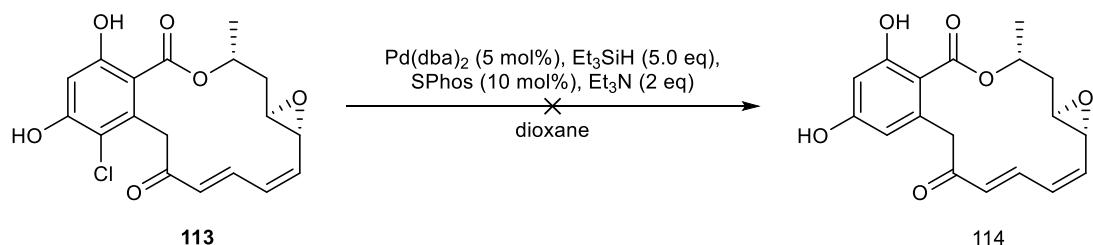
Unfortunately on treating **133** with the conditions described in the literature for the protection of the *ortho*-phenol decomposition occurred (Scheme 65).<sup>128</sup>



**Scheme 69.** The unsuccessful protection of **133** towards the RCM precursor **134**.

### 3.2.1.1. Dehalogenation of radicicol

Radicicol (**113**) is commercially available but is expensive (£108 per five milligrams, Apollo scientific biochemicals). Considering the route towards it was lengthy, it was to be seen if the radicicol could be hydrodehalogenated to obtain monocillin I (**114**, Scheme 70). Subjecting radicicol to Pd(dba)<sub>2</sub>, SPhos, Et<sub>3</sub>SiH and NEt<sub>3</sub> with dioxane, did give dehalogenated product determined by the presence of the two aromatic doublets but mass spectrometry confirmed that the molecule was halogenated elsewhere on the molecule. Further data obtain to elucidate the formed product proved inconclusive.

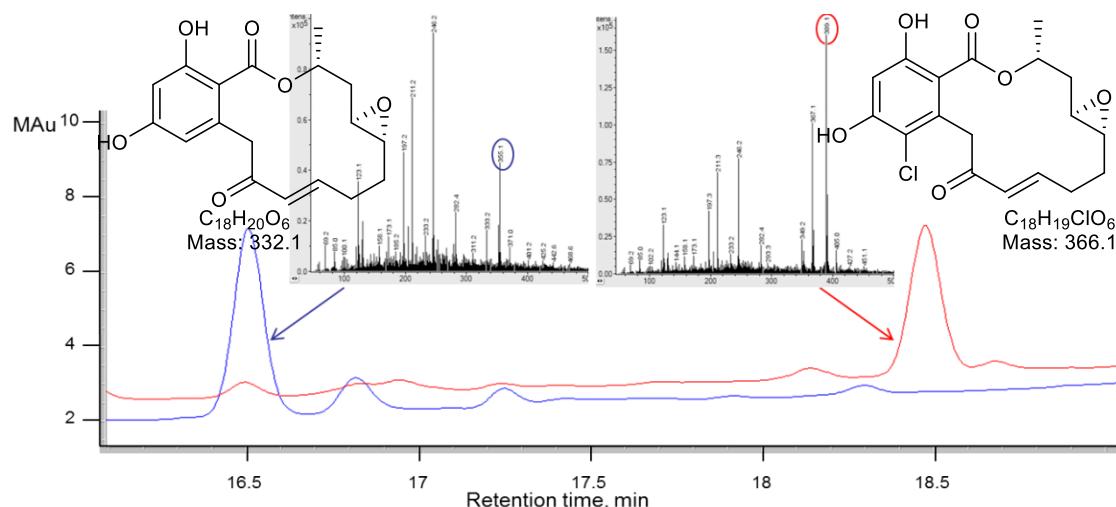


**Scheme 70.** Attempted dehalogenation of radicicol to form monocillin I.

### 3.2.4. Halogenation of Monocillin II and Monocillin III

With the natural products **115** and **116** in hand, they were then subjected to the halogenation reactions with RadH, gratifyingly they were both found to be substrates for the halogenase. Attempts to measure the Michaelis-Menten kinetics study of RadH with monocillin II was attempted by B.R.K.M. but unfortunately was found to have slow kinetics and saturation could not be obtained for the determination of the  $k_{\text{cat}}/K_m$  values.

Due to limited amounts of material and the acute instability of epoxide **116** under the reaction conditions, the kinetics for the epoxide **116** with RadH was not tested. Interestingly it was found to be a quicker substrate than monocillin II (**115**) and the reaction was almost complete (82% conversion) after one hour (Figure 12).



**Figure 12.** The HPLC trace of the chlorination reaction of monocillin II with RadH, the Mass spectra of the peaks have been overlayed. The blue line shows the control reaction without RadH and the red trace is the RadH containing reaction.

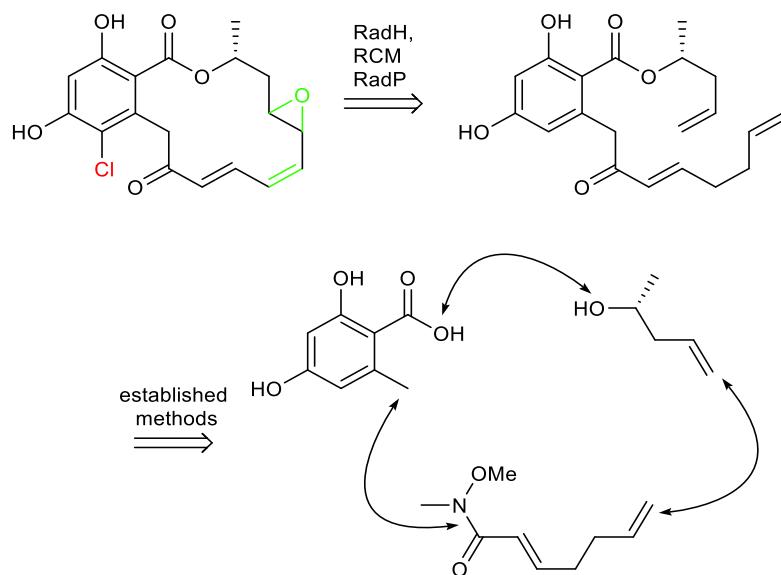
The formation of the product was confirmed by the isotope distribution pattern in LCMS (Figure 12). The unnatural diastereoisomer which was taken from the opposite enantiomer was also tested; this gave only trace halogenated product (<5%). After 18

hours the reaction becomes extremely messy, this is probably due to decomposition of the substrate and/or products.

The deprotected metathesis precursor **143** was also tested with RadH and was seen to be converted quicker than monocillin II (**115**). Furthermore, no dihalogenation was detected when it was incubated with RadH for longer times.

### 3.2.5. RCM and halogenation in one pot

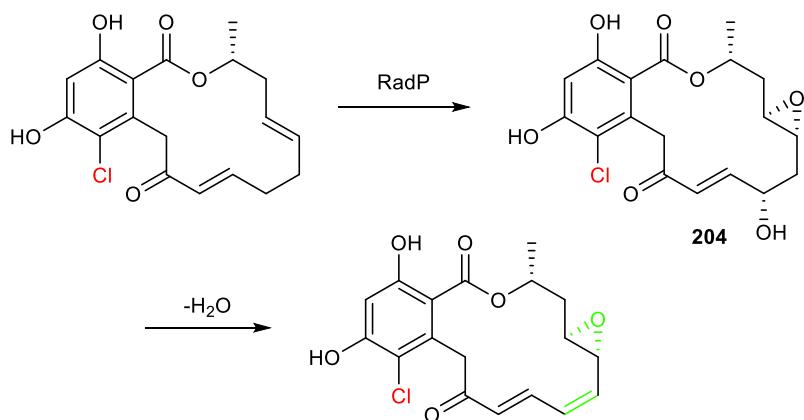
Following the pleasing result with the halogenation of **183** investigations into a RCM-halogenation one pot protocol were commenced. This chemoenzymatic strategy towards the total synthesis of radicicol (Scheme 71) would present an interesting prospect, because even though enzymes have been employed in total synthesis; to date they have not been used as part of an end game strategy for the synthesis of natural products.<sup>141–145</sup>



**Scheme 71.** Retrosynthetic analysis of radicicol using a chemoenzymatic endgame strategy.

### 3.2.6. RadP

As mentioned earlier, RadP is believed to be a cytochrome P450 enzyme responsible for installing the epoxide and the *cis*-double bond. It is also thought that the RadH forms intermediate **204** and a dehydration step yields the *cis* double bond (Scheme 68).

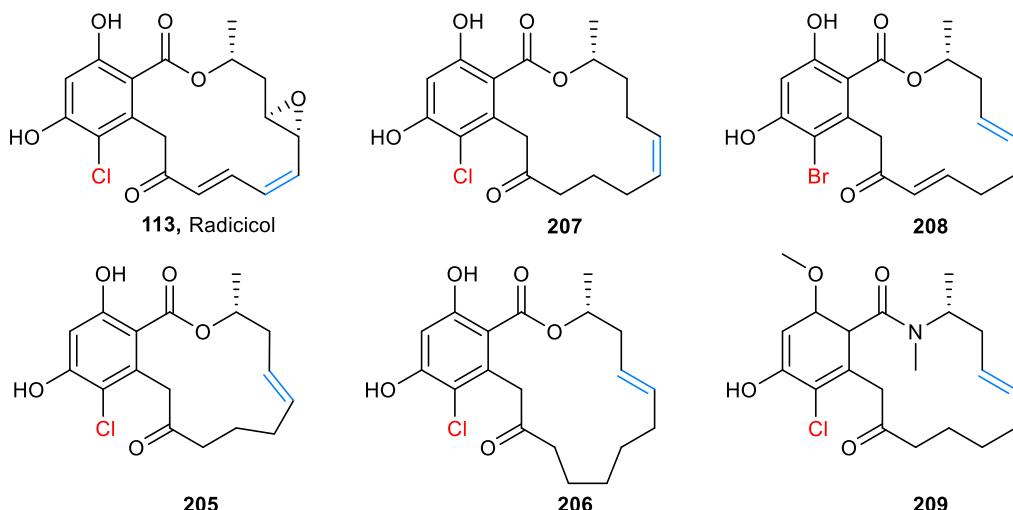


**Scheme 72.** Proposed function of RadP in the radicicol biosynthesis.

To fully furnish the radicicol natural product from monocillin II, RadP would need to be isolated and expressed. Isolation and expression of soluble protein for testing was attempted by B.R.K.M. and regrettably proved futile. This is probably due to the enzyme being a fungal derived P450 and these are widely believed to be difficult to express as they are generally associated to membranes.<sup>146</sup>

### 3.2.7. Synthesis of analogues

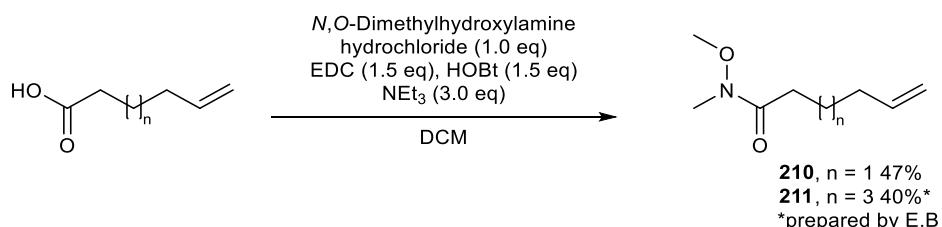
With the halogenation and RCM reaction now optimised (see later section 3.2.8.), efforts then turned to the scope of the total synthesis. The structures shown in Figure 17 looked to be interesting targets to pursue *via* the optimised process. Compound **113** is the natural product Radicicol, and the other structures (**205-209**) in Figure 13 are novel. We believed that the bromide could be prepared from **115** using RadH. Radicicol analogues containing bromide has not been explored before.



**Figure 13.** Target products from RadH.

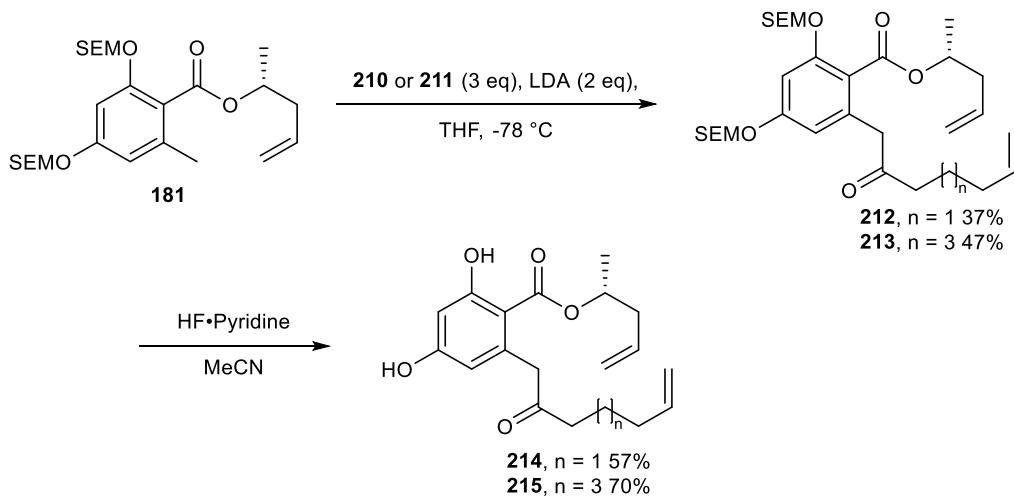
### 3.2.7.1. Ring contracted and ring expanded synthesis

The advantage of the convergent synthetic route developed is that analogues can be accessed expediently by modification of one of the fragments. By altering the length of the Weinreb amide to a carbon shorter **210** or longer **211** the size of macrocycle can be altered (Figure 17, **205** and **206**). The Weinreb amides were synthesised from the corresponding carboxylic acids (Scheme 69) in good yields.



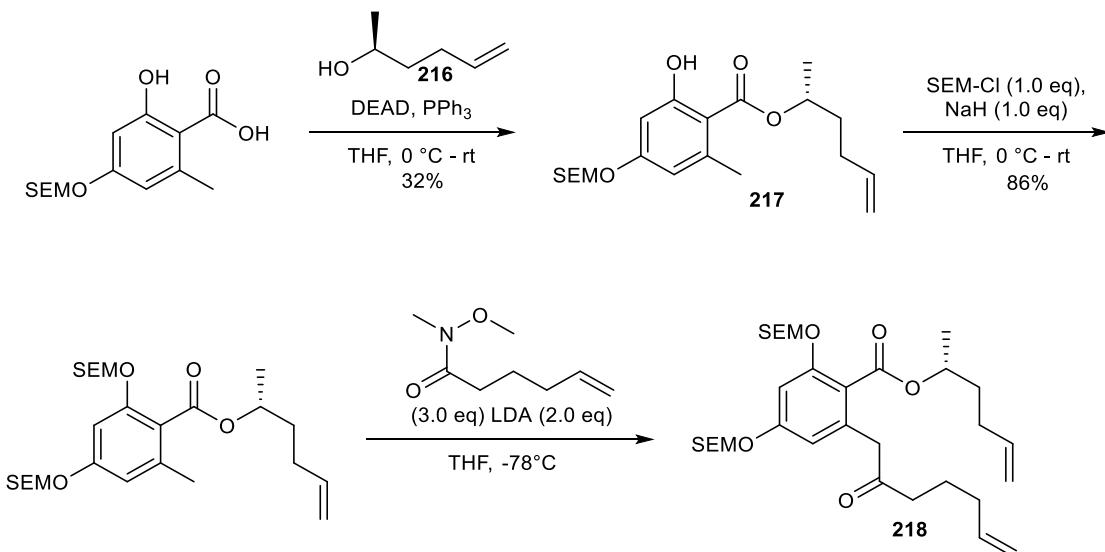
**Scheme 73.** Synthesis of the contracted and extended Weinreb amides **210** and **211**.

These could be attached to **181** in high yields using the same conditions previously optimised. Using these Weinreb amides (**210-211**) in the lateral lithiation step gave the desired acyclic compounds **212** and **213** in good yields (Scheme 74). Deprotection with HF•Pyridine then gave compounds **214** and **215**.



**Scheme 74.** Synthesis towards the homologated (**215**) and dehomologated (**214**) acyclic RCM-metathesis reaction precursors

Another way of achieving a larger macrocycle is by switching the alcohol coupling partner to a longer one (**176**). Mitsunobu esterification with chiral alcohol **176** gave **177** in 48% yield, which was then protected in 96% yield. Formation of a larger macrocycle from **177** was not pursued but instead it decided upon treating it with a 6-carbon chained Weinreb amide (**172**) to form compound **178** (Scheme 70).



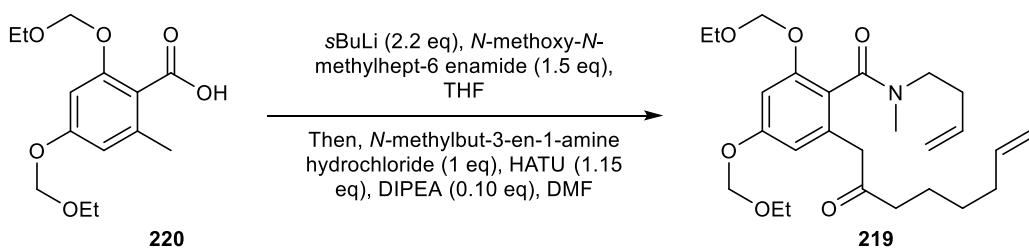
**Scheme 75.** Synthesis of the acyclic precursor **218** using **216** for the synthesis of isomer of radicicol analogue **217**.

Once **218** undergoes RCM and halogenation it would give the same ring size as radicicol, but the double bond would be in a different position (**207**). This small change could have dramatic effect on the conformation of the macrocycle. The synthesis of this substrate has not been completed and only requires two additional steps.

### 3.2.7.2. Radicicol lactam analogue synthesis

In 2010, Moody and coworkers prepared various lactams that were analogous to radicicol in the hope that they would show greater stability *in vivo*.<sup>123,130,132,136</sup> These compounds were found to be indeed more stable than radicicol and had stronger binding to HSP90 than their respective macrolactones. The synthesis of these compounds follow a route reminiscent of Winssinger's<sup>134</sup> and Danishefsky's route<sup>127,128</sup>, on which the synthesis described earlier is based on.

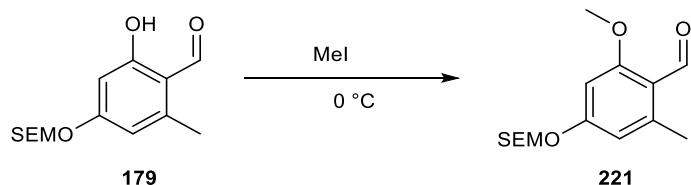
In the paper, it was stated that the desired amide RCM precursor **219**, (Scheme 76) could not be achieved by forming the amide bond before the benzylic acylation of **220**.<sup>132</sup> This is probably due to the steric congestion around the benzylic CH<sub>3</sub>. The lateral acylation reaction was conducted on EMOM protected orsellinic acid, before HATU mediated coupling in a one pot process. The overall yield obtained for the two steps was 33% (Scheme 76).<sup>132</sup>



**Scheme 76.** The reported synthesis of **219** from **220** using a one pot lateral lithiation and amide coupling reaction.

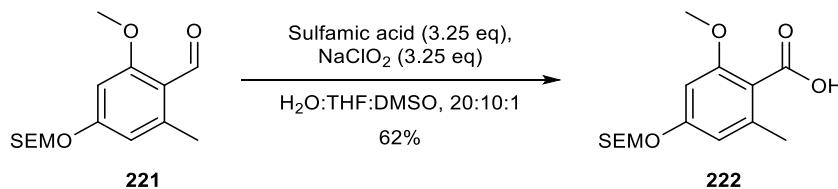
Following the ease of use of the SEM protecting group it was decided that it would continue to be used for the synthesis of this substrate. As the lateral lithiation seemed to be sensitive to bulky *ortho*-protecting groups, it was thought that by methylating at this position, the methyl group would be small enough to not prevent the reaction. The methoxy at this position would give another point of deviation from the literature. Furthermore, it was decided that only the *para*-phenol was necessary for the halogenation reaction with RadH. When treating the mono-SEM protected orcinaldehyde with conditions used earlier to protect this phenol (Table 17, entry 1) only 16% yield was obtained the remaining material was starting material. When switching the solvent to a DMF/THF mixture then **221** was isolated in a higher yield of 68%.

**Table 17 -** *O*-methylation of 4-SEM protected orcinaldehyde **179**



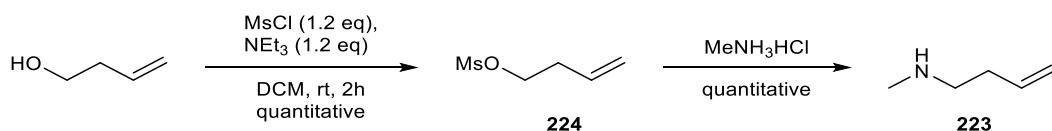
Entry	Methylation conditions	Yield
1	MeI (1.5 eq), NaH (1.2 eq), THF	16%
2	MeI (2 eq), NaH (1.0 eq), DMF:THF (4:1)	68%

This could then be oxidised to **222** using the conditions described earlier for the synthesis of **178**. The yield of **178** was not as high as for this methylated substrate (Scheme 77).



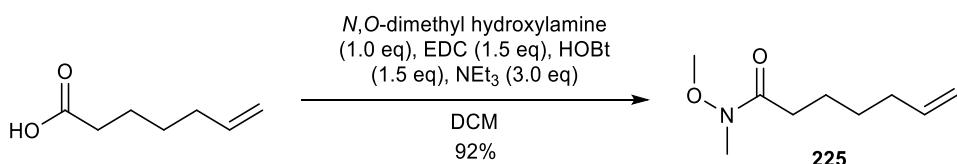
**Scheme 77.** Pinnick oxidation of **211** to **222**.

Efforts began on obtaining the coupling partner **213**; this proved to be more difficult than expected.  $S_N2$  reaction of 4-bromobutene with *N*-methylamine gave the desired product. The difference of the boiling points of 4-bromobutene and *N*-methyl butenamine (**183**) was small and separation of these two compounds by distillation failed. It was thought that by converting the amine **213** to the hydrochloride salt it could be separated from the starting material. Very little of the desired amine was recovered from the acidic extraction. Efforts then turned to obtaining the amine from the mesylated butene **214** (Scheme 78). This seemed to be an easier route and the amine could be obtained without purification however it was also found that this amine was hygroscopic.



**Scheme 78.** Synthesis of **223** via mesylate **224**.

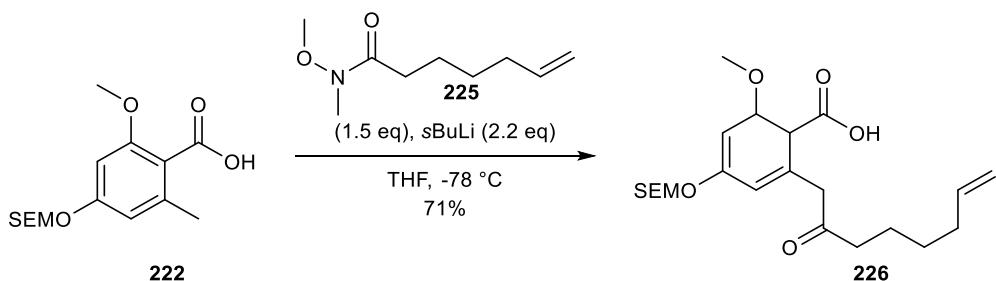
The Weinreb amide **225** could be synthesised in one step from the corresponding carboxylic acid using the methods described earlier (Scheme 79).



**Scheme 79.** Synthesis of **225** using a EDC coupling reaction,

Once the amine and the Weinreb amide coupling partners were obtained, the one pot reaction to the metathesis precursor optimised by Dutton *et al.* was attempted.<sup>132</sup> Unfortunately the reaction gave a complex mixture and so only the first step was tried to investigate if this was the limiting step. Treating the methoxy benzoic acid (**222**) with *s*BuLi, on a 0.6 mmol scale, gave a complex mixture but the desired acylated product (**226**) was isolated with an impurity in 71% yield (Scheme 80). This was then treated with HATU for an amide coupling reaction. Unfortunately this reaction yielded

only small amounts of product and the synthesis could not proceed. Further endeavour would be required to optimise and complete the synthesis of this structure.

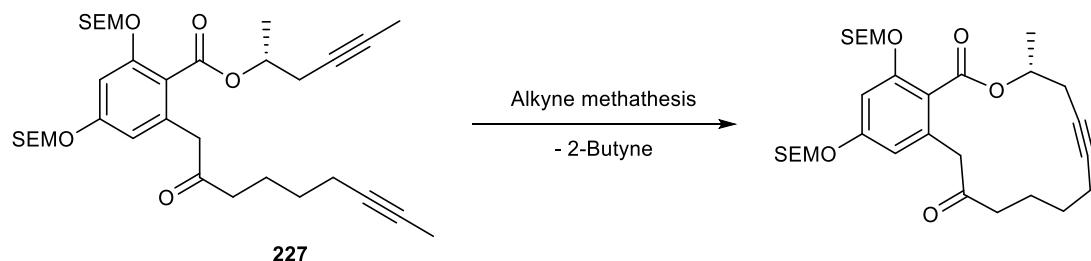


**Scheme 80.** Synthesis of **226** using the conditions described by Moody.<sup>132</sup>

### 3.2.7.3. Ring closing alkyne metathesis substrate

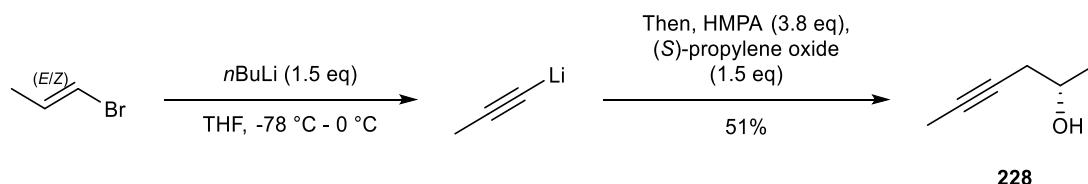
Ring closing alkyne metathesis (RCAM) is a useful way to install an internal alkyne in the ring. It was proposed that the macrocycle would be large enough to accommodate an alkyne within the ring. The chemistry of alkyne metathesis is different to that of metathesis of two alkenes. This is due to a metallocarbyne having different reactivity to a metallocarbene.<sup>147–149</sup> RCAM is not as well developed as RCM and it is for this reason that there are far fewer catalysts available for this reaction and the catalysts that are commercially available, are sensitive to moisture and oxygen. Nevertheless, being able to form the alkyne using the methodology developed would give rise to a novel structure which could be subsequently functionalised and tested for HSP90 activity.

Alkyne metathesis is known to work better with internal alkynes as opposed to terminal ones (Scheme 81). The alkyne metathesis precursor (**227**) was synthesised using alcohol **228** and Weinreb amide **229** from the same methodology described earlier.



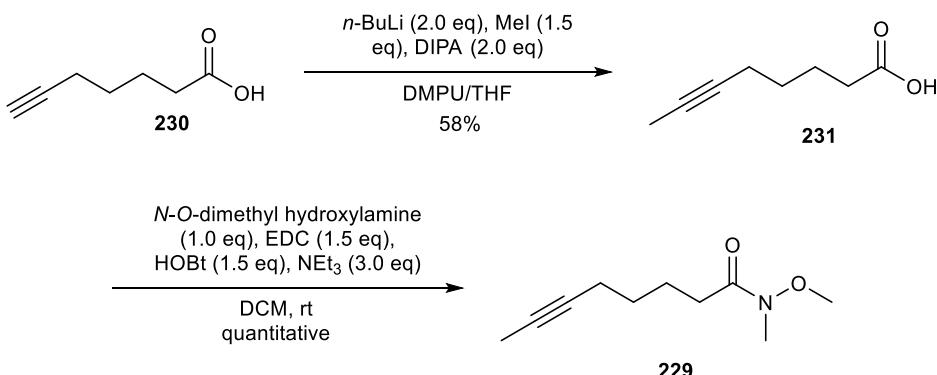
**Scheme 81.** Proposed product from a RCAM reaction of **227**.

The alcohol **228** coupling partner could be synthesised in a yield of 51%, by ring opening of (*S*)-propylene oxide with propynyl lithium (Scheme 82). Propynyl lithium was generated by reaction of *n*BuLi with (*Z/E*)-l-bromopropene at -78 °C.



**Scheme 82.** Synthesis of the chiral alcohol **228**.

The alkyne Weinreb amide bearing an alkyne handle required to make the desired alkyne metathesis substrate was made using EDC coupling reaction of the corresponding acid. The starting carboxylic acid **230** was methylated to **231** on the *sp*-carbon with iodomethane (Scheme 83).



**Scheme 83.** Synthesis of the Weinreb amide **229**.

These two side arms could then be attached onto the orsellinic acid core using the route developed. As there was little precedent for alkyne metathesis on substrates bearing free hydroxyls, the substrate was not deprotected.

Unfortunately due to the time restraints, studies on this substrate did not progress beyond this point.

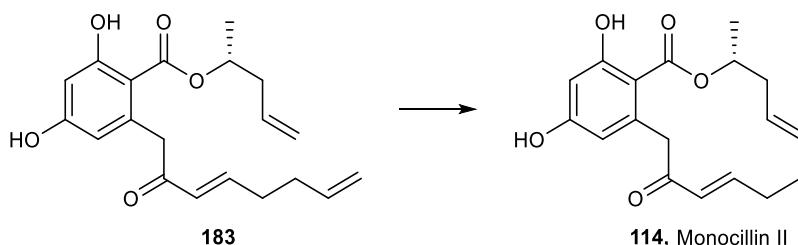
### 3.2.8. A one pot RCM-halogenation towards the synthesis of Pochonin D and related analogues

Encouraged by the relative ease at which the macrocycle formed, it was to be seen if this could be combined with the halogenase reaction. Hartwig had already shown that the Grubbs-Hoveyda catalysts were active in aqueous conditions.<sup>51</sup> Furthermore RCM

reaction for this synthesis was conducted in high dilution conditions (2.0 mM) so the issues with concentrations faced earlier would no longer be a problem.

When lowering the temperature of RCM reaction from 80 °C to 40 °C using the Grubbs II catalyst, no conversion was seen (Table 18, entry 2). But when using Hoveyda-Grubbs II catalyst at 40 °C then complete conversion is observed (Table 18, entry 4). Using Dioxane as the solvent gives less product (Table 18, entry 5). The reaction also works well using a biphasic aqueous toluene media (Table 18, entry 6), however using the iso-octane buffer mixture (Table 18, entry 7) as used in Hartwig's reaction<sup>51</sup> gave only traces of product, this is most likely due to the insolubility of the substrate in this solvent. In neat buffer decomposition of the substrate was observed (Table 18, entry 8).

**Table 18** – Condition screening for the RCM reaction of **183** to **114**.



Entry	Catalyst	Solvent	T/ °C	Conversion <sup>a</sup>
1	Grubbs II	Toluene	80	quantitative
2	Grubbs II	Toluene	40	Trace
3	Grubbs II	DCM	40	n.p.
4	HG II	Toluene	40	quantitative
5	HG II	Dioxane	40	21%
6	HG II	Toluene:Buffer (1:1)	40	quantitative
7	HG II	Isooctane:Buffer (1:1)	40	trace
8	HG II	Buffer	40	decomposition

<sup>a</sup>conversion determined by GC by integration of the starting material and product. reactions conducted on 0.1 mmol scale with 10 mol% metathesis catalyst and 2 mM in solvent and run for 18 hours. HGII = Hoveyda-Grubbs 2<sup>nd</sup> generation catalyst.

The RCM reaction of **183** gave monocillin II (**114**) in quantitative yield even when reducing the temperature from 40 °C to 30 °C (Table 19), the temperature at which RadH is known to be optimal at. Gratifyingly the RCM also tolerated oxygen and

conducting the reaction without prior purging of oxygen with nitrogen gave quantitative conversions.

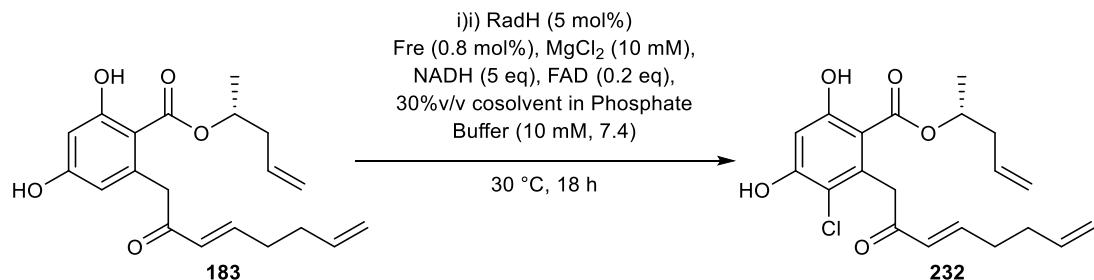
**Table 19** - Optimisation of the RCM of **183** in ambient conditions

Entry	Solvent	Under N <sub>2</sub>	Conversion
1	Toluene: Buffer (1:1)	Yes	quantitative
2	Toluene	No	quantitative
3	Toluene: Buffer (1:1)	No	quantitative

<sup>a</sup>conversion determined by GC by integration of the starting material and product. reactions conducted on 0.1 mmol scale with 10 mol% metathesis catalyst and 2 mM in solvent mentioned.

### 3.2.8.1 Tolerance of RadH to metathesis conditions

The chlorination reaction of **183** with RadH was tested in the presence of solvents and it was found that it was not affected by toluene or isoctane as cosolvent (Scheme 84). Although the reaction tolerated dioxane and DMSO as cosolvent but the conversions were seen to be lower. DCM caused precipitation of the protein and no product conversion was seen.



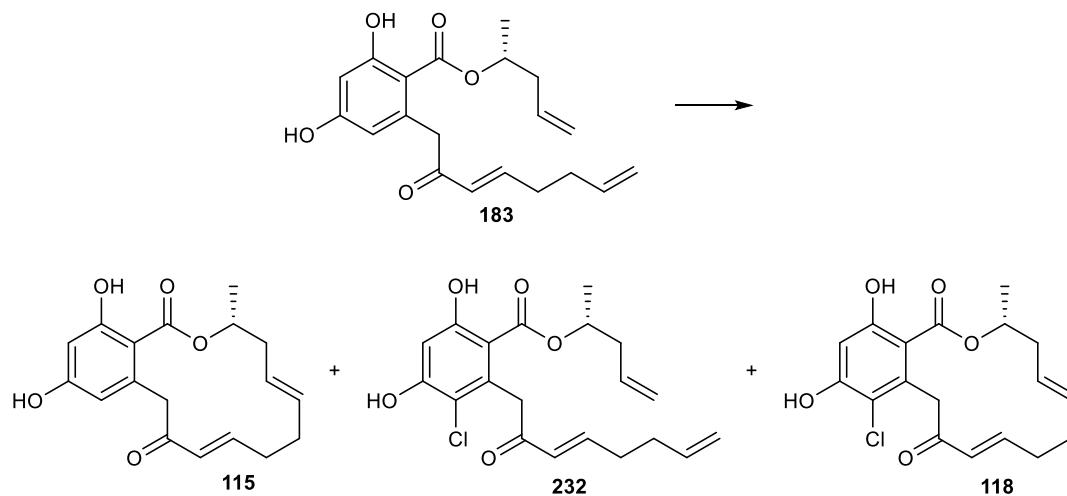
**Scheme 84.** Biohalogenation of **183** with RadH in the presence of 30% v/v solvent.

### 3.2.8.2 Integrated RadH and RCM reaction

The conditions optimised for the RCM (Table 19, entry 3) were then applied to the reaction with RadH (Table 20). The control reaction without the Grubbs-Hoveyda II catalyst showed that RadH could convert the acyclic substrate by 30% (Table 20, entries 1 and 2). The control reaction contained all of the cofactors and enzymes present in the enzymatic reaction but not RadH (Table 20, entry 3): the high conversion in this reaction indicates that the metathesis catalyst was not inhibited by these. Additionally in combination with RadH trace amounts of chlorinated macrocycle was observed (Table 20, entries 4 and 5). From these observations it was hypothesised that the metathesis catalyst had a much faster catalytic turnover than the halogenase and to

get the highest yields it was suggested that it would be necessary to add the catalyst once **183** had been completely converted to **232**.

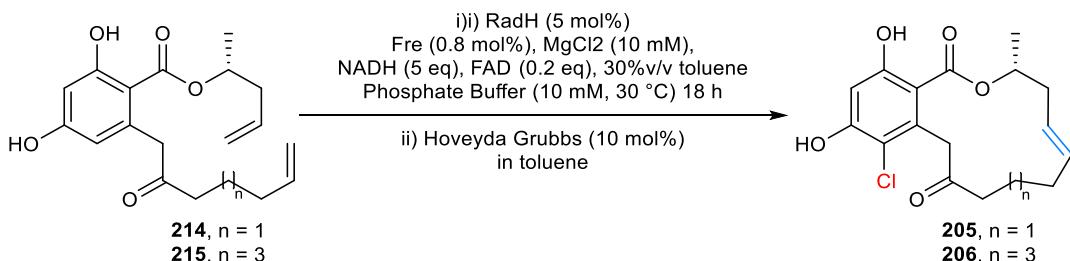
**Table 20** - Attempted one pot reaction with RadH and RCM and the control reactions



Entry	HG II loading	RadH loading	Toluene (v/v %)	HPLC conversion		
				<b>76</b>	<b>191</b>	<b>79</b>
1	-	4 mol%	0	-	~30%	-
2	-	4 mol%	30	-	~30%	-
3	10 mol%	-	30	>99%	-	-
4	20 mol%	4 mol%	30	>90%	nd	trace
5	10 mol%	4 mol%	30	>90%	nd	trace

<sup>a</sup>Apparent conversion determined by HPLC by integration of the starting material and product no calibration curves were prepared, reactions conducted on 200µL scale with 0.5 mM substrate in 100 mM phosphate buffer, pH 7.4.concentration Reaction conditions: RadH (15 µM) Fre (2.5 µM), FAD (1 µM), NADH (2.5 mM), MgCl<sub>2</sub> (10 mM).

Once compounds **214** and **215** were obtained, they were subjected to the optimised one pot two step conditions (Scheme 85). Pleasingly they were found to be both halogenated with RadH and LCMS confirmed that chlorinated **175** underwent the ring closing metathesis but isolation of these compounds has proven difficult.

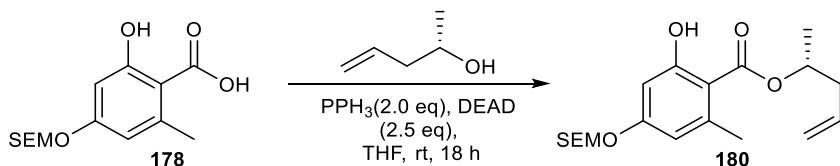


**Scheme 85.** Large scale reaction of **214** and **215** towards **205** to **206** using the RadH-RCM one pot reaction.\* reactions conducted by E.B. on 0.075 mmol scale.

### 3.2.9. Conclusions

Monocillin II (**115**) and monocillin III (**116**) could be synthesised using routes adapted from literature. The natural products **115** and **116** were prepared from the common intermediate **183**. **183** was used to prepare **115**, in good yields, using a ring closing metathesis reaction of **183**.

The metathesis precursor **183** was prepared using a mitsunobu reaction of orselinic acid derivative **178** the chiral intermediate **168**. The weinreb amide **136** was attached by treating it with the benzylic anion.



**Scheme 86.** Mitsunobu Reaction of Orselinic acid **176** and alcohol **168**.

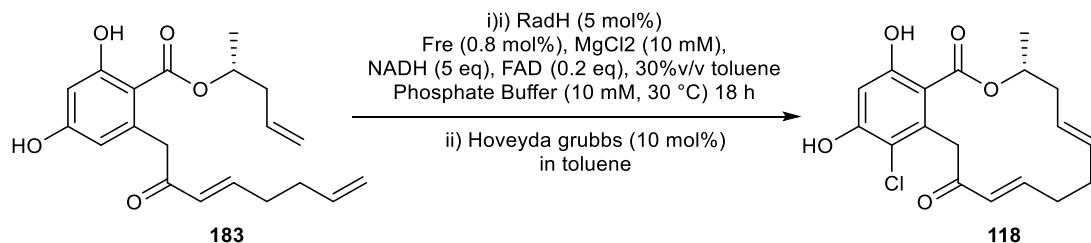
The compounds **115** and **116** prepared were tested with RadH. Unfortunately, no solid evidence could be obtained to confirm the role RadH in nature or its role in the biosynthesis Radiciol and related structures in its compound class. This was due to the instability of these compounds in the reaction conditions.

Monocillin II was found to be a much slower substrate for RadH than the other substrates tested.<sup>150</sup> We hypothesised that this could mean that Monocillin II has a high binding affinity to RadH and dissociation out of the active site is slow. Computational docking studies have been started within our group to probe the binding of monocillin II to RadH.

During the synthesis of these compounds a one pot metathesis halogenation procedure to **118** was optimised from **183**. The RadH catalysed halogenase was not affected by

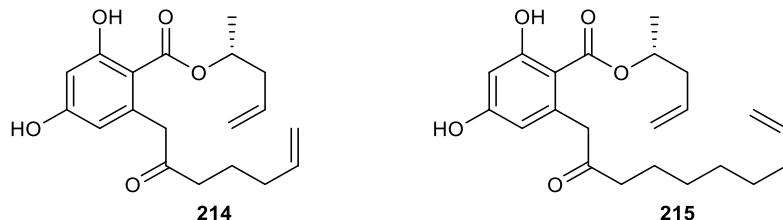
the components of the halogenase, but it was deemed more efficient to simply add the catalyst after halogenation, this is because the Grubbs-Hoveyda catalysed step is quicker than the halogenase and **183** was found to be a better substrate for RadH.

Attempts at isolating **118** from the preparative scale combined RadH-Grubbs Hoveyda reaction have been unsuccessful (Scheme 87).



**Scheme 87.** Large scale reaction of **183** towards **118** using the RadH-RCM one pot reaction.

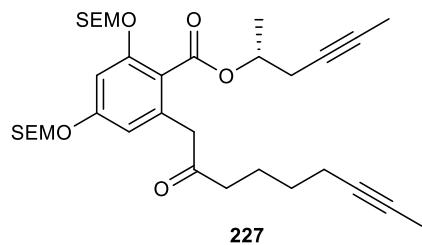
As the synthesis route is divergent a library of compounds can be synthesised from common intermediates. Progress towards the synthesis of compounds as precursors to novel radicicol related analogues has been achieved. Preliminary studies (HPLC and LCMS) have shown that compounds **214** and **215** (Figure 14) have indeed been halogenated by RadH. Furthermore **215** underwent the ring closing metathesis.



**Figure 14.** Compounds **214** and **215**.

These compounds have also been prepared in sufficient quantities for the preparative biohalogenation and RCM reaction. The products have been difficult to isolate from the large-scale reactions in a pure state.

A substrate for alkyne metathesis (**227**), using the optimised route, was also prepared (Figure 15) but studies of the alkyne metathesis could not be started.



**Figure 15.** Precursor for the alkyne metathesis reaction

The lactam analogue **219** has been more difficult to prepare and further optimisation towards this compound is needed. It is hoped that this could also be a substrate for RadH.

We anticipate that once these compounds are isolated from the halogenation reactions they can be evaluated for their medicinal activity against HSP90

## **4. Future perspectives**

Although it has been addressed in part, with this work, compatibility of biocatalysis with chemocatalysis still is an issue. Work within the group is ongoing to demonstrate some of the underexploited potential in combining chemocatalysis with biocatalysis. The use of flow chemistry is an obvious route into finding new reactivities on combining these two systems. Our group is now involved in developing flow reactors for this purpose.

Methods for the development of more robust catalysts are becoming more prominent<sup>7,9</sup>. Advances have been made in the biocatalysis and biochemistry community and as a result a wider range of biocatalysts are becoming more suitable for use in organic synthesis. This is encouraging and may mean that many more examples employing the combination of these two important areas could be found.

## 5. Experimental

### 5.1. Molecular Biology/Biocatalysis procedures

LB and 2YT pre-mix powders were purchased from ForMedium or were prepared by the media service at the Manchester Institute of Biotechnology. All chemicals, molecular biology kits were purchased from New England BioLabs, Qiagen or Sigma-Aldrich unless otherwise stated.

Antibiotics were added where appropriate to the following final concentrations: ampicillin 50 µg mL<sup>-1</sup>, chloramphenicol 50 µg mL<sup>-1</sup>, kanamycin 50 µg mL<sup>-1</sup> and gentamycin 50 µg mL<sup>-1</sup>. Solid media was prepared by addition of agarose (1.5% w/v) to liquid media.

#### 5.1.1. Instruments

Cell lysis was achieved using a Sonopuls HD 2070, Bandelin ultrasound apparatus equipped with an ultrasound probe. The cells were sonicated for 0.5 s on, 0.5 s off for 600 cycles at 70 % power. The protein concentration was determined using A280 setting on a Thermo Scientific Nanodrop 2000 spectrophotometer. The bench-top centrifuges were from Eppendorf, Beckmann or Thermo scientific. Small scale reactions were conducted in Eppendorf shaking incubators with block temperature control.

#### 5.1.2. Buffers and Solutions

*LB media:* The powdered medium (25 g) was dissolved in distilled H<sub>2</sub>O (1 L) and sterilized by autoclaving. The medium was used as provided by the supplier (Formedium,UK), containing tryptone (10 g), yeast extract (5 g), and NaCl (10 g).

*2×YT media:* The powdered medium (31 g) was dissolved in distilled H<sub>2</sub>O (1 L) and sterilized by autoclaving. The medium was used as provided by the supplier (Formedium,UK), Difco Bacto tryptone 16.0 g, Difco Yeast Extract 10.0 g, NaCl 5.0 g, dH<sub>2</sub>O to 1.0 litre (15.0 g Difco Bacto agar).

*TB media:* Tryptone (12 g), yeast extract (24 g), and glycerol (4 mL) was dissolved in distilled H<sub>2</sub>O (900 mL) and sterilised by autoclaving. KH<sub>2</sub>PO<sub>4</sub> (2.31 g) and K<sub>2</sub>HPO<sub>4</sub>

(12.54 g) was dissolved in distilled H<sub>2</sub>O (100 mL) and sterilised by filtration through a sterile filter. The two components were then combined under sterile conditions.

*10× SDS running buffer:* 250 mM Glycine, 25 mM tris base, 0.1% w/v SDS.

*4× loading dye:* provided by Dr Brian Law, 200 mM Tris-Cl (pH 6.8), 400 mM DTT, 8% SDS, 0.4% bromophenol blue, 40% glycerol

*10 % separating gel:* distilled H<sub>2</sub>O (11.5 mL), 40% acrylamide (6mL), tris

*lysis buffer:* 50 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub>, pH 7.2, 500 mM NaCl, 10 mM imidazole, pH adjusted to 7.2 and protease inhibitor tablet 1 per 20 mL.

*wash buffer 1:* 50 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub>, pH 7.2, 500 mM NaCl and 10 mM imidazole. pH adjusted to 7.2 with 1 M HCl.

*wash buffer 2:* 50 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub>, pH 7.2, 500 mM NaCl and 80 mM imidazole. pH adjusted to 7.2 with 1 M HCl.

*elution buffer:* 50 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub>, pH 7.2, 500 mM NaCl and 300 mM imidazole. pH adjusted to 7.2 with 1 M HCl.

### **5.1.3. Transformation of PyrH**

The pET 28a (+) vector containing the PyrH was available in the Micklefield Lab. *E.Coli* ArcticExpress (DE3)RP was used as an expression host for protein production. The DNA construct (1 µL) was added to competent cells (20 µL) and placed on ice for 1 h before heat shock (42 °C, 45 s). LB medium (1 mL) with kanamycin was then added and the cells were incubated for 1 h at 37 °C. The cells were then plated onto LB-agarose plates containing kanamycin. The plate was incubated overnight at 37 °C.

A single transformant was used to inoculate LB medium (10 mL) which was incubated at 37 °C and 180 rpm until an OD<sub>600</sub> = 0.5-0.6 was reached. A glycerol stock was prepared from 750 µL of culture diluted with 750 µL of water/glycerol (50% v/v). This was then flash frozen in liquid nitrogen and stored at -80 °C until needed.

### **5.1.4. Expression and purification of PyrH**

Starter cultures were grown, in LB (10 mL) at 37 °C and 200 rpm overnight, from the glycerol stock prepared as described above. The starter culture (5 mL) was used to inoculate LB (400 mL) supplemented with kanamycin which was incubated at 37 °C

and 180 rpm until an  $OD_{600} = 0.5\text{-}0.6$  was reached. The cells were then cold shocked at 4 °C for 30 min and then induced using IPTG (final concentration 1 mM). The culture was incubated further at 15 °C and 180 rpm overnight. The *E. coli* cells were then harvested by centrifugation (4500 rpm, 20 min, 4 °C). The cells were then resuspended in lysis buffer (60 mL) and sonicated on ice. The lysate was centrifuged twice (10,000 rpm, 30 min, 4°C) and the supernatant was filtered (0.45 µm syringe filter) before being loaded onto Ni-NTA affinity column (Qiagen, 2 mL solid phase). The column was washed with wash buffer 1 (6 mL) and wash buffer 2 (6 mL) before elution of the his-tagged protein with elution buffer (20 mL). The eluted fraction was concentrated using Vivaspin centricon (30,000 MWCO). The protein was diluted with phosphate buffer (100mM KPi, pH 7.2) and then concentrated; this process was repeated three times to remove residual imidazole. The purity was judged by SDS-PAGE analysis and the protein stored at -20 °C until further use.

### **5.1.5. Expression and purification of RadH**

*E.coli* Rosetta (DE3) was used as an expression host for protein production of RadH and a glycerol stock was provided by Jonathan Latham or Binuraj Menon from the Micklefield group.

This glycerol stock was used to inoculate LB (10 mL) supplemented with kanamycin and chloramphenicol which was incubated at 37 °C and 180 rpm overnight. This starter culture (5 mL) was used to inoculate LB (400 mL) containing with kanamycin which was incubated at 37 °C and 180 rpm. Once an  $OD_{600} = 0.5\text{-}0.6$  was achieved, the cells were then induced using IPTG (final concentration 0.1 mM) and left shaking at 18°C for 20 h. The cells were harvested by centrifugation and resuspended in lysis buffer (60 mL). The cells were lysed by sonication on ice and centrifuged (10,000 rpm, 30 min, 4°C). The supernatant was centrifuged again and then filtered (0.45 µm syringe filter). The clarified lysate was loaded onto an Ni-NTA column (Qiagen, 4 mL solid phase) and washed with wash buffer 1 (5 mL) and wash buffer 2 (6 mL). The protein was then eluted using the elution buffer (20 mL) and concentrated in a Vivaspin Centricon (30,000 MWCO). The concentrated protein was washed with phosphate buffer (100mM KPi, pH 7.2) and concentrated again using a Vivaspin Centricon (30,000 MWCO), this cycle was repeated three times. The purity of the protein was judged by SDS-PAGE analysis.

### **5.1.6. Transformation of RebH**

The pET 28a (+) vector containing RebH was available in the Micklefield Lab. *E.Coli* ArcticExpress (DE3)RP was used as an expression host for protein production. The DNA (1 µL) was added to competent cells (20 µL) and placed on ice for 1 h before heat shock (42°C, 45 s). LB medium (1 mL) with kanamycin was then added and the cells were incubated for 1 h at 37 °C. The cells were then plated onto LB-agarose plates containing kanamycin. The plate was then incubated overnight at 37 °C.

### **5.1.7. Expression and purification of RebH**

A single colony of *E. coli* carrying the desired plasmid was then picked from the plate and used to inoculate LB medium (10 mL), containing kanamycin and gentamycin, which was incubated at 37 °C and 180 rpm until an  $OD_{600} = 0.5\text{-}0.6$  was reached. This starter culture (1 mL) was then used to inoculate LB (100 mL) with kanamycin and was incubated at 37 °C and 180 rpm overnight. This subsequent culture (5 mL) was then used to inoculate TB (440 mL) supplemented with kanamycin which was incubated at 37 °C and 180 rpm until an  $OD_{600} = 0.5\text{-}0.6$  was reached. The cells were harvested by centrifugation and resuspended in lysis buffer (120 mL). The cells were sonicated on ice, centrifuged (10,000 rpm, 30 min, 4°C) and the supernatant was then filtered (0.45 µm syringe filter). The clarified lysate was loaded onto a Ni-NTA column (Qiagen, 4 mL solid phase) and washed with wash buffer 1 (5 mL) and wash buffer 2 (6 mL). The protein was then eluted using the elution buffer (20 mL) and was diluted with phosphate buffer (100mM KPi, pH 7.2) and dialysed against phosphate buffer (4 L, 100mM KPi, pH 7.2) overnight before being concentrated using a Vivaspin Centricon (30,000 MWCO). The purity of the protein was judged by SDS-PAGE analysis.

### **5.1.8. Transformation of Fre**

Chemically competent *E. coli* BL21 (DE3) was used as an expression host for the pET 45b (+) vector containing the Fre gene. The plasmid (1 µL) available in the Micklefield Lab, was added to the competent cells (20 µL) and placed on ice for 1 h before heat shock (42°C, 45 s) this was then added to LB (1 mL) with ampicillin. The cells were incubated for 1h at 37 °C before being plated onto LB-agarose plates containing ampicillin. The plate was then incubated overnight at 37 °C.

### **5.1.9. Expression and purification of Fre**

A single colony of *E.coli* carrying the Fre plasmid was picked from the plate and used to inoculate LB (10 mL) supplemented with ampicillin. This was then incubated at 30 °C and 180 rpm overnight, this starter culture was then used to inoculate LB (400 mL) with ampicillin which was incubated at 37 °C and 180 rpm until an  $OD_{600} = 0.5-0.6$  was reached. The cultures were then induced with IPTG (final concentration 1 mM) and incubated further at 30 °C and 180 rpm for 5 h before being harvested by centrifugation. The cells were then resuspended in lysis buffer (40 mL) before being sonicated on ice. The soluble protein was collected by centrifugation (10,000 rpm, 30 min, 4°C) and filtered (0.45 µm syringe filter) before being loaded onto Ni-NTA affinity column (Qiagen, 2 mL solid phase). The column was then washed with wash buffer 1 (5 mL) and then wash buffer 2 (6 mL) before being eluted with elution buffer (20 mL). The eluted protein was concentrated in a Vivaspin Centricon (10,000 MWCO) washed with phosphate buffer (100mM KPi, pH 7.2) and concentrated again in a Vivaspin Centricon. This process was repeated 3 times to remove residual imidazole; the protein purity was then assessed by SDS-PAGE. The protein was stored at -20 °C until further use.

### **5.1.10. Transformation of GDH2**

The pET 21b (+) vector containing the GDH2 was available in the Micklefield Lab and *E.Coli* BL21 (DE3) was used as an expression host for protein production. The plasmid (1 µL) was added to competent cells (20 µL) and placed on ice for 1 h before heat shock (42°C, 45 s). LB (1 mL) with ampicilin was then added and the cells were incubated for 1 h at 37 °C. The cells were then plated onto LB-agarose plates containing ampicillin. The plate was incubated overnight at 37 °C.

A single colony of *E.coli* carrying the desired gene was selected from the plate and used to inoculate LB (10 mL) with ampicillin which was incubated at 37 °C and 180 rpm until an  $OD_{600} = 0.5-0.6$  was reached. A glycerol stock was then prepared by dilution 750 µL of culture with 750 µL of water/glycerol (50% v/v) and then flash freezing in liquid nitrogen before storing at -80 °C until needed.

### **5.1.11. Expression and purification of GDH2**

Starter cultures were grown in LB (10 mL) containing ampicillin from the glycerol stock prepared as described by incubation at 37 °C and 180 rpm overnight. These starter cultures were then used to inoculate LB (400 mL) with ampicillin which were

incubated at 37 °C and 180 rpm until an OD<sub>600</sub> = 0.5-0.6 was noted. The cells were then induced with IPTG (final concentration 1 mM) and incubated at 30 °C and 180 rpm for 4 h. The cells were then harvested by centrifugation, resuspended in lysis buffer (40 mL) and sonicated on ice. The lysate was centrifuged twice (10,000 rpm, 30 min, 4°C) and the supernatant was filtered (0.45 µm syringe filter) before being loaded onto Ni-NTA affinity column (Qiagen, 2 mL solid phase). The column was washed with wash buffer 1 (6 mL) and wash 2 (6 mL) before elution of the his-tagged protein with elution buffer (20 mL). The eluted fraction was concentrated using Vivaspin centricon (10, 000 MWCO). The protein was resuspended in phosphate buffer (10 mL, 100mM KPi, pH 7.2) and then concentrated. The process was repeated 3 times to remove residual imidazole. The purity was judged by SDS-PAGE analysis and the protein was stored at –20 °C until needed.

### **5.1.12. Analytical Scale Biotransformations for testing the activity**

#### ***PyrH***

To a solution containing tryptophan (2.0 mM), NaBr (100 mM), glucose (20 mM), FAD (1.0 µM), PyrH (20 µM), Fre (2.0 µM) and GDH2 (12 µM) in 10 mM potassium phosphate buffer was added NADH (100 µM) to a total volume of 200 µL. After incubation at 20 °C overnight, reactions were boiled 10 min and then centrifuged. The supernatant was then analysed by analytical HPLC method 1.

#### ***RebH***

To a solution containing tryptophan (2.0 mM), NaBr (100 mM), glucose (20 mM), FAD (1.0 µM), PyrH (20 µM), Fre (2.0 µM) and GDH2 (12 µM) in 10 mM potassium phosphate buffer was added NADH (100 µM) to a total volume of 200 µL. After incubation at 20 °C overnight, reactions were boiled 10 min and then centrifuged. The supernatant was then analysed by analytical HPLC method 1.

#### ***RadH***

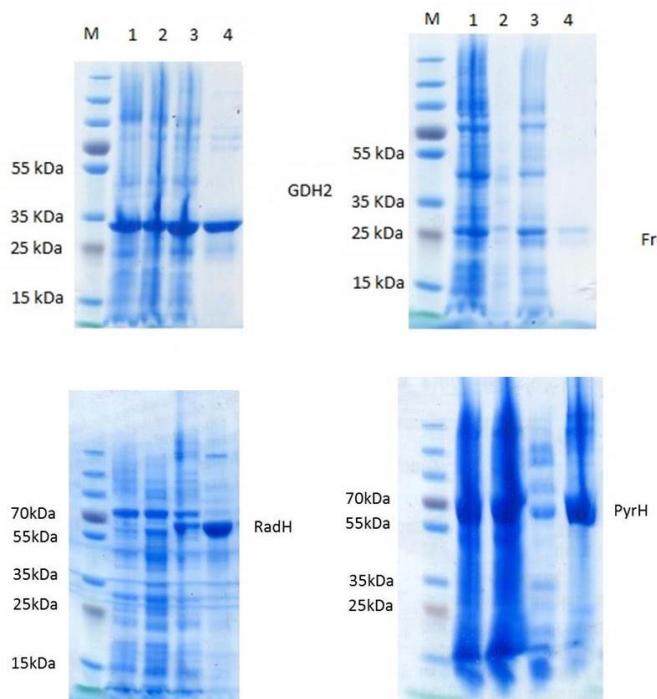
To a solution containing 6-hydroxyisoquinoline (0.50 mM), NaBr (100 mM), FAD (1.0 µM), RadH (25 µM), Fre (4.0 µM) in 10 mM potassium phosphate buffer and 1% ethanol NADH (2.5 mM) was added and the total volumne was made to 200 µL. The resulting solution was incubated at 30 °C overnight. The reactions were stopped

by addition of ethanol and then boiling for 10 minutes. The reactions were centrifuged and the supernatant was analysed by analytical HPLC using method 2

### 5.1.13. Analytical Scale Biotransformations with substrates 76, 183 and 148

Purified RadH (15  $\mu$ M) was incubated at 30 °C with shaking at 800 rpm with Fre (2.5  $\mu$ M), FAD (1  $\mu$ M), MgCl<sub>2</sub> (10 mM) and substrate (0.5 mM) to a total volume of 200  $\mu$ L in 10 mM potassium phosphate buffer (pH 7.4). The reactions were initiated by addition NADH (2.5 mM). The reactions were quenched by boiling at 95 °C for 5 min and precipitated protein was separated by centrifugation and then washed by resuspension in MeCN. The protein was then removed by centrifugation and the supernatant was added to the supernatant after the first centrifugation. The reaction were analysed by HPLC method 3.

### 5.1.14. SDS Page gels



**M** – molecular weight ladder

**1** – lysate

**2** – column flowthrough

**3** – column wash with 80 mmol imidazole

**4** – protein elution with 300 mmol imidazole

### 5.1.15. Analytical HPLC Semi preparative HPLC and LCMS and methods

Method	Conditions
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1	Phenomenex Eclipse Plus® C18 analytical column (3.5 $\mu$ packing, (Analytical 4.6 mm x 100 mm).Mobile phase: 5% MeCN/H <sub>2</sub> O for 2 mins, to HPLC) 95% MeCN/H <sub>2</sub> O over 10 min and held at this gradient for 2 mins, to 5% MeCN/H <sub>2</sub> O over 3 mins and held at this gradients for 2 min.  Flow rate: 1 mL/min. UV absorbance was monitored at 254 nm, 280 nm and 310 nm.
2	Phenomenex Eclipse Plus® C18 analytical column (3.5 $\mu$ packing, (Analytical 4.6 mm x 100 mm).  HPLC) H <sub>2</sub> O with 0.05% TFA, MeCN with 0.05% TFA.  Mobile phase: 5% MeCN/H <sub>2</sub> O for 2 mins, to 95% MeCN/H <sub>2</sub> O over 7 min and held at this gradient for 2 mins, to 5% MeCN/H <sub>2</sub> O over 3 mins and held at this gradients for 2 min. Flow rate: 1 mL/min. UV absorbance was monitored at 254 nm, 280 nm and 325 nm.  Flow rate: 1 mL min <sup>-1</sup> . Column temperature: 30 °C. UV absorbance was detected at 280 nm and 325 nm.
3	Phenomenex Eclipse Plus® C18 analytical column (3.5 $\mu$ packing, (Analytical 4.6 mm x 100 mm).  HPLC) H <sub>2</sub> O with 0.05% TFA, MeCN with 0.05% TFA.  Mobile phase: 5% MeCN/H <sub>2</sub> O for 5 mins, to 45% MeCN/H <sub>2</sub> O over 10 min, to 65% MeCN/H <sub>2</sub> O over 15 mins to 95% MeCN/H <sub>2</sub> O over 5 mins and held at this gradient for 2 min.  Flow rate: 1 mL/min. Column temperature: 30 °C. UV absorbance was monitored at 254 nm, 280 nm and 325 nm.
4	Phenomenex Gemini® semi-preparative C18 HPLC column (5 $\mu$ (Semi- packing, 250 x 10 mm).  preparative H <sub>2</sub> O with 0.05% TFA, MeCN with 0.05% TFA.  HPLC) Mobile Phase: 5% MeCN/H <sub>2</sub> O for 3 min to 35% MeCN/H <sub>2</sub> O over 23 min to 95% MeCN/H <sub>2</sub> O over 3 min then held for 3 min to 5% MeCN/H <sub>2</sub> O over 3 min.  Flow rate: 5 mL/min. UV absorbance was detected at 280 nm and 325 nm.
5	Phenomenex Luna® preparative C18 HPLC column (5 $\mu$ packing, 250 x 21.2 mm).

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(Preparative HPLC)	H <sub>2</sub> O with 0.2% formic Acid, MeCN with 0.2% formic acid. Mobile Phase: 5% MeCN/H <sub>2</sub> O to 40% MeCN/H <sub>2</sub> O over 40 min to 95% MeCN/H <sub>2</sub> O over 10 min to 5% MeCN/H <sub>2</sub> O over 5 min. Flow rate: 15 mL/min. UV absorbance was detected at 254 nm, 275 nm, and 325 nm
6 (LCMS)	Agilent 1200 series fitted with a 3.0 x 20 mm, C18, 3.0 µm column, and mass analysis performed by a single quadrupole Agilent 6100, 183 with ESI ionisation. Standard run conditions were a 4 minute gradient cycle with two solvents, H <sub>2</sub> O with 0.2% formic Acid, and 9:1 Methanol:IPA with 0.2% formic acid.

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## 5.2. General procedures

All reactions were carried out under a nitrogen atmosphere unless otherwise stated. All glassware was kept in an oven at 140 °C overnight and cooled under vacuum unless otherwise stated. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl immediately before use. DCM, toluene, diisopropylamine (DIPA) and triethylamine were distilled over calcium hydride immediately before use. All other solvents used were anhydrous grade purchased from commercial sources, used as received and stored under nitrogen in Sure/Seal™, AcroSeal™ or ChemSeal™ bottles unless otherwise stated. Reagents were either purchased from commercial sources or prepared according to literature procedures. Compositions of solvent mixtures are quoted as percentages of volume. Potassium carbonate was dried by heat under vacuum prior to being weighed. 'ether' refers to diethyl ether and 'Petroleum' refers to a fraction of light petroleum, b.p. 60–80 °C. For all reactions requiring the use of *n*BuLi, the concentration was determined prior to use by titration against *N*-(*o*-tolyl)pivalamide at 0 °C. 'brine' refers to a saturated solution of NaCl in dH<sub>2</sub>O, 'NH<sub>4</sub>Cl' refers to a saturated solution of NH<sub>4</sub>Cl in dH<sub>2</sub>O and 'bicarbonate' refers to a saturated solution of NaHCO<sub>3</sub> in dH<sub>2</sub>O unless otherwise stated.

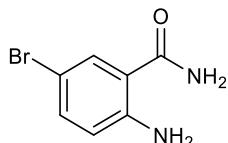
<sup>1</sup>H NMR spectra were recorded on 400, or 500 MHz Bruker NMR spectrometers in CDCl<sub>3</sub>, (CD<sub>3</sub>)<sub>2</sub>CO, CD<sub>3</sub>OD or (CD<sub>3</sub>)<sub>2</sub>SO at 298 K; <sup>13</sup>C NMR spectra were recorded at 101 or 126 MHz. <sup>1</sup>H and <sup>13</sup>C NMR data for the products isolated from the biotransformations were measured on a NMR fitted with a CryoProbe™. Chemical shift values are reported in parts per million (ppm) relative to the solvent signal ((<sup>1</sup>H NMR: δ = 7.26 ppm, <sup>13</sup>C NMR: δ = 77.16 ppm for CDCl<sub>3</sub>; <sup>1</sup>H NMR: δ = 2.05 ppm, <sup>13</sup>C NMR: δ = 98.8 ppm for (CD<sub>3</sub>)<sub>2</sub>CO; <sup>1</sup>H NMR: δ = 3.31 ppm, <sup>13</sup>C NMR: δ = 49.0 ppm for CD<sub>3</sub>OD; <sup>1</sup>H NMR: δ = 2.50 ppm, <sup>13</sup>C NMR: δ = 39.5 ppm for (CD<sub>3</sub>)<sub>2</sub>SO) with coupling constant (*J*) values reported to the nearest 0.1 Hz. The <sup>1</sup>H NMR data is presented as follows: chemical shift (in ppm on the δ scale), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin. = quintet, sept. = septet, m = multiplet, br s = broad singlet and combinations thereof), the coupling constant (*J*, in Hertz, Hz), integration and assignment. The <sup>13</sup>C NMR data is recorded as the ppm on the δ scale. For overlapping signals a range of shifts is reported. NMR spectra were assigned with the aid of 2-D correlation and DEPT-135 spectra where appropriate.

Low resolution and high resolution mass spectra were obtained from the University of Manchester, mass spectrometry service using either positive and/or negative electrospray ionisation (ESI), electron impact ionisation (EI) or chemical ionisation (CI) techniques. High resolution mass spectrometry was performed on a Waters QTOF with ESI/APCI ionisation and a Thermo Finnigan MAT95XP (EI). Melting points were measured on a variable heater apparatus and are uncorrected. IR spectra were recorded on an ATR FTIR spectrometer as evaporated films or neat and wavelengths of maximum absorbance ( $\nu_{\text{max}}$ ) are quoted in wave numbers ( $\text{cm}^{-1}$ ). Optical rotations were measured on a Rudolph Research Analytical Autopol I Automatic Polarimeter.

Reactions were monitored by TLC: Machery-Nagel, TLC plates Alugram® Sil G/UV254. Plates were visualised using 254 nm ultraviolet light and/or dipped in aqueous basic potassium permanganate, *p*-anisaldehyde or phosphomolybdic acid. The compounds were purified by flash chromatography using silica gel (Sigma Aldrich, 40-60  $\mu\text{m}$ , 60  $\text{\AA}$ ) under positive pressure.

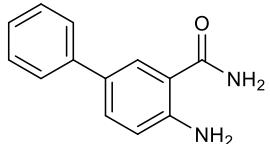
### 5.3. Experimental for Chapter 2

#### 95, 2-amino-5-bromobenzamide<sup>151</sup>



Bromine (0.52 mL, 10 mmol) was added dropwise over 30 min to a stirred suspension of 2-aminobenzamide (1.36 g, 10 mmol) in chloroform at 0 °C. The solution was warmed to room temperature and stirred for a further 1 hour. The solid was filtered and washed twice with water. Column chromatography (40% EtOAc in hexanes) yielded the product **2** as white crystals (1.41 g, 6.6 mmol, 66%). Data is consistent with literature values.<sup>151</sup> **1H NMR** (500 MHz, Dimethyl sulfoxide-*d*<sub>6</sub>) δ 7.68 (d, *J* = 2.3 Hz, 1H, Ar*H*), 7.29 (dd, *J* = 8.8, 2.3 Hz, 1 H, Ar*H*), 6.70 (d, *J* = 8.8, Ar*H*); **13C NMR** (101 MHz, Dimethyl sulfoxide-*d*<sub>6</sub>) δ 170.0 (C=O), 149.4 (Ar*C*<sub>q</sub>), 134.3 (CH<sub>Ar</sub>), 130.8 (CH<sub>Ar</sub>), 118.5 (CH<sub>Ar</sub>), 115.2 (Ar*C*<sub>q</sub>), 104.7 (Ar*C*<sub>q</sub>); **LRMS:** (ESI+) *m/z*: 215.2, (100%, M + H), 217.2, (100, M + H).

#### 96, 4-amino-[1,1'-biphenyl]-3-carboxamide<sup>152</sup>

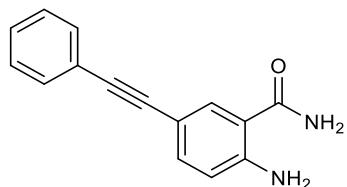


A microwave tube containing 2-amino-5-bromobenzamide (4.30 mg, 0.020 mmol), phenylboronic acid (2.92 mg, 0.024 mmol) and potassium carbonate (5.50 mg, 0.040 mmol) was evacuated and back-filled with N<sub>2</sub> three times before addition of phosphate buffer (10 mL, 10 mM, pH 7.2), sodium tetrachloropalladate(II) (0.15 mg, 0.50 μmol) in water (10 μL) and TPPTS (0.71 mg, 1.3 μmol) in water (10 μL). The vial was then sealed and the reaction was heated for 14 hours at 50 °C. Column chromatography then afforded the desired compound as a white solid (3.8 mg, 89%). Data is consistent with literature values. **1H NMR** (500 MHz, Chloroform-*d*) δ 8.38 (d, *J* = 2.4 Hz, 1H, Ar*H*), 7.67-7.70 (m, 2H, Ar*H*), 7.64 (dd, *J* = 8.6, 2.4, 1H, Ar*H*), 7.47-7.54 (m, 3H, Ar*H*), 7.02 (d, *J* = 8.6 Hz, 1H, Ar*H*), 6.78 (s, 1H, Ar*H*); **13C NMR** (101 MHz, Methanol-*d*<sub>4</sub>) δ 174.6 (C=O), 150.5 (Ar*C*<sub>q</sub>), 141.9 (CH<sub>Ar</sub>), 132.2 (CH<sub>Ar</sub>), 130.3 (Ar*C*<sub>q</sub>),

129.7 ( $\text{CH}_{\text{Ar}}$ ), 128.0 ( $\text{ArC}_q$ ), 127.3 ( $\text{CH}_{\text{Ar}}$ ), 127.1 ( $\text{CH}_{\text{Ar}}$ ), 118.8 ( $\text{CH}_{\text{Ar}}$ ), 116.0 ( $\text{ArC}_q$ );

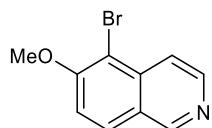
**LRMS:** (ESI+)  $m/z$  212.8 (100%, M + H).

### 97, 2-amino-5-(phenylethyynyl)benzamide



A microwave tube containing 2-amino-5-bromobenzamide (24.1 mg, 0.10 mmol) and  $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$  (7.00 mg, 0.01 mmol) and copper(I) iodide (1.90 mg, 0.01 mmol) was evacuated and back-filled with  $\text{N}_2$  three times before addition of diethylamine (5.0 mL) and phenyl acetylene (16.5  $\mu\text{L}$ , 0.71 mg, 0.10 mmol). The vial was sealed and the reaction was then heated at 50 °C for 14 hours. Column chromatography (20% EtOAc in hexanes to 40% EtOAc in hexanes) then afforded the desired compound as a yellow solid (18 mg, 0.076 mmol, 76%). Data is consistent with literature values.  **$^1\text{H NMR}$**  (400 MHz, Chloroform-*d*)  $\delta$  7.58 (d, I 1.5 Hz, 1H, Ar*H*), 7.49 (dd, *J* = 7.8, 1.7, 1H, Ar*H*), 7.39 (dd, *J* = 8.6, 1.7, 1H, Ar*H*), 7.26-7.36 (m, 3H, Ar*H*), 6.65 (d, *J* = 8.6 Hz, 1H, Ar*H*), 5.94 (br.s, 2H, ArCONH<sub>2</sub>), 5.75 (br.s, 2H, ArNH<sub>2</sub>);  **$^{13}\text{C NMR}$**  (101 MHz, Chloroform-*d*)  $\delta$  170.9 (C=O), 149.6 (Ar*C*<sub>q</sub>), 136.2 ( $\text{CH}_{\text{Ar}}$ ), 136.2 ( $\text{CH}_{\text{Ar}}$ ), 131.8 ( $\text{CH}_{\text{Ar}}$ ), 131.5 ( $\text{CH}_{\text{Ar}}$ ), 128.5 ( $\text{CH}_{\text{Ar}}$ ), 128.1 ( $\text{CH}_{\text{Ar}}$ ), 123.6 (Ar*C*<sub>q</sub>), 117.5 (Ar*C*<sub>q</sub>), 113.6 (Ar*C*<sub>q</sub>), 110.8 (Ar*C*<sub>q</sub>), 89.3 (C≡C), 87.8 (C≡C); **LRMS:** (ESI+)  $m/z$  237.3 (100%, M + H).

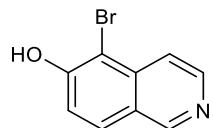
### 5-bromo-6-methoxyisoquinoline



6-methoxyisoquinoline (540 mg, 3.43 mmol) was added to conc.  $\text{H}_2\text{SO}_4$  (3.5 mL) at –5 °C. Once addition was complete the reaction was cooled to –25 °C and *N*-bromosuccinimide (785 mg, 4.41 mmol) was added portionwise whilst carefully maintaining the temperature at –22 °C. After complete addition, the reaction was stirred for a further 4 hours between -22 °C to –18 °C. The reaction was poured onto ice water and 10 % ammonium hydroxide was added until a pH of 9 was reached. The

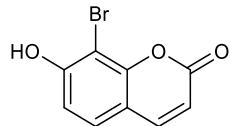
green slurry was extracted with ether three times and then washed with 1 M NaOH aq. then twice with water dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated. Column chromatography gave the bromide as a yellow solid (548 mg, 2.3 mmol, 67%). Data is consistent with literature values.  **$^1\text{H NMR}$**  (400 MHz, Chloroform-*d*)  $\delta$  9.13 (s, 1H, ArH), 8.56 (d,  $J = 6.0$  Hz, 1H, ArH), 8.00 – 7.93 (m, 2H, ArH), 7.38 (d,  $J = 8.9$  Hz, 1H, ArH), 4.09 (s, 3H, OMe);  **$^{13}\text{C NMR}$**  (101 MHz, Chloroform-*d*)  $\delta$  157.0 (ArC<sub>q</sub>), 152.4 (CH<sub>Ar</sub>), 144.7 (CH<sub>Ar</sub>), 136.5 (CH<sub>Ar</sub>), 129.3 (CH<sub>Ar</sub>), 125.3 (ArC<sub>q</sub>), 118.8 (CH<sub>Ar</sub>), 114.5 (CH<sub>Ar</sub>), 107.0 (ArCq), 57.1 (OMe).

### 5-bromoisoquinolin-6-ol



A solution of 5-bromo-6-methoxyisoquinoline (536 mg, 2.3 mmol) in DCM (18 mL) was cooled to 0 °C. Boron tribromide (1M in hexanes, 18 mL, 18 mmol) was added dropwise before being allowed to be warmed to room temperature over a period of 2 hours. The resultant solution was then warmed up to reflux for 70 hours. The reaction was quenched with dropwise addition of methanol at 0 °C and after evaporation of the volatiles the solid was filtered through a plug of silica and washed with (9: 1, DCM: methanol) to give a cream solid (497 mg, 2.2 mmol, 99%). Data is consistent with literature values.  **$^1\text{H NMR}$**  (400 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  9.56 (s, 1H, ArH), 8.49 – 8.53 (m, 2H, ArH), 8.41 (d,  $J = 8.8$  Hz, 1H, ArH), 7.66 (d,  $J = 8.8$  Hz, 1H, ArH);  **$^{13}\text{C NMR}$**  (100 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  163.9 (ArCOH), 146.9 (CH<sub>Ar</sub>), 141.7 (ArC<sub>q</sub>), 133.7 (CH<sub>Ar</sub>), 133.4 (CH<sub>Ar</sub>), 124.3 (ArC<sub>q</sub>), 124.0 (CH<sub>Ar</sub>), 123.4 (CH<sub>Ar</sub>), 105.4 (ArC<sub>q</sub>).

### 8-bromo-7-hydroxy-2*H*-chromen-2-one<sup>153</sup>



This product was made using one of the two methods;

To a solution of 7-hydroxycoumarin (810 mg, 5.0 mmol) in acetonitrile (20 mL), 2,4,4,6-Tetrabromo-2,5-cyclohexadienone (2.2g, 5.5 mmol) was added portionwise and the resulting solution was heated at reflux for 3 hours. The volatiles were removed

and the crude mixture was loaded onto column. Column chromatography (40% - 60% Et<sub>2</sub>O in hexanes) yielded the desired product as a white solid (62.0 mg, 0.31 mmol, 5%).

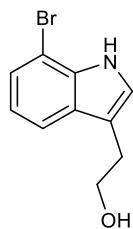
To a solution of 2-bromoresorcinol (1.13g, 6.0 mmol) in conc. sulphuric acid (3.5 mL), propiolic acid (1.47 mL, 1.68 g, 24 mmol) was added. The reaction was heated to 100 °C for 30 mins. Once the reaction was cooled to room temperature, it was quenched with ice water (20 mL) and extracted into ethyl acetate. Column chromatography (40% - 60% Et<sub>2</sub>O in hexanes) yielded the product as a white solid (75.2 mg, 9 %). Data is consistent with literature values.<sup>153</sup> **1H NMR** (400 MHz, Methanol-*d*<sub>4</sub>) δ 9.84 (d, *J* = 9.8 Hz, 1H, pyranone*H*), 7.45 (d, *J* = 8.6 Hz, 1H, Ar*H*), 6.91 (d, *J* = 8.6 Hz, 1H, Ar*H*), 6.24 (d, *J* = 9.4 Hz, 1H, pyranone*H*); **13C NMR** (100 MHz, Dimethyl sulfoxide-*d*<sub>6</sub>) δ 159.7 (C=O), 158.0 (ArC<sub>q</sub>), 152.0 (ArC<sub>q</sub>), 144.4 (CH<sub>Ar</sub>), 128.1 (CH<sub>Ar</sub>), 112.6 (CH<sub>Ar</sub>), 112.1 (CH<sub>Ar</sub>) 111.7 (ArC<sub>q</sub>), 111.7 (ArC<sub>q</sub>); **LRMS:** (ESI-) *m/z* 238.9 (100%, M + H), 241.0 (100%, M + H); **HRMS:** (ESI-) C<sub>9</sub>H<sub>4</sub>O<sub>3</sub>Br calculated 238.9344 found 238.9345.

### 7-bromo-1*H*-indole<sup>154</sup>



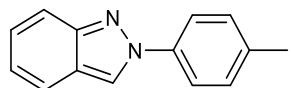
To a solution of 1-bromo-2-nitrobenzene (6.00 g, 28 mmol) in THF (180 mL) at -45 °C, vinyl magnesium bromide (1 M in THF, 90 mL, 90 mmol) was added dropwise over a duration of 30 minutes and the reaction was stirred this temperature for a further 30 minutes. The reaction was warmed to room temperature and quenched with NH<sub>4</sub>Cl and extracted with ether three times. The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent removed *in vacuo*. Column chromatography (10% DCM in hexanes) afforded the title compound as a cream solid (2.81 g, 14 mmol, 52%). Data is consistent with literature values.<sup>154</sup> **1H NMR** (400 MHz, Chloroform-*d*) δ 8.14 (s, 1H, NH), 7.48 (d, 1H, *J* = 7.9 Hz, Ar*H*), 7.25 (d, 1H, *J* = 7.6 Hz, Ar*H*), 7.05 (m, 1H, Ar*H*), 6.90 (t, 1H, *J* = 7.7 Hz, Ar*H*), 6.40 – 6.56 (m, 1H, Ar*H*); **13C NMR** (100 MHz, Chloroform-*d*) δ 134.6 (ArC<sub>q</sub>), 129.0 (ArC<sub>q</sub>), 124.9 (CH<sub>Ar</sub>), 124.4 (CH<sub>Ar</sub>), 121.1 (CH<sub>Ar</sub>), 120.0 (CH<sub>Ar</sub>), 104.7 (ArC<sub>q</sub>) 103.9 (CH<sub>Ar</sub>); **LRMS:** (ESI+) *m/z* (100%, M + H).

**105, 2-(7-bromo-1*H*-indol-3-yl)ethan-1-ol<sup>155</sup>**



Oxalyl chloride (3.0 mL, 36 mmol) was added dropwise to a solution of 7-bromoindole (2.60 g, 13 mmol) in THF (60 mL) at 0 °C and the resulting solution was allowed to stir for 10 hours at room temperature. The volatiles were removed under reduced pressure before anhydrous ethanol (40 mL) was added and the solution allowed to stir at room temperature overnight. EtOAc was added and the resulting solution was separated with brine. The combined organic phases were dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The solid containing mainly glyoxylate (590 mg, 2.0 mmol) was dissolved in THF (42 mL) and Lithium aluminium hydride (500 mg, 15 mmol) was added portionwise. The reaction was heated at reflux for 4 hours before dilution with DCM. The reaction was washed with brine dried over MgSO<sub>4</sub> and filtered before being concentrated *in vacuo*. Column chromatography (20-50% EtOAc in hexanes) afforded alcohol **105** as a yellow oil (339 mg, 1.4 mmol, 71%). Data is consistent with literature values.<sup>155</sup> **1H NMR** (400 MHz, Chloroform-*d*) δ 8.26 (br.s, 1H, NH), 7.58 (dt, 1H, *J* = 7.8, 0.8 Hz, ArH), 7.37 (dd, 1H, *J* = 7.6, 0.8 Hz, ArH), 7.17 (d, *J* = 2.3 Hz, 1H, ArH), 7.03 (t, 1H, *J* = 7.8 Hz, ArH), 3.92 (t, 2H, *J* = 6.3 Hz, CH<sub>2</sub>) 3.03 (td, 2H, *J* = 6.3, 0.8 Hz, CH<sub>2</sub>).

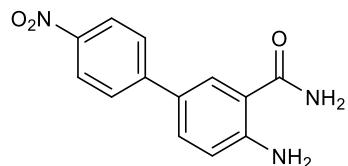
**100, 2-(*p*-tolyl)-2*H*-indazole<sup>156</sup>**



A solution of 2-bromobenzaldehyde (287 mg, 1.5 mmol), *p*-toluidine (193 mg, 1.8 mmol) copper(I) iodide (29.0 mg, 0.15 mmol) and sodium azide (196 mg, 3.0 mmol) in DMSO (5.0 mL) was heated at 120 °C. After heating at this temperature for 12 hours the reaction was allowed to cool to room temperature and diluted with EtOAc, the reaction was washed with water three times and then brine three times. The organic layer was dried over MgSO<sub>4</sub> and filtered through Celite. Concentration of the reaction *in vacuo* followed by column chromatography (5% EtOAc in hexanes) afforded the

title compound as a brown solid (201 mg, 0.97 mmol, 64%). Data is consistent with literature values.<sup>156</sup> **1H NMR** (500 MHz, Chloroform-*d*) δ 8.38 (d, *J* = 1.0 Hz, 1H, Ar*H*), 7.77 – 7.80 (m, 3H, Ar*H*), 7.37 (dt, 1H, *J* = 8.5, 1.0 Hz, Ar*H*), 7.30 – 7.34 (m, 3H, Ar*H*), 7.11 (ddd, 1H, *J* = 8.5, 6.6, 0.9 Hz, Ar*H*), 2.43 (s, 3H, ArCH<sub>3</sub>); **13C NMR** (126 MHz, Chloroform-*d*) δ 149.8 (ArC<sub>q</sub>), 138.1 (ArC<sub>q</sub>), 130.3 (CH<sub>Ar</sub>), 126.8 (CH<sub>Ar</sub>), 122.9 (ArC<sub>q</sub>), 122.5 (CH<sub>Ar</sub>), 121.0 (CH<sub>Ar</sub>), 120.5 (2CH<sub>Ar</sub>), 118.0 (CH<sub>Ar</sub>), 21.2 (ArCH<sub>3</sub>); **LRMS:** (ESI+) *m/z* 209.3 (100%, M + H).

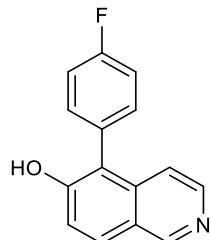
### **101, 4-amino-4'-nitro-[1,1'-biphenyl]-3-carboxamide**



Anthranilamide (800 μL, 50 mM in EtOH, 40 μmol), glucose (800 μL, 1.0 M in KPi buffer, 0.80 mmol), sodium bromide (1.0 mL, 1.0 M in KPi buffer, 1.0 mmol), FAD (20 μL, 1.0 mM in KPi buffer, 0.02 μmol), PyrH (0.4 μmol), Fre (0.040 μmol) and GDH (0.24 μmol) were added to phosphate buffer (10 mL, 10 mM pH 7.2) at 0 °C. The total volume was then made up to 20 mL with phosphate buffer (10 mM, pH 7.2) and then NADH (20 μL, 0.10 M, 2.0 μmol) was added. The reaction was left on a rotary shaker (100 rpm) at room temperature for 18 hours. The reaction was then filtered through a 10 kDa MWCO filter (Vivaspin 10) by centrifugation. The filtrate was then freeze-pump-thawed three times and then backfilled with N<sub>2</sub>. Sodium tetrachloropalladate(II) (6.00 mg, 20 μmol), TPPTS (23.0 mg, 40 μmol), potassium phosphate (10.0 mg, 48 μmol) and 4-nitrophenylboronic acid (200 mg, 1.2 mmol) were then added under N<sub>2</sub> and the reaction was thawed and then heated at 80 °C for 24 hours. 2 M NaOH (20 mL) was added and the organics separated with EtOAc three times. The combined organic phases were then dried over MgSO<sub>4</sub>, filtered and solvent removed *in vacuo*. Flash column chromatography (20% EtOAc in hexanes to 50% in hexanes) yielded the desired product as a yellow oil (4.7 mg, 46%): **1H NMR** (500 MHz, Acetonitrile-*d*<sub>3</sub>) δ 8.32 – 8.17 (m, 2H, Ar*H*), 7.88 – 7.73 (m, 3H, Ar*H*), 7.62 (dd, *J* = 8.6, 2.2 Hz, 1H, Ar*H*), 6.84 (d, *J* = 8.6 Hz, 1H, Ar*H*), 6.29 (s, 2H, ArCONH<sub>2</sub>), 5.92 (br. s, 2H, ArNH<sub>2</sub>); **13C NMR** (126 MHz, Acetonitrile-*d*<sub>3</sub>) δ 172.1 (C=O), 151.8 (ArC<sub>q</sub>), 147.8 (ArC<sub>q</sub>), 147.1 (ArC<sub>q</sub>), 132.2 (CH<sub>Ar</sub>), 128.8 (CH<sub>Ar</sub>), 127.3 (CH<sub>Ar</sub>), 126.0

( $CH_{Ar}$ ), 125.1 ( $CH_{Ar}$ ), 115.0 ( $ArC_q$ ); **LRMS:** (ESI-)  $m/z$  256 (100%, M – H); **HRMS:** (ESI+)  $C_{13}H_{11}O_3N_3$  calculated 257.0752 found 257.0771; **UV**  $\lambda_{max}$  (EtOH) 325 nm.

### **104, 5-(4-fluorophenyl)-6-methoxyisoquinoline**

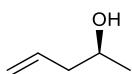


6-hydroxy isoquinoline (800  $\mu$ L, 50 mM in EtOH, 40  $\mu$ mol), sodium bromide (5.0 mL, 1.0 M in KPi buffer, 5.0 mmol), FAD (50  $\mu$ L, 1.0 mM in KPi buffer, 0.05  $\mu$ mol), RadH (1.0  $\mu$ mol) and Fre (0.20  $\mu$ mol) were added to phosphate buffer (10 mL, 10 mM pH 7.2) at 0 °C. The total volume was then made up to 50 mL with phosphate buffer (10 mM, pH 7.2) and then NADH (1.25 mL, 0.10 M, 13  $\mu$ mol) was added. The reaction was left on a rotary incubator (180 rpm) at 30 °C for 20 hours. The reaction was then filtered through a 10 kDa MWCO filter (Vivaspin 10) by centrifugation. The filtrate was then freeze-pump-thawed three times and then backfilled with  $N_2$ . Sodium tetrachloropalladate(II) (6.00 mg, 20  $\mu$ mol), TPPTS (23.0 mg, 40  $\mu$ mol), potassium phosphate (10.0 mg, 48  $\mu$ mol) and 4-fluorophenylboronic acid (168 mg, 1.2 mmol) were then added under  $N_2$ , the reaction was thawed and then heated at 80 °C for 24 hours. The solvent was removed by rotary evaporation and MeCN (1.5 mL) was added. The filtered through a syringe filter. After washing the filter with MeCN the clear yellow filtrate was concentrated to a smaller volume it was purified by reverse phase semi prep HPLC to yield the desired biaryl (1.80 mg, 19%):  **$^1H$  NMR** (400 MHz, Acetonitrile- $d_3$ )  $\delta$  9.30 (s, 1H, ArH), 8.26 (d,  $J$  = 9.0 Hz, 1H, ArH), 8.22 (d,  $J$  = 6.9 Hz, 1H, ArH), 7.65 (d,  $J$  = 9.0 Hz, 1H, ArH), 7.57 (d,  $J$  = 6.9 Hz, 1H, ArH), 7.40 (m, 2H, ArH), 7.32 (m, 2H, ArH); the amount isolated was too low for full characterisation and so the  $^{13}C$ ,  $^{19}F$  and mass data reported was collected by J.L.  **$^{13}C$  NMR** (101 MHz, Acetonitrile- $d_3$ )  $\delta$  163.7 (d,  $^1J_{CF}$  = 245.4 Hz,  $CH_{Ar}$ ), 162.6 ( $ArC_q$ ), 146.1 ( $ArC_q$ ), 141.2 ( $ArC_q$ ), 133.9 (d,  $^3J_{CF}$  = 8.4 Hz,  $CH_{Ar}$ ), 133.4 ( $CH_{Ar}$ ), 132.2 ( $CH_{Ar}$ ), 130.0 (d,  $^4J_{CF}$  = 3.2 Hz,  $ArC_q$ ), 124.3 ( $CH_{Ar}$ ), 123.2 ( $ArC_q$ ), 121.8 ( $CH_{Ar}$ ), 116.8 (d,  $^3J_{CF}$  = 21.8 Hz,  $CH_{Ar}$ );  **$^{19}F$  NMR** (470 MHz, Acetonitrile- $d_3$ ) -115.5; **LRMS:** (ESI+)  $m/z$  240.4 (M + H); **HRMS:** (ESI+)  $C_{15}H_{11}FNO$  calculated 240.0819 found 240.0814.



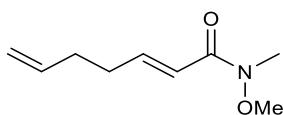
## 5.4. Experimental for Chapter 3

### 168, (S)-pent-4-en-2-ol<sup>157</sup>



A suspension of dried CuI (4.10g, 21.5 mmol) in THF (200 mL) was stirred for 15 minutes at -78 °C before vinyl magnesium bromide (1.0 M in THF, 215 mL, 215 mmol) was added dropwise, stirring was continued for a further 15 minutes and (S)-propylene oxide (5.00 g, 86.0 mmol) was added in one go and allowed to stir. After 30 minutes the temperature was raised to -40 °C and stirred for 1 hour before the temperature was raised again to -10 °C. The reaction was quenched with NH<sub>4</sub>Cl and then water. The aqueous layer was extracted with ether three times and the combined organic phases were dried over MgSO<sub>4</sub> and concentrated to 10 mL. Kugelrohr short path distillation at 120 °C and ambient pressure, afforded the desired homoallylic alcohol **128** as a colourless liquid 68% in THF. Data is consistent with literature values.<sup>157</sup> **1H NMR** (500 MHz, Chloroform-*d*) δ 5.81 (m, 1H, CH=CH<sub>2</sub>), 5.13 (m, 1H, CH=CH<sub>2</sub>), 5.11 (m, 1H, CH=CH<sub>2</sub>), 3.83 (m, 1H, CHCH<sub>3</sub>), 2.25 (ddt, *J* = 12.6, 6.1, 3.3 Hz, 1H, CH<sub>2</sub>), 2.16 (dt, *J* = 14.3, 7.6 Hz, 1H, CH<sub>2</sub>), 1.84 (m, 1H, OH), 1.19 (d, *J* = 6.1 Hz, 3H, CH<sub>3</sub>); **13C NMR** (126 MHz, Chloroform-*d*) δ 134.9 (CH=CH<sub>2</sub>), 118.2 (CH=CH<sub>2</sub>), 67.0 (CHCH<sub>3</sub>), 43.8 (CH<sub>2</sub>), 22.9 (CH<sub>3</sub>); **LRMS:** EI *m/z* 71.1 (M - CH<sub>3</sub>), 85.1 (M - H); [α]<sub>D</sub><sup>25</sup> =

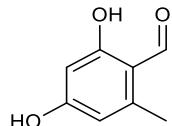
### 136, (E)-*N*-methoxy-*N*-methylhepta-2,6-dienamide<sup>133</sup>



To a suspension of NaH (60% dispersion in mineral oil, 168 mg, 4.18 mmol) in THF (30 mL) at 0 °C diethyl (*N*-methoxy-*N*-methylcarbamoylmethyl)phosphonate (0.86 mL, 1.00 g, 4.18 mmol) was added dropwise and stirred for 30 minutes. 4-pentenal (370 μL, 316 mg, 3.75 mmol) was added dropwise and the resulting solution was stirred for 7 hours before water was added. The reaction mixture was extracted with EtOAc three times and the combined organic layers were then washed with brine. The crude product was purified by column chromatography (40% EtOAc in hexanes) to

yield the desired Weinreb amide **97** as a pale yellow liquid (626 mg, 3.7 mmol, 99%). Data is consistent with literature values.<sup>133</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 3077w, 2936wbr., 1662s, 1632s, 1414m, 1379s, 1177m, 1150w, 1116w, 992s, 913m, 843w; **1H NMR** (400 MHz, Chloroform-*d*)  $\delta$  6.85 (m, 1H, CH=CHCON(OMe)Me), 6.32 (dt, *J* = 15.4, 1.6 Hz, 1H, CH=CHCON(OMe)Me), 5.70 (m, 1H, CH=CH<sub>2</sub>), 4.94 (dq, *J* = 17.1, 1.6 Hz, 1H, CH=CH<sub>2</sub>), 4.88 (m, 1H, CH=CH<sub>2</sub>), 3.59 (s, 3H, NOCH<sub>3</sub>), 3.12 (s, 3H, NCH<sub>3</sub>), 2.23 (m, 2H, CH<sub>2</sub>), 2.12 (dtd, *J* = 7.9, 6.4, 1.3 Hz, 2H, CH<sub>2</sub>); **13C NMR** (101 MHz, Chloroform-*d*)  $\delta$  166.6 (C=O), 146.6 (CH=CHCON(OMe)Me), 137.2 (CH=CH<sub>2</sub>), 118.9 (CH=CHCON(OMe)Me), 115.2 (CH=CH<sub>2</sub>), 61.5 (NOMe), 32.2 (CH<sub>2</sub>), 32.1 (NMe), 31.6 (CH<sub>2</sub>); **LRMS**: (ESI+) *m/z* 170.0 (100%, M + H), 192.0 (92, M + Na).

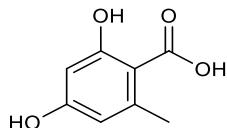
### **167, 2,4-dihydroxy-6-methylbenzaldehyde**<sup>158</sup>



POCl<sub>3</sub> (5.40 mL, 8.83 g, 57.6 mmol) was added dropwise into DMF (30 mL) at -10 °C and allowed to stir for 10 minutes before adding a solution of 3,5-dihydroxytoluene (5.70 g, 48.0 mmol) in DMF (20 mL) portionwise over a period of 45 minutes. The reaction was then slowly brought up to room temperature and the resulting solution was left to stir overnight. The reaction was quenched with ice water and then the pH was adjusted to 10 using 2 M NaOH. The solution containing newly formed precipitate was then boiled for 10 minutes and then cooled to room temperature. Reprecipitation was then achieved by adding 1 M HCl to the solution until a pH of 3 was reached and leaving the solution at -20 °C. After 20 hours the precipitate was collected by Büchner filtration and washed with copious amounts of water and dried in a vacuum oven. An orange solid was obtained as the title compound (4.82 g, 16 mmol, 66%) The filtrate was also extracted with EtOAc three times and after evaporation of the volatiles flash column chromatography (40% EtOAc in hexanes) gave aldehyde **127** as a cream solid (1.59 g, 5.3 mmol, 22%, overall yield 88%). Data is consistent with literature values.<sup>158</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2970w, 1625wbr., 1625s, 1604s, 1481m, 1465m, 1298m, 1273m, 1232m, 1168s, 1060s, 984w; **1H NMR** (400 MHz, Dimethyl sulfoxide-*d*<sub>6</sub>)  $\delta$  12.06 (br. s, 1H, ArOH), 10.73 (br. s, 1H, ArOH), 10.05 (s, 1H, CHO), 6.20 (m, 1H, ArCH), 6.12 (d, *J* = 2.3 Hz, 1H, ArCH), 2.45 (s, 3H, ArCH<sub>3</sub>); **13C NMR** (101 MHz, Dimethyl

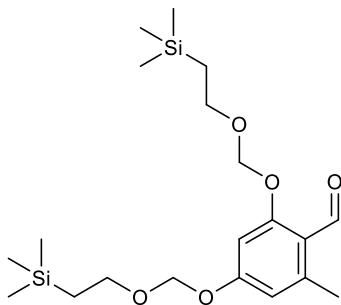
sulfoxide -*d*<sub>6</sub>) δ 193.1 (C=O), 165.4 (ArC<sub>q</sub>), 165.3 (ArC<sub>q</sub>), 144.8 (ArC<sub>q</sub>), 112.69 (ArC<sub>q</sub>), 110.8 (CH<sub>Ar</sub>), 100.3 (CH<sub>Ar</sub>), 18.7 (ArCH<sub>3</sub>); **LRMS:** (ESI+) *m/z* 152.9 (100 %, M + H); **m.p.** 158 °C.

**165, 2,4-dihydroxy-6-methylbenzoic acid<sup>159</sup>**



Orcinaldehyde **167** (5.00 g, 32.9 mmol) was dissolved in DMSO (100 mL) and cooled to −10 °C. A solution of NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O (82.2 mmol), in water (25 mL) was added dropwise using a glass pipette. NaClO<sub>2</sub> (78.4 mmol) in water (25 mL) was then added dropwise over a period of 15–20 minutes and the reaction was left to warm slowly overnight. Saturated Na<sub>2</sub>CO<sub>3</sub> aq. was added and the aqueous layer was washed with EtOAc. The aqueous layer was then acidified to pH 1 using 1 M HCl and the solution was kept at 5 °C for 14 hours. The precipitate was collected by Büchner filtration and the solid was washed with ice water to give orsellinic acid **125** as a white solid. Data is consistent with literature values.<sup>159</sup>  $\nu_{\text{max}}$  / cm<sup>−1</sup> 2950mbr., 1619s, 1574w, 1410w, 1324m, 1458m. **<sup>1</sup>H NMR** (400 MHz, Methanol-*d*<sub>4</sub>) δ 6.14 – 6.19 (m, 2H, ArH), 2.48 (s, 3H, ArCH<sub>3</sub>); **<sup>13</sup>C NMR** (100 MHz, Chloroform-*d*) δ 167.0 (C=O), 163.7 (ArC<sub>q</sub>), 159.3 (ArC<sub>q</sub>), 145.3 (ArC<sub>q</sub>), 112.3 (ArC<sub>q</sub>), 108.5 (CH<sub>Ar</sub>), 101.5 (CH<sub>Ar</sub>), 24.3 (ArCH<sub>3</sub>).

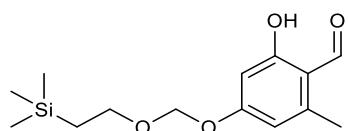
**177, 2-methyl-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzaldehyde**



To a suspension of NaH (60 % dispersion in mineral oil, 1.05 g, 26 mmol) in THF (40 mL) at 0 °C a solution of orcinaldehyde (2.00 g, 13 mmol) in THF (20 mL) was added dropwise and then 2-(trimethylsilyl)ethoxymethyl chloride (4.39 g, 26 mmol) was added. The ice bath was removed and the resulting solution was stirred for 40 minutes before quenching with NH<sub>4</sub>Cl. It was then extracted with EtOAc three times.

The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The crude residue was then purified by flash column chromatography (10% EtOAc in hexanes) to yield the title compound (5.09 g, 12 mmol, 94%) as a colourless oil: **<sup>1</sup>H NMR** (400 MHz, Chloroform-d) δ 10.51 (d, *J* = 0.5 Hz, 1H, CHO), 6.72 (d, *J* = 2.2 Hz, 1H, ArH), 6.52 (app. dt, *J* = 2.2, 0.5 Hz, 1H, ArH), 5.29 (s, 2H, OCH<sub>2</sub>O), 5.24 (s, 2H, OCH<sub>2</sub>O), 3.75 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>TMS), 2.57 (s, 3H, ArCH<sub>3</sub>), 0.96 (m, 4H, CH<sub>2</sub>TMS), 0.00 (s, 18H, TMS); **<sup>13</sup>C NMR** (101 MHz, Chloroform-d) δ 190.9 (C=O), 163.2 (ArC<sub>q</sub>), 162.2 (ArC<sub>q</sub>), 144.3 (ArC<sub>q</sub>), 118.5 (ArC<sub>q</sub>), 112.2 (CH<sub>Ar</sub>), 100.5 (CH<sub>Ar</sub>), 93.3 (OCH<sub>2</sub>O), 92.6 (OCH<sub>2</sub>O), 67.0 (CH<sub>2</sub>CH<sub>2</sub>TMS), 66.9 (CH<sub>2</sub>CH<sub>2</sub>TMS), 22.4 (ArCH<sub>3</sub>), 18.2 (CH<sub>2</sub>TMS), 1.3 (TMS); **LRMS**: (ESI+) *m/z* 435 (100%, M + Na); **HRMS**: (ESI+) C<sub>20</sub>H<sub>36</sub>O<sub>5</sub>NaSi<sub>2</sub> calculated 435.1993 found 435.1994.

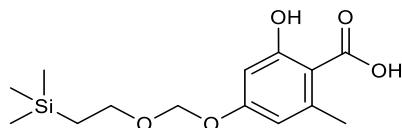
### 179, 2-hydroxy-6-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)benzaldehyde



A rigorously stirred suspension of K<sub>2</sub>CO<sub>3</sub> (3.05 g, 22 mmol) in DMF (23 mL) was cooled to 0 °C and to it a solution of orcinaldehyde (2.80 g, 18.4 mL) in DMF (23 mL) was added dropwise. Once addition was complete stirring was continued for 30 minutes. 2-(trimethylsilyl)ethoxymethyl chloride (3.90 mL, 3.68 g, 22 mmol) was then added and the reaction was warmed to room temperature diluted with EtOAc washed with NH<sub>4</sub>Cl and filtered through a pad of silica (washed with 10% ether in hexanes). Evaporation of the solvent gave the desired benzaldehyde **139** containing doubly protected orcinaldehyde (<10%). This material was carried on to the next step without further purification as the second protecting group cleaves in the next step. Yield of mixture as a white solid (4.31 g, 15 mmol, 83%). A small sample for characterisation was purified by column chromatography (10% ether in hexanes). Exact melting point could not be determined as it melts at temperatures less than 30 °C. **<sup>1</sup>H NMR** (500 MHz, Chloroform-d) δ 12.33 (s, 1H, ArOH), 10.12 (s, 1H, CHO), 6.43 (d, *J* = 2.2 Hz, 1H, ArH), 6.37 (d, *J* = 2.2, 1H, ArH), 5.24 (s, 2H, OCH<sub>2</sub>O), 3.74 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>TMS), 2.54 (s, 3H, ArCH<sub>3</sub>), 0.95 (m, 2H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS); **<sup>13</sup>C NMR** (101 MHz, Chloroform-d) δ 190.9 (C=O), 163.2 (ArC<sub>q</sub>), 162.2 (ArC<sub>q</sub>), 144.3 (ArC<sub>q</sub>), 118.5 (ArC<sub>q</sub>), 112.2 (CH<sub>Ar</sub>), 100.5 (CH<sub>Ar</sub>), 93.3 (OCH<sub>2</sub>O), 92.6

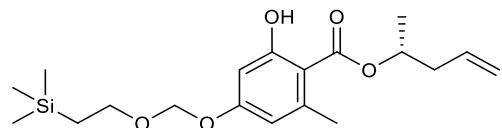
(OCH<sub>2</sub>O), 67.0 (CH<sub>2</sub>CH<sub>2</sub>TMS), 66.9 (CH<sub>2</sub>CH<sub>2</sub>TMS), 22.4 (ArCH<sub>3</sub>), 18.2 (CH<sub>2</sub>TMS), 1.3 (TMS); **LRMS:** (ESI+) *m/z* 435 (100 %, M + Na); **HRMS:** (ESI+) C<sub>20</sub>H<sub>36</sub>O<sub>5</sub>NaSi<sub>2</sub> calculated 435.1993 found 435.1994.

**138, 2-hydroxy-6-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)benzoic acid**



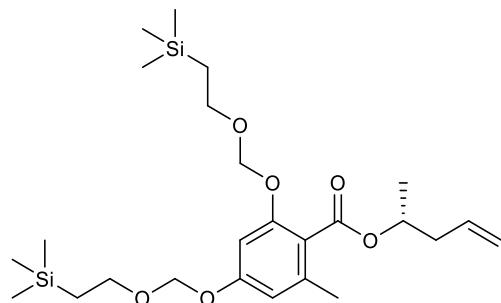
Sulfamic acid (4.78 g, 49.3 mmol) was dissolved in water (60 mL) and added dropwise using a glass pipette to benzaldehyde **179** (4.28 g, 15.0 mmol) which was dissolved in a solution of THF (60 mL) and DMSO (6.0 mL) and cooled to -10 °C. The solution was added at such a rate as to maintain the temperature below 10 °C. NaClO<sub>2</sub> (4.45 g, 49.3 mmol) dissolved in water (60 mL) was then added dropwise, using a glass pipette, at such a rate to maintain the temperature below 10 °C. The resulting stirred solution was then allowed to warm slowly overnight (16 h) to room temperature. The organics were partitioned into EtOAc and the aqueous phase was extracted three times with EtOAc. The combined organic layers were dried over MgSO<sub>4</sub>, filtered and then solvent was removed *in vacuo*. The residue was adsorbed onto silica and column chromatography (2% AcOH in chloroform) yielded the titled compound as a pale yellow solid (4.84 g, 16 mmol, 94%).  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2950m-br., 1619s., 1575m, 1489w, 1458m, 1411w, 1361w; **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*)  $\delta$  11.44 (s, 1H, CO<sub>2</sub>H), 6.51 (d, *J* = 2.5 Hz, 1H, ArH), 6.43 (dd, *J* = 2.6, 0.9 Hz, 1H, ArH), 5.23 (s, 2H, OCH<sub>2</sub>O), 3.74 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>TMS), 2.58 (s, 3H, ArCH<sub>3</sub>), 0.96 (m, 2H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*)  $\delta$  175.9 (C=O), 166.3 (ArC<sub>q</sub>), 162.8 (ArC<sub>q</sub>), 145.0 (ArC<sub>q</sub>), 112.5 (CH<sub>Ar</sub>), 104.8 (ArC<sub>q</sub>), 101.6 (CH<sub>Ar</sub>), 92.5 (OCH<sub>2</sub>O), 66.9 (CH<sub>2</sub>CH<sub>2</sub>TMS), 24.6 (ArCH<sub>3</sub>), 18.1 (CH<sub>2</sub>TMS), -1.3 (TMS); **LRMS:** (ESI-) *m/z* 297.1 (100%, M - H); **HRMS:** (ESI-) C<sub>14</sub>H<sub>21</sub>O<sub>5</sub>Si calculated 297.1164 found 297.1154. **m.p.** 79 °C

**180, (*R*)-pent-4-en-2-yl 2-hydroxy-6-methyl-4-((2-trimethylsilyl)ethoxy)methoxybenzoate**



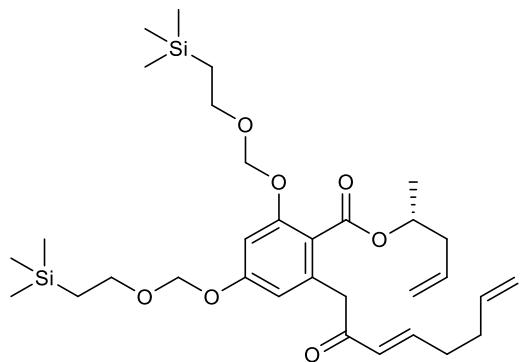
To a flask containing acid **178** (800 mg, 2.68 mmol) and triphenylphosphine (2.82 g, 10.7 mmol), THF (120 mL) was added and cooled to 0 °C. To this was added (*S*)-4-penten-2-ol (462 mg, 5.37 mmol) in one portion. Diethyl azodicarboxylate (1.70 mL, 1.87 g, 10.8 mmol) was then added slowly to ensure the internal temperature is maintained below 5 °C. Once addition was complete the reaction was allowed to warm to room temperature and stirring was continued for 14 hours. The reaction mixture was concentrated *in vacuo* and the residue adsorbed onto silica before flash column chromatography (100% hexanes to 5% ether in hexanes) yielded the desired ester **180** as a colourless oil (412 mg, 1.1 mmol, 42%).  $\nu_{\text{max}}$  / cm<sup>-1</sup> 3019m, 1646s, 1258m, 1214s, 750s, 668m; <sup>1</sup>H NMR (500 MHz, Chloroform-*d*) δ 11.77 (s, 1H, ArOH), 6.49 (d, *J* = 2.6 Hz, 1H, ArH), 6.37 (dd, *J* = 2.6, 0.9 Hz, 1H, ArH), 5.82 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H, CH=CH<sub>2</sub>), 5.28 (m, 1H, CHCH<sub>3</sub>), 5.21 (s, 2H, OCH<sub>2</sub>O), 5.17 – 5.09 (m, 2H, CH=CH<sub>2</sub>), 3.73 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>TMS), 2.55 – 2.40 (m, 5H, ArCH<sub>3</sub>, CH<sub>2</sub>), 1.37 (d, *J* = 6.3 Hz, 3H, CHCH<sub>3</sub>), 0.95 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS); <sup>13</sup>C NMR (126 MHz, Chloroform-*d*) δ 171.3 (C=O), 165.4 (ArC<sub>q</sub>), 161.7 (ArC<sub>q</sub>), 143.4 (ArC<sub>q</sub>), 133.5 (CH=CH<sub>2</sub>), 118.4 (CH=CH<sub>2</sub>), 112.0 (CH<sub>Ar</sub>), 106.6 (ArC<sub>q</sub>), 101.7 (CH<sub>Ar</sub>), 92.5 (CH<sub>2</sub>), 71.8 (CH), 66.8 (CH<sub>2</sub>CH<sub>2</sub>TMS), 40.5 (CH<sub>2</sub>), 24.8 (ArCH<sub>3</sub>), 19.8 (CH<sub>3</sub>), 18.2 (CH<sub>2</sub>TMS), -1.3 (TMS); LRMS: (ESI+) m/z 367.2 (100%, M + H); HRMS (ESI+) C<sub>19</sub>H<sub>31</sub>O<sub>5</sub>Si calculated 367.1935 found 367.1935, [α]<sub>D</sub><sup>23</sup> = -11.05 (c = 1.00).

**181, (*R*)-pent-4-en-2-yl 2-methyl-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzoate**



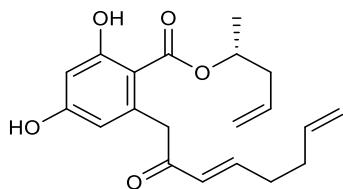
To a suspension of NaH (60 % dispersion in mineral oil, 82 mg, 2.04 mmol) in THF (6.0 mL) at -10 °C, a solution of ester **180** (622 mg, 1.70 mmol) in THF (4.0 mL) was added and the resulting mixture was stirred at this temperature for 20 minutes and effervescence had stopped before addition of 2-(trimethylsilyl)ethoxymethyl chloride (300 µL, 283 mg, 1.70 mmol). The ice/brine bath was removed and the solution was stirred for a further hour before quenching with NH<sub>4</sub>Cl. The reaction was extracted with ether three times and the combined organic phases dried over MgSO<sub>4</sub>. After filtration the solvent was removed *in vacuo* and the residue was filtered through a plug of silica and washed (10% ether in hexanes). After evaporation the colourless oil obtained was used in the next step without further purification.  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2953m br., 1709s, 1605s, 1249s, 1162m, 1101m, 1020s, 858s, 750s, <sup>1</sup>H NMR (400 MHz, Chloroform-*d*) δ 6.69 (d, *J* = 2.2 Hz, 1H, ArH), 6.52 (app. dd, *J* = 2.2, 0.8 Hz, 1H, ArH), 5.83 (ddt, *J* = 17.1, 10.2, 7.0 Hz, 1H, CH=CH<sub>2</sub>), 5.22 (q, *J* = 6.3 Hz, 1H, CHCH<sub>3</sub>), 5.18 (s, 2H, OCH<sub>2</sub>O), 5.17 (s, 2H, OCH<sub>2</sub>O), 5.09 (m, 2H, CH=CH<sub>2</sub>), 3.72 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>TMS ), 2.40 (m, 2H, CH<sub>2</sub>), 2.27 (s, 3H, ArCH<sub>3</sub>), 1.32 (d, *J* = 6.3 Hz, 3H, CH<sub>3</sub>), 0.94 (m, 4H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS), -0.01 (s, 9H, TMS); LRMS (ESI+) *m/z* 497.3 (100%, M + H), 519.3 (100, M + Na); HRMS C<sub>25</sub>H<sub>43</sub>O<sub>6</sub>Si<sub>2</sub> calculated 495.2604 found 495.2585; [α]<sub>D</sub><sup>23</sup> = -12.69 (c = 1.50).

**182, (*R*)-pent-4-en-2-yl (*E*)-2-(2-oxoocta-3,7-dien-1-yl)-4,6-bis((2-trimethylsilyl)ethoxy)methoxybenzoate**



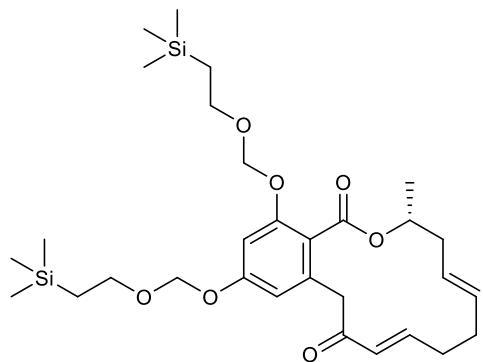
To a solution of freshly prepared LDA (15.5 mL, 0.22 M, 3.41 mmol) at  $-78\text{ }^{\circ}\text{C}$ , a solution of ester **181** (327 mg, 1.70 mmol) in THF (17 mL) was added dropwise and stirred for 10 minutes in which time a deep red colour was observed. The Weinreb amide **136** was then added and allowed to stir for a further 30 minutes before quenching with  $\text{NH}_4\text{Cl}$  (6.0 mL). The reaction was allowed to warm to room temperature before dilution with EtOAc. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over  $\text{MgSO}_4$  filtered and concentrated *in vacuo*. Column chromatography (5% EtOAc in hexanes to 10% EtOAc in hexanes) yielded the desired ketone **182** as a colourless to pale yellow oil (711 mg, 1.2 mmol, 69% over two steps).  **$^1\text{H NMR}$**  (400 MHz, Chloroform-*d*)  $\delta$  6.89 (dt,  $J = 15.8, 6.7\text{ Hz}$ , 1H,  $\text{COCH}=\text{CH}$ ), 6.80 (d,  $J = 2.2\text{ Hz}$ , 1H, ArH), 6.50 (d,  $J = 2.2\text{ Hz}$ , 1H, ArH), 6.17 (dt,  $J = 15.7, 1.4\text{ Hz}$ , 1H,  $\text{C(O)CH=CH}_2$ ), 5.88 – 5.72 (m, 2H,  $\text{CH=CH}_2$ ), 5.24 – 4.89 (m, 9H,  $\text{OCH}_2\text{O}$ ,  $\text{CHCH}_3$ ,  $\text{CH=CH}_2$ ), 3.85 (m, 2H, ArCH<sub>2</sub>), 3.73 (m, 4H,  $\text{CH}_2\text{CH}_2\text{TMS}$ ), 2.43 (dtt,  $J = 14.5, 6.5, 1.4\text{ Hz}$ , 1H, CH<sub>2</sub>), 2.47 – 2.18 (m, 6H, CH<sub>2</sub>), 1.28 (d,  $J = 6.3\text{ Hz}$ , 3H, CHCH<sub>3</sub>), 0.95 (m, 4H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS), 0.00 (s, 9H, TMS);  **$^{13}\text{C NMR}$**  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  196.3 (C=O), 167.4 (C=O), 159.3 (ArC<sub>q</sub>), 156.5 (ArC<sub>q</sub>), 147.3 (COCH=CH), 137.2 (COCH=CH), 135.0 (ArC<sub>q</sub>), 134.0 (CH=CH<sub>2</sub>), 129.6 (CH=CH<sub>2</sub>), 118.6 (ArC<sub>q</sub>), 117.8 (CH=CH<sub>2</sub>), 115.7 (CH=CH<sub>2</sub>), 111.1 (CH<sub>Ar</sub>), 102.6 (CH<sub>Ar</sub>), 93.4 (OCH<sub>2</sub>O), 92.9 (OCH<sub>2</sub>O), 71.2 (CHCH<sub>3</sub>), 66.6 (CH<sub>2</sub>), 45.6 (CH<sub>2</sub>), 40.4 (CH<sub>2</sub>), 32.2 (CH<sub>2</sub>CH<sub>2</sub>TMS), 31.9 (CH<sub>2</sub>CH<sub>2</sub>TMS), 19.6 (CH<sub>3</sub>), 18.2 (CH<sub>2</sub>TMS), 18.1 (CH<sub>2</sub>TMS), -1.3 (TMS); **LRMS:** (ESI-)  $m/z$  603.4 (100%, M – H), 639.4 (30, M + Cl); **HRMS** (APCI+)  $\text{C}_{32}\text{H}_{52}\text{O}_7\text{NaSi}_2$  calculated 627.3149 found 627.3130;  $[\alpha]_D^{23} = 2.21$  (c = 1.46).

**183, (*R*)-pent-4-en-2-yl (*E*)-2,4-dihydroxy-6-(2-oxoocta-3,7-dien-1-yl)benzoate**



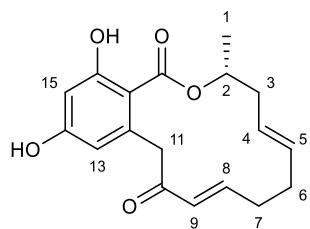
To a solution of **142** (316 mg, 0.52 mmol) in MeCN (23 mL) HF-Pyridine (~70% hydrogen fluoride basis, 2.8 mL was added). The reaction was left to stir for 16 hours at room temperature or until the reaction was determined to be complete by TLC. The reaction was quenched by addition of bicarbonate dropwise. The addition was continued until the pH reached between 7–8. The reaction was extracted with EtOAc three times and the organic layers were combined dried over MgSO<sub>4</sub> filtered and concentrated under vacuum. The crude residue was then purified by column chromatography (20% EtOAc in hexanes to 30% EtOAc in hexanes) to yield the title compound as a pale yellow oil (159 mg, 0.46 mmol, 89%). **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*) δ 11.78 (s, 1H, ArOH), 6.96 (dt, *J* = 15.8, 6.7 Hz, 1H, C(O)CH=CH), 6.77 (br. s, 1H, ArOH), 6.27 (d, *J* = 2.5 Hz, 1H, ArH), 6.20 (dt, *J* = 15.8, 1.5 Hz, 1H, C(O)CH=CH), 6.08 (d, *J* = 2.5 Hz, 1H, ArH), 5.89 – 5.63 (m, 2H, CH=CH<sub>2</sub>), 5.22 (q, *J* = 6.3 Hz, 1H, CHCH<sub>3</sub>), 5.12 – 5.01 (m, 4H, CH=CH<sub>2</sub>), 4.18 (d, *J* = 17.7 Hz, 1H, ArCH<sub>a</sub>H<sub>b</sub>), 3.99 (d, *J* = 17.7 Hz, 1H, ArCH<sub>a</sub>H<sub>b</sub>), 2.44 – 2.20 (m, 6H, CH<sub>2</sub>), 1.23 (d, *J* = 6.3 Hz, 3H, CH<sub>3</sub>); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*) δ 198.0 (C=O), 170.3 (C=O), 165.7 (ArC<sub>q</sub>), 161.2 (ArC<sub>q</sub>), 147.7(C(O)CH=CH), 138.9 (ArC<sub>q</sub>), 137.0 (C(O)CH=CH), 133.5 (CH=CH<sub>2</sub>), 129.6 (CH=CH<sub>2</sub>), 118.3 (CH=CH<sub>2</sub>), 116.0 (CH=CH<sub>2</sub>), 113.3 (ArC<sub>q</sub>), 105.8 (CH<sub>Ar</sub>), 103.2 (CH<sub>Ar</sub>), 72.1 (CHCH<sub>3</sub>), 49.2 (ArCH<sub>2</sub>), 40.2 (CH<sub>2</sub>), 32.2 (CH<sub>2</sub>), 31.9 (CH<sub>2</sub>), 19.6 (CH<sub>3</sub>); **LRMS:** (ESI+) *m/z* 345.2 (100%, M + H); **HRMS:** (ESI+) C<sub>20</sub>H<sub>23</sub>O<sub>5</sub> calculated 343.1540 found 343.1548.

**184, (R,5E,9E)-3-methyl-14,16-bis((2-(trimethylsilyl)ethoxy)methoxy)-3,4,7,8-tetrahydro-1*H*-benzo[c][1]oxacyclotetradecine-1,11(12*H*)-dione**



Grubbs 2<sup>nd</sup> generation catalyst (8.5 mg, 0.01 mmol) was added to a solution of metathesis precursor **182** (60 mg, 0.10 mmol) in toluene (50 mL) and the reaction sealed. The resulting solution was then stirred at 80 °C for 12 hours. Once the solution was allowed to cool down the solution was filtered through silica and washed with DCM and the filtrate was concentrated. The residue was further purified by column chromatography (15% EtOAc in hexanes to 20% EtOAc in hexanes) to yield macrocycle **144** as a clear oil (53 mgs, 0.92 mmol, 92%). **<sup>1</sup>H NMR** (500 MHz, Chloroform-*d*) δ 6.84 – 6.68 (m, 2H, C(O)CH=CH, ArH), 6.55 (d, *J* = 2.2 Hz, 1H, ArH), 5.99 (d, *J* = 16.1 Hz, 1H, C(O)CH=CH), 5.35 (m, 1H, CHCH<sub>3</sub>), 5.23 – 5.11 (m, 6H, OCH<sub>2</sub>O, alkene), 4.06 (d, *J* = 14.7 Hz, 1H, ArCH<sub>a</sub>H<sub>b</sub>), 3.83 – 3.65 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>TMS), 3.47 (d, *J* = 14.7 Hz, 1H, ArCH<sub>a</sub>H<sub>b</sub>), 2.42 – 1.96 (m, 6H, CH<sub>2</sub>), 1.39 (d, *J* = 6.1 Hz, 3H, CH<sub>3</sub>), 0.94 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS), -0.01 (s, 9H, TMS); **<sup>13</sup>C NMR** (126 MHz, Chloroform-*d*) δ 197.7 (C=O), 167.9 (C=O), 159.3 (ArC<sub>q</sub>), 156.4 (ArC<sub>q</sub>), 149.0 (C(O)CH=CH), 135.2 (ArC<sub>q</sub>), 131.9 (CH=CH), 130.0 (CH=CH), 128.7 (CH=CH), 118.6 (ArC<sub>q</sub>), 109.7 (CH<sub>Ar</sub>), 102.2 (CH<sub>Ar</sub>), 93.1 (OCH<sub>2</sub>O), 92.9 (OCH<sub>2</sub>O), 71.5 (CHCH<sub>3</sub>), 44.6 (CH<sub>2</sub>), 39.7 (CH<sub>2</sub>), 31.1 (CH<sub>2</sub>CH<sub>2</sub>TMS), 30.8 (CH<sub>2</sub>CH<sub>2</sub>TMS), 20.4 (CHCH<sub>3</sub>), 18.1 (CH<sub>2</sub>TMS), 18.1 (CH<sub>2</sub>TMS), -1.3 (TMS), -1.3 (TMS).

**115, (R,5E,9E)-14,16-dihydroxy-3-methyl-3,4,7,8-tetrahydro-1H-benzo[c][1]oxacyclotetradecine-1,11(12H)-dione, Monocillin II<sup>119</sup>**



The title compound was prepared using one of the two procedures.

*From acyclic compound 183:*

To a solution of **183** (24.6 mg, 0.072 mmol) in toluene (36 mL) Hoveyda-Grubbs catalyst 2<sup>nd</sup> generation (9.1 mg, 7.1  $\mu$ mol) was added. The resulting solution was left to stir at room temperature for 18 hours, after this time the solvent was removed and column chromatography (20% EtOAc in hexanes) yielded macrocycle **115** as a light yellow oil that solidified on standing (1.40 mg, 44%).

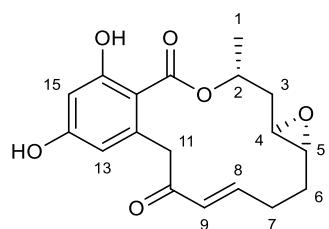
*From macrocycle 184:*

To a solution of **184** (50 mg, 0.087 mmol) in MeCN (4.3 mL) HF-Pyridine (~70% hydrogen fluoride basis, 0.48 mL) was added. The reaction was left to stir for 16 hours at room temperature or until the reaction was determined to be complete by TLC. The reaction was quenched by addition of NaHCO<sub>3</sub> dropwise. The addition was continued until the pH reached between 7–8. The reaction was extracted with EtOAc three times and the organic layers were combined dried over MgSO<sub>4</sub> filtered and concentrated under vacuum. The crude residue was then purified by column chromatography (20% EtOAc in hexanes to 30% EtOAc in hexanes) to yield the title compound as a pale yellow oil (17 mg, 62%).

Further purification by semi-preparative HPLC gave the desired compound as a white solid (9.2 mg, 0.029 mmol, 33%). Data is consistent with literature values.<sup>119</sup> **1H NMR** (500 MHz, Acetone-*d*<sub>6</sub>):  $\delta$  11.52 (br. s, 1H, ArOH), 9.25 (br. s, 1H ArOH), 6.64 (m, 1H, H-8), 6.33 (d, *J* = 2.5 Hz, 1H, ArH), 6.32 (d, *J* = 2.5 Hz, 1H, ArH), 5.83 (d, *J* = 15.5 Hz, 1H, H-9), 5.23-5.36 (m, 3H, H-2, H-4, H-5), 4.06 (d, *J* = 17.0 Hz, 1H, H-11), 3.89 (d, *J* = 17.0 Hz, 1H, H-11'), 2.65 (ddd, *J* = 14.5, 8.0, 4.0 Hz, 1H, CH<sub>2</sub>), 2.13-2.30 (m, 5H, CH<sub>2</sub>), 1.30 (d, *J* = 6.5 Hz, 3H, CH<sub>3</sub>); **13C NMR** (126 MHz, CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$  198.2

(ArCH<sub>2</sub>CO), 170.6 (ArCOOR), 166.1(ArC<sub>q</sub>), 161.5 (ArC<sub>q</sub>), 148.4 (C-8), 140.6 (ArC<sub>q</sub>), 132.5 (ArC<sub>q</sub>), 130.6, (C-9), 127.6 (alkene), 113.0 (CH<sub>Ar</sub>), 106.4 (alkene), 103.1 (CH<sub>Ar</sub>), 73.0 (C-2), 49.1 (ArCH<sub>2</sub>), 37.4 (CH<sub>2</sub>), 31.7 (CH<sub>2</sub>), 31.6 (CH<sub>2</sub>), 18.6 (CH<sub>3</sub>); **LRMS:** (ESI-) *m/z* 315.1 (100%, M – H); **HRMS:** (ESI-) *m/z* calculated for C<sub>18</sub>H<sub>19</sub>O<sub>5</sub>, 315.1238; found, 315.1232.

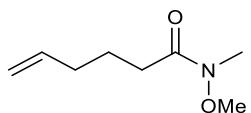
**116, (1aR,14R,15aR,E)-9,11-dihydroxy-14-methyl-1a,2,3,14,15,15a-hexahydro-6H-benzo[c]oxireno[2,3-k][1]oxacyclotetradecine-6,12(7H)-dione<sup>119</sup>**



*meta*-Chlorobenzoic acid (88 % , 0.22 mmol, 1 equiv) was added to solution of **183** (7 mg, 0.22 mmol) in DCM (8.0 M) and then stirred until starting material was consumed (4.5 h). The crude was diluted in diethyl ether and washed with 5 % bicarbonate (10 mL). The organic layer was dried, filtered and concentrated. The mixture of diastereoisomers (1:1) was then subjected to preparative TLC (75% Et<sub>2</sub>O in hexanes) to give the desired diastereomer title compound as a white solid (3.2 mg, 9.6 µmol, 44%). Data is consistent with literature values.<sup>119</sup> **1H NMR** (500 MHz, Acetone-*d*<sub>6</sub>) δ 11.96 (s, 1H, ArOH), 9.26 (br. s, 1H, ArOH), 6.98 (ddd, *J* = 15.5, 10.9, 4.5 Hz, 1H, H-8), 6.33 (d, *J* = 2.6 Hz, 1H, ArH), 6.30 (d, *J* = 2.6 Hz, 1H, ArH), 6.08 (dd, *J* = 15.9, 1.6 Hz, 1H, H-9), 5.25 (qt, *J* = 6.5, 3.3 Hz, 1H, H-2), 4.72 (d, *J* = 17.6 Hz, 1H, H-11), 3.72 (d, *J* = 17.6 Hz, 1H, H-11'), 2.87 (m, 1H, H-4), 2.57 (dt, *J* = 9.8, 2.8 Hz, 1H, H-5), 2.53 (m, 1H, H-7), 2.42 – 2.31 (m, 2H, H-6, H-7'), 2.12 (ddd, *J*=16.1, 5.6, 3.2 Hz, 1 H, H-3), 1.70 (dt, *J* = 16.1, 3.2 Hz, 1 H, H-3'), 1.44 (d, *J* = 6.5 Hz, 3H, CH<sub>3</sub>), 1.24 (td, *J* = 9.9, 4.1 Hz, 1H, H-6); **13C NMR** (126 MHz, Acetone-*d*<sub>6</sub>) δ 197.0 (ArCH<sub>2</sub>CO), 166.9 (ArC<sub>q</sub>), 163.0 (ArC<sub>q</sub>), 148.6 (C-8), 141.3 (ArC<sub>q</sub>), 131.9 (C-9), 113.9 (CH<sub>Ar</sub>), 107.7 (ArC<sub>q</sub>), 102.8 (CH<sub>Ar</sub>), 72.6 (CH), 57.0 (C-5), 55.7 (C-4), 48.3 (ArCH<sub>2</sub>), 37.0 (CH<sub>2</sub>), 31.8 (CH<sub>2</sub>), 29.9 (CH<sub>2</sub>), 18.1 (CH<sub>3</sub>); **LRMS:** (ESI-) *m/z* 331.2 (100%, M – H) 367.2 (21, M + Cl); **HRMS:** (ESI-) *m/z* calculated for C<sub>18</sub>H<sub>19</sub>O<sub>6</sub>, 331.1187; found 331.1189.

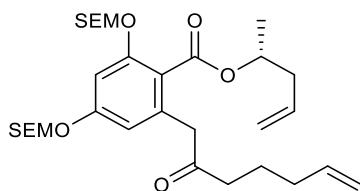
### 5.4.1. Preparation of analogues

#### 210, *N*-methoxy-*N*-methylhex-5-enamide<sup>160</sup>



A solution of 5-hexenoic acid (4.60 g, 40.0 mmol), *N,O*-dimethyl hydroxylaminehydrochloride (3.90 g, 40.0 mmol), triethylamine (17.0 mL, 12.1 g, 120 mmol) and EDC (11.5g, 60 mmol) in DCM (400 mL) was stirred at room temperature for 16 hours. The solvent was removed and the crude residue was dissolved in EtOAc and washed consecutively with H<sub>2</sub>O, bicarbonate and brine. Column chromatography (50% EtOAc in hexanes) yielded the desired compound as a yellowish liquid (2.96 g, 19 mmol, 47%). Data is consistent with literature values.<sup>160</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 3077m, 2938m br., 1661s, 1441m, 1414s, 1177m, 994s, 911s; **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*)  $\delta$  5.80 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H, CH=CH<sub>2</sub>), 5.03 (dq, *J* = 17.1, 1.7 Hz, 1H, CH=CH<sub>2</sub>), 4.97 (ddt, *J* = 10.2, 2.3, 1.3 Hz, 1H, CH=CH<sub>2</sub>), 3.67 (s, 3H, NOME), 3.17 (s, 3H, NMe), 2.42 (t, *J* = 7.6 Hz, 2H, CH<sub>2</sub>), 2.11 (m, 2H, CH<sub>2</sub>), 1.74 (app. p, *J* = 7.5 Hz, 2H, CH<sub>2</sub>); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*)  $\delta$  138.3 (CH=CH<sub>2</sub>), 115.2 (CH=CH<sub>2</sub>), 61.4 (NOME), 33.4 (CH<sub>2</sub>), 32.3 (NMe), 31.3 (CH<sub>2</sub>), 23.8 (CH<sub>2</sub>): **LRMS:** (ESI+) m/z 158.0 (100%, M + H), 180.1 (100, M + Na); **HRMS:** (ESI+) C<sub>8</sub>H<sub>16</sub>NO<sub>2</sub> calculated 158.1176 found 158.1168.

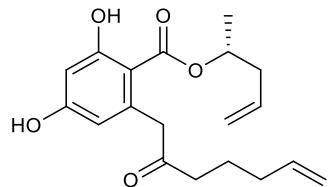
#### 212, (*R*)-pent-4-en-2-yl 2-(2-oxohept-6-en-1-yl)-4,6-bis((2-trimethylsilyl)ethoxy)methoxybenzoate



A solution of ester **181** (184 mg, 0.37 mmol) in THF (6.5 mL) was added dropwise to a solution of freshly prepared LDA (4.4 mL, 0.74 mmol) at -78 °C and the solution stirred for 10 minutes. Weinreb amide **210** (149 mg, 0.95 mmol) dissolved in THF (0.50 mL) was then added dropwise and stirring was continued for 25 minutes. The reaction was quenched at -78 °C with NH<sub>4</sub>Cl and the organics was extracted with ether three times. The combined organic layers were then dried over MgSO<sub>4</sub> filtered

and concentrated. Column chromatography (20% ether in hexanes) yielded the title compound as a colourless oil (81 mg, 0.14 mmol, 37%). **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*) δ 6.80 (d, *J* = 2.2 Hz, 1H, ArH), 6.50 (d, *J* = 2.2 Hz, 1H, ArH), 5.84 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H, CH=CH<sub>2</sub>), 5.74 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H, CH=CH<sub>2</sub>), 5.23 – 4.90 (m, 8H, OCH<sub>2</sub>O, CH=CH<sub>2</sub>), 3.80 – 3.60 (m, 6H, ArCH<sub>2</sub>, CH<sub>2</sub>CH<sub>2</sub>TMS), 2.50 – 2.25 (m, 4H, CH<sub>2</sub>), 2.02 (m, 2H, CH<sub>2</sub>), 1.65 (p, *J* = 7.5 Hz, 2H, CH<sub>2</sub>), 1.30 (d, *J* = 6.3 Hz, 3H, CH<sub>3</sub>), 0.95 (m, 4H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS), -0.00 (s, 9H, TMS); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*) δ 207.2 (C=O), 167.4 (C=O), 159.3 (ArC<sub>q</sub>), 156.6 (ArC<sub>q</sub>), 138.2 (CH=CH<sub>2</sub>), 135.1 (ArC<sub>q</sub>), 134.0 (CH=CH<sub>2</sub>), 118.4 (ArC<sub>q</sub>), 117.8 (CH=CH<sub>2</sub>), 115.2 (CH=CH<sub>2</sub>), 111.2 (CH<sub>Ar</sub>), 102.7 (CH<sub>Ar</sub>), 93.4 (OCH<sub>2</sub>O), 92.9 (OCH<sub>2</sub>O), 71.2 (CHCH<sub>3</sub>), 66.6 (CH<sub>2</sub>CH<sub>2</sub>TMS), 48.1 (ArCH<sub>2</sub>), 41.3 (CH<sub>2</sub>), 40.4 (CH<sub>2</sub>), 33.2 (CH<sub>2</sub>), 22.8 (CH<sub>2</sub>), 19.6 (CH<sub>3</sub>), 18.2 (CH<sub>2</sub>TMS), 18.1 (CH<sub>2</sub>TMS), -1.3 (TMS); **LRMS:** (ESI-) *m/z* 627.5 (100 %, M + Cl), 629.6 (16, M + Cl) 591.6 (35, M – H); **HRMS:** (ESI+) calculated 610.3590 found 610.3581.

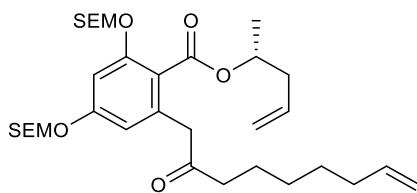
#### 174, (*R*)-pent-4-en-2-yl 2,4-dihydroxy-6-(2-oxohept-6-en-1-yl)benzoate



To a solution of diprotected acyclic compound **212** (81 mg, 0.14 mmol) in MeCN (7.0 mL), HF-Pyridine (~70% hydrogen fluoride basis, 0.7 mL) was added dropwise and was stirred at room temperature until the SM was consumed (6 hours). Purification by column chromatography (30% EtOAc in hexanes) gave the title compound as a white solid (25 mg, 0.075 mmol, 57%). **<sup>1</sup>H NMR** (500 MHz, Chloroform-*d*) δ 11.60 (br. s, 1H), 6.78 (br. s, 1H), 6.29 (d, *J* = 2.5 Hz, 1H, ArH), 6.11 (d, *J* = 2.5 Hz, 1H, ArH), 5.83 – 5.67 (m, 2H, CH=CH<sub>2</sub>), 5.26 (h, *J* = 6.4 Hz, 1H, CHCH<sub>3</sub>), 5.17 – 4.94 (m, 4H, CH=CH<sub>2</sub>), 4.02 (d, *J* = 17.5 Hz, 1H, ArCH<sub>a</sub>H<sub>b</sub>), 3.84 (d, *J* = 17.4 Hz, 1H, ArCH<sub>a</sub>H<sub>b</sub>), 2.47 (t, *J* = 7.5 Hz, 2H, CH<sub>2</sub>), 2.42 (m, 1H, CH<sub>a</sub>H<sub>b</sub>), 2.35 (m, 1H, CH<sub>a</sub>H<sub>b</sub>), 2.06 (m, 2H, CH<sub>2</sub>), 1.69 (p, *J* = 7.4 Hz, 2H, CH<sub>2</sub>), 1.29 (d, *J* = 6.3 Hz, 3H, CH<sub>3</sub>); **<sup>13</sup>C NMR** (126 MHz, Chloroform-*d*) δ 209.35 (C=O), 169.97 (C=O), 165.35 (ArC<sub>q</sub>), 161.17 (ArC<sub>q</sub>), 139.1 (ArC<sub>q</sub>), 137.96 (CH=CH<sub>2</sub>), 133.31 (CH=CH<sub>2</sub>), 118.56 (CH=CH<sub>2</sub>), 115.53 (CH=CH<sub>2</sub>), 113.19 (CH<sub>Ar</sub>), 105.77 (ArC<sub>q</sub>), 103.18 (CH<sub>Ar</sub>), 72.24 (CHCH<sub>3</sub>),

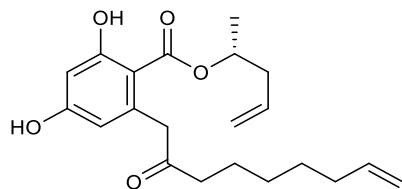
51.26 (CH<sub>2</sub>), 41.33 (CH<sub>2</sub>), 40.26 (CH<sub>2</sub>), 33.12 (CH<sub>2</sub>), 22.66 (CH<sub>2</sub>), 19.62 (CH<sub>3</sub>); **LRMS:** (ESI-) *m/z* 245.1 (100 %, M – CH<sub>2</sub>CHCH<sub>2</sub>CHCH<sub>3</sub>OH), 331.3 (66, M – H); **HRMS:** (ESI+) C<sub>19</sub>H<sub>25</sub>O<sub>5</sub> calculated 333.1697 found 333.1694.

**213, (*R*)-pent-4-en-2-yl 2-(2-oxonon-8-en-1-yl)-4,6-bis((2-trimethylsilyl)ethoxy)methoxybenzoate**



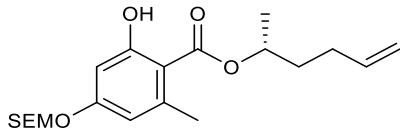
A solution of diprotected ester **181** (186 mg, 0.38 mmol), was added dropwise to a solution of freshly prepared LDA (0.22 M, 0.80 mmol) at -78 °C and the resulting mixture was stirred at this temperature for 10 minutes. The Weinreb amide **211** was then added and the reaction was stirred for 30 minutes. The reaction was quenched by addition of NH<sub>4</sub>Cl (2 mL) at -78 °C on warming to room temperature the reaction was diluted with ether, the organic phase separated, dried over MgSO<sub>4</sub> and filtered. The filtrated was concentrated *in vacuo* and then purified by column chromatography to yield the title compound as a colourless oil (109 mg, 0.18 mmol, 47%). **<sup>1</sup>H NMR** (500 MHz, Chloroform-*d*) δ 6.80 (d, *J* = 2.2 Hz, 1H, ArH), 6.51 (d, *J* = 2.2 Hz, 1H, ArH), 5.80 (app. dddt, *J* = 32.1, 17.0, 10.2, 6.8 Hz, 2H, CH=CH<sub>2</sub>), 5.22 – 5.05 (m, 7H, OCH<sub>2</sub>O, CH=CH<sub>2</sub>, CHCH<sub>3</sub>), 4.97 (dq, *J* = 17.2, 1.8 Hz, 1H, CH=CH<sub>2</sub>), 4.91 (ddt, *J* = 10.2, 2.3, 1.2 Hz, 1H, CH=CH<sub>2</sub>), 3.78 – 3.60 (m, 6H, CH<sub>2</sub>CH<sub>2</sub>TMS, ArCH<sub>2</sub>), 2.50 – 2.25 (m, 4H, CH<sub>2</sub>), 2.02 (m, 2H, CH<sub>2</sub>), 1.55 (app. p, *J* = 7.5 Hz, 2H, CH<sub>2</sub>), 1.36 (m, 2H, CH<sub>2</sub>), 1.41 – 1.19 (m, 7H, CH<sub>3</sub>, CH<sub>2</sub>, CH<sub>2</sub>), 0.95 (m, 4H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS), 0.00 (s, 9H, TMS); **<sup>13</sup>C NMR** (126 MHz, Chloroform-*d*) δ 207.4 (C=O), 167.3 (C=O), 159.3 (ArC<sub>q</sub>), 156.6 (ArC<sub>q</sub>), 139.0 (CH=CH<sub>2</sub>), 135.1 (ArC<sub>q</sub>), 134.0 (CH=CH<sub>2</sub>), 118.6 (ArC<sub>q</sub>), 117.7 (CH=CH<sub>2</sub>), 114.5 (CH=CH<sub>2</sub>), 111.3 (CH<sub>Ar</sub>), 102.7 (CH<sub>Ar</sub>), 93.4 (OCH<sub>2</sub>O), 92.9 (OCH<sub>2</sub>O), 71.2 (CHCH<sub>3</sub>), 66.6 (CH<sub>2</sub>CH<sub>2</sub>TMS), 66.0 (CH<sub>2</sub>CH<sub>2</sub>TMS), 48.0 (CH<sub>2</sub>), 42.0 (CH<sub>2</sub>), 40.4 (CH<sub>2</sub>), 33.7 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 28.7 (CH<sub>2</sub>), 23.6 (CH<sub>2</sub>), 19.6 (CH<sub>3</sub>), 18.2 (CH<sub>2</sub>TMS), 18.1 (CH<sub>2</sub>TMS), -1.3 (TMS); **LRMS:** (ESI-) *m/z* 619.5 (100%, M – H); **HRMS:** (ESI-) C<sub>33</sub>H<sub>55</sub>O<sub>7</sub>Si<sub>2</sub> calculated 619.3481 found 619.3485.

**215, (*R*)-pent-4-en-2-yl 2,4-dihydroxy-6-(2-oxonon-8-en-1-yl)benzoate**



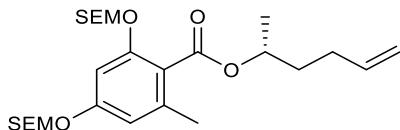
HF-Pyridine (~70% hydrogen fluoride basis, 0.48 mL) was added dropwise to a solution of **213** (109 mg, 0.18 mmol) in MeCN (9.0 mL), the reaction was allowed to stir at room temperature for 18 hours. The reaction was quenched by addition of bicarbonate, addition was continued until a pH of 7-8 was reached and then the reaction was extracted three times with EtOAc. The combined organic phases were then dried over MgSO<sub>4</sub> filtered and concentrated. The title compound was purified by column chromatography (20% EtOAc in hexanes to 40% EtOAc in hexanes) as a white solid (43 mg, 0.12 mmol, 70%): **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*) δ 11.63 (s, 1H, OH), 6.35 (d, *J* = 2.5 Hz, 1H, ArH), 6.16 (d, *J* = 2.6 Hz, 1H, ArH), 5.77 (app. dddt, *J* = 17.2, 14.0, 10.2, 6.8 Hz, 2H, CH=CH<sub>2</sub>), 5.26 (h, *J* = 6.3 Hz, 1H, CH), 5.13 (dq, *J* = 24.5, 1.5 Hz, 1H, CH=CH<sub>2</sub>), 5.12 (dq, *J* = 3.2, 1.3 Hz, 1H, CH=CH<sub>2</sub>), 4.98 (dq, *J* = 17.2, 1.7 Hz, 1H, CH=CH<sub>2</sub>), 4.93 (ddt, *J* = 10.2, 2.3, 1.2 Hz, 1H, CH=CH<sub>2</sub>), 4.01 (d, *J* = 17.3 Hz, 1H, ArCH<sub>2</sub>), 3.82 (d, *J* = 17.3 Hz, 1H, ArCH<sub>2</sub>), 2.52 – 2.29 (m, 4H, CH<sub>2</sub>), 2.03 (m, 2H, CH<sub>2</sub>), 1.57 (p, *J* = 7.5 Hz, 2H, CH<sub>2</sub>), 1.37 (m, 2H, CH<sub>2</sub>), 1.30 (d, *J* = 6.4 Hz, 3H, CH<sub>3</sub>), 1.29 (m, 2H, CH<sub>2</sub>); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*) δ 209.1 (C=O), 170.1 (C=O), 165.5 (ArC<sub>q</sub>), 161.3 (ArC<sub>q</sub>), 139.3 (ArC<sub>q</sub>), 139.0 (ArC<sub>q</sub>), 138.9 (CH=CH<sub>2</sub>), 133.4 (CH=CH<sub>2</sub>), 118.5 (CH<sub>Ar</sub>), 114.6 (CH<sub>Ar</sub>), 113.2 (CH=CH<sub>2</sub>), 105.8 (C), 103.1 (CH=CH<sub>2</sub>), 72.2 (CH), 51.3 (ArCH<sub>2</sub>), 42.1 (CH<sub>2</sub>), 40.3 (CH<sub>2</sub>), 33.7(CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 23.5 (CH<sub>2</sub>), 19.6 (CHCH<sub>3</sub>); **LRMS:** (ESI<sup>-</sup>) *m/z* 273.1 (100 %, M – CH<sub>2</sub>CHCH<sub>2</sub>CHCH<sub>3</sub>OH), 359.2 (87, M – H); **HRMS:** (ESI<sup>+</sup>) C<sub>21</sub>H<sub>29</sub>O<sub>5</sub> calculated 361.2010 found 361.2013.

**217, (*R*)-hex-5-en-2-yl 2-hydroxy-6-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)benzoate**



To a flask containing acid **178** (200 mg, 0.67 mmol) and triphenylphosphine (262 mg, 1.0 mmol), THF (120 mL) was added and cooled to 0 °C. To this was added (*S*)-(+)-5-hexen-2-ol (121 µL, 100 mg, 1.0 mmol) in one portion. DEAD (350 mg, 2.0 mmol) was then added slowly to ensure the internal temperature is maintained below 5 °C. Once addition was complete the reaction was allowed to warm to room temperature and stirring was continued for 14 hours. The reaction mixture was concentrated *in vacuo* and the residue adsorbed onto silica before flash column chromatography (hexanes to 2% ether in hexanes) yielded the desired ester **177** as a colourless oil (192 mg, 0.51 mmol, 75%): **1H NMR** (500 MHz, Chloroform-*d*) δ 11.83 (s, 1H, ArOH), 6.50 (d, *J* = 2.6 Hz, 1H, ArH), 6.38 (dd, *J* = 2.6, 0.9 Hz, 1H, ArH), 5.81 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.24 (m, 1H, CHCH<sub>3</sub>), 5.21 (s, 2H, OCH<sub>2</sub>O), 5.03 (dq, *J* = 17.2, 1.6 Hz, 1H, CH=CH<sub>2</sub>), 4.98 (m, 1H, CH=CH<sub>2</sub>), 3.73 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>TMS), 2.52 (s, 3H, ArCH<sub>3</sub>), 2.16 (m, 2H, CH<sub>2</sub>), 1.87 (dddd, *J* = 13.6, 9.0, 7.3, 6.2 Hz, 1H, CH<sub>2</sub>), 1.72 (m, 1H, CH<sub>2</sub>), 1.37 (d, *J* = 6.3 Hz, 3H, CH<sub>3</sub>), 0.96 (m, 2H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS); **13C NMR** (126 MHz, Chloroform-*d*) δ 171.4 (C=O), 165.4 (ArC<sub>q</sub>), 161.6 (ArC<sub>q</sub>), 143.2 (ArC<sub>q</sub>), 137.5 (CH=CH<sub>2</sub>), 115.4 (CH=CH<sub>2</sub>), 112.0 (CH<sub>Ar</sub>), 106.5 (ArC<sub>q</sub>), 101.7 (CH<sub>Ar</sub>), 92.5 (OCH<sub>2</sub>O), 72.1 (CHCH<sub>3</sub>), 66.7 (CH<sub>2</sub>CH<sub>2</sub>TMS), 35.3 (CH<sub>2</sub>), 29.8 (CH<sub>2</sub>), 24.7(CH<sub>3</sub>), 20.2 (CH<sub>3</sub>), 18.1 (CH<sub>2</sub>TMS), -1.3 (TMS); [α]<sub>D</sub><sup>23</sup> = -12.7 (c = 1.50).

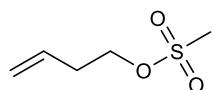
**(*R*)-hex-5-en-2-yl 2-methyl-4,6-bis((2-(trimethylsilyl)ethoxy)methoxy)benzoate**



To a suspension of NaH (60 % dispersion in mineral oil, 22 mg, 0.55 mmol) in THF (6.0 mL) at -10 °C, a solution of ester **177** (190 mg, 0.50 mmol) in THF (2.5 mL) was added and the resulting mixture was stirred at this temperature for 20 minutes and effervescence had stopped before addition of 2-(Trimethylsilyl)ethoxymethyl chloride

(97.0  $\mu$ L, 91.7 mg, 0.55 mmol). The ice/brine bath was removed and the solution was stirred for a further hour before quenching with NH<sub>4</sub>Cl. The reaction was extracted with ether three times and the combined organic phases dried over MgSO<sub>4</sub>. After filtration the solvent was removed *in vacuo* and the residue was purified by column chromatography (hexanes to 10% ether in hexanes) to obtain the title compound as a colourless oil (202 mg, 0.39 mmol, 89%).  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2953m br., 1709s, 1605s, 1249s, 1162m, 1101m, 1020s, 858s, 750s, **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*)  $\delta$  6.71 (d, *J* = 2.2 Hz, 1H, ArH), 6.54 (d, *J* = 2.1 Hz, 1H, ArH), 5.83 (ddt, *J* = 16.9, 10.3, 6.7 Hz, 1H, CH=CH<sub>2</sub>), 5.18 (m, *J* = 2.7 Hz, 5H, CHCH<sub>3</sub>, OCH<sub>2</sub>O), 5.04 (dq, *J* = 17.1, 1.7 Hz, 1H, CH=CH<sub>2</sub>), 4.98 (ddt, *J* = 10.2, 2.2, 1.3 Hz, 1H, CH=CH<sub>2</sub>), 3.73 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>TMS), 2.28 (s, 3H, ArCH<sub>3</sub>), 2.19 (m, 2H, CH<sub>2</sub>), 1.80 (dddd, *J* = 13.9, 9.1, 8.1, 5.9 Hz, 1H, CH<sub>2</sub>), 1.64 (dddd, *J* = 14.1, 9.4, 6.7, 4.9 Hz, 1H, CH<sub>2</sub>), 1.34 (d, *J* = 6.2 Hz, 3H, CH<sub>3</sub>), 0.95 (m, 4H, CH<sub>2</sub>TMS), 0.01 (s, 9H, TMS), -0.01 (s, 9H, TMS); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*)  $\delta$  167.9 (C=O), 158.9 (ArC<sub>q</sub>), 155.5 (ArC<sub>q</sub>), 137.9 (CH=CH<sub>2</sub>), 137.5 (ArC<sub>q</sub>), 119.0 (ArC<sub>q</sub>), 115.2 (CH=CH<sub>2</sub>), 110.6 (CH<sub>Ar</sub>), 101.3 (ArCH), 93.2 (OCH<sub>2</sub>O), 92.9 (OCH<sub>2</sub>O), 71.1 (CHCH<sub>3</sub>), 66.4 (CH<sub>2</sub>CH<sub>2</sub>TMS), 66.4 (CH<sub>2</sub>CH<sub>2</sub>TMS), 35.4 (CH<sub>2</sub>), 29.8 (CH<sub>2</sub>), 20.3 (CH<sub>3</sub>), 19.7 (ArCH<sub>3</sub>), 18.2 (CH<sub>2</sub>TMS), 18.1 (CH<sub>2</sub>TMS), -1.3 (TMS); **LRMS:** (ESI<sup>+</sup>) m/z 511.4 (100 %, M + H); **HRMS:** ESI+ 511.2904 found calc 511.2906 for C<sub>26</sub>H<sub>47</sub>O<sub>6</sub>Si<sub>2</sub>.

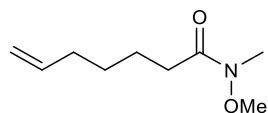
### 224, but-3-en-1-yl methanesulfonate<sup>161</sup>



Methanesulfonyl chloride (3.2 mL, 4.77 g, 42 mmol) was added dropwise to a solution of a solution of buten-1-ol (2.50 g, 35 mmol) and triethylamine (5.8 mL, 4.21 g, 42 mmol) in DCM (50 mL) and the resulting solution was allowed to stir at room temperature after 2 hours the reaction was quenched with bicarbonate and the organic layer separated. The aqueous layer was extracted with DCM twice and the combined organic phases were dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. Column chromatography (50% EtOAc in petrol) gave the desired product as a yellow liquid (5.25g, quantitative). Data is consistent with literature values.<sup>161</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2981wbr., 1643s, 1348s, 1169s, 1041w, 973m, 947s, 905m, 832m, 801m, 733w; **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*)  $\delta$  5.78 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H,

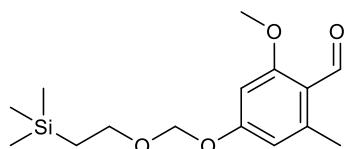
$\text{CH}=\text{CH}_2$ ), 5.26 – 5.12 (m, 2H,  $\text{CH}=\text{CH}_2$ ), 4.27 (t,  $J = 6.7$  Hz, 2H,  $\text{CH}_2$ ), 3.01 (s, 3H,  $\text{CH}_3$ ), 2.51 (qt,  $J = 6.7, 1.4$  Hz, 2H,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR (101 MHz, Chloroform-*d*)  $\delta$  132.5 ( $\text{CH}=\text{CH}_2$ ), 118.7 ( $\text{CH}=\text{CH}_2$ ), 69.0 ( $\text{CH}_2$ ), 37.7 ( $\text{CH}_3$ ), 33.6 ( $\text{CH}_2$ ).

**185, *N*-methoxy-*N*-methylhept-6-enamide**<sup>162</sup>



Heptenoic acid (1.0 mL, 946 mg, 7.4 mmol), *N*-*O* dimethyl hydroxylamine (722 mg, 7.4 mmol), EDC (2.13 g, 11 mmol), HOBr (1.50 g, 11 mmol) and triethylamine (3.0 mL, 2.25 g, 22 mmol) was dissolved in DCM (74 mL) and stirred at room temperature overnight. The solvent was removed and the crude was redissolved in EtOAc and the reaction was washed consecutively with water, bicarbonate and brine. The organic phase was then dried over  $\text{MgSO}_4$ , filtered and then concentrated under reduced pressure. Column chromatography then yielded the desired product as a clear yellow liquid (1.16g, 6.8 mmol, 92%). Data are in accordance with literature reports.<sup>162</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2970s, 2883m, 1467s, 1379s, 1340m, 1305m, 1160m, 1128m, 950s, 816m  $^1\text{H}$  NMR (400 MHz, Chloroform-*d*)  $\delta$  5.80 (ddt,  $J = 16.9, 10.1, 6.7$  Hz, 1H,  $\text{CH}=\text{CH}_2$ ), 5.00 (ddt,  $J = 17.1, 2.1, 1.6$  Hz, 1H,  $\text{CH}=\text{CH}_2$ ), 4.94 (ddt,  $J = 10.2, 2.2, 1.2$  Hz, 1H,  $\text{CH}=\text{CH}_2$ ), 3.67 (s, 3H, *NOMe*), 3.17 (s, 3H, *NMe*), 2.42 (t,  $J = 7.6$  Hz, 2H,  $\text{CH}_2$ ), 2.11 (m, 2H,  $\text{CH}_2$ ), 1.67 (m, 2H,  $\text{CH}_2$ ), 1.44 (m, 2H,  $\text{CH}_2$ );  $^{13}\text{C}$  NMR (101 MHz, Chloroform-*d*)  $\delta$  138.3 ( $\text{CH}=\text{CH}_2$ ), 115.2 ( $\text{CH}=\text{CH}_2$ ), 61.4 (*NOMe*), 33.4 ( $\text{CH}_2$ ), 32.3 (*NMe*), 31.3 ( $\text{CH}_2$ ), 23.8 ( $\text{CH}_2$ ); LRMS: (ESI<sup>+</sup>) m/z 158.0 (100 %, M + H), 180.1 (100, M + Na); HRMS: (ESI<sup>+</sup>) calculated 158.1176 found 158.1168.

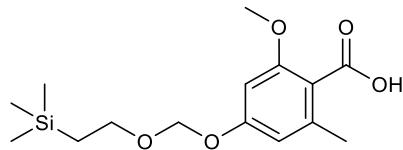
**221, 2-methoxy-6-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)benzaldehyde**



A solution of benzaldehyde **179** (1.41 g, 5.0 mmol) in THF (5.0 mL) was added to a suspension of NaH (60 % dispersion in mineral oil, 82 mg, 2.04 mmol) in THF (6.0 mL) and DMF (1.5 mL) at 0 °C. Methyl iodide (620  $\mu\text{L}$ , 1.42 g, 10 mmol) was then added, the reaction was allowed to warm to room temperature and stirring was

continued for 3 hours before dilution with ether. The organics were washed with water before being dried over MgSO<sub>4</sub>, filtered and then concentrated. The crude residue was then filtered through silica and the solvent evaporated to yield the title compound as a colourless oil (1.01g, 3.4 mmol, 68%); the compound was deemed pure enough for use in the next reaction.  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2970br, 1602s, 1465s, 1161s, 1127s, 949m, 816s; **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*)  $\delta$  10.50 (d, *J* = 0.6 Hz, 1H, CHO), 6.48 – 6.43 (m, 2H, ArH), 5.26 (s, 2H, OCH<sub>2</sub>O), 3.87 (s, 3H, OMe), 3.76 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>TMS), 2.57 (t, *J* = 0.6 Hz, 3H, ArCH<sub>3</sub>), 1.01 – 0.92 (m, 2H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*)  $\delta$  190.8 (C=O), 165.2 (ArC<sub>q</sub>), 162.4 (ArC<sub>q</sub>), 144.6 (ArC<sub>q</sub>), 118.1 (ArC<sub>q</sub>), 111.2 (CH<sub>Ar</sub>), 97.4 (CH<sub>Ar</sub>), 92.7 (CH<sub>2</sub>CH<sub>2</sub>TMS), 66.9 (CH<sub>2</sub>TMS), 55.9 (OMe), 22.3, 18.2, -1.3 (TMS); **LRMS:** (ESI-) *m/z* 331.2 (100%, M + H) 367.2 (21, M + Cl); **HRMS:** (ESI-) C<sub>18</sub>H<sub>19</sub>O<sub>6</sub> calculated 331.1187 found 331.1189.

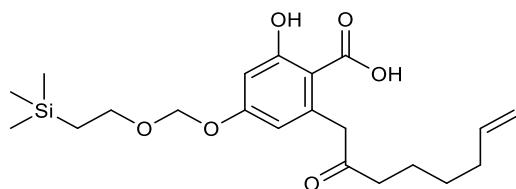
### 222, 2-methoxy-6-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)benzoic acid



A solution of benzaldehyde **181** (888 mg, 3.0 mmol) in THF (15 mL) and DMSO (1.0 mL) was cooled to -10 °C and to it a solution of sulfamic acid (947 mg, 9.8 mmol) in water (10 mL) was added *via* a glass pipette dropwise whilst maintaining the temperature below 5 °C. NaClO<sub>2</sub> (882 mg, 9.3 mmol) was then added dropwise using a glass pipette ensuring the temperature remained below 5 °C. The resulting solution was allowed to warm up to room temperature slowly and stirring was continued for 12 hours. The reaction mixture was extracted with EtOAc three times and the combined organic layers were then dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. Column chromatography (2% AcOH in chloroform) gave the desired product as an orange oil (583 mg, 1.9 mmol, 62%);  $\nu_{\text{max}}$  / cm<sup>-1</sup> 3016m, 2952mbr., 1695s, 1609s, 1583s, 1465m, 1315s, 1281s, 1029s, 914s, 860s, 833s 759m; **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*)  $\delta$  10.36 (br. s, 1H, CO<sub>2</sub>H), 6.60 (d, *J* = 2.2 Hz, 1H, ArH), 6.54 (d, *J* = 2.2 Hz, 1H, ArH), 5.24 (s, 2H, OCH<sub>2</sub>O), 3.95 (s, 3H, OMe), 3.76 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>TMS), 2.55 (t, *J* = 0.6 Hz, 3H, ArCH<sub>3</sub>), 0.96 (m, 2H, CH<sub>2</sub>TMS), 0.01 (s, 9H, TMS); **<sup>13</sup>C NMR** (126 MHz, Chloroform-*d*)  $\delta$  167.0 (C=O), 160.4 (ArC<sub>q</sub>), 159.7

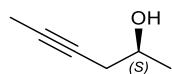
( $\text{ArC}_\text{q}$ ), 144.9 ( $\text{ArC}_\text{q}$ ), 112.4 ( $\text{ArC}_\text{q}$ ), 112.3 ( $\text{CH}_{\text{Ar}}$ ), 98.0 ( $\text{CH}_{\text{Ar}}$ ), 92.8 ( $\text{OCH}_2\text{O}$ ), 66.9 ( $\text{CH}_2\text{CH}_2\text{TMS}$ ), 56.8 ( $\text{OMe}$ ), 23.0 ( $\text{ArCH}_2$ ), 18.2 ( $\text{CH}_2\text{TMS}$ ), -1.3 (TMS).

**226, 2-hydroxy-6-(2-oxooct-7-en-1-yl)-4-((2-trimethylsilyl)ethoxy)methoxybenzoic acid**



A solution of benzoic acid **222** (312 mg, 1.0 mmol) in THF (5.0 mL) at  $-78^\circ\text{C}$  was treated dropwise with *s*BuLi (1.3M in cyclohexane, 1.7 mL, 2.2 mmol) and the resultant solution was left to stir for 20 minutes before Weinreb amide **225** (257 mg, 1.5 mmol) was added as a solution in THF (0.5 mL). The reaction mixture was stirred for 50 minutes. The reaction was quenched by addition of  $\text{NH}_4\text{Cl}$ . The mixture was allowed to warm before extraction with EtOAc three times. The combined organic layers were dried over  $\text{MgSO}_4$ , filtered and concentrated *in vacuo*. Column chromatography afforded the title compound contaminated with **225** as a dark yellow oil (291mg, 0.71 mmol, 71%). Extensive attempts to completely purify this compound were unsuccessful.  $\nu_{\text{max}} / \text{cm}^{-1}$  2934sbr., 1732s, 1663s, 1599m, 1571w, 1460m, 1439s, 1413, 1383, 1247w, 1192w, 1167m, 1120m, 1091m, 1028m, 992s, 911m, 858m, 836s, 691w; **1H NMR** (500 MHz, Chloroform-*d*)  $\delta$  6.65 (d,  $J = 2.0$  Hz, 1H, ArH), 6.54 (d,  $J = 2.0$  Hz, 1H, ArH), 5.81 (m, 1H,  $\text{CH}=\text{CH}_2$ ), 5.25 (s, 2H,  $\text{OCH}_2\text{O}$ ), 5.05 – 4.91 (m, 2H,  $\text{CH}=\text{CH}_2$ ), 3.99 (s, 3H,  $\text{OMe}$ ), 3.80 – 3.72 (m, 2H,  $\text{ArCH}_2$ ), 2.43 (t,  $J = 7.7$  Hz, 2H,  $\text{CH}_2$ ), 2.09 (m, 2H,  $\text{CH}_2$ ), 1.64 (dp,  $J = 15.0, 7.7$  Hz, 2H,  $\text{CH}_2$ ), 1.44 (m, 2H,  $\text{CH}_2$ ), 0.95 (m, 2H,  $\text{CH}_2$ ), 0.01 (s, 9H, TMS); **LRMS:** (ESI+)  $m/z$  423.2 (100%, M + H) 445.2 (83, M + Na); **HRMS:** (ESI+)  $\text{C}_{22}\text{H}_{33}\text{O}_6\text{Si}$  calculated 423.2197 found 423.2196.

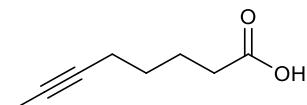
**228, (S)-hex-4-yn-2-ol<sup>163</sup>**



*n*-BuLi (1.6 M in hexanes, 13.4 mL, 21 mmol) was added to a solution of (*Z/E*)-1-bromopropane (1.87 g, 15 mmol) in THF (20 mL) at  $-78^\circ\text{C}$  for 1.5 hours. The reaction was warmed to  $-20^\circ\text{C}$  and (S)-propylene oxide (1.31 g, 23 mmol) in HMPA (15 mL)

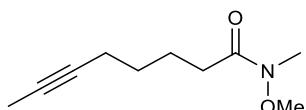
was added. The resulting solution was then stirred for a further 30 minutes before the ice/brine bath was removed and stirring was continued for another hour. The reaction mixture was then poured onto ice and the concentrated HCl was added. The reaction was extracted with ether three times and the combined organic phases were dried over MgSO<sub>4</sub>, filtered and concentrated. The crude product was purified by column chromatography (20% ether in pentane) to give a colourless oil (773 mg, 7.9 mmol, 51%). Data are in accordance with literature. **<sup>1</sup>H NMR** (500 MHz, Chloroform-*d*): δ 3.88 (m, 1H, CHOH), 2.37 – 2.20 (m, 2H, CH<sub>2</sub>), 1.79 (t, *J* = 2.6 Hz, 3H, CH<sub>3</sub>), 1.22 (d, *J* = 6.2 Hz, 3H, CH<sub>3</sub>CHOH); **<sup>13</sup>C NMR** (126 MHz, Chloroform-*d*): δ 78.5 (C≡C), 75.5 (C≡C), 66.7 (CH), 29.5 (CH<sub>2</sub>), 22.3 (CH<sub>3</sub>), 3.6 (CH<sub>3</sub>); [α]<sub>D</sub><sup>25</sup> = 95 (*c* 1.00 in CHCl<sub>3</sub>).

**231, oct-6-yneic acid**<sup>164</sup>



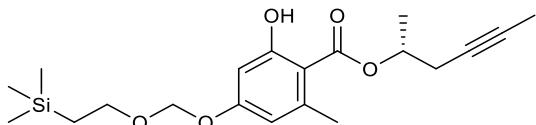
To a freshly prepared solution of LDA (8.0 mmol) in THF (80 mL) cooled to -78 °C, 6-heptynoic acid (504 mg, 4.0 mmol) was added and stirred for 1 hour before DMPU (0.96 mL) was added followed by methyl iodide (0.37 mL, 6.0 mmol). The temperature was then allowed to warm to room temperature and stirred overnight. Column chromatography (10% EtOAc in hexane + 3% AcOH to 15% EtOAc in hexane + 3% AcOH) gave the desired internal alkyne **231** as a white solid (322 mg, 2.3 mmol, 58%). Data are in accordance with literature.  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2946sbr., 1683s, 1640m, 1458s, 1440s, 1413s, 1386w, 1358w, 1317s, 1295w, 1265m, 1178m, 993m, 911s; **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*): δ 2.38 (t, *J* = 7.3 Hz, 2H, CH<sub>2</sub>), 2.16 (tq, *J* = 7.3, 2.5 Hz, 2H, CH<sub>2</sub>), 1.77 (t, *J* = 2.5 Hz, 3H) 1.68 (m, 2H, CH<sub>2</sub>), 1.54 (m, 2H, CH<sub>2</sub>); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*): 179.7 (C=O), 78.6 (C≡C), 76.1 (C≡C), 33.7 (CH<sub>2</sub>), 28.5 (CH<sub>2</sub>), 24.0 (CH<sub>2</sub>), 18.6 (CH<sub>2</sub>), 3.6 (CH<sub>3</sub>); **LRMS:** (ESI-) *m/z* 139.0 (100%, M – H).

**229, N-methoxy-N-methyl-6-ynamide**



A solution of acid **231** (250 mg, 1.8 mmol), *N,O*-dimethylhydroxylamine (174 mg, 1.8 mmol), EDC (513 mg, 2.7 mmol), 1-hydroxybenzotriazole hydrate (362 mg, 2.7 mmol) and triethylamine (0.75 mL, 542 mg, 5.4 mmol) in DCM (18 mL) was stirred overnight at room temperature. The solvent was removed *in vacuo* and EtOAc was added. The organic phase was washed consecutively with water, bicarbonate and brine. The organic phase was dried over MgSO<sub>4</sub>, filtered and solvent removed *in vacuo*. The crude product was purified by column chromatography (50% EtOAc in hexanes) to yield the title compound as colourless liquid (329 mg, quantitative).  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2938mbr., 1661s, 1440s, 1415m, 1320w, 1177m, 996s; **1H NMR** (400 MHz, Chloroform-*d*)  $\delta$  3.67 (s, 3H, NO*Me*), 3.17 (s, 3H, N*Me*), 2.43 (t, *J* = 7.6 Hz, 2H, CH<sub>2</sub>), 2.15 (app. tq, *J* = 7.2, 2.5 Hz, 2H, CH<sub>2</sub>), 1.76 (t, *J* = 2.5 Hz, 3H, CH<sub>3</sub>), 1.73 (m, 2H, CH<sub>2</sub>), 1.53 (m, 2H, CH<sub>2</sub>); **13C NMR** (101 MHz, Chloroform-*d*)  $\delta$  79.0 (C≡C), 75.8 (C≡C), 61.3 (CH<sub>2</sub>), 28.9 (CH<sub>2</sub>), 24.0 (CH<sub>2</sub>), 18.7 (CH<sub>2</sub>), 3.6 (CH<sub>3</sub>); **LRMS:** (ESI+) *m/z* 206.1 (100%, M + Na), 184.1 (68, M + H); **HRMS:** (ESI+) C<sub>10</sub>H<sub>17</sub>O<sub>2</sub>NNa calculated 206.1152 found, 206.1149.

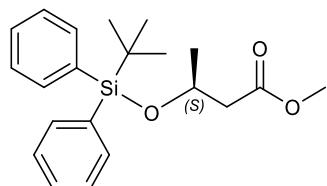
**(R)-hex-4-yn-2-yl 2-hydroxy-6-methyl-4-((2-(trimethylsilyl)ethoxy)methoxy)benzoate**



To mono-SEM-protected orsellinic acid **178** (999 mg, 3.3 mmol) and PPh<sub>3</sub> (1.32 g, 5.0 mmol) in THF (50 mL) at 0 °C, alkyne 188 (361 mg, 3.7 mmol) was added in one portion. Diethyl azodicarboxylate (1.75 g, 10 mmol) was added dropwise keeping the temperature below 10 °C. The resulting mixture was then allowed to warm to room temperature and stirred for 19 hours. The solvent was then removed, and the crude was adsorbed onto silica before column chromatography (100% hexane to 5% ether in hexanes) to yield the desired compound as a colourless oil (667 mg, 1.8 mmol, 53%). **1H NMR** (400 MHz, Chloroform-*d*):  $\delta$  11.67 (s, 1H, ArOH), 6.49 (d, *J* = 2.6

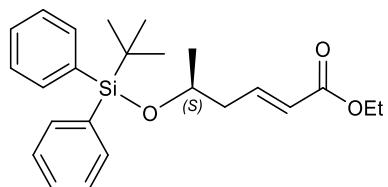
Hz, 1H, ArH), 6.38 (dd,  $J = 2.6, 0.8$  Hz, 1H, ArH), 5.24 (m, 1H, CHCH<sub>3</sub>), 5.21 (s, 2H, OCH<sub>2</sub>O), 3.73 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>TMS), 2.56 – 2.51 (m, 5H, ArCH<sub>3</sub>, CH<sub>2</sub>), 1.78 (t,  $J = 2.5$  Hz, 3H, C≡CCH<sub>3</sub>), 1.45 (d,  $J = 6.3$  Hz, 3H, CHCH<sub>3</sub>), 0.95 (m, 2H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*):  $\delta$  171.1 (C=O), 165.3 (ArC<sub>q</sub>), 161.7 (ArC<sub>q</sub>), 143.7 (ArC<sub>q</sub>), 112.0 (CH<sub>Ar</sub>), 106.5 (ArC<sub>q</sub>), 101.6 (CH<sub>Ar</sub>), 92.5 (OCH<sub>2</sub>O), 78.4 (C≡C), 74.4 (C≡C), 70.8 (CHCH<sub>3</sub>), 66.8 (CH<sub>2</sub>CH<sub>2</sub>TMS), 26.1 (CH<sub>2</sub>), 24.7 (ArCH<sub>3</sub>), 19.4 (CH<sub>3</sub>), 18.2 (CH<sub>2</sub>TMS), 3.6 (C≡CCH<sub>3</sub>), -1.3 (TMS); **LRMS:** (ESI+) *m/z* 379.2 (100%, M + H), 401.2 (95, M + Na), 299.1 (93, M – CH<sub>3</sub>CCCH<sub>2</sub>CHCH<sub>3</sub>); **HRMS:** (ESI+) C<sub>20</sub>H<sub>31</sub>O<sub>5</sub>Si calculated 379.1935 found 379.1935.

**1911, methyl (*S*)-3-((tert-butyldiphenylsilyl)oxy)butanoate<sup>128</sup>**



Methyl (*S*)-3-hydroxy butyrate (3.6 mL, 3.78g, 32 mmol) was dissolved in DCM (34 mL) cooled to 0 °C and imidazole (4.36 g, 64 mmol) was added. After 10 minutes *tert*-butyldiphenylchlorosilane (12 mL, 13.0 g, 48 mmol) was added dropwise and the reaction was allowed to warm to room temperature. Stirring was continued for 4 hours before dilution with ether. The reaction was then washed consecutively with 5% NH<sub>4</sub>Cl and then brine, dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (hexanes to 5% EtOAc in hexanes) and isolated as a colourless oil (10.1 g, 28 mmol, 89%). Data is consistent with literature values.<sup>128</sup>  $\nu_{\text{max}} / \text{cm}^{-1}$  2930m, 2857m, 1739s, 1427m, 1193m, 1175m, 1135m, 1104m, 1080m, 996m, 822m, 738m, 700s, 684m; **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*)  $\delta$  7.72 – 7.63 (m, 4H, ArH), 7.47 – 7.34 (m, 6H, ArH), 4.30 (app. dp,  $J = 7.1, 6.1$  Hz, 1H, CHCH<sub>3</sub>), 3.59 (s, 3H, CO<sub>2</sub>CH<sub>3</sub>), 2.56 (dd,  $J = 14.6, 7.0$  Hz, 1H, CH<sub>2</sub>), 2.39 (dd,  $J = 14.6, 5.8$  Hz, 1H, CH<sub>2</sub>), 1.11 (d,  $J = 6.1$  Hz, 3H, CH<sub>3</sub>), 1.03 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*)  $\delta$  172.0 (C=O), 136.0 (CH<sub>Ar</sub>), 136.0 (CH<sub>Ar</sub>), 134.4 (ArC<sub>q</sub>), 134.0 (ArC<sub>q</sub>), 129.8 (CH<sub>Ar</sub>), 129.7 (CH<sub>Ar</sub>), 127.7 (CH<sub>Ar</sub>), 127.6 (CH<sub>Ar</sub>), 67.0 (CHCH<sub>3</sub>), 51.6 (CO<sub>2</sub>Me), 44.6 (CH<sub>2</sub>), 27.0 (C(CH<sub>3</sub>)<sub>3</sub>), 23.8 (CH<sub>3</sub>), 19.3 (C(CH<sub>3</sub>)<sub>3</sub>); **LRMS:** (ESI+) *m/z* 379.2 (100%, M + Na).

**193, ethyl (*S,E*)-5-((*tert*-butyldiphenylsilyl)oxy)hex-2-enoate<sup>128</sup>**

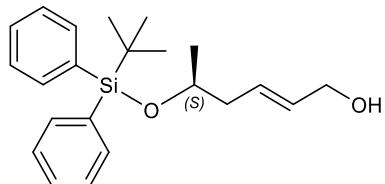


A solution of TBDPSO protected methyl ester **151** (8.40 g, 24 mmol) in toluene (150 mL) was cooled to  $-78^{\circ}\text{C}$  and DIBAL (1 M in THF, 26 mL, 26 mmol) was added dropwise. The reaction was stirred for 10 minutes before consecutive addition of MeOH (18 mL) and NH<sub>4</sub>Cl. Stirring was continued for 50 minutes at room temperature. The reaction mixture was diluted with ether and after stirring for an additional 1 hour, it was filtered through celite and concentrated under reduced pressure. The crude aldehyde **192** was taken directly onto the next step without further purification.<sup>1</sup>**H NMR** (500 MHz, Chloroform-*d*)  $\delta$  9.77 (ddd, *J* = 2.9, 2.1, 0.8 Hz, 1H, aldehyde), 7.68 (m, 4H, ArH), 7.44 – 7.34 (m, 6H, ArH), 4.36 (hd, *J* = 6.1, 1.2 Hz, 1H, CHCH<sub>3</sub>), 2.54 (dddd, *J* = 15.8, 6.0, 2.9, 1.1 Hz, 1H, CH<sub>2</sub>), 2.47 (dddd, *J* = 15.8, 5.6, 2.1, 0.9 Hz, 1H, CH<sub>2</sub>), 1.19 (dd, *J* = 6.3, 1.1 Hz, 3H, CHCH<sub>3</sub>), 1.06 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>): Data is consistent with literature values.<sup>128</sup>

The crude from the previous step was dissolved in MeCN (12 mL) and added dropwise to a solution containing LiCl (1.17 g), triethylphosphonoacetate (5.50 g), Hünig's base (4.0 mL) in MeCN (120 mL). The reaction was allowed to stir for 14 hours at room temperature. The reaction mixture was diluted with ether and washed consecutively with H<sub>2</sub>O and brine. The organic phase was dried over MgSO<sub>4</sub>, filtered and solvent removed *in vacuo*. Column chromatography (2% EtOAc in hexanes) yielded the title compound as a colourless oil (7.20 g, 18 mmol, 77% over two steps). Data is consistent with literature values.<sup>128</sup> <sup>1</sup>**H NMR** (400 MHz, Chloroform-*d*)  $\delta$  7.74 – 7.62 (m, 4H, ArH), 7.49 – 7.30 (m, 6H, ArH), 6.92 (dt, *J* = 15.7, 7.5 Hz, 1H, CH<sub>2</sub>CH=CHCO<sub>2</sub>Me), 5.76 (dt, *J* = 15.7, 1.4 Hz, 1H, CH<sub>2</sub>CH=CHCO<sub>2</sub>Me), 4.17 (q, *J* = 7.1 Hz, 2H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 3.96 (h, *J* = 6.1 Hz, 1H, CHCH<sub>3</sub>), 2.31 (dtd, *J* = 7.6, 5.7, 1.5 Hz, 2H, CH<sub>2</sub>), 1.28 (t, *J* = 7.1 Hz, 3H, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.09 (d, *J* = 6.1 Hz, 3H, CH<sub>3</sub>), 1.05 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>**C NMR** (101 MHz, Chloroform-*d*)  $\delta$  166.5 (C=O), 145.7 (CH<sub>2</sub>CH=CHCO<sub>2</sub>Et), 136.0 (CH<sub>Ar</sub>), 136.0 (CH<sub>Ar</sub>), 134.5 (ArC<sub>q</sub>), 134.1 (ArC<sub>q</sub>), 129.8 (CH<sub>Ar</sub>), 129.7 (CH<sub>Ar</sub>), 127.7(CH<sub>Ar</sub>), 127.6 (CH<sub>Ar</sub>), 123.6 (CH<sub>2</sub>CH=CHCO<sub>2</sub>Et), 68.7

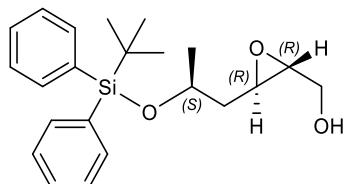
(CHCH<sub>3</sub>), 60.3 (CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 42.3 (CH<sub>2</sub>), 27.1 (C(CH<sub>3</sub>)<sub>3</sub>), 23.4 (CH<sub>3</sub>), 19.4 (C(CH<sub>3</sub>)<sub>3</sub>), 14.4 (CO<sub>2</sub> CH<sub>2</sub>CH<sub>3</sub>); **LRMS:** (ESI+) *m/z* 419.3 (100%, M + Na).

**194, (S,E)-5-((tert-butyldiphenylsilyl)oxy)hex-2-en-1-ol<sup>128</sup>**



A solution of ethyl ester **193** (7.00 g, 20 mmol) in DCM (160 mL) was cooled to -20 °C and DIBAL (1.0 M in THF, 42 mL, 42 mmol) was added dropwise. The reaction was stirred at -20 °C for 2 hours the reaction was warmed to 0 °C and stirred at this temperature for 1 hour. The reaction was quenched by addition of NH<sub>4</sub>Cl (13 mL), warmed to room temperature and stirred at room temperature. The reaction mixture was diluted with ether and stirred for an additional 1 hour before MgSO<sub>4</sub> was added and the suspension was filtered through celite. The solvent was removed and column chromatography (hexanes to 20% EtOAc in hexanes) yielded the title compound as a colourless oil (5.25 g, 15 mmol, 74%). Data is consistent with literature values.<sup>128</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2930m, 2857m, 1472w, 1426m, 1104m, 996m, 821m, 738m, 700s, 688m; **1H NMR** (500 MHz, Chloroform-*d*)  $\delta$  7.68 (m, 4H, ArH), 7.43 (m, 2H, ArH), 7.38 (m, 4H, ArH), 5.58 (m, 2H, alkene), 4.01 (m, 2H, CH<sub>2</sub>OH), 3.91 (h, *J* = 6.0 Hz, 1H, CHCH<sub>3</sub>), 2.19 (m, 2H, CH<sub>2</sub>), 1.10 (d, *J* = 6.1 Hz, 3H, CH<sub>3</sub>), 1.06 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>); **LRMS:** (ESI+) *m/z* 377.2 (100%, M + Na).

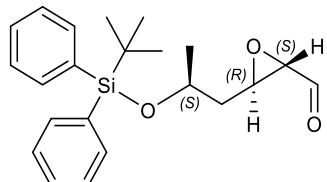
**195, ((2*R*,3*R*)-3-((S)-2-((tert-butyldiphenylsilyl)oxy)propyl)oxiran-2-yl)methanol<sup>128</sup>**



To DCM (20 mL) containing powdered 4 Å molecular sieves (100 mg) cooled to -30 °C, (L)-(+)-diethyl tartrate (120 µL, 0.68 mmol) was added followed by titanium(IV) isopropoxide (170 µL, 0.56 mmol). *tert*-butylhydroperoxide (5.5 M in nonane, 1.6 mL, 8.5 mmol) was then added and stirred for 30 minutes. Allylic alcohol **154** (2.00 g, 5.6

mmol) and the resulting solution was stirred at  $-30\text{ }^{\circ}\text{C}$  for 13 hours. The reaction was quenched by addition of 10% NaOH saturated with NaCl (2.0 mL) and the reaction was diluted with ether. MgSO<sub>4</sub> (2.00 g) and Celite (500 mg) were added and the solution was stirred at  $-10\text{ }^{\circ}\text{C}$  for 15 minutes. The resulting suspension was then filtered through Celite and washed with ether. The solvent was then removed under reduced pressure and column chromatography (30% EtOAc in hexanes) then afforded the title compound as a colourless oil (1.74 g, 4.7 mmol, 83%). Data is consistent with literature values.<sup>128</sup> **1H NMR** (400 MHz, Chloroform-*d*)  $\delta$  7.67 (m, 4H, ArH), 7.49 – 7.32 (m, 6H, ArH), 4.05 (qd, *J* = 6.2, 5.1 Hz, 1H, CHCH<sub>3</sub>), 3.85 (ddd, *J* = 12.5, 5.6, 2.6 Hz, 1H), 3.56 (ddd, *J* = 12.5, 7.3, 4.4 Hz, 1H), 3.07 (td, *J* = 5.9, 2.3 Hz, 1H), 2.83 (dt, *J* = 4.4, 2.5 Hz, 1H), 1.78 (ddd, *J* = 14.0, 6.2, 5.0 Hz, 1H), 1.64 (dt, *J* = 14.0, 5.8 Hz, 1H, CH<sub>2</sub>), 1.56 (dd, *J* = 7.3, 5.7 Hz, 1H, CH<sub>2</sub>), 1.16 (d, *J* = 6.2 Hz, 3H, CH<sub>3</sub>), 1.06 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>); **13C NMR** (101 MHz, Chloroform-*d*)  $\delta$  136.0 (CH<sub>Ar</sub>), 135.9 (CH<sub>Ar</sub>), 134.5 (ArC<sub>q</sub>), 134.2 (ArC<sub>q</sub>), 129.8 (CH<sub>Ar</sub>), 129.8 (CH<sub>Ar</sub>), 127.8 (CH<sub>Ar</sub>), 127.7 (CH<sub>Ar</sub>), 67.6 (CHCH<sub>3</sub>), 61.8, 58.2, 53.1, 41.3, 27.1 (C(CH<sub>3</sub>)<sub>3</sub>), 23.5 (CH<sub>3</sub>), 19.3, (C(CH<sub>3</sub>)<sub>3</sub>); **LRMS:** (ESI+) *m/z* 393.2 (100%, M + Na).

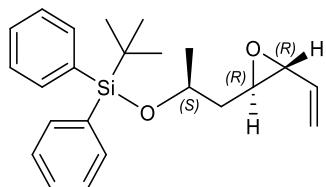
**196, (2*S*,3*R*)-3-((*S*)-2-((*tert*-butyldiphenylsilyl)oxy)propyl)oxirane-2-carbaldehyde<sup>128</sup>**



Alcohol **194** (1.81 g, 4.9 mmol) and triethylamine (3.4 mL, 24 mmol) was dissolved in DCM (40 mL) and DMSO (10 mL) and sulfur trioxide pyridine complex (2.70 g, 17 mmol) was added. The reaction was allowed to stir at room temperature for 30 minutes before dilution with EtOAc and washing with water three times. The organic phase was washed consecutively with bicarbonate and brine, dried over MgSO<sub>4</sub> and filtered. The solvent was evaporated to give **196** as a yellow oil (1.67 g, 4.5 mmol, 77%) and was used directly in the next step without further purification. Data is consistent with literature values.<sup>128</sup> **1H NMR** (500 MHz, Chloroform-*d*)  $\delta$  8.95 (d, *J* = 6.3 Hz, 1H, CHO), 7.66 (dd, *J* = 8.0, 4.2, 3.0, 1.6 Hz, 4H), 7.49 – 7.34 (m, 6H), 4.08 (m, 1H, CHCH<sub>3</sub>), 3.37 (ddd, *J* = 6.3, 5.1, 2.0 Hz, 1H, epoxide CHCHO), 3.04 (dd, *J* =

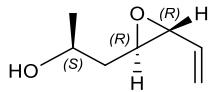
6.3, 2.0 Hz, 1H, epoxide  $\text{CHCH}_2$ ), 1.74 (m, 2H,  $\text{CH}_2$ ), 1.18 (d,  $J = 6.2$  Hz, 3H,  $\text{CH}_3$ ), 1.06 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ); **LRMS:** (ESI+)  $m/z$  391.2 (100%, M + Na).

**197, *tert*-butyldiphenyl((*S*)-1-((2*R*,3*R*)-3-vinyloxiran-2-yl)propan-2-yl)oxy)silane<sup>128</sup>**



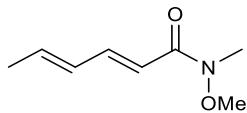
Methyl triphenylphosphonium bromide (2.95 g, 8.3 mmol) was flame dried and THF (46 mL) was added and the suspension cooled to 0 °C. NaHMDS (1.0 M in THF, 7.8 mL, 7.8 mmol) was added dropwise and the reaction mixture was warmed to room temperature and stirred for 30 minutes. The reaction was then recooled to -10 °C and **196** (1.60 g, 4.3 mmol) in THF (8.0 mL) was added dropwise and stirring was continued for a further 10 minutes. The reaction was quenched by the addition of NH<sub>4</sub>Cl and extracted with ether three times. The combined organic phase was washed consecutively with water and brine and dried over MgSO<sub>4</sub>. Filtration and the concentration under reduced pressure before column chromatography (5% EtOAc in hexanes) afforded the title compound as a colourless oil (1.32g, 3.6 mmol, 83%). Data is consistent with literature values.<sup>128</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2931m, 2857m, 1427m, 1379m, 1265m, 1110s, 1082w, 1036w, 997w, 911w, 822m, 736s, 701s, 622m; **<sup>1</sup>H NMR** (500 MHz, Chloroform-*d*)  $\delta$  7.67 (m, 4H, ArH), 7.43 (m, 2H, ArH), 7.37 (m, 4H, ArH), 5.54 (ddd,  $J = 17.5, 10.1, 7.6$  Hz, 1H,  $\text{CH}=\text{CH}_2$ ), 5.44 (dd,  $J = 17.2, 1.5$  Hz, 1H  $\text{CH}=\text{CH}_a\text{H}_b$ ), 5.26 (dd,  $J = 10.2, 1.6$  Hz, 1H,  $\text{CH}=\text{CH}_a\text{H}_b$ ), 4.06 (h,  $J = 6.1$  Hz, 1H,  $\text{CHCH}_3$ ), 3.04 (dd,  $J = 7.6, 2.1$  Hz, 1H, epoxide CH), 2.97 (td,  $J = 5.8, 2.2$  Hz, 1H, epoxide CH), 1.79 (dt,  $J = 13.9, 5.4$  Hz, 1H,  $\text{CH}_a\text{H}_b$ ), 1.65 (dt,  $J = 13.8, 5.9$  Hz, 1H, ,  $\text{CH}_a\text{H}_b$ ), 1.14 (d,  $J = 6.2$  Hz, 3H,  $\text{CH}_3$ ), 1.05 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*)  $\delta$  136.99 (ArCH), 136.0 ( $\text{CH}_{\text{Ar}}$ ), 135.9 ( $\text{CH}=\text{CH}_2$ ), 134.6 ( $\text{ArC}_q$ ), 134.2 ( $\text{ArC}_q$ ), 129.8 ( $\text{CH}_{\text{Ar}}$ ), 129.7 ( $\text{CH}_{\text{Ar}}$ ), 127.8 ( $\text{CH}_{\text{Ar}}$ ), 127.6 ( $\text{CH}_{\text{Ar}}$ ), 119.3 ( $\text{CH}=\text{CH}_2$ ), 67.6 ( $\text{CHCH}_3$ ), 58.7 (epoxide), 57.5 (epoxide), 41.7 ( $\text{CH}_2$ ), 27.1 ( $\text{C}(\text{CH}_3)_3$ ), 23.4 ( $\text{CHCH}_3$ ), 19.4 ( $\text{C}(\text{CH}_3)_3$ ).

**131, (*S*)-1-((2*R*,3*R*)-3-vinyloxiran-2-yl)propan-2-ol<sup>128</sup>**



TBAF (1.0 M in THF, 1.6 mL, 1.6 mmol) was added to a solution of **197** (491 mg, 1.3 mmol) in THF (13 mL). After concentration *in vacuo*, column chromatography (hexanes to 50% EtOAc in hexanes) yielded the title compound as a colourless oil (130 mg, 1.0 mmol, 76%). Data is consistent with literature values.<sup>128</sup> **1H NMR** (400 MHz, Chloroform-*d*) δ 5.73 – 5.41 (m, 2H, CH=CH<sub>2</sub>, CH=CH<sub>a</sub>H<sub>b</sub>), 5.29 (dd, *J* = 9.8, 1.9 Hz, 1H, CH=CH<sub>a</sub>H<sub>b</sub>), 4.09 (h, *J* = 6.1 Hz, 1H, CHCH<sub>3</sub>), 3.14 (dd, *J* = 7.1, 2.2 Hz, 1H, epoxide CH), 2.99 (m, 1H, epoxide CH), 1.86 (dt, *J* = 14.2, 4.3 Hz, 1H, CH<sub>a</sub>H<sub>b</sub>), 1.59 (dt, *J* = 14.8, 7.7 Hz, 1H, CH<sub>a</sub>H<sub>b</sub>), 1.25 (d, *J* = 6.3 Hz, 3H, CH<sub>3</sub>);

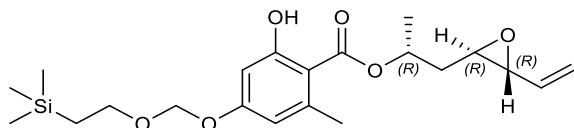
**189, (2E,4E)-N-methoxy-N-methylhexa-2,4-dienamide**<sup>165</sup>



CDI (3.73 g, 23 mmol) was added in portions to a solution of sorbic acid (2.24 g, 20 mmol) in DCM (45 mL). Once addition was complete the solution stirred for 1 hour and *N,O*-dimethylhydroxylamine hydrochloride (2.54g, 26 mmol) was added. The resulting solution was then left to stir for 24 hours. NH<sub>4</sub>Cl was added and the organic layer separated and the aqueous phase was extracted twice more with DCM. The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered and then concentrated *in vacuo*. The product was then purified by column chromatography (60% ether in petroleum ether) as a yellow oil (2.67g, 17 mmol, 86%). Data is consistent with literature values.<sup>165</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2937mbr., 1658s, 1630s, 1606s, 1411m, 1371s, 1178m, 1078s, 996s, 813m, 793m, 620s; **1H NMR** (400 MHz, Chloroform-*d*) δ 7.29 (dd, *J* = 15.2, 10.8 Hz, 1H, COCH=CH), 6.30 (m, 1H, CH), 6.24 (m, 1H, CH), 6.12 (m, 1H, CH), 3.69 (s, 3H, NOMe), 3.24 (s, 3H, NMe), 1.86 – 1.81 (m, 3H, CH<sub>3</sub>); **13C NMR** (101 MHz, Chloroform-*d*) δ 167.6 (C=O), 143.9 (CH), 138.6 (CH), 130.4

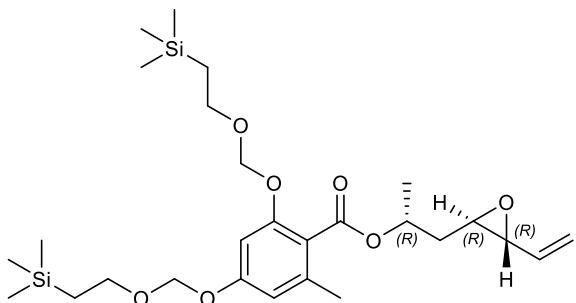
(CH), 116.8 (CH), 61.8 (NOMe), 32.6 (NMe), 18.8 (CH<sub>3</sub>).; **LRMS:** (ESI+) *m/z* 156.1 (100%, M + H).

**135, (R)-1-((2*R*,3*R*)-3-vinyloxiran-2-yl)propan-2-yl 2-hydroxy-6-methyl-4-((2-trimethylsilyl)ethoxy)methoxybenzoate**



DIAD (140  $\mu$ L, 0.71 mmol) was added dropwise to a solution triphenylphosphine (186 mg, 0.71 mmol) and epoxy alcohol **131** (91.0 mg, 0.71 mmol) in THF (5 mL) and was stirred for 10 minutes at 0 °C. To this mixture acid **130** (212 mg, 0.71 mmol) in THF (1.0 mL) was then added dropwise. The resulting solution was allowed to stir at room temperature for 1.5 hours. The reaction mixture was concentrated *in vacuo* and adsorbed onto silica prior to column chromatography (hexanes to 5% EtOAc in hexanes) to yield the desired compound as colourless oil (132mg, 0.32 mmol, 35%).  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2953mbr., 1647s, 1616m, 1577m, 14210w, 1378m, 1316m, 1254s, 1212m, 1164m, 1108m, 1014s, 940m, 834s, 802m, 757w, 698m; **<sup>1</sup>H NMR** (400 MHz, Chloroform-*d*)  $\delta$  11.71 (s, 1H, ArOH), 6.49 (d, *J* = 2.5 Hz, 1H, ArH), 6.38 (m, 1H, ArH), 5.63 – 5.16 (m, 6H, CHCH<sub>3</sub>, CH=CH<sub>2</sub>, CH=CH<sub>2</sub>, OCH<sub>2</sub>O), 3.73 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>TMS), 3.10 (dd, *J* = 7.5, 2.1 Hz, 1H, epoxide CH), 2.95 (ddd, *J* = 7.0, 5.1, 2.1 Hz, 1H, epoxide CH), 2.52 (s, 3H, ArCH<sub>3</sub>), 2.04 (ddd, *J* = 14.4, 7.7, 5.0 Hz, 1H, CH<sub>a</sub>H<sub>b</sub>), 1.87 (ddd, *J* = 14.4, 6.7, 4.8 Hz, 1H, CH<sub>a</sub>H<sub>b</sub>), 1.45 (d, *J* = 6.4 Hz, 3H, CHCH<sub>3</sub>), 0.95 (m, 2H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS); **<sup>13</sup>C NMR** (101 MHz, Chloroform-*d*)  $\delta$  171.2 (C=O), 165.5 (ArC<sub>q</sub>), 161.8 (ArC<sub>q</sub>), 143.2 (ArC<sub>q</sub>), 135.1 (CH=CH<sub>2</sub>), 119.7 (CH=CH<sub>2</sub>), 112.1 (CH<sub>Ar</sub>), 106.3 (ArC<sub>q</sub>), 101.7 (CH<sub>Ar</sub>), 92.5 (OCH<sub>2</sub>O), 70.0 (CHCH<sub>3</sub>), 66.8 (CH<sub>2</sub>CH<sub>2</sub>TMS), 58.9 (epoxide CH), 57.1 (epoxide CH), 39.0 (CH<sub>2</sub>), 24.8 (ArCH<sub>3</sub>), 20.6 (CHCH<sub>3</sub>), 18.2 (CH<sub>2</sub>TMS), -1.3 (TMS); **LRMS:** (ESI+) *m/z* 409.2 (100%, M + H), 431.2 (78, M + Na); **HRMS:** (ESI+) C<sub>21</sub>H<sub>33</sub>O<sub>6</sub>Si calculated 409.2041 found 409.2038.

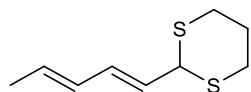
**(*R*)-1-((2*R*,3*R*)-3-vinyloxiran-2-yl)propan-2-yl 2-methyl-4,6-bis((2-trimethylsilyl)ethoxy)methoxybenzoate**



To a solution containing the epoxy ester **135** (127 mg, 0.31 mmol) in THF (1.3 mL) at 0 °C, NaH (60 % dispersion in mineral oil, 14.0 mg, 0.34 mmol) was added in one portion and then 2-(trimethylsilyl)ethoxymethyl chloride (60 µL, 57.0 mg, 0.34 mmol) was added and the resulting solution was allowed to room temperature. The reaction was quenched by dilution with ether and the addition of NH<sub>4</sub>Cl. The organic phase was separated and the aqueous phase extracted twice with ether. The combined organic layers were then dried over MgSO<sub>4</sub> filtered and solvent removed under reduced pressure. Column chromatography (hexanes to 5% EtOAc in hexanes) yielded the title compound as a colourless oil (133 mg, 0.25 mmol, 80%): **1H NMR** (400 MHz, Chloroform-*d*) δ 6.69 (d, *J* = 2.2 Hz, 1H, ArH), 6.53 (d, *J* = 2.2 Hz, 1H, ArH), 5.62 – 5.11 (m, 8H, CHCH<sub>3</sub>, CH=CH<sub>2</sub>, CH=CH<sub>2</sub>, OCH<sub>2</sub>O), 3.72 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>TMS), 3.13 (dd, *J* = 7.3, 2.2 Hz, 1H, epoxide CH), 2.99 (ddd, *J* = 6.8, 4.5, 2.2 Hz, 1H, epoxide CH), 2.27 (s, 3H, ArCH<sub>3</sub>), 2.04 (m, 1H, CH<sub>a</sub>H<sub>b</sub>), 1.73 (m, 1H, CH<sub>a</sub>H<sub>b</sub>), 1.40 (d, *J* = 6.3 Hz, 3H, CHCH<sub>3</sub>), 0.94 (m, 4H, CH<sub>2</sub>TMS), 0.00 (s, 9H, TMS), -0.01 (s, 9H, TMS); **13C NMR** (101 MHz, Chloroform-*d*) δ 167.7 (C=O), 159.0 (ArC<sub>q</sub>), 155.7 (ArC<sub>q</sub>), 137.7 (ArC<sub>q</sub>), 135.5 (CH=CH<sub>2</sub>), 119.5 (CH=CH<sub>2</sub>), 118.6 (ArC<sub>q</sub>), 110.6 (CH<sub>Ar</sub>), 101.4 (CH<sub>Ar</sub>), 93.3 (OCH<sub>2</sub>O), 92.9 (OCH<sub>2</sub>O), 69.5 (CHCH<sub>3</sub>), 66.5 (CH<sub>2</sub>CH<sub>2</sub>TMS), 66.5 (CH<sub>2</sub>CH<sub>2</sub>TMS), 58.7 (epoxide CH), 57.3 (epoxide CH), 38.9 (CH<sub>2</sub>), 20.5 (ArCH<sub>3</sub>), 19.9 (CHCH<sub>3</sub>), 18.2 (CH<sub>2</sub>TMS), 18.1 (CH<sub>2</sub>TMS), -1.2 (TMS), -1.3 (TMS); **LRMS:**

(ESI+)  $m/z$  561.3 (100%, M + Na); **HRMS:** (ESI+) C<sub>27</sub>H<sub>47</sub>O<sub>7</sub>Si<sub>2</sub> calculated 539.2855 found 539.2856.

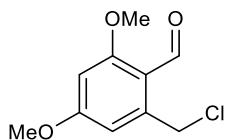
**2-((1*E*,3*E*)-penta-1,3-dien-1-yl)-1,3-dithiane<sup>128</sup>**



Mg(ClO<sub>4</sub>)<sub>2</sub> (600 mg, 2.5 mmol), concentrated sulfuric acid (20  $\mu$ L) and 1,3 propanedithiol (5.0 mL, 50 mmol) was added to CHCl<sub>3</sub> (80 mL) at -10 °C. To this solution 2,4-hexadienal (5.5 mL, 50 mmol) in CHCl<sub>3</sub> (80 mL) was added dropwise to this solution. The reaction was then allowed to warm to room temperature and was stirred at this temperature for 2 hours before being poured onto ice cooled 10% KOH (100 mL). The mixture was stirred for 15 minutes before separation. The organic phase was washed with 10% KOH and then water before being dried over MgSO<sub>4</sub> and filtered through a pad of celite. The solvent was then removed *in vacuo* and column chromatography (4% EtOAc in hexanes) afforded the title compound as a colourless oil (6.79 g, 37 mmol, 73%).

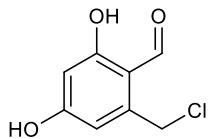
The title compound decomposes readily and becomes yellow on standing and needs to be filtered through silica immediately before the next step and cannot be stored. Data obtained is consistent with literature values.<sup>165</sup> **1H NMR** (500 MHz, Chloroform-*d*)  $\delta$  6.35 (dd, *J* = 15.1, 10.4 Hz, 1H, CH), 6.03 (dd, *J* = 14.8, 10.4 Hz, 1H, CH), 5.76 (dd, *J* = 14.8, 7.1 Hz, 1H, CH), 5.60 (dd, *J* = 15.1, 7.5 Hz, 1H, CH), 4.66 (d, *J* = 7.8 Hz, 1H, dithiane CH), 2.99 – 2.78 (m, 6H, SCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>S), 1.76 (d, *J* = 6.8 Hz, 3H, CH<sub>3</sub>); **13C NMR** (126 MHz, Chloroform-*d*)  $\delta$  134.0 (CH), 131.6 (CH), 130.5 (CH), 126.6 (CH), 47.7 (dithiane CH), 30.5 (CH<sub>2</sub>), 25.4 (CH<sub>2</sub>), 18.3 (CH<sub>3</sub>); **LRMS:** (EI)  $m/z$  186.1 (100%, M).

**201, 2-(chloromethyl)-4,6-dimethoxybenzaldehyde<sup>128</sup>**



POCl<sub>3</sub> (18.6 mL 200 mmol) was added dropwise *via* a syringe pump to DMF (30 mL) at 0 °C. Once addition was complete the solution was allowed to warm to room temperature and stirred for 20 minutes. A solution of 3,5 dimethoxybenzyl alcohol (8.41g, 50 mmol) in DMF (5.0 mL) was then added dropwise and the reaction was then heated to 75 °C and stirred for 2 hours before allowing to cool to room temperature. The reaction mixture was quenched by pouring onto ice water. The pH was adjusted to 7 by addition of 2M NaOH and stirred for 1.5 hours. The formed precipitate was collected by Büchner filtration and washed with ice cold water. The solid was dried in a vacuum oven to give the title compound as a white solid (8.21 g, 38 mmol, 88%). Data obtained is consistent with literature values.<sup>128</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2945w, 2897w, 2846w, 1669s, 1594s, 1575m, 1462w, 1455m, 1428m, 1412m, 1317s, 1278w, 1263w, 1207s, 1191w, 1185w, 1164m, 1149s, 1090m, 1046m, 970w, 942m, 911w, 869m, 791m, 748m, 690m, 617m; **1H NMR** (400 MHz, Chloroform-*d*) δ 10.46 (s, 1H, CHO), 6.76 (d, *J* = 2.2 Hz, 1H, ArH), 6.44 (d, *J* = 2.2 Hz, 1H, ArH), 5.05 (s, 2H, ArCH<sub>2</sub>), 3.90 (s, 3H, ArOCH<sub>3</sub>), 3.90 (s, 3H, ArOCH<sub>3</sub>); **13C NMR** (101 MHz, Chloroform-*d*) δ 190.0 (CHO), 165.4 (ArC<sub>q</sub>), 165.2 (ArC<sub>q</sub>), 142.5 (ArC<sub>q</sub>), 116.1 (ArC<sub>q</sub>), 107.7 (CH<sub>Ar</sub>), 97.8 (CH<sub>Ar</sub>), 56.1 (OCH<sub>3</sub>), 55.8 (OCH<sub>3</sub>), 45.0 (CH<sub>2</sub>); **LRMS:** (ESI<sup>+</sup>) *m/z* m.p. 89 °C

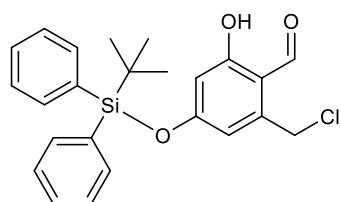
**202, 2-(chloromethyl)-4,6-dihydroxybenzaldehyde<sup>128</sup>**



Chloromethyl benzaldehyde **201** (7.00 g, 33.0 mmol) was dissolved in DCM (100 mL) and cooled to -78 °C and BBr<sub>3</sub> (13 mL) was added dropwise. After stirring at this temperature for 10 minutes the reaction was stirred at room temperature for 20 hours. 1 M HCl aq. was then added to the solution and the reaction was extracted with ether three times. The combined organic layers were dried over MgSO<sub>4</sub>, filtered and the

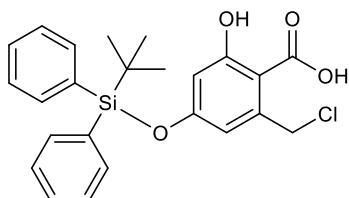
solvent removed. Column chromatography yielded the desired deprotected product as a black solid (4.97 g, 27 mmol, 81%). Data obtained is consistent with literature values.<sup>128</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 3083mbr., 1615s., 1574s, 1485m, 1403s, 1381s, 1340, 1310, 1150m, 1201m, 11705, 1150m, 997m; **1H NMR** (400 MHz, Dimethyl sulfoxide-*d*<sub>6</sub>)  $\delta$  11.69 (s, 1H, ArOH), 10.89 (s, 1H, ArOH), 10.18 (s, 1H, CHO), 6.50 (d, *J* = 2.3 Hz, 1H, ArH), 6.31 (d, *J* = 2.3 Hz, 1H, ArH), 5.02 (s, 2H, CH<sub>2</sub>); **13C NMR** (101 MHz, Dimethyl sulfoxide-*d*<sub>6</sub>)  $\delta$  191.7 (CHO), 165.3 (ArC<sub>q</sub>), 164.7 (ArC<sub>q</sub>), 142.5 (ArC<sub>q</sub>), 111.3 (ArC<sub>q</sub>), 110.9 (CH<sub>Ar</sub>), 102.6 (CH<sub>Ar</sub>), 43.0 (CH<sub>2</sub>); **LRMS:** (ESI-) *m/z* 185.0 (M - H).

**203, 4-((tert-butylidiphenylsilyl)oxy)-2-(chloromethyl)-6-hydroxybenzaldehyde**<sup>128</sup>



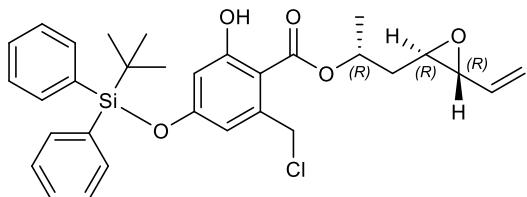
Imidazole (600 mg 8.8 mmol) was added to a solution of benzaldehyde **202** (1.50 g, 8.0 mmol) in DCM (100 mL) and THF (100 mL) at 0 °C. *tert*-butyldiphenylchlorosilane (2.21 g, 8.0 mmol) was then added and stirring was continued for 12 hours at room temperature. The reaction was quenched by addition of 1M HCl aq. and extracted with ether three times. The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. The product was then purified by column chromatography to afford the title compound as an yellow oil (2.02g, 4.8 mmol, 60%). Data obtained is consistent with literature values.<sup>128</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2932m br., 2858w, 1735w, 1668w, 1634m, 1608m, 1489m, 1471m, 1414w, 1426w, 1353w, 1317w, 1262s, 1208m, 1170s, 1114m, 1034w, 1006w, 999w, 942w, 871m, 825m, 772w, 738m, 710m, 694s; **1H NMR** (400 MHz, Chloroform-*d*)  $\delta$  12.16 (s, 1H), 10.16 (s, 1H), 7.74 – 7.61 (m, 4H), 7.52 – 7.34 (m, 7H), 6.36 (d, *J* = 2.3 Hz, 1H), 6.22 (d, *J* = 2.3 Hz, 1H), 4.59 (s, 2H), 1.10 (s, 9H); **13C NMR** (101 MHz, Chloroform-*d*)  $\delta$  192.5 (CHO), 166.3 (ArC<sub>q</sub>), 163.2 (ArC<sub>q</sub>), 141.9 (ArC<sub>q</sub>), 135.5 (CH<sub>Ar</sub>), 131.6 (ArC<sub>q</sub>), 130.6 (CH<sub>Ar</sub>), 128.2 (CH<sub>Ar</sub>), 115.2 (CH<sub>Ar</sub>), 112.2 (ArC<sub>q</sub>), 108.9 (CH<sub>Ar</sub>), 42.0 (CH<sub>2</sub>), 26.5 (C(CH<sub>3</sub>)<sub>3</sub>), 19.6 (C(CH<sub>3</sub>)<sub>3</sub>); **LRMS:** (ESI+) *m/z* 185.0 (100%, M - H), 187.0 (21, M - H), 427.1 (16, M + H).

**130, 4-((tert-butyldiphenylsilyl)oxy)-2-(chloromethyl)-6-hydroxybenzoic acid<sup>128</sup>**



A solution of benzaldehyde **203** (2.00 g, 4.7 mmol) in THF (52 mL), H<sub>2</sub>O (100 mL) and DMSO (5.2 mL) was cooled to 0 °C and to it sulfamic acid (1.28 g, 13 mmol) was added portionwise. NaClO<sub>2</sub> (1.10 g, 12 mmol) was then added and the resulting solution was then allowed to warm up to room temperature slowly and stirring was continued for 12 hours. The reaction mixture was quenched with 1 M HCl and extracted with ether three times. The combined organic layers were then dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* affording the title compound as a sticky yellow oil. This product was used further without any purification. Data obtained is consistent with literature values.<sup>128</sup>  $\nu_{\text{max}}$  / cm<sup>-1</sup> 2962w, 2857w, 1635m, 1609m, 1457w, 1425w, 1353w, 1260s, 1215m, 1171s, 1114m, 1105w, 1034m, 872w, 825s, 772m, 738m, 824m, 711s; <sup>1</sup>H NMR (500 MHz, Chloroform-*d*)  $\delta$  11.22 (s, 1H, ArOH), 7.73 – 7.66 (m, 4H, ArH), 7.47 – 7.43 (m, 2H, ArH), 7.39 (t, *J* = 7.2 Hz, 4H, ArH), 6.53 (d, *J* = 2.5 Hz, 1H, ArH), 6.28 (d, *J* = 2.5 Hz, 1H, ArH), 4.77 (s, 2H, ArCH<sub>2</sub>), 1.10 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>); <sup>13</sup>C NMR (101 MHz, Chloroform-*d*)  $\delta$  166.2 (ArC<sub>q</sub>), 161.8 (ArC<sub>q</sub>), 141.9 (ArC<sub>q</sub>), 135.5 (CH<sub>Ar</sub>), 131.8 (ArC<sub>q</sub>), 130.5 (CH<sub>Ar</sub>), 128.2 (CH<sub>Ar</sub>), 116.4 (CH<sub>Ar</sub>), 108.6 (CH<sub>Ar</sub>), 66.0 (ArC<sub>q</sub>) 46.1 (CH<sub>2</sub>), 26.7 (C(CH<sub>3</sub>)<sub>3</sub>), 19.6 (C(CH<sub>3</sub>)<sub>3</sub>). Peak corresponding to C=O was not observed in the <sup>13</sup>C NMR spectra; LRMS: (ESI+) *m/z* 405.2 (100%, M – Cl), 427.1 (53, M – Cl + Na), 427.1 (16, M + H).

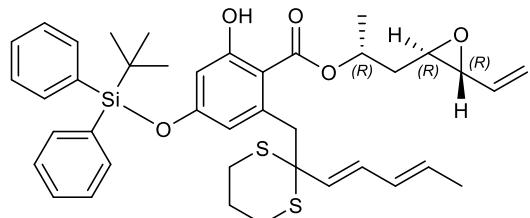
**135, (*R*)-1-((2*R*,3*R*)-3-vinyloxiran-2-yl)propan-2-yl 4-((tert-butyldiphenylsilyl)oxy)-2-(chloromethyl)-6-hydroxybenzoate<sup>128</sup>**



2-furyl phosphine (540 mg, 2.3 mmol) was added to a solution of diisopropyl azodicarboxylate (0.46 mL, 2.3 mmol) in benzene (20 mL) at 25 °C for 10 minutes. Alcohol **131** (299 mg, 2.3 mmol) was then added in benzene (5.0 mL). After stirring

for 15 minutes a solution of benzoic acid **130** in benzene (5.0 mL) was added and stirring was continued for 12 hours. The solvent was removed under reduced pressure and the crude product was adsorbed onto silica before column chromatography yielded the desired compound as a colourless oil (513 mg, 0.93 mmol, 41%). Data obtained is consistent with literature values.<sup>128</sup> **1H NMR** (400 MHz, Chloroform-*d*):  $\delta$  11.54 (s, 1H, ArOH), 7.75 – 7.64 (m, 4H, ArH), 7.48 – 7.35 (m, 8H, ArH), 6.43 (d, *J* = 2.5 Hz, 1H, ArH), 6.27 (d, *J* = 2.5 Hz, 1H, ArH), 5.59 – 5.31 (m, 3H, CHCH<sub>3</sub>, CH=CH<sub>2</sub>, CH=CH<sub>2</sub>), 5.20 (dd, *J* = 10.2, 1.4 Hz, 1H, CH=CH<sub>2</sub>), 4.70 (d, *J* = 2.9 Hz, 2H, ArCH<sub>2</sub>), 3.11 (dd, *J* = 7.6, 2.1 Hz, 1H, epoxide CH), 2.97 (ddd, *J* = 6.9, 4.9, 2.1 Hz, 1H, epoxide CH), 2.08 (m, 1H, CH<sub>a</sub>H<sub>b</sub>), 1.86 (ddd, *J* = 14.4, 6.8, 4.7 Hz, 1H, CH<sub>a</sub>H<sub>b</sub>), 1.46 (d, *J* = 6.4 Hz, 3H, CHCH<sub>3</sub>), 1.10 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>); **13C NMR** (101 MHz, Chloroform-*d*):  $\delta$  170.0 (C=O), 165.5 (ArC<sub>q</sub>), 160.7 (ArC<sub>q</sub>), 140.6 (ArC<sub>q</sub>), 135.5 (CH<sub>Ar</sub>), 135.1 (CH=CH<sub>2</sub>), 131.9 (ArC<sub>q</sub>), 130.4 (CH<sub>Ar</sub>), 128.1 (CH<sub>Ar</sub>), 119.8 (CH=CH<sub>2</sub>), 116.4 (CH<sub>Ar</sub>), 108.8 (CH<sub>Ar</sub>), 105.0 (ArC<sub>q</sub>), 70.6 (CHCH<sub>3</sub>), 58.8 (epoxide), 57.0 (epoxide), 46.5 (CH<sub>2</sub>), 38.7 (CH<sub>2</sub>), 26.6 (C(CH<sub>3</sub>)<sub>3</sub>), 20.3 (CHCH<sub>3</sub>), 19.6 (C(CH<sub>3</sub>)<sub>3</sub>).

**133, (R)-1-((2*R*,3*R*)-3-vinyloxiran-2-yl)propan-2-yl 4-((tert-butyl diphenylsilyl)oxy)-2-hydroxy-6-((2-((1*E*,3*E*)-penta-1,3-dien-1-yl)-1,3-dithian-2-yl)methyl)benzoate<sup>128</sup>**



A solution of ester **135** (513 mg, 0.93 mmol) in THF (0.80 mL) was treated with *n*-BuLi (1.2 M in hexanes, 0.77 mL, 0.93 mmol) and stirred for 5 minutes at -78 °C. To this dithiane (294 mg, 1.6 mmol) in THF (5.3 mL), which was treated with *n*-BuLi (1.2 M in hexanes, 1.35 mL, 1.6 mmol) and stirred at -20 °C for 30 minutes was added dropwise. The resulting solution was then stirred at -78 °C for 2 hours before being quenched by the addition NH<sub>4</sub>Cl. The reaction was then extracted with ether three times. The combined organic phases were then washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and filtered. The solvent was then evaporated under reduced pressure and column chromatography (hexanes to 5% EtOAc in hexanes) gave the desired compound as a pale yellow oil (139 mg, 0.20 mmol, 22%). Data obtained is consistent with literature

values.<sup>128</sup> **1H NMR** (400 MHz, Chloroform-*d*): δ 11.01 (s, 1H, ArOH), 7.71 (dddd, *J* = 7.0, 5.1, 3.5, 2.0 Hz, 4H, Ar*H*), 7.55 – 7.30 (m, 6H, Ar*H*), 6.34 (d, *J* = 2.5 Hz, 1H, Ar*H*), 6.25 (d, *J* = 2.5 Hz, 1H, Ar*H*), 6.11 (dd, *J* = 14.9, 10.5 Hz, 1H, CH), 5.98 (ddd, *J* = 14.9, 10.6, 1.8 Hz, 1H, CH), 5.65 (dd, *J* = 14.9, 6.8 Hz, 1H, CH), 5.52 (ddd, *J* = 17.6, 10.2, 7.6 Hz, 1H, CH), 5.39 – 5.20 (m, 3H, CH<sub>2</sub>), 3.61 (d, *J* = 13.4 Hz, 1H, ArCH<sub>a</sub>H<sub>b</sub>), 3.36 (d, *J* = 13.4 Hz, 1H, ArCH<sub>a</sub>H<sub>b</sub>), 3.10 (dd, *J* = 7.6, 2.2 Hz, 1H, epoxide CH), 3.00 (ddd, *J* = 7.0, 5.0, 2.1 Hz, 1H, epoxide CH), 2.83 – 2.72 (m, 2H, CH<sub>2</sub>), 2.57 – 2.46 (m, 2H, CH<sub>2</sub>), 2.11 (ddd, *J* = 14.3, 7.0, 5.0 Hz, 1H, CH<sub>a</sub>H<sub>b</sub>), 2.02 – 1.91 (m, 2H, CH<sub>2</sub>), 1.84 (ddd, *J* = 14.4, 6.8, 5.5 Hz, 1H), 1.74 (dd, *J* = 6.6, 1.1 Hz, 3H, CH<sub>3</sub>), 1.61 (dt, *J* = 6.3, 1.2 Hz, 1H), 1.48 (d, *J* = 6.3 Hz, 3H, CHCH<sub>3</sub>), 1.08 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>); **13C NMR** (101 MHz, Chloroform-*d*) δ 170.8 (C=O), 163.6 (ArC<sub>q</sub>), 159.3 (ArC<sub>q</sub>), 138.0 (ArC<sub>q</sub>), 135.6 (CH=CH<sub>2</sub>), 135.5, 135.3, 135.3, 134.9, 134.6, 132.6, 132.3, 130.5, 130.3, 130.1, 129.8, 128.0 (CH<sub>Ar</sub>), 127.9, 119.7 (CH=CH<sub>2</sub>), 118.3, 108.2 (ArC<sub>q</sub>), 107.4 (CH<sub>Ar</sub>), 70.9, 58.9 (epoxide CH), 57.1 (epoxide CH), 55.7 (dithiane C<sub>q</sub>), 46.4 (ArCH<sub>2</sub>), 38.9 (CH<sub>2</sub>), 27.5 (dithiane CH<sub>2</sub>), 27.4 (dithiane CH<sub>2</sub>), 26.6 (C(CH<sub>3</sub>)<sub>3</sub>), 26.54, 25.4, 20.4, 19.6 (C(CH<sub>3</sub>)<sub>3</sub>), 18.3 (CH=CHCH<sub>3</sub>), 15.4; **LRMS:** (ESI<sup>+</sup>) *m/z* 701.3 (100%, M + H); **HRMS:** (ESI<sup>+</sup>) C<sub>40</sub>H<sub>48</sub>O<sub>5</sub>SiNaS<sub>2</sub>Si calculated 723.2593 found 723.2605.

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