Alkyne Probes and Natural Products

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Abstract

This thesis is split into two projects, both of which involve efforts to synthesise compounds containing alkynes. The first project is related to the synthesis of probes to label plant CYP450s, a key superfamily of enzymes involved in plant metabolism. The second project details efforts undertaken towards the synthesis of natural products isolated from the *Laurencia* genus of red algae.

Chapters 1 and 3 provide introductions to the respective projects. CYP450 enzymes and activity-based protein profiling (ABPP) are introduced in chapter 1. Chapter 3 provides some background to the *Laurencia* natural products; the biosynthetic postulates proposed for the formation of the diverse skeletons seen in the chemical structures are presented and a selection of interesting total syntheses carried out by numerous groups are also set forth.

Chapter 2 details the synthesis of CYP450 probes of a number of generations (termed G1 through G4) and outlines some of the challenges faced not only in the synthesis of these probes, but also with respect to observing labelling of the CYP450 enzymes *in planta*.

Chapters 4 and 5 concern the synthetic endeavours undertaken towards the total synthesis of *ent*-laurepinnacin, a natural product from the *Laurencia* genus, using a key silver(I)-mediated skeletal rearrangement involving complex oxonium ions as key intermediates. Chapter 6 concludes the thesis by detailing the development of an alternative protocol for the skeletal rearrangement, allowing access to alternative natural products *ent*-(*E*)-notoryne and laurendecumenyne B.

Declaration

The work described in this thesis is entirely the work of the author except where specifically indicated. All biological testing was carried out by Maria Font-Farre under the supervision of Prof. Renier van der Hoorn. The authorship of the biological testing is explicitly stated in the following chapters of the thesis, and permission has been obtained from the authors for the use of the following figures within this thesis:

1. Figure 2.2.1, page 36
2. Figure 2.3.1, page 44
3. Figure 2.3.3, page 53

This thesis has not been previously submitted for a degree, diploma or any other qualification at the University of Oxford or elsewhere.

Daniel Brown.

Acknowledgements

Well, the end has come, and with it there are many people that need thanking. Without their help this thesis would not have been completed, primarily because I would have left the lab' long before having enough content to actually write about. I apologise in advance to anyone that thinks they deserve a mention and does not get one, I hopefully will have already thanked you in person.

Without further ado, I would like to thank my supervisors both in academia and industry, without your intellectual input, none of the projects would have even got off the ground. Thanks especially to Prof. Jonathan Burton for just about steadying the ship when it reached the extremely stormy waters of the last two years, I will remain forever grateful. Thanks also to Prof. Renier van der Hoorn and the ABPP contingent from the group for integrating me into the biology side of the project, I am glad to have had the chance to be part of discussions at group meetings and hopefully haven't bored you all too much with chemistry. On the industry side, thanks to Mell Morris for being the primary day to day contact for the project and also spotting all of the double spaces in the thesis body during your thorough examination of the content. In addition, thanks to Sam Hall and Rich Dale for your input on the projects at our quarterly meetings and also dealing with the interfacing of the academic and industry streams.

Given the collaborative nature of this work, I would like to thank Maria Font-Farre for her unrelenting determination at getting CYP450 labelling with the probes. I valued our discussions throughout our time working together, and learnt a lot of biology throughout, I wish you well for your future endeavours.

F6 has provided a fantastic playground from which to carry out research, with the group members being instrumental to the friendly and supportive environment. The group is now vastly different to when I started and I guess it makes sense to start at the beginning. So thank you to 'the old guard'; Manjeet, Bruno, Kilian, Joe, Sam, and Sean for helping me to settle into the group and honing my skills in the first years. A special mention to Sam and Kilian, without the former my project wouldn't exist, and without the latter my practical skills would be a fraction of what they are now.

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Let's go back to the lab and 'the new guard'; Harry, Nicoleta and Diana. I hope you will continue the Burton group spirit, you have all been a pleasure to work with. I hope that Coldplay classics such as Original Nuttah continue to be played from the lab speaker long into the future. Thank you to all of the people above for the proof-reading, if there are any mistakes remaining, they are all my fault. From a lab' perspective, all that remains is to thank the temporary members of the group; Denis, Claire, Maxime, Penny, Steve, Maryn, Anna and Scott you have all been excellent people to work alongside.

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Contents

Selected Abbreviations

2-EN	2-ethylene naphthoate
Ac	acetyl
Ar	aryl
aq	aqueous
Bn	benzyl
n-Bu	*n*-butyl
t-Bu	*t*-butyl
BPO	bromoperoxidase
cat.	catalytic
COSY	correlation spectroscopy
DIAD	diisopropyl azodicarboxylate
DIBAl-H	diisobutylaluminium hydride
DIPA	diisopropylamine
DIPEA	*N*, *N*-diisopropylethylamine, Hünig's base
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
d.r.	diastereomeric ratio
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EtOAc	ethyl acetate
equiv.	equivalent(s)
ESI	electrospray ionisation
h	hour(s)
HMBC	heteronuclear multiple bond correlation spectroscopy
HMDS	bis(trimethylsilyl)amide
HMPA	hexamethylphosphoramide
HOBt	1-Hydroxybenzotriazole
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence spectroscopy
Hz	Hertz

IR	infrared
KF	potassium fluoride
LPO	lactoperoxidase
LRMS	low resolution mass spectrometry
m-CPBA	*meta*-chloroperbenzoic acid
MeOH	methanol
Ms	methanesulfonyl
MTPA	Mosher's acid
NADH	reduced nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NBS	*N*-bromosuccinimide
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
PFPOTfa	pentafluorophenyl trifluoroacetate
Ph	phenyl
R_f	retention factor
rt	room temperature
sat.	saturated
$TBABH_4$	tetrabutylammonium borohydride
TBACl	tetrabutylammonium chloride
TES	triethylsilyl
Tf	trifluoromethanesulfonyl
TFA	trifluoracetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	4-methylbenzenesulfonyl

1. Cytochrome P450s and Activity Based Protein Profiling

1.1. Significance of Cytochrome P450 Enzymes

The oxidising cytochrome P450 (CYP450) enzymes are ubiquitous in the natural world and essential within biological detoxification pathways and biosynthesis.[1] CYP450s have been in the spotlight since their isolation from the microsomal fraction of animal livers in 1961.[2] Although it is important to study these enzymes in drug discovery platforms, from the perspective of plants, the study of this superfamily of enzymes is key (*vide infra*) in order to tackle the increasing problem of food security for a growing global population. Factors such as global warming, shrinking availability of farming land and a scarcity of water are compounded by the issue of herbicide resistance.[3] The issue of herbicide resistance was first reported in the late 1950s; since then, several mechanisms of resistance to herbicides from a number of modes of action have continued to enter the literature.[3–5] There are two main avenues by which resistance can arise: target-site resistance and nontarget-site resistance.[6] Target-site resistance generally encompasses gene mutations of the enzyme of interest causing the binding efficiency of ligands to be reduced. Nontarget-site resistance, on the other hand, is caused by a lower amount of the herbicide reaching the target enzyme, either by increased elimination by translocation or by more effective metabolism.[7] Metabolism by CYP450s falls into the category of nontarget-site resistance; a further discussion of resistance modes is beyond the scope of this thesis.

Herbicide detoxification in plants can be categorised into four main phases. During phase I, compounds are oxidised, usually by CYP450 enzymes (Figure 1.1.1).[8] This has the effect of introducing a polar handle from which further detoxification can occur in latter phases; the synthesis of probes for this class of enzymes forms the bulk of chapter II. In phase II metabolism, the polarity of the compound is further increased by tagging it to a bulky and hydrophilic residue; this is most commonly achieved by glutathione *S*-transferases (GSTs) or UDP-glycosyltransferases (UGTs). The synthesis of a chemical probe for GST enzymes is provided in the appendix (10.1 Synthesis of Additional Probes).[9] The metabolism of xenobiotics proceeds with the effect of nullifying toxicity, however there are instances when oxidation can cause their potency to increase, prodrugs are a particular example of this.[10] The remaining phases of metabolism are concerned with the transport of the tagged molecule to the vacuole by ABC transporters and further degradation once compartmentalised.[8,9,11]

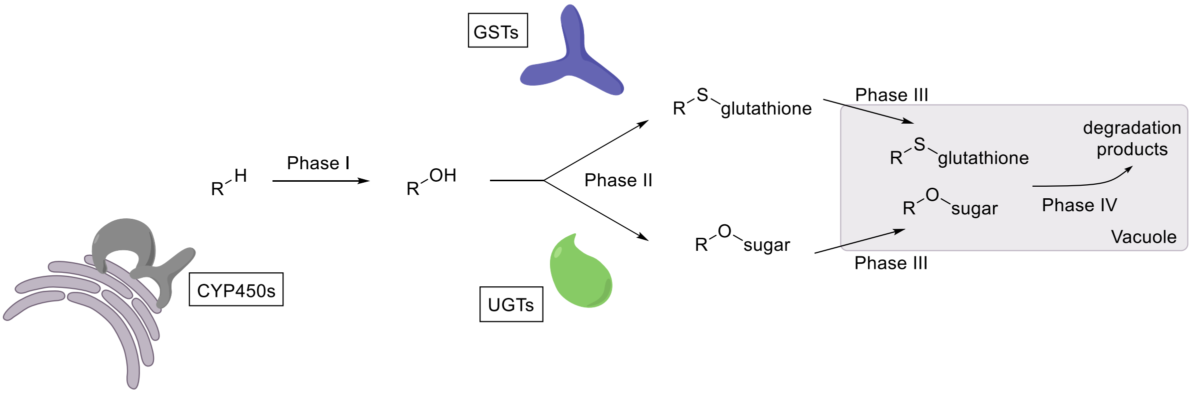


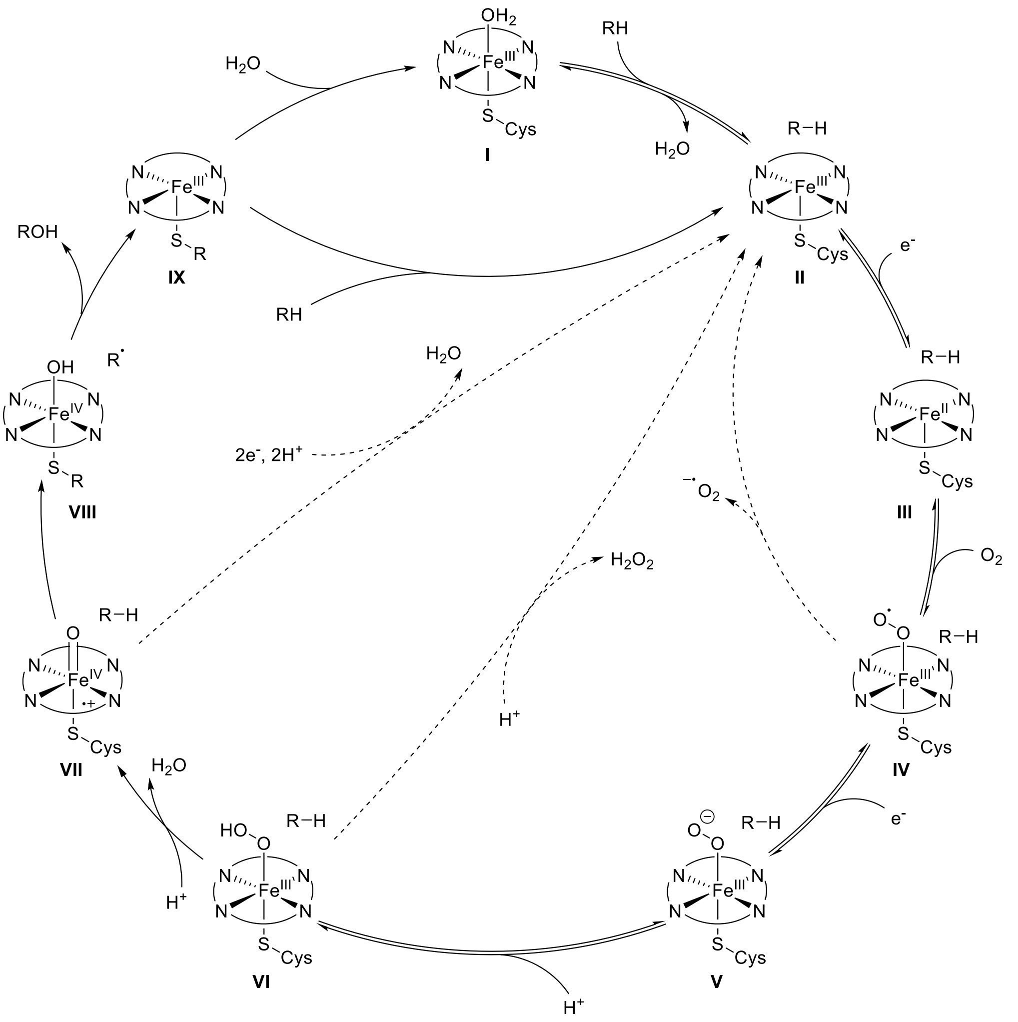
Figure 1.1.1: Phases I-IV of metabolism in plants. Phase I consisting of oxidation by CYP450s, Phase II involving tagging by GSTs (see text) or UGTs (not mentioned further), and phases III and IV whereby transport to the vacuole instigates further degradation.

The number of CYP450s present in plants far outweighs that of their mammalian counterparts, it is suspected that this is due in part to the sessile nature of plants but can also be attributed to the polyploidy often observed in plant species.[12,13] In addition to this, they mediate the biosynthesis of plant defence compounds, fatty acids, lignins and secondary metabolites.[14] Given the vast number of CYP450s in plant species - there are 272 CYP450 encoding genes in Arabidopsis Thaliana, versus only 57 in humans - it is surprising that at the time of writing there are only two examples of plant CYP450 crystal structures.[12,15–17] CYP450s are in general difficult to isolate, with the majority of X-ray structures still being from prokaryotic sources on account of their simplicity in comparison to their eukaryotic counterparts.[18]

The membrane-bound nature of eukaryotic CYP450s and their coupling to CYP450 reductases complicates their purification and can partially account for this lack of data. For this reason, their use as biocatalysts is also limited. CYP450 enzymes used for biocatalysis are typically those from bacterial species due to their non-membrane bound nature and the fact that that the CYP450 itself is bound to its cofactor as a single entity.[19] Cost also remains a challenging problem to overcome, with the need for external cofactors in the case of eukaryotic CYP450 enzymes being an additional consideration when using them as catalysts.

Given the large number of CYP450 enzymes in plants, their gene sequence similarity is incredibly low, with some being less than 20% identical. The enzyme's active site structure is well conserved however, with the active site consisting of an iron centre bound by heme at four coordination sites. The other two sites are occupied by the thiolate of a conserved cysteine residue and a molecule of water.[12,20] It is suspected that only a dozen CYP450 enzymes from the 57 in humans are responsible for up to 80% of drug metabolism.[15] The exception to the promiscuity of CYP450 oxidations is that of enzymes with anabolic roles, whose substrates are often better defined.[21]

The prototypical reaction of these enzymes is the insertion of a single oxygen atom from molecular oxygen into a C-H bond with the other oxygen atom being reduced to water, although alternative reactions are also possible.[21] The catalytic cycle has been debated since the 1970s with new iterations being presented throughout the proceeding decades. Today, the catalytic cycle is broadly agreed upon and summarised below (Scheme 1.1.1).[21]



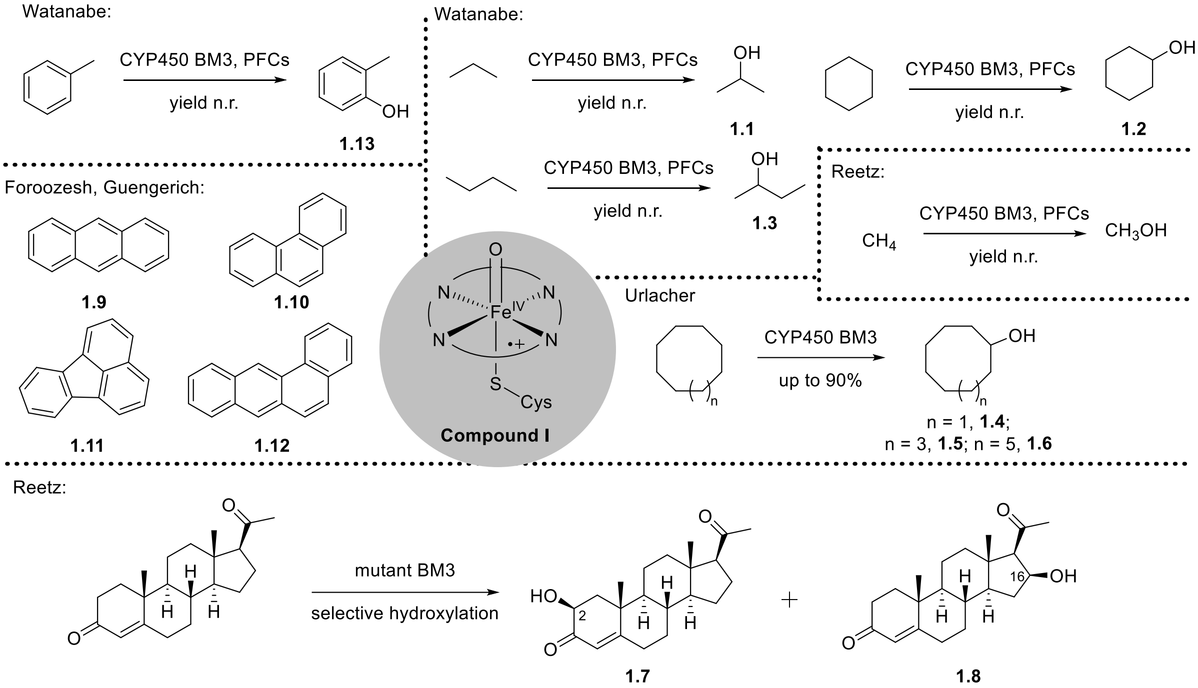
Scheme 1.1.1: Catalytic cycle adapted from Wong *et al.* for oxidation by CYP450 enzymes showing intermediates **VI** and **VII** commonly referred to as 'compound 0' and 'compound I' respectively.[21] Intermediate **VI** has been implicated as a putative oxidant whilst intermediate **VII** is thought to be responsible for the majority of CYP450 oxidations.[21] Non-productive termination pathways are shown with dashed arrows.

In the resting state **I**, iron(III) is flanked on all sides by the heme system (shown above in simplified form) which provides four of the six ligands. The axial ligands are a thiolate anion from a conserved cysteine and a molecule of water; in this state, the iron centre is low spin. When a substrate enters the active site, the water ligand is displaced giving species **II**. This is associated with a change in spin state of the iron centre from low spin to high spin and an increase in reduction potential allowing the first electron transfer (likely from NADH or NADPH *via* the cytochrome P450 reductase), forming the iron (II) species **III**. Oxygen rapidly binds to this species to give the iron(III) oxy-species **IV**, which is further reduced to the peroxy-species **V** by a second electron transfer.

Protonation events first form hydroperoxyl species **VI**, widely termed ‘compound 0’, which is proposed as a putative oxidant in its own right. This species can undergo a second protonation and heterolytic O-O cleavage to release water and give ‘compound I’ **VII**. ‘Compound I’ is the oxidant in the majority of CYP450 oxidations, which proceed through a radical rebound sequence. First, a hydrogen atom is abstracted from the substrate, giving species **VIII** and $R^{\bullet}$. Recombination of this radical with the hydroxy radical from the iron(IV) species **VIII** has the net effect of inserting an oxygen atom into a C-H bond in the substrate. This has the effect of simultaneously returning the iron species back to the +3 oxidation state (entity **IX**). This intermediate can then return to either the resting state **I** or to intermediate **II** following ligation by either a molecule of water or another substrate molecule, respectively.[13,20,21]

At various points in the mechanism it is possible for the catalytic cycle to terminate, returning back to species **II**. For instance, if the second electron is not supplied rapidly enough to intermediate **IV**, it is possible for superoxide $O_2^{\bullet -}$ to be lost. Likewise, the iron-bound oxygen atom of species **VI** can be protonated when the substrate fit is not ideal, causing hydrogen peroxide to be lost and catalytic intermediate **II** to be reformed. ‘Compound I’ **VII** may be formed in the presence of substrates with no suitable hydrogens for abstraction; in this case, reduction gives intermediate **II** similarly to the above cases.

The summary provided here is by no means exhaustive. A vast amount of work has been conducted in the elucidation of the CYP450 catalytic cycle and a full discussion is beyond the scope of this thesis. Despite the primary research focus on human CYP450s being understanding drug metabolism pathways for drugs and biosynthesis, utilisation of CYP450s as catalysts for selective oxidations in synthesis and to provide alternative routes towards feedstock chemicals is sought (Scheme 1.1.2).[22] CYP450s from various organisms have been used to this end, with CYP450 BM3 (isolated from *Bacillus megaterium*) receiving special interest, likely due to its nature as a single soluble polypeptide unit. The difficulties associated with cofactor dependence, stability of the enzymes and cost are still detrimental to the wide adoption of CYP450s as industrial biocatalysts.[23]



Scheme 1.1.2: Representative examples of CYP450 catalysed oxidations.

Beginning with alkanes, notoriously hard substrates to selectively oxidise by chemical methods, the Watanabe group showed the ability of CYP450 BM3 to oxidise simple alkanes such as propane, butane, and cyclohexane to the alcohols **1.1** to **1.3**. The Reetz group expanded this work enabling the oxidation of methane, giving methanol as the product. Interestingly, the terminal position is not oxidised for the longer alkyl chains; in both of the above cases, the addition of perfluorinated carboxylic acids was required to act as an inert reaction partner to trick the active site of the enzyme.[24] The catalytic cycle could not be initiated in the presence of the short chain alkanes exclusively with the oxidatively inert perfluorinated carboxylic acids being required to sit in the active site to mimic the native substrate, allowing the short chain alkanes to be tolerated.[24,25]

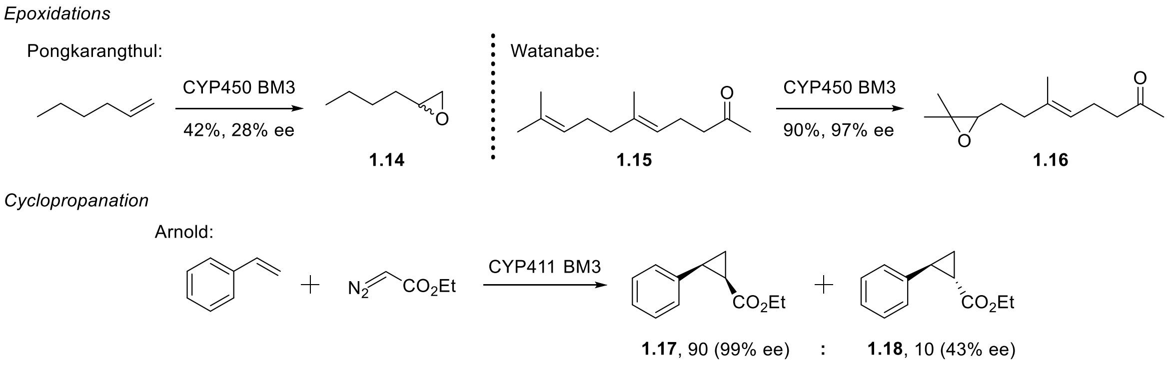
Similarly, Urlacher and co-workers have demonstrated the use of CYP450 BM3 mutants for the oxidation of cycloalkanes. Cyclooctane, cyclodecane and cyclododecane were oxidised to their respective monoalcohols **1.4** to **1.6** in up to 90% yield. With the substrates here being closer in size to the fatty acid native substrates for the enzymes, the addition of inert reaction partners was not required.[26] The hydroxylation of C-H bonds is not limited to simple alkanes; Reetz and co-workers also demonstrated that using CYP450 BM3 mutants selective oxidation of the C-2 and C-16 positions of progesterone could be achieved giving the respective alcohols **1.7** and **1.8**.[27]

Polyaromatic molecules are also known substrates for CYP450s, with the Guengerich and Faroozesh groups having identified a number of CYP450 inhibitors **1.9** to **1.12**.[28,29] Although they did not identify the oxidation products of these aromatic compounds, monohydroxylation would appear to be a sensible pathway. In a similar vein, the Watanabe group showcased the oxidation of toluene giving primarily *otho*-cresol **1.13**.[30] Again, this research required the addition of perfluorinated decoy molecules to allow the oxidation to proceed.

A whole cell biocatalysis approach, although still in its infancy for oxidations, was utilised for the epoxidation of 1-hexene to its respective epoxide **1.14** in a moderate yield of 42% by Pongkarangthul *et al.* (Scheme 1.1.3).[31] This transformation was found to proceed with a very slight preference for formation of the (*R*)-enantiomer. The Watanabe group demonstrated the regio- and enantioselective epoxidation of the monoterpene geranylacetone **1.15** with

CYP450 BM3, although the absolute configuration of epoxide enantiomer **1.16** was not elucidated. This oxidation protocol was also applicable to related monoterpenes and no over oxidation is observed in any of the examples.[32]

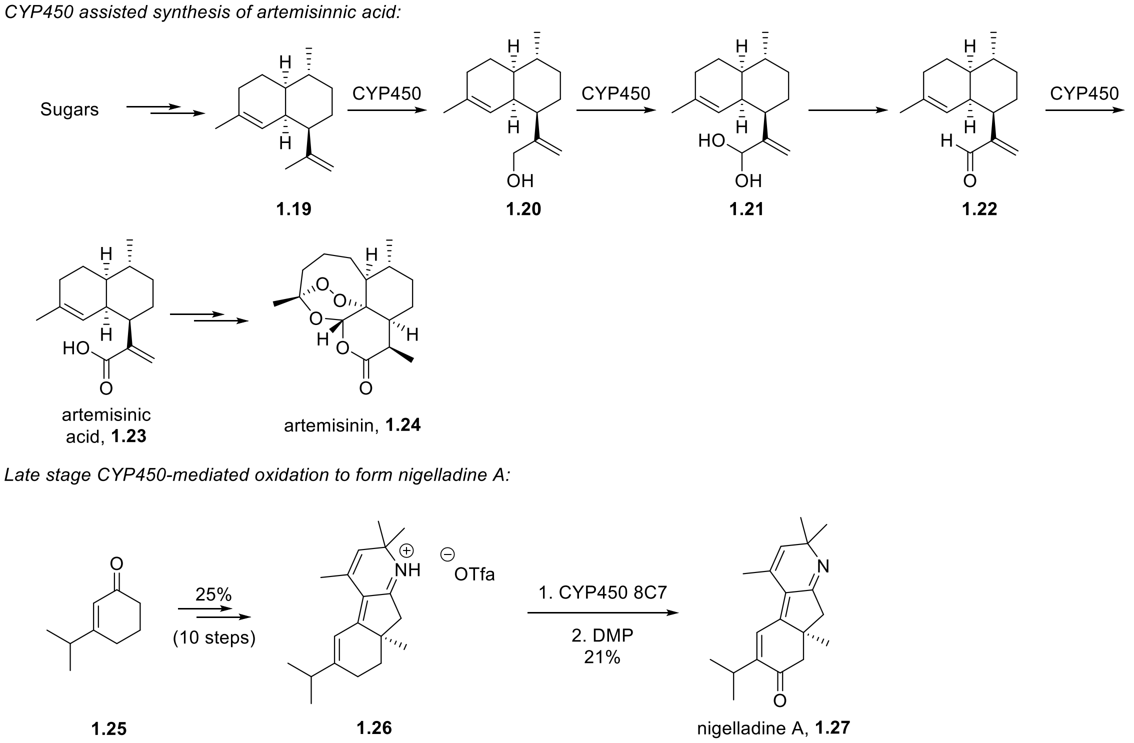
Perhaps the most striking example of the versatility of CYP450s and the power of gene editing, Arnold and co-workers demonstrated carbene transfer by enzymes dubbed CYP411s, mutated CYP450 enzymes from CYP450 BM3.[33] Their pioneering work enabled formation of 1,2-substituted cyclopropanes **1.17** and **1.18** from styrene (Scheme 1.1.3).



Scheme 1.1.3: Further examples of CYP450 oxidations not limited to hydroxylation and use of engineered CYP411 for the formation of cyclopropanes.

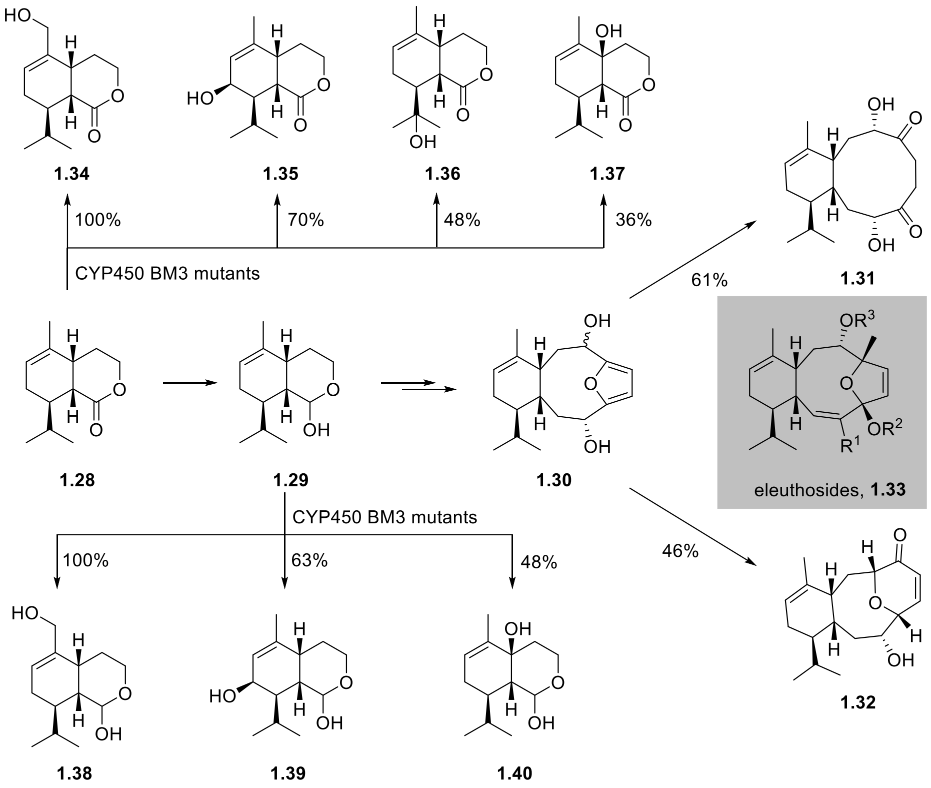
CYP450 oxidations have also found use in natural product synthesis; the most notable example being in the industrial synthesis of artemisinic acid **1.23**, a precursor to the widely used antimalaria drug, artemisinin **1.24** (Scheme 1.1.4).[34] CYP71AV1, isolated from *Artemisia annua*, catalysed three consecutive steps on the biosynthetic pathway to artemisinic acid. From alkene **1.19**, two successive oxidation events form the alcohol **1.20** and then acetal **1.21**, which undergoes dehydration *in situ* to form aldehyde **1.22**. This aldehyde undergoes a third CYP450-mediated oxidation giving artemisinic acid **1.23**, which can be chemically converted to artemisinin **1.24**. This methodology has since been expanded to enable the robust synthesis of artemisinic acid **1.23** on an industrial scale.[35,36]

More recently, Stoltz and co-workers used CYP450 8C7, engineered from CYP450 BM3, to enact a late-stage oxidation on their route to nigelladine A **1.27** (Scheme 1.1.4).[37] Standard chemical oxidation conditions were unsuccessful in their route but the use of biocatalysis gave access to their desired natural product from iminium salt **1.26**, derived from enone **1.25** over 10 steps.



Scheme 1.1.4: Synthesis of artemisinic acid **1.23** using CYP71AV1 for three successive oxidation steps from alkene **1.19**. Synthesis of nigelladine A **1.27** using late stage CYP450 oxidation where other chemical methods failed.

Similarly, the Robertson and Wong groups have reported the use of mutant CYP450 BM3 libraries for the generation of intermediates useful for total synthesis (Scheme 1.1.5).[38] Although oxidation of tricyclic intermediate **1.30** led to products **1.31** and **1.32** having undergone structural rearrangements not conducive to the synthesis of analogues of the eleuthoside **1.33** core, analogues **1.28** and **1.29** from earlier in the route were demonstrated to be amenable to numerous oxidations by various CYP450 BM3 mutants giving alcohols **1.34** to **1.40** which could find use in the synthesis of the desired analogues of the eleuthosides.



Scheme 1.1.5: Robertson and Wong use of CYP450 BM3 mutants in the oxidation of intermediates relevant for the synthesis of the eleuthoside **1.33** natural products.

CYP450 enzymes are not only integral in biosynthesis and metabolism and thus require an intense amount of study in the context of drug development, but also their versatility as enzymes for oxidation has been highlighted with a number of examples above. There is a great deal of untapped potential however given that there over 7500 annotated plant CYP450s across the genomes of various plant species, the presence of only two X-ray crystal structures from plants shows the limit to current understanding. Understanding the oxidation events carried out by this superfamily of enzymes across multiple species remains elusive. With this in mind, the next section will introduce the use of activity-based protein profiling (ABPP) with a view to using this method for the study of plant CYP450 enzymes.

1.2. Activity-Based Protein Profiling and Chemical Probes

Following the genome sequencing projects, the assignment of function to the newly-identified proteins in organisms has become a challenge.[39] Although genome editing methods improve the understanding of the interconnectivity of cellular processes, they are limited by the fact that many proteins are regulated by a host of post-translational modifications.[40,41] Activity-based protein profiling emerged at the beginning of this century as a way to natively study the activity of proteins by inactivation using chemically reactive molecules.[42]

The power of ABPP is in its ability to target subsets of the active proteome at a given time within biological systems, which is achieved thanks to the inherent reactivity of the chemical probes utilised. These probes reach the active site of a target protein and covalently bind through a reactive handle. From the perspective of the enzymes, they must be in an active conformation; ABPP allows for evaluation of protein activity rather than just the expression level as is the case for the genome editing methods (Figure 1.2.1).

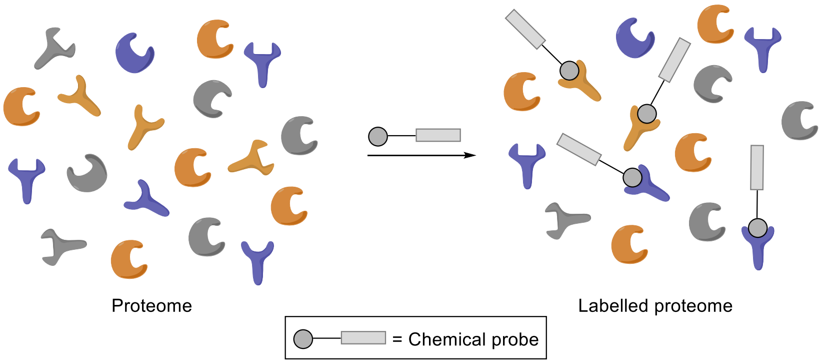


Figure 1.2.1: Graphical representation of ABPP showing the labelling of only a subset of the total possible proteins in a native system.

The general structure of the probes used for ABPP consist of four main elements: the warhead, directing group, linker portion (often optional), and finally the reporter tag (Figure 1.2.2).[41] Each of these elements enables the probe to carry out its role; the warhead unit is the group that undergoes chemical reaction with the enzyme of choice, with the directing group enabling the probe to make it to the active site. The reporter tag is critical for analysis, with the option to either directly observe labelling using fluorophores, or use pull-down experiments with biotin. The biotin pull-down method relies upon the tight binding affinity between biotin and streptavidin to purify the labelled proteins from their unlabelled counterparts.

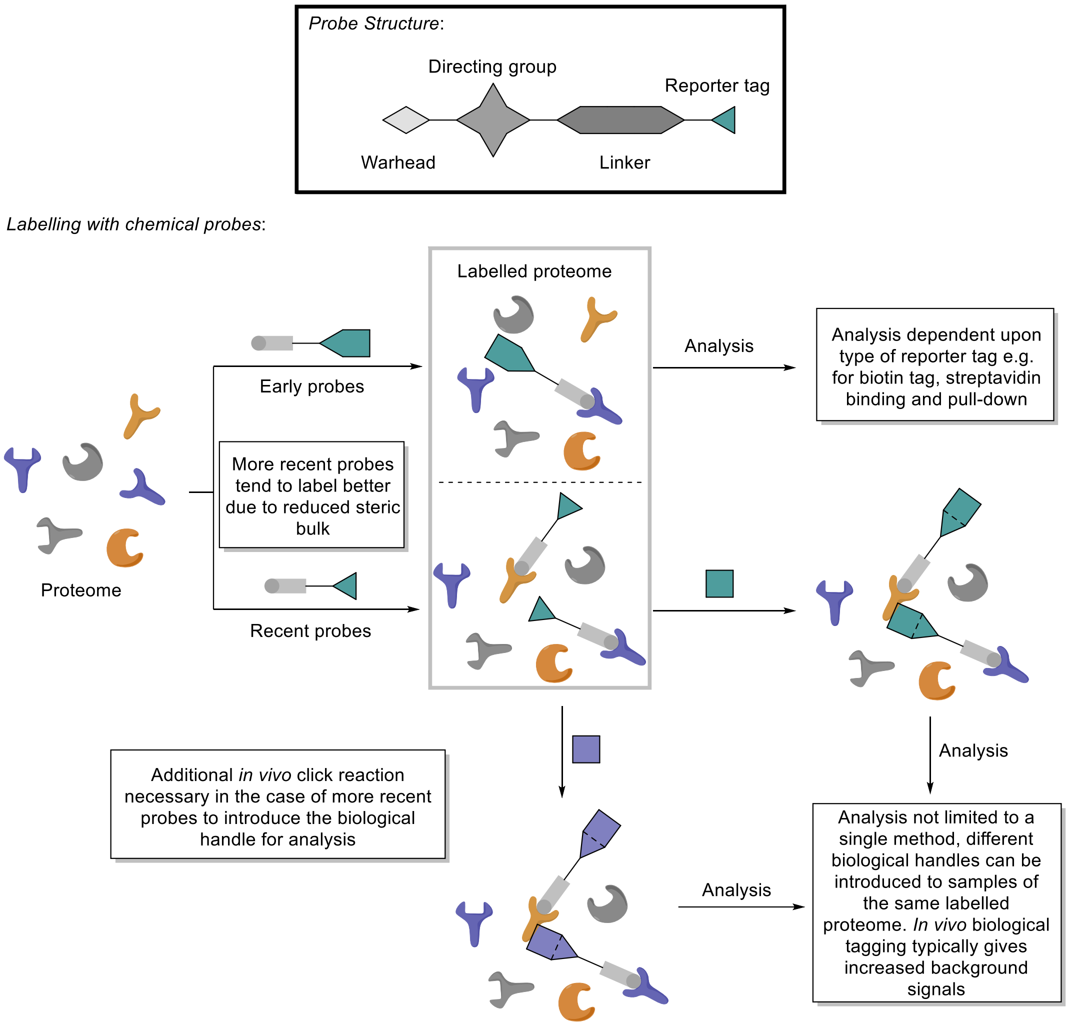


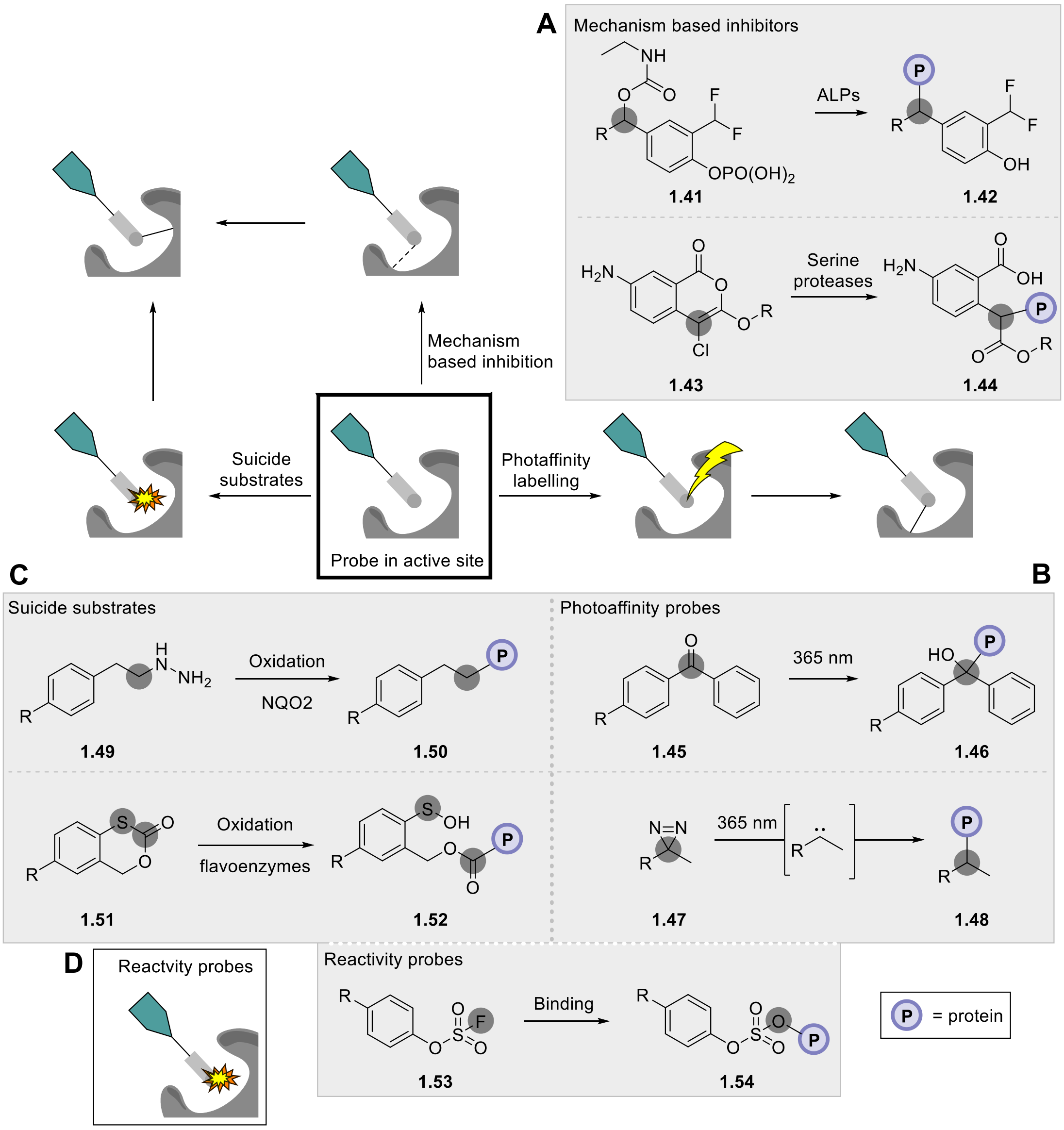
Figure 1.2.2: Structure of probes containing reporter tags or handles for further functionalisation and their use in ABPP labelling.

Early probes typically incorporated the fluorophore or biotin tag within the structure as the reporter tag. This can present problems due to the steric bulk of the tags, with probes of this type typically having molecular weights between 700-1000 Da.[43] More recently, efforts have been made to incorporate this tag after labelling of the proteome using *in vivo* click chemistry methods. The reporter tag then functions as a handle for this derivatisation rather than the tag itself.[44,45] It should be noted that the method of *in vivo* labelling, whilst useful in negating the steric problems of the early probes, faces its own challenges. The background contamination observed in the gel analysis in this case can often obscure the observation of bands corresponding to proteins expressed at low levels.

The warheads can be classified into four categories: mechanism-based inhibitors, photoaffinity probes, suicide substrates, and reactivity probes (Scheme 1.2.1).[46,47] The first and most common are mechanism-based inhibitors, which rely on the exploitation of the native reactivity of the enzyme (Scheme 1.2.1, **A**).[48–50] Alkaline phosphatases (ALPs) were labelled successively by the Xie group using carbamate probes of the type **1.41** that form conjugates **1.42** *via* the *p*-quinone methide.[48] In another example, probes with the scaffold **1.43** could be hydrolysed by serine proteases giving probe conjugates akin to **1.44**.[49] Given that many enzymes tend to have covalent intermediates in their pathways, mechanism-based inhibitors lock the enzyme in the covalently bound state by mimicking the native substrates.

Secondly, photoaffinity probes whose activation is dependent upon irradiation by light, have also found wide use (Scheme 1.2.1, **B**). Probes of this type are typically modified reversible inhibitors, containing photoreactive groups that can be triggered by irradiation with light initiating binding to proximal reactive groups in the active site.[51–53] In two examples, benzophenones **1.45** and diazirenes **1.47** were utilised under irradiation with 365 nm light to give their respective bound conjugates **1.46** and **1.48**.

Thirdly, suicide substrates operate in a similar manner to mechanism-based probes (Scheme 1.2.1, **C**). However, the warhead undergoes modification by the target enzyme and a highly-reactive (not covalently bound) intermediate is formed.[54,55] This then reacts with nearby residues, the risk here being that the intermediate could diffuse and label other proteins instead of the target protein. In the case of the examples shown, *N*-ribosyldihydronicotinamide quinone reductase 2 (NQO2) was able to oxidise hydrazine probes **1.49**, their respective radical species being quickly trapped to the conjugate **1.50** in the active site.[55] Additionally, flavoenzymes oxidised 1,3-oxathiin-2-one probes **1.51** to give acyl sulfoxides that were readily trapped as their adduct **1.52**.[54]



Scheme 1.2.1: Summary of different warhead classes and examples of probes used in each of the classes for ABPP purposes.

The final type of probe, and perhaps the least selective, are reactivity probes (Scheme 1.2.1, **D**). These consist of a reactive chemical moiety (typically iodoacetamide or sulfonyl fluoride **1.53** shown) that can react with exposed nucleophilic residues from a variety of enzyme classes.[56–58] These probes are prone to off-target labelling by virtue of their high reactivity.

A number of classes of enzyme have been targeted using ABPP, which include but are not limited to: hydrolases, kinases, glycosidases and oxidoreductases.[59–64] Of particular interest are the oxidoreductase class of enzymes, into which CYP450s fall. Despite reports in the mid-1980s as to the applicability of aryl alkynes **1.55** to **1.58** as mechanism-based inhibitors of CYP450s, it was not until 2007 that Wright and co-workers published the first chemical probe 2-EN ABPP **1.59** for the CYP450 superfamily (Figure 1.2.3).[65–68]

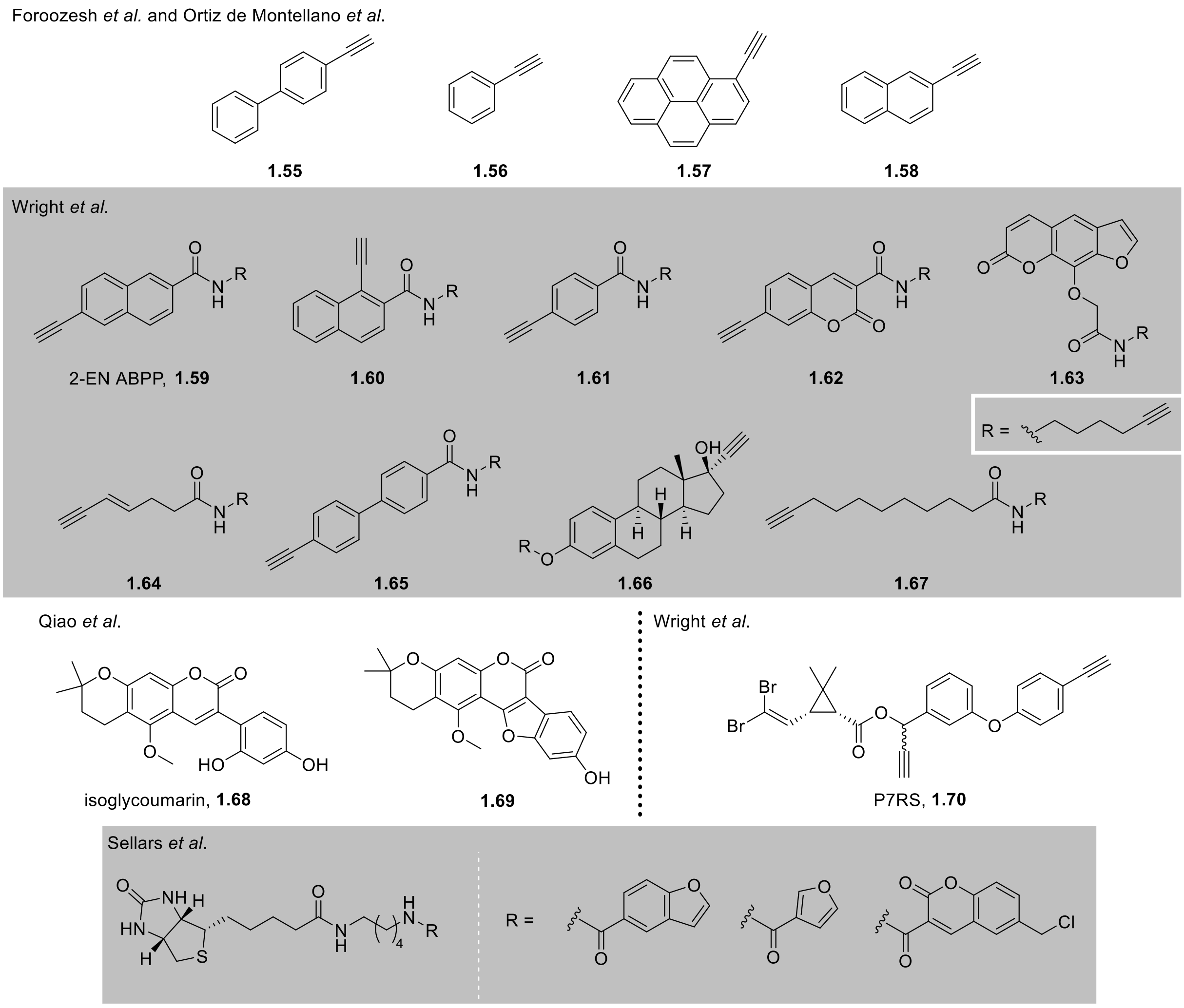
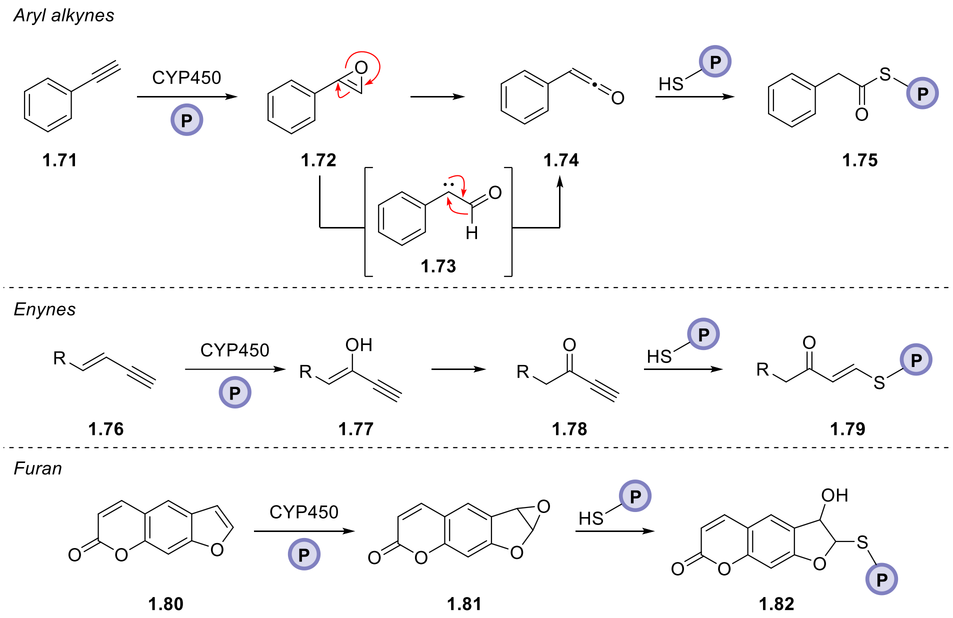


Figure 1.2.3: Aryl alkyne structures **1.55** to **1.58** found to inhibit CYP450s in the late 1900s and Wright and co-workers' probe library for human CYP450 enzymes.[69] Probes for CYP450s by Qiao, Wright and Sellars *et al*.[70–72]

In the first instance, Wright selected 2-ethylenenaphthalene **1.58** as the warhead group based upon structures previously identified.[73] Following successful labelling, it was demonstrated that a suite of nine chemical probes **1.59** to **1.67** were able to differentially label CYP450s.[69] However, the labelling of CYP450s by ABPP methods is still limited. Further studies showcased the labelling ability of a small coumarin-based library against human CYP450s and a set of pyrethroid-type probes against mosquito CYP450s, the best examples of which are shown **1.68, 1.69** and **1.70** respectively.[70,71] Recent work by Sellars and co-workers demonstrated that the biotin group is tolerated in chemical probes targeting CYP450s. In their work, a small library of probes were shown to label human CYP450s, namely CYP3A4.[72] As seen in these examples, the labelling of CYP450s by chemical probes is still limited largely to human CYP450s, with research into other organisms being limited.

The probes outlined above are expected to inactivate CYP450s by one of three mechanisms depending on their structure. In the first case, oxidation of the terminal aryl alkyne **1.71** to the short-lived oxirene intermediate **1.72** is thought to be followed by rearrangement to the reactive ketene species **1.74** *via* carbene **1.73**, readily trapped by cysteine in the CYP450 active site giving probe-protein conjugate **1.75**. For general enynes **1.76**, the internal olefin position is first hydroxylated to enol **1.77**, this is followed by tautomerisation to the conjugated ketone **1.78** that can undergo a Michael-type addition, again with residues in the active site giving α,β-unsaturated ketone **1.79**. Finally, the benzofuran reactivity is similar to the aryl alkynes. Oxidation of the furan of **1.80** to epoxide **1.81** is followed by trapping with nucleophilic residues giving **1.82** (Scheme 1.2.2).[74,75]

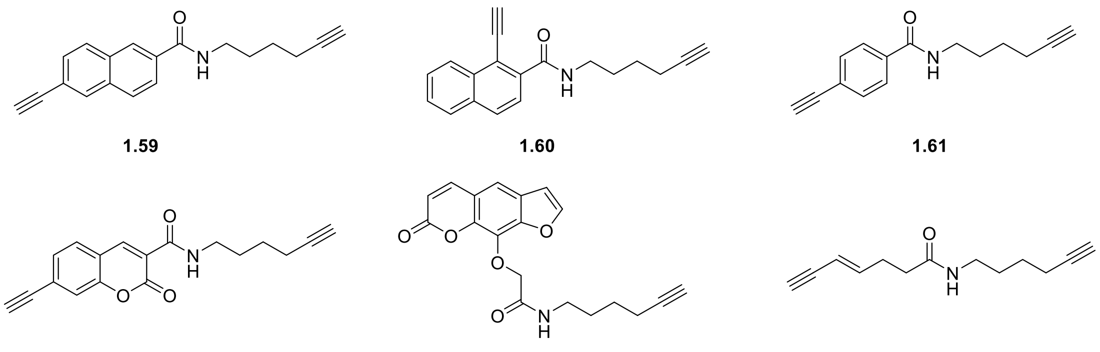


Scheme 1.2.2: Inactivation mechanism of chemical probe warheads **1.71**, **1.76** and **1.80** for labelling of CYP450s.

1.3. Project Aims

Given the importance and versatility of CYP450 enzymes in biosynthesis and metabolism of xenobiotics, twinned with their increasing use both industrially and in natural product synthesis, the further study of this superfamily of enzymes was an attractive target. Due to the large amount of CYP450s present in plant species versus their mammalian counterparts and their role in herbicide metabolism and resistance, a more comprehensive understanding of their metabolic pathways is necessary.

ABPP was selected as a viable method for this task. The power of ABPP to identify only active enzymes within the proteome marked it as the prime candidate for deconvoluting the key players in herbicide metabolism. With several probe structures known to be effective in labelling human CYP450s, a number of these were selected for synthesis and evaluation against plant CYP450s (Figure 1.3.1).


1.59 **1.60** **1.61**

1.62 **1.63** **1.64**

Figure 1.3.1: Initial probes **1.59** to **1.64** selected for synthesis and evaluation of their activity against plant CYP450s.

Provided that the labelling translated well into plant CYP450s, an area not yet explored in the literature, the library of probes could be expanded to help identify the specific enzymes responsible for herbicide metabolism in plants. This was hoped to be achieved by utilising the established ABPP procedures (*vide* supra). The probe library could be used in a number of plant species of interest to the agrochemical industry with potential expansion into other agrochemically-relevant organisms such as fungi.

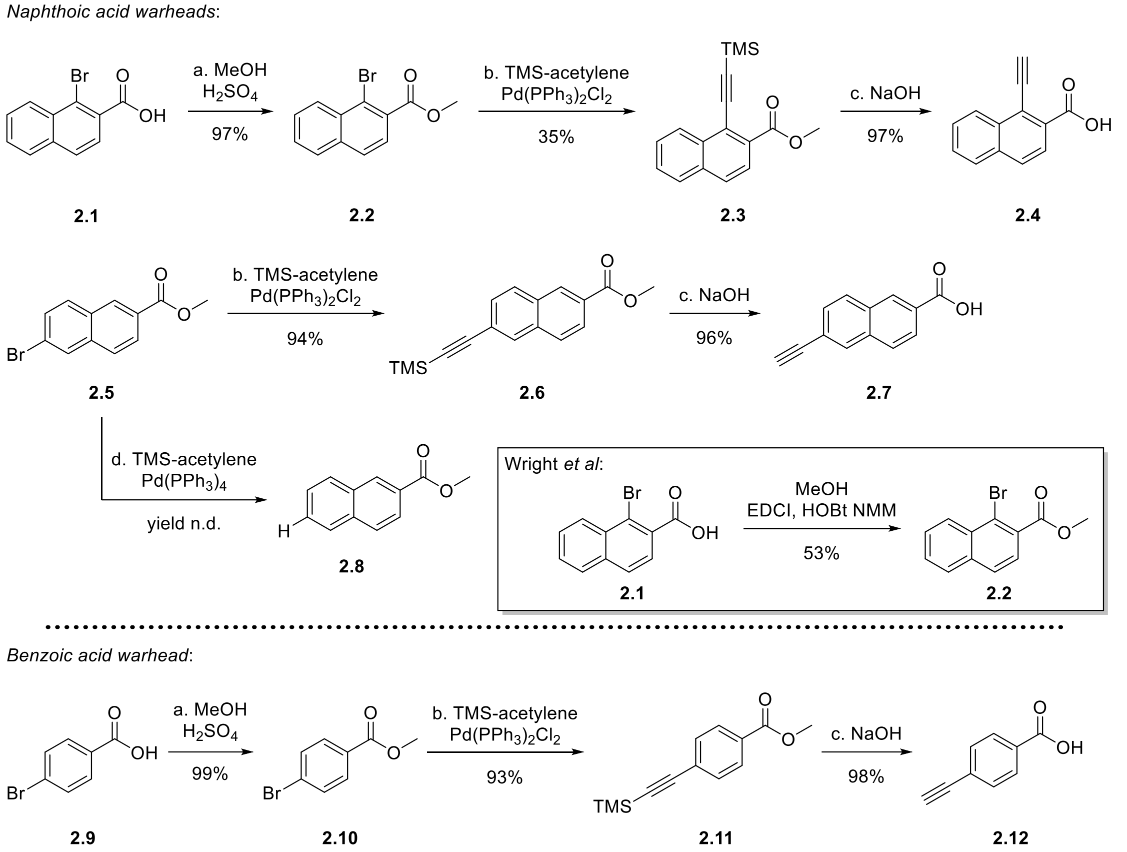
Ultimately, and with a suite of probes in hand to label plant CYP450s, the metabolic pathways of plants could be better understood. This could find application in the continued efforts to grant food security to a growing population. Chapter 2 outlines the synthesis of chemical probes across a number of generations in both click tag and biotinylated forms to enable biological studies to commence.

2. Synthesis of Probes

2.1. Synthesis and Optimisation of Warheads

As identified previously (1.3 Project Aims), several of the initial probes used by Wright *et al*. in their study demonstrating successful labelling of human CYP450s expressed in insect cells had generated interest as being possible candidates for use in the pursuit of CYP450 labelling in plants.[69] The synthetic endeavours in this area began with the synthesis of the warheads necessary to make these probes. Although the syntheses of several of the probes themselves had been presented in the work by Wright *et al*., many of the intermediates within the routes were lacking in experimental detail.[69] Therefore, these gaps in the experimental protocols were plugged with newly developed conditions presented in the section below; alterations were made to the reported experimental conditions where applicable, in order to provide the project with robust routes to key intermediates for subsequent probe derivatisation.

The synthetic studies towards the naphthoic acid warheads **2.4** and **2.7** were relatively simple and hence these warheads were tackled first (Scheme 2.1.1, top).



Scheme 2.1.1: Synthesis of naphthoic acid warheads **2.4** and **2.7** *via* an esterification (for 1-bromo-2-napthoic acid), Sonogashira, and deprotection sequence (top). Synthesis of benzoic acid warhead **2.12** (bottom). Esterification conditions employed by Wright *et al*. (boxed). Reagents and conditions: a) H_2SO_4 (cat.), MeOH, reflux, 18 h, 97% (1-Br naphthoate), 99% (4-Br benzoate); b) TMS-acetylene (2.0 equiv.), $Pd(PPh_3)_2Cl_2$ (5.0 mol%), CuI (10 mol%), Et_3N (2.0 equiv.), MeCN, 90 °C, 16 h, 35% (1-(trimethylsilyl)ethynyl naphthoate), 94% (6-(trimethylsilyl)ethynyl naphthoate), 93% (4-(trimethylsilyl)ethynyl benzoate); c) 1 M NaOH (aq.), EtOH/CH_2Cl_2 (2:1), rt, 16 h, 97% (1-ethynyl naphthoic acid), 96% (6-ethynyl naphthoic acid), 98% (4-ethynyl benzoic acid); d) TMS-acetylene (1.2 equiv.), $Pd(PPh_3)_4$ (5.0 mol%), CuI (10 mol%), Et_3N (2.0 equiv.), rt, 1 h, yield n.d.

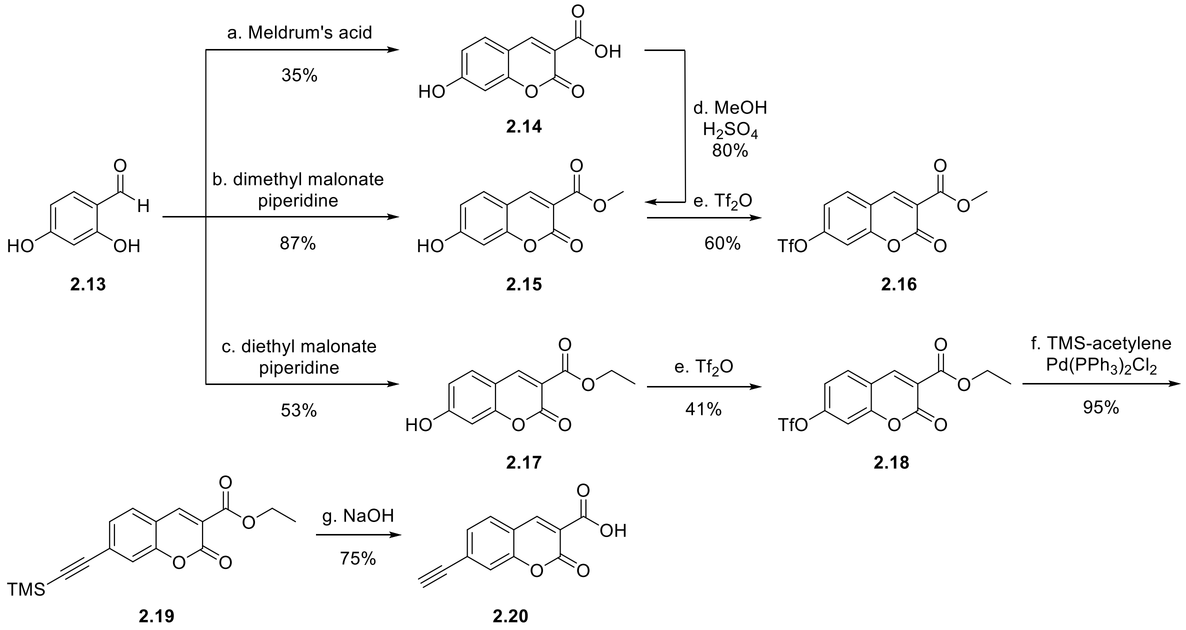
Given the commercial availability of methyl 6-bromo-2-naphthoate **2.5**, the first step of this sequence was the synthesis of the analogous methyl ester **2.2** from 1-bromo-2-napthoic acid **2.1** in order to set up the intermediates required for the Sonogashira reaction. This was achieved in a facile manner; simply heating the parent acid **2.1** at reflux in methanol under acid catalysis gave the desired methyl ester **2.2** in a near quantitative yield. This was in contrast to the 53% observed in procedure conducted by Wright *et al*. previously, wherein they utilised the coupling reagent EDCI in the presence of HOBt and a base (NMM).[69]

The Sonogashira reaction was initially attempted on methyl 6-bromo-2-naphthoate **2.5** using $Pd(PPh_3)_4$ as the palladium source; however, under these test reaction conditions the protodehalogenated compound **2.8** appeared to be the only isolable product from the reaction mixture after stirring for 1 h at rt; this was however not fully characterised (Scheme 2.1.1). The lack of the desired mass for product in the mass spectrum nor a peak with the 1:1 bromide isotope pattern in the LRMS spectrum ruled out either starting material or product, with presence of a peak at *m/z* 187.1 (corresponding to the $M+H^+$ ion of protonated naphthoate **2.8**, $C_{12}H_{11}O_2^+$) giving a good indication that protodehalogenation had occurred instead.

Switching to conditions more akin to those employed by Wright *et al*. (despite their exact conditions not being reported) and using $Pd(PPh_3)_2Cl_2$ with acetonitrile as the solvent, gave the desired 6-(trimethylsilyl)acetylene **2.6** in a 94% yield (Scheme 2.1.1). This reaction required heating to 90 °C; stirring at rt led to no conversion. The conditions were repeated in order to synthesise the analogous 1-(trimethylsilyl)acetylene **2.3**, albeit with a significant drop in yield likely due to the increased steric bulk at the reactive position. The methyl esters **2.3** and **2.6** were saponified with concomitant deprotection of the TMS-protecting groups by utilising the conditions previously reported by Wright *et al*. (NaOH, EtOH, CH_2Cl_2), giving the respective warheads **2.7** and **2.4** in near quantitative yields.

Given the ease of access to the naphthoate warheads, the corresponding warhead from benzoic acid was synthesised in identical fashion (Scheme 2.1.1, bottom). Esterification of the benzoic acid **2.9** gave methyl ester **2.10**, which was then subjected to the established Sonogashira conditions to give 4-(trimethylsilyl)acetylene **2.11** with both of these steps providing the products in excellent yields. The Sonogashira product **2.11** underwent clean saponification and deprotection giving the warhead **2.12**, again in near quantitative yield. The probe from this warhead had been shown to have good activity against human CYP450s, and the low cost of benzoic acid in comparison to the naphthoic acid/ester starting materials also made it a valuable intermediate not only as a probe, but also as a substrate for future test reactions in the event of synthetic issues.

With the simplest of the all-carbon containing aromatic warheads in hand, attention was turned to the coumarin warhead **2.20** (Scheme 2.1.2). The general strategy for the synthesis of this compound bore resemblance to the warheads already accessed; protection as the methyl ester, Sonogashira coupling to install the TMS-protected acetylene, and saponification/deprotection to give the warhead itself. A number of additional steps were required in order enact this; namely, formation of the coumarin core itself and activation of the phenolic position in order to carry out the Sonogashira coupling.

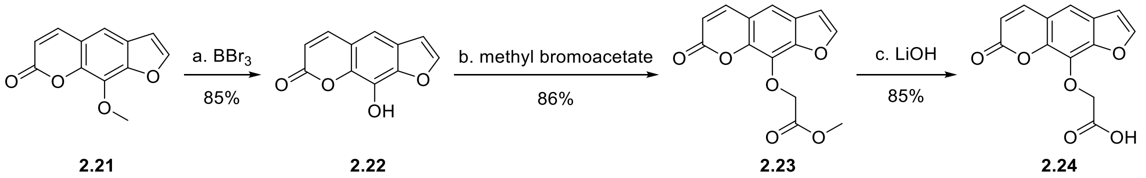


Scheme 2.1.2: Synthesis of coumarin warhead **2.20**. Reagents and conditions: a) Meldrum's acid (1.3 equiv.), H_2O, reflux, 18 h; b) dimethyl malonate (1.5 equiv.), piperidine (cat.), MeOH, 60 °C, 48 h, 87%; c) diethyl malonate (2.0 equiv.), piperidine (cat.), rt, 18 h, 53%; d) H_2SO_4 (cat.), MeOH, reflux, 18 h, 80%; e) triflic anhydride (1.2 equiv.), pyridine (1.2 equiv.), CH_2Cl_2, 0 °C, 1 h, 60% (methyl ester), 41% (ethyl ester); f) TMS-acetylene (2.0 equiv.), $Pd(PPh_3)_2Cl_2$ (5.0 mol%), CuI (10 mol%), Et_3N (2.0 equiv.), MeCN, 90 °C, 16 h, 95%; g) 1 M NaOH (aq.), EtOH/CH_2Cl_2 (2:1), rt, 16 h, 75%.

The coumarin core was tackled as the first step in the synthesis of this warhead. In the first instance, Knoevenagel condensation between 2,4-dihydroxybenzaldehyde **2.13** and Meldrum's acid gave acid **2.14** in a moderate yield of 35%. This could be protected as the methyl ester with the same procedure employed in the synthesis of warheads **2.7** and **2.12** (shown previously), giving the methyl ester **2.15** in good yield (87%). By using malonates as the Knoevenagel partner, similarly to Wright *et al.*, the coumarin synthesis could be expedited and the low yielding first step avoided.[69]

Using dimethyl malonate or diethyl malonate in this reaction allowed access to methyl ester **2.15** and ethyl ester **2.17** respectively, the disparity in yields in the formation of these compounds can be attributed to the workup and purification required for each of them. The reaction to form the methyl ester **2.15** could be worked up by washing with ice cold methanol to give material of acceptable purity for use in the next step, however this was not the case for the ethyl ester **2.17** which had to be purified by flash column chromatography. Given that phenols frequently streak during purification on normal phase silica, with this compound being no exception, a problematic purification led to a lower than hoped for yield. Triflation could be achieved on both the methyl and ethyl esters using triflic anhydride and pyridine to give the triflates **2.16** and **2.18**. Arbitrarily, only triflate **2.18** derived from the ethyl ester was carried through the remaining Sonogashira and saponification/deprotection sequence, giving the TMS-alkyne **2.19** and ultimately the coumarin warhead **2.20**.

With a number of warheads bearing the aryl alkyne moiety already obtained, the next focus was to move into warheads activated by CYP450s according to different mechanisms. The first of the probes of this type was psoralen warhead **2.24**, synthesised from the skin sensitiser methoxsalen, used in the treatment of various skin conditions (Scheme 2.1.3).

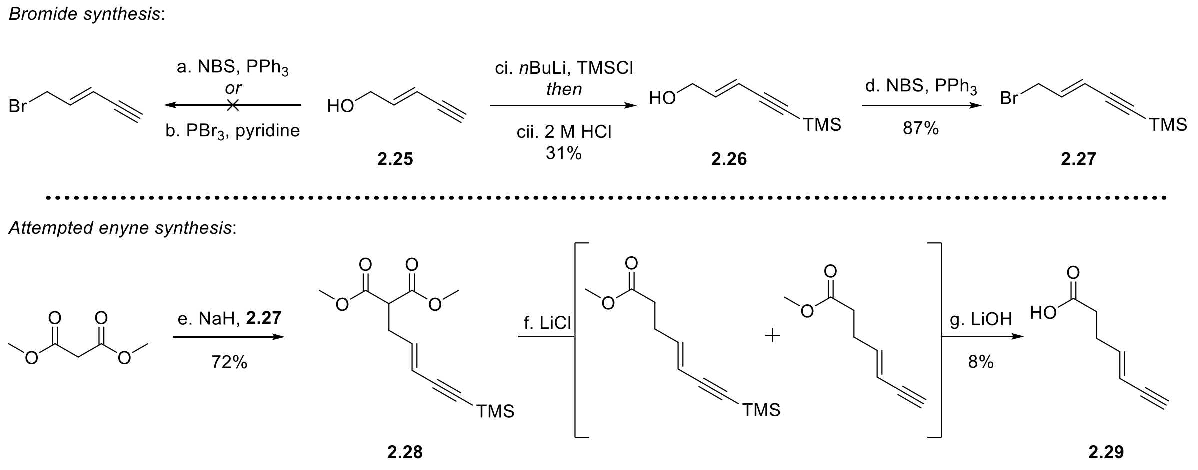


Scheme 2.1.3: Column-free synthesis of psoralen warhead **2.24**. Reagents and conditions: a) boron tribromide (2.0 equiv.), CH_2Cl_2, 0 °C – rt, 16 h, 85%; b) methyl bromoacetate (1.2 equiv.), K_2CO_3 (3.0 equiv.), DMF, rt, 16 h, 86%; c) lithium hydroxide monohydrate (2.0 equiv.), THF/H_2O (2:1), rt, 16 h, 85%.

The synthesis of the psoralen warhead **2.24** was carried out without the need for any column chromatography, with aqueous workups at each step providing material of sufficient purity to be used in the next step. Demethylation of methoxsalen **2.21** was carried out with boron tribromide to give free alcohol **2.22** which was derivatised into methyl ester **2.23** through S_N2 displacement of bromide from methyl bromoacetate and then deprotected with lithium hydroxide to give the psoralen warhead **2.24**.

The lithium hydroxide deprotection procedure was trialled at this stage as it was expected to provide more consistent results than the sodium hydroxide procedure used up until now. There had been isolated instances where the yield for saponification/deprotection had been lower than expected, it was thought that this might be due to insufficient mixing of the ternary system employed by Wright *et al*. Addition of solid lithium hydroxide to a mixture of THF and H_2O was expected to be a more miscible system and overcome the irregularities observed up until now. This procedural alteration was also accompanied by a change in workup; an aqueous wash with pH 1 sulfate buffer rather than acidification with hydrochloric acid was hoped to aid the successful retention of the product acid in the organic phase, on account of the higher ionic strength of pH 1 sulfate buffer versus hydrochloric acid, and therefore lower propensity of the polar organic material to partition into the aqueous phase. The yield of this reaction was slightly better than that obtained by Wright *et al.* in their report and it was operationally more straightforward; moving forward, this was used as the gold standard procedure for the saponification/deprotection sequence.[69]

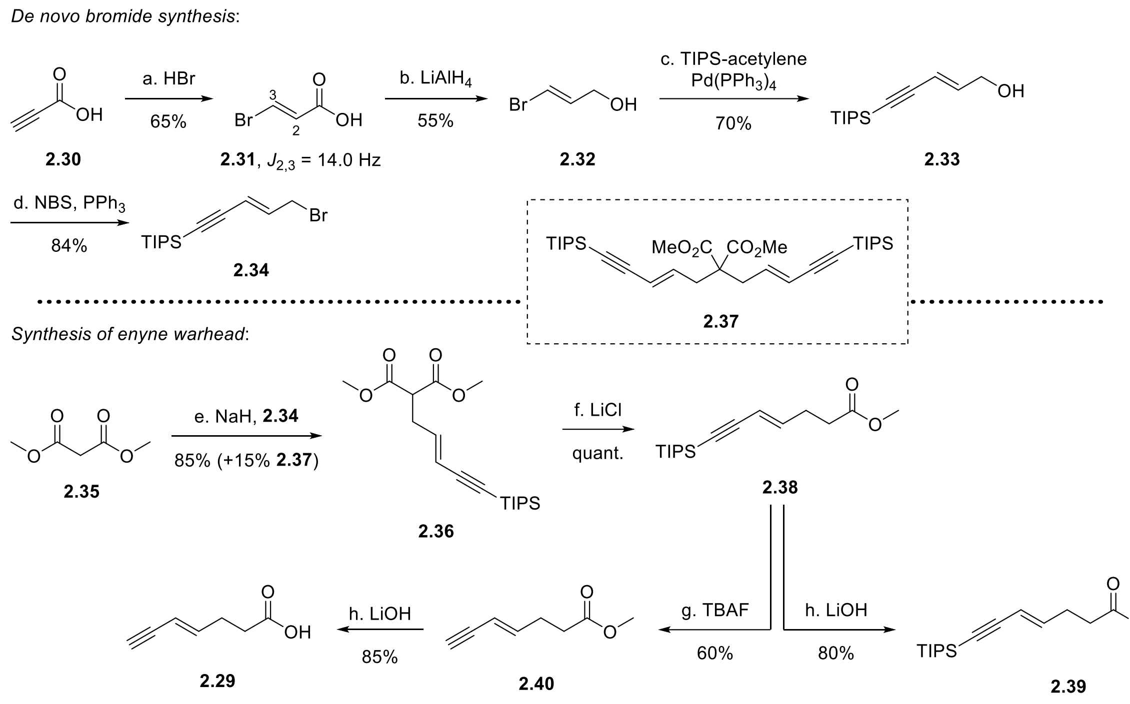
Next, the synthesis of the enyne warhead **2.29** was attempted, this warhead required the most deviation from the procedures set out in the work by Wright *et al*. on account of low yields found for a number of the steps using Wright's procedures.[69] The synthesis of bromide **2.27** necessary in the malonate alkylation was the first undertaking in the synthesis of this warhead (Scheme 2.1.4, top).



Scheme 2.1.4: Synthesis of bromide **2.27** for malonate alkylation (top), and attempted synthesis of enyne warhead **2.29** (bottom). Reagents and conditions: a) *N*-bromosuccinimide (1.2 equiv.), triphenylphosphine (1.2 equiv.), CH_2Cl_2, -30 °C – rt, 0.5 h, no product isolated; b) phosphorous tribromide (1.2 equiv.), pyridine (1.2 equiv.), CH_2Cl_2, 0 °C – rt, 1 h, no product isolated; ci) *n*-butyllithium (1.5 M in hexanes, 2.1 equiv.), trimethylsilyl chloride (2.1 equiv.), THF, -78 °C – rt, 2.5 h *then* cii) 2 M HCl (aq.), 0 °C, 1 h, 31%; d) *N*-bromosuccinimide (1.2 equiv.), triphenylphosphine (1.2 equiv.), CH_2Cl_2, -30 °C – rt, 0.5 h, 87%; e) NaH (0.66 equiv.), **2.27** (0.66 equiv.), DMF/THF (2:1), 0 °C – rt, 16 h, 72% (based on **2.27**); f) lithium chloride (5.0 equiv.), H_2O (0.1 equiv.), DMF, 150 °C, intermediate product not isolated; g) lithium hydroxide monohydrate (5.0 equiv.), THF/H_2O (2:1), rt, 16 h, 8%.

Despite a limited amount of literature precedent for this transformation, direct bromination of (*E*)-pent-2-en-4-yn-1-ol **2.25** could not be achieved with either *N*-bromosuccinimide and triphenylphosphine nor with phosphorous tribromide in the presence of pyridine, with intractable mixtures being formed in both cases.[76,77] Protection of the enyne group as the TMS-alkyne **2.26** *via* double deprotonation with *n*-butyllithium followed by quenching with trimethylsilyl chloride gave alcohol **2.26**, albeit in low yield. Alcohol **2.26** was then cleanly converted into the corresponding bromide **2.27** using the initial protocol with *N*-bromosuccinimide, attempted on the unprotected analogue. Alkylation of dimethyl malonate proceeded smoothly with bromide **2.27** to give malonate **2.28** (Scheme 2.1.4, bottom).

Problems were encountered at the following decarboxylation step; the reaction was attempted under classical Krapcho decarboxylation conditions, with the hope of isolating the intermediate methyl ester.[78] This compound could not be obtained cleanly and robustly, and the reaction proceeded with partial deprotection of the TMS-group, leaving the reaction for a longer time merely caused decomposition of the starting material/product. Telescoping the subsequent saponification step on the partially deprotected material was hoped to allow direct access to the enyne warhead **2.29**. However, the resulting crude material was not clean enough to be used in subsequent reactions and the material was difficult to purify by flash column chromatography on account of its high polarity. It was envisaged that a less labile alkyne protecting group would obviate the problems encountered in the Krapcho decarboxylation step; to this end, the TIPS-group was selected and the synthesis of bromide **2.34** was undertaken (Scheme 2.1.5, top).



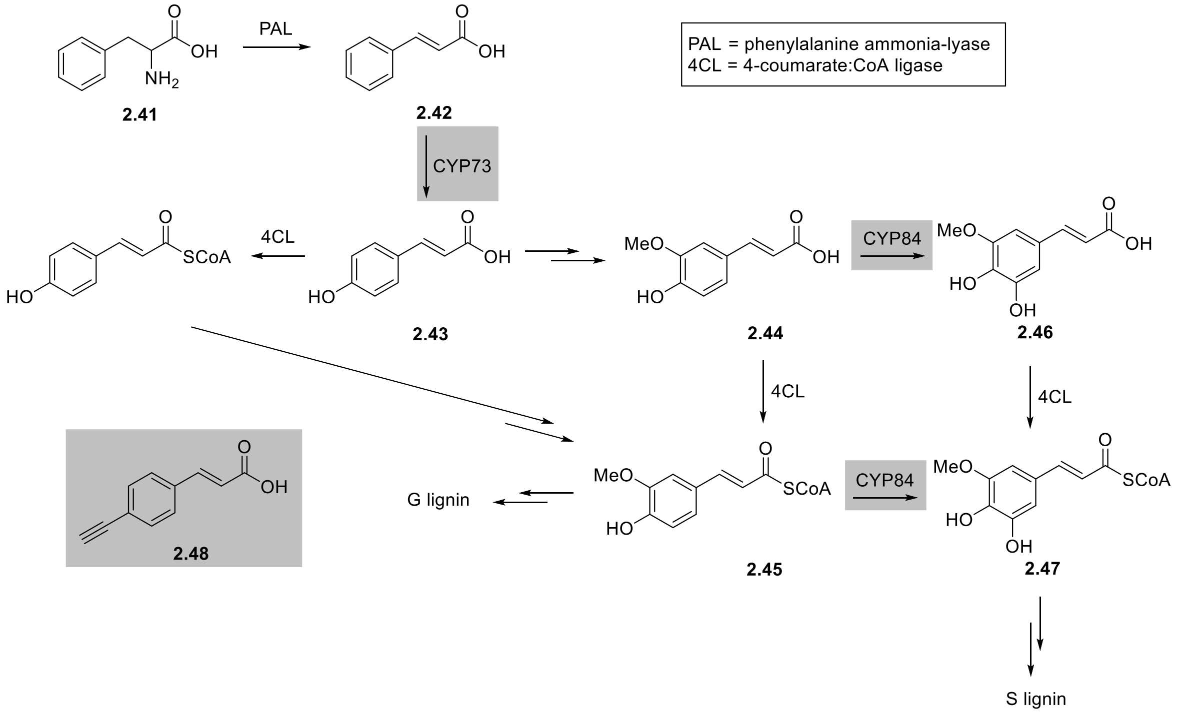
Scheme 2.1.5: *De novo* synthesis of bromide **2.34** (top), for derivatisation into enyne warhead **2.29** (bottom). Reagents and conditions: a) hydrobromic acid (48% in H_2O), 95 °C, 2 h, 65%; b) $LiAlH_4$ (4 M in Et_2O, 2.0 equiv.), Et_2O, 0 °C, 2 h, 55%; c) TIPS-acetylene (1.2 equiv.), BHT (0.10 equiv.), $Pd(PPh_3)_4$ (5.0 mol%), CuI (10 mol%), DIPA, rt, 2 h, 70%; d) NBS (1.2 equiv.), PPh_3 (1.2 equiv.), CH_2Cl_2, -30 °C – rt, 0.5 h, 84%; e) NaH (0.66 equiv.), **2.34** (0.66 equiv.), DMF/THF (1:1), 0 °C – rt, 18 h, 85% (+15% **2.37**); f) lithium chloride (5.0 equiv.), H_2O (0.10 equiv.), DMF, 150 °C, 4 h, quant.; g) TBAF (1 M in THF, 1.8 equiv.), glacial acetic acid (2.0 equiv.), THF, 0 °C – rt, 24 h, 60%; h) lithium hydroxide monohydrate (5.0 equiv.), THF/H_2O (2:1), rt, 16 h, 85% (free enyne), 80% (TIPS-enyne).

Owing to the limited commercial availability of (*E*)-pent-2-en-4-yn-1-ol **2.25**, a *de novo* approach to bromide **2.34** was carried out based on work by Zeng *et al.*[79] Propiolic acid **2.30** underwent hydrobromination to give the (*E*)-vinyl bromide **2.31** ($J_{2,3}$ = 14.0 Hz), with simple filtration and evaporation giving analytically pure material without the need for further purification. The acid could be readily reduced to the alcohol **2.32** using standard lithium aluminium hydride conditions, with the workup procedure pioneered by Mićović and Mihailović, commonly termed the Fieser workup employed to facilitate easier removal of aluminium species by filtration followed by aqueous workup.[80] Despite the initial steps of this sequence having mediocre yields (65% and 55% respectively), the chemistry was robust and scalable, allowing access to ample material for the remaining synthetic campaign.

The vinyl bromide **2.32** was next subjected to a Sonogashira reaction with TIPS-acetylene under conditions employed by Cho *et al.*, which gave the TIPS-protected alcohol **2.33** in 70% yield and provided the alcohol **2.33** as an analogue to alcohol **2.26** used previously.[81] This alcohol was routinely converted to the corresponding bromide **2.34** in comparable yield to the TMS-variant and then subjected to the identical malonate alkylation conditions (Scheme 2.1.5, bottom). Malonate **2.36** was formed in excellent yield (85%), along with its dialkylated counterpart **2.37**, following the alkylation of dimethyl malonate **2.35**. Pleasingly, this compound was amenable to Krapcho decarboxylation conditions, giving the ester **2.38** in quantitative yield and without evidence of the significant decomposition that had been observed in the previous system.

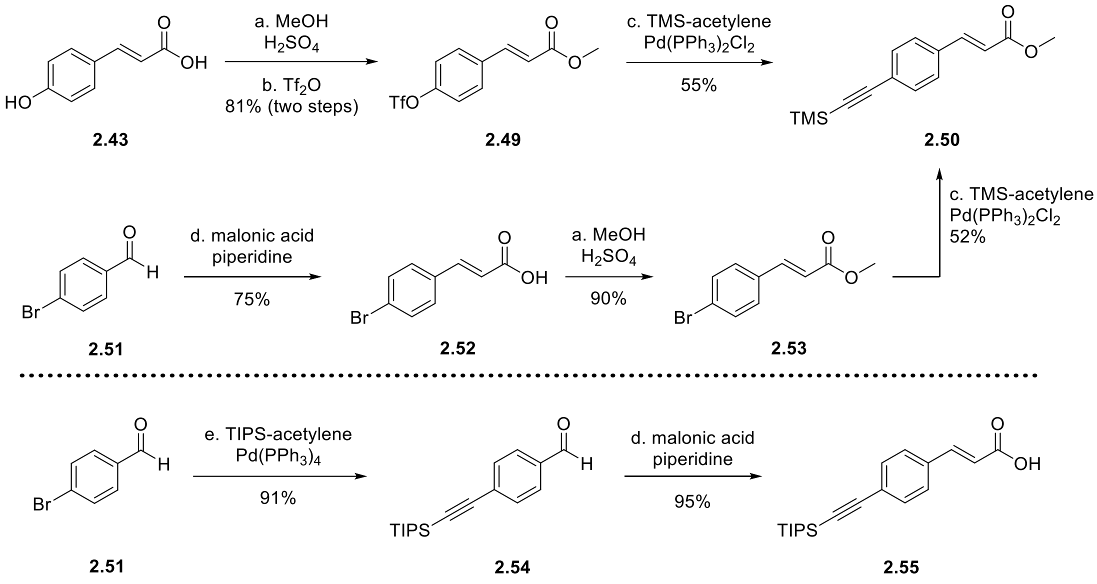
The ester **2.38** could be hydrolysed under the standard lithium hydroxide conditions to the free acid **2.39** in good yield, however this was not accompanied by concomitant deprotection of the TIPS-group. This is entirely as one might expect for this protecting group due to its higher stability towards basic conditions than the TMS-group. A deprotection step with TBAF was required to get access to the enyne warhead **2.29** with the step order of the above deprotection and ester hydrolysis reactions making no difference to the formation of the desired compound **2.29**. Free enyne **2.40** could just as easily be formed from ester **2.38**, followed by hydrolysis to give warhead **2.29** or *vice versa*. Due to concerns about the stability of the free enyne moiety, it was decided ultimately to deprotect the enyne after formation of the probe itself (see 2.2 Synthesis of Generation One – Generation Three Probes).

The final warhead **2.48**, based upon cinnamic acid **2.42**, was of interest as an example of a warhead outside of the suite of probes used by Wright *et al*. Cinnamic acid **2.42** itself is involved in phenylpropanoid metabolism in plants and formed from the reaction of phenylalanine **2.41** with phenylalanine ammonia-lyase (PAL). Hydroxylation of the 4-position of cinnamic acid **2.42** is carried out by CYP73 giving *p*-coumaric acid **2.43** (Scheme 2.1.6).[82] This compound is then converted into phenols **2.44** and **2.45** which undergo further oxidation by CYP450s to **2.46** and **2.47**, the latter of which is converted to S lignin, whereas phenol **2.45** is converted over a number of steps to G lignin.



Scheme 2.1.6: Summary of G and S lignin biosynthesis from phenylalanine **2.41** involving - amongst other CYP450 mediated oxidations - the formation of *p*-coumaric acid **2.43** from cinnamic acid **2.42**, providing inspiration for the cinnamic acid warhead **2.48**.

With this in mind, it was proposed that a warhead based on ethynyl cinnamic acid **2.48** would be suitable for oxidation by the same CYP450 enzyme on account of the promiscuity of CYP450s. To this end, the ethynyl cinnamic warhead synthesis was carried out (Scheme 2.1.7).



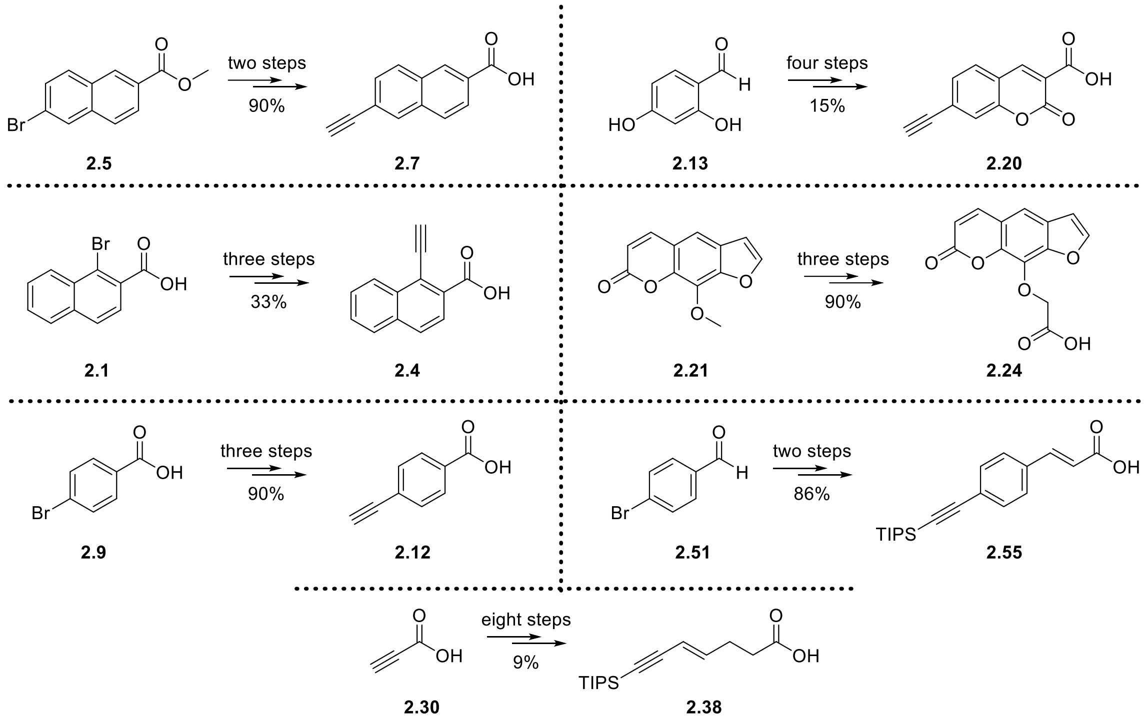
Scheme 2.1.7: Methods for the synthesis of cinnamic acid warhead **2.55**. Reagents and conditions: a) H_2SO_4 (cat.), MeOH, reflux, 16 h, 98% (4-OH), 90% (4-Br); b) triflic anhydride (1.2 equiv.), pyridine (2.2 equiv.), CH_2Cl_2, 0 °C, 1 h, 85%; c) TMS-acetylene (2.0 equiv.), $Pd(PPh_3)_2Cl_2$ (5.0 mol%), CuI (10 mol%), Et_3N (2.0 equiv.), MeCN, 90 °C, 16 h, 55% (4-OTf), 52% (4-Br); d) malonic acid (2.2 equiv.), piperidine (cat.), pyridine, reflux, 2 h, 75% (4-Br), 95% (4-(triisopropylsilyl)ethynyl); e) TIPS-acetylene (1.5 equiv.), $Pd(PPh_3)_4$ (5.0 mol%), CuI (10 mol%), DIPA (1.0 equiv.), toluene, rt, 4 h, 91%.

The sequence leading to the formation of the cinnamic warhead **2.55** was reached after a short period of optimisation. Initially, due to availability, *p*-coumaric acid **2.43** itself was chosen as the starting point for this venture. Protection of *p*-coumaric acid **2.43** as its methyl ester (not shown) and triflation under standard conditions gave the triflate **2.49** for use in the Sonogashira reaction.

Disappointingly, the precedented conditions for the following Sonogashira reaction were less effective than for the other warheads synthesised up to this point giving the TMS-acetylene **2.50** in a yield of 55%. It was hypothesised that the palladium catalyst used in this reaction was being sequestered by the alkene and therefore unable to react at the desired site in the molecule. To determine whether this was the case rather than the stability of the triflate being the problematic factor, the complementary aryl bromide **2.53** was also synthesised. 4-Bromobenzaldehyde **2.51** was reacted under Knoevenagel conditions with malonic acid to give the cinnamic acid **2.52** which was esterified to give methyl ester **2.53** in a 90% yield, in line with other substrates. Aryl bromide **2.53** was subjected to identical Sonogashira coupling conditions as for the triflate **2.49** and again the product yield was very similar, lending weight to the above hypothesis. Nevertheless, a solution to this problem presented itself during this sequence.

Switching the step order and forming TIPS-protected acetylene **2.54** from 4-bromobenzaldehyde **2.51** in the first step *via* Sonogashira reaction, led to much improved yield (the TIPS-group was once again selected due to doubts about the TMS-group stability in the Knoevenagel step). Treating this aldehyde **2.54** under the same Knoevenagel conditions as before gave the desired cinnamic acid **2.55** both in excellent yield (95%) and requiring only an aqueous wash to give material of acceptable purity. Similarly to the enyne warhead **2.38**, this was stored as the protected compound for deprotection at the time of use.

With the desired warheads in hand and their syntheses allowing access to material in a robust manner and at the required scale for derivatisation (Scheme 2.1.8), the synthesis of probes could begin. An iterative process for the probe synthesis was aimed for, with testing results at each stage allowing for informed decisions to be made so that the probes could be tailored as necessary to facilitate the successful labelling of plant CYP450s. This is discussed in detail below (2.2 Synthesis of Generation One – Generation Three Probes).

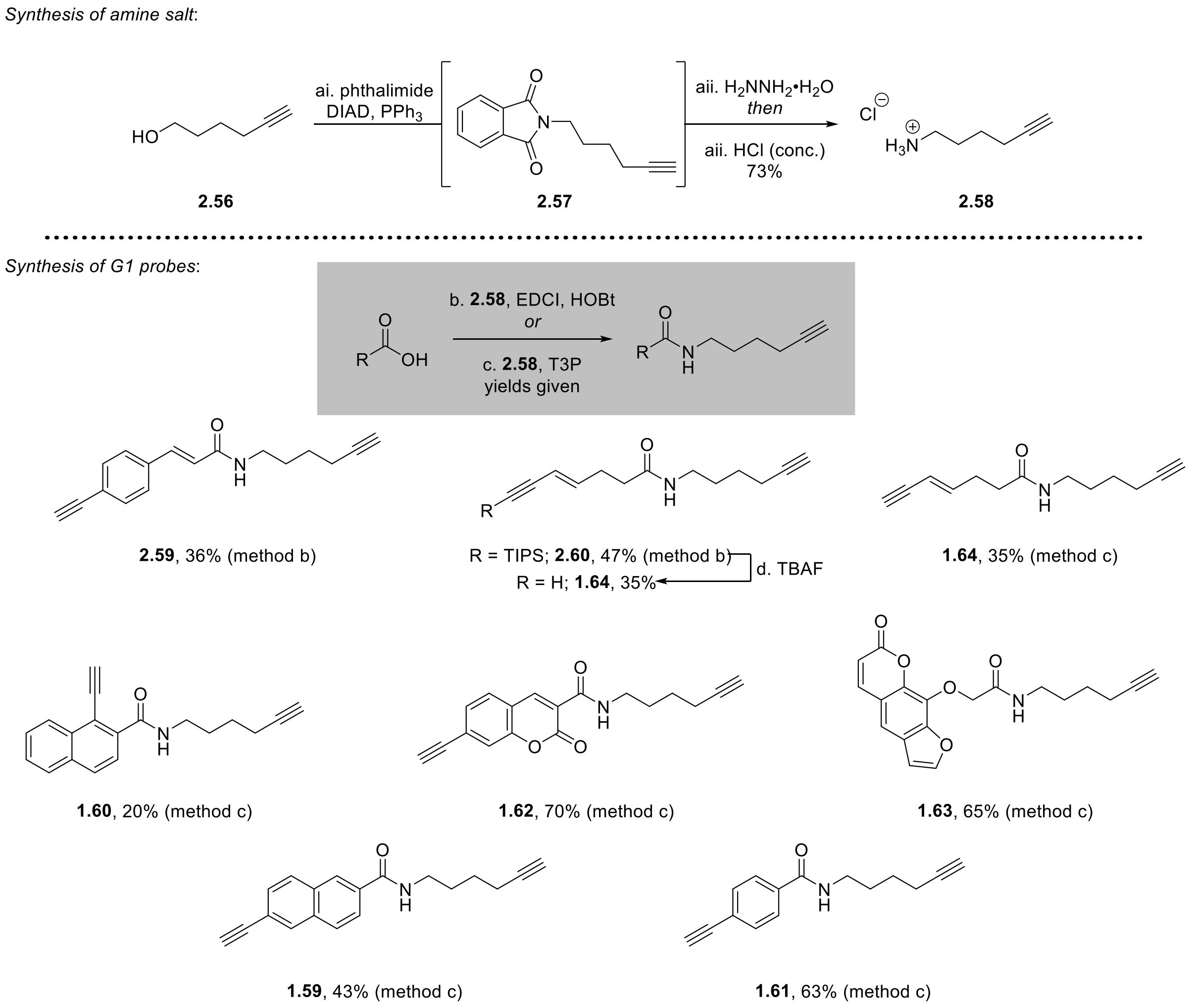


Scheme 2.1.8: Summary of warhead synthesis for use in derivatisation into plant CYP450 probes.

2.2. Synthesis of Generation One – Generation Three Probes

With the required warheads now in-hand, this section details the synthesis of probes termed generation one (G1), generation two (G2) and generation three (G3) which may be referred to collectively as early generation probes. For the sake of clarity, G1 probes were those directly comparable to the probes detailed in the work by Wright *et al*. (see 1.3 Project Aims).[69] G2 and G3 probes were sought later in the project; these probes are biotinylated and contain ester and amide linkages respectively. Their syntheses and the rationale behind their conception are outlined later in this section.

The primary amine **2.58**, required for the final amide coupling to make G1 probes, formed the initial synthetic efforts in this area. This was accomplished by the reaction of 5-hexyn-1-ol **2.56** under well known Gabriel conditions *via* the phthalimide intermediate **2.57** shown, and ultimately provided the amine **2.58** as its hydrochloride salt in a 73% yield over two steps (Scheme 2.2.1, top). It was imperative to conduct the hydrazine hydrolysis under a thoroughly inert atmosphere to prevent oxidation of the hydrazine to diimide which would have reduced some of the terminal acetylene to the respective alkene.

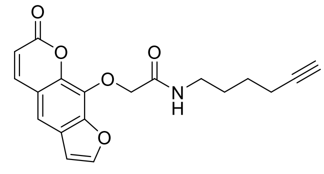


Scheme 2.2.1: Synthesis of amine salt **2.58** *via* Gabriel reaction (top). Synthesis of G1 probes by the amide coupling between previously synthesised warheads and newly synthesised amine salt **2.58**. Reagents and conditions: ai) phthalimide (1.0 equiv.), DIAD (1.0 equiv.), triphenylphosphine (1.0 equiv.), THF, 0 °C – rt, 16 h *then* aii) hydrazine hydrate (0.60 equiv.) reflux, 6 h *then* aiii) HCl (conc.), reflux, 2 h, 73% (over three steps); b) **2.58** (1.0 equiv.), EDCI (1.5 equiv.), HOBt (1.5 equiv.), triethylamine (2.0 equiv.), CH_2Cl_2, rt, 18 h, yields given; c) **2.58** (1.2 equiv.), T3P (50% in EtOAc, 1.4 equiv.), DIPEA (3.0 equiv.), CH_2Cl_2, rt, 18 h, yields given; d) TBAF (1 M in THF, 1.8 equiv.), acetic acid (1.9 equiv.), THF, rt, 18 h, 35%.

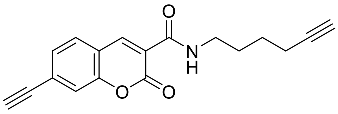
Amine **2.58** was then utilised in the respective amide couplings enabling the formation of the G1 probes (Scheme 2.2.1, bottom). Very little optimisation was carried out on this reaction given that the main aim at this juncture of the study was the generation of a sufficient amount of material for each of the substrates to enable the first round of testing on as large a library as possible. To this end, an experimentally straightforward procedure was prioritised, rather than having a highly optimised amide coupling procedure, developed at the expense of some of the desired generation one probes.

Two methods are shown (Scheme 2.2.1), the first much closer to the conditions of Wright *et al*. utilising EDCI and the second using the cyclic phosphonic anhydride T3P, both of which provided comparable yields with the latter being generally slightly superior. In the case of enyne warhead **2.60**, an additional deprotection of the TIPS-protecting group was required, which was carried out with TBAF and gave probes **1.64**. Additionally, enyne probe **1.64** was also accessible from its unprotected alkyne *via* method one (with EDCI and HOBt), albeit in slightly lower yield.

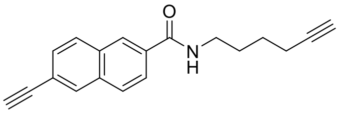
A detailed discussion of the testing protocol and its results is beyond the scope of this thesis and can be found in the work of Maria Font-Farre, the collaborator for the biological testing mentioned throughout this chapter. Nevertheless, the results of G1 testing can be summarised very briefly as follows. Six of the seven G1 probes (cinnamic probe **2.61** was omitted) were tested on total plant extracts, with none of the probes showing labelling after fluorescent tagging (Figure 2.2.1). Despite this, the probes were shown to label mouse liver microsomes after the same labelling protocol.



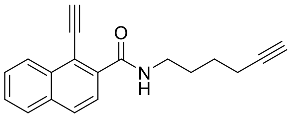
DB080, **1.63**



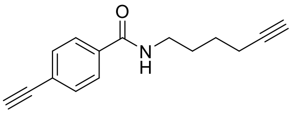
DB086, **1.62**



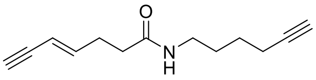
DB088, **1.59**



DB089, **1.60**



DB096, **1.61**



DB143, **1.64**

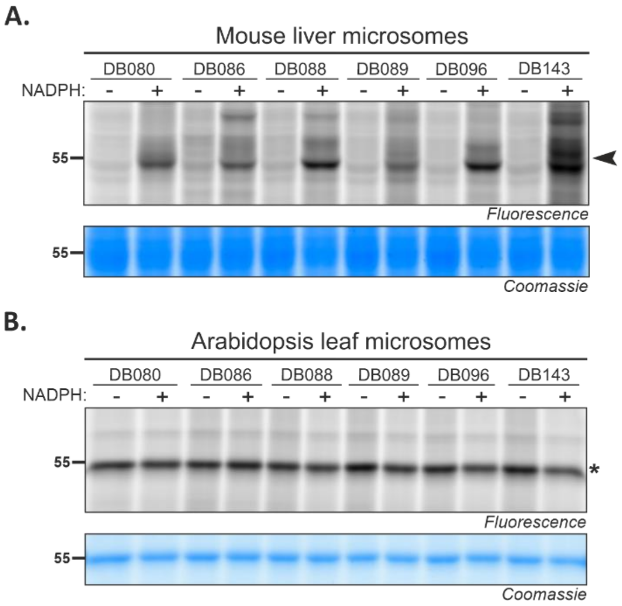


Figure 2.2.1: Labelling results for the testing of G1 probes against mouse liver microsomes and *Arabidopsis* leaf microsomes showing NADPH-dependent labelling in **A** and no detectable NADPH-dependent labelling in **B**. Microsomes were labelled for 1 h with 10 µM probe with or without 1 mM NADPH. Labelled proteins were coupled to picolyl azide fluorophore *via* click chemistry, separated on SDS-PAGE gels and visualised by in-gel fluorescence scanning. Coomassie staining is shown as loading control. Labelling indicated by the asterisk in **B** corresponds to background NADPH-independent rubisco labelling. Reproduced with permission from Maria Font-Farre.[83]

It was hypothesised that the concentration of CYP450s present in the total plant extracts was far lower than that seen in the mouse liver cells – this was later confirmed by carrying out total mass spectrometry analysis of the mouse liver cells versus the plant extracts, the details of which are not discussed further here (see thesis of Maria Font-Farre).[83]

Given the results, a more sensitive detection method was required for the labelling of plant CYP450s. Thus, a series of biotinylated probes were designed based upon the existing warheads in order for pulldown and mass spectrometry analysis to be carried out. The previously used *in vivo* click reaction to form fluorescence tag labelled probe-protein conjugates pioneered by Wright and co-workers was not suitable as the click reaction to form the corresponding biotinylated analogues was accompanied by a strong click chemistry background, consequently hampering analysis. Taking inspiration from work from Sellars *et al.*, the G2 (ester) and G3 amide probes were conceived (Figure 2.2.2).[72] Fears about the stability of the ester bond *in vivo* were present from the outset, however, these probes were the easiest to synthesise of the two generations and were hoped to provide some initial results whilst work continued on the synthesis of the more valuable G3 probes.

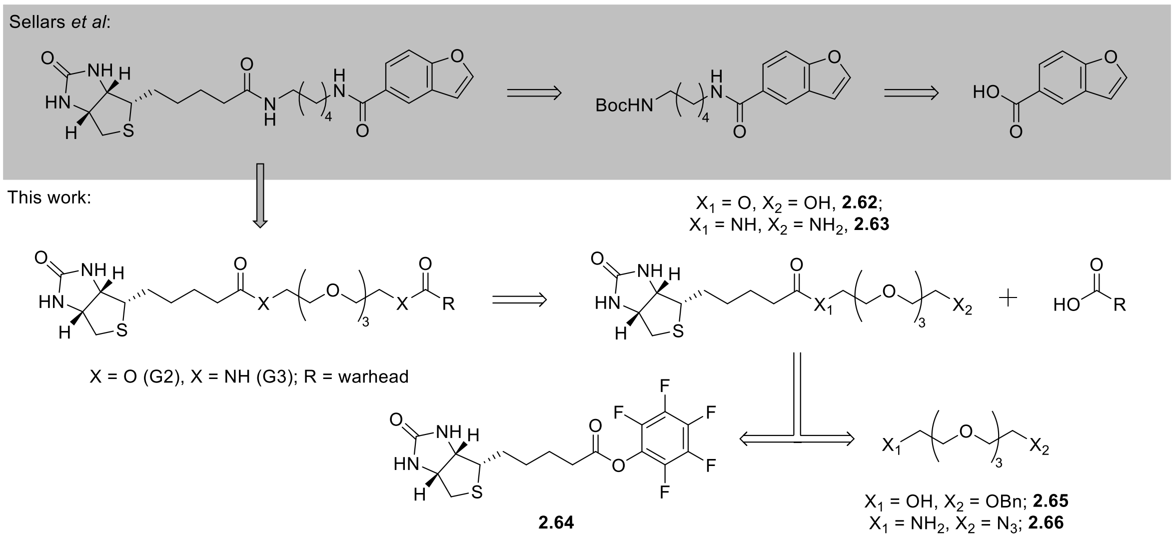
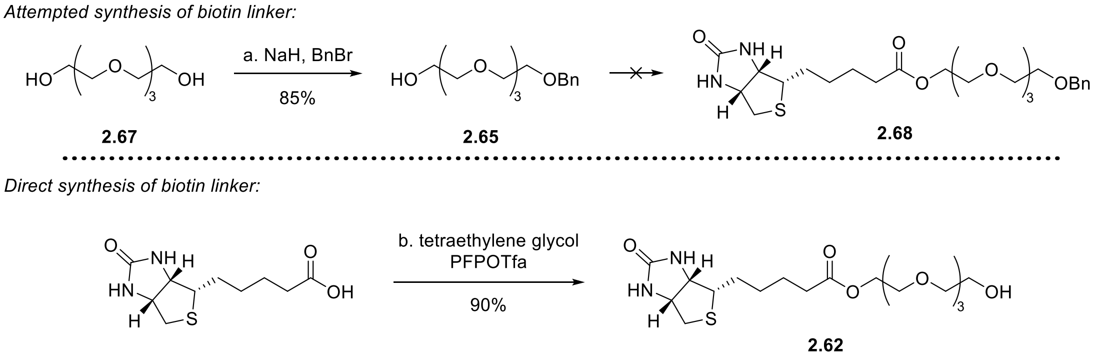


Figure 2.2.2: Sellars' biotinylated CYP450 probe for human CYP450 isoforms, providing inspiration for the design of G2 and G3 probes for plant CYP450s based upon this structure. Disconnection of Sellars' probe and the desired disconnection for the synthesis of G2 and G3 probes leading back to intermediates **2.64** to **2.66**.

The desired disconnection in the synthesis of the G2 and G3 probes was between the warhead itself and a biotin linker which required synthesis. This would have provided the most streamlined procedure for the formation of probes of this type since the already accessed warheads could be functionalised as biotinylated probes in one step. It was hoped that this would allow for the expansion of the probe library without the necessity for a laborious derivatisation procedure early in the respective probe syntheses.

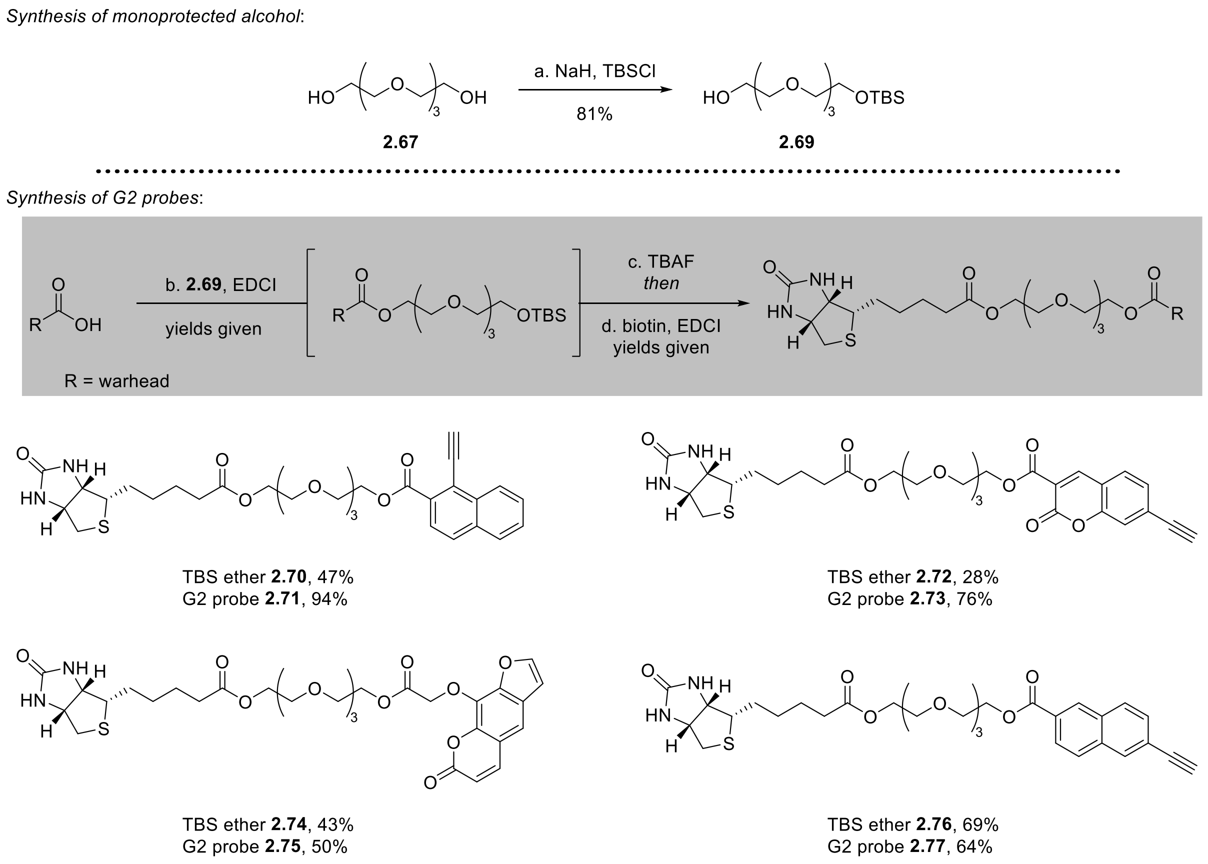
With this in mind, the synthesis of PEG-linked biotin alcohol **2.62** was attempted (Scheme 2.2.2). To begin, tetraethylene glycol **2.67** was monobenzylated to give alcohol **2.65** following the procedure of McDougal.[84] Despite various attempts, the desired benzylated intermediate **2.68** could not be accessed cleanly from biotin and alcohol **2.65**, as purification from unreacted starting material and the coupling reagents themselves was difficult due to the polar nature of the biotin containing compound and the respective starting materials and by-products.



Scheme 2.2.2: Attempted synthesis of biotin linker **2.68** and direct synthesis of biotin linker **2.62** facilitated by reverse-phase column chromatography. Reagents and conditions: a) NaH (60% in mineral oil, 1.1 equiv.), benzyl bromide (0.6 equiv.), THF, 0 °C – rt, 4.5 h, 85% (based on benzyl bromide equiv.); b) tetraethylene glycol (10 equiv.), pentafluorophenyl trifluoroacetate (1.4 equiv.), triethylamine (5.0 equiv.), DMF, 60 °C, 18 h, 90%.

It was not until much later into the project that a procedure for the formation of PEG-linked biotin alcohol **2.62** could be achieved (Scheme 2.2.2). This was facilitated largely by the reverse-phase purification capabilities utilised in an industrial setting, and allowed access to the desired compound directly from tetraethylene glycol **2.67** in a single step.

Due to the inability to access the required coupling partner at the necessary time, the eventual route for the synthesis of G2 probes moved closer to that used by Sellars *et al*. in their synthesis of biotinylated CYP450 probes (Scheme 2.2.3).[72]


Synthesis of monoprotected alcohol:

2.67

2.69

Synthesis of G2 probes:

R = warhead

TBS ether **2.70**, 47%
G2 probe **2.71**, 94%

TBS ether **2.72**, 28%
G2 probe **2.73**, 76%

TBS ether **2.74**, 43%
G2 probe **2.75**, 50%

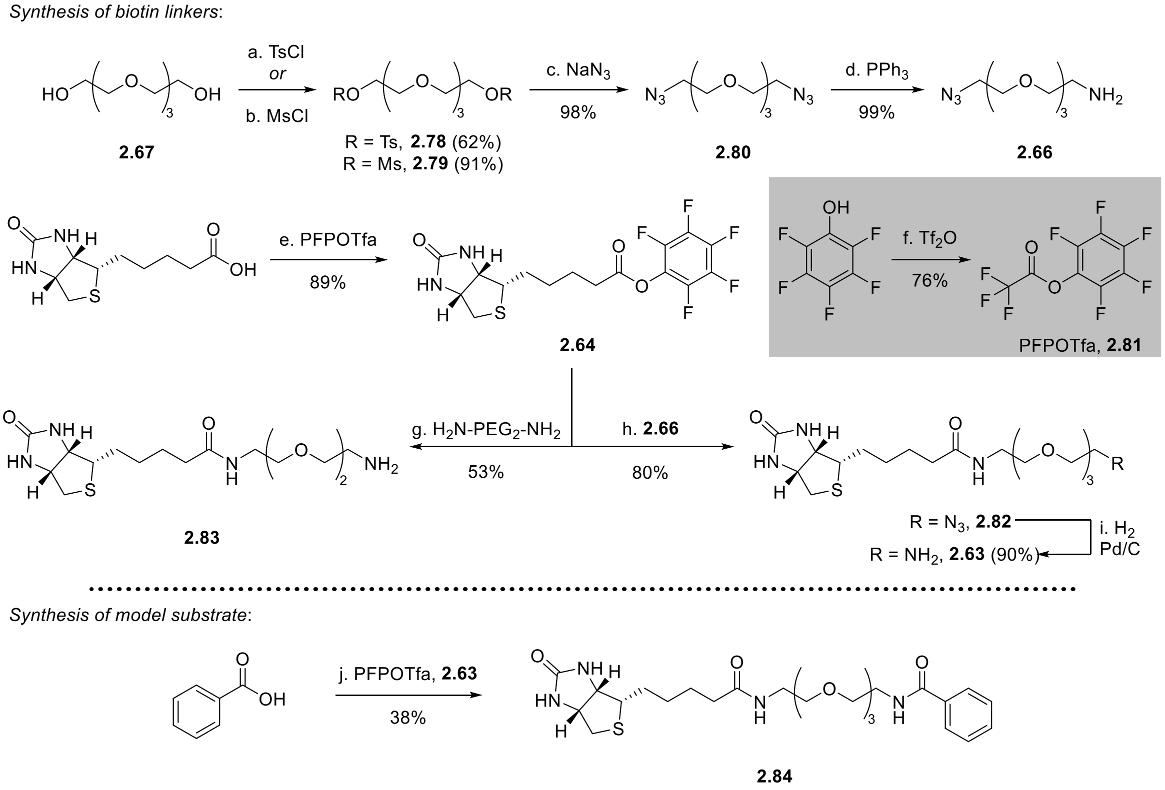
TBS ether **2.76**, 69%
G2 probe **2.77**, 64%

Scheme 2.2.3: Synthesis of silylated alcohol **2.69** and derivatisation into G2 probes *via* ester formation, followed by telescoped deprotection and biotinylation sequence. Reagents and conditions: a) NaH (1.0 equiv.), *tert*-butyldimethylsilyl chloride (1.1 equiv.), THF, rt, 1.5 h, 81%; b) **2.69** (1.0 equiv.), EDCI (1.5 equiv.), DMAP (1.5 equiv.), CH_2Cl_2, 40 °C, 18 h, yields given; c) TBAF (1 M in THF, 1.2 equiv.), acetic acid (1.5 equiv.), THF, rt, 18 h, yields given; d) D-biotin (1.0 equiv.), EDCI (1.5 equiv.), DMAP (1.5 equiv.), CH_2Cl_2, rt, 40 °C, 18 h, yields given.

Formation of silylated alcohol **2.69** was possible under the conditions developed by McDougal and co-workers in the 1980s.[84] This was then used under coupling conditions for the formation of the respective TBS-protected alcohols **2.70**, **2.72**, **2.74**, and **2.76**. These compounds were subjected to deprotection with TBAF and then further coupling to biotin in a telescoped manner, giving the desired G2 probes **2.71**, **2.73**, **2.75**, and **2.77**.

As feared, the ester moiety itself was not hydrolytically stable enough to garner promising labelling results *in vivo* (results not shown). At this point, no further time was spent on the synthesis of G2 probes and attention was turned solely to the delivery of the G3 probes that were proving challenging to synthesise in their own right.

Similarly to the G2 probes, the desired disconnection for the formation of the G3 probes led to the necessity to synthesise biotin linker **2.63**. Unlike the linker required for the G2 probes, the synthesis of this biotin linker was realised early on into the synthetic effort towards G3 probes (Scheme 2.2.4). Formation of the necessary PEG-amine **2.66** could be carried out in three steps. Forming either ditosylate **2.78** or dimesylate **2.79** could be achieved easily from the corresponding diol **2.67**, with the dimesylate being preferred on account of the easier purification procedure. The diazide **2.80** was then formed in near quantitative yield from the respective dimesylate **2.79** and then underwent monoreduction to the desired PEG-amine **2.66** under the conditions of Okoth *et al*.[85] The success of this step depended upon utilising a biphasic reaction medium, with the acidic aqueous phase removing the protonated amine from the organic phase upon formation and preventing over reduction.



Scheme 2.2.4: Synthesis of biotin linkers **2.63**, **2.83** and formation of model substrate **2.84** *via* activation of the parent acid as the pentafluorophenyl ester. Reagents and conditions: a) tosyl chloride (3.0 equiv.), potassium hydroxide (8.0 equiv.), CH_2Cl_2, 0 °C – rt, 4 h, 62%; b) methanesulfonyl chloride (3.4 equiv.), triethylamine (4.8 equiv.), CH_2Cl_2, 0 °C – rt, 18 h, 91%; c) sodium azide (4.0 equiv.), EtOH/DMF (4:1), reflux, 6 h, 98%; d) triphenylphosphine (1.0 equiv.), hydrochloric acid (2 M),

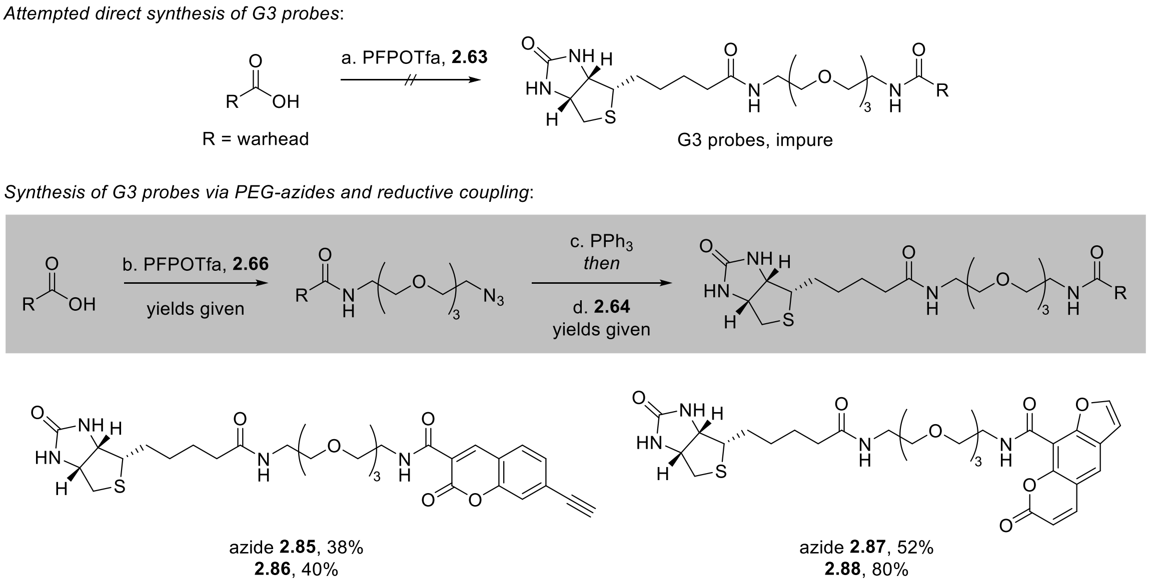
Et_2O, rt, 18 h, 99%; e) pentafluorophenyl trifluoroacetate (1.4 equiv.), triethylamine (1.8 equiv.), DMF, rt, 1 h, 89%; f) trifluoracetic anhydride (1.5 equiv.), 40 °C, 18 h, 76%; g) H_2N-PEG2-NH_2 (10 equiv.), triethylamine (5.0 equiv.), DMF, rt, 18 h, 53%; h) **2.66** (2.0 equiv.), triethylamine (1.5 equiv.), DMF, 40 °C, 18 h, 80%; i) Pd/C (15 mol%), H_2, EtOH, rt, 18 h, 90%; j) pentafluorophenyl trifluoroacetate (1.3 equiv.), triethylamine (1.2 equiv.), CH_2Cl_2, rt 1 h *then* **2.63** (1.0 equiv.), rt, 2 h, 38%.

With the required amine in hand, activation of biotin as the respective pentafluorophenyl ester **2.64** was carried out smoothly with pentafluorophenyl trifluoroacetate **2.81** (formed in one step from reaction between pentafluorophenol and trifluoracetic anhydride) in the presence of triethylamine. Trituration of the resulting pentafluorophenyl ester with Et_2O gave clean material which could be converted into the biotin linker precursor **2.82** in 80% yield upon treatment with amine **2.66**. Presumably, the increased nucleophilicity of the amine with respect to the corresponding alcohol used in the synthesis of linker **2.65**, enabled this reaction to go readily to completion. The azide was hydrogenated and gave the linker **2.63** without any need for column purification. A related biotin linker **2.83** could be formed directly according to the procedure developed during industrial placement, this gave biotin linker **2.83** in 53% yield. The yield for this transformation was somewhat lower than for the ester-containing biotin linker **2.62** and thus the original route was retained despite the additional synthetic steps.

It was buoying to observe that this linker was able to undergo amide formation with benzoic acid (after activation as its respective pentafluorophenyl ester), albeit in a 38% yield giving model probe **2.84**. With this promising initial result in hand the newly established protocol was tested on the synthesis of the G3 probes of interest.

The pentafluorophenyl esters derived from the warheads (shown in Scheme 2.1.8) were readily synthesised upon exposure of the warheads themselves with PFPOTfa **2.81**. However, they were soluble in Et_2O, meaning that trituration was not a viable procedure for accessing the activated esters. This resulted in products contaminated with trifluoroacetic acid after the following amide formation with biotin linker **2.63** as demonstrated in Scheme 2.2.4, even after column chromatography. Aqueous washing of the products was hoped to remove these water soluble by-products, but the products themselves were also extracted into the aqueous phase. Unfortunately, the acid chlorides of the warheads could not be reliably formed, with intractable mixtures formed after reaction with biotin linker **2.63**. Similarly, amide coupling conditions with either EDCI or T3P, conditions that had enabled the synthesis of G1 probes, led to similar purification issues.

The G3 probe polarity proved to be the Achilles heel in the direct formation of G3 probes; however, using a longer route *via* the warhead amide derived from the coupling between warheads and the PEG-amine **2.66** allowed the synthesis of probes **2.86** and **2.88** (Scheme 2.2.5).



Scheme 2.2.5: Failed direct synthesis of G3 probes and synthesis of G3 probes **2.86** and **2.88** ultimately *via* a longer route involving coupling of warheads to amine **2.66**, followed by reduction and coupling to biotin, giving G3 probes. Reagents and conditions: a) pentafluorophenyl trifluoroacetate (1.3 equiv.), triethylamine (1.2 equiv.), CH_2Cl_2, rt 1 h *then* **2.63** (1.0 equiv.), rt, 2 h, no product isolated; b) pentafluorophenyl trifluoroacetate (1.4 equiv.), triethylamine (1.2 equiv.), CH_2Cl_2, rt, 1 h *then* **2.66** (1.0 equiv.), rt, 18 h; yields given; c) triphenylphosphine (1.1 equiv.), THF, rt, 2 h *then* d) **2.64** (1.0 equiv.), triethylamine (2.0 equiv.), CH_2Cl_2, rt, 18 h, yields given.

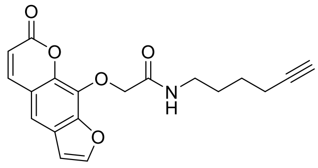
Activation of the warheads **2.20** and **2.24** was possible with pentafluorophenyl trifluoroacetate, with the subsequently formed products used directly in the coupling to PEG-amine **2.66**. These intermediates could be reduced by triphenylphosphine *in situ* before addition of activated biotin ester **2.64** forging the amide bond to give compounds **2.86** and **2.88** in moderate yields. Naturally, the purification step was difficult, again on account of the polarity of the compounds.

Decomposition of a number of the synthesised warheads during this period of optimisation and the forced closure of the department at the outbreak of the COVID-19 pandemic led to the full library not being synthesised at this point. Ultimately these probes became redundant at the advent of a unified probe synthesis involving a new generation of probes and the realisation of a route allowing access to a more comprehensive probe library, which is the subject of the following section.

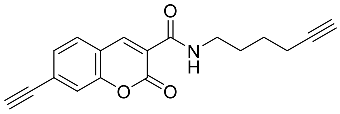
2.3. Generation Four Probes and Unified Probe Synthesis for Applicability to Library Synthesis

Cognisant of the fact that the initial biotinylated probes (G2 and G3) were not fit for purpose on account of their non-modular synthetic routes and difficult purification, a new generation of probes (G4) aimed to address some of the aforementioned issues that had been faced up until this point. Additionally, storing the warheads over long periods of time led to significant degradation and a necessity to resynthesise them. This was thought to be associated with the free alkynes since psoralen warhead **2.24** and the two TIPS-protected warheads **2.38** and **2.55** fared better than the rest in terms of stability.

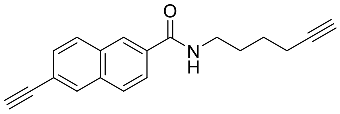
The desire for a new generation of probes was accompanied with renewed interest in the G1 probes on account of them having been recently demonstrated to successfully label fungal CYP450s from *Zymoseptoria tritici* by project collaborator Maria Font-Farre (Figure 2.3.1).[83]



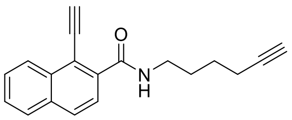
DB080, **1.63**



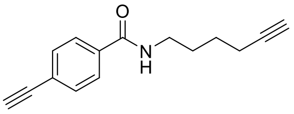
DB086, **1.62**



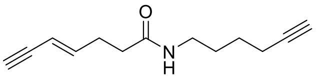
DB088, **1.59**



DB089, **1.60**



DB096, **1.61**



DB143, **1.64**

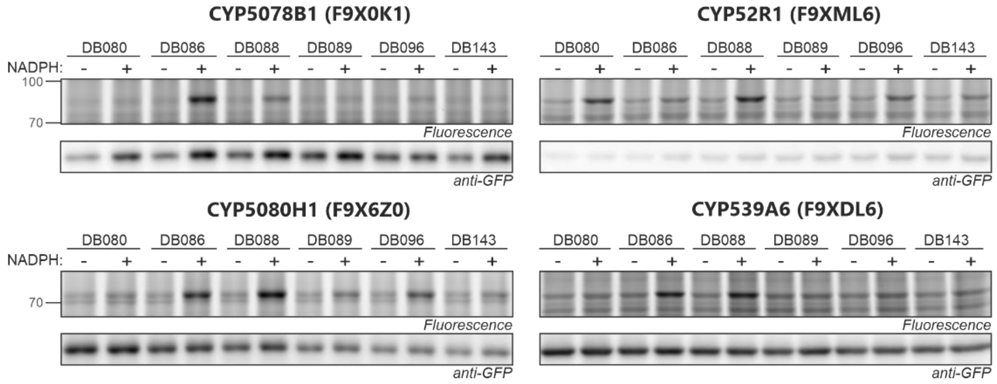


Figure 2.3.1: Testing results of G1 probes against four *Zymoseptoria tritici* CYP450 enzymes transiently over expressed in *Nicotiana benthamiana* showing NADPH-dependent labelling. Agroinfiltrated *N. benthamiana* leaf microsomes were isolated *via* ultracentrifugation and resuspended microsomes were labelled with 5 µM probe concentration in the absence or presence of 1 mM NADPH for 1 h. Labelled proteins were coupled to a fluorophore via click chemistry and visualised by in-gel fluorescence scanning. Reproduced with permission from Maria Font-Farre.[83]

This pleasing testing result led to a more modular approach in which biotinylated (G4) and non-biotinylated (G1) probes could be accessed from a later stage intermediate. This desire was accentuated by the existing biotinylated probe synthesis being rather cumbersome and the final purification of G3 probes tricky on account of the polarity of the probes. Rather than derive a new series of probes from scratch, a new generation of probes was sought to utilise existing compounds in the project and unify the current synthetic methodology, enabling the probe synthesis to be applied to larger scale library synthesis of G1 and G4 probes.

To achieve this, it was hoped that the existing route to biotinylated probes could be streamlined further, and the synthesis of G4 probes carried out by a click reaction (pioneered by Sharpless and others at the turn of the century) from analogues close to G1 probes (Figure 2.3.2).[86,87] Carrying out the amide coupling as the first step would enable the esterification and deprotection sequence to be dropped, provided that the Sonogashira reaction could be optimised in order to tolerate the additional alkyne present in the molecule. From intermediates of this type, the click reaction with biotin linker **2.82** (synthesised previously on route to biotin linker **2.63**), was envisaged to go on the unprotected alkyne leading to biotinylated probes (G4). Simply deprotecting the intermediates rather than subjecting them to the click reaction would allow easy access to G1 probes.

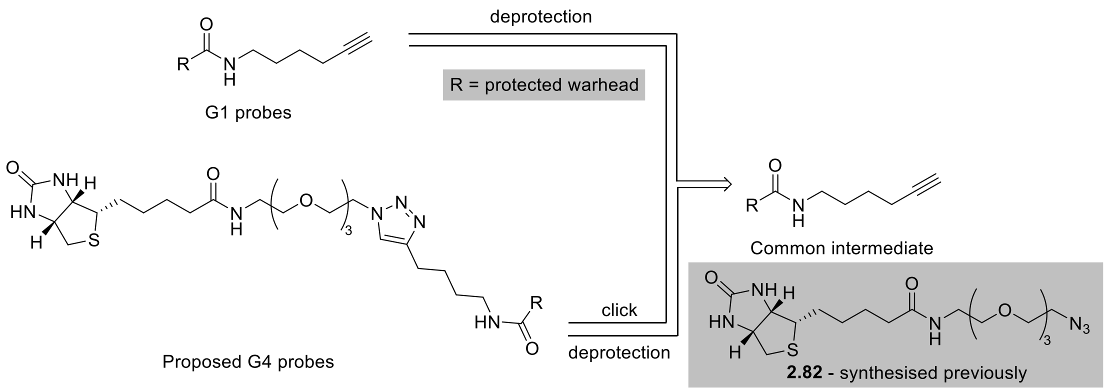
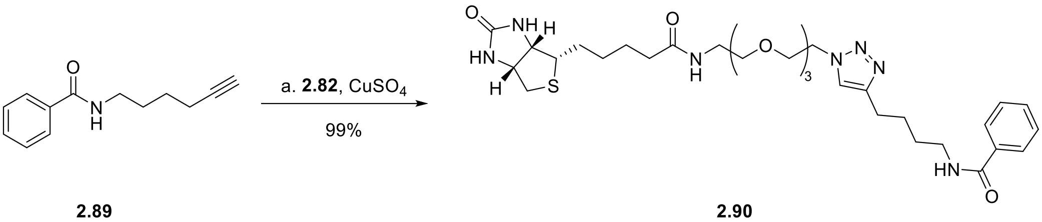


Figure 2.3.2: Strategy to enable easier synthesis of G1 probes and the derivatisation into new biotinylated G4 probes.

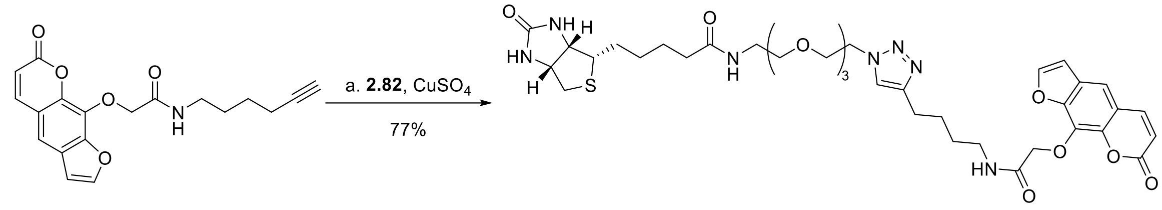
To enact this strategy, it was deemed pertinent to assess the efficacy of the key reaction necessary for the formation of biotinylated probes of this generation. Pleasingly, a model amide **2.89** performed well under the first set of conditions tried and enabled access to triazole **2.90** near quantitatively under standard click conditions (Scheme 2.3.1).[86] These conditions were utilised for the synthesis of G4 psoralen probe **2.91** from its G1 analogue **1.63**, albeit with a slight drop in yield.

Model substrate synthesis:



2.89 **2.90**

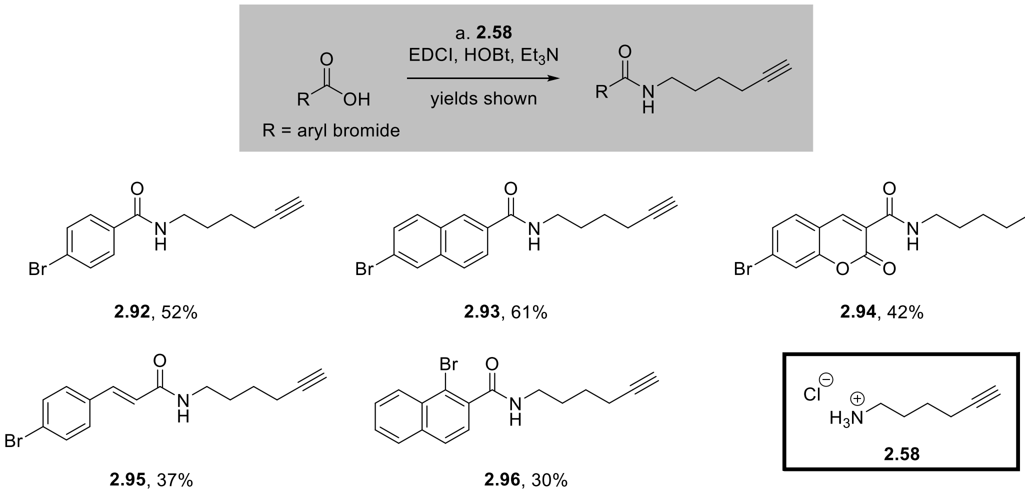
Synthesis of psoralen G4 probe:



1.63 **2.91**

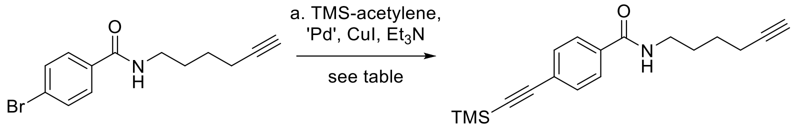
Scheme 2.3.1: Synthesis of model click probe **2.90** (top). Synthesis of psoralen G4 probe **2.91** (bottom). Reagents and conditions: a) **2.82** (1.0 equiv.), sodium-L-ascorbate (10 mol%), copper sulfate hydrate (10 mol%), DMF/H_2O (5:1), rt, 48 h, 99% (benzyl analogue); 77% (psoralen analogue).

Following this early success, efforts were undertaken to deliver the remaining substrates based upon existing warheads for their respective derivatisation into G4 probes (Scheme 2.3.2). Amides **2.92** to **2.96** were synthesised *via* established EDCI coupling procedures. From this pool, amide **2.92** was selected as the optimisation substrate for the desired Sonogashira reaction due to its respective simplicity compared to the others.



Scheme 2.3.2: Synthesis of amides **2.92** to **2.96** for use in the synthesis of G4 probes. Reagents and conditions: a) **2.58** (1.1 equiv.), EDCI (1.5 equiv.), HOBt (1.5 equiv.), triethylamine (2.2 equiv.), CH_2Cl_2, rt, 18 h, yields given.

A brief survey of the literature did not return any examples of Sonogashira reactions being carried out in the presence of additional alkynes present in the starting materials. Glaser-type couplings between the terminal alkynes were thought to be the main competitors to the desired reaction pathway in the presence of oxidants and copper (I) salts.[88–90] With rigorous air exclusion the potential problems with competing reactions might be circumvented. A round of optimisation was carried out (Table 2.3.1).



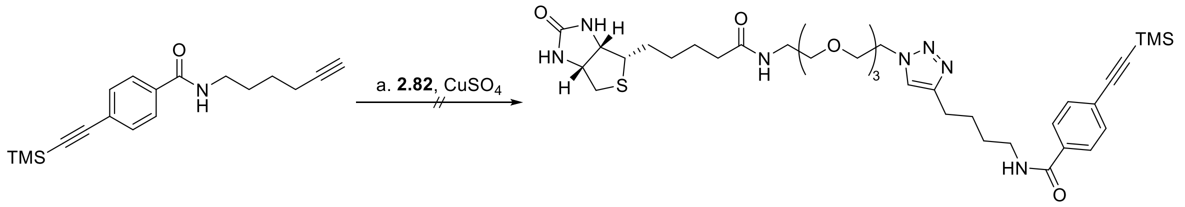
2.92 **2.97**

Entry	Palladium catalyst	Catalyst loading/ mol%	TMS-acetylene/ equiv.	Temp/ °C	Time/h	Yield/%	Observation(s)
1	$Pd(PPh_3)_2Cl_2$	1.0	1.2	25	2	n.d.	No conversion
2	$Pd(PPh_3)_2Cl_2$	1.0	1.2	60	2	n.d.	No conversion
3	$Pd(PPh_3)_2Cl_2$	5.0	1.2	60	16	38	39% SM recovery
4	$Pd(PPh_3)_2Cl_2$	5.0	2.0	60	16	44	SM not isolated, present by TLC analysis
5	$Pd(PPh_3)_4$	5.0	2.0	60	16	n.d.	Product spot faint by TLC analysis
6[a]	$Pd(PPh_3)_2Cl_2$	5.0	2.0	85	16	66	

Table 2.3.1: Optimisation of Sonogashira reaction between amide **2.92** and TMS-acetylene to form TMS-acetylene **2.97** for use in click reaction towards G4 probes. Unless specified otherwise reactions were carried out at a 0.20 mmol scale in N_2 sparged MeCN with Et_3N (2.0 equiv.) as the base. Yields given are isolated. [a]Reaction carried out at 0.50 mmol scale.

Initially the Sonogashira reaction was attempted under standard conditions with a slight excess of TMS-acetylene over bromide **2.92** using a Pd(II) precatalyst, copper(I) iodide and triethylamine in acetonitrile (entry 1). The reaction was first attempted at rt for only a short time in comparison to the previously used Sonogashira conditions (see 2.1 Synthesis and Optimisation of Warheads), this unfortunately led to no conversion of the starting material. Likewise, heating the reaction to 60 °C for the same time also gave the same result (entry 2); a pleasing result was obtained by both increasing the catalyst loading and leaving the reaction overnight (entry 3). Although the yield of TMS-acetylene **2.97** was only 38%, it was also accompanied by the recovery of 39% of unreacted starting material **2.92**. This result provided a platform for further optimisation in the expectation that the feared Glaser-coupling could be avoided on the warhead substrates. Changing the source of palladium to $Pd(PPh_3)_4$ led to no notable improvement in yield, nor did increasing the equivalents of TMS-acetylene two-fold (entries 4 and 5 respectively). Further increasing the temperature of the reaction, twinned with carrying out the reaction in a sealed tube (in order to alleviate concerns with loss of the TMS-acetylene by evaporation at this temperature) led to the desired product being formed in a respectable yield of 66% (entry 6).

Disappointingly, substrate **2.97** performed poorly under the click reactions employed in the synthesis of model substrate **2.90** and psoralen probe **2.91** (Scheme 2.3.3).



2.97

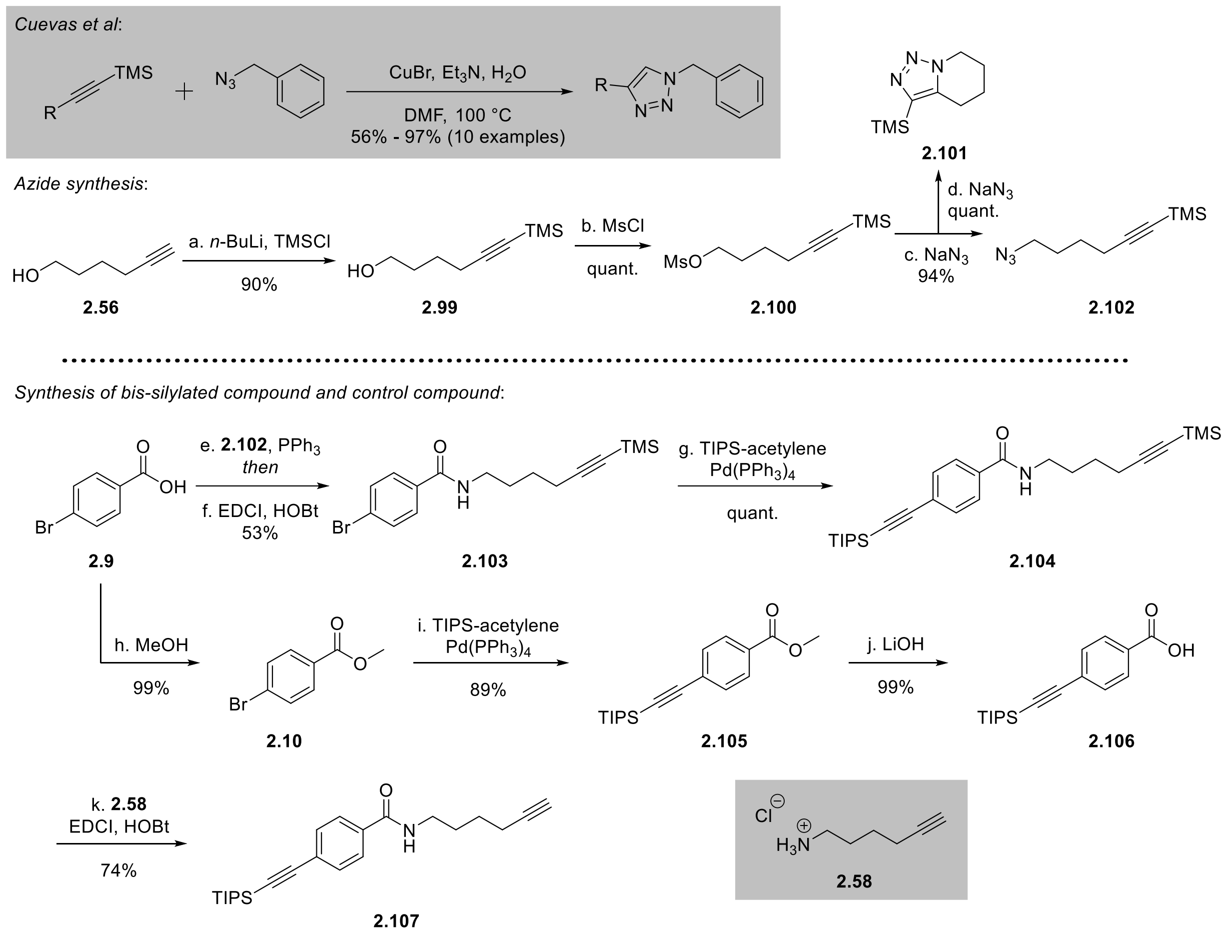
2.98, impure

Scheme 2.3.3: Attempted synthesis of G4 precursor **2.98** *via* click reaction. Reagents and conditions: a)) **2.82** (1.0 equiv.), sodium-L-ascorbate (10 mol%), copper sulfate hydrate (10 mol%), DMF/H_2O (5:1), rt, 48 h, product not isolated.

The reaction did not go to completion, leading to an inseparable mixture of products and biotin linker **2.82**. This was also the case upon increasing the equivalents of alkyne **2.97** and led to the hypothesis that the TMS-acetylene moiety was inhibiting the click reaction, perhaps by sequestering the copper catalyst. In efforts to counteract this, attention was once again turned to the TIPS-acetylene group, which had proved useful previously and in this case was recruited as steric bulk to mask the acetylene on which reaction was not desired. Under the optimised Sonogashira conditions, it was not possible to install the TIPS-acetylene group selectively in the presence of the free alkyne in **2.92** and, despite a lengthy optimisation effort which is not discussed here, the formation of the respective TIPS-acetylene was unsuccessful.

Given that a late stage intermediate was still desired for the formation of G1 and G4 probes, instead, it was hoped that the bis-silylated intermediate **2.104** could be reacted exclusively at the TMS-acetylene position to give the desired click scaffold according to conditions utilised by Cuevas and co-workers (Scheme 2.3.4, top).[91]

In order to synthesise the bis-silylated compound **2.104** of interest, the synthesis of azide **2.102** was required. It was expected that the azide **2.102** could be reduced *in situ* with triphenylphosphine and then coupled to 4-bromobenzoic acid **2.9**. With this in mind, alcohol **2.56**, used previously in the synthesis of amine **2.58**, was protected as its TMS-acetylene **2.9** following double deprotonation with *n*-butyllithium and quench with trimethylsilyl chloride and then subjected to mesylation conditions giving mesylate **2.100**. The mesylate **2.100** was initially reacted with sodium azide at reflux as previously and surprisingly bicyclic triazole **2.101** was isolated in quantitative yield. Fortunately, reducing the reaction time considerably from 18 h to 15 minutes enabled the isolation of azide **2.102** prior to the thermal [3+2]-cycloaddition (Scheme 2.3.4, top).

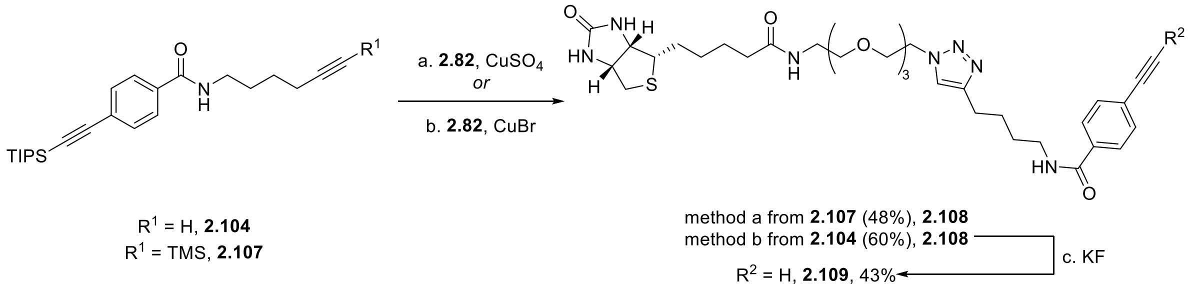


Scheme 2.3.4: Cuevas and co-workers' direct click reaction using silylated alkynes and synthesis of azide **2.102** (top). Synthesis of bis-silylated intermediate **2.104** as a G4 precursor candidate and TIPS-acetylene to be used as a control for the efficacy of the new click reaction conditions along with mono-protected **2.107** to be used as a control for the original conditions. Reagents and conditions: a) *n*-butyllithium (2.5 M in hexanes, 2.2 equiv.), DMAP (0.20 equiv.), trimethylsilyl chloride (3.6 equiv.), THF, -78 °C – rt, 3 h, followed by HCl workup, 90%; b) methanesulfonyl chloride (1.7 equiv.), triethylamine (2.4 equiv.), CH_2Cl_2, 0 °C – rt, quant.; c) sodium azide (1.2 equiv.), DMF, 65 °C, 15 mins, 94%; d) sodium azide (2.0 equiv.), EtOH/DMF (4:1), 80 °C, 18 h, quant.; e) **2.102** (1.0 equiv.), triphenylphosphine (1.2 equiv.), THF, H_2O, 60 °C, 4 h *then* f) EDCI (1.3 equiv.), HOBt (1.3 equiv.), triethylamine (2.0 equiv.), CH_2Cl_2, rt, 18 h, 53%; g) triisopropyl acetylene (2.0 equiv.), $Pd(PPh_3)_4$ (2.5 mol%), copper(I) iodide (5.0 mol%), triethylamine (2.0 equiv.), THF, 60 °C, 18 h, quant.; h) H_2SO_4 (cat.), MeOH, reflux, 18 h, 99%; i) triisopropyl acetylene (2.0 equiv.), $Pd(PPh_3)_4$ (2.5 mol%), copper(I) iodide (5.0 mol%), triethylamine (2.0 equiv.), THF, 60 °C, 18 h, 89%; j) lithium hydroxide hydrate (5.0 equiv.), THF/H_2O (2:1), rt, 16 h, 99%; k) **2.58** (1.2 equiv.), EDCI (1.5 equiv.), HOBt (1.5 equiv.), triethylamine (2.0 equiv.), CH_2Cl_2, rt, 18 h, 74%.

With azide **2.102** in hand, the *in situ* reduction with triphenylphosphine and amide formation sequence was enacted for the formation of amide **2.103** in 53% yield (Scheme 2.3.4, bottom). The Sonogashira reaction was then conducted on aryl bromide **2.103** with TIPS-acetylene and pleasingly gave the desired bis-silylated compound **2.104** in quantitative yield. In order to provide a direct comparison in the click reaction, the free alkyne **2.107** was also synthesised according to the established route. 4-Bromobenzoic acid **2.9** was esterified to methyl ester

2.10 as previously and then subjected to the Sonogashira reaction with TIPS-acetylene, providing the respective TIPS-protected compound **2.105** in excellent yield. The methyl ester was hydrolysed to acid **2.106** and then subjected to amide forming conditions with amine **2.58** to give mono-protected acetylene **2.107** in a 74% yield.

The acetylenes **2.104** and **2.107** were then subjected to the respective click reactions, under the original conditions in the case of acetylene **2.107**, and the Cuevas conditions for the TMS-protected analogue **2.104**. Pleasingly, the desired triazole **2.108** was formed in both cases, with the Cuevas conditions at 100 °C in the presence of copper(I) bromide proving slightly better (Scheme 2.3.5). To evaluate whether this reaction was now purely proceeding under thermal conditions, the reaction was repeated in the absence of copper(I) bromide and none of the desired compound was isolated.



R^1 = H, **2.104**
R^1 = TMS, **2.107**

method a from **2.107** (48%), **2.108**
method b from **2.104** (60%), **2.108**
R^2 = H, **2.109**, 43%

Scheme 2.3.5: Click reaction to form triazole **2.108** and subsequent deprotection to G4 probe **2.109**. Reagents and conditions: a) **2.82** (1.0 equiv.), sodium-L-ascorbate (10 mol%), copper sulfate hydrate (10 mol%), DMF/H_2O (5:1), rt, 48 h, 48%; b) **2.82** (1.0 equiv.), copper(I) bromide (15 mol%), triethylamine (1.0 equiv.), H_2O (cat.), DMF, 100 °C, 1 h, 60%; c) potassium fluoride (15 equiv.), MeOH, rt, 336 h, 43%.

The deprotection of the TIPS-group of **2.108** was achieved using potassium fluoride rather than the more conventional TBAF. Using TBAF led to further purification issues as tetrabutylammonium salts were found to coelute with G4 probe **2.109**. The less soluble KF enabled clean conversion to the probe, however the reaction time for this transformation was significantly longer than when using TBAF.

The G4 benzyl probe **2.109** was tested against plant CYP450s by collaborator Maria Font-Farre and pleasingly showed labelling (Figure 2.3.3).[83] A band at ~55 KDa, corresponding to CYP450 enzymes after mass spectrometry analysis had been carried out, showed the upregulation of four CYP450 enzymes versus the six observed for the G1 probe following the *in vivo* click reaction. Although the labelling was weaker for the G4 probe, likely on account of increased steric bulk, the successful labelling of plant CYP450s with the biotinylated probe **2.109** had been demonstrated.[83]

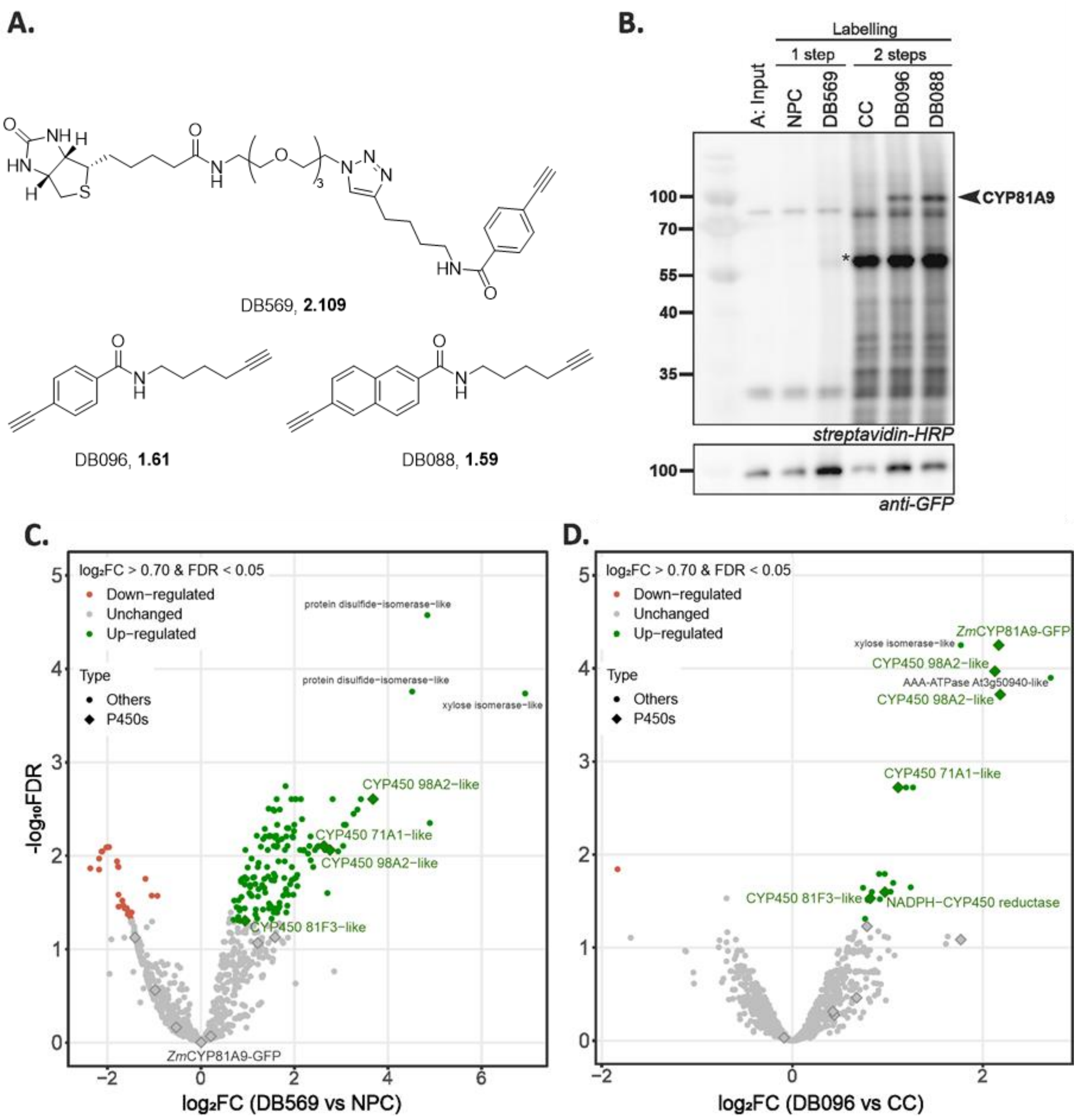
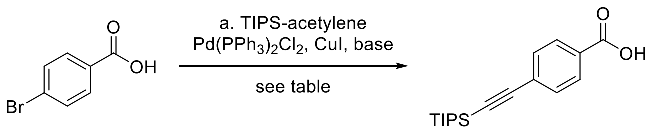


Figure 2.3.3: **A**: Structure of G4 probe DB569 **2.109** and G1 probes **1.61** and **1.59** used for testing. **B**: Labelling comparison of one step, DB569 **2.109**, and two steps, DB096 **1.61** and DB088 **1.59**, CYP450 labelling by protein blot. NPC corresponds to the no probe control of DB569. CC corresponds to the click chemistry control of DB096. **C** and **D**: Volcano plots highlighting proteins depleted and enriched by DB569 **2.109** and DB096 **1.61** labelling of CYP81A9 overexpressing *N. benthamiana* microsomes. Microsomes were incubated with 5 µM of DB569 **2.109** and DB096 **1.61**, or an equal volume of DMSO, in the presence of 1 mM NADPH. DB096-labelled proteins were subjected to click chemistry for biotin attachment and, DB569-labelled and DB096-labelled proteins were enriched *via* streptavidin pull-down, enzymatically digested and subjected to LC-MS/MS analysis. Enrichment is represented by volcano plots where the $\log_2$FC (CYP450 probe/control) where FC indicates the fold-change, against the significance ($-\log_{10}$FDR) where FDR indicates the false-discovery rate between the control and each CYP450 probe, DB569 **2.109** or DB096 **1.61**, using four or three independent biological replicates, respectively. The colour of the dots means whether proteins (dots) were enriched (green), unchanged (grey) or depleted (red) in the CYP450 probe containing sample. Reproduced with permission from Maria Font-Farre.[83]

With this promising result, the aim was to develop a more comprehensive library of probes with the use of the newly developed route. This would allow access a much greater number of probes, with various directing groups having been identified in order to explore the chemical space available for future probes. To this end, a final round of optimisation was required in order to make the route robust enough to be used in the automated synthesis platform at Syngenta where the library synthesis was to be undertaken.

The inability to synthesise the gram scale quantities of azide **2.102** reliably and safely in industry led to the possibility of moving the Sonogashira step to the front end of the synthesis. This would circumvent the need for azide **2.102** altogether and enable use of the original amine **2.58** as in the synthesis of G1 probes, this would be accompanied with a switch back to the original click reaction conditions for use with the free alkynes. A small optimisation of the desired Sonogashira reaction was conducted on 4-bromobenzoic acid **2.9** with microwave conditions being employed with the hope of bringing the reaction time down for this step (Table 2.3.2).



2.9 **2.110**

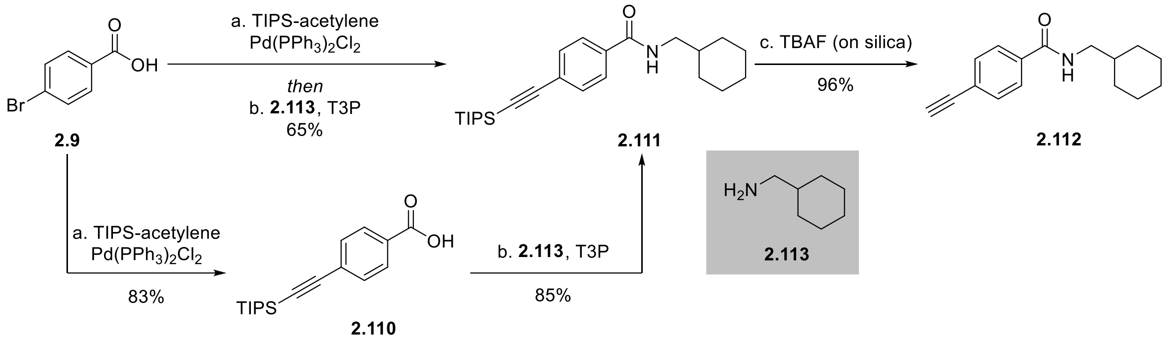
Entry	TIPS-acetylene/ equiv.	Temp/ °C	Time/mins	Solvent	Base	LCMS yield (isolated yield)/%
1	1.5	130	15	MeCN	Et_3N	26
2	5.0	130	15	MeCN	Et_3N	19
3	1.5	130	15	MeCN	DIPA	32
4	1.5	140	15	MeCN	DIPA	34
5	1.5	140	25	MeCN	DIPA	61
6	1.5	140	25	DMF	DIPA	100
7	1.5	120	25	DMF	DIPA	86
8	1.1	140	25	DMF	DIPA	95
9[a]	1.2	140	25	DMF	DIPA	89 (83)
10[b]	1.2	140	25	DMF	DIPA	83 (61)

Table 2.3.2: Reaction condition optimisation for the Sonogashira between 4-bromobenzoic acid and TIPS-acetylene. All reactions were carried out with 10 mol% $Pd(PPh_3)_2Cl_2$, 20 mol% CuI and with were possible without use of inert atmosphere (N_2) although this was still preferred. Reactions were carried out on a 0.10 mmol scale unless specified otherwise. [a]Reaction carried out on 1.0 mmol scale. [b]Reaction carried out on 2.0 mmol scale.

Since the reaction temperature was likely to exceed the boiling point of THF, and the final temperature required for this transformation on a reasonable time scale was unknown, acetonitrile was chosen as the solvent. This was in part due to its wide use in Sonogashira couplings generally, but also its successful use for previous couplings within the project. Attempting the reaction at 130 °C for 15 mins as a starting point (entry 1) gave only a 26% yield of TIPS-acetylene **2.110** as judged by LCMS analysis. Increasing the equivalents of TIPS-acetylene (entry 2), offered no significant improvement to the yield. Changing the base to DIPA as opposed to Et_3N gave a small increase in yield (entry 3), however increasing the temperature to 140 °C (entry 4) offered no further boost to the respective yield. The first of the more significant breakthroughs were realised upon increasing the reaction time to 25 mins (entry 5), which had the effect of doubling the observed yield. Changing the solvent to DMF (entry 6) led to 100% LCMS yield of TIPS-acetylene **2.110**, presumably the increased solubilising power of DMF as opposed to MeCN with respect to the starting material solubilities, allowed for a greater level of conversion. The crude mixture in this case appeared far more soluble than the previous entries where MeCN had been the solvent of choice. Dropping the temperature to 120 °C (entry 7) had detrimental effects on the yield observed, however significantly dropping the TIPS-acetylene equivalents (entry 8) to near equimolar with respect to the starting material still gave a very high yield of **2.110**.

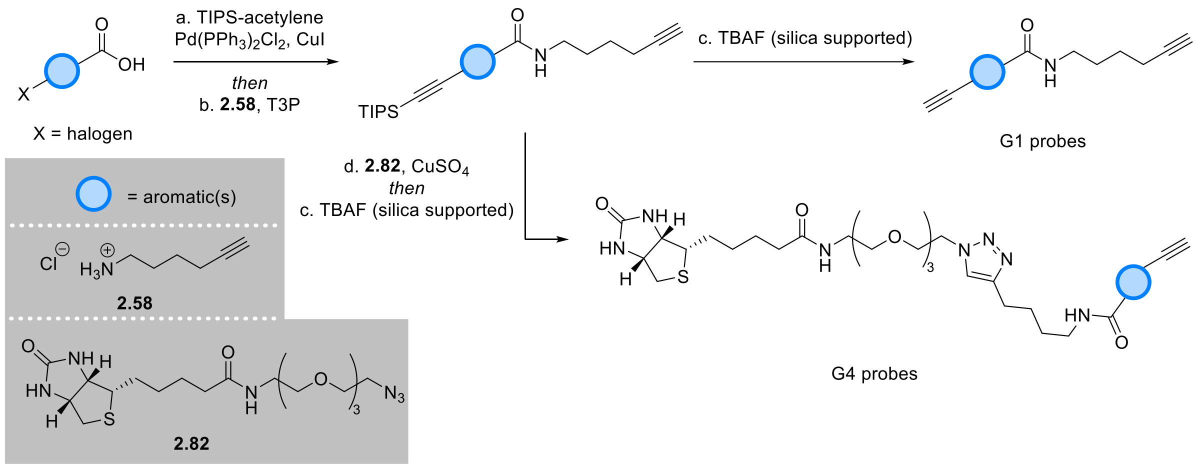
The reaction was scaled up from 0.1 mmol to 1.0 mmol as this was the scale required for the library synthesis (entry 9), to ensure full consumption of the starting material and thus enable more easy purification, the equivalents of TIPS-acetylene was marginally increased from 1.1 to 1.2, this was still lower than the original conditions (entry 6). Isolation of the TIPS acetylene **2.110** was achieved in 83% yield on this scale, with further scaling of the reaction from 1.0 mmol to 2.0 mmol (entry 10) leading to a decreased yield. Although there appears to be an issue with further scaling up of the reaction, for the purpose of the library synthesis, the conditions were deemed acceptable.

In order to streamline the process further, telescoping the amide formation was trialled. Due to unavailability at the time of the actual amine **2.58** (shown previously) for the formation of probes, cyclohexylmethylamine **2.113** was used as a model substrate for this step (Scheme 2.3.6).



Scheme 2.3.6: Comparison of two-step procedure to form amide **2.112** *via* acid **2.110** *versus* telescoped procedure to form amide **2.112** and subsequent deprotection with solid supported TBAF for use in library synthesis. Reagents and conditions: a) triisopropylsilyl acetylene (1.2 equiv.), $Pd(PPh_3)_2Cl_2$ (10 mol%), copper(I) iodide (20 mol%), DIPA (5.0 equiv.), DMF, µW, 140 °C, 25 mins, yields given; b) **2.113** (1.5 equiv.), propylphosphonic anhydride (50% in EtOAc, 1.1 equiv.), triethylamine (2.0 equiv.), CH_2Cl_2, rt, 16 h, yields given; c) TBAF (silica supported, 1.1 equiv.), THF, rt, 1 h, 96%.

The synthesis of amide **2.112** was shown to be comparable in yield either by direct synthesis from the purified acid **2.110** or *via* the telescoped procedure directly from 4-bromobenzoic **2.9** acid giving 65% yield over two steps or 70% yield respectively of amide **2.111**. It was then possible to greatly shorten the reaction time of the deprotection sequence with the use of solid supported TBAF rather than the KF (previously necessary in the synthesis of G4 probe **2.109**); no evidence of any tetrabutylammonium salts were present in the crude ^{1}H NMR obtained after this step that gave acetylene **2.112** in excellent yield. This gave confidence that the final deprotection step would now be amenable to automation without the need for prohibitively long reaction times in an industrial setting. A summary of the proposed library route towards G1 and G4 probes is given below (Scheme 2.3.7).



Scheme 2.3.7: Summary of industry route towards G1 and G4 probes.

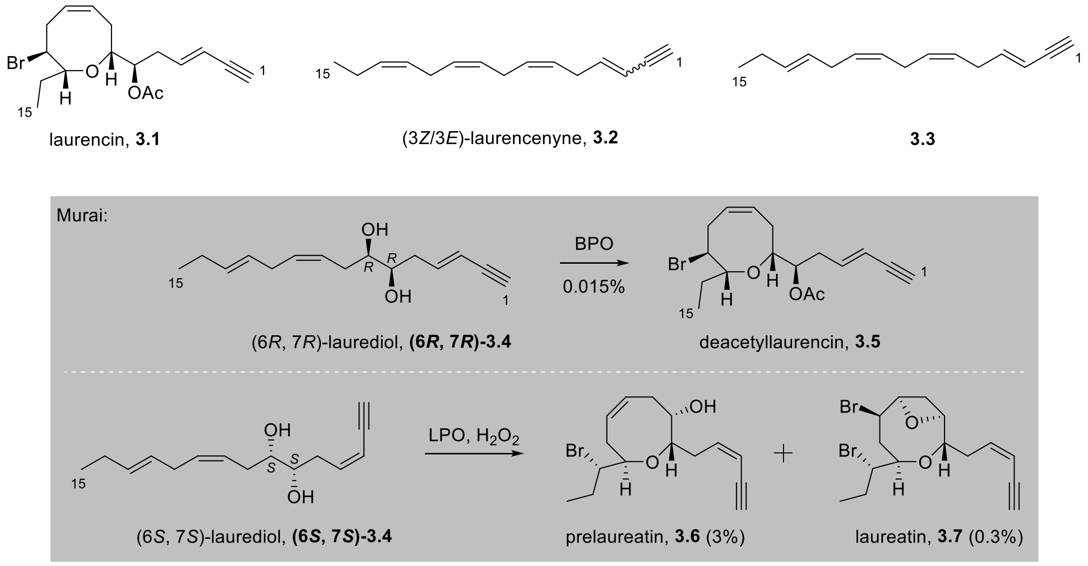
Treatment of halogenated aromatic acids under the established Sonogashira reaction and amide coupling sequence with amine **2.58** would give access to the TIPS protected probe precursors that can either be deprotected immediately to G1 probes or subjected to the click reaction with **2.82** and then deprotected to G4 probes. Unfortunately, due to the ongoing COVID-19 pandemic, it was not possible to conduct any of the library synthesis.

This chapter has detailed the synthetic efforts towards probes targeting plant CYP450 enzymes, a number of probes of different types (G1 – G4) were synthesised to meet criteria that evolved following probe testing. The G1 probes, although easy to synthesise, were not suitable for testing on plants; they instead found use as probes for fungal CYP450s later in the project. G2 and G3 probes (ester and amide linked biotinylated probes) allowed the biotinylation chemistry required to be trialled comprehensively, ultimately these probes remained only test substrates for the chemistry and had little use in CYP450 labelling. Upon conception of the G4 probes, the synthesis of CYP450 probes was streamlined and common intermediates to both G1 and G4 were accessed. This allows access to both of the probe generations of interest in a rapid and modular manner, although a full library could not be synthesised. For a full conclusion of this work see chapter 7.

3. *Laurencia* Natural Products

3.1. Isolation and Biosynthetic Postulates

The *Laurencia* genus, found in temperate waters across the globe is but one genus of red algae in the Rhodomelaceae family with significant interest to both biologists and chemists, and is estimated to be one of the largest marine algal families, containing over 700 recognised species worldwide.[92] Natural products from the *Laurencia* genus have been widely studied, and since the first report of the isolation of laurencin **3.1** from *Laurencia gladulifera* in 1965, the number of isolated natural products has grown extensively (Scheme 3.1.1).[92–96] The interesting biological profiles exhibited by numerous halogenated natural products of this type include: protein phosphatase inhibition, antitumour function, cytotoxic properties and general antiviral and antimicrobial activity.[92] Combining their medicinal properties with their interesting structural motifs and complex stereochemical relationships makes them attractive targets for study.[94–98]



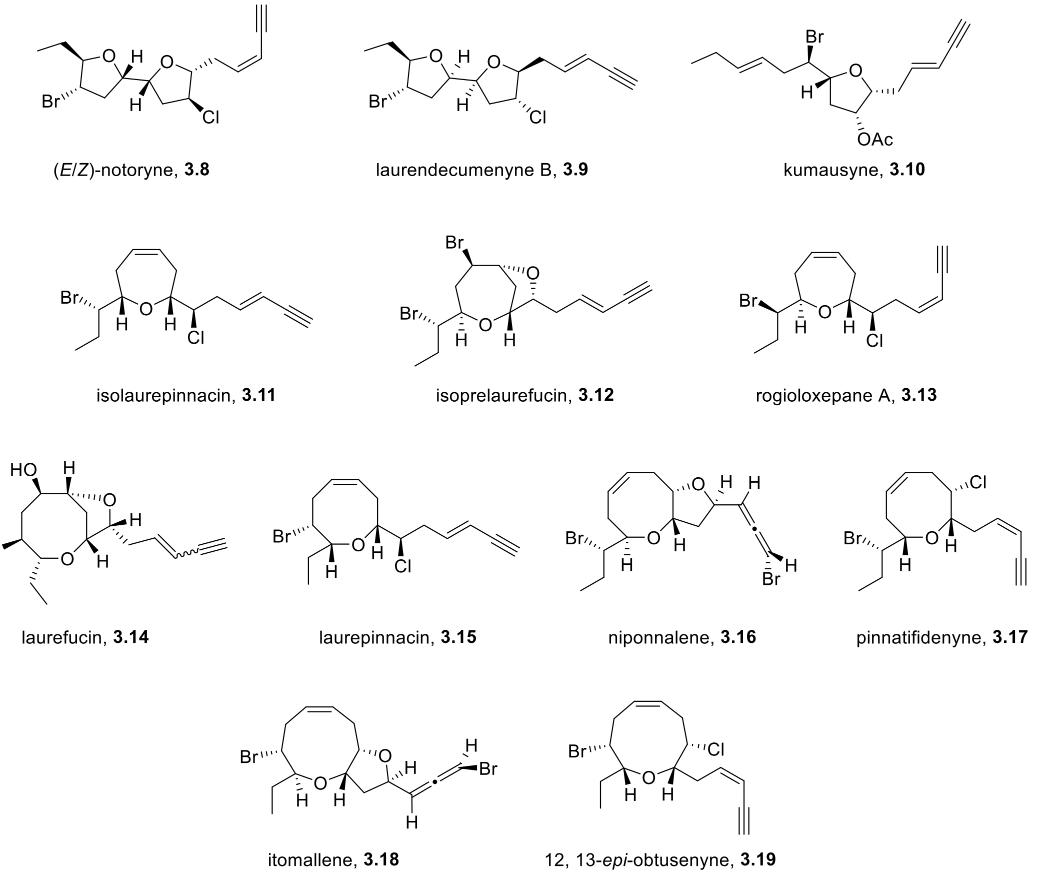
Scheme 3.1.1: Structures of Laurencin **3.1**, (3*Z*/3*E*)-laurencenynes **3.2** and selected related linear C_{15}-acetogenins **3.3** and **3.4**. Formation of deacetyllaurencin **3.5** under enzymatic conditions with partially purified BPO from *Laurencia nipponica* by Murai and co-workers and similar formation of prelaureatin **3.6** and laureatin **3.7** with LPO.

The compounds isolated from these algal species are mostly C_{15}-acetogenins, thought to be derived ultimately from a C_{16} fatty acid. However, linear C_{15} metabolite laurencenyne **3.2** and its related analogues **3.3**, **(6*R*, 7*R*)-3.4**, and **(6*S*, 7*S*)-3.4**, have been isolated in their own right and form the basis for a number of biosynthetic postulates in the literature (*vide infra*).[99] A number of other metabolites with similar linear scaffolds have also been isolated but have been omitted for clarity of discussion. Murai and co-workers demonstrated in the early 1990s that laurediol **(6*R*, 7*R*)-3.4** could be converted into deacetyllaurencin **3.5** under enzymatic conditions using a bromoperoxidase enzyme (BPO) isolated from *Laurencia nipponica* (Scheme 3.1.1).[100,101] Additionally, they were able to form prelaureatin **3.6** and laureatin **3.7** from the related laurediol **(6*S*, 7*S*)-3.4** with the opposite diol stereochemistry. This transformation required a similar enzyme to BPO, lactoperoxidase (LPO), since the same transformation with BPO led to only trace amounts of prelaureatin **3.6**.

The C_{15}-acetongenins are typically halogenated medium ring ethers bearing enyne or bromoallene functionality and are thought to be forged from their linear precursors *via* bromocyclization reactions utilising BPOs and inorganic bromide as a source of Br^+ (Figure 3.1.1).[95,97,102] The examples shown highlight the structural diversity that can be obtained from the linear precursors and they typically contain five to nine-membered rings.

For the five-membered ring containing species, (*E*/*Z*)-notoryne **3.8** and laurendecumenyne B **3.9** bear the bis-THF motif whilst kumausyne **3.10** has a single THF unit with both bromine and chlorine atoms present in the former examples. Moving into the seven-membered rings, isolaurepinnacin **3.11** and rogioloxepane A **3.13** similarly contain bromine and chlorine atoms, whilst isoprelaurefucin **3.12** contains a bicyclic structure and two bromine atoms. The eight-membered rings make up the most numerous examples of natural products from the

Laurencia genus, again with laurefucin **3.14** and niponnalene **3.16** bearing bicyclic scaffolds and niponnalene **3.16** containing the bromoallene functionality. Further examples, laurepinnacin **3.15** and pinnatifidenyne **3.17** instead consist of only a single ring and the bromine and chlorine atoms as seen in previous examples. Structures with larger rings, itomallene **3.18** and 12, 13-*epi*-obtusenyne **3.19** have also been isolated.



(*E*/*Z*)-notoryne, **3.8**

laurendecumenyne B, **3.9**

kumausyne, **3.10**

isolaurepinnacin, **3.11**

isoprelaurefucin, **3.12**

rogioloxepane A, **3.13**

laurefucin, **3.14**

laurepinnacin, **3.15**

niponnalene, **3.16**

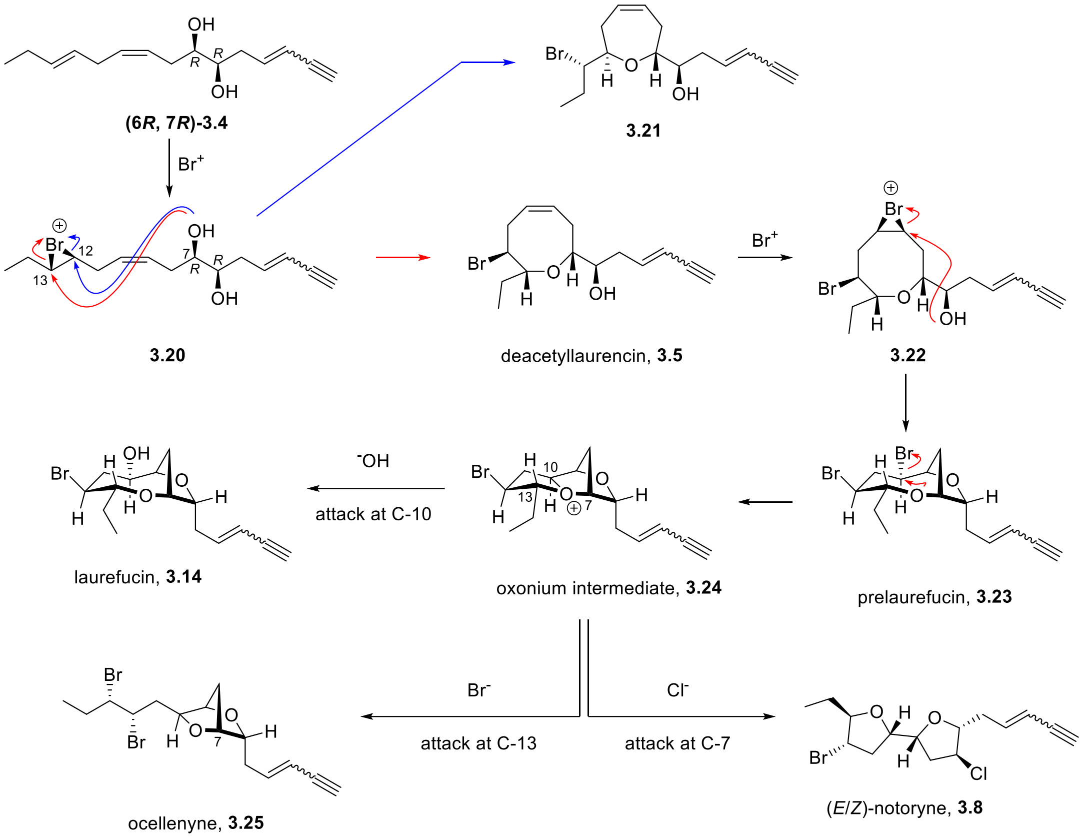
pinnatifidenyne, **3.17**

itomallene, **3.18**

12, 13-*epi*-obtusenyne, **3.19**

Figure 3.1.1: Representative examples of *Laurencia* natural products **3.8** to **3.19** showcasing the diversity in chemical structures.

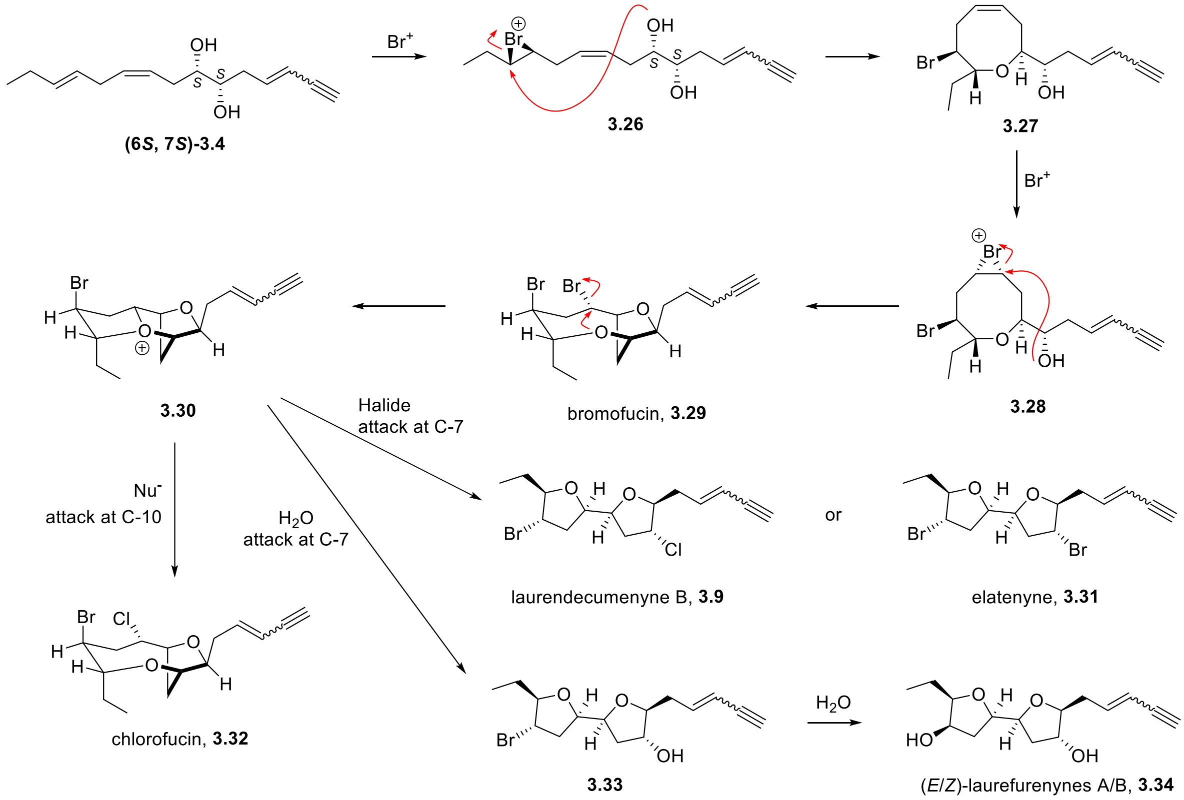
The isolation and structural elucidation of (*Z*)-notoryne **(*Z*)-3.8** was published in 1991 by Suzuki and co-workers.[103] Within this paper, one of the first postulates for the biosynthesis of a number of *Laurencia* natural products is presented; interestingly, they invoke oxonium intermediates to account for a number of natural products isolated previously (Scheme 3.1.2).[104]



Scheme 3.1.2: Suzuki biosynthetic postulate for formation of *Laurencia* natural products.

Beginning from (6*R*, 7*R*)-laurediol **(6*R*, 7*R*)-3.4**, Suzuki proposed bromonium ion **3.20** formation followed by cycloetherification at C-12 (blue route) or C-13 (red route), using the alcohol at C-7, giving either medium ring ether **3.21** – termed deacetylisolaurencin by Suzuki and co-workers – or deacetyllaurencin **3.5** respectively. From deacetyllaurencin **3.5**, bromonium ion formation on the endocyclic alkene leads to intermediate **3.22** which can undergo a second cyclisation event, this time from the C-6 alcohol giving prelaurefucin **3.23**. Transannular displacement of the bromide group gives the oxonium intermediate **3.24** first postulated by Suzuki *et al*. in their work and quenching of this intermediate, at either the C-7, C-10 or C-13 positions with a variety of nucleophiles, gives rise to the structurally diverse compounds laurefucin **3.14**, (*E*/*Z*)-notoryne **3.8** and ocellenyne **3.25**.[105,106]

Building upon this initial biosynthetic postulate, the Kim and Burton groups have invoked a different laurediol stereoisomer **(6*S*, 7*S*)-3.4** to account for the formation of another set of structurally related natural products including chlorofucin **3.32** and laurendecumenyne B **3.9**; laurediol exists naturally as unequal mixtures of 3*E*/*Z*, 12*E*/*Z*, *RR*/*SS* stereoisomers.[106–109] Their postulate is closely related to the original case presented by Suzuki and again utilises an oxonium ion intermediate **3.30** to account for the variety of structurally diverse natural products observed (Scheme 3.1.3).

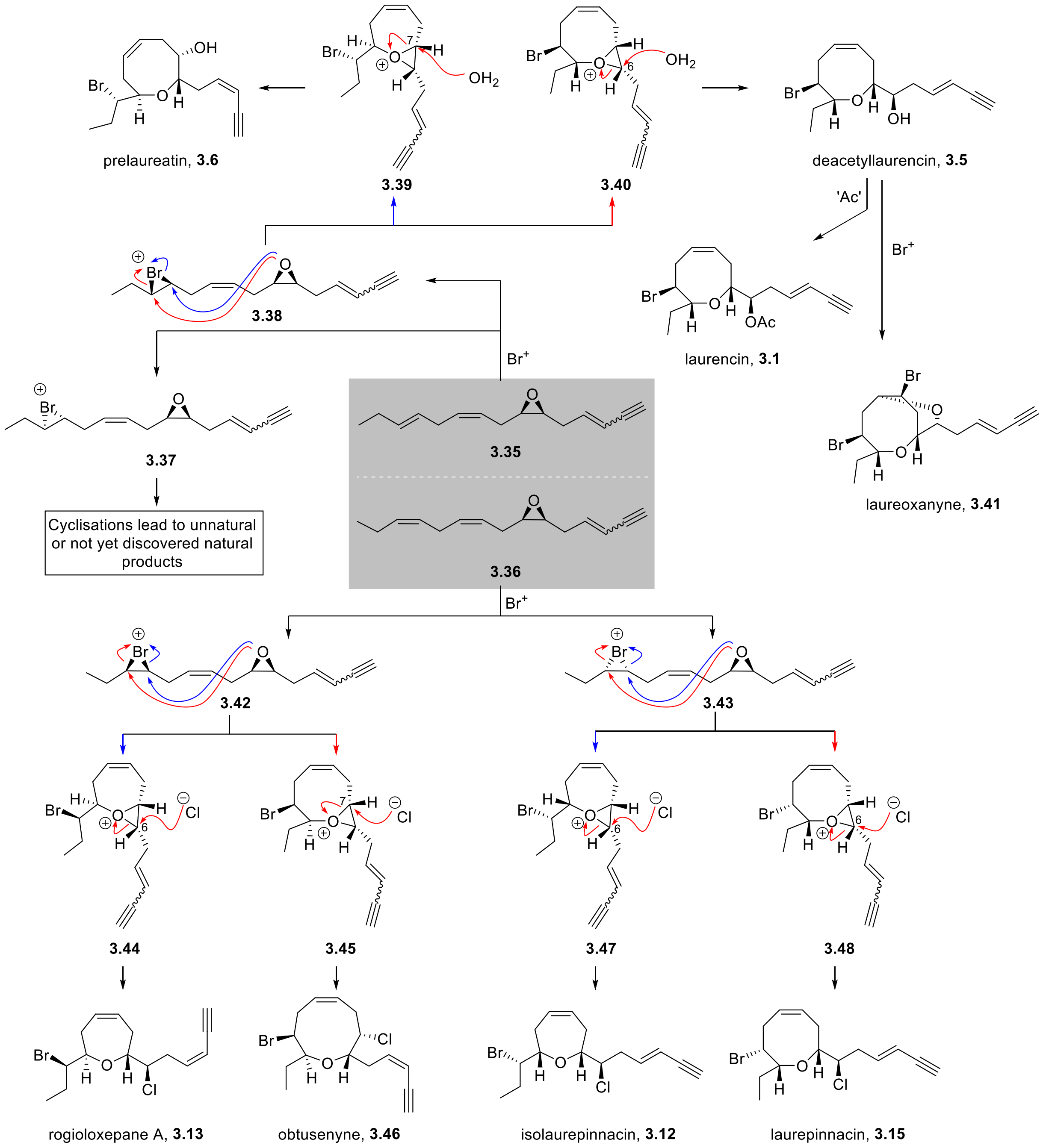


Scheme 3.1.3: Kim and Burton's biosynthetic proposals from laurediol **(6*S*, 7*S*)-3.4** for the formation of bromofucin **3.29**, chlorofucin **3.32**, and bis-THFs **3.9**, **3.31**, **3.33** and **3.34**.

This time, starting from (6*S*, 7*S*)-laurediol **(6*S*, 7*S*)-3.4**, an analogous route is taken. It is proposed that bromonium ion **3.26** is first formed which undergoes cyclisation to give deacetyllaurencin diastereoisomer **3.27**, this is proposed to undergo a second bromonium ion formation to give **3.28** akin to the Suzuki intermediate. Cyclisation through the C-6 alcohol gives bromofucin **3.29**, proposed to react in a similar fashion by displacement of the bromide and transannular cyclisation to give the oxonium ion intermediate **3.30**. Nucleophilic attack at C-7 by chloride or bromide gives laurendecumenyne B **3.9** or elatenyne **3.31** respectively whilst C-10 quench gives chlorofucin **3.32**.[110–114] This biosynthetic pathway is thought to also account for the formation (E/*Z*)-laurefurenynes A/B **3.34**. C-7 quench of the oxonium ion by water gives alcohol **3.33** which undergoes subsequent displacement of the remaining bromide *via* an S_N2 reaction with water, giving rise to (E/*Z*)-laurefurenynes A/B **3.34**, whose structures were reassigned by the Burton and Britton groups in 2013.[115,116] C-10 attack of H_2O gives laurefurenynes C – F (not shown).[117]

More recently, Braddock and co-workers have attempted to unify the biosynthetic pathway towards the medium ring ether natural products isolated from *Laurencia* species. To this end, they have proposed epoxides **3.35** and **3.36** derived from linear C_{15}-acetogenins **3.2** shown previously, as the starting point for biosynthetic derivatisation (Scheme 3.1.4).[98]

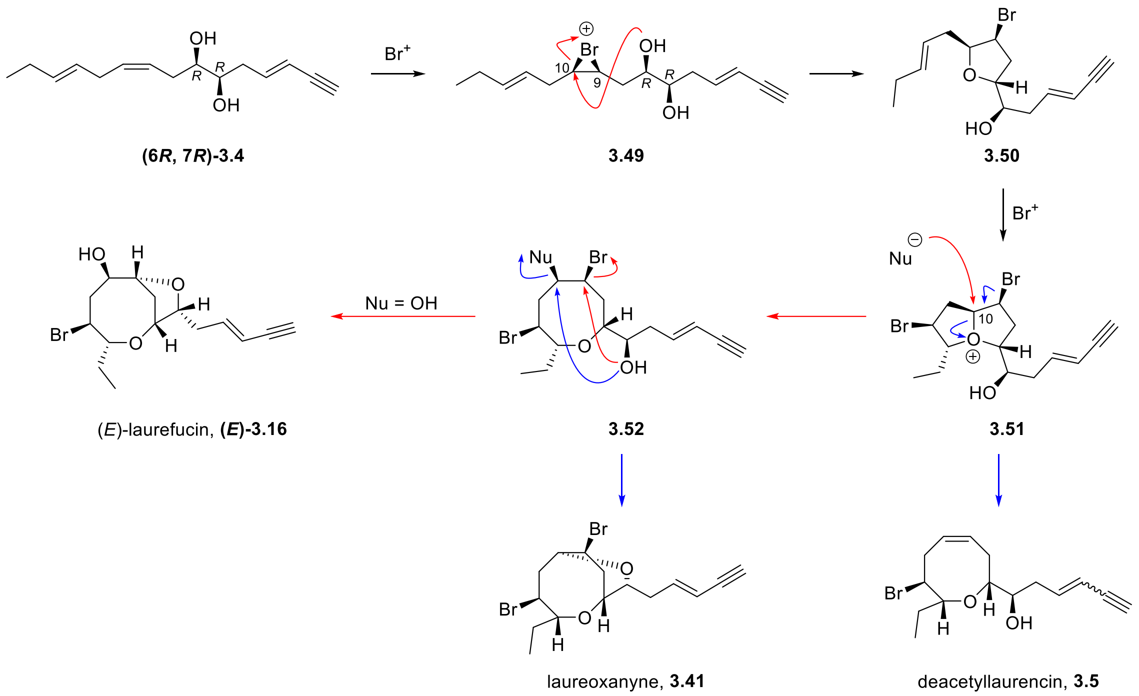
Beginning from epoxide **3.35** in the C-12,-13 (*E*)-configuration, diastereomeric bromonium ions **3.37** and **3.38** are proposed to form under enzymatic conditions. Cyclisation of bromonium ion **3.37** at either C-12 or C-13 from the epoxide oxygen gives epoxide containing oxonium intermediates where the quench products have not yet been isolated, these are not shown. On the other hand, cyclisation of the other bromonium ion diastereoisomer **3.38** leads to formation of seven and eight-membered oxonium ion intermediates **3.39** and **3.40**, these are proposed to quench at either C-7 or C-6 with water giving prelaureatin **3.6** or deacetyllaurencin **3.5**. Deacetyllaurencin **3.5** can undergo either acylation to give laurencin **3.1** or react with another equivalent of Br^+ to give laureoxenyne **3.41**.



Scheme 3.1.4: Braddock's proposal of a unified biosynthesis of medium ring ether natural products from epoxides **3.35** and **3.36**, several reaction pathways leading to alternative natural products are not show for clarity.

Alternatively, the C-12,-13 (*Z*)-epoxide **3.36** can undergo similar bromonium ion formation to give bromonium ions **3.42** and **3.43** (Scheme 3.1.4). In this case, both diastereoisomers are shown to lead to natural products isolated from *Laurencia* species. In the case of oxonium ion intermediates **3.44** and **3.45**, quenching at C-6 or C-7 with chloride gives either rogioloxepane A **3.13** or obtusenyne **3.46** respectively. For the other diastereoisomers **3.47** and **3.48**, quenching of the seven-membered ring oxonium ion **3.47** at C-6 gives isolaurepinnacin **3.11** whereas the eight-membered ring oxonium ion **3.48** quenches similarly at C-6, this time giving laurepinnacin **3.15**. For the sake of brevity, not all of the quench products of oxonium ion intermediates **3.44** to **3.48** are shown despite the fact that several of the quench events lead to natural products in their own right. Similarly, several of the intermediates and natural products shown can be converted into other natural products not shown in a single step. This proposal of unsaturated epoxides leading to medium ring ether natural products in the presence of Br^+ is backed up by experimental evidence carried out by Braddock and co-workers.[98]

Orthogonally to the above postulates, Snyder *et al*. proposed that the 7-*endo* and 8-*endo* cyclisations proposed in the above biosyntheses might be unfavoured, citing both existing literature concerning the closure of rings of various sizes and Murai's exceptionally low yield of deacetyllaurencin as possible reasons for this.[100,118] Instead, they propose a series of more favourable 5-*endo* cyclisations from (6*R*, 7*R*)-laurediol **3.4** in order to form the bicyclic oxonium ion intermediate **3.51** *via* bromonium ion **3.49** and THF **3.50**. From oxonium ion **3.51**, a number of natural products **3.5**, **(*E*)-3.16** and **3.41** can be obtained (Scheme 3.1.5).[119]



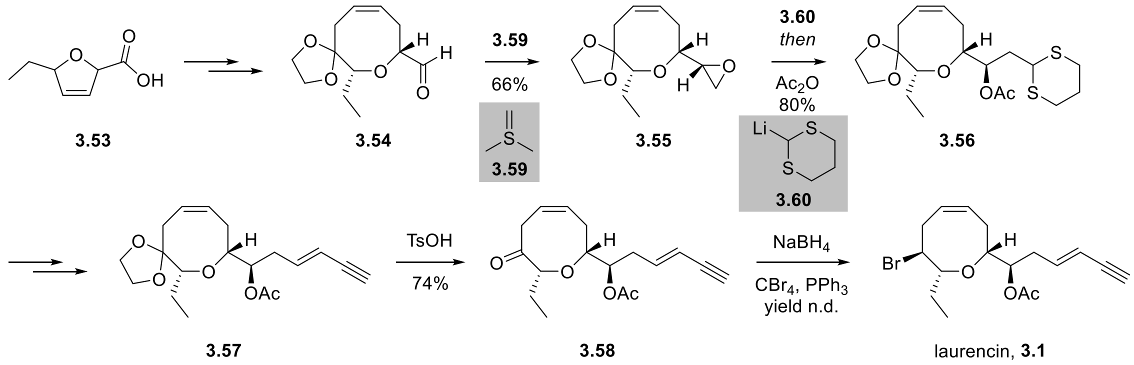
Scheme 3.1.5: Snyder biosynthetic proposal for the formation of deacetyllaurencin **3.5**, (*E*)-laurefucin **(*E*)-3.16** and laureoxanyne **3.41**.

Similarly to Suzuki's proposal (*vide supra*), Snyder's proposal begins from (6*R*, 7*R*)-laurediol **(6*R*, 7*R*)-3.4**, with bromonium ion formation at the C-9/C-10 alkene followed by a 5-*endo*-cyclisation using the C-7 alcohol giving THF **3.50**. A second 5-*endo*-cyclisation following the formation of another bromonium ion intermediate (not shown) leads to the formation of bicyclic oxonium ion **3.51**. Nucleophilic attack at C-10 leads to medium ring ether **3.52**, whereas loss of Br^+ and opening of the oxonium ion **3.51** at the transannular position gives deacetyllaurencin **3.5**. From medium ring ether **3.52**, intramolecular reactions of the C-6 alcohol can lead to the natural products (*E*)-laurefucin **(*E*)-3.16** and laureoxenyne **3.41** shown.

3.2. Synthetic Endeavours Towards *Laurencia* Natural Products and Recent Highlights from the Burton Group

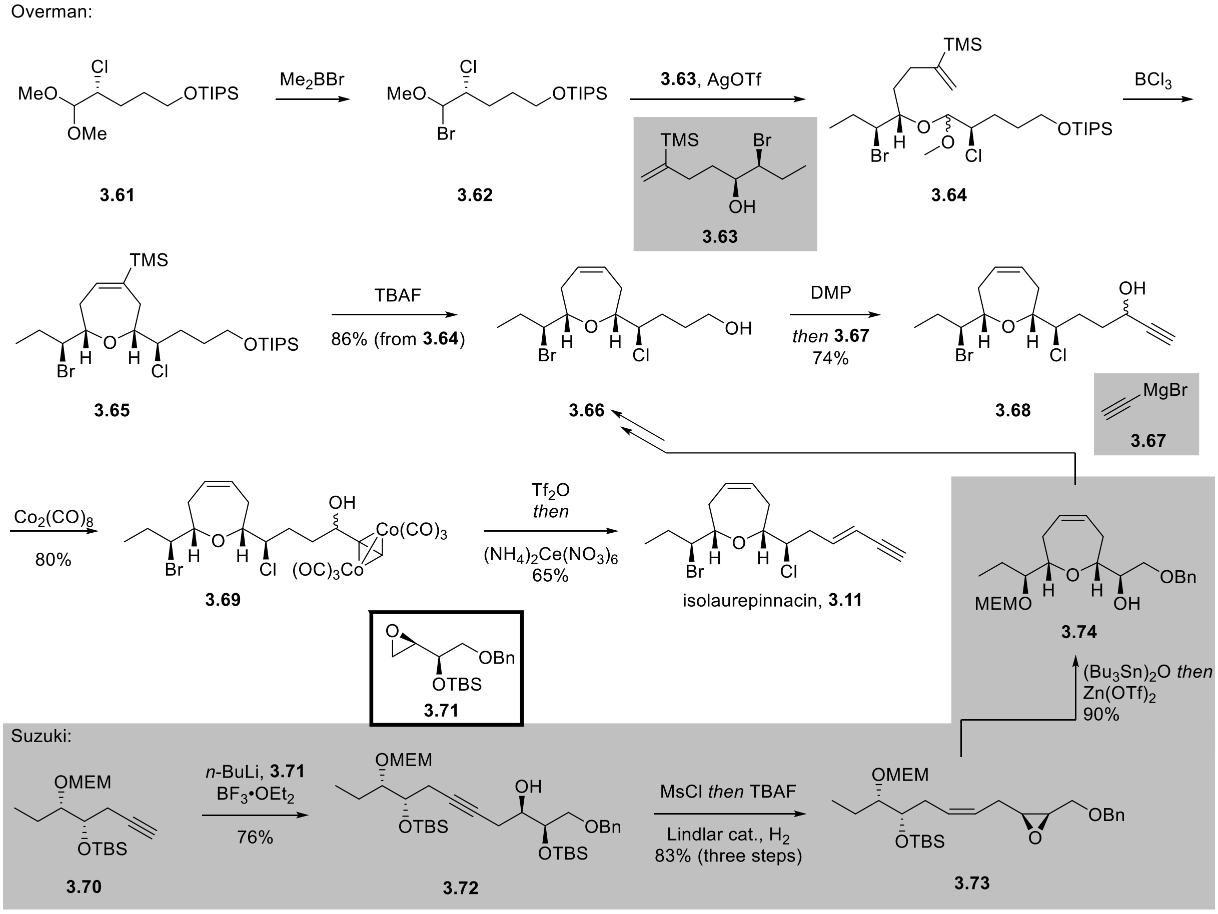
Given the interesting biomimetic postulates put forward to rationalise the structures of natural products isolated from the *Laurencia* species, it is perhaps unsurprising that their *de novo* syntheses by organic chemists have long been pursued.[120–128] The functionalised medium ring ether scaffold, along with the intricate stereochemical relationships found in the compounds have led to the *Laurencia* natural products being attractive targets not only for the development of new chemical methods, but also to confirm the proposed structures of the natural products themselves.

Given the vast number of syntheses of natural products from *Laurencia* species only a few examples are covered here. To date, there have been over 50 syntheses reported beginning with Masamune's pioneering synthesis of (±)-laurencin **3.1** in the late 1970s.[102] Masamune's synthesis utilises aldehyde **3.54**, prepared from 5-ethyl-2-furoic acid **3.53**, for their derivatisation into (±)-laurencin **3.1** (Scheme 3.2.1).[129,130] Formation of the respective epoxide **3.55** was achieved by treatment with the Corey-Chaykovsky ylide **3.59**. Reaction of this epoxide with lithiated dithiane **3.60**, followed by *in situ* acylation allowed for isolation of the acetate **3.56**. The dithiane moiety could be hydrolysed with mercury(II) oxide and the resulting aldehyde immediately converted into enyne **3.57** using a Wittig reaction. (±)-laurencin **3.1** was then accessed following the cleavage of the acetal to ketone **3.58**, reduction of this ketone with sodium borohydride followed by bromination of the resulting alcohol with carbon tetrabromide and triphenylphosphine.



Scheme 3.2.1: Masamune synthesis of (±)-laurencin **3.1** from aldehyde **3.54.**

The Overman and Suzuki groups have both contributed heavily to the literature on the synthesis of *Laurencia* natural products, with both groups presenting routes towards (+)-isolaurepinnacin **3.11** in the 1990s, with the Suzuki synthesis being a formal synthesis (Scheme 3.2.2).[131–133] Orthogonal strategies were employed by both the Overman and Suzuki groups; in the first case, the Overman group used an alkene-acetal cyclisation to form the seven-membered ether whereas the Suzuki group employed a more classical epoxide opening reaction.



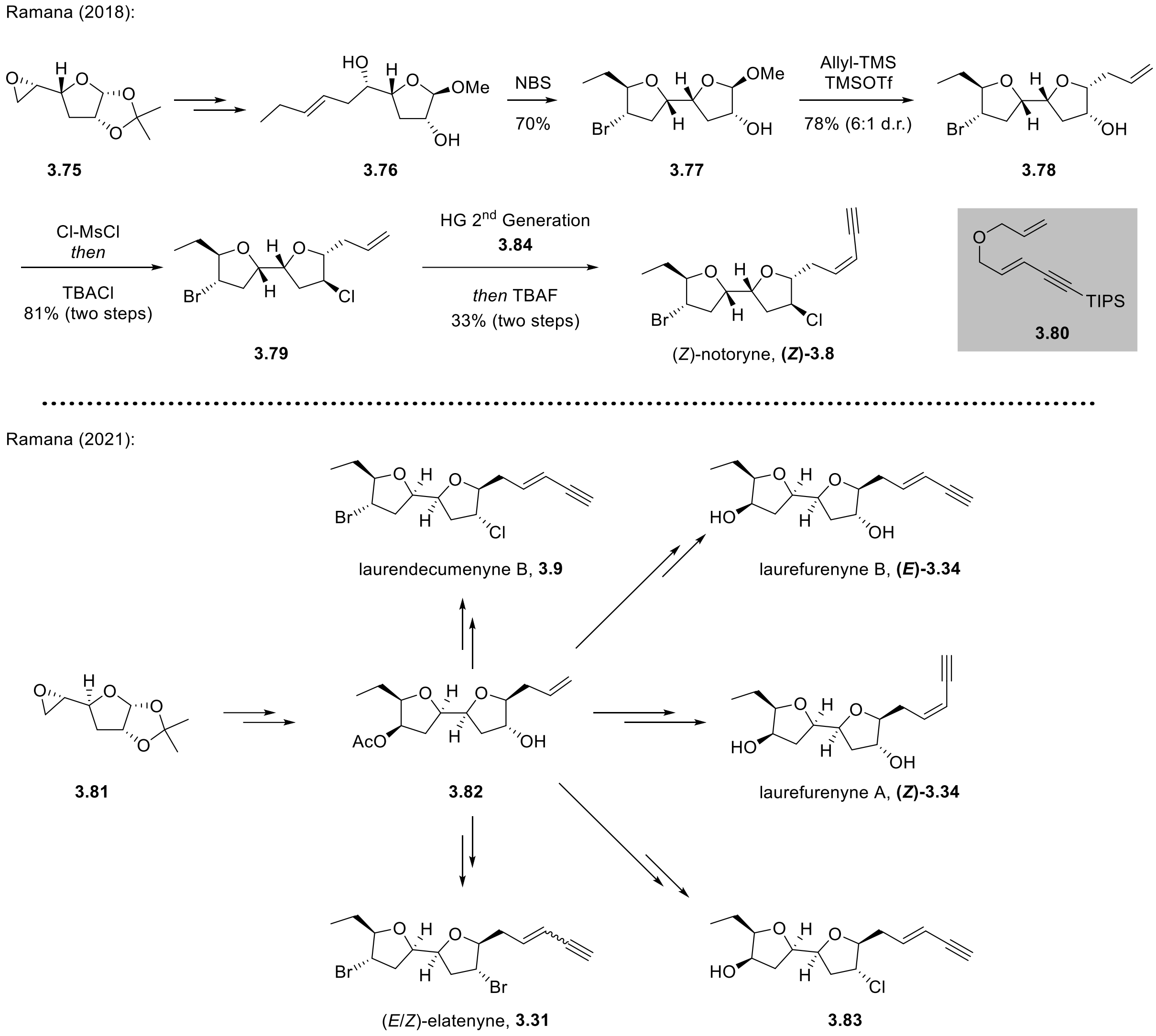
Scheme 3.2.2: Synthesis of (+)-isolaurepinnacin **3.11** by Overman and co-workers and formal synthesis by Suzuki *et al.* (grey box).

To access cyclisation precursor **3.64**, Overman and co-workers demonstrated that acetal **3.61** could be brominated to give bromide **3.62** and then coupled to alcohol **3.63** in the presence of silver(I). This compound could then be treated with BCl$_3$ to enable chlorination of the methoxy-ether position of **3.64** and cyclisation through the tethered alkene to give medium ring ether **3.65** which could be deprotected by TBAF giving medium ring ether **3.66**. Oxidation of the free alcohol followed by reaction with ethynylmagnesium bromide **3.67** gave propargyl alcohol **3.68**. The necessary dehydration had to be conducted after complexation of the alkyne with dicobalt octacarbonyl giving protected alkyne **3.69**, otherwise both the (*E*)- and (*Z*)-isomers of (+)-isolaurepinnacin **3.11** were formed.[132]

In the case of the Suzuki strategy (Scheme 3.2.2, grey box), alkyne **3.70** was lithiated on reaction with *n*-butyllithium and the respective anion added into epoxide **3.71** in the presence of boron trifluoride etherate to give alkyne **3.72**. Formation of the cyclisation precursor **3.73** was achieved *via* a mesylation, deprotection of the TBS-alcohols and epoxide formation followed by Lindlar reduction of the alkyne to give (*Z*)-olefin **3.73**. This substrate was subjected to cyclisation conditions in the presence of tin and zinc salts to give seven-membered ring **3.74**. Conversion of **3.74** over a number of steps gave alcohol **3.66**, from which Overman had previously carried out the remaining steps towards (+)-isolaurepinnacin **3.11** as described above.[133]

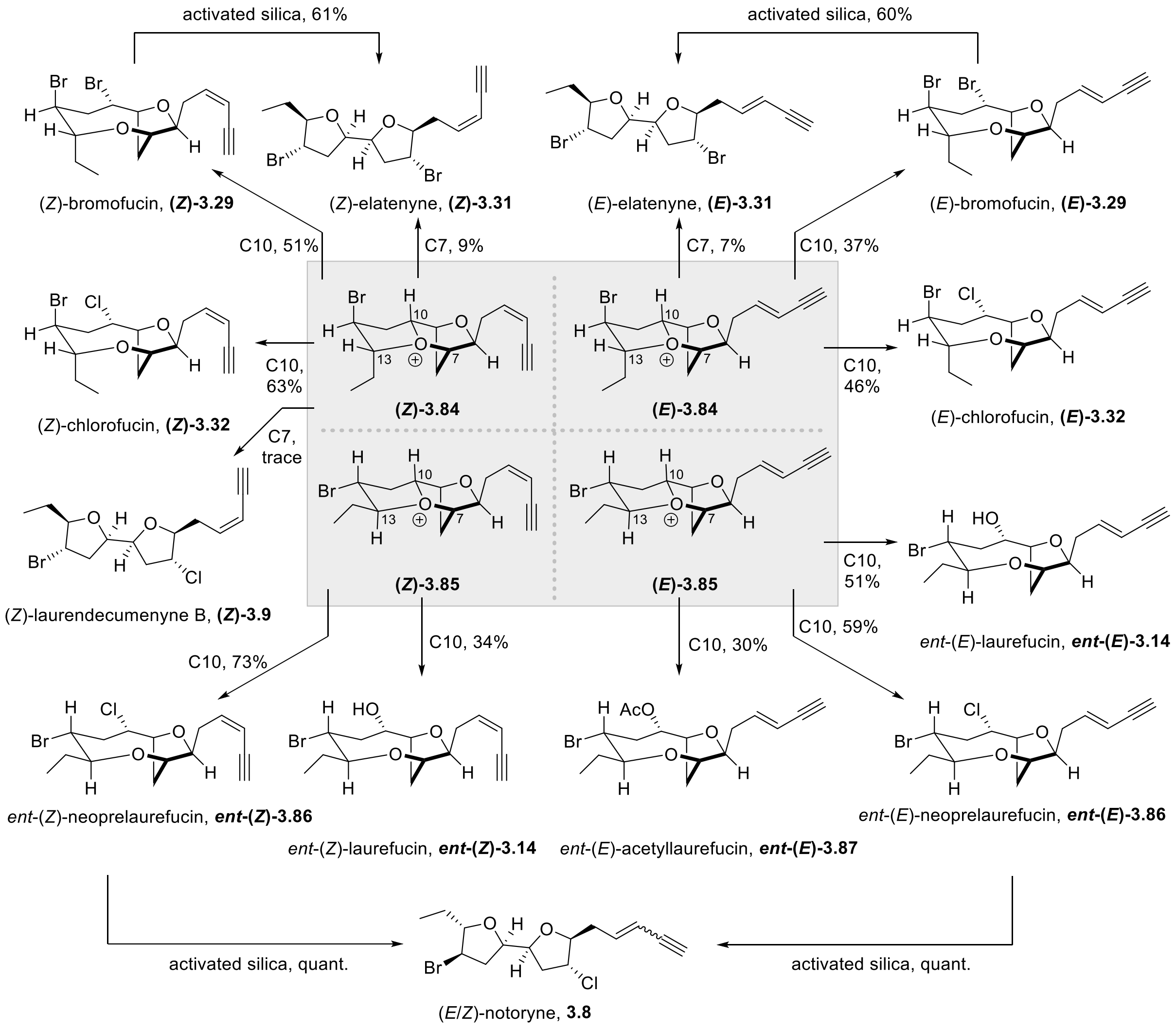
More recently, the Ramana group detailed the first total synthesis of (*Z*)-notoryne **(*Z*)-3.8** (Scheme 3.2.3).[134] In this work, epoxide **3.75**, derived from a glucose diacetonide was converted into furan **3.76** which was shown to undergo bromocyclisation with NBS to give bis-THF **3.77**. Sakurai olefination gave allylic intermediate **3.78** with good diastereoselectivity (6:1 for the 1,2-*cis* compound). Chlorination of the alcohol could be achieved with inversion of configuration by treating with chloromethanesulfonyl chloride and then TBACl to give chloride **3.79**. This was then subjected to relay-Grubbs metathesis with ether **3.80** in the presence of 2nd generation Hoveyda-Grubbs catalyst and the TIPS-group deprotected to give (*Z*)-notoryne **(*Z*)-3.10**.

Building upon these studies, the same group published an extension to this work and unified the synthesis of a number of bis-THF natural products from a closely related epoxide intermediate **3.81** (Scheme 3.2.3, bottom).[135] Key bis-THF **3.82** was accessed following a similar sequence to their previous work (not shown). From bis-THF **3.82**, the synthesis of laurefurenynes A/B **(*E*/*Z*)-3.34**, chloroenyne **3.83**, laurendecumenyne B **3.9** and elatenyne **3.31** was achieved.



Scheme 3.2.3: Ramana's synthesis of (*Z*)-notoryne **(*Z*)-3.8** and unified synthesis of bis-THF natural products **3.9**, **3.31**, **(*E*/*Z*)-3.34**, and **3.83.**

The Burton group has had an interest in natural products from the *Laurencia* genus for a number of years, with early work leading to the structural reassignment of a number of the bis-THF natural products shown above.[116,136] More recently, work has been centred around the characterisation and reactivity of the complex oxonium ions **3.84** and **3.85** proposed as key intermediates in the biosynthesis of *Laurencia* natural products (Scheme 3.2.4).[102] As part of this work, Burton and co-workers have synthesised a range of natural products, or enantiomers of natural products upon treatment of the oxonium ions with a range of nucleophiles.



Scheme 3.2.4: Complex oxonium ions **3.85** to **3.86** characterised by Chan *et al*. and the synthesis of a number of *Laurencia* natural products. Adapted from the Work of Chan and co-workers.[102]

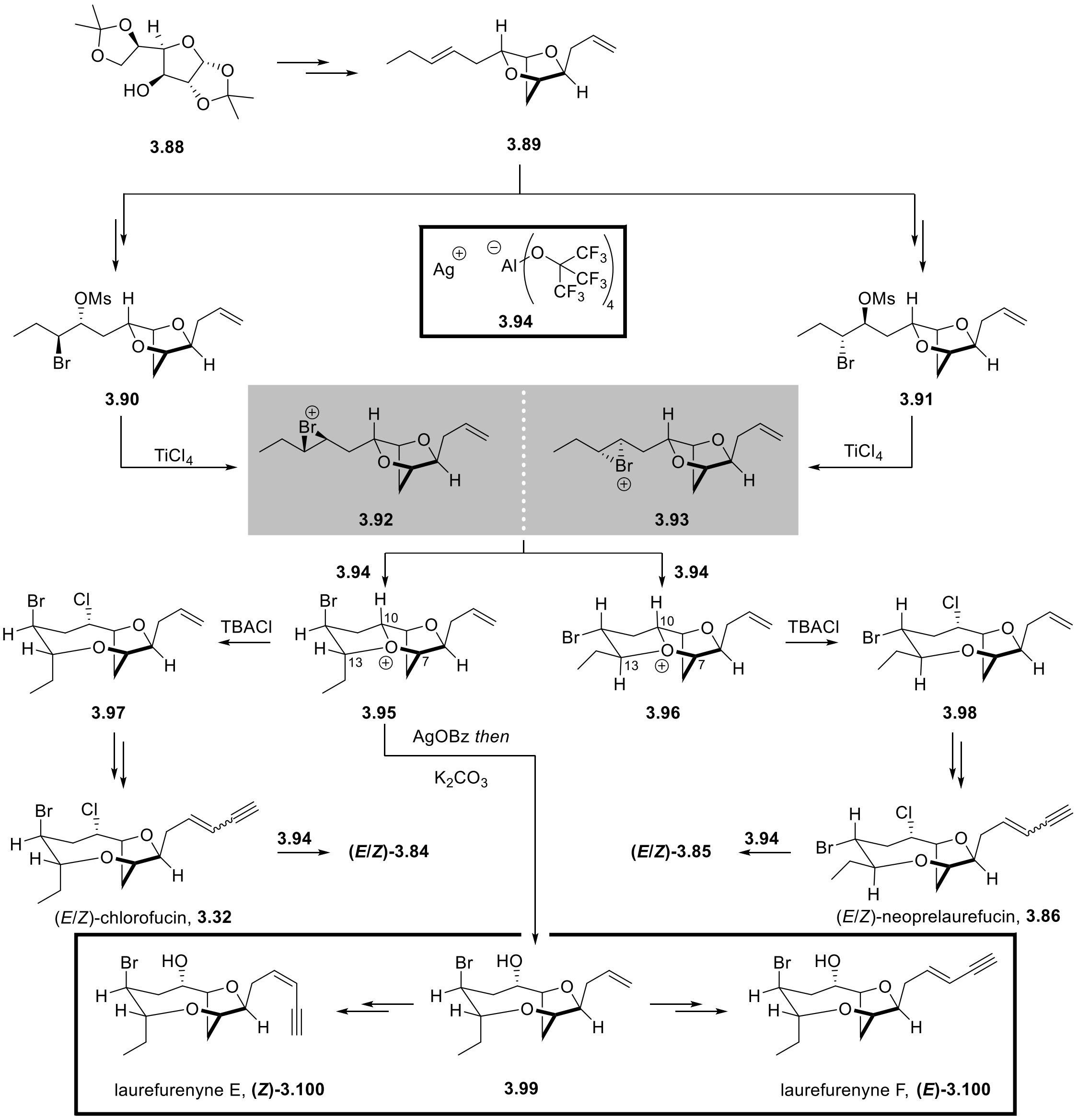
For example, quenching of the well characterised oxonium ion **(*Z*)-3.84**, by either chloride or bromide at C-10 leads to formation of (*Z*)-chlorofucin **(*Z*)-3.32** or (*Z*)-bromofucin **(*Z*)-3.29** in 63% yield and 51% yield respectively. The chloride quench is accompanied by the formation of trace amounts of laurendecumenyne B **3.9** by the quench at C-7. Similarly, quenching of oxonium ion **(*E*)-3.84** with the same halogens leads to their respective (*E*)-isomer natural products, (*E*)-chlorofucin **(*E*)-3.32** in 46% yield and (*E*)-bromofucin **(*E*)-3.29** in 37% yield.

Concomitant formation of minor (but isolable) amounts of (*E*/*Z*)-elatenyne **3.31** were observed by similar quenching at C-7 from oxonium ions **(*E*/*Z*)-3.84** respectively. These bis-THF natural products could be formed independently from the C-10 quench products by treatment with activated silica.[106]

From the other oxonium ion diastereoisomers **(*E*/*Z*)-3.85** a similar array of natural products can be formed. Oxonium ion **(*Z*)-3.85** was shown to undergo quenching by either chloride or water to *ent*-(*Z*)-neoprelaurefucin ***ent*-(*Z*)-3.86** or *ent*-(*Z*)-laurefucin ***ent*-(*Z*)-3.14**. Oxonium ion **(*E*)-3.85** on the other hand could be quenched by either chloride, acetate or water to give *ent*-(*E*)-acetyllaurefucin ***ent*-(*E*)-3.87**, *ent*-(*E*)-neoprelaurefucin ***ent*-(*E*)-3.86** or *ent*-(*E*)-laurefucin ***ent*-(*E*)-3.14** respectively. A similar skeletal rearrangement from (*E*/*Z*)-neoprelaurefucins **3.86** granted access to (*E*/*Z*)-notoryne **3.8** quantitatively.

The linchpin to this work was the ability to synthesise the key oxonium ions, which ultimately relied upon the generation of enantiomerically pure bromonium ions which was achieved using a method pioneered by Braddock and co-workers.[137] From [2.2.1]-dioxabicycloheptane intermediate **3.89** (prepared from diacetone-D-glucose **3.88**), both bromonium ion diastereoisomers **3.92** and **3.93** could be accessed after treatment of the respective bromomesylates **3.90** and **3.91** with titanium tetrachloride (Scheme 3.2.5). Addition of silver salt(I) **3.94** to the *in situ* generated bromonium ions gave oxonium ions **3.95** and **3.96**.[138] The choice of silver(I) salt **3.94** was vital for the clean formation of oxonium ions **3.95** and **3.96** from the respective bromonium ions. Silver (I) salt **3.94** was selected as its anion is very non-coordinating and non-nucleophilic, with silver(I) being necessary to sequester excess chloride ions that would inhibit formation of the two oxonium ion species of interest **3.95** and **3.96**.

Quenching of the oxonium ion species at C-10 could be achieved with TBACl to give C-10 chlorides **3.97** and **3.98**, from these C-10 chlorides, derivatisation into the (*E*/Z)-chlorofucins **3.32** and (*E*/*Z*)-neoprelaurefucins **3.86** was possible and allowed for formation of the oxonium ion species **3.84** and **3.85** (shown in Scheme 3.2.4). Notably, in the quenching of oxonium ions **3.95** and **3.96**, minor amounts of the C-7 products were also observed (not shown). A key limitation of this work was the incompatibility of the bromomesylates bearing an enyne moiety to the titanium tetrachloride/silver(I) mediated cyclisation meaning that the lengthier route shown was required for the direct generation of *Laurencia* natural products from oxonium ions **3.84** and **3.85**.



Scheme 3.2.5: Use of bromomesylates **3.90** and **3.91** for the formation of enantiomerically pure bromohydrins **3.92** and **3.93** by $TiCl_4$ and the subsequent formation of oxonium species **3.95** and **3.96** with silver(I) salt **3.94**. Quenching of oxonium species **3.95** and **3.96** with chloride to give C-10 chlorides **3.97** and **3.98** for further derivatisation into chlorofucin **3.32** and neoprelaurefucin **3.86**. Direct quenching of oxonium species **3.95** with AgOBz to give C-10 alcohol **3.99** following hydrolysis in order to facilitate the synthesis and reassignment of the structures of laurefurenynes C – F **3.100**, laurefurenynes C and D are not shown (boxed).

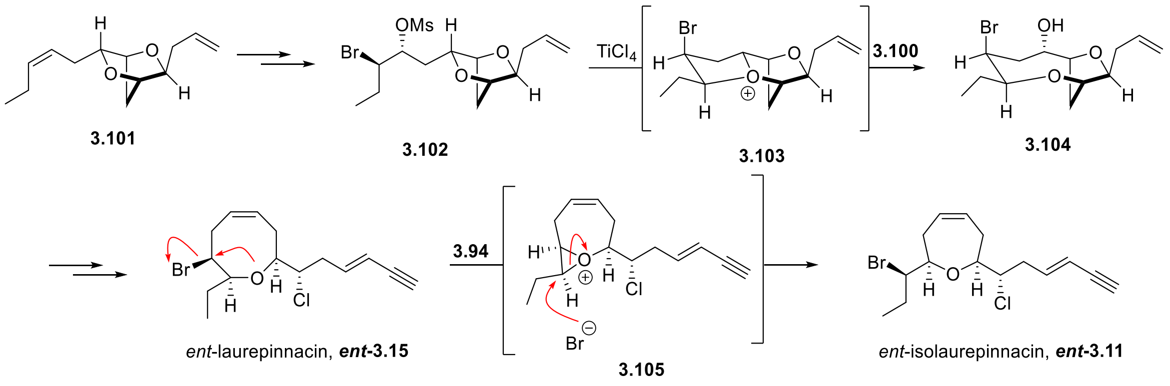
The work was recently expanded to tolerate an oxygen nucleophile (AgOBz) in the quenching of oxonium ion **3.95**. This allowed direct access to C-10 alcohol product **3.99**, from which laurefurenynes C – F could be accessed and their structures reassigned accordingly.[117] The oxonium rearrangement protocol was, however, only possible on a small scale (50 mg) of starting material since there were problems associated with the solubility of the silver(I) salt

3.94. In addition to this, large quantities of silver (I) salt with respect to starting material (3.2 equiv.) were required to effect the transformation.

3.3. Approach and Aims of the Project

Given the knowledge accrued in the group surrounding the synthesis of *Laurencia* natural products from complex oxonium species, this project aimed to expand the work further and target a further two natural products, *ent*-laurepinnacin ***ent*-3.15** and *ent*-isolaurepinnacin ***ent*-3.11** (Scheme 3.3.1). It was envisaged that *ent*-laurepinnacin ***ent*-3.15** could be accessed from a closely related [2.2.1]-dioxabicyloheptane **3.101** to that used in previous studies, with the (*Z*)-configuration of the olefin rather than the (*E*)-olefin shown previously.[102,117]

The synthesis of bromomesylate **3.102** would follow precedented procedures from [2.2.1]-dioxabicycloheptane **3.101** and then silver(I) mediated oxonium ion formation was expected to grant access to oxonium ion **3.103**.[102,139] From this species, quenching with AgOBz would give C-10 alcohol **3.104** which was expected to be converted over a number of steps to *ent*-laurepinnacin ***ent*-3.15**. From *ent*-laurepinnacin ***ent*-3.15**, a silver(I) mediated skeletal rearrangement could be envisaged, granting access to *ent*-isolaurepinnacin ***ent*-3.11** *via* an epoxide oxonium ion species **3.105** - closely related to those proposed by Braddock and co-workers in their biosynthesis, and aimed to be characterised as part of this work - provided that suitable conditions could be found.[98,140]



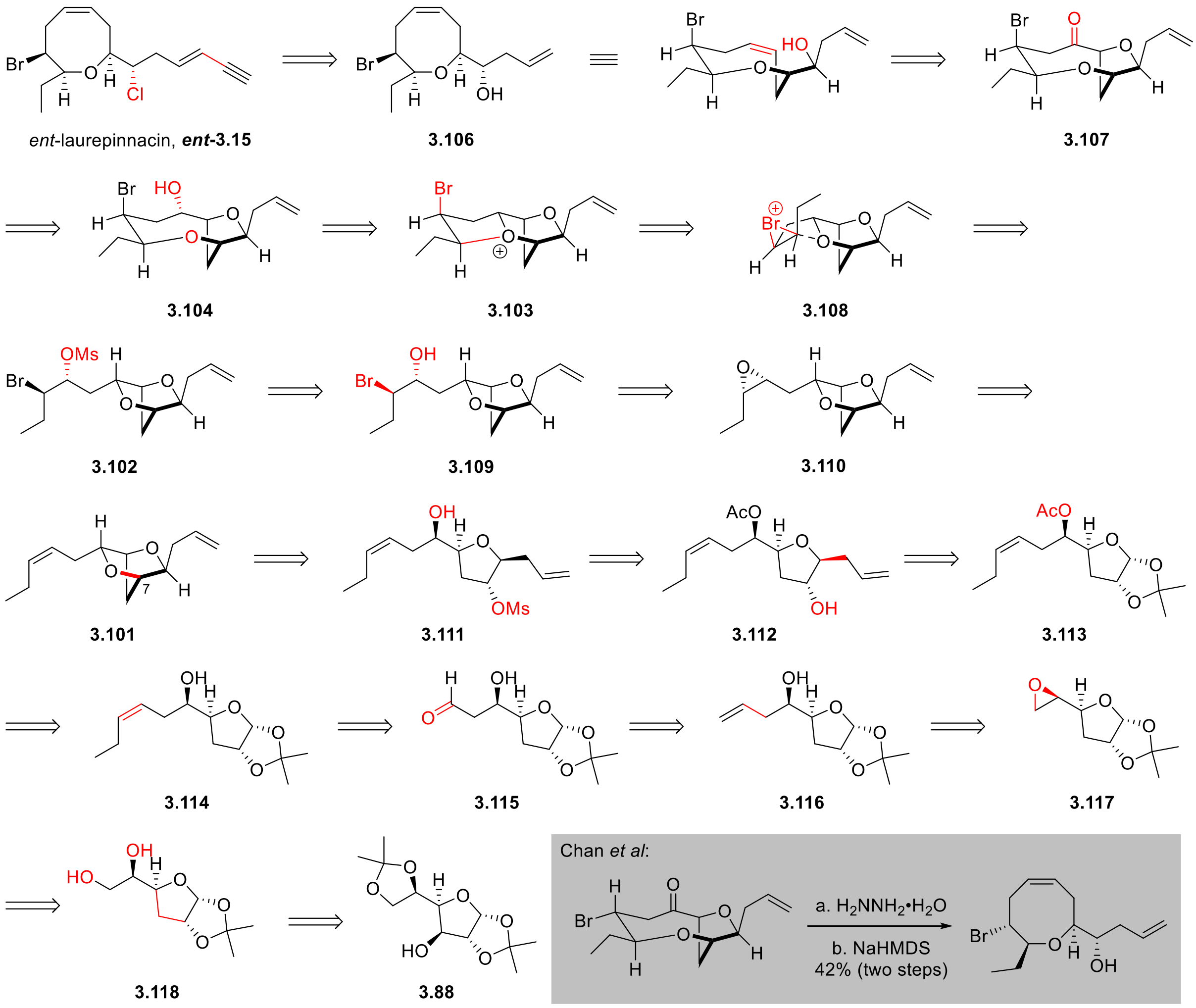
Scheme 3.3.1: Proposed synthesis of *ent*-laurepinnacin ***ent*-3.15** from [2.2.1]-dioxabicycloheptane **3.101** and rearrangement of *ent*-laurepinnacin ***ent*-3.15** to *ent*-isolaurepinnacin ***ent*-3.11** under silver(I) conditions.

ent-Laurepinnacin ***ent*-3.15** was selected as the first target on account of starting material availability. Given the success of previous work in this area, a similar retrosynthetic strategy was implemented for the initial synthesis attempts towards the natural product.

The (*E*)-enyne functionality and chloride were expected to be installed by precedented procedures.[139,141,142] Oxidative cleavage of the terminal alkene of medium ring ether **3.106** and Wittig reaction with the semi-stabilised phosphonium ylide derived from triphenyl(3-(trimethylsilyl)prop-2-yn-1-yl)phosphonium bromide would install the enyne (Scheme 3.3.2).[141,142] For the chloride, treatment of the free alcohol with thionyl chloride, as per the original isolation paper, was expected to be sufficient.[143]

The core structure itself was envisaged to be accessible from ketone **3.107** following Kishner-Leonard elimination, whilst the ketone **3.107** would arise from the oxidation of the C-10 alcohol **3.104** obtained from the silver(I)-mediated skeletal rearrangement of bromomesylate **3.102** *via* the bromonium ion **3.108** and oxonium ion **3.103** according to Braddock's method and previous work by Chan (Scheme 3.3.2, boxed).[117,137,144,145] The bromomesylate **3.102** of interest could be generated from its respective bromohydrin **3.109**, accessed by the magnesium assisted opening of the mixed epoxides formed in the reaction of key [2.2.1]-

dioxabicycloheptane **3.101** with *m*-CPBA. Utilising this methodology would also grant access to the diastereomeric bromohydrin **5.9** (not shown), although not directly related to any natural products, study of the oxonium generated in this case was also sought so that the respective oxonium reactivities could be evaluated.



Scheme 3.3.2: Retrosynthetic analysis of *ent*-laurepinnacin ***ent*-3.15** leading back initially to key [2.2.1]-bicycloheptane **3.101** and ultimately diacetone-D-glucose **3.88**. Chan *et al*. Kishner-Leonnard elimination.[139]

From [2.2.1]-dioxabicycloheptane **3.101**, disconnection of the C7-O bond would lead back to mesylate **3.111**, accessed from acetate **3.112** following deprotection of the acetate group and mesylation of the free alcohol (Scheme 3.3.2). The allyl group was envisaged to be introduced by reacting the acetonide **3.113** under Sakurai allylation conditions. In order to synthesise the (*Z*)-olefin **3.114**, Wittig reaction with the aldehyde **3.115**, derived from the alkene **3.116** following Lemieux-Johnson oxidation, was expected to allow access to the desired olefin **3.114** with the correct alkene geometry.

Alkene **3.116** was expected to be accessible according to previously reported conditions from diacetone-D-glucose **3.88**. A deoxygenation, and primary acetonide deprotection would lead to diol **3.118**. Mitsunobu reaction on the primary alcohol, followed by *in situ* trapping would give epoxide **3.117**, from which vinylmagnesium bromide addition in the presence of copper(I) would lead to alkene **3.116**.

Chapters IV to VI detail the synthetic endeavours towards *ent*-laurepinnacin ***ent*-3.15**. Chapter IV details the validation of steps involved in the synthesis of key [2.2.1]-dioxabicycloheptane **3.101** addressing of problems associated with the (*Z*)-alkene synthesis for a model substrate. Chapter V outlines the synthesis of [2.2.1]dioxabicycloheptane **3.110** and the subsequent steps in the synthesis of *ent*-laurepinnacin ***ent*-3.15**, with the route ultimately stalling at the Kishner-Leonard elimination. Chapter VI details efforts undertaken to mitigate issues pertaining to the silver(I)-mediated cyclisation as mentioned above and concludes with the synthesis of related *Laurencia* natural products, *ent*-(*E*)-notoryne **(*E*)-3.8** and laurendecumenyne B **3.9** *via* a silver(I)-free skeletal rearrangement.

4. Initial Attempts to Synthesise Key [2.2.1]-Dioxabicycloheptane

4.1. Attempted Synthesis *via* Wittig Route

The campaign to synthesise the key [2.2.1]-dioxabicycloheptane **3.101** from protected sugar **3.88** aimed to remain close to the original route developed in the group, with the major alteration in the synthetic sequence being use of the Wittig reaction to append the respective ethyl chain in the (*Z*)-configuration giving alcohol **3.114** rather than the Julia-Kocienski or Grubbs metathesis giving the (*E*)-isomer **4.1** used in the synthesis of [2.2.1]-dioxabicycloheptane **3.89** (Figure 4.1.1).[102]

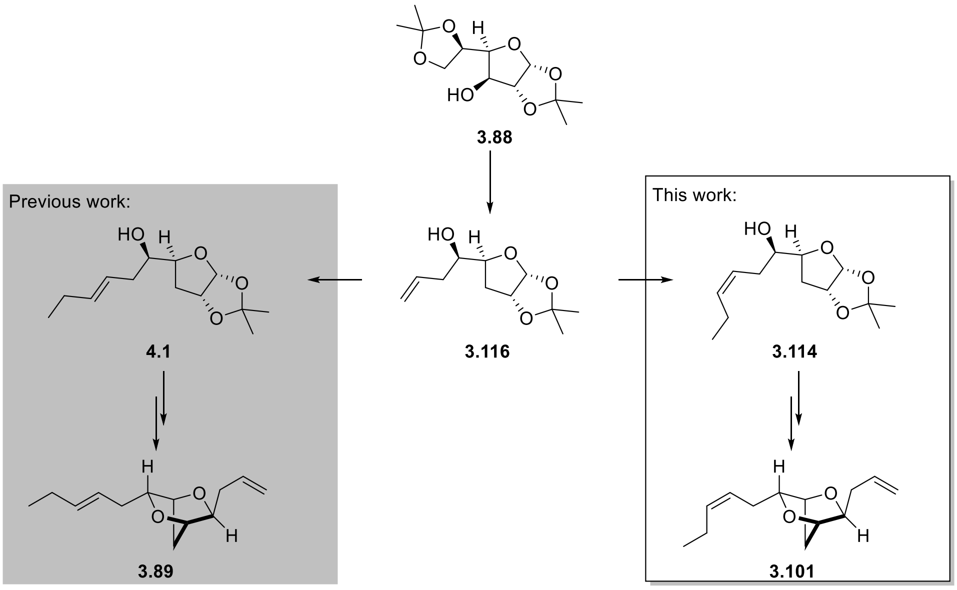
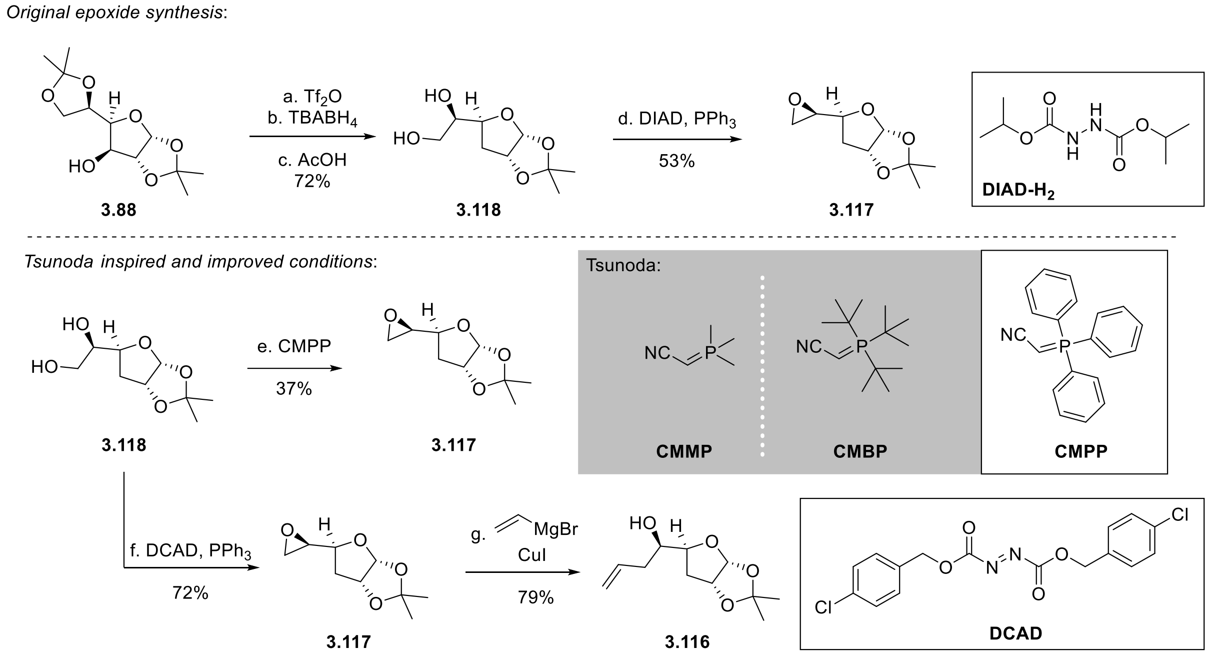


Figure 4.1.1: Formation of (*Z*)-alkene *via* Wittig reaction as opposed to (*E*)-alkene synthesised previously in the group.

Synthesis of olefin **3.116**, required in the oxidative cleavage and Wittig sequence began from readily available diacetone-D-glucose **3.88**. protected sugar **3.88** was subjected to the previously developed triflation, deoxygenation, and acetonide deprotection sequence to give diol **3.118** in 52% yield over three steps (Scheme 4.1.1).[146,147] This was subjected to Mitsunobu conditions giving epoxide **3.117** in 53%. The main issue here was the difficult purification of epoxide **3.117** from the reduced DIAD-H_2 by-product shown due the propensity of the reduced DIAD to streak during column chromatography and coelute with the epoxide **3.117**; thus, alternative conditions were sought for this troublesome step.



Scheme 4.1.1: Telescoped synthesis of diol **3.118** and formation of epoxide **3.117** according to original Mitsunobu conditions (top). Tsunoda Mitsunobu conditions for the formation of epoxide **3.117** and successful synthesis using DCAD followed by Grignard conditions for the formation of alcohol **3.116** (bottom). Reagents and conditions: a) triflic anhydride (1.2 equiv.), pyridine (2.2 equiv.), CH_2Cl_2, 0 °C – rt, 1 h *then* b) tetrabutylammonium borohydride (2.5 equiv.), toluene, 80 °C, 8 h *then* c) 75% AcOH (aq.), rt, 18 h, 72%; d) DIAD (1.0 equiv.), triphenylphosphine (1.2 equiv.), toluene, 110 °C, 18 h, 53%; e) (triphenylphosphoranylidene)acetonitrile (2.0 equiv.), toluene, 150 °C, 22 h, 37%; f) DCAD (1.1 equiv.), triphenylphosphine (1.1 equiv.), reflux, 18 h, 72%; g) vinylmagnesium bromide (2.0 equiv.), copper(I) iodide (10 mol%), THF, -20 °C, 1 h, 79%.

Work by Tsunoda and co-workers at the turn of the last century cast (cyanomethylene)trimethylphosphorane (CMMP) into the spotlight as an alternative reagent for carrying out the desired Mitsunobu reaction.[148,149] The use of cyanomethylenephosphoranes generally for Mitsunobu reactions of this type has been documented in a review by Dembinski.[150] The Tsunoda reagents have acetonitrile and a phosphine oxide as the only by-products and hence it was hoped that (cyanomethylene)triphenylphosphorane (CMPP), a bench-stable reagent already employed as an ylide in Wittig reactions, would allow epoxide formation from diol **3.118** without the aforementioned purification issues associated with the use of azodicarboxylates.[151]

Utilising CMPP in the reaction rather than the DIAD and triphenylphosphine system under otherwise identical conditions, gave only a very moderate yield of epoxide **3.117** (30%). Prolonged heating at a higher temperature in a sealed tube only slightly improved the product yield to 37%. Notably, in both cases with CMPP, the purification was much easier than with DIAD. Given the poor yields and prior to the synthesis of further Tsunoda reagents, the use of di-(4-chlorobenzyl)azodicarboxylate (DCAD) was trialled. Reduced DCAD is reported to be highly crystalline and insoluble in toluene and another group member had reported success in the same transformation with DCAD.[152] Indeed, it was found that using DCAD in the Mitsunobu reaction gave epoxide **3.117** in 72% after a simpler column purification. This was readily converted into the homoallylic alcohol **3.116** using vinylmagnesium bromide in the presence of catalytic copper(I) iodide, allowing for the key Wittig step to be explored.[102]

The mechanism of the Wittig reaction has been hotly debated in the literature for several decades. Several undergraduate text books still invoke betaine intermediates, despite no longer expected to be present in the mechanism, at least in the absence of lithium salts.[153] The mechanism for the lithium salt-free Wittig reaction is now generally accepted and thought to proceed exclusively *via* a [2+2] cycloaddition of the respective ylide and carbonyl species to form the respective oxaphosphetane (Figure 4.1.2).[153,154] This intermediate then undergoes a subsequent [2+2] cycloreversion giving the requisite olefin, usually accompanied by formation of a phosphine oxide by-product. The mechanism of the reaction in the presence of lithium salts is still not well understood; the possibility of isolating lithium betaines and the observation by Vedejs and co-workers of the drop in (*E/Z*)-selectivity of the Wittig reaction in the presence of lithium bromide imply other reaction mechanisms are possible in these cases.[155]

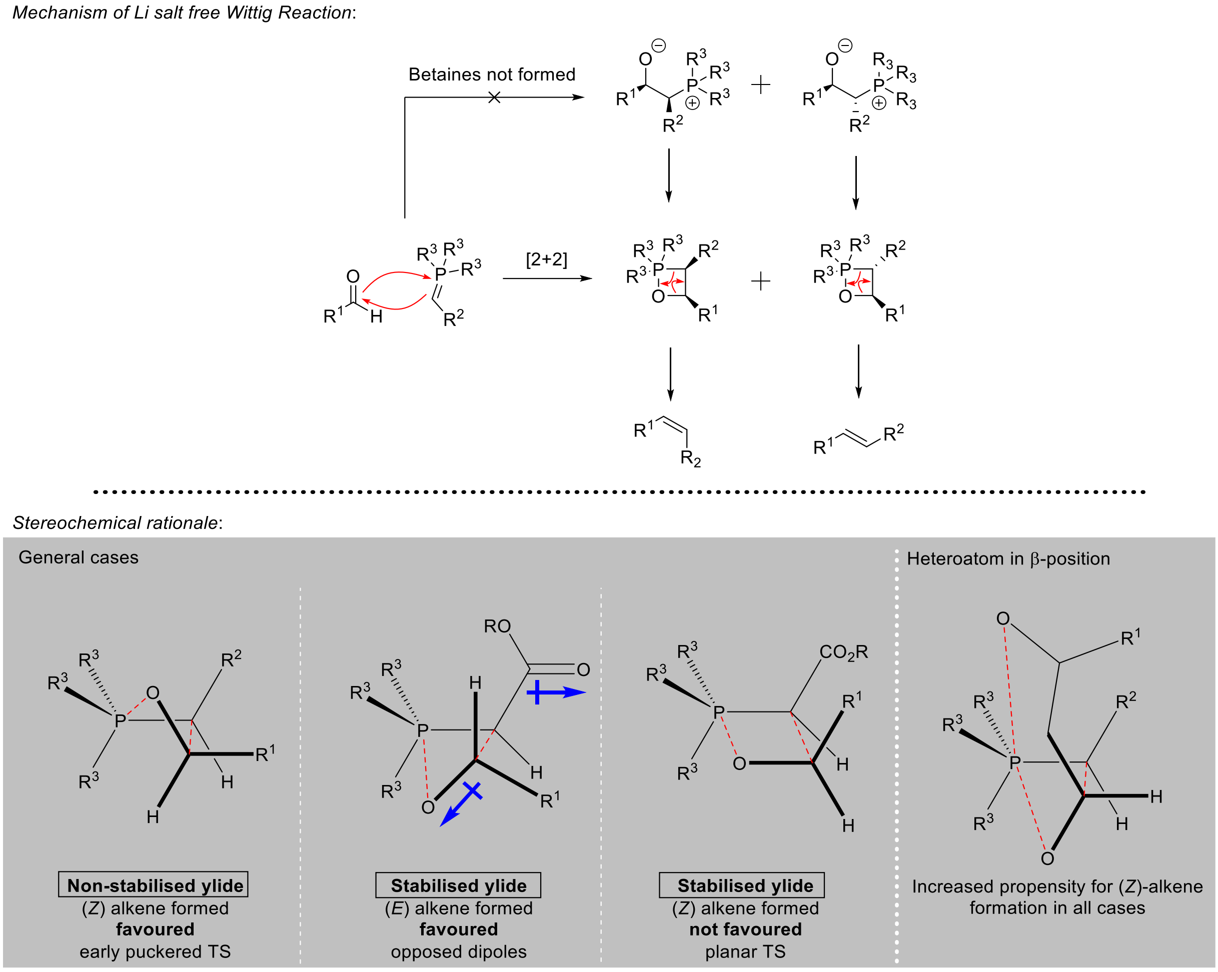
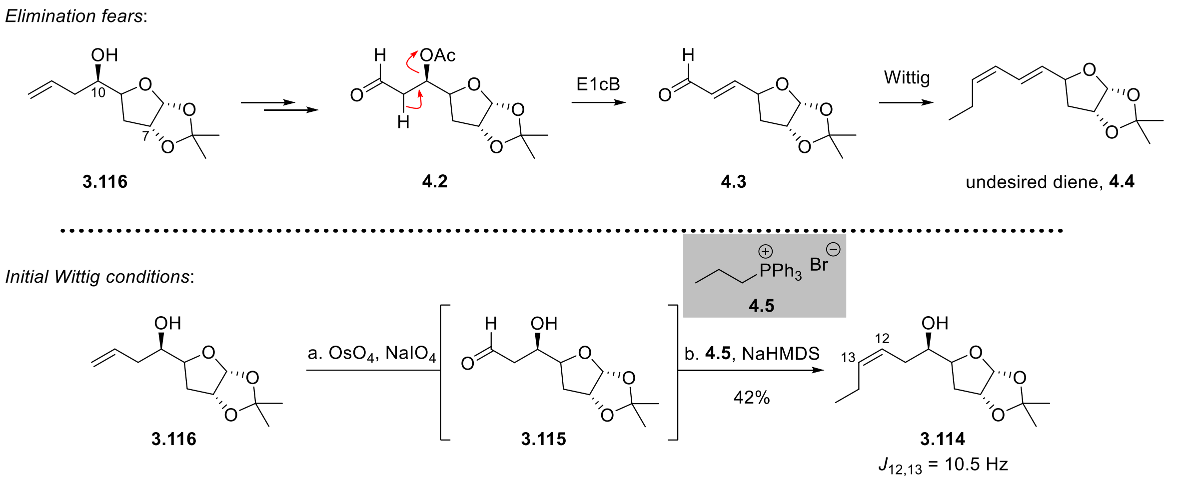


Figure 4.1.2: Widely accepted mechanism of the lithium salt free Wittig reaction and rationale of stereochemical outcome of the reaction in the case of different ylide types and aldehydes bearing β-heteroatoms.

In addition to the elucidation of the mechanism itself, Byrne *et al*. offer a rationale for the observed stereochemical outcome of the Wittig reaction. For the case involving non-stabilised ylides (relevant for this project), in the absence of a β-oxygen substituent on the carbonyl substrate, an early-puckered transition is invoked (Figure 4.1.2, general cases). This reduces steric clash between the substituent on the aldehyde (R_1) and the groups on phosphorus (R_3) resulting in the (*Z*)-olefin being formed. In the case of stabilised ylides, the formation of the (*E*)-alkenes is favoured (Figure 4.1.2, general cases). By opposing the dipoles of the carbonyl group in the ester and aldehyde, formation of the (*E*)-alkene dominates. (*Z*)-Alkene formation is not favoured in the case of stabilised ylides and can be rationalised by the planar transition state model, with R_1 occupying the same face as the stabilising group there is increased torsional strain in the transition state.[154] For cases involving β-oxygen substituents on the aldehyde, an additional phosphorus-oxygen interaction confers a boost to the (*Z*)-selectivity with all ylide types (Figure 4.1.2, heteroatom in β-position).

In order to form the key [2.2.1]-dioxabicycloheptane **3.101**, protection of the homoallylic secondary alcohol at C-10 was necessary in order to differentiate this position from the alcohol unmasked at C-7 upon Sakurai olefination of the acetonide **3.113** (shown previously). The ideal protecting group at C-10 would be an acetate which had been previously shown to be unmasked under mild conditions later in the route to analogous [2.2.1]-dioaxabicycloheptane **3.89**.[102]

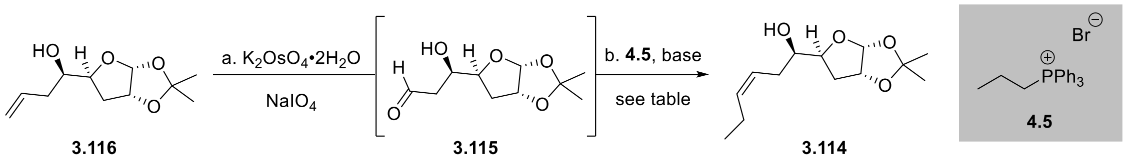
However, the stability of the acetate of homoallylic alcohol **4.2** could be problematic, as β-acetoxy aldehydes can readily undergo E1cB elimination to give α-,β-unsaturated aldehydes under the basic conditions employed in Wittig reactions. E1cB elimination in the case of aldehyde **4.2** would lead to the formation of the undesired diene **4.4** *via* the α-,β-unsaturated aldehyde **4.3** and lead to homoallylic alcohol **3.116** itself being selected as the substrate for the initial attempt at the desired Wittig transformation (Scheme 4.1.2).



Scheme 4.1.2: Potential E1cB elimination of aldehyde **4.2** prior to the desired Wittig reaction forming undesired diene **4.4**. Initial conditions employed for the Wittig olefination of aldehyde **3.124** formed in situ by Lemieux-Johnson oxidation of homoallylic alcohol **3.116**. Reagents and conditions: a) osmium tetroxide (2.5% in *t*-BuOH, 1mol%), sodium periodate (4.0 equiv.), 2,6-lutidine (2.0 equiv.), dioxane/H_2O (3:1), rt, 2 h *then* b) **4.5** (2.0 equiv.), NaHMDS (1 M in THF, 2.7 equiv.), THF, -78 °C – rt, 4 h, 42%.

Lemieux-Johnson oxidation of the homoallylic alcohol to form aldehyde **3.115** was achieved with substoichiometric osmium tetroxide and sodium periodate according to the procedure of Jin and co-workers.[141] This aldehyde was used without purification in the subsequent Wittig olefination with the ylide formed by the reaction of propyltriphenylphosphonium bromide **4.5** with NaHMDS at 0 °C. The addition of the crude aldehyde was carried out at -78 °C to the pre-formed ylide and stirred for a period of 2 h, with the reaction then warmed to rt for a further 2 h before isolation of the desired (*Z*)-olefin **3.114** in a modest yield of 42%. The configuration of the *Z*-olefin was confirmed by analysis of the coupling constants of CH-12 and CH-13 (J = 10.5 Hz) and by comparison to the analogous *E*-olefin reported by Chan *et al*.[102] The formation of this compound was accompanied by observation of an additional non-starting material spot by TLC analysis; although this was suspected to be one or both of the undesired diene side products postulated above owing to the presence of an increased number of resonances in the olefin region of the NMR spectrum, its identity could not be ascertained with certainty due to an inability to obtain a clean sample.

The yield of the Wittig step was consistently around 40% independent of the method used for the formation of aldehyde **3.115**. A number of methods for oxidative cleavage were attempted, including ozonolysis followed by reductive workup and also cleavage with ruthenium(III) chloride and sodium periodate as pioneered by Yang *et al*. in the early 2000s.[156] Although initially trialled in an attempt to move away from highly toxic osmium tetroxide, these methods served a dual purpose in that they highlighted a necessity to optimise the Wittig step itself. Unless all of the aldehyde forming reactions were not only poor yielding but identically so within experimental error, the Wittig reaction itself could be attributed to the poor yield observed. Ultimately, osmium(VI) and sodium periodate were used for the formation of aldehyde **3.115**. The use potassium osmate dihydrate rather than osmium(VIII) tetroxide directly was deemed a safer alternative. Optimisation of the Wittig step was attempted, *vide infra* (Table 4.1.1).



Entry	**4.5**/equiv.	Base	Base/equiv.	T/°C[a]	Time/h	Yield/%	*Z*/*E*-ratio[b]
1	2.0	NaHMDS	2.0	-78	1	35	>9:1
2	2.0	KHMDS	2.0	-78	1	22	>9:1
3	2.0	NaHMDS	2.0	-78	4	39	>9:1
4	2.0	LiHMDS	2.7	-78	4	53	2:1
5	2.0	LiHMDS	2.7	-78 then 25	4	56	2:1
6	2.0	LiHMDS	2.7	0 then 40	4	70	2:1
7	2.0	NaHMDS	2.7	0 then 40	4	46	>9:1
8	1.6	NaHMDS	1.5	0 then 40	4	43	>9:1

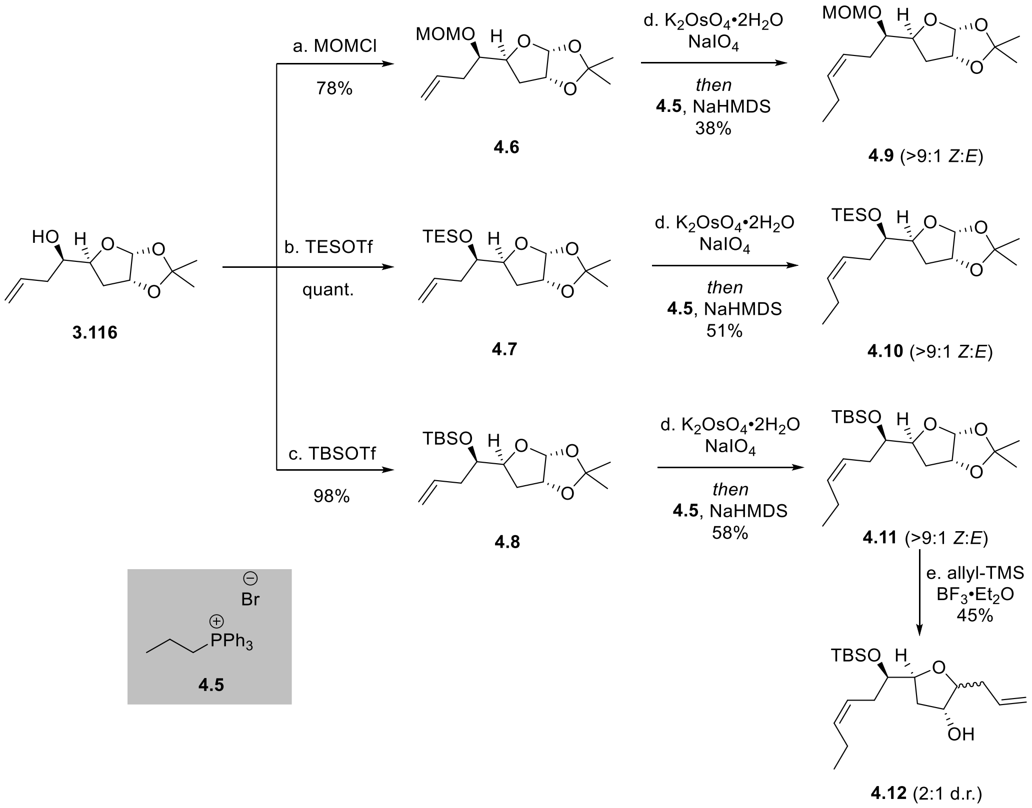
Table 4.1.1: Optimisation conditions for the formation of **3.114** *via* Wittig reaction. All reactions were conducted on a 0.2 mmol scale according to the reaction conditions set out above. [a]For reactions with two temperatures, the first temperature was held for a period of 2 h and then the second temperature held for the remainder of the reaction time. [b]*Z*/*E*-ratio assigned by comparison of the CH-13 and CH-12 ratios in the ^{1}H NMR spectrum. Reagents and conditions: a) potassium osmate dihydrate (1.0 mol%), sodium periodate (4.0 equiv.), 2,6-lutidine (2.0 equiv.), dioxane/H_2O (3:1), rt, 2 h *then* b) see table.

The optimisation of the Wittig was initiated under the pretext that excess equivalents of base were causing increased amounts of the undesired diene **4.4** to be formed over the course of the reaction. To evaluate whether this was the case, the excess equivalents of base versus the phosphonium salt were equalised and the reaction time shortened; disappointingly, this was not accompanied by the increase in yield that had been hoped for (entry 1). As a control, KHMDS was used as the base (entry 2); this had the effect of giving more of the side product, at the expense of formation of the (*Z*)-olefin **3.114**, lending weight to the postulate that high basicity was leading to increased formation of the suspected diene side products. The reaction time had very little effect on the yield (entry 3), as stirring the reaction for 4 h at -78 °C in fact led to a slight increase in the yield.

To probe the effect of the base, LiHMDS was trialled (entry 4); although a moderate increase in yield was observed in this case, the presence of lithium ions in solution had a deleterious effect on the (*Z*/*E*)-ratio of the product. This is in accordance with the observation by Vedejs and co-workers that alternative mechanistic pathways are facilitated by lithium ions in solution.[155] Nevertheless, it was decided that optimisation should continue, with the hope of a set of conditions being developed for a return to non-lithium bases. To this end, the temperature of the reaction was adjusted accordingly (entries 5 and 6). Stirring at rt for 4 h (entry 5) offered no significant boost to the yield of (*Z*)-olefin **3.114**, with the biggest boost in yield being observed with stirring at 40 °C for a period of 4 h (entry 6). Stirring at reflux or leaving the reaction for a longer period of time (not shown) offered no further boost to the yield.

Returning to NaHMDS with the newly developed conditions (entry 7) was again associated with a drop in yield back within the range observed previously. Further attempts to reduce the base equivalents at the optimised temperature (entry 8) and appeared to have little to no effect on the yield of (*Z*)-olefin **3.114**. The optimisation process was at this point stalling; with the yield of the desired compound not greatly exceeding the conditions trialled in the first instance, unless at the expense of (*Z*/*E*)-selectivity, a new tactic was needed.

To this end, it was hoped that it would be possible to inhibit formation of the putative diene by-product by protecting the free alcohol (Scheme 4.1.3). Homoallylic alcohol **3.116** was protected as the silyl ethers **4.6** and **4.7**, and also MOM-ether **4.8** in good yields. Notably, TBSOTf was required for the protection of the somewhat hindered secondary alcohol centre as the usual TBSCl conditions used routinely for transformations of this type offered only a mediocre yield of 47%. The triflate was used for the analogous reaction to form TES-ether **4.7**.



Scheme 4.1.3: Protection of homoallylic alcohol **3.116** as silyl ethers **4.6** and **4.7**, and MOM-ether **4.8**. Subjection of these intermediates to the Wittig reaction and subsequent Sakurai allylation of (*Z*)-olefin **4.11**. Reagents and conditions: a) chloromethyl methyl ether (2.0 equiv.), diisopropylamine (2.0 equiv.), CH_2Cl_2, 0 °C – rt, 24 h, 78%; b) triethylsilyl triflate (1.5 equiv.), 2,6-lutidine (2.0 equiv.), CH_2Cl_2, 0 °C – rt, 1 h, quant.; c) *t*-butyldimethylsilyl triflate (1.5 equiv.), 2,6-lutidine (2.0 equiv.), CH_2Cl_2, 0 °C – rt, 1 h, 98%; d) potassium osmate dihydrate (1.0 mol%), sodium periodate (4.0 equiv.), 2,6-lutidine (2.0 equiv.), dioxane/H_2O (3:1), rt, 2 h *then* **4.5** (1.6 equiv.), NaHMDS (1 M in THF, 1.5 equiv.), THF, -78 °C – rt, 16 h, 38% (MOM-protected **4.9**), 51% (TES-protected **4.10**), 58% (TBS-protected **4.11**); e) allyltrimethylsilane (5.0 equiv.), boron trifluoride etherate (2.0 equiv.), CH_2Cl_2, 0 °C – rt, 18 h, 45% (2:1 d.r.).

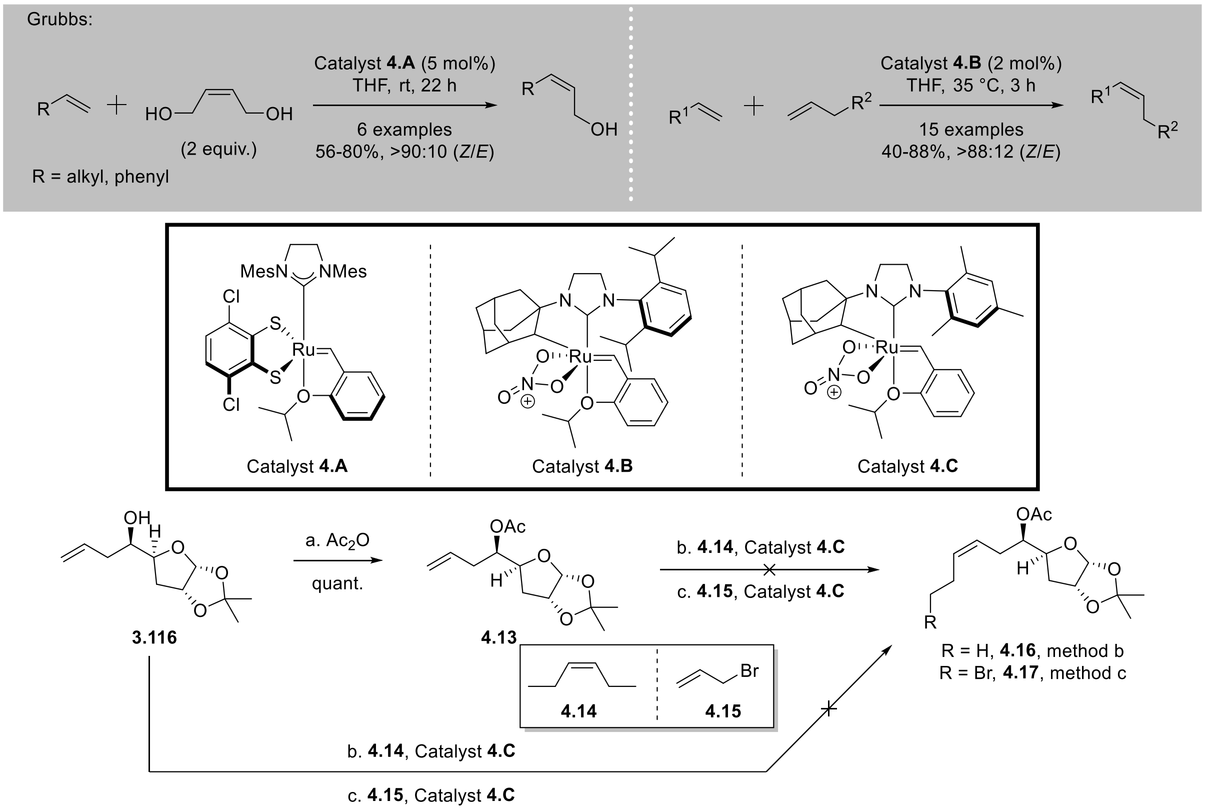
Subjection of the protected alcohols **4.6** to **4.8** to the alkene cleavage/ Wittig reaction using NaHMDS as the base gave mixed results, none of which coming close to the yield observed under the LiHMDS conditions (Scheme 4.1.3). Only TBS-ether **4.8** performed significantly better than the unprotected alcohol **3.116**, with MOM-ether **4.6** performing worse (38%) and TES-ether **4.7** being only marginally better (51%). Disappointingly, the newly synthesised (*Z*)-olefin **4.11** also performed badly in the subsequent Sakurai allylation. Not only was the yield only 45%, a significant baseline component was observed by TLC analysis indicated the potential deprotection of the TBS-group under the reaction conditions. The observed diastereoselectivity of the Sakurai was also lower than in the substrate used in previous work within the group.[102]

Given the difficulties encountered in optimising the Wittig reaction, and with a desire to access the [2.2.1]-dioxabicycloheptane **3.101** for further study, alternative methods for accessing the (*Z*)-olefin **3.114** were sought.

4.2. Attempts to Access the (*Z*)-Olefin by Alternative Routes

Since the respective (*E*)-olefin of interest in previous projects in the group had been accessible by both Julia-Kocienski olefination and Grubbs metathesis, the analogous reaction with (*Z*)-selective Grubbs catalyst seemed a sensible starting point for the continued efforts to access (*Z*)-olefin **3.114**.[102]

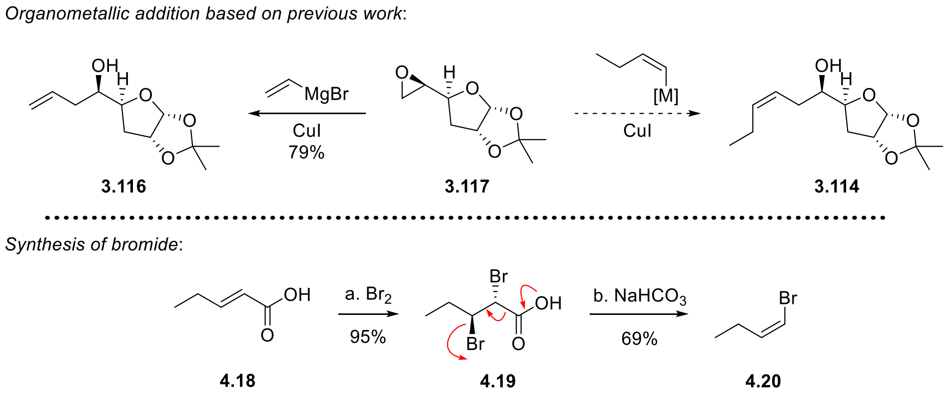
Recent reports by Grubbs, utilising (*Z*)-selective catalysts **4.A** and **4.B** had indicated that this transformation may be possible (Scheme 4.2.1, top).[157–159] To this end, (*Z*)-hex-3-ene **4.14** was selected with the aim of enabling desired cross-metathesis with catalyst **4.C** due to limited availability of catalysts **4.A** and **4.B** (Scheme 4.2.1). Failing this, use of 4-bromobut-1-ene **4.15** provided a possible alternative substrate, albeit with an extra step necessary for the removal of the terminal bromide.



Scheme 4.2.1: Application of (*Z*)-selective Grubbs catalysts **4.A** and **4.B** for the formation of (*Z*)-olefins. Synthesis of homoallylic acetate **4.13**, reaction of this and homoallylic alcohol **3.116** with modified conditions using catalyst **4.C** to access (*Z*)-olefins **4.X** and **4.X**. Reagents and conditions: a) acetic anhydride (3.0 equiv.), DMAP (3.0 equiv.), CH_2Cl_2, rt, 2 h, quant.; b) **4.14** (10 equiv.), (*Z*)-selective Grubbs catalyst **4.C** (5.0 mol%), THF, 40 °C, 18 h, no conversion; c) **4.15** (10 equiv.), (*Z*)-selective Grubbs catalyst **4.C** (5.0 mol%), THF, 40 °C, 18 h, no conversion.

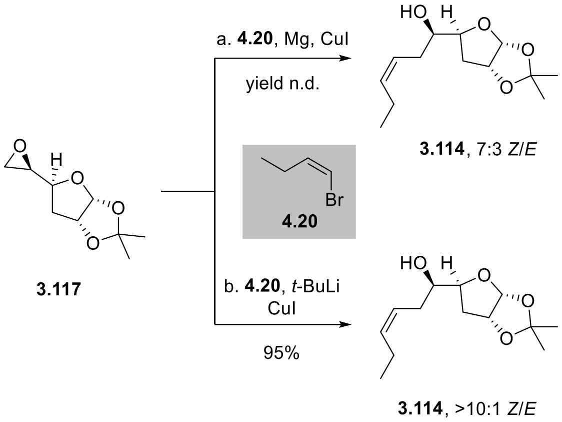
Since the (*Z*)-olefin **3.114** (not shown) would necessarily be acylated prior to the Sakurai allylation in order to differentiate the newly formed alcohol from that already present, both the unprotected homoallylic alcohol **3.116** and its acetate protected counterpart **4.13** (synthesised in one step using widely utilised acylation conditions) were subjected to metathesis conditions with catalyst **4.C**. No conversion was observed for either homoallyl acetate **4.13** with the desired metathesis partners **4.14** and **4.15**, nor for homoallyl alcohol **3.116** under the same conditions, based on those reported by Grubbs *et al*. in their seminal work with the (*Z*)-selective catalyst **4.C**.[160,161] The lack of easy access to the array of ruthenium catalysts available to enact this transformation on account of their high cost and limited commercial availability, twinned with the relatively expensive nature of the metathesis partner **4.14** and the high excess of **4.14** and **4.15** used in the reaction meant that this avenue was pursued no further in favour of cheaper alternatives.

The successful addition of organometallic reagents to epoxide **3.117** had been demonstrated already in this project and previously. With this in mind, the next plausible route to install the required fragment directly, required the synthesis of bromide **4.20** and then its subsequent transformation into an organometallic species of the type shown without scrambling of the alkene stereochemistry (Scheme 4.2.2). Thus treatment of pentenoic acid **4.18** with bromine in CH_2Cl_2 gave the desired dibromide **4.19** in a 95% yield. The dibromide **4.19** was then converted into the *Z*-alkenyl bromide **4.20** on addition to mildly basic hot DMF under reduced pressure.[162]



Scheme 4.2.2: Organometallic strategy based upon previous success and synthesis of bromide **4.20** necessary for the direct addition of the desired fragment to epoxide **3.117**. Reagents and conditions: a) bromine (1.0 equiv.), CH_2Cl_2, 0 °C – rt, 3 h, 95%; b) sodium bicarbonate (1.7 equiv.), DMF, 70 °C, 120 mbar, product distilled from reaction mixture, 69%.

With the bromide **4.20** in hand, attention was turned to the addition of an organometallic species derived from it into epoxide **3.117**. The initial desire, from a practical ease perspective, was the formation of the corresponding Grignard reagent and then use of the already precedented conditions for epoxide opening with no further optimisation being required. In reality, this could not be achieved since the formation of the respective Grignard reagent could not be achieved without partial scrambling of the alkene stereochemistry (Scheme 4.2.3). This reaction was not explored further due to the loss of stereochemical integrity of the olefin being deemed insurmountable and expected to be even more problematic on scale due to the heterogenous nature of Grignard formation.

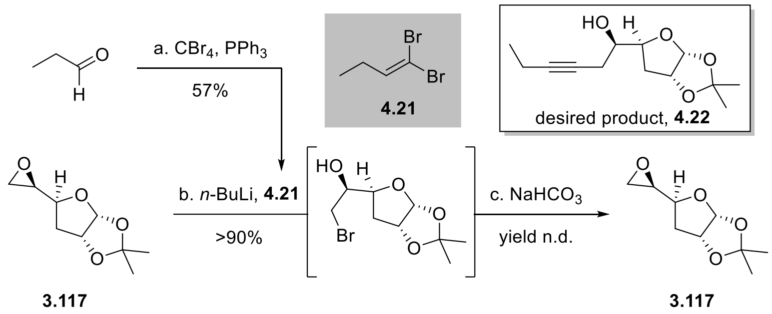


Scheme 4.2.3: Formation of (*Z*)-olefin **3.114** by addition of Grignard and organolithium species derived from bromide **4.20**. Reagents and conditions: a) **4.20** (2.0 equiv.), magnesium (2.0 equiv.), copper(I) iodide (10 mol%), THF, -20 °C, 1 h, yield n.d. (7:3 *Z*/*E*); b) **4.20** (4.0 equiv.), *t*-butyllithium (8.0 equiv.), copper(I) iodide (10 mol%), Et_2O, -78 °C – -20 °C, 2 h, 95% (>10:1 *Z*/*E*).

Pleasingly, formation of the corresponding alkenyl lithium reagent by lithium halogen exchange of alkenyl bromide **4.2** with *t*-butyllithium followed by addition of the epoxide **3.117** gave the desired (*Z*)-olefin **3.114** in 95% yield with greater than 10:1 *Z*:*E* selectivity ($J_{12,13}$ = 10.5 Hz). *t*-Butyllithium was necessary for the lithium-halogen exchange, with the reaction returning exclusively starting material in the presence of *n*-buyllithium; this necessitated the use of two equivalents of base versus the bromide on account of the first equivalent of *t*-butyl bromide reacting with the base to give isobutylene.

Carrying out the reaction in THF rather than diethyl ether led to an intractable mixture most likely caused by the *t*-butyllithium deprotonating the solvent; likewise, a complex mixture was also formed when the reaction was conducted in the absence of CuI. Interestingly, dropping the equivalents of bromide led to the appearance of a side-product that couldn't be separated from the desired compound, even after extensive column purification. Despite copious efforts at purification, these artifacts could not be removed from the sample. Ultimately, the inability to carry out the reaction in the presence of fewer than four equivalents of bromide (and therefore eight equivalents of *t*-butyllithium), meant that scale up of the reaction for the synthesis of multigram quantities of product would be challenging.

Due to the continued success at adding organometallic species to epoxide **3.117**, this was retained as the strategy moving forward. Since addition of the alkene to the epoxide directly seemed beyond reach, addition of the desired fragment as the alkyne, rather than the alkene, seemed pertinent. It was envisaged that this could be followed by reduction of the resulting internal alkyne to the desired (*Z*)-olefin **3.114** using well documented hydrogenation procedures (Scheme 4.2.4).



Scheme 4.2.4: Synthesis of dibromide for Corey-Fuchs procedure and resulting formation of bromide **4.21** and reformation of epoxide **3.117**. Reagents and conditions: a) tetrabromomethane (1.0 equiv.), triphenylphosphine (2.0 equiv.), CH_2Cl_2, 0 °C – rt, 1 h, 57%; b) *n*-butyllithium (2.1 equiv.), **4.21** (2.0 equiv.), THF, -20 °C, 1 h, >90%; c) $NaHCO_3$ (5.0 equiv.), CH_2Cl_2, rt, 48 h, yield n.d.

Initially, the alkyne anion was formed *in situ* on account of the gaseous nature of 1-butyne itself at rt. Thus, dibromide **4.21** was synthesised in one step from propanal, with the procedure required to be done in the dark on account of the light sensitivity of the product. The subsequent formation of the respective alkyne anion and addition into epoxide **3.117** was not successful. Treatment of the dibromide **4.21** with excess *n*-butyllithium and then addition of the epoxide **3.117** into this reaction mixture led to the formation of a significantly more polar product which was suspected to be the bromide addition product shown, in excellent yield (>90%). Although this compound was not characterised fully, it was possible to reform epoxide **3.117** upon treatment under mildly basic conditions. Taking into consideration the light-sensitivity of the respective dibromide, and inability to add in the desired fragment *via* this method in the presence of bromide, direct lithiation of an alkyne was preferred.

The simplest alkyne fragment, acetylene itself, can be obtained commercially as lithium acetylide diamine complex; this had been shown to add to the terminal end of epoxides by Danishefsky *et al.* in their 2003 synthesis of panaxytriol (Figure 4.2.1).[163] A similar substrate to epoxide **3.117** had been reacted under similar conditions by Makabe and co-workers in their synthesis of pyranicin and the exact transformation from epoxide **3.117** had also been reported in a recent patent.[164] The details of the exact conditions from this patent were unclear; however, DMSO was proposed as the solvent for this transformation. Given these promising reports detailing the use of lithium acetylide in natural a product synthesis, and epoxide **3.117** having been reported in patent literature as being amenable to this transformation, this avenue of research was pursued.

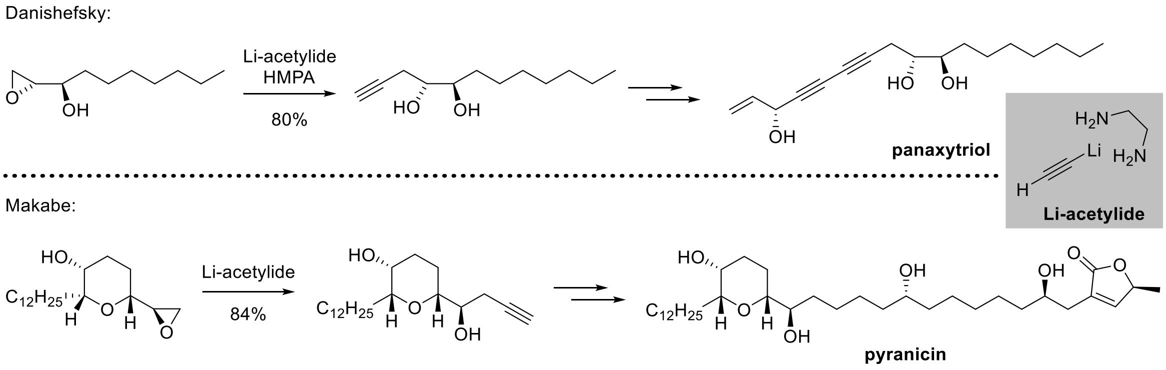
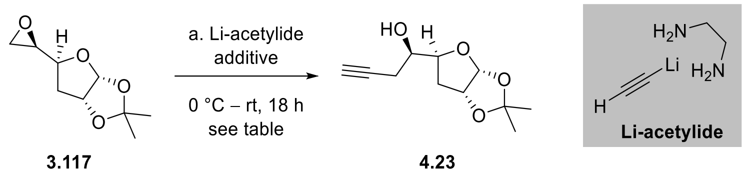


Figure 4.2.1: Use of lithium acetylide in natural product synthesis by the groups of Danishefsky and Makabe for the synthesis of panaxytriol and pyranicin respectively.

By merging the conditions employed in the literature reports and simply stirring the epoxide **3.117** in 1:1 mixture of THF and DMSO in the presence of two equivalents of lithium acetylide ethylenediamine complex, a reasonable yield of alkyne **4.23** was obtained (Table 4.2.1, entry 1). A rapid optimisation period followed, with each successive alteration to the aforementioned conditions providing a considerable boost to the yield.



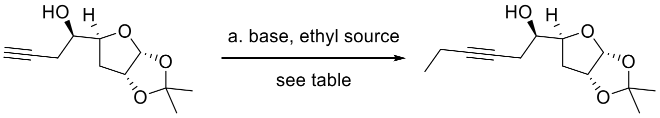
Entry	Scale/mmol	Solvent	Li-acetylide/ equiv.	Conc./ M	Additive/ mmol	Yield/%
1	0.2	THF/DMSO (1:1)	2.0	0.1	-	54
2	0.2	THF/DMSO (1:1)	5.0	0.1	-	75
3	1	THF/DMSO (1:1)	5.0	0.5	-	91
4	1.5	THF/DMSO (1:1)	5.0	0.7	-	86
5	0.2	THF	2.0	0.1	HMPA/ 0.3	35

Table 4.2.1: Optimisation conditions for the addition of Li-acetylide into epoxide **3.117** to give alkyne **4.23**.

Increasing the equivalents of lithium acetylide from two to five gave a substantial increase to the yield (entry 2); this was followed by an increase in reaction concentration from 0.1 M to 0.5 M which also granted a similar yield increase (entry 3) and providing the desired alkyne **4.23** in excellent yield and at a reasonable scale. Upon further increasing the concentration (entry 4), a slight drop in yield was observed but this was not significant. In the report by Danishefsky, HMPA had been employed as an additive rather than the co-solvent DMSO, which was trialled for completeness (entry 5). Use of these conditions proved detrimental to the yield and given the toxicity of HMPA, these conditions were not pursued further.

Despite the ease by which the alkyne itself was installed, accessing alkyne **4.24** proved impossible. Attempts to *C*-alkylate intermediate alkyne **4.23** were unsuccessful, contrary to a number of reports in the literature detailing that transformations of this type are possible.[165–

[167] A comprehensive exploration of this process was undertaken in an effort to form the substituted alkyne **4.24** from terminal alkyne **4.23** (Table 4.2.2).

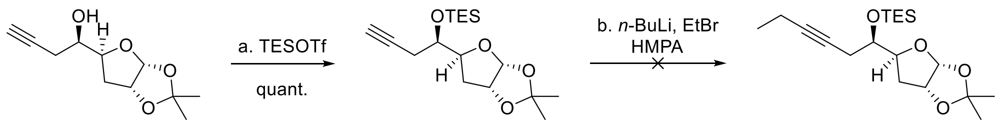


4.23 **4.24**

Entry	Base^a	Ethyl source^b	Solvent	Additive^c	T/°C	Remarks
1^d	*n*-BuLi	EtI	THF	-	-78	SM returned
2^d	*n*-BuLi	EtI	THF	-	-30	SM returned
3^d	*n*-BuLi	EtI	THF	-	25	SM returned
4^d	*n*-BuLi	EtI	THF	TMEDA	25	SM returned
5^d	EtMgBr	EtI	THF	-	25	SM returned
6^e	*n*-BuLi	EtI	THF/DMI	-	25	SM returned
7^e	*n*-BuLi	EtBr	THF	-	25	SM returned
8^e	*n*-BuLi	EtBr	THF/DMI	-	25	SM returned
9^e	*n*-BuLi	EtI	THF/DMI	TMEDA	25	SM returned
10^e	*n*-BuLi	EtBr	THF/DMI	TMEDA	25	SM returned
11^e	*n*-BuLi	EtBr	THF/HMPA	-	25	*O*-Alkylation

Table 4.2.2: Attempted alkylation of alkyne **4.23** to give internal alkyne **4.24**. Reactions were conducted on a 0.2 mmol scale. ^{a}2.2 Equivalents used. ^{b}2.2 Equivalents used. ^{c}2.2 Equivalents used. ^{d}Base added at -78 °C and stirred for 30 mins before addition of ethyl source also at -78 °C prior to warming to specified temperature for a period of 18 h. ^{e}Base added at 0 °C and stirred for 30 mins before addition of ethyl source also at 0 °C prior to warming to specified temperature for a period of 18 h.

Gradually increasing the temperature of the reaction (entries 1 to 4) had no effect on product formation, with starting material being recovered in each case, even with the addition of TMEDA (entry 4) to replicate the lithium acetylide conditions. Switching the base from *n*-butyllithium to ethylmagnesium bromide (entry 5) was also ineffective. Use of DMI, a safer alternative to HMPA, as co-solvent for the reaction did not result in product formation (entry 6). Changing the electrophile to ethyl bromide (entry 7) provided the same outcome as observed previously, even with use of DMI (entry 8). Likewise, addition of TMEDA, gave no sign of product with either of the electrophiles used up until this point (entries 9 and 10). Finally, using HMPA as the co-solvent (entry 11) looked promising, with ethyl resonances present in the crude ^{1}H NMR spectrum. However, the ^{1}H NMR spectrum also contained the peak corresponding to the terminal alkyne proton, indicating *O*-alkylation had been the likely outcome. This by-product was not isolated from the crude mixture. Attempting the alkylation on the TES-protected substrate **4.25**, readily prepared from alcohol **4.23** also gave no sign of the desired product **4.26** (Scheme 4.2.5).



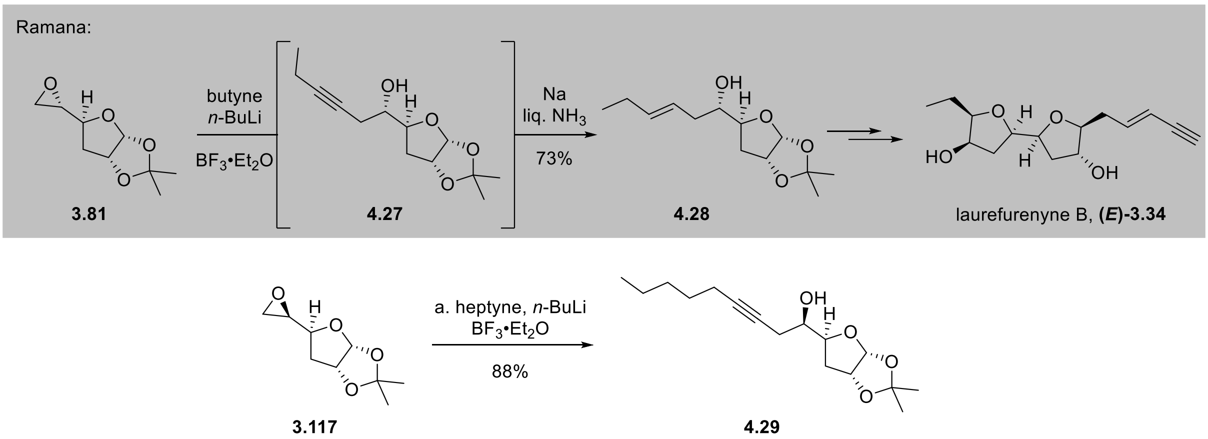
4.23 **4.25** **4.26**

Scheme 4.2.5: Formation of TES-protected alcohol **4.25** and subsequent failed alkylation. Reagents and conditions: a) triethylsilyl triflate (1.5 equiv.), 2,6-lutidine (2.0 equiv.), CH_2Cl_2, 0 °C – rt, 1 h, quant.; b) *n*-butyllithium (2.2 equiv.), ethyl bromide (2.2 equiv.), THF/HMPA, 0 °C – rt, 18 h, no product formed.

At this point, the options for accessing (*Z*)-olefin **3.114** in a robust and scalable way were becoming sparse. The alkyne route still appeared to be the best way forward; however, the addition of butyne itself to epoxide **3.117** looked like the only viable way to proceed. Prior to embarking on this route, necessitating the use of butyne gas, confirmation of the reduction aptitude of the internal alkyne to form the (*Z*)-olefins was sought. For practical ease, addition of a longer chain alkyne (hept-1-yne) to epoxide **3.117** was undertaken in order to validate the reduction itself and allow the subsequent steps in the synthesis to be established for the (*Z*)-olefin system.

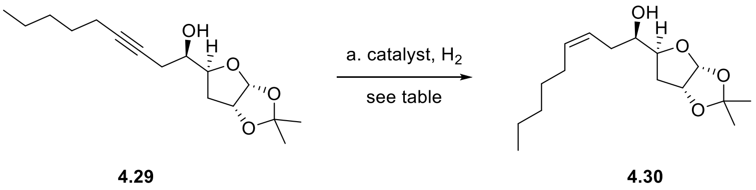
4.3. Development of Alkyne Reduction Protocol and Synthesis of Model [2.2.1]-Bicycloheptane

In a very recent report by Ramana *et al*. for the synthesis of laurefurenyne B and other bis-THF natural products, the addition of butyne directly into epoxide **3.117** had been reported.[135] At this point, their synthesis diverged with reduction to the (*E*)-alkene being favoured. Despite this, the precedent for the desired butyne addition was encouraging and offered a set of conditions to validate using the model system proposed (Scheme 4.3.1).



Scheme 4.3.1: Ramana procedure for addition of butyne to epoxide **3.81** and their derivatisation into laurefurenyne B **(*E*)-3.38**. Addition of heptyne to epoxide **3.117** using Ramana conditions. Reagents and conditions: a) 1-heptyne (1.8 equiv.), *n*-butyllithium (1.8 equiv.), boron trifluoride etherate (0.8 equiv.), THF, 0 °C – -78 °C, 1 h, 88%.

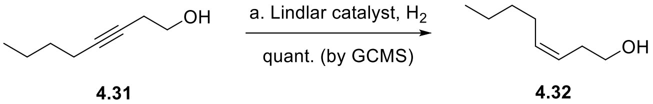
Pleasingly, the conditions developed by Ramana *et al*. required no additional optimisation and alkyne **4.29** could be accessed with ease and in excellent yield (88%). This procedure enabled access to ample material to evaluate the reduction step deemed to be viable using the well-precedented Lindlar hydrogenation procedure.[168–171] A number of Lindlar hydrogenation conditions were screened and the results are summarised below (Table 4.3.1).



Entry	Catalyst	Loading/ wt%	Additive	Solvent[a]	Remarks[b]
1	Lindlar	10	-	EtOH	No conversion
2	Lindlar	10	Quinoline[c]	EtOH	No conversion
3	Lindlar	15	-	EtOH	No conversion
4	Pd/C	10	-	EtOH	No conversion
5	Pd/$BaSO_4$	10	-	EtOH	No conversion

Table 4.3.1: Optimisation attempts for the reduction of alkyne **4.29** to (*Z*)-olefin **4.30**. Reactions were carried out on a 0.05 mmol scale at rt for a period of 8 h. [a]Solvent dried over 4Å molecular sieves. [b]As judged by crude ^1H NMR and GCMS analysis. [c]0.08 mmol additive added to the reaction prior to catalyst.

The progress of the hydrogenation reaction was determined by crude ^1H NMR analysis and GCMS. Neither Lindlar conditions in the presence of quinoline nor without (entries 1 and 2) provided any evidence for reduction of the alkyne. This trend continued with increased catalyst loading (entry 3); hydrogenation in the presence of palladium on carbon (entry 4) also gave none of the desired alkene, nor the corresponding alkane that might be expected. This lack of reactivity continued with the more esoteric Pd/$BaSO_4$ system (entry 5). The complete lack of reactivity of the starting material under any of the conditions employed cast doubt on the applicability of heterogenous hydrogenation to this substrate; these fears were confirmed by the facile reduction of test substrate oct-3-yn-1-ol **4.31** to (*Z*)-olefin **4.32** under the original conditions used for the desired substrate (Scheme 4.3.2).

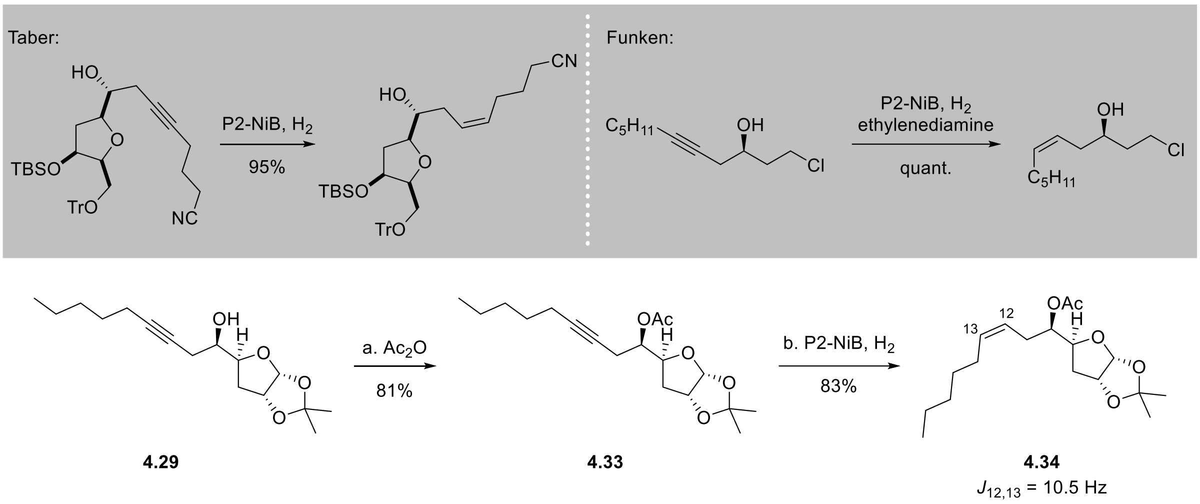


4.31

4.32

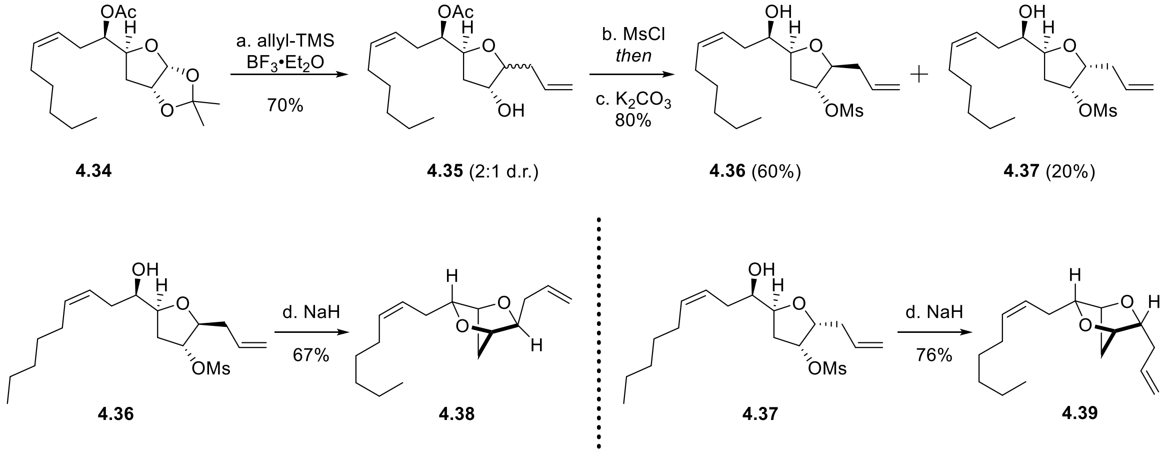
Scheme 4.3.2: Reduction of oct-3-yn-1-ol **4.31** by Lindlar conditions. Reagents and conditions: a) Lindlar catalyst (10 wt%), H_2, EtOH, rt, 6 h, quant (by GCMS).

Recognising the value of the alkyne addition in terms of its ease and scalability, other hydrogenation methods were sought. P2-nickel boride, a catalyst pioneered by Brown *et al*. in the 1970s and prepared by formation *in situ* from nickel(II) acetate and sodium borohydride, has been shown to be effective in the formation of (*Z*)-olefins from their respective alkynes.[172] There had been only a smattering of reports of this system being used since its introduction, at least by comparison to other methods known for this transformation. Nevertheless, Taber and co-workers had reported its use in their synthesis of the Δ^{13}-9-isofurans (Scheme 4.3.3). In addition Funken *et al*. had demonstrated the use of this catalyst system for a substrate bearing a free alcohol in the β-position to the alkyne, similar to alkyne **4.29**.



Scheme 4.3.3: Literature conditions showcasing the use of P2-nickel boride in synthesis. Formation of (*Z*)-olefin **4.X** *via* this method. Reagents and conditions: a) acetic anhydride (3.0 equiv.), DMAP (3.0 equiv.), CH_2Cl_2, rt, 2 h, 81%; b) nickel acetate tetrahydrate (20 mol%), sodium borohydride (20 mol%), ethylenediamine (40 mol%), H_2, EtOH, rt, 18 h, 83%.

Prior to subjecting alkyne **4.29** to the reduction conditions, the alcohol was protected as the corresponding acetate **4.33**. Pleasingly, on exposure of alkyne **4.33** to nickel boride and H_2, the (*Z*)-olefin **4.34** was formed in excellent yield. This procedure ultimately proved instrumental in the accessibility of large quantities of [2.2.1]-bicycloheptane **3.101** (see 5.1 Synthesis of [2.2.1]-Bicycloheptane) and also allowed the remainder of the route to be validated for the model system (Scheme 4.3.4).



Scheme 4.3.4: Synthesis of model system [2.2.1]-dioxabicycloheptanes **4.38** and **4.39** following Sakurai allylation, mesylation, deprotection and cyclisation sequence. Reagents and conditions: a) allyltrimethylsilane (5.0 equiv.), boron trifluoride etherate (3.0 equiv.), CH_2Cl_2, 0 °C – rt, 18 h, 70% (2:1 d.r.); b) methanesulfonyl chloride (1.1 equiv.), triethylamine (1.1 equiv.), CH_2Cl_2, 0 °C – rt, 1 h *then* c) potassium carbonate (2.0 equiv.), MeOH/H_2O (7:1), rt, 2 h, 80% (major **4.36**, 60%; minor **4.37**, 20%); d) NaH (60% in mineral oil, 7.0 equiv.), DMF, 0 °C – rt, 18 h, 67% (desired diastereoisomer **4.37**), 76% (undesired diastereoisomer **4.39**).

Treatment of acetate **4.34** under Sakurai olefination conditions with allyl-TMS in the presence of boron trifluoride diethyl etherate as used over the course of previous projects, allowed access to an inseparable 2:1 mixture of diastereomeric alcohols **4.35**.[102] Only a moderate diastereoselectivity of 2:1 was observed in this reaction as compared to previous cases, however, rather than optimise this step at this point, it was decided to wait for the real system in order to see whether this was general for the (*Z*)-olefin system or an artifact of the long chain model system.

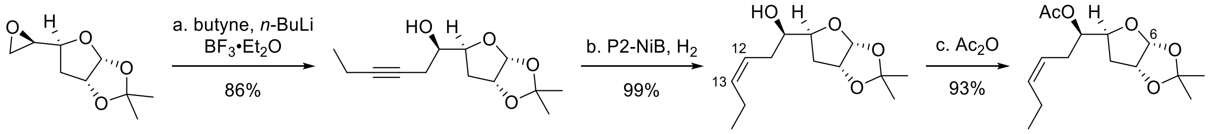
The mesylation and deprotection sequence of the mixture of Sakurai diastereoisomers **4.35** proceeded smoothly and separation of alcohols **4.36** and **4.37** was possible. Subjecting the respective alcohols to cyclisation conditions gave the model [2.2.1]-dioxabicycloheptanes **4.38** and **4.39** in good yield. The configuration of the [2.2.1]-dioxabicycloheptanes **4.38** and **4.39** was confirmed by close analogy to previously reported compounds with the same [2.2.1]-dioxabicycloheptane system.[102]

Having spent a considerable amount of time taking efforts to forge the (*Z*)-olefin **3.114**, and ultimately finding it necessary to resort to the model system described in this chapter, it was time to return to the real system to address the remaining challenges and push forward into the latter stages of the synthesis.

5. Efforts to Synthesise *ent*-Laurepinnacin

5.1. Synthesis of [2.2.1]-Bicycloheptane

With the synthesis of model [2.2.1]-dioxabicycloheptanes **4.38** and **4.39** having been carried out previously (see 4.3 Development of Alkyne Reduction Protocol and Synthesis of Model [2.2.1]-Bicycloheptane), it was envisaged that the same methodology could be applied once more in order to access to key intermediate **5.1** from 1-butyne. Deprotonation of 1-butyne, and subsequent addition of the respective anion into epoxide **3.117** in the presence of boron trifluoride etherate gave alkyne **5.1** in 86% yield, analogously to the heptyne addition used previously in the model substrate synthesis (Scheme 5.1.1). Having now gained access to alkyne **5.1**; the synthesis towards *ent*-laurepinnacin ***ent*-3.15** could be further advanced.



3.117 **5.1** **3.114**, $J_{12,13}$ = 10.5 Hz **3.113**

Scheme 5.1.1: Reaction sequence for the formation of protected (*Z*)-olefin **3.113** from epoxide **3.117** according to established procedure. Reactions and conditions: a) butyne (1.9 equiv.), *n*-butyllithium (1.9 equiv.), boron trifluoride etherate (0.75 equiv.), THF, -78 °C – rt *then* -78 °C, 1 h, 86%; b) nickel acetate tetrahydrate (20 mol%), sodium borohydride (20 mol%), ethylenediamine (40 mol%), H_2, EtOH, rt, 18 h, 99%; c) acetic anhydride (3.0 equiv.), DMAP 3.0 equiv.), CH_2Cl_2, rt, 2 h, 93%.

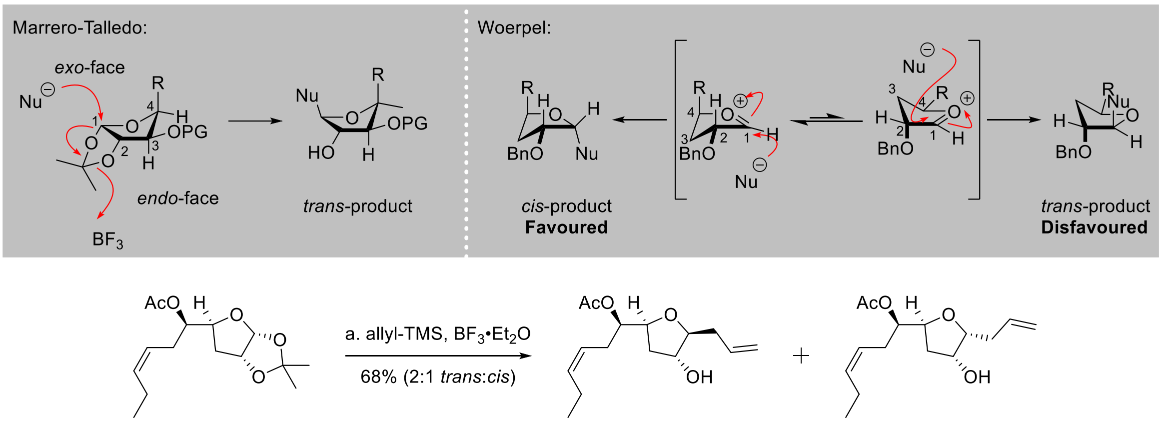
In order to measure the amount of 1-butyne used in the synthesis of **5.1**, the reaction flask was cooled in an ice bath. This allowed for the rapid weighing of pre-condensed 1-butyne directly from the freezer; after addition, the reaction flask was immediately cooled to -78 °C to prevent any loss by evaporation. The remainder of the manipulations were carried out as per the previously employed procedure. A slight excess of 1-butyne was used to ensure complete consumption of the epoxide, this proved not to be detrimental to the yield of this step. Pleasingly, this alkyne **5.1** performed well in the reduction step with nickel boride to give

(*Z*)-olefin **3.114** in near quantitative yield, with formation of exclusively the (*Z*)-geometry being observed by comparison of the ^{1}H NMR spectrum to the previous Wittig method ($J_{12,13}$ = 10.5 Hz). This was a vast improvement to the initial Wittig conditions employed (4.1 Attempted Synthesis *via* Wittig Route), due to the better *Z*:*E* ratio and was procedurally much simpler to carry out, giving access to a large quantity of the (*Z*)-olefin **3.114** (5.4 g synthesised) previously unattainable on this scale. Acylation of the free alcohol gave protected (*Z*)-olefin **3.113** in excellent yield, giving access to ample material to enable further study of the following Sakurai reaction.

The Sakurai reaction of (*Z*)-olefin **3.113** would ideally provide alcohol **3.112** with stereocontrol such that the nucleophile reacts at C-6 with inversion of the configuration at this centre with concomitant unmasking of the alcohol position at C-7 as demonstrated in previous work.[102]

A number of groups have attempted to rationalise the *cis*- and *trans*- ratios of the products formed in the reaction of similar sugar derived acetonides with nucleophiles under Lewis acid catalysis. Notably, Marrero-Telledo's *exo*-face templating model provides suitable justification for the observed *trans*-selectivity for their system and is reported to be independent of substrate substitution and existing stereochemistry (Scheme 5.1.2).[173] Use of the acetonide protecting group is thought to protect the *endo* face from nucleophilic attack leading exclusively to the *trans*-products, provided that the mechanism proceeds without intermediate glycosyl cations. They propose that exceptions to this rule exist only in the case of particularly demanding steric bulk in the 4-position. Their report exclusively details substrates bearing oxygen substituents at the C-3 position, which is not the case for the substrate of interest here, and opens the possibility of the competing glycosyl cation mechanism proposed by Woerpel.[174,175]

Woerpel's proposal in his inside attack model is that the nucleophile preferentially attacks on the inside face of the envelope conformation of an oxocarbenium (glycosyl cation). In the case of C-2 oxygen substituents, these preferentially occupy the equatorial position in order to best align the C-2 $\sigma_{C\text{-}H}$ donating orbital with the vacant $\pi^*_{C=O}$ orbital of the oxocarbenium ion. C-4 substitution, as is the case for the system here, is thought to have little effect on the outcome of the reaction. This position exerts little effect on the observed stereochemistry of the product since it biases the conformational equilibrium very little in comparison to the C-2 substituent.

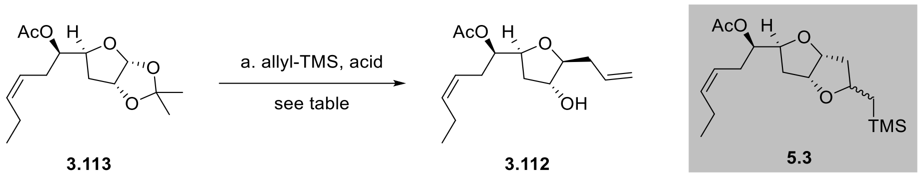


3.113 **3.112** (desired) **5.2** (undesired)

Scheme 5.1.2: Marrero-Talledo *exo*-templating and Woerpel's inside attack model to explain observed stereochemistry of Sakurai allylation in sugars. Sakurai reaction of protected (*Z*)-olefin **3.122** giving only moderate diastereoselectivity. Reagents and conditions: a) allyltrimethylsilane (5.0 equiv.), boron trifluoride etherate (3.0 equiv.), CH_2Cl_2, 0 °C – rt, 18 h, 68% (2:1 d.r, *trans*:*cis*).

Treatment of acetonide **3.113** with allyltrimethylsilane in the presence of BF_3•OEt_2 gave the allylated products **3.112** and **5.2** as a 2:1 mixture of desired : undesired stereoisomers. The observed diastereoselectivity for the system of interest can be thought of as the outcome of the competing models, with only a slight preference for the Marerro-Talledo *exo*-facial templating effect due, in part to the lack of substituent at C-3. This poor diastereoselectivity, twinned with difficult separation of the respective diastereoisomers **3.112** and **5.2** made advancing significant quantities of material problematic. Fortunately, a solution to the problem had been developed by another group member (H. Hicks) working on a related project.[152]

Work on a similar system had identified tetrafluoroboric acid as a suitable acid for this transformation. Rather than try to overcome the inherent diastereoselectivity of the reaction itself, this method enabled for removal of the undesired diastereoisomer as intermediate **5.3**, stable under the reaction conditions and separable by column chromatography due to the vastly different polarity to that of the desired compound **3.112** (Table 5.1.1).



Entry	Scale/ mmol	Allyl-TMS/ equiv.	Acid	Acid/ equiv.	T/ °C	Time/ h	Yield/ %	d.r.[a]
1	0.9	5.0	$BF_3\cdot OEt_2$	3.0	25	16	68	2:1
2	0.1	8.0	$HBF_4\cdot OEt_2$	0.7	40	16	41	2:1
3[b]	0.1	8.0	$HBF_4\cdot OEt_2$	0.5	25	16	n.d	n.d
4	0.1	8.0	$HBF_4\cdot OEt_2$	0.5	40	3	40	5:1
5	1.0	8.0	$HBF_4\cdot OEt_2$	0.5	40	3	66[c]	>5:1

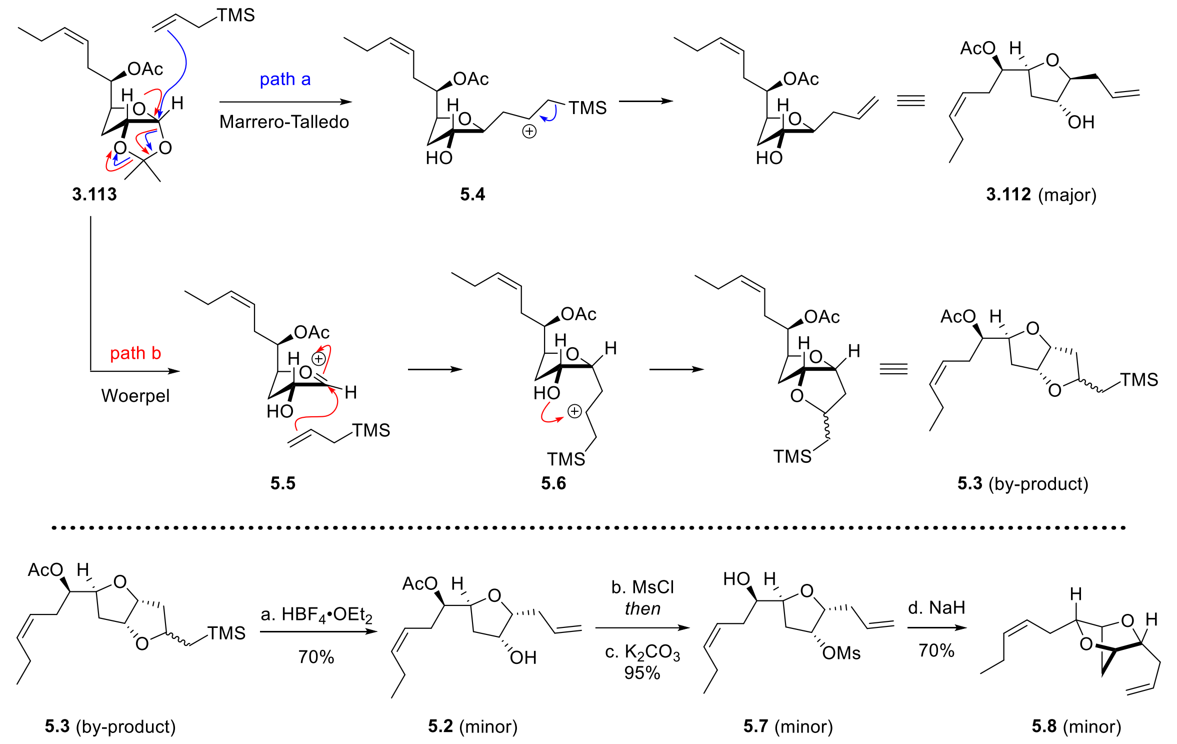
Table 5.1.1: Optimisation results for the Sakurai reaction to form **3.112**. [a]d.r. Calculated from integrals of the ^{1}H NMR spectra by comparison of the olefinic protons. [b]Reaction abandoned as incomplete after the designated time. [c]By-product **5.3** was isolated in 21% yield, total yield for the reaction 87%.

For comparison, the original boron trifluoride etherate conditions are shown (entry 1). Utilisation of the previously developed conditions using tetrafluoroboric acid, led to a disappointing outcome, both in terms of yield and diastereoselectivity (entry 2).[152] Similarly, reducing the reaction temperature led to incomplete conversion over the course of the same reaction time (entry 3). This was quickly remedied by using reflux conditions with TLC monitoring. Under these conditions, the reaction was found to be complete after only 3 h (entry 4), and the diastereomeric ratio of Sakurai product **3.112** was greatly improved to >5:1

from a poor 2:1 observed previously. This was accompanied by the formation of by-product **5.3** that was not isolated in this case. Pleasingly, this reaction was found to be scalable, and a slight increase to the yield observed (entry 5). At this point, by-product **5.3** was isolated and the overall yield of the reaction found to be excellent. Obviously, in all these cases, the improved diastereoselectivity observed arises at the expense of total yield; however, the ease of purification for this new system was deemed to be an acceptable compromise.

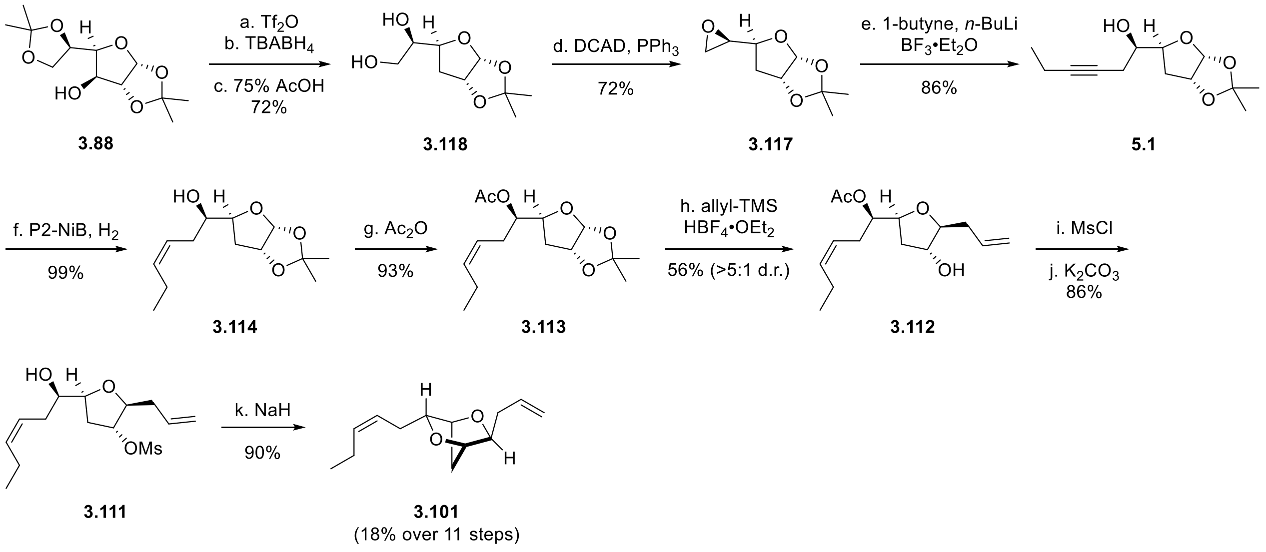
A rationalisation of the outcome of this reaction is considered below (Scheme 5.1.3). By path a (blue), the nucleophile attacks the glycosidic position of acetonide **3.113** according to the Marrero-Tellado proposal leading to the *trans*-product **3.112** following loss of the TMS-group from intermediate **5.4**. In path b (red), the glycosyl cation **5.5** is formed first; arranging this such that the overlap of the C-2 $\sigma_{C\text{-}H}$ is best with the $\pi^*_{C=O}$ (i.e. placing the hydroxyl group equatorial) leads to the 1,2-*cis* intermediate **5.6** after nucleophilic attack from the inside of the envelope. In this case, the stereochemical arrangement of the C-1 and C-2 positions allows for the formation of bicyclic intermediate **5.3** which is stable under the reaction conditions, this is not possible from the 1,2-*trans* intermediate since the groups are too distant and the formation of the *trans*-fused ring system is not favoured.

The bicyclic intermediate **5.3** was formed as a (1:1) mixture of diastereoisomers which made assignment of the NMR spectra very difficult. However, resubjecting this intermediate to the Sakurai conditions for an extended period of time enabled the minor Sakurai product **5.2** to be obtained and fully characterised (Scheme 5.1.3). On account of the mechanistic proposal and by comparison to Sakurai products of the (*E*)-olefin made in a previous project by Chan *et al.*, the configuration of the stereochemistry of the two Sakurai products **3.112** and **5.2** can be stated with reasonable confidence.[102]



Scheme 5.1.3: Mechanism accounting for the formation of **3.112** *via* Marrero-Talledo mechanism and bicyclic by-product **5.3** from the Woerpel pathway. Formation of the minor Sakurai diastereoisomer **5.2** after resubjecting **5.3** to the original reaction conditions and subsequent derivitisaiton to minor [2.2.1]-dioxabicycloheptane **5.8**. Reactions and conditions: a) tetrafluoroboric acid etherate (3.0 equiv.), CH_2Cl_2, rt, 18 h, 70%; b) methanesulfonyl chloride (1.2 equiv.), triethylamine (1.2 equiv.), CH_2Cl_2, 0 °C – rt, 1 h *then* c) potassium carbonate (2.0 equiv.), $MeOH/H_2O$ (7:1), rt, 2 h, 95%; d) sodium hydride (60% in mineral oil, 7.0 equiv.), DMF, 0 °C – rt, 18 h, 70%.

The minor product **5.2** could be taken through the established mesylation/deprotection sequence to form mesylate **5.7** and subsequently cyclised to the [2.2.1]-dioxabicycloheptane **5.8** in good yield (70%). Similarly subjecting major diastereoisomer **3.112** to these conditions allowed access to the key [2.2.1]-dioxabicycloheptane **3.101** for further derivatisation. A full summary of the route to [2.2.1]-dioxabicycloheptane **3.101** is provided, *vide infra* (Scheme 5.1.4). The configuration of the [2.2.1]-dioxabicycloheptanes **3.101** and **5.8** was confirmed by analogy to previously reported compounds of this type.[102]

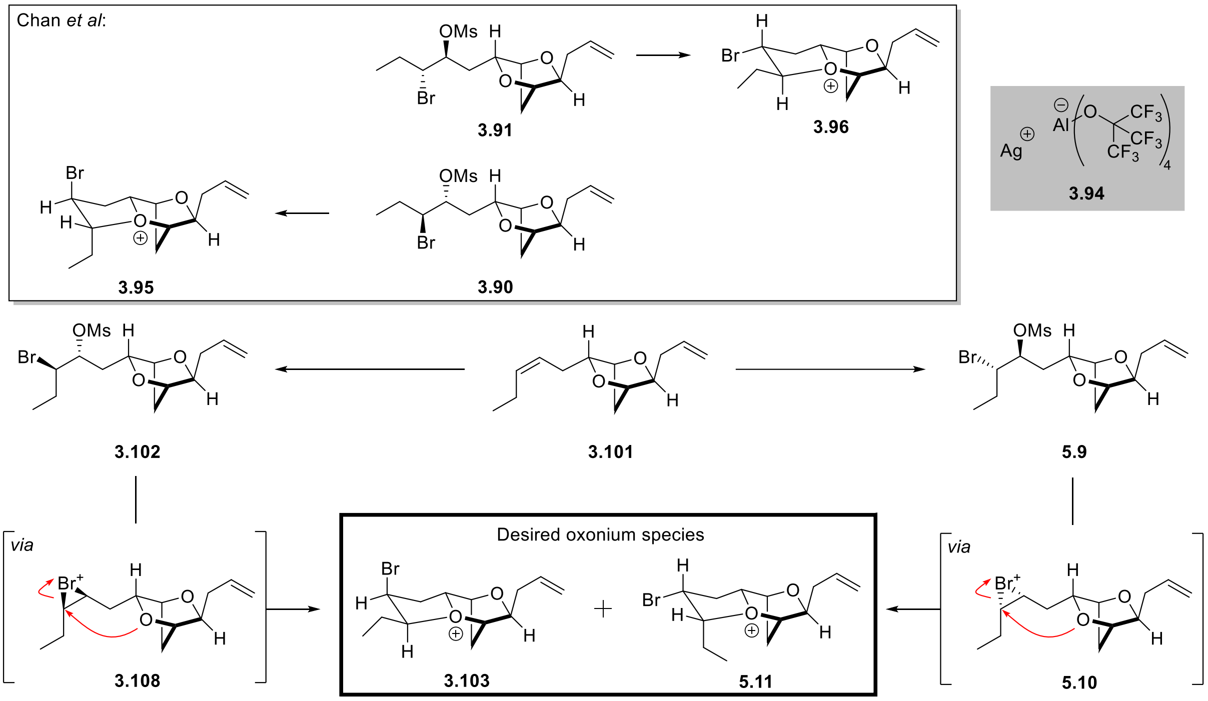


Scheme 5.1.4: Complete route to enable access to [2.2.1]-dioxabicycloheptane **3.101** from diacetone-ᴅ-glucose, yields quoted are starting from 15 g diacetone-ᴅ-glucose in the first step. Reagents and conditions: a) triflic anhydride (1.2 equiv.), pyridine (2.2 equiv.), CH_2Cl_2, 0 °C – rt, 1 h *then* b) tetrabutylammonium borohydride (2.5 equiv.), toluene, 80 °C, 8 h *then* c) 75% AcOH (aq.), rt, 18 h, 72%; d) DCAD (1.1 equiv.), triphenylphosphine (1.1 equiv.), reflux, 18 h, 72%; e) 1-butyne (1.9 equiv.), *n*-butyllithium (1.9 equiv.), boron trifluoride etherate (0.75 equiv.), THF, -78 °C – rt *then* -78 °C, 1 h, 86%; f) nickel acetate tetrahydrate (20 mol%), sodium borohydride (20 mol%), ethylenediamine (40 mol%), H_2, EtOH, rt, 18 h, 99%; g) acetic anhydride (3.0 equiv.), DMAP 3.0 equiv.), CH_2Cl_2, rt, 2 h, 93%; h) allyltrimethylsilane (8.0 equiv.), tetrafluoroboric acid etherate (0.50 equiv.), CH_2Cl_2, reflux, 3 h, 56% (>5>1 d.r.); i) methanesulfonyl chloride (1.2 equiv.), triethylamine (1.2 equiv.), CH_2Cl_2, 0 °C – rt, 1 h *then* c) potassium carbonate (2.0 equiv.), MeOH/H_2O (7:1), rt, 2 h, 86%; d) sodium hydride (60% in mineral oil, 5.0 equiv.), DMF, 0 °C – rt, 18 h, 90% .

Much of the route has been detailed extensively in the previous sections, so further discussion is not provided here. The key steps optimised as part of this project were: the 1-butyne addition, the formation of the (*Z*)-olefin **3.114** *via* nickel boride reduction, and the Sakurai allylation optimisation giving the significantly improved d.r. by virtue of removing the minor diastereoisomer as a by-product. This route enabled access to the desired [2.2.1]-dioxabicycloheptane **3.101** on multigram scale in an overall yield of 18% (over 11 steps from 15 g of starting material **3.88**).

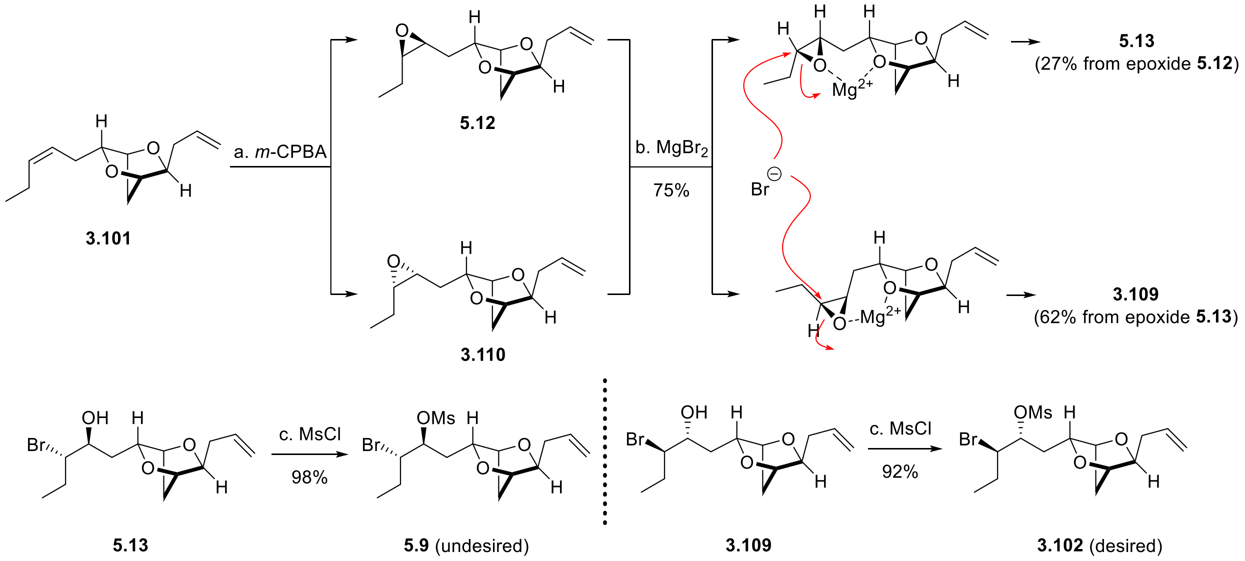
5.2. Reactivity of [2.2.1]-Dioxabicycloheptane System and Initial Silver(I)-Mediated Cyclisation Results

In order to test whether this system would cyclise to form the oxonium ion intermediate under the established conditions with silver(I) salt **3.94** used in previous work by Chan *et al*. for the formation of oxonium ion intermediates **3.95** and **3.96**, cyclisation precursors **3.102** and **5.9** were sought from [2.2.1]-dioxabicycloheptane **3.101** (Scheme 5.2.1).[102] From bromomesylates **3.102** and **5.9**, formation of the enantiopure bromonium ion intermediates **3.108** and **5.10** according to Braddock's method was envisaged to allow access to the desired oxonium ions **3.103** and **5.11**.[137]



Scheme 5.2.1: Chan *et al*. oxonium ions **3.95** and **3.96**. Bromomesylates **3.102** and **5.9** required for the silver(I)-mediated cyclisation to form oxonium ions **3.103** and **5.11** *via* enantiopure bromonium ions **3.108** and **5.10**.

Due to limited literature methods for the enantioselective synthesis of epoxides from isolated (*Z*)-olefins, epoxides **3.110** and **5.12** were synthesised from the [2.2.1]-dioxabicycloheptane **3.101** unselectively with *m*-CPBA. Substrate control provided a 2:1 ratio in favour of the desired diastereoisomer (Scheme 5.2.2).

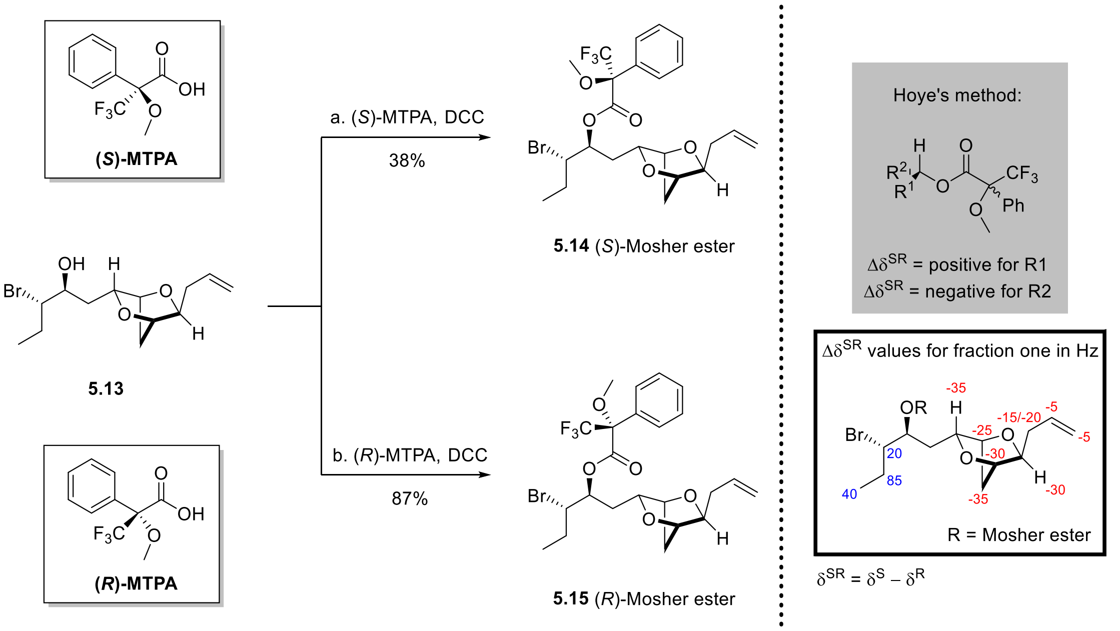


Scheme 5.2.2: Non-selective formation of diastereomeric epoxides and subsequent opening to bromohydrins **5.13** and **3.109**. Mesylation of bromohydrins **5.13** and **3.109** to form the respective bromomesylates **5.9** and **3.102**. Reagents and conditions: a) *m*-chloroperoxybenzoic acid (1.1 equiv.), sodium bicarbonate (aq.), CH_2Cl_2, rt, 2 h *then* b) magnesium bromide etherate (2.0 equiv.), Et_2O, rt, 2 h, 75% (two steps, yields of respective bromohydrins given).

These epoxides could be opened regioselectively in the presence of magnesium bromide, with the regioselectivity most likely arising from the magnesium ion chelation from the ether oxygen from the [2.2.1]-dioxabicycloheptane and the epoxide oxygen, to give bromohydrins **5.13** and **3.109** in good yield and a 2.3:1 ratio of **3.109**:**5.13**. Examination of the HSQC spectrum of the respective bromohydrins **5.13** and **3.109** provided evidence for the regioselectivity of this reaction. Although the epoxidation was carried out without conditions to bias the formation of one epoxide over the other, reaction of the inseparable mixture of epoxides led to a higher yield of bromohydrin **3.109** rather than bromohydrin **5.13** after column chromatography. Mesylation of the bromohydrins was readily achieved to giving both mesylates **5.9** and **3.102** respectively.

The absolute configuration of the bromohydrins **5.13** and **3.109** was confirmed by analysis using Kakisawa and Hoye's extension to Mosher's method.[176,177] The method relies on the synthesis of both the (*S*)- and (*R*)-derivatives of a chiral auxillary, in this case (*S*)-MTPA and (*R*)-MTPA. In this case, the ester carbonyl group and CF_3 group are proposed to preferentially sit in the same plane which leaves the substrate R groups flanked by either the phenyl or methoxy groups, given that the configuration of the MTPA groups is known, a comparison of the difference in chemical shifts of R^1 and R^2 in each of the derivatives based upon their shielding/deshielding by the MTPA groups enables the absolute configuration of chiral secondary alcohols to be assigned.[178]

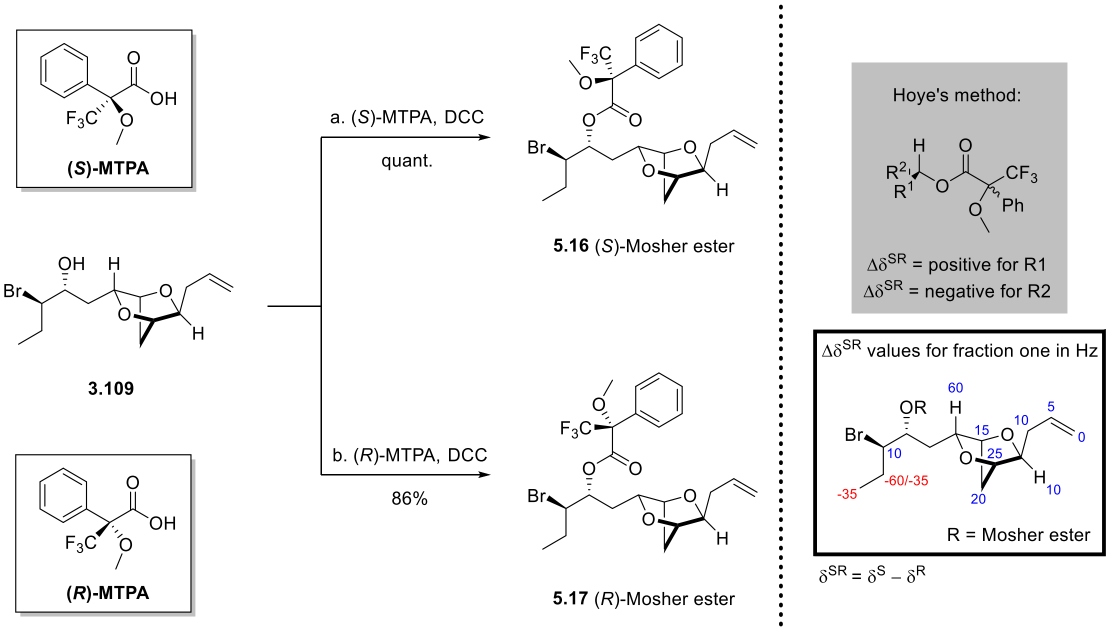
Assuming arbitrarily that fraction one after column chromatography (the minor product) had configuration corresponding to the undesired diastereoisomer **5.13**, assignment of the absolute configuration of the C-12 could be conducted after derivatisation into (*R*)- and (*S*)-Mosher esters **5.14** and **5.15** respectively (Scheme 5.2.3).



Scheme 5.2.3: Formation of (*R*)- and (*S*)-Mosher esters **5.14** and **5.15** respectively and subsequent analysis by Kakisawa and Hoye's extension to Mosher's method. Reagents and conditions: a) (*S*)-MTPA (2.5 equiv.), DCC (2.5 equiv.), DMAP (2.5 equiv.), CH_2Cl_2, rt, 18 h, 38%; b) (*R*)-MTPA (2.5 equiv.), DCC (2.5 equiv.), DMAP (2.5 equiv.), CH_2Cl_2, rt, 18 h, 87%.

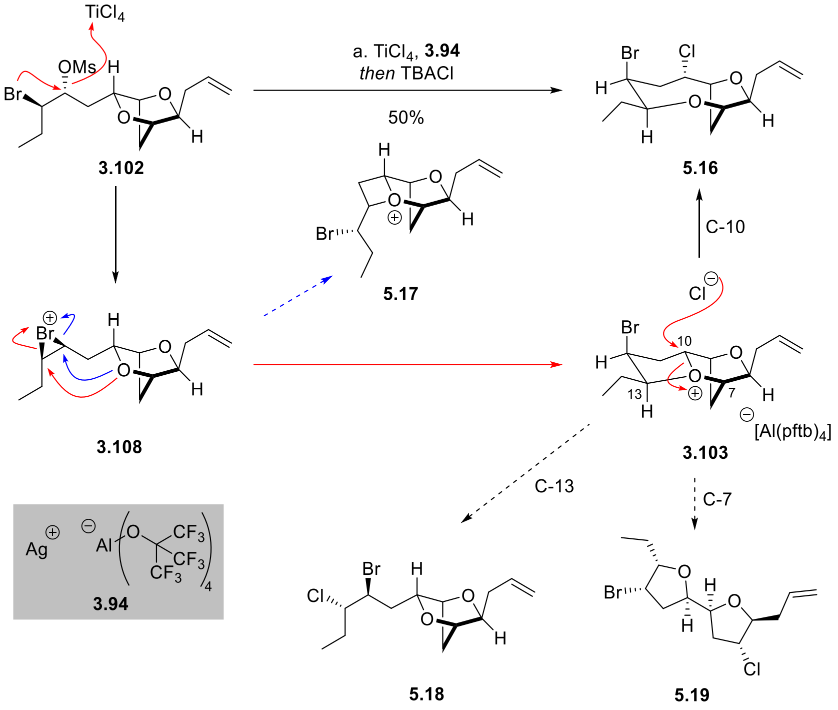
With the $\Delta\delta^{SR}$ values confirming the [2.2.1]-bicycloheptane moiety to be R^1 and the propyl group to be R^2, the stereochemistry of fraction one could be assigned as being the undesired diastereoisomer. Similarly (*R*)- and (*S*)-Mosher esters of the second fraction, thought to contain diastereoisomer **3.109** were synthesised, and the same analysis performed (Scheme

5.2.4). In this case, the Δδ^{SR} values showed the opposite signs, providing evidence that the propyl group was R^1 and the [2.2.1]-dioxabicycloheptane was R^2. This result was entirely consistent with the assumption that the two bromohydrins had opposite configurations at C-12 and also provided evidence that diastereoisomer **3.109** had the desired configuration for derivatisation into *ent*-laurepinnacin ***ent*-3.15**.



Scheme 5.2.4: Formation of (*R*)- and (*S*)-Mosher esters **5.16** and **5.17** respectively and subsequent analysis by Kakisawa and Hoye's extension to Mosher's method. Reagents and conditions: a) (*S*)-MTPA (2.5 equiv.), DCC (2.5 equiv.), DMAP (2.5 equiv.), CH_2Cl_2, rt, 18 h, quant.; b) (*R*)-MTPA (2.5 equiv.), DCC (2.5 equiv.), DMAP (2.5 equiv.), CH_2Cl_2, rt, 18 h, 86%.

Having identified the absolute configuration of the respective bromohydrins **5.13** and **3.109**, the mesylate **3.102** of the desired diastereoisomer **3.109** was subjected the established silver(I)-mediated cyclisation for the formation of oxonium ion **3.103**, as developed by Chan *et al*. (Scheme 5.2.5).[102]



Scheme 5.2.5: Subjection of bromomesylate **3.102** to established silver(I)-mediated cyclisation conditions to gain access to [5.2.1]-dioxabicyclic chloride **5.16**. Reagents and conditions: a) titanium tetrachloride (2.0 equiv.), **3.94** (3.1 equiv.), CH_2Cl_2, -40 °C, 2 h *then* TBACl (10 equiv.), -78 °C, 1 h, 50%.

Under the reaction conditions used, chloride **5.16** was expected to be the major product according to the mechanism outlined above. Formation of the bromonium ion **3.108** through activation of the bromomesylate **3.102** with titanium chloride, followed by intramolecular trapping of this species by the ether oxygen of the [2.2.1]-dioxabicycloheptane in a 5-*exo* manner rather than the alternative 4-*exo* cyclisation would provide access to the oxonium ion species **3.103** rather than **5.17**. On addition of chloride, nucleophilic quench at C-10 would give [5.2.1]-dioxabicyclic product **5.16**. This pathway was expected to dominate on the basis that in related systems, the C-10 quenching pathway giving [5.2.1]-dioxabicyclic chlorides **3.97** and **3.98** (not shown), has the lowest energy barrier in comparison to attack at C-7 or C-13 to give the [2.2.1]-bicycloheptane **5.18** or bis-THF **5.19** respectively (Scheme 5.2.5). Quenching at C-10 involves the least structural rearrangement in the transition state.[102]

The presence of silver(I) salt **3.94** was instrumental in providing a stable environment for this highly reactive species; the excess silver cations scavenge chloride from the titanium tetrachloride, whilst the extremely low nucleophilicity of the anion itself led to the oxonium ion species **3.103** itself being stable in solution. Quenching by addition of chloride in the form of TBACl gave 50% yield of a compound tentatively assigned as the C-10 chloride **5.16**.

Analysis of the mass spectrometry data indicated that a chloride quench product had been formed (Figure 5.2.1). HRMS *m/z* (MSS$^+$) peaks were found at 323.0409 and 325.0386 ($C_{13}H_{21}{}^{79}Br^{35}ClO_2$ and $C_{13}H_{21}{}^{81}Br^{35}ClO_2$, M+H$^+$ adducts require 323.0408 and 325.0385 respectively) with the correct isotope pattern being observed for a compound containing both bromide and chloride (^{37}Cl isotope peaks are not annotated).

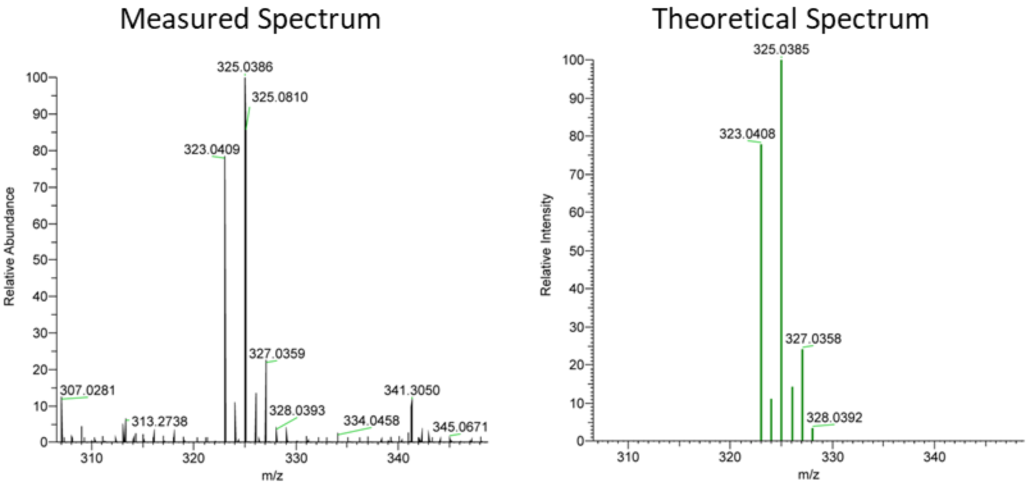
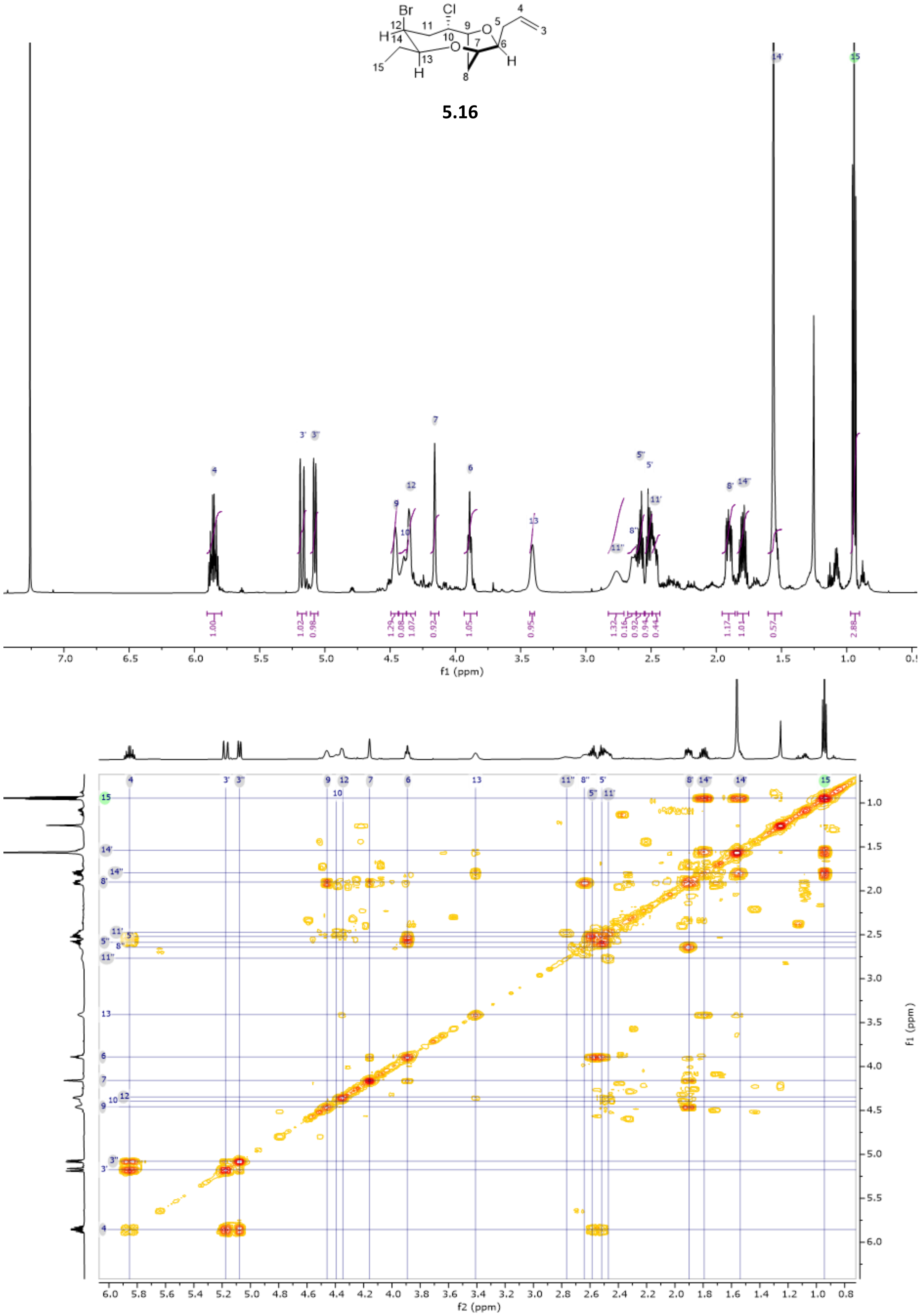


Figure 5.2.1: Measured HRMS spectrum for C-10 chloride **5.16** and comparison to the theoretical spectrum expected.

In addition to this, analysis of the ^1H NMR spectrum with the aid of the $^1H^1H$ COSY spectrum enabled the connectivity of the species to be established and a tentative assignment of the structure to be made (Figure 5.2.2).

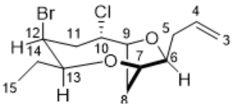


5.16

Figure 5.2.2: ^{1}H NMR and ^{1}H^{1}H COSY (600 MHz, CDCl$_3$) spectra of C-10 chloride **5.16**.

Analysis of the ^{1}H NMR spectra was possible on account of the straightforward assignment of the C-3 and C-15 protons due to their distinct chemical shifts. From these initial assignments, it was then possible to assign the remainder of the spectrum based on the $^{1}H^{1}H$ COSY correlations with the entire backbone carbon backbone of the molecule being a single spin system. The assignments of CH-15 through CH-11 were achieved easily by analysis of the $^{1}H^{1}H$ COSY correlations. The assignment of CH-12 (δ 4.38 – 4.31 ppm) was corroborated by its associated ^{13}C resonance (δ 57.5 ppm) indicative of a proton on a carbon also bearing a bromide. Similarly, beginning at CH-3 enabled identical $^{1}H^{1}H$ COSY analysis to be preformed for the assignment through to CH-9, leaving the remaining resonance to be assigned as CH-10.

The analysis of the spectral data for C-10 chloride **5.16** was somewhat hampered by the broadness of several resonances. This broadness can be explained by the interconversion of conformations of the medium ring ether being in the intermediate exchange regime on the NMR timescale.[179] This also lends weight to the assignment of the NMR data since the protons of the medium ring undergoing slow conformational exchange are most affected, with the protons of the right hand of the molecule (CH-3 – CH-6) and the ethyl chain (CH-14 and CH-15) still being well resolved. The $^{1}H^{1}H$ NOE data was also consistent for the proposed structure (Figure 5.2.3).



5.16

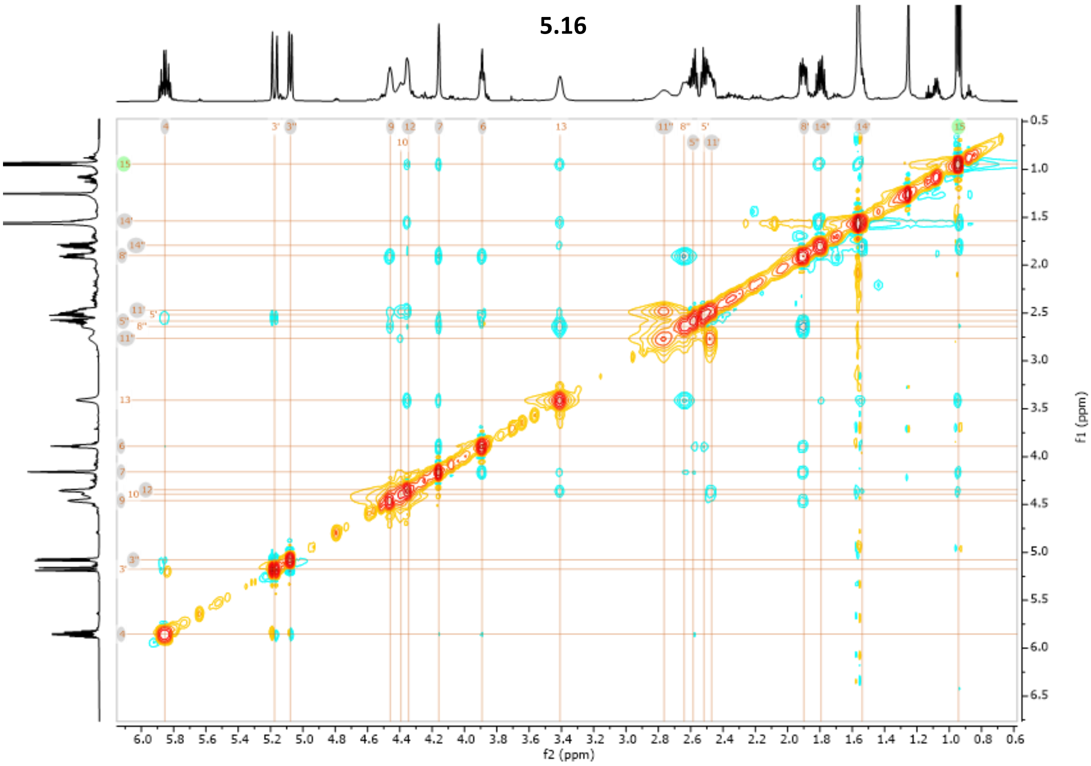


Figure 5.2.3: ^{1}H^{1}H NOE spectrum of C-10 chloride **5.16**.

The correlation of the broad CH-8 ↔ CH-13 (δ 2.64 ppm and 3.41 ppm respectively) indicates a close proximity of these protons, arising from the presence of the medium ring ether scaffold shown. Likewise, CH-13 also has correlations to CH-14 and CH-15 of the ethyl chain and also CH-7, one of the CH-11s and CH-12 of the medium ring ether as expected. The well resolved CH-8 resonance (1.96 – 1.86 ppm) corresponding to the proton facing away from the medium ring at this position has a correlation to CH-6. Full analysis of the NOE spectrum is not provided, but these key correlations provide further evidence for the structure of the proposed C-10 chloride **5.16**.

However, the spectroscopic data was limited and full assignment of the ^{13}C NMR data was not possible on account of severe broadening of the peaks causing a lack of resolution of many of the medium ring ether carbons. Additionally, C-10 chloride **5.16** was not stable to column chromatography, and hence could not be isolated with 100% purity with the isolated material not suitable for further experiments to try and regenerate the oxonium species **3.103** and study it by low temperature NMR spectrometry.

Given that the data for the C-10 chloride **5.16** could not be assigned fully, it was envisaged that quenching with other nucleophiles may allow access to compounds with less conformational flexibility, enabling the identity and stereochemistry of the cyclisation products to be determined/confirmed with more confidence since direct study of the oxonium ion could not be carried out.

In addition to this, non-chloride quench products, with handles for elimination were required in order to access the core of *ent*-laurepinnacin itself.

5.3. Continued Cyclisation Attempts Towards the Synthesis of *ent*-Laurepinnacin

Despite the setbacks outlined above, the aim for the project remained to synthesise *ent*-laurepinnacin using the oxonium ion rearrangement methodology developed previously in the group.[102] During the course of this endeavour, confirmation of the structure and relative configuration of the oxonium ion quench products was aimed for. To this end, it was necessary to generate intermediates **3.107** and **5.20** to probe the required elimination to form the medium ring ether core of the natural product itself (Figure 5.3.1).

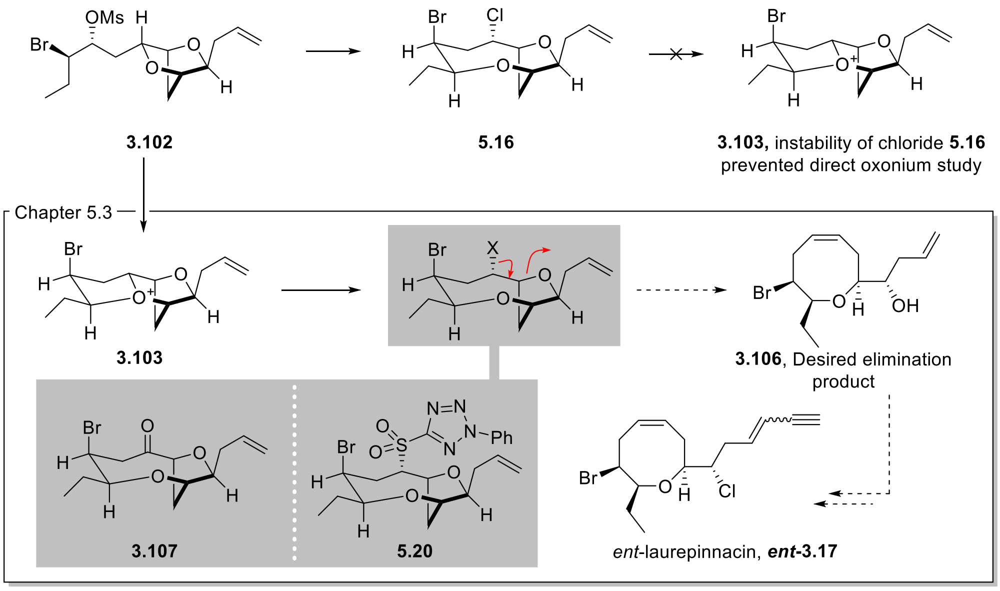
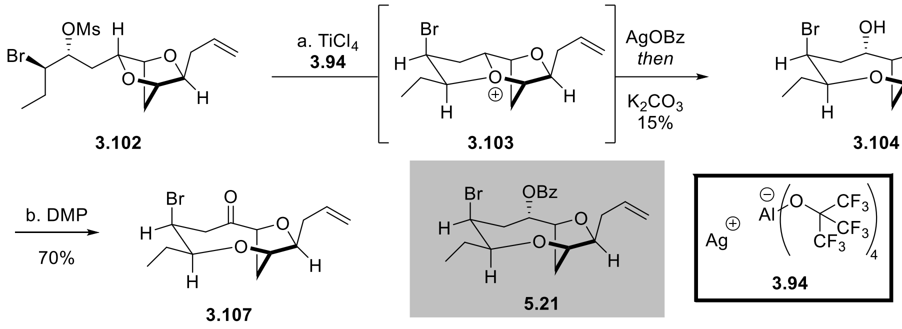


Figure 5.3.1: Previous work detailing the unsuccessful attempt to directly study the oxonium ion species **3.103**. Elimination strategy to form the core of *ent*-laurepinnacin ***ent*-3.15** involving intermediates **3.107** and **5.20** chosen for their propensity to undergo this transformation in related systems.[139]

Since the quenching of oxonium ions with oxygen based nucleophiles had recently been published in the group, this methodology was adopted for the initial elimination attempt, with ketone **3.107** being expected to undergo the desired Kishner-Leonard elimination.[117]

The previously successful silver(I)-mediated cyclisation was again employed, however, applying the previously developed conditions for oxonium ion formation, namely treatment of bromomesylate **3.102** with titanium tetrachloride and silver(I) salt **3.94** and subsequent oxygen nucleophile quench led to a very disappointing yield of the C-10 alcohol **3.104** in pursuit of ketone **3.107** (Scheme 5.3.1).



Scheme 5.3.1: Utilisation of the silver(I) mediated cyclisation method and subsequent quench with oxygen nucleophile (silver benzoate) to form C-10 alcohol **3.113** after *in situ* deprotection and oxidation to the desired ketone **3.116**. reagents and conditions: a) titanium tetrachloride (2.0 equiv.), **3.100** (3.2 equiv.), silver benzoate (3.3 equiv.), -40 °C – -78 °C, 3 h *then* potassium carbonate (5.0 equiv.), MeOH, rt, 2 h, 15%; b) Dess-Martin periodinane (3.0 equiv.), sodium bicarbonate (10 equiv.), CH_2Cl_2, rt, 1.5 h, 70%.

Despite the oxonium ion formation proceeding with the same colour changes as observed previously, the quench with silver benzoate and TBAI repeatedly led to a poor yield of alcohol **3.104** after benzoate deprotection. The reasons for this are not clear but evidently the oxygen nucleophile quench of the oxonium ion with silver(I) benzoate is not tolerated by this related oxonium system, isolation of the intermediate benzoate ester **5.21** could not be achieved either. It is possible that the high excess equivalents of silver introduced upon addition of the silver(I) benzoate react with the secondary bromide present in the molecule, however, these are the conditions used previously in a closely related system with success.[102]

The characterisation of alcohol **3.104** proceeded in much the same way as for the C-10 chloride **5.16**. Assignment of the ^1H NMR spectrum could once again be carried out with assistance from the $^1H^1H$ COSY spectrum with the ^1H NMR spectrum resembling the previously obtained spectrum of the C-10 chloride **5.16**, albeit with slightly less broadness (Figure 5.3.2).

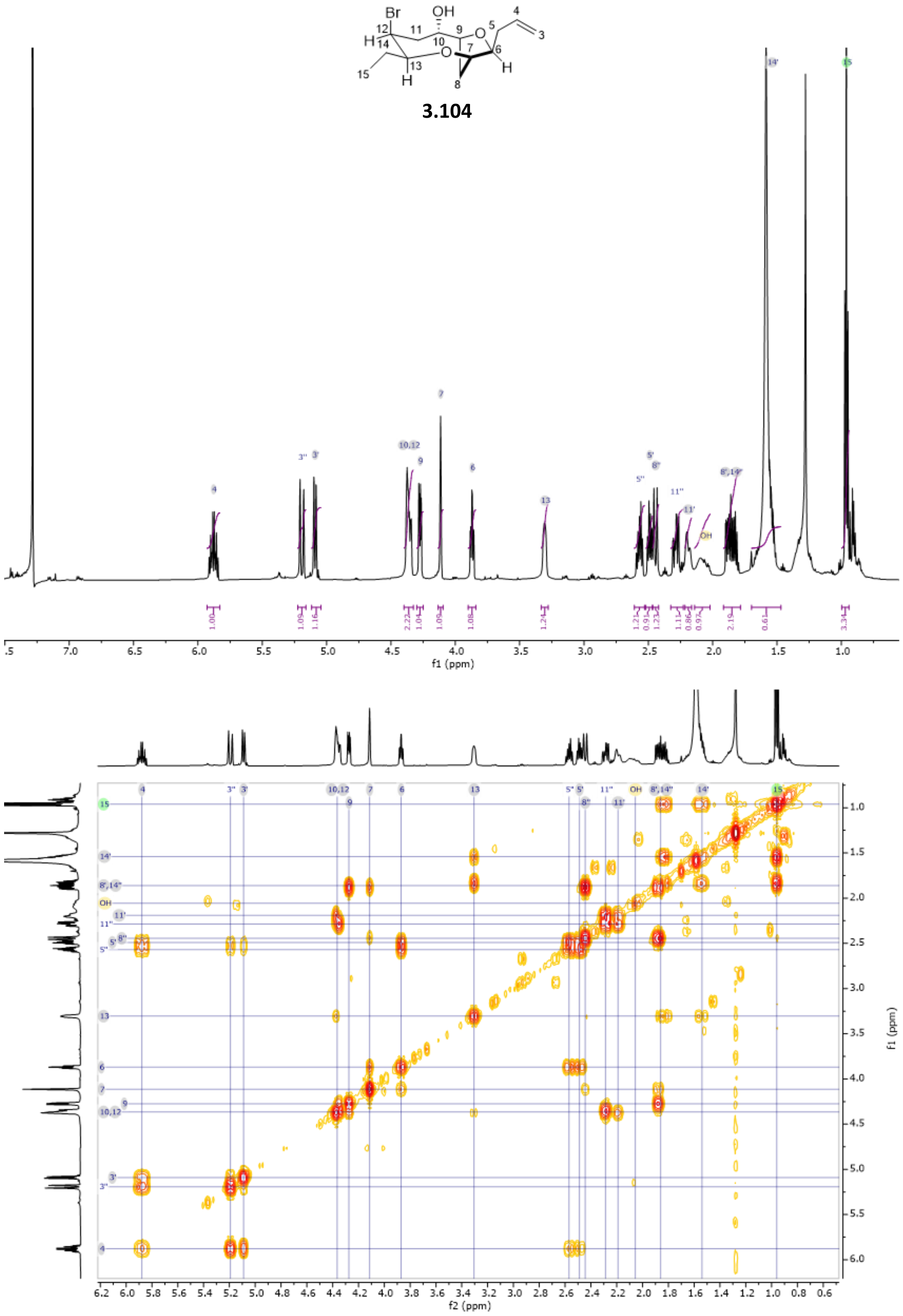
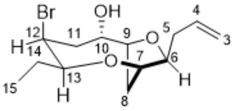

3.104

Figure 5.3.2: ^{1}H and ^{1}H^{1}H COSY (600 MHz, CDCl$_3$) spectra of C-10 alcohol **3.104**.

Beginning again with the terminal positions CH-3, CH-3’ and CH-15 resonances (δ 5.19, 5.09 and 0.94 ppm respectively), with the aid of the ^{1}H^{1}H COSY spectrum the connectivity of the compound could be established. Pleasingly, in this case the ^{1}H NMR resonances were suitably well resolved and had less overlap than for the C-10 chloride, so it was possible to follow the correlations in the ^{1}H^{1}H COSY spectrum from either of the terminal positions all the way through the compound in either direction. The proposed structure was once again corroborated by the ^{1}H^{1}H NOE spectrum showing key resonances expected for the medium ring ether product (Figure 5.3.3).



3.104

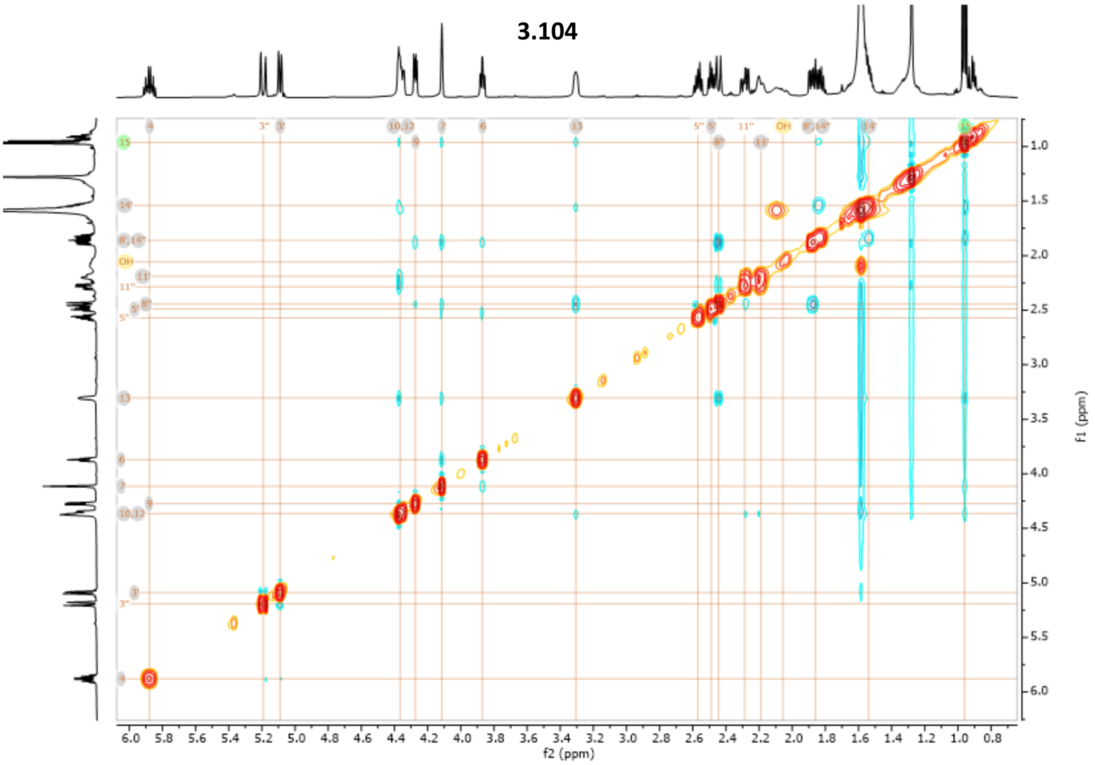
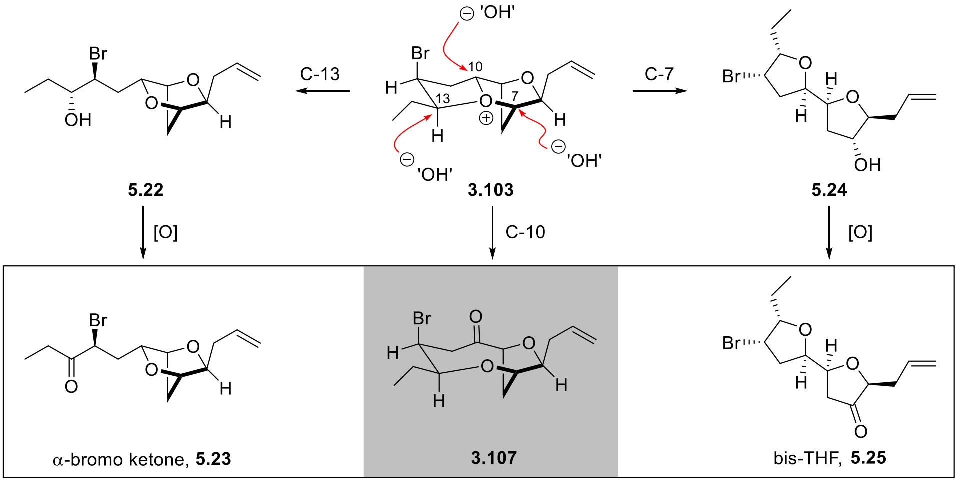


Figure 5.3.3: ^{1}H^{1}H NOE spectrum of C-10 alcohol **3.104**.

Once again, the key ^{1}H^{1}H NOE interaction between CH-13 and CH-8 (δ 3.33 – 3.28 ppm and 2.29 ppm respectively) lent weight to the proposed structure of C-10 alcohol **3.104**. Similarly, the CH-13 correlations to CH-12 and CH-7 of the medium ring further corroborated this view. Unfortunately, although the ^{13}C NMR resolved enough for assignment of the resonances to be conducted from the HSQC, the clarity of the HMBC data was still lacking such that the structure of the C-10 product could not be stated with absolute certainty. Given that the next step of the synthesis was oxidation of the C-10 alcohol **3.104** to ketone **3.107**, a transformation expected to limit the conformational flexibility of the medium ring ether even further. It was hoped that this would provide the final pieces of data necessary for the assignment of the oxonium ion quench products to be validated.

Taking the limited amount of alcohol **3.104** that had been obtained and subjecting it to DMP oxidation allowed access to ketone **3.107** in good yield for this step (*vide supra*). Fortunately, the oxidation to the ketone, led to even better resolved NMR spectra and therefore the final assignments of the proposed oxonium products could be carried out.

Considering the possible products of the oxonium cyclisation and oxidation sequence led to three potential products; the first being C-13 quenching to give alcohol **5.22** and oxidation to give the α-bromo ketone **5.23**, the second of which being the desired C-10 quench followed by oxidation to desired ketone **3.107**. Finally, C-7 quenching to give bis-THF **5.24** and oxidation of the resulting alcohol would give the ketone containing bis-THF product **5.25** (Scheme 5.3.2).



Scheme 5.3.2: Desired oxonium quench and oxidation product **3.107** and alternative structures for products possible by the cyclisation and oxidation sequence (α-bromo ketone **5.23** and bis-THF **5.25**).

Pleasingly, the mass spectrometry data were consistent for the ketone products proposed (Figure 5.3.4). HRMS *m/z* (ESI$^+$) peaks were found at 303.0592 and 305.0571 ($C_{13}H_{20}{}^{79}BrO_3$ and $C_{13}H_{20}{}^{81}BrO_3$, the $M+H^+$ adducts require 303.0590 and 305.0570 respectively). In addition to this the resonance at δ 211.8 ppm in the ^{13}C NMR spectrum along with the IR stretch at 1705 cm^{-1} were also indicative of the presence of a carbonyl group.

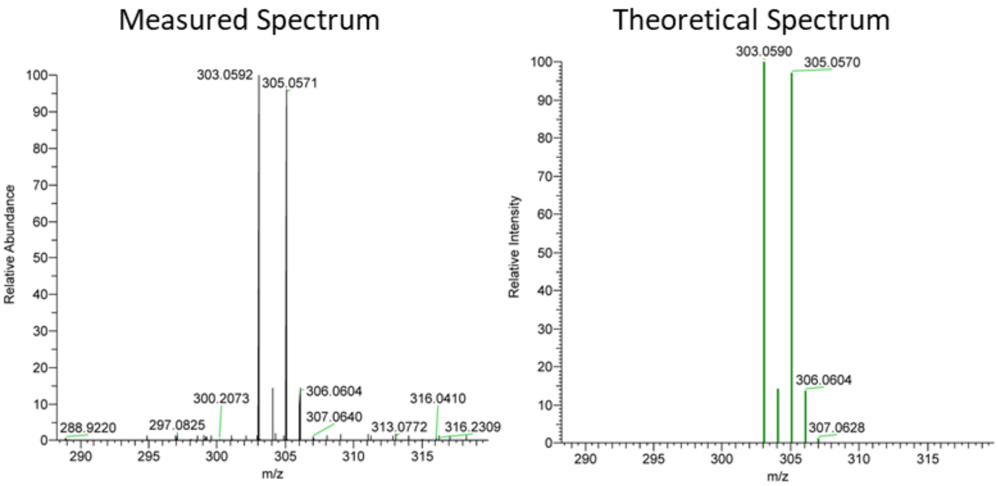
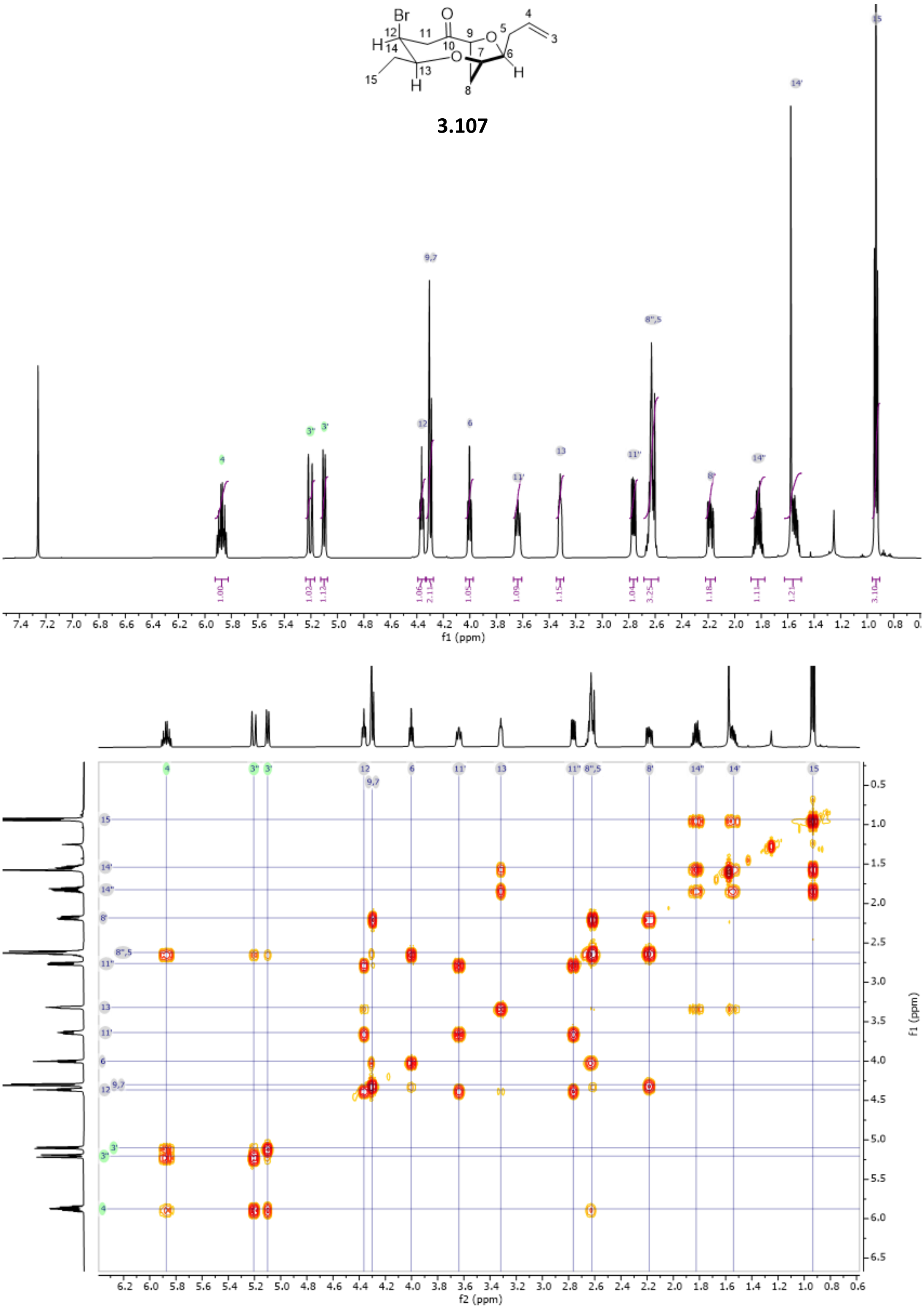


Figure 5.3.4: HRMS data for C-10 ketone **3.107**.

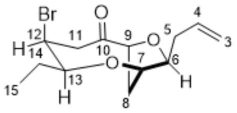
Introduction of the ketone into the molecule had the benefit not only of resolving the NMR spectra, it also increased the number of spin systems within the molecule from one to two. With this in mind, an inspection of the ^{1}H^{1}H COSY spectrum enabled the bis-THF **5.23** and α-bromo ketone **5.25** to be ruled out (Figure 5.3.5). If the bis-THF **5.5** had been formed in the reaction, the CH-3 to CH-6 spin system would have been isolated; likewise, formation of the α-bromo ketone **5.23** would have led to isolation of the CH-15 and CH-14 positions as a single spin system. From the ^{1}H^{1}H COSY spectrum neither of these possibilities were observed, the diastereotopic CH-14s (δ 1.54 ppm and 1.88 – 1.77 ppm) had correlations to both CH-15 (δ 0.93 ppm) and CH-13 (δ 3.32 ppm), and CH-6 (δ 4.00 ppm) had interactions to CH-7 (4.33 – 4.27 ppm) and CH-5 (2.69 – 2.58 ppm). The remainder of the ^{1}H NMR spectrum was assigned by an analogous method to the C-10 chloride **5.16** and C-10 alcohol **3.104** and is not discussed further.



3.107

Figure 5.3.5: ^{1}H and ^{1}H^{1}H COSY (600 MHz, CDCl$_3$) spectra for C-10 ketone **3.107**.

At this point, it was becoming increasingly evident that the C-10 pathway had prevailed. The final pieces of evidence to suggest that this had been the case were provided by the $^1H^{13}C$ HMBC and $^1H^1H$ NOE spectra (Figure 5.3.6).



3.107

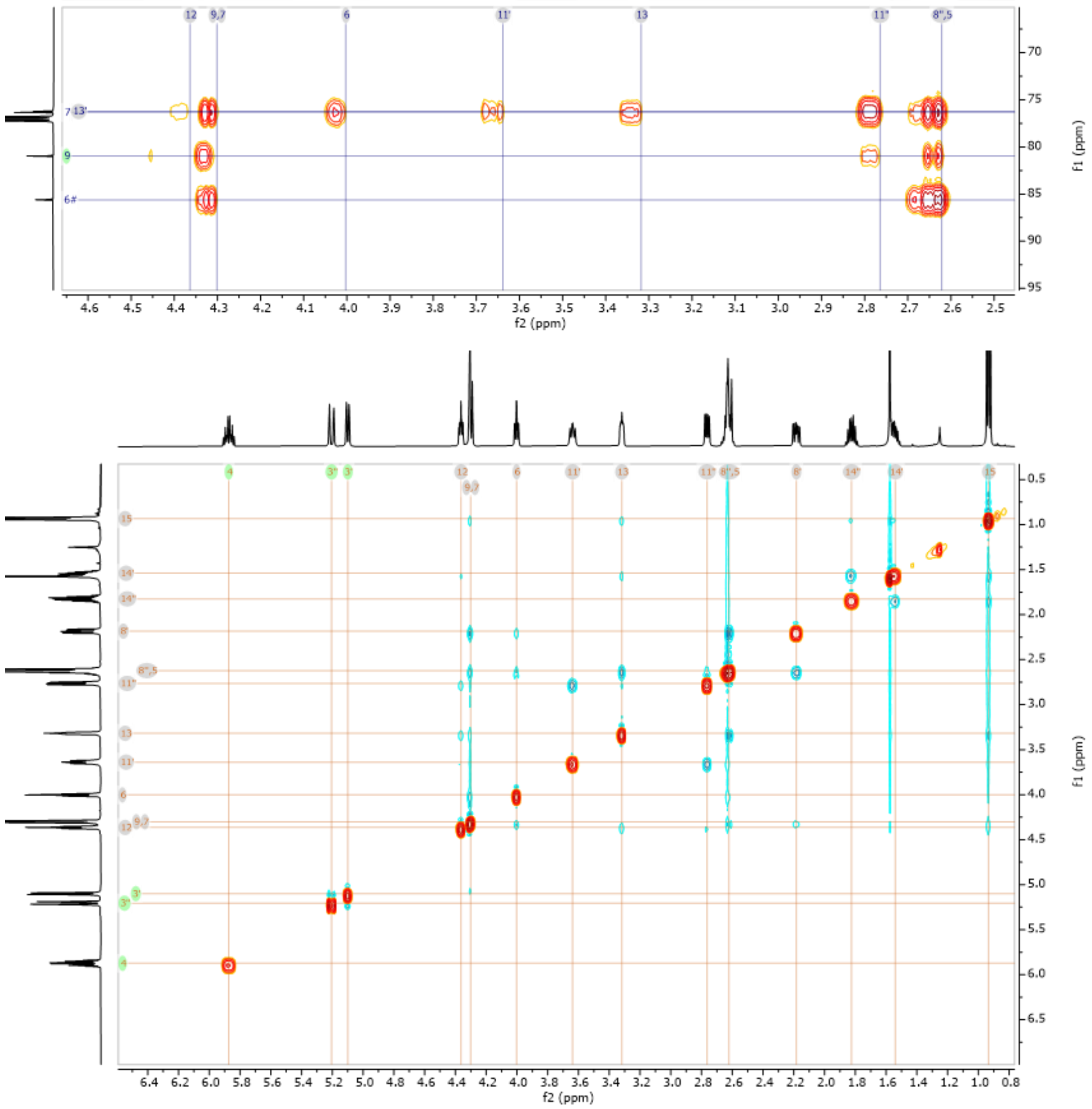
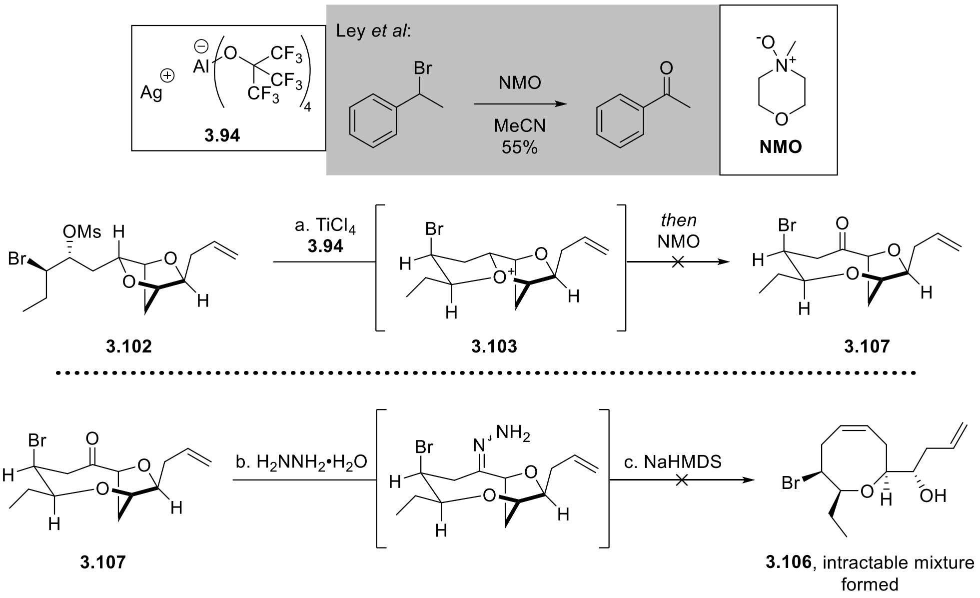


Figure 5.3.6: $^1H^{13}C$ HMBC and $^1H^1H$ NMR spectra (600 MHz, $CDCl_3$) for C-10 ketone **3.107**.

The $^1H^{13}C$ HMBC spectrum shows the CH-7 ↔ C-13 and CH-13 ↔ C-7 correlation exclusively possible in the case of the formation of the C-10 ketone **3.107** over the other possibilities. The $^1H^1H$ NOE spectrum reinforces the identity of C-10 ketone **3.107** as per the previous C-10 quench products. The correlation once more between CH-13 and other positions of the medium ring (CH-14, CH-12 and CH-8) are indicative of the proposed structure. Similarly, the correlation between CH-11 ↔ CH-12 along with the CH-8’ ↔ CH-6 further back up this structural assignment.

Given that the spectroscopic data for ketone **3.107** were providing good evidence that the analogous C-10 products **5.16** and **3.104** had been formed as the major products in the oxonium ion quench, a direct quench from the oxonium ion to form ketone **3.107** was sought. Since the current sequence was proving ineffective at granting access to suitable quantities of material to push the synthesis forward.

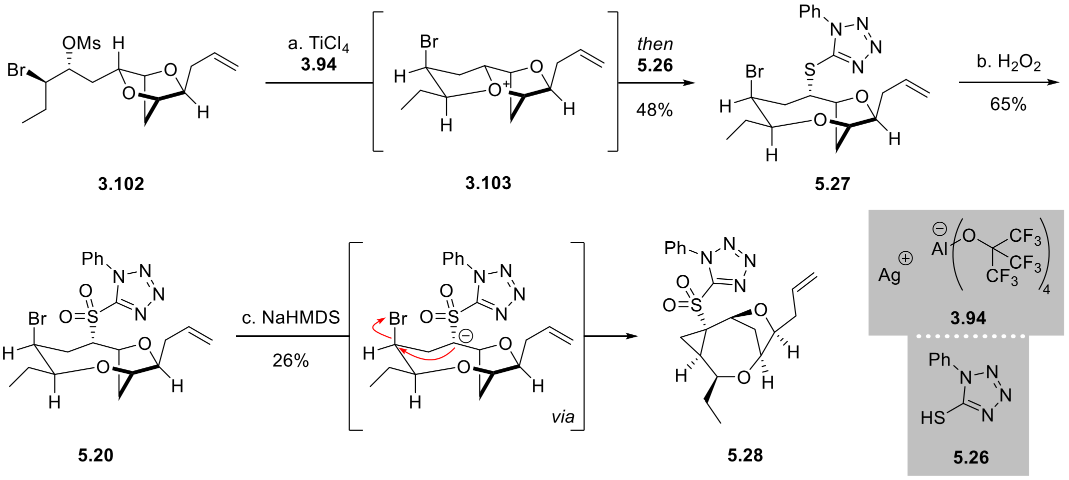
Taking inspiration from work by the Ley group in the early 1990s, use of their modified Kornblum/Ganem oxidation conditions by using *N*-methylmorpholine-*N*-oxide (NMO) as the oxonium ion quench substrate, it was hoped to enable the formation of ketone **3.107** directly.[180] This had proven successful in the oxidation of halides to their respective carbonyl compounds without elimination issues, so it was envisaged that NMO would act as a C-10 quench nucleophile rather than a base. Unfortunately, stirring at -78 °C or alternatively warming to rt after the quench with NMO led in both cases to an intractable mixture, the highly reactive nature of the respective oxonium ion was not compatible with this oxidation protocol (Scheme 5.3.3).



Scheme 5.3.3: Ley conditions for the formation of aldehydes and ketones from halides and attempt to form ketone **3.107** directly from oxonium ion **3.103** quench by NMO. Failed Kishner-Leonard elimination to access the core of ent-laurepinnacin. Reagents and conditions: a) titanium tetrachloride (2.0 equiv.), **3.94** (3.2 equiv.), CH_2Cl_2, -40 °C, 2 h *then* NMO (10 equiv.), -78 °C, 1 h, no product formed; b) hydrazine hydrate (2.5 equiv.), EtOH, rt, 1 h; *then* c) NaHMDS (1 M in THF, 1.1 equiv.), THF, -78 °C – rt, 1 h, no product formed.

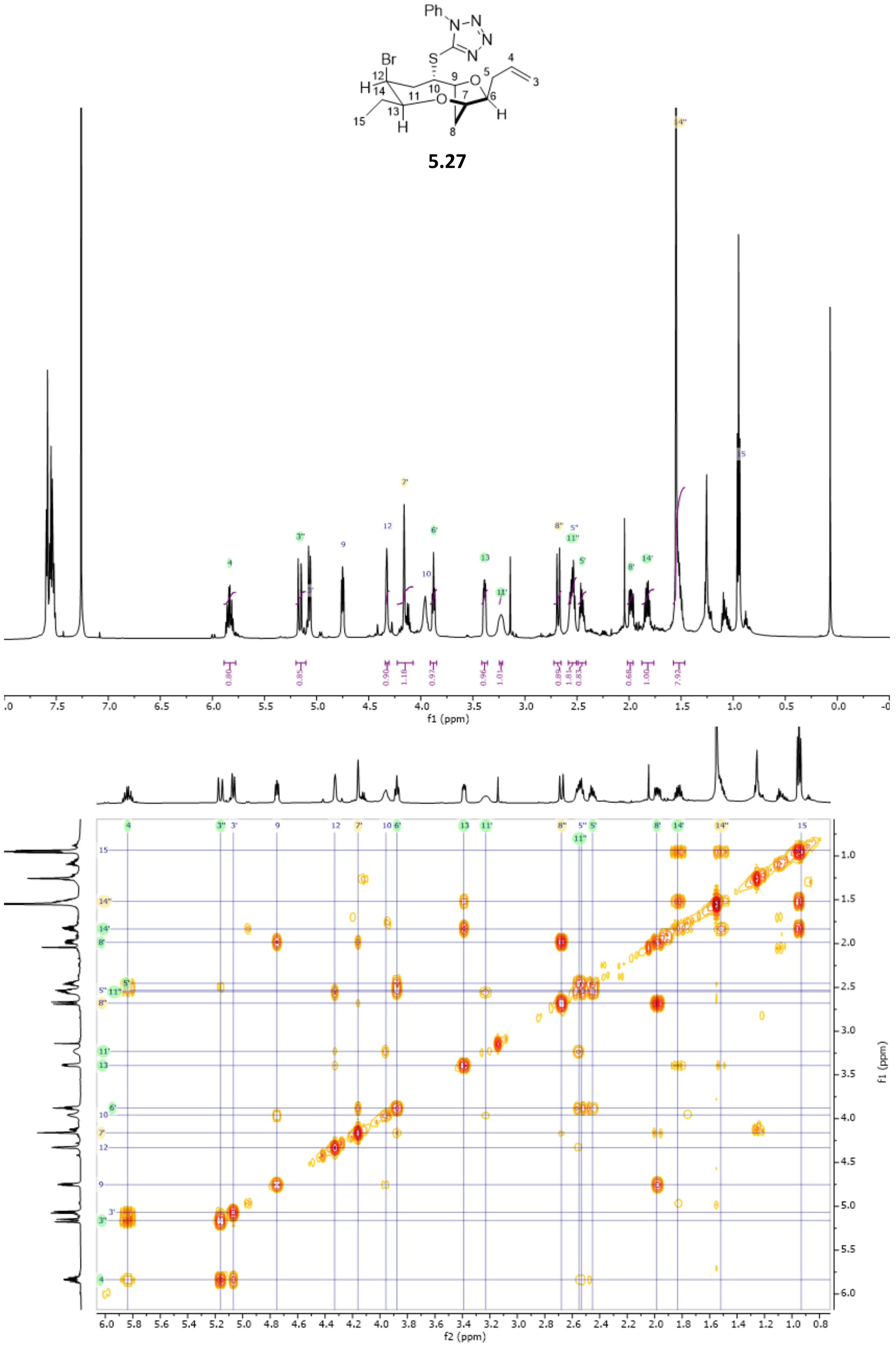
With the ketone **3.107** in hand, attention was turned to the elimination itself. A two-step procedure was required to enable this transformation using Kishner-Leonard elimination conditions as reported by Chan and co-workers.[102] Formation of the hydrazone for the Kishner-Leonard elimination itself could be achieved by stirring the ketone with hydrazine hydrate, full consumption of the starting material was observed after 1 h at rt. The polar nature of the hydrazone limited the purification options available at this point and the intermediate was used without further purification. It was expected that treating the hydrazone under basic conditions, starting at -78 °C with warming to rt to eliminate the ether oxygen with concomitant loss of nitrogen would occur, as had been observed previously.[102] Unfortunately in this case, complete decomposition of the starting material was observed with no evidence of the desired compound **3.106** in the crude ^{1}H NMR spectrum and a complex TLC profile.

Options were very limited with respect to the optimisation of this reaction; primarily due to the lack of ketone material accessible on account of the low yield of product formed during the oxonium cyclisation, and also the limited scale at which the reaction could be performed. The second option for this desired elimination, involving sulfone **5.20** seemed an attractive avenue to pursue despite the previously reported system having issues with cyclopropane formation.[102] Revisiting the oxonium ion chemistry, this time quenching with 1-phenyl-1*H*-tetrazole-5-thiol **5.26** allowed access to thioether **5.27** in a respectable yield of 48%. The yield for this quench was far higher than the oxygen nucleophile quench and more in line with the yield observed for the quench with chloride (Scheme 5.3.4).



Scheme 5.3.4: Oxonium ion quench with thiol **5.26** for the formation of thioether **5.27** and subsequent oxidation to sulfone **5.20** *via* molybdenum-mediated oxidation with hydrogen peroxide. Elimination attempt leading to formation of cyclopropane **5.28**. Reagents and conditions: a) titanium tetrachloride (2.0 equiv.), **3.94** (3.2 equiv.), CH_2Cl_2, -40 °C, 2 h *then* 1-phenyl-1*H*-tetrazol-5-thiol (10 equiv.), -78 °C, 1 h, 48%; b) ammonium molybdate tetrahydrate (0.30 equiv.), hydrogen peroxide solution (30% in H_2O), EtOH, rt, 18 h, 65%; c) NaHMDS (0.1 M in THF, 1.1 equiv.), THF, -78 °C – rt, 20 min, 26%.

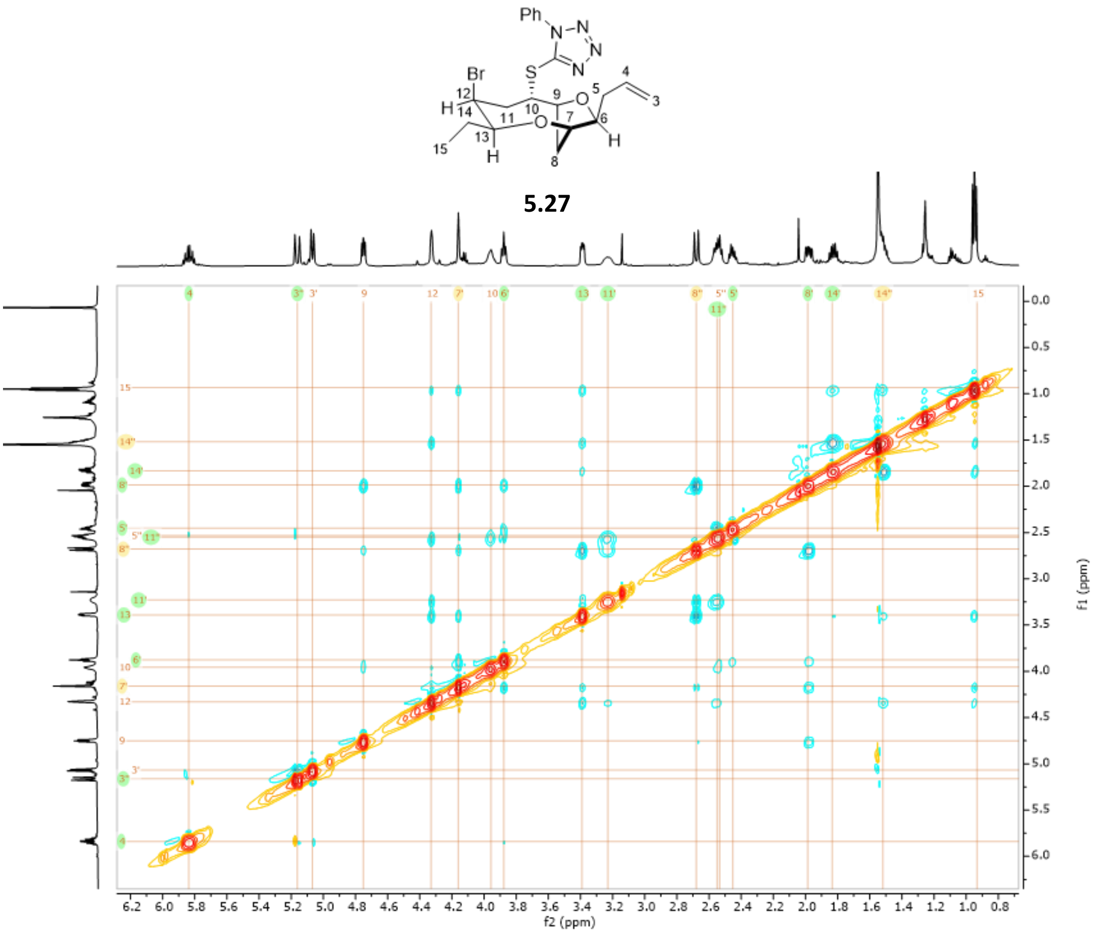
The C-10 quench product **5.27** was assigned according to the previous strategy utilised in the analysis of other quench products. Beginning from the terminal positions CH-3, CH-3' and C-15 (δ 5.19, 5.13 – 5.06, and 0.97 ppm respectively), with the aid of the $^1H^1H$ COSY spectrum, the connectivity of the compound could be established, a full discussion is not provided here (Figure 5.3.7).



5.27

Figure 5.3.7: ${}^{1}H$ and ${}^{1}H{}^{1}H$ COSY spectra (600 MHz, $CDCl_3$) of sulfide **5.27**.

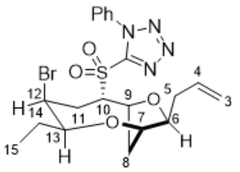
Similarly, the $^1H^1H$ NOESY spectrum for sulfide **5.27** corroborated the previous data and provided evidence for the proposed structure. The critical $^1H^1H$ NOE interaction between CH-8 and CH-13 (δ 2.68 and 3.39 ppm respectively) was once again present (Figure 5.3.8). Furthermore correlations between CH-13 to CH-11 and CH-12 lent additional weight to the proposed structure.



5.27

Figure 5.3.8: $^1H^1H$ NOESY spectrum (600 MHz, $CDCl_3$) for sulfide **5.27**.

Thioether **5.27** was cleanly converted to the corresponding sulfone **5.20** under oxidation conditions with hydrogen peroxide in the presence of molybdate. Although a full discussion of the assignment of this compound is not provided due to its close similarity to the previous C-10 quench products, an interesting NOE is highlighted below (Figure 5.3.9). The NOE correlation between CH-10 and CH-5 enables the configuration of the CH-10 proton to be stated with reasonable confidence, lending further evidence for the formation of the oxonium ion **3.103** as proposed by the mechanism outlined previously. This NOE correlation had previously been ambiguous due to overlapping resonances in the previous C-10 quench products.



5.20

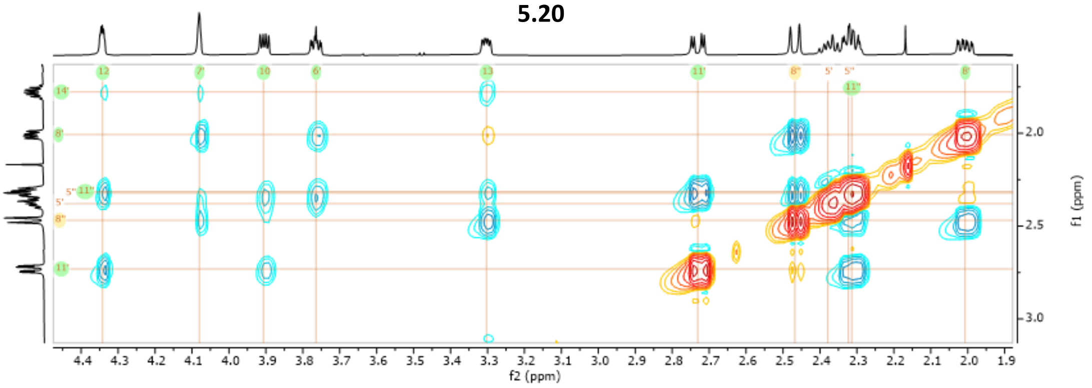
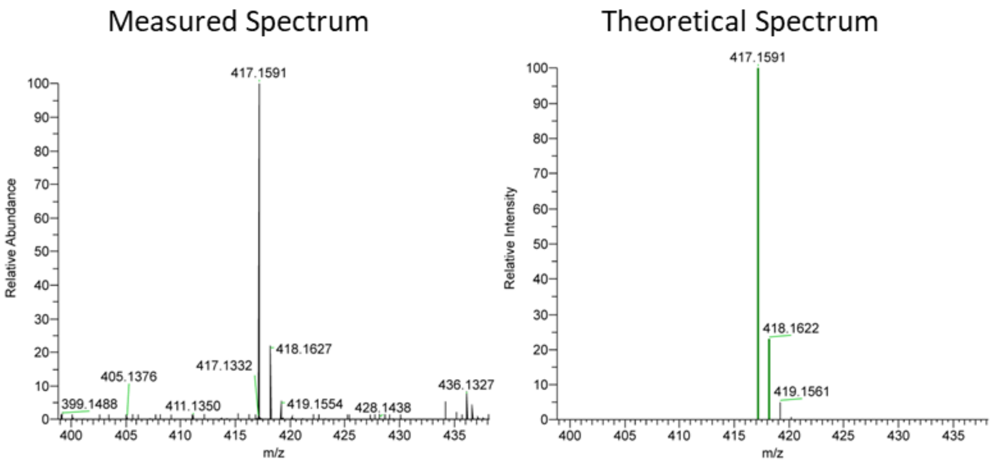


Figure 5.3.9: ^{1}H^{1}H NOESY spectrum (600 MHz, CDCl$_3$) for sulfone **5.20** showing CH-10 ↔ CH-5 correlation to support the proposed mechanism for the oxonium ion chemistry.

Disappointingly, this compound did not undergo the desired elimination, under basic conditions, to give the core structure of *ent*-laurepinnacin ***ent*-3.15**. Instead, the major isolated product was cyclopentane **5.28** *via* an S$_N$2 reaction onto the bromide.

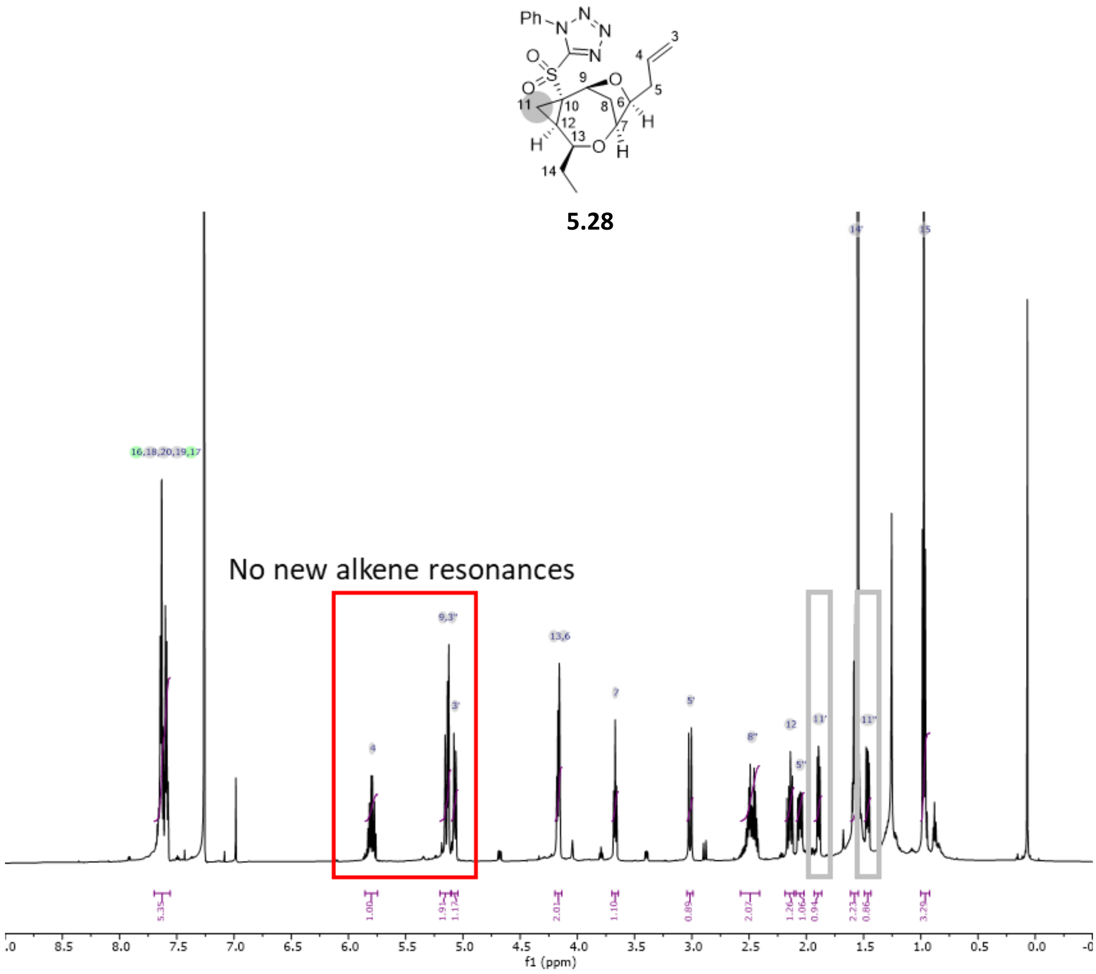
The identity of cyclopropane **5.28** was confirmed by its analogy to the respective compound from previous projects along with analysis of the spectral data. Loss of bromide was confirmed by examination of the HRMS spectrum obtained. HRMS *m/z* (ESI$^+$) was found for 417.1591 ($C_{20}H_{25}N_4O_4S$, the M+H$^+$ adduct for the cyclopropane requires 417.1591) with no bromide isotope pattern observed (Figure 5.3.10).


Measured Spectrum

Theoretical Spectrum

Figure 5.3.10: Measured and theoretical HRMS *m/z* (ESI$^+$) spectra for cyclopropane **5.28**.

This loss of bromide was corroborated by the loss of the ^{13}C resonance at δ 56.4 ppm observed for the sulfone **5.27** previously. Similarly, the lack of additional resonances in the alkene region indicated that the desired elimination had not occurred (Figure 5.3.11). The presence of new resonances at δ 1.46 ppm and δ 1.87 ppm, bound to the same carbon provided further evidence for the formation of a cyclopropane through loss of bromide.



5.28

Figure 5.3.11: ^{1}H NMR spectrum (600 MHz, $CDCl_3$) for cyclopropane **5.28** showing no new alkene resonances (red box) and the presence of new diastereotopic alkyl C-H resonances (grey boxes).

Similarly to previous examples, the connectivity of the cyclopropane could be established through analysis of the ^{1}H^{1}H COSY spectrum beginning again from the CH-3, CH-3' and CH-15 protons (δ 5.14, 5.07 and 0.97 ppm) due to their distinctive chemical shifts (Figure 5.3.12). From these protons, their respective ^{1}H^{1}H COSY correlations enabled the remainder of the ^{1}H NMR spectrum to be assigned.

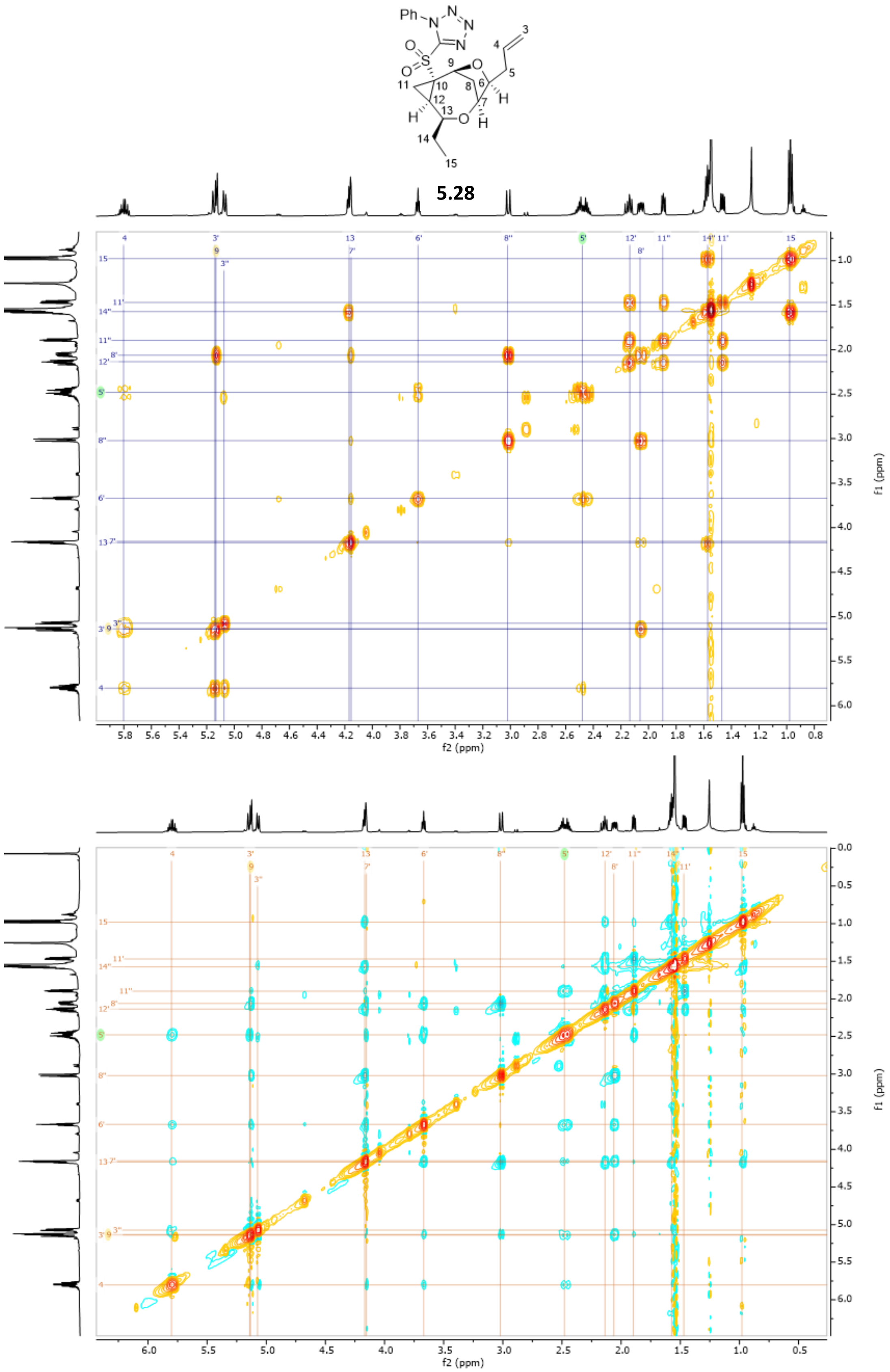


Figure 5.3.12: ^{1}H^{1}COSY and ^{1}H^{1}H NOESY spectra (600 MHz, CDCl$_3$) for cyclopropane **5.28**.

Additionally, a number of $^1H^1H$ NOESY correlations help to support the proposed structure (Figure 5.3.12). The correlation between CH-11 to CH-15 and CH-5 demonstrates that the cyclopropane is on the same face of the molecule as the ethyl group and the allyl group as shown. Similarly, the correlation between CH-6 and CH-8 also provides evidence to support the proposed structure.

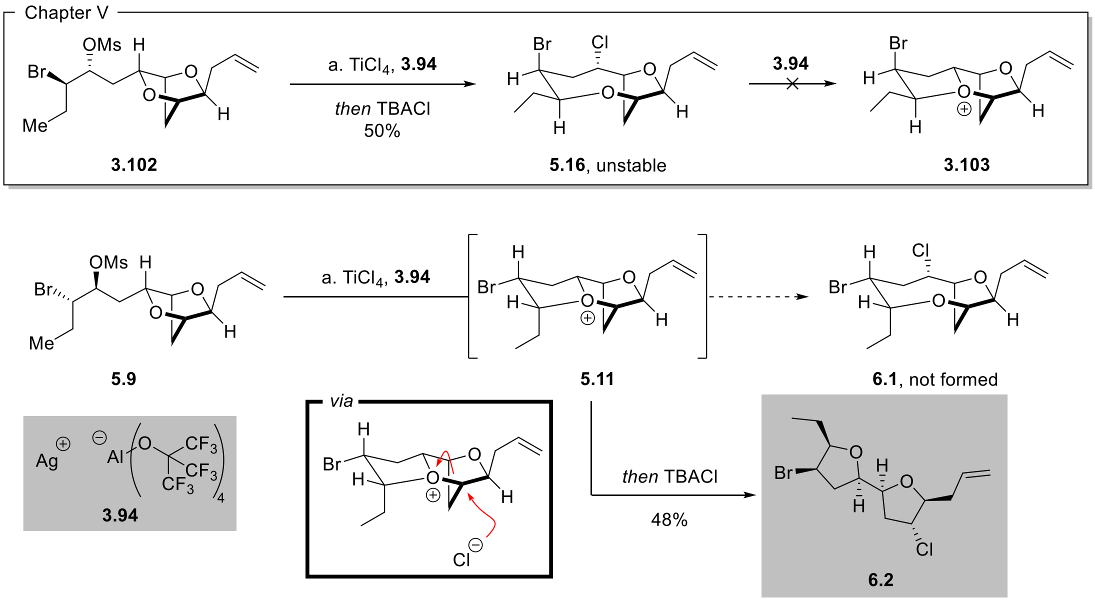
While the inherent reactivity of the system appeared to disfavour the desired elimination, accessing enough material to conduct optimisation of the elimination methodology also proved to be particularly challenging. This was due to the low yields and small maximum scale (50 mg of starting material) of the oxonium ion formation reactions and the large quantities of silver(I) salt **3.94** (shown previously) required to effect the transformation.

With this in mind, it was necessary to take steps to carry out the rearrangement under alternative reaction conditions with the aim of gaining access to a larger quantity of material, post-cyclisation, to attempt to push forward with the remaining steps of the synthesis. Although this was set to be the main focus of work going forward, observing the behaviour of the minor bromomesylate diastereoisomer **5.9** under the established cyclisation conditions had thus far been neglected. Elucidating the reactivity of the minor diastereoisomer, and the efforts to move away from the silver(I)-mediated cyclisation to allow the formation of oxonium ions form the bulk of the upcoming and final chapter.

6. Development of a Silver(I)-Free Cyclisation Protocol

6.1. Silver(I)-Mediated Cyclisation of the Minor Diastereoisomer

Given the inability to directly study the oxonium ion **3.103** (see 5.2 Reactivity of [2.2.1]-Dioxabicycloheptane System and Initial Silver(I)-Mediated Cyclisation Results) of interest due to the instability of C-10 chloride **5.16**, it was hoped that the cyclisation of the minor bromomesylate diastereoisomer **5.9** would give a more stable C-10 chloride **6.1** *via* oxonium ion **5.11**. If successful, then analysis of the closely related oxonium ion species **5.11** rather than the desired oxonium ion **3.103** could be conducted (Scheme 6.1.1).



Scheme 6.1.1: Attempts to directly study oxonium ions derived from the major bromomesylate diastereoisomer **3.103** and the minor bromomesylate **5.9** instead. Reagents and conditions: a) titanium tetrachloride (2.0 equiv.), **3.94** (3.2 equiv.), CH_2Cl_2, -40 °C, 2 h *then* TBACl (10 equiv.), -78 °C, 1 h, 50% (C-10 product **5.16** from **3.102**), 48% (C-7 product **6.2** from **5.9**).

Bromomesylate **5.9** was subjected to standard cyclisation conditions with titanium tetrachloride and silver(I) salt **3.94**. Pleasingly, the product isolated from this reaction was found to be stable, with the C-7 quench pathway dominating over quenching at C-10 leading to formation of bis-THF **6.2** rather than C-10 chloride **6.1**. This unexpected result not only required validation, but also rationalisation.

Given the presence of HRMS (ESI$^+$) *m/z* peaks at 345.0227 and 347.0204 ($C_{13}H_{20}{}^{79}BrClO_2Na$ and $C_{13}H_{20}{}^{81}BrClO_2Na$, the product sodium adducts, require *m/z* 345.0227 and 347.0205 respectively) a chloride quench product was the suspected product. In addition to this, the correct isotope pattern for a compound bearing both a bromide and chloride was observed (Figure 6.1.1).

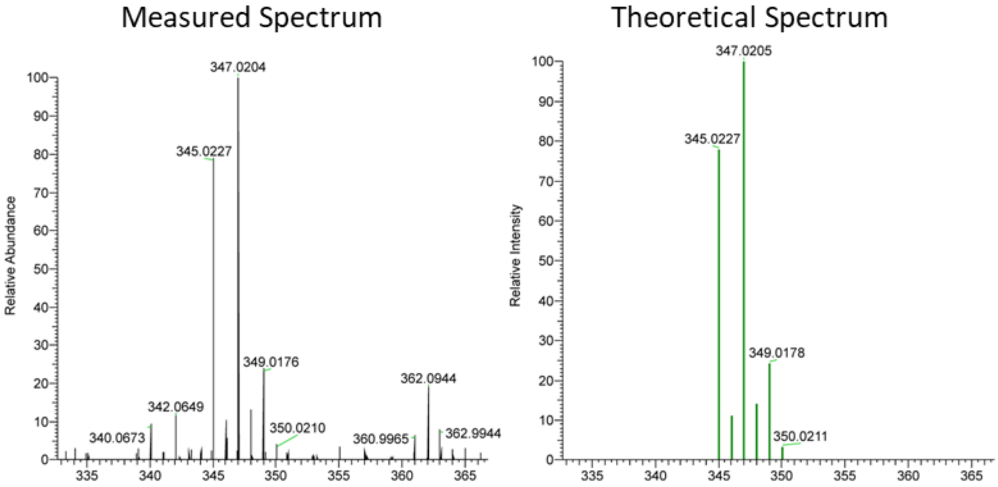
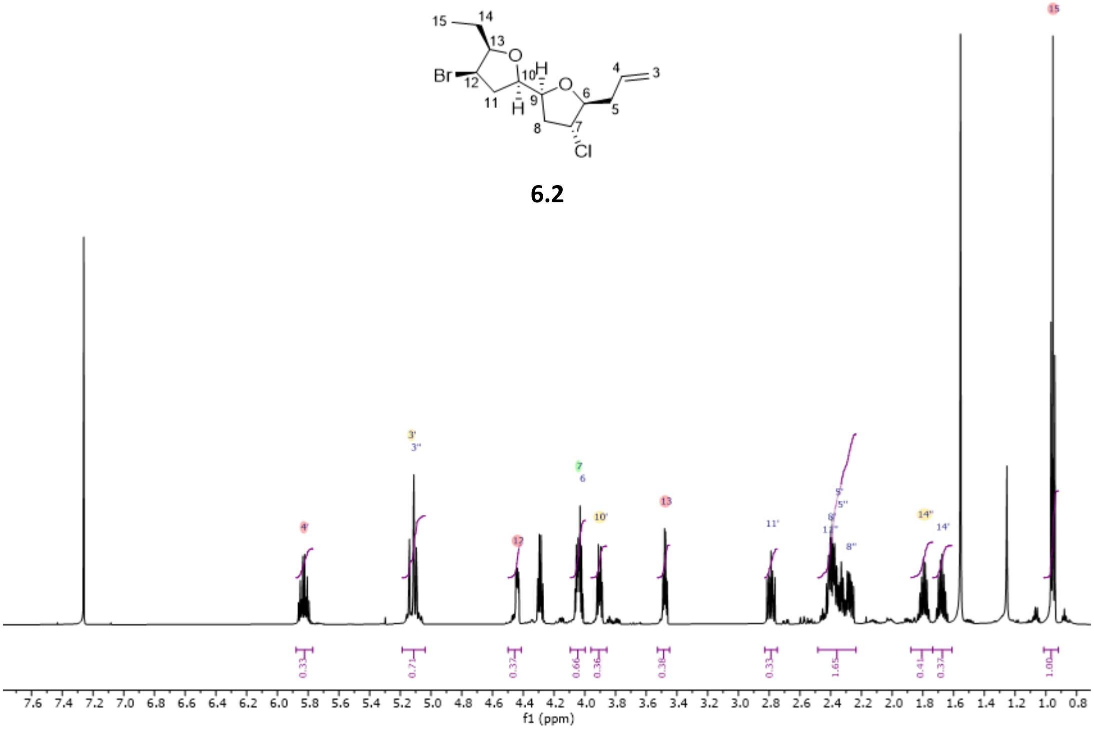


Figure 6.1.1: Measured high resolution mass spectrum (ESI$^+$) of **6.2** and theoretical high resolution mass spectrum (ESI$^+$) of **6.2**.

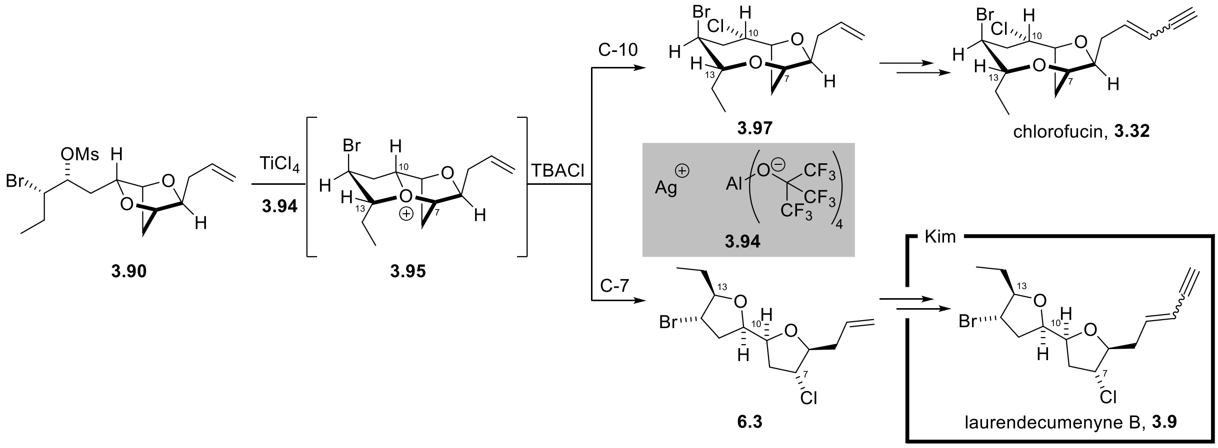
Assignment of the ^{1}H NMR spectrum of the bis-THF species **6.2** with aid from the ^{1}H^{1}H COSY and ^{1}H^{13}C HSQC NMR spectra enabled the connectivity of the compound to be established. From the ^{1}H NMR of the product it was clear that the product was not a [2.2.1]-dioxabicycloheptane as it lacked the distinctive broad singlet resonances at 4.18 and 4.47 ppm for the C-7 and C-9 protons observed previously in the C-10 chloride **5.16** and C-10 alcohol **3.104** (Figure 6.1.2).



6.2

Figure 6.1.2: ^{1}H NMR spectrum of chloride quench product **6.2**.

The key to assigning a bis-THF structure to the product over the C-10 [5.2.1]-dioxabicyclodecane and the C-13 [2.2.1]-dioxabicycloheptane structures comes from unambiguous assignment of the chlorine bearing carbon. Furthermore, it has been assumed that the relative configuration of the bis-THF is as shown in keeping with the likely mechanism of this transformation and previous precedent. For example, Chan demonstrated, that on exposure of the mesylate **3.90** to titanium tetrachloride and silver(I) salt **3.94** followed by addition of tetrabutylammonium chloride gave previously reported bis-THF **6.3** and the [5.2.1]-dioxabicyclodecane **3.97** in about 1:7 ratio (Scheme 6.1.2).[102] The structure and configuration of **3.97** was proven by conversion to chlorofucin **3.32**, with the structure and configuration of the bis-THF **6.3** being demonstrated by Kim in related work through the synthesis of the natural product laurendecumenyne B **3.9**.[107]



Scheme 6.1.2: Chan synthesis of [5.2.1]-dioxabicyclodecane **3.97** and bis-THF **6.3** and derivatisation into chlorofucin **3.32** by Chan *et al*. and bis-THF structure validation by Kim.[102,107]

The product **6.2** from the bromomesylate **5.9** of interest had two resonances in the ^{13}C NMR at 59.3 and 53.1 ppm which, on the basis or chemical shift correspond to chlorine and bromine-bearing carbon atoms. The resonance at 59.3 ppm was unambiguously assigned as the chlorine bearing carbon as it was possible to observe the separate ^{13}C NMR resonances of the carbon attached to ^{35}Cl and ^{37}Cl. These resonances were in a 3:1 ratio and separated by 9 ppb which is a typical value for an sp^3-hybridised carbon bound to a chlorine atom (Figure 6.1.3).[181]

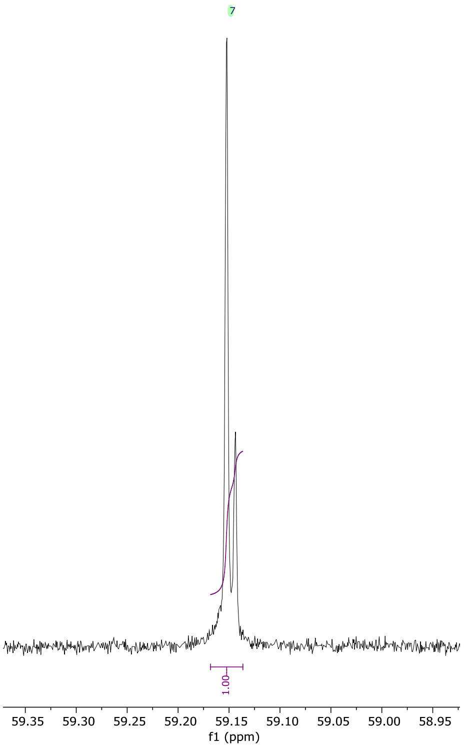


Figure 6.1.3: High resolution ^{13}C NMR showing 3:1 splitting pattern expected for a chlorine-bearing carbon (151 MHz, $CDCl_3$).

Having unambiguously assigned the chlorine-bearing carbon and from inspection of the ^{1}H-^{13}C HSQC, the proton on that carbon; it was clear from the ^{1}H-^{1}H COSY spectrum that the chlorine atom was not bonded to C-10 or to C-13 but was bonded to C-7 as would be the case for a bis-THF structure **6.2**. Additionally all of the ^{1}H and ^{13}C NMR data were in keeping with a bis-THF structure. Previously the Burton group have reported a general trend that for bis-THF structures, the central oxygen bearing carbons resonate at >76 ppm.[182,183] For the bis-THF reported the C-9 and C-10 carbons resonate at 79.9 and 79.7 ppm in keeping with this correlation.

Furthermore, the ^{1}H^{1}H NOESY spectrum showed a number of strong NOE correlations, H-13 ↔ H-12, H-13 ↔ H-10 - in keeping with the expected configuration of the bromine-containing THF ring. Given the partial overlapping nature of the ^{1}H NMR resonances for H-6 and H-7 along with their closeness in chemical shift to H-9 and H-10 it was not possible to tell whether their were any NOEs between H-6 and H-9 or H-7 and H-10 which would be in keeping with the expected configuration (Figure 6.1.4).

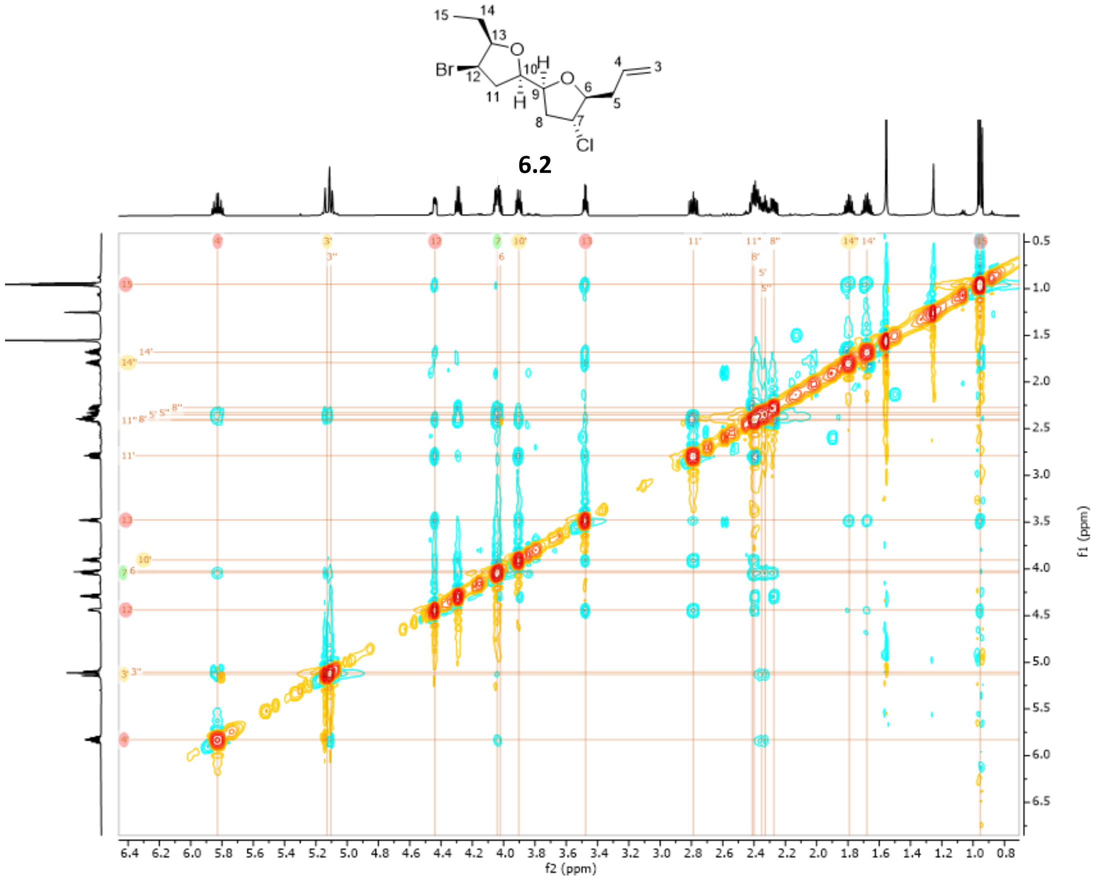


Figure 6.1.4: ^{1}H^{1}H NOE spectrum (600 MHz, $CDCl_3$) for bis-THF **6.2**.

The use of NOE correlations to assign the relative configuration in 5-membered rings is prone to error as it is possible to see NOE correlations between both vicinal-*cis* protons and vicinal-*trans* protons on account of the high degree of conformational flexibility and low energy barrier to interconversion of the respective envelope conformers, hence, the above analysis should be viewed with caution.[184] It is however clear that there is no NOE correlation between H-14 and H-8 and if the compound were C-10 product **6.1** an NOE correlation between C-14 and one of the H-8 protons would be expected. Further evidence of the bis-THF nature of **6.2** came from EI mass spectrometry which showed *m/z* peaks at 176.99198 and 178.99081 with $C_6H_{10}{}^{79}BrO^+$ and $C_6H_{10}{}^{81}BrO^+$ requiring *m/z* 176.9910 and 178.9980 respectively, indicative of a bis-THF with fragmentation of the central C-C bond under EI conditions (Figure 6.1.5).[103,183]

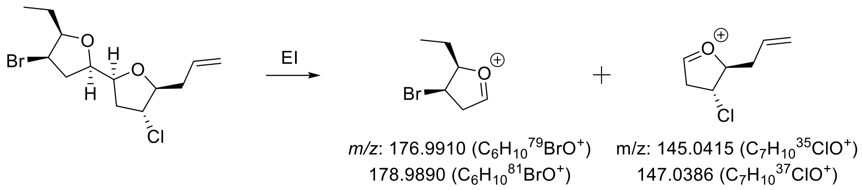


Figure 6.1.5: EI mass spectrum fragments giving evidence for bis-THF **6.2** on account of peaks corresponding to bromide-containing oxocarbenium fragment.

A similar chlorobromo-bis-THF to **6.2** had previously been synthesised in the group en route to a total synthesis of notoryne **3.10** and has been named "notorene" **6.4** (Figure 6.1.6).[102] The ^{13}C NMR chemical shifts of the chlorine-containing THF and side chain (C-3 to C-9) are virtually identical to those of the bis-THF **6.2** providing further evidence to support the assigned configuration; similarly the chlorine containing THF of chlorobromo-bis-THF **6.3** prepared by Kim and in the Burton group had ^{13}C NMR chemical close in value to those of the bis-THF **6.2** for C-3 to C-9.[107]

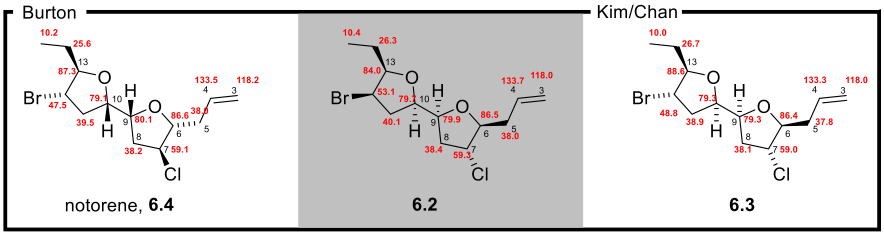
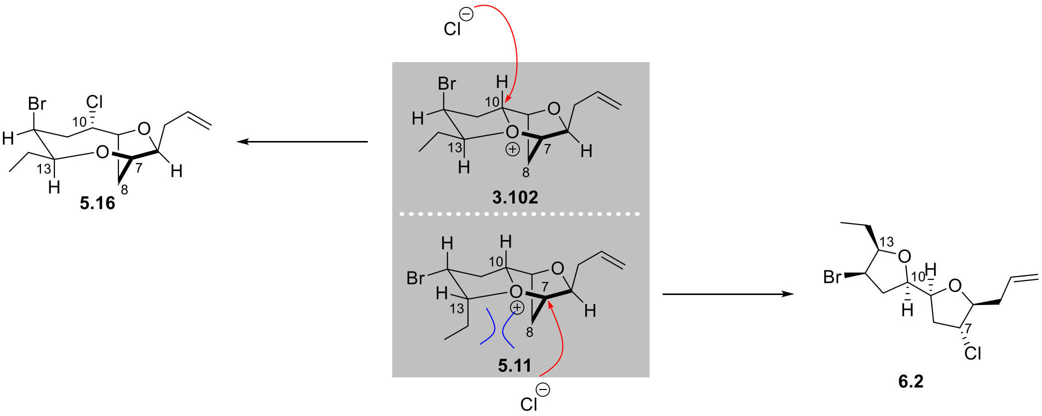


Figure 6.1.6: ^{13}C NMR shift comparison between bis-THF **6.2** and similar known compounds notorene **6.4** and bis-THF **6.3**.

Considering the proposed structures of the oxonium ions **3.103** and **5.11** formed from the cyclisation of the major and minor diastereoisomers of the bromomesylates **3.102** and **5.9**, a tentative explanation for the preference of the C-7 product **6.2** from the minor diastereoisomer **5.9** can be offered (Scheme 6.1.3). In the case of the major diastereoisomer **3.102**, there is little strain between H-8 and H-14, since the latter is likely in an equatorial position (as shown by the CH-13 ↔ CH-8 NOE correlation shown in previous examples). Conversely, in the minor diastereoisomer **5.9**, the ethyl substituent probably sits axially and on the same side of the ring as H-8. This conformation, twinned with the bromide also being on the *endo* face of the molecule could be expected to generate transannular strain. Although C-10 quenching may occur in the first instance, this is reversible and given the strain release upon C-10 quench being minimal likely reforms the oxonium **5.11**. In order to alleviate this strain, another possibility is that the (C-7)–O^+ bond length increases, thus weakening the C-7 – O^+ bond and increasing the rate of C-7 opening causing the C-7 product to dominate. Since the C-7 quench is likely irreversible by virtue of the entropic freedom gained during this process and the release of strain (enthalpy) in breaking the [2.2.1]-bicyclic structure, the C-7 product **6.2** is isolated as the major product.



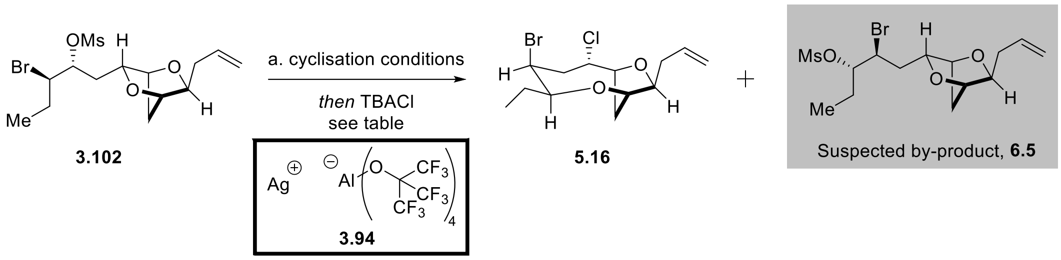
Scheme 6.1.3: Proposed mechanistic rationale for the different outcomes observed from oxonium ion quenching, leading to the formation of C-10 and C-7 products **5.16** and **6.2** respectively.

As a result of the unexpected reactivity of the minor diastereoisomer, the ability to directly study the oxonium ion of interest remained out of reach. Regenerating the oxonium intermediate from the bis-THF product **6.2** would be energetically unfavourable both enthalpically and entropically. Recognising that with the current protocol study of the oxonium ions of interest was not possible, attention was instead diverted to discovering an alternative procedure for accessing the C-10 quench products.

6.2. Initial Alternative Cyclisation Conditions

It was hoped that an alternative to silver(I) mediated cyclisation could be found. Although the silver salt **3.94** (shown previously) could enable the desired transformation to form the oxonium ions for quenching with various nucleophiles, it was accompanied by a number of practical problems. Primarily, the high cost of silver(I) salt **3.94** and excess equivalents of the reagent required for the cyclisation severely impacted material throughput. Secondly, there were scalability issues, with the solubility of the silver salt generating mixing problems; carrying out the cyclisation on a larger scale than 0.1 mmol with respect to the bromomesylates **3.102** and **5.9** led to poor yields. Ultimately, although the silver(I)-mediated cyclisation proved viable on small scale, it was not suitable for the preparing enough material in this case to advance the total synthesis.

Efforts to move away from silver(I) salt **3.94** for the cyclisation naturally began with an evaluation of commercially available silver salts that could also potentially promote the desired transformation (Table 6.2.1).

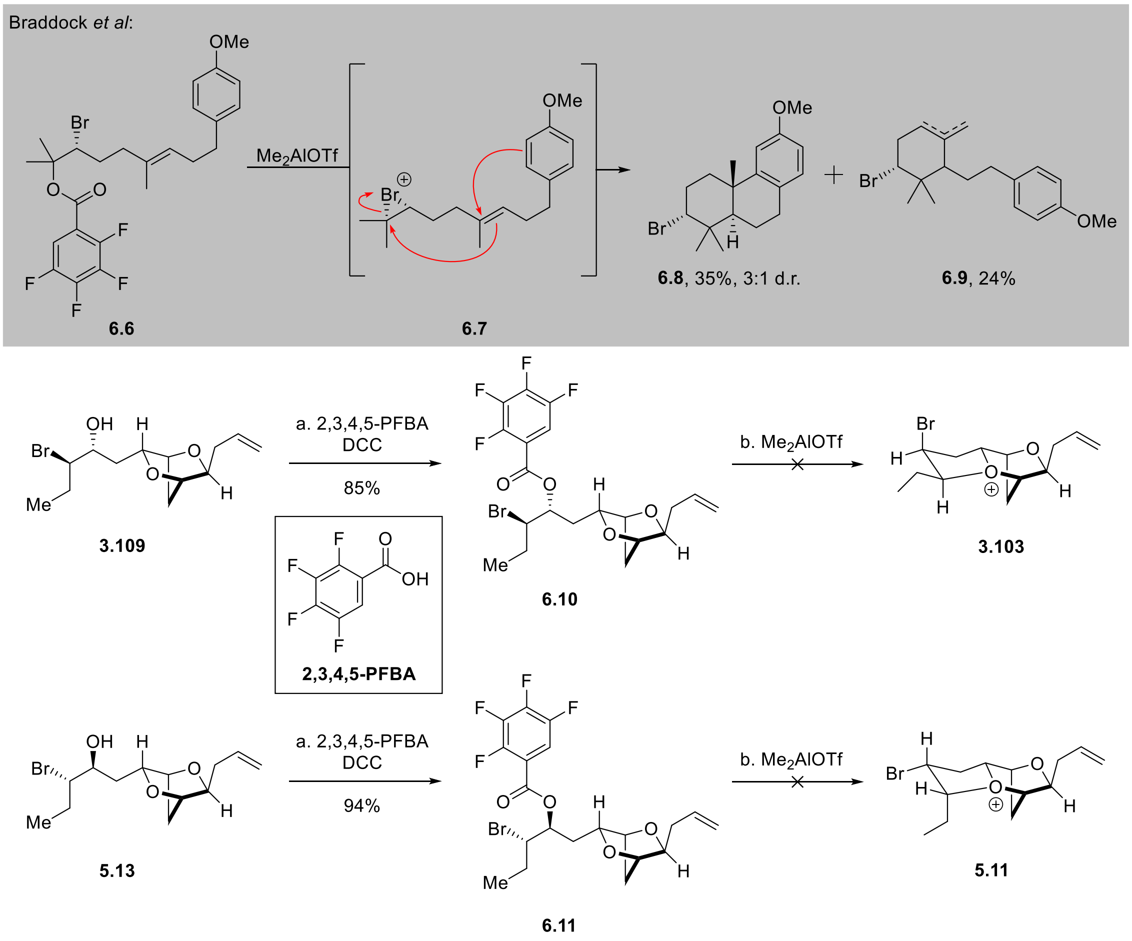


Entry	Lewis Acid	Silver source	Recovered SM/ %	**5.16**/ %	By-product/ %
1[a]	$TiCl_4$	$AgBF_4$	n.d.	n.d.	n.d.
2	$TiCl_4$	$AgSbF_6$	58	14	8
3[b]	$AlMe_3$	-	n.d.	n.d.	n.d.

Table 6.2.1: Initial attempts to move away from the silver(I)-mediated cyclisation protocol. [a]Reaction not conducted due to insolubility of silver source. [b]TLC analysis showed inseparable mixture of SM and by-product, reaction abandoned. Reagents and conditions: a) lewis acid (2.0 equiv.), silver source (3.2 equiv.), CH_2Cl_2, -40 °C, 2 h *then* TBACl (10 equiv.), -78 °C, 1 h, see table.

In the first case, silver(I) tetrafluoroborate was trialled, however the salt proved to be completely insoluble in CH_2Cl_2 (entry 1). Fortunately, silver(I) hexafluoroantimonate was found to be soluble in CH_2Cl_2, however after addition of this salt to the reaction mixture, only a low yield of the desired compound was isolated (entry 2). This was accompanied by the suspected formation of a respective bromomesylate **6.5** formed by C-13 quenching of the bromonium by the mesylate anion in low yield. Unfortunately, it was not possible to isolate this by-product cleanly from the starting material for further characterisation. However the presence of a singlet resonance corresponding to mesylate in the crude ^{1}H NMR spectrum and similarity of the by-product to the starting material led to this as the proposed structure of by-product **6.5**. The large amount of starting material returned from this reaction indicated an incompatibility of the silver(I) hexafluoroantimonate and titanium tetrachloride. In an attempt to try and enable the cyclisation in the absence of a silver(I) species altogether, trimethylaluminium was trialled as a Lewis acid (entry 3). It was hoped that if the bromonium ion could form, then subsequent intramolecular cyclisation to generate the oxonium would follow. Disappointingly, this was not observed.

Continuing on this tangent, inspiration was drawn from the work of Braddock and co-workers. In this work, a polyene cyclisation was triggered *via* the enantiopure bromonium ion **6.7** generated by the treatment of α-bromo-tetrafluorobenzoate ester **6.6** with dimethylaluminium triflate. This gave the tricyclic compound **6.8** and the partially cyclised compound **6.9** associated with a lack of cyclisation involving the aromatic ring.[185,186] It was hoped that bromohydrins **3.109** and **5.13** of interest would be amenable bromonium ion precursors after derivatisation into their respective tetrafluorobenzoates **6.10** and **6.11** under these conditions, facilitating access to the desired oxonium ions **3.103** and **5.11** (Scheme 6.2.1).

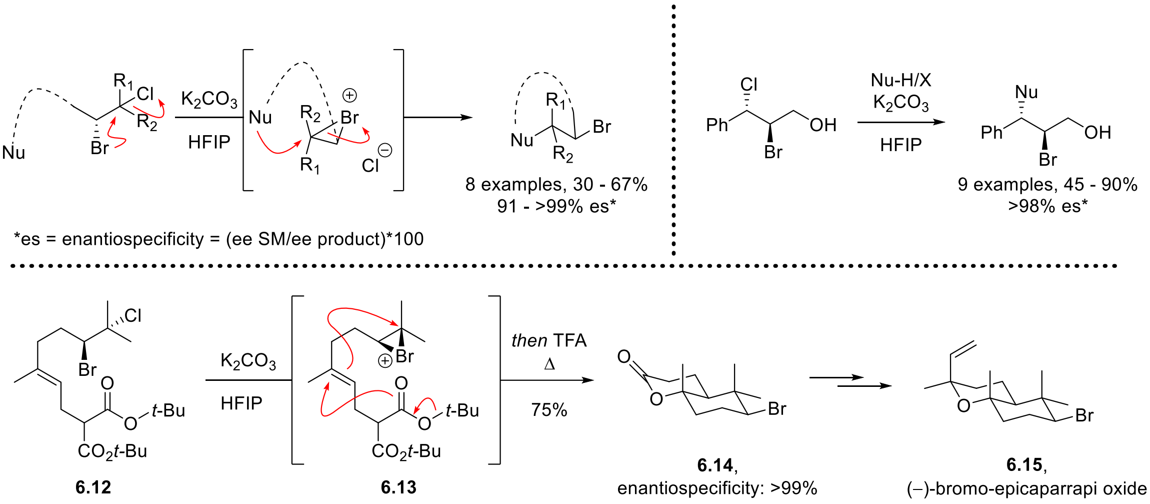


Scheme 6.2.1: Polyene cyclisation by Braddock *et al*. from α-bromo tetrafluorobenzoate esters. Synthesis of tetrafluorobenzoate esters **6.10**, **6.11** and subsequent non-reactivity under the Braddock conditions. Reagents and conditions: a) 2,3,4,5-tetrafluorobenzoic acid (2.5 equiv.), DCC (2.5 equiv.), DMAP (2.5 equiv.), CH_2Cl_2, rt, 18 h, 85% (major

diastereoisomer **6.10**), 94% (minor diastereoisomer **6.11**); b) dimethylaluminium triflate (3.0 equiv.), CH_2Cl_2, -78 °C – rt, 18 h, SM not consumed.

The initial synthesis of the tetrafluorobenzoate esters **6.10** and **6.11** proceeded in excellent yields. Disappointingly, neither of these compounds underwent the proposed cyclisation, with starting material being recovered in both cases. Even at room temperature, no reactivity was observed with dimethylaluminium triflate. As Braddock and co-workers allude to in their work, the right combination of Lewis acid and leaving group are crucial to enabling these transformations. Potentially, the right combination could be found for the system of interest, however with limited time remaining other avenues for bromonium ion formation were investigated instead.

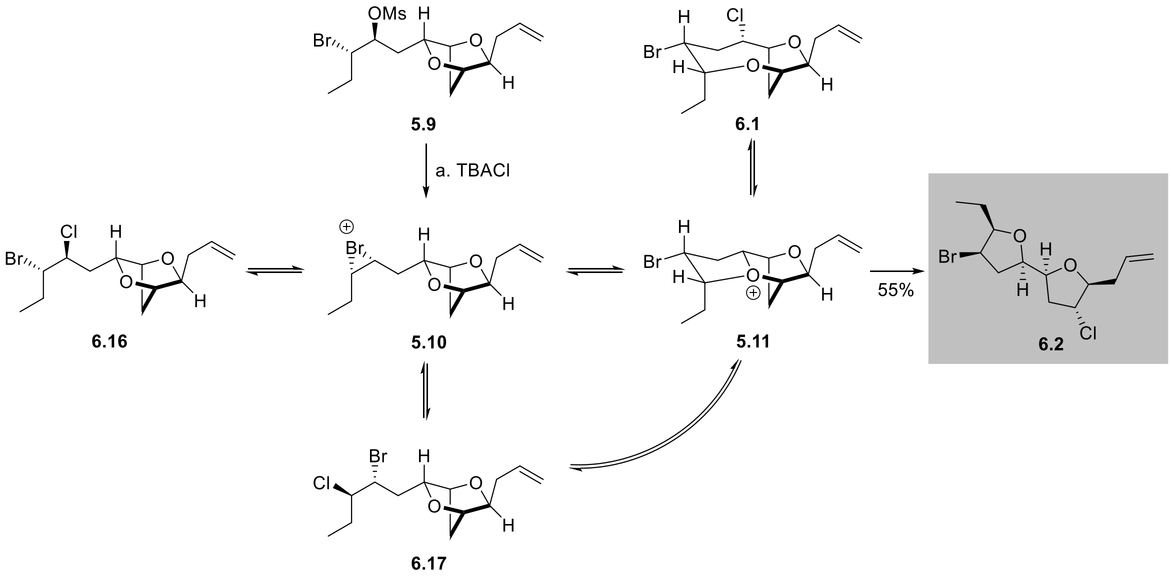
The discovery by Noah Burns *et al*.; that vicinal chlorobromides were able to undergo bromonium ion formation and subsequent trapping, both *via* intramolecular and exogenous nucleophiles, under very mild conditions, generated interest for the potential use on the desired system.[187] Burns found that a variety of substrates could be treated with potassium carbonate in hexafluoroisopropanol - known to be excellent at stabilising cationic intermediates - generating enantiopure bromonium ions *in situ*, which upon nucleophilic quenching often gave products in excellent ee (Scheme 6.2.2).[188] The methodology had also been demonstrated on scale, allowing access to (–)-bromo-epicaparrapi oxide **6.15** from vicinal bromochloride **6.12**. Treatment of vicinal bromochloride **6.12** with potassium carbonate allowed access to fused lactone **6.14** *via* bromonium **6.13** which was subsequently derivatised into (–)-bromo-epicarrapi oxide **6.15**. This appeared to be a promising alternative to the silver(I)-mediated cyclisation employed up until now.



Scheme 6.2.2: Summary of Noah Burns *et al*. HFIP mediated bromonium ion formation and trapping/cyclisation.

Despite the use of vicinal chlorobromides in the literature, it was envisioned that bromomesylates **3.102** and **5.9** might be amenable to this chemistry. In the first instance, minor diastereoisomer **5.9** was subjected to slightly modified conditions with a view to forming bis-THF **6.2** *via* bromonium ion **5.10** (Scheme 6.2.3). Rather than using potassium carbonate, it was hypothesised that addition of TBACl would enable chloride quenching of any transient oxonium ion **5.11** formed. In previous oxonium ion quenching experiments the minor diastereoisomer **5.9** had been shown to exclusively form the thermodynamically stable C-7 chloride quench product **6.2**.

As formation of the bis-THF product is highly likely to be an irreversible process from the oxonium quench at C-7, with the preceding steps all being reversible, the minor diastereoisomer was selected as a reasonable starting point for investigation and the bis-THF **6.2** was expected to be the sole product of the reaction (Scheme 6.2.3). The proposed mechanism for the formation of C-7 chloride **6.2** is based on the high probability that the C-13, C-12 and C-10 chloride quenched oxonium/bromonium products **6.16**, **6.17** and **6.1** respectively would all be able to reform the oxonium ion **5.11**. In theory this would enable all of the material to be shuttled through to the C-7 product **6.2**. As noted by Burns, one of the interesting features of this methodology is that if the bromonium ion is reopened by chloride at either end, bromonium formation with loss of chloride will lead to the same bromonium ion intermediate. Hence, the bromochlorides serve as non-racemising enantioenriched bromonium ion surrogates.[187]



Scheme 6.2.3: Proposed HFIP-mediated rearrangement of minor diastereoisomer **5.9** leading to bis-THF **6.2.** Reagents and conditions: a) TBACl (1.2 equiv.), hexafluoroisopropanol, rt, 48 h, 55%.

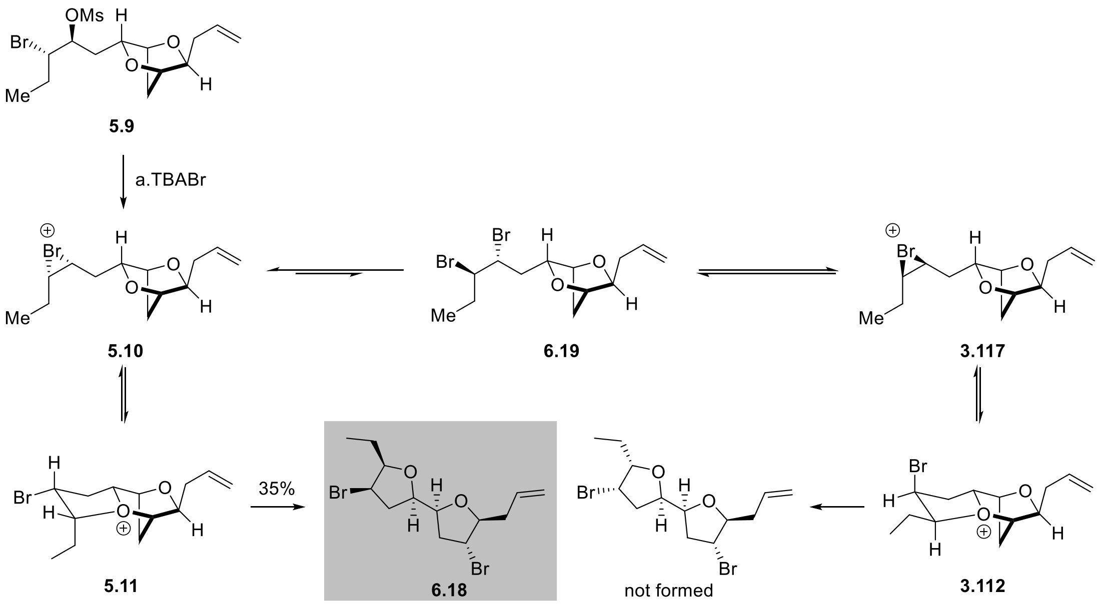
In the above case, bromonium ion formation from bromomesylate **5.9** would occur in HFIP to give bromonium ion **5.10**, which would be trapped by chloride reversibly to give **6.16** and **6.17** or by the oxygen atom of the [2.2.1]-bicycloheptane to give oxonium ion **5.11**. This oxonium ion would either be trapped at C-10 reversibly to give **6.1** or C-7 irreversibly leading ultimately to bis-THF **6.2**.

Pleasingly, bromomesylate **5.9** was tolerated by these reaction conditions, with the expected bis-THF product **6.2** being formed in comparable yield to the silver(I) mediated cyclisation and with identical data to the previously employed conditions. Presumably, the formation of the bromonium and oxonium ions are reversible processes and a low concentration of the oxonium is maintained. Likewise, quenching by chloride at the C-10 of the oxonium ion **5.11** could also be envisaged to be reversible, with the C-7 chloride acting as a thermodynamic sink; from previous work on related [5.2.1]-dioxabicyclic oxonium ions, opening of the oxonium ion at C-13 is highly unlikely.[102,106] The use of HFIP as the solvent for this reaction was instrumental for enabling this rearrangement to proceed; switching to an alternative fluorinated solvent, trifluoroethanol, led to the formation of an intractable mixture.[188]

With this promising initial result, it was hoped that the scope of the HFIP mediated rearrangement could be expanded in order to enable other cyclisation products to be accessed. The final section of this chapter details efforts to expand the HFIP rearrangement and the synthesis of *ent*-(*E*)-notoryne **(*E*)-3.8** and laurendecumenyne B **3.9** from related bromomesylates (not shown).

6.3. Expansion of the HFIP Rearrangement and Synthesis of *ent*-(*E*)-Notoryne and Laurendecumenyne B

To expand the scope of the rearrangement with HFIP, and to provide further evidence to support the mechanism of the reaction, it was decided to test the quenching protocol with other nucleophiles. The first obvious change to make was the use of bromide for quenching. Initially, it was unknown whether this would result in a single diastereoisomer of the C-7 bromide **6.18** being formed (Scheme 6.3.1).



Scheme 6.3.1: Proposed mechanism for the potential scrambling bromide stereochemistry during the course of the HFIP rearrangement with tetrabutylammonium bromide and isolation of single diastereoisomer **6.18** *via* this protocol. Reagents and conditions: a) TBABr (1.2 equiv.), hexafluoroisopropanol, rt, 48 h, 35%.

Unlike in the chloride case, where the bromonium ion stereochemistry is retained by the preferential loss of chloride versus bromide. When using bromide as the external nucleophile both bromonium intermediates **5.10** and **3.117** could be envisaged forming *via* vicinal dibromide **6.19** and thus access to both diastereomers of the oxonium ion **5.11** and **3.112** could be possible. The effect of this would be formation of products corresponding to the C-7 quench of the major and minor diastereomers of the cyclisation precursors in the same pot.

Pleasingly however, exposure of the bromomesylate **5.9** to TBABr in HFIP led to the formation of the bis-THF **6.18** which was isolated as a single diastereoisomer in 35% yield. Assignment of the structure of the dibromide **6.18** followed a similar course to that of bis-THF **6.2**. The mass spectrum of **6.18**, had the measured mass corresponding to the molecular formula for the sodium adduct of the product ($C_{13}H_{20}{}^{79}Br^{79}BrO_2Na$ requires 388.9722, $C_{13}H_{20}{}^{79}Br^{81}BrO_2Na^+$ requires 390.9702 and $C_{13}H_{20}{}^{81}Br^{81}BrO_2Na$ requires 388.9682) and the isotope distribution corresponding to two bromine atoms (Figure 6.3.1).

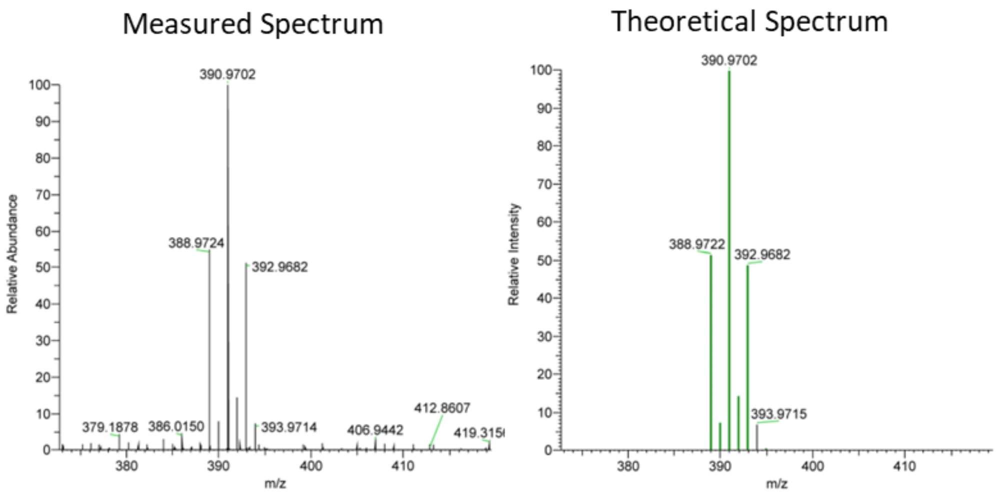


Figure 6.3.1: Measured and theoretical HRMS (ESI$^+$) spectra for dibromide **6.18**.

As with the chlorobromo bis-THF, it was clear from the ^{1}H NMR of the product that it was not a [2.2.1]-dioxabicycloheptane as it lacked the distinctive broad singlet resonances at 4.18 and 4.47 ppm for the C-7 and C-9 protons (Figure 6.3.2). The key to assigning the structure as a bis-THF rather than the [5.2.1]-dioxabicyclodecane and the C-13 [2.2.1]-dioxabicycloheptane structures came from assignment of the bromine bearing carbon atoms in this case. Furthermore, it has been assumed that the relative configuration of the bis-THF is as shown in keeping with the likely mechanism of this transformation and previous precedent.

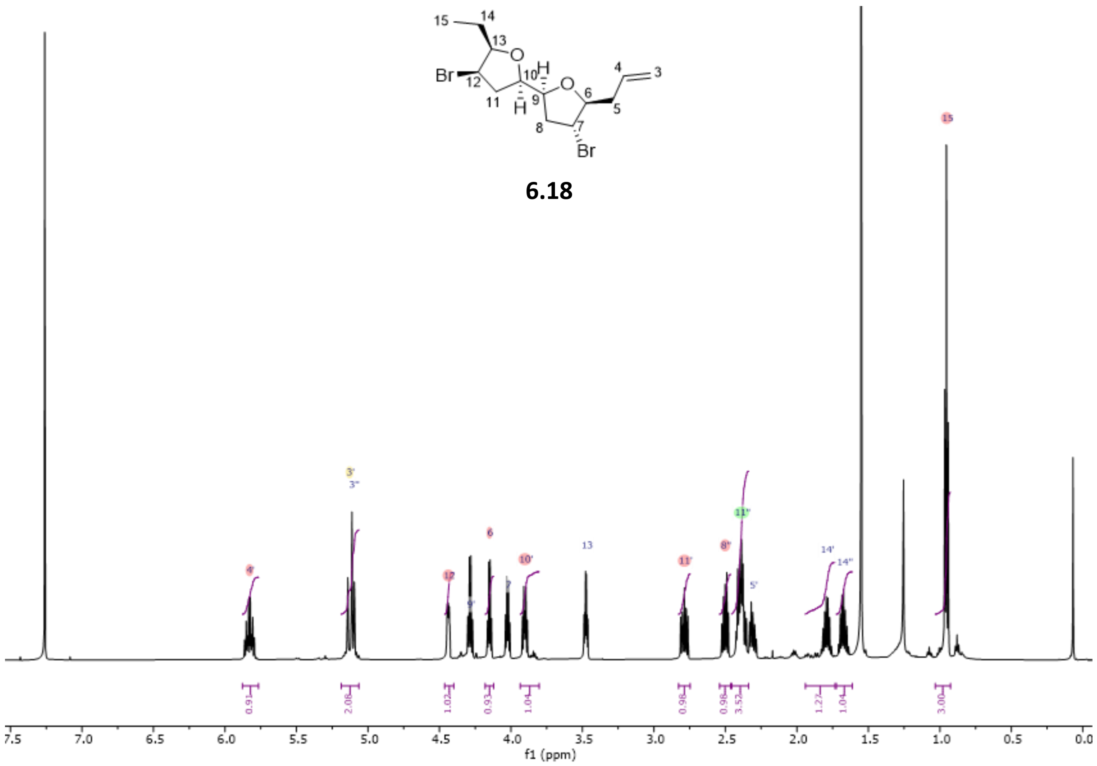


Figure 6.3.2: ^1H NMR spectrum (600 MHz, $CDCl_3$) of bis-THF **6.18**.

The product **6.18** from the bromomesylate **5.9** had two resonances in the ^{13}C NMR at 53.1 and 48.6 ppm which, on the basis or chemical shift correspond to the two bromine-bearing carbon atoms. The resonance at 53.1 ppm was readily assigned to C-12 and that at 48.6 ppm to C-7 from unambiguous ^1H-^1H COSY correlations and ^1H-^{13}C HSQC correlations. These assignments of the bromine bearing carbon atoms ruled out either of the dioxabicyclic structures. Additionally all of the ^1H and ^{13}C NMR data were in keeping with a bis-THF structure. Furthermore, the C-9 and C-10 carbons resonated at 80.0 and 79.6 ppm in keeping with a bis-THF structure.

Furthermore, the $^1H$$^1H$ NOESY spectrum showed a number of strong NOE correlations, H-13 ↔ H-12, H-13 ↔ H-10 - in keeping with the expected configuration of the bromine-containing ethyl-substituted THF ring **6.18** (Figure 6.3.3). Given the closeness of the resonances corresponding to H-6 and H-9 it was not possible to determine whether or not there was an NOE between these protons.

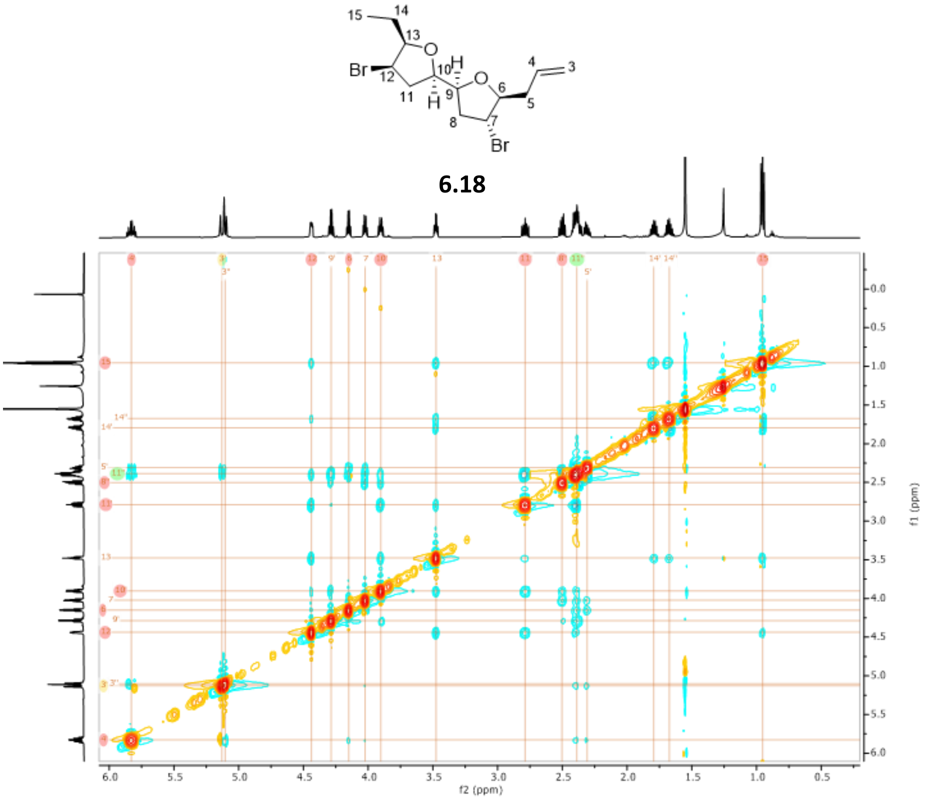

6.18

Figure 6.3.3: ^{1}H^{1}H NOE spectrum (600 MHz, $CDCl_3$) for bis-THF **6.18** showing key correlations.

As noted above, the use of NOE correlations to assign the relative configuration in 5-membered rings is prone to error and assignment of the configuration of the ethyl-bearing THF by NOE must be viewed with caution. It is however clear that there is no NOE correlation between H-14 and H-8 and if the compound were a C-10 quench product then this correlation between C-14 and one of the H-8 protons would be expected. The dibromobis-THF **6.18** has been assigned the configuration shown and, as such it would be expected that the ^{13}C NMR chemical shifts of the ethyl bearing THF would be very close to the ^{13}C NMR chemical shifts of the ethyl bearing THF ring of the bis-THF **6.2** (Figure 6.3.4). Indeed, the corresponding ^{13}C NMR resonances for C-15 to C-10 are within 0.1 ppm.

The dibromo-bis-THF **6.20** had previously been synthesised in the group and independently by Kim en-route to a total synthesis of elatenyne **3.31** and has been named "elatene".[107] This bis-THF has the same relative configuration for the allyl bearing THF ring as that assigned to the dibromobis-THF **6.18**. Here the corresponding ^{13}C NMR resonances for C-3 to C-9 are within 0.3 ppm (C-8 resonates at either 39.1 or 39.4 ppm). Given this chemical shift structure correlation between known bis-THFs and the two bis-THFs synthesised above, the configuration of the bis-THFs synthesised can be stated with a reasonable degree of confidence.

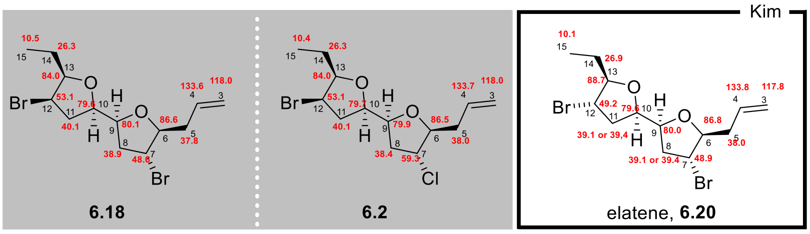
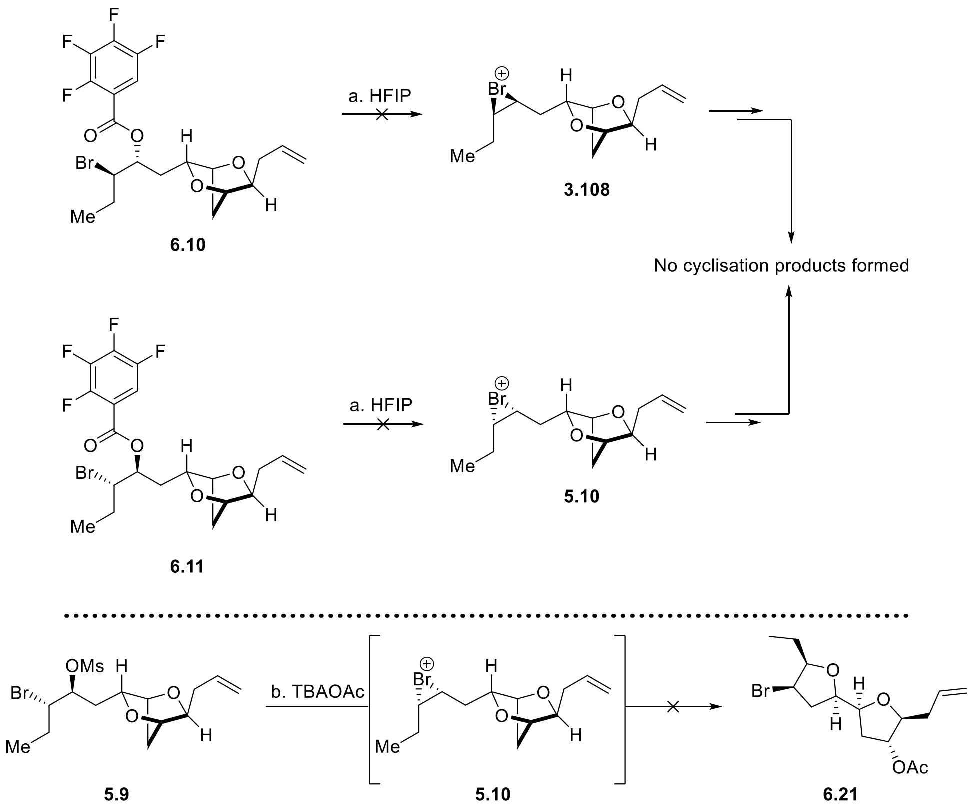


Figure 6.3.4: Comparison of ^{13}C NMR shifts of bis-THFs **6.18** and **6.2** and correlation to known bis-THF **6.20** synthesised by Kim.[107]

For the two cases of halide quenching so far, the C-7 products **6.2** and **6.18** had been exclusively formed from the minor bromomesylate diastereoisomer **5.9**. It had been hoped that the rearrangement itself could be expanded further, not only to the major diastereoisomer but also to incorporate quenching by oxygen nucleophiles.

As a first attempt, the Braddock tetrafluorobenzoates **6.10** and **6.11** were trialled to undergo this rearrangement. The carboxylate that would result from the formation of the bromonium ions **3.108** and **5.10**, was hoped to be nucleophilic enough to trap out the oxonium ion generated from the respective bromonium ions (Scheme 6.3.2). This however was not found to be the case and it is likely that the bromonium ions themselves were never formed, as starting material was returned in both cases.

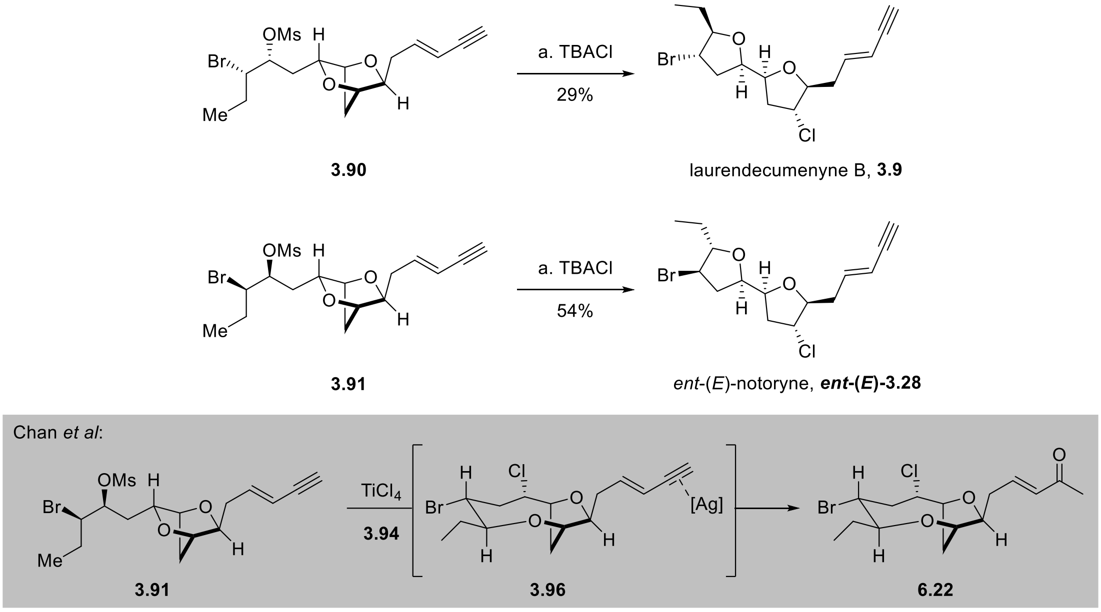


Scheme 6.3.2: Attempts to rearrange tetrafluorobenzoates **6.10** and **6.11** using HFIP (top). Attempted HFIP rearrangement of bromomesylate **5.9** with TBAOAc under the previously established protocol. Reagents and conditions: a) HFIP, rt, 48 h, no reaction; b) TBAOAc (1.2 equiv.), HFIP, rt, 48 h, intractable mixture formed.

Returning to the minor bromomesylate diastereoisomer **5.9** as the reaction substrate, TBAOAc was trialled as the quenching nucleophile. Unfortunately this led to the formation of an intractable mixture of uncharacterisable products.

The HFIP rearrangement was increasingly looking like a valuable tool for accessing oxonium-mediated-rearrangement products, albeit with the current limitation being quenching at the C-7 position with halide nucleophiles. With this in mind; it was expected that two natural products, *ent*-(*E*)-notoryne ***ent*-(*E*)-3.8** and laurendecumenyne B **3.9** could be accessed from their respective bromomesylates using the developed methodology.

Excitingly, the bromomesylates **3.90** and **3.91**, synthesised previously by Sam Chan, underwent smooth conversion to *ent*-(*E*)-notoryne ***ent*-(*E*)-3.28** and laurendecumenyne B **3.9** respectively (Scheme 6.3.3).[139] The value of the rearrangement protocol with HFIP cannot be overstated, as previously the bromomesylate was found to be incompatible with the silver(I)-mediated cyclisation on account of enyne reactivity leading to α-,β-unsaturated ketone **6.22**. This led to a much longer route being necessary for synthesis of the natural products themselves.



Scheme 6.3.3: Formation of *ent*-(*E*)-notoryne ***ent*-(*E*)-3.8** and laurendecumenyne **3.9** from their respective bromomesylates and the incompatibility of the bromomesylate **3.91** to the silver(I)-mediated cyclisation. Reagents and conditions: a) TBACl (1.2 equiv.), hexafluorisopropanol, rt, 48 h, (laurendecumenyne B, 29%), (*ent*-notoryne, 54%).

The NMR spectra of the natural products obtained by HFIP rearrangement are identical to the published data obtained *via* the alternative route with use of the silver(I)-mediated cyclisation. This observation lends further weight to the proposed reaction mechanism being analogous to that with silver(I) salt **3.94** (shown previously), at least in the case of chloride as the external nucleophile. Additional experiments are required to expand this method further and elucidate the mechanism for all of the cases presented in this chapter.

7. Conclusions and Future Work

7.1. CYP450 Probes

Within the first two chapters of this thesis, the CYP450 superfamily was introduced and their catalytic cycle and metabolic prowess were demonstrated. The importance of understanding these enzymes was demonstrated and with this in mind, ABPP was offered as a strategy for studying plant species relevant in crop protection.

Chapter 2 presented the efforts towards the development of ABPP probes for plant CYPP450s. An initial library of probes **1.59** to **1.64** (G1) was synthesised based upon the work of Wright *et al.*[69] A cinnamic acid based probe **2.60** was added to this library due a CYP450 mediated oxidation being responsible for the oxidation of cinnamic acid to *p*-coumaric acid in the biosynthesis of lignin (Figure 7.1.1).[82] Disappointingly these were shown to be unsuccessful at labelling plant CYP450s.[83]

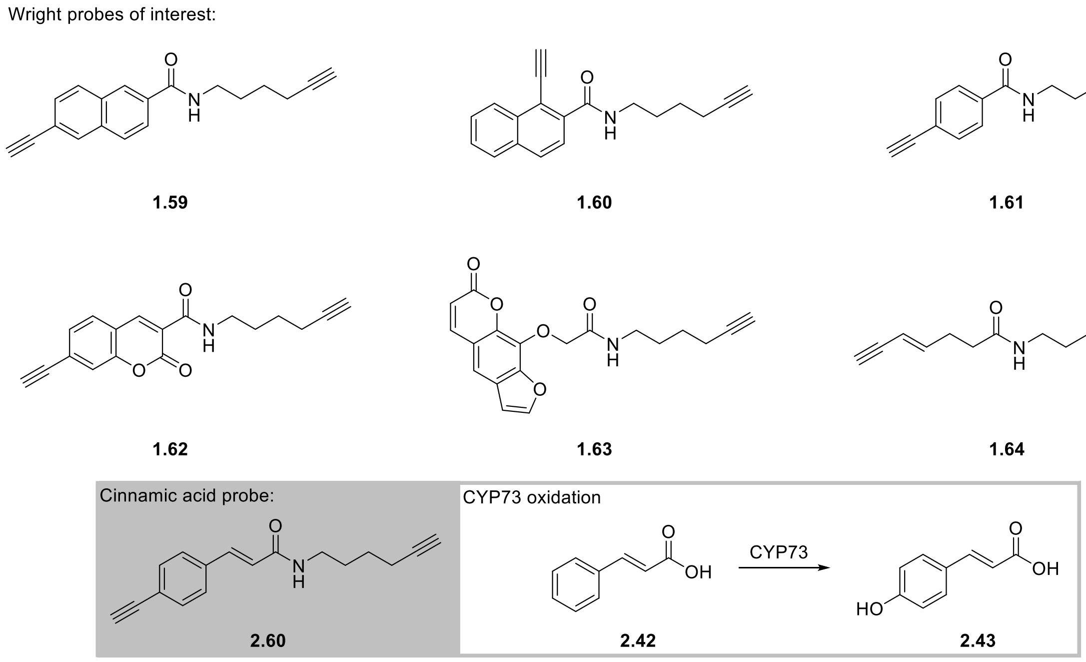
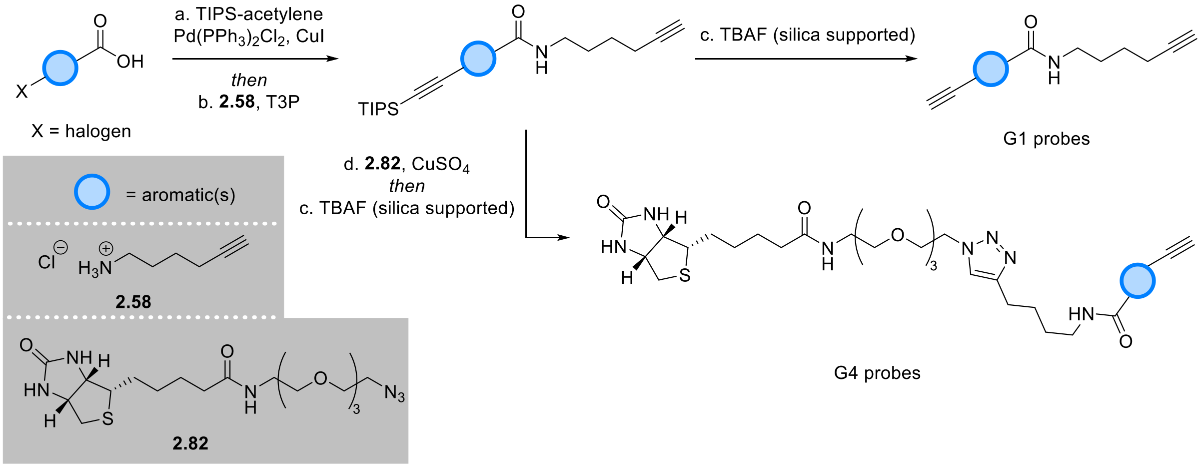


Figure 7.1.1: Initial G1 probes **1.59** to **1.64** synthesised with a view to enable labelling of plant CYP450s and cinnamic acid probe **2.60** added to this library due the known oxidation of cinnamic acid **2.42** to *p*-coumaric acid **2.43** by CYP73.

Moving forward, generations of biotinylated probes (G2 and G3) were synthesised. These probes sought to enable detection of labelled CYP450s by utilising more sensitive pull-down experiments. Unfortunately, their synthetic routes were laborious and non-modular and hence efforts were undertaken to unify the probe synthesis and provide access to both non-biotinylated (G1) and a new iteration of biotinylated (G4) probes. This was in light of both of these types of probes having been demonstrated to have use in labelling fungal CYP450s and plant CYP450s respectively.[83]

A streamlined route to the non-biotinylated (G1) and biotinylated (G4) probes was developed with a view to enable a comprehensive library of CYP450 probes to be synthesised (Scheme 7.1.1). Unfortunately, the library synthesis itself could not be achieved within the time frame of the project and thus remains a priority for future work. Upon completion of the library synthesis, a more comprehensive understanding of CYP450 labelling can be established through testing, with a view to develop additional probes to characterise the key CYP450 players involved in metabolism and detoxification.

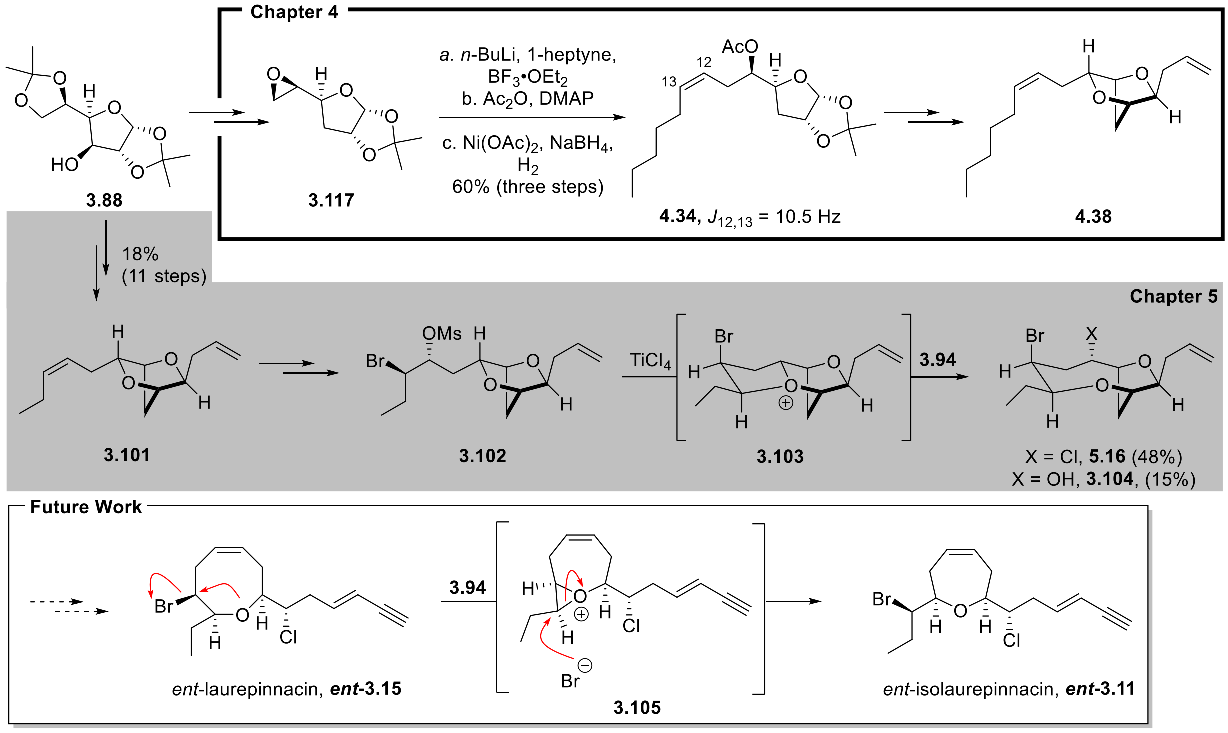


Scheme 7.1.1: Summary of route towards G1 and G4 probes.

7.2. *Laurencia* Natural Products

Orthogonally, chapter 3 introduced the *Laurencia* natural products, typically halogenated medium ring ethers containing five to nine-membered rings with enyne or bromoallene functionality. The key biosynthetic postulates were presented with a view to account for the large amount of structural diversity observed in the natural products isolated from the *Laurencia* genus.[98,100,107,189] Building upon this, a number of syntheses of natural products isolated from the genus sought to augment the biosynthetic postulates and provide evidence for them, whilst showcasing the interesting chemistries utilised in the building of the medium ring ether scaffolds.

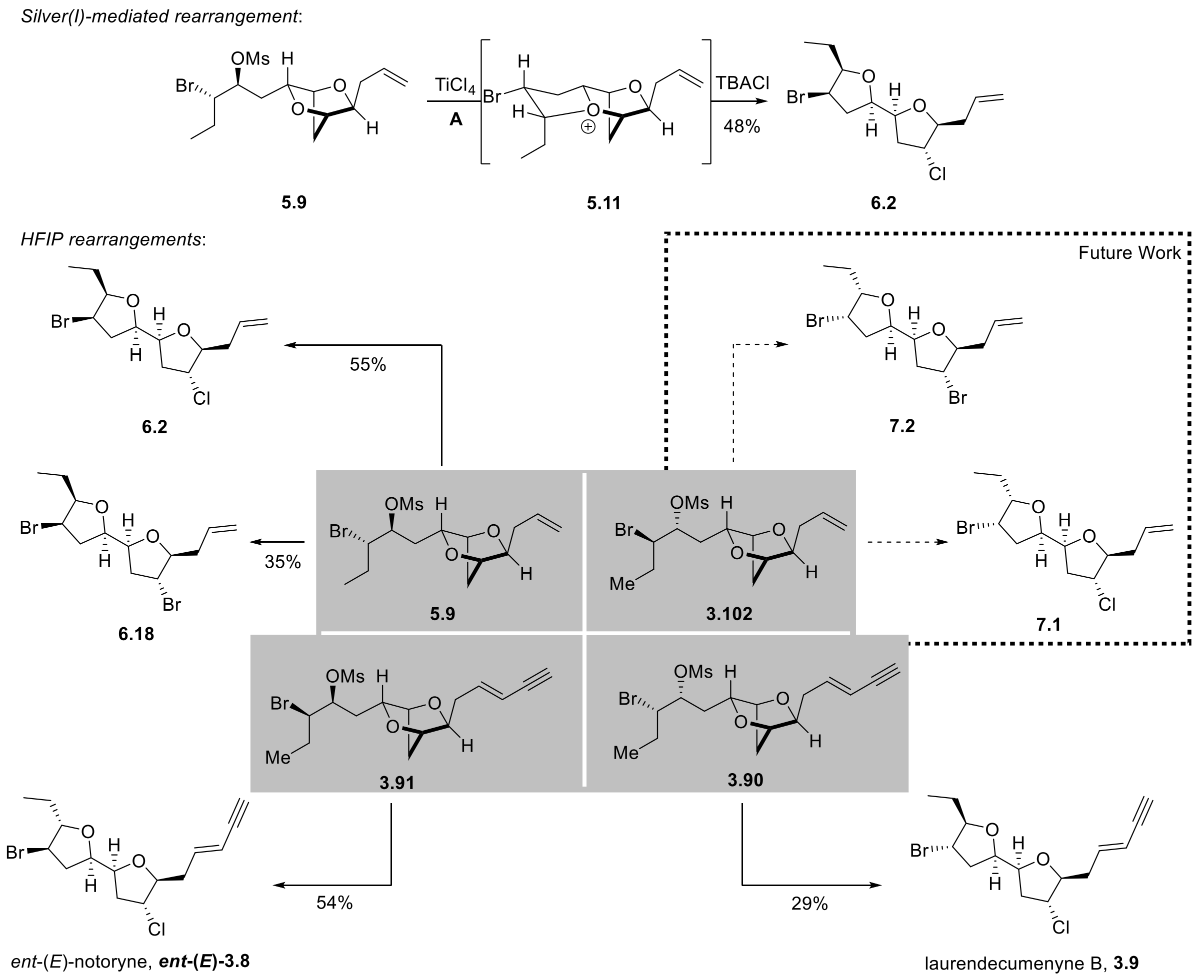
In the latter part of chapter three, a plausible strategy for the synthesis of *ent*-laurepinnacin from diacetone-D-glucose, invoking [2.2.1]-dioxabicycloheptane **3.101** as one of the key intermediates was proposed (Scheme 7.2.1). From this, formation of the bromomesylate **3.102** enabled the silver(I)-mediated skeletal rearrangement *via* bromonium ion **3.108** followed by oxonium ion quench at C-10 to be undertaken. The synthetic campaign from [2.2.1]-bicycloheptane to C-10 products **5.16** and **3.104** was explored in detail throughout the middle of chapter five, with chapter four dedicated to addressing the challenges faced in the synthesis of [2.2.1]-bicycloheptane **3.101** and the necessary use of a model system. A proposal for the silver(I)-mediated rearrangement of *ent*-laurepinnacin ***ent*-3.15** into *ent*-isolaurepinnacin ***ent*-3.11** was also presented at the end of chapter three.



Scheme 7.2.1: Summary of chapter 4 detailing the synthesis of model substrate **4.38**, chapter 5 detailing the synthesis of key [2.2.1]-dioxabicycloheptane **5.101** and the subsequent steps towards alcohol **3.104** and future work involving the completion of the synthesis of *ent*-laurepinnacin ***ent*-3.15** and the subsequent silver-mediated rearrangement to *ent*-isolaurepinnacin ***ent*-3.11.**

The elimination to the core of *ent*-laurepinnacin was ultimately not possible from either ketone **3.107** or **5.20** (not shown) and difficulties in bringing meaningful quantities of material to the front of the route hampered progress, comprehensive details of this endeavour were outlined towards the end of chapter five.

In chapter six, the final chapter, the silver(I)-mediated skeletal rearrangement was extended to the minor bromomesylate diastereoisomer **5.9** and interestingly, the C-7 quench product **6.2** was isolated as the major product. With this result, efforts were undertaken to take steps to provide alternative conditions for the skeletal rearrangement given that the route was not able to adequately facilitate the latter stages of the route (Scheme 7.2.2).



Scheme 7.2.2: Silver(I)-mediated skeletal rearrangement for minor diastereoisomer **5.9** and HFIP rearrangement of bromomesylates **5.9**, **3.111**, **3.96** and **3.97**.

Inspired by the work of Burn's *et al.* a protocol involving the generation of the desired bromonium ions was developed using HFIP as the solvent of choice.[187] With the addition of tetrabutylammonium salts, the C-7 oxonium quench products shown could be accessed without the need for the aforementioned silver(I) salt **3.94**. Building upon this, the synthesis of two natural products *ent*-notoryne ***ent*-(*E*)-3.8** and laurendecumenyne B **3.9** were achieved using the newly developed protocol from bromomesylates **3.90** and **3.91** synthesised in a previous project.[139] The key future work in this area is the expansion of the protocol to the major diastereoisomer **3.102** for the formation of C-7 products **7.1** and **7.2** and the further expansion of the protocol in order to enable C-10 products and access to material on route to *ent*-laurepinnacin ***ent*-3.15** so that the silver(I)-mediated rearrangement toward *ent*-isolaurepinnacin ***ent*-3.11** as originally planned can be explored in more detail.

8. Experimental

8.1. General Experimental

NMR spectra: Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker AV 600 (600/126 MHz), Bruker AV 500 (500/125 MHz), Bruker AV 400 (400/100 MHz) or Bruker DPX 200 (200/50 MHz) spectrometer. Proton and carbon chemical shifts are quoted in ppm and referenced to residual protonated solvent. Assignments were made on the basis of chemical shifts, coupling constants, COSY, HSQC, HMBC data and comparison with spectra of related compounds. Resonances are described as s (singlet), d (doublet), t (triplet), q (quartet), sept (septet), m (multiplet), br (broad), dd (double doublet) and so on. Coupling constants (*J*) are given in Hz and are rounded to the nearest 0.5 Hz. Diastereotopic protons and carbons are distinguished with (‘) for example CHH’ refers to a CH_2 group with diastereotopic protons.

Mass spectra: Low resolution mass spectra were recorded on a Fisons Platform spectrometer (ES). High resolution mass spectra were recorded by mass spectrometry staff at the Chemistry Research Laboratory, University of Oxford, using a Bruker Daltronics microTOF spectrometer (ES) or a Micromass GCT (FI). m/z values are reported in Daltons with their percentage abundances and, where known, the relevant fragment ions in parentheses. High resolution values are calculated to four decimal places from the molecular formula, all found values being within a tolerance of 5 ppm.

Infrared spectra: Infrared spectra were recorded on a Bruker Tensor 27 Fourier Transform spectrometer, using diamond ATR. Absorption maxima (ν_{max}) are quoted in wavenumbers (cm^{-1}).

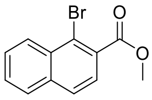
Optical rotation: Optical rotations were measured using a Perkin-Elmer 241 polarimeter in a cell of 1 dm path length (l). Specific rotations denoted as $[\alpha]_D^T$ where T = temperature in °C, D refers to the 145 line of a sodium lamp (λ = 589 nm), and are calculated according to the equation below where α is the observed rotation: $[\alpha]_D^T = \frac{100a}{lc}$.

Chromatography techniques: TLC was performed on Merck DC-Alufolien 60 F254 0.2 mm precoated plates and visualised using an acidic vanillin or basic potassium permanganate dips. Flash column chromatography was performed on Merck 60 silica (particle size 40–63 µm, pore diameter 60 Å) and the solvent system used is recorded in parentheses.

Reactions: All non-aqueous reactions were carried out in flame dried glassware under an inert atmosphere of nitrogen and employing standard techniques for handling air-sensitive materials unless specified otherwise. Solvents and commercially available reagents were dried and purified before use, as appropriate. All water used experimentally was distilled and the term 'brine' refers to a saturated solution of sodium chloride in water.

8.2. Experimental Procedures for Chapter 2

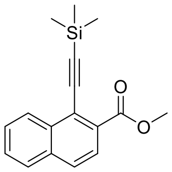
Methyl 1-bromo-2-naphthoate (2.2)



According to the modified procedure of Krätzschmar *et al.*:[190] sulfuric acid (5 drops) was added to a stirred suspension of 1-bromo-2-naphthoic acid **2.1** (1.0 g, 4.0 mmol, 1.0 equiv.) and MeOH (10 mL) and the reaction mixture was heated under reflux for 18 h. The reaction mixture was cooled to rt and then solvent was removed *in vacuo* before being redissolved in EtOAc (15 mL). The organic layer was washed with sat. $NaHCO_3$ solution (20 mL) and H_2O (2 ×

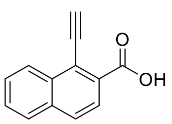
20 mL), dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give the title compound as a colourless oil (1.1 g, 3.9 mmol, 97%) which was used without further purification; R_f 0.40 (20% EtOAc/ petroleum ether 40 – 60); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (ddt, *J* = 8.5, 1.5, 1.0 Hz, 1H, ArC**H**), 7.88 – 7.81 (m, 2H, 2 × ArC**H**), 7.71 – 7.57 (m, 3H, 3 × ArC**H**), 4.01 (s, 3H, C**H$_3$**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9 (**C**=O), 135.2 (**C**CO_2Me), 132.3 (Ar**C**), 131.3 (Ar**C**), 128.6 (Ar**C**H), 128.2 (Ar**C**H), 128.1 (Ar**C**H), 127.8 (Ar**C**H), 125.8 (Ar**C**H), 122.6 (Ar**C**H), 52.7 (**C**H_3). One aromatic carbon resonance is unresolved. Data are in accordance with the literature.[190]

Methyl 1-((trimethylsilyl)ethynyl)-2-naphthoate (2.3)



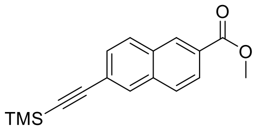
According to the modified procedure of Wright *et al.*: bis(triphenylphosphine)palladium(II) chloride (0.04 g, 0.096 mmol, 2.0 mol%) was added to a solution of methyl 1-bromo-2-naphthoate **2.2** (0.50 g, 1.9 mmol, 1.0 equiv.), ethynyltrimethylsilane (0.55 mL, 3.8 mmol, 2.0 equiv.), triethylamine (0.51 mL, 3.8 mmol, 2.0 equiv.), and copper(I) iodide (0.020 g, 0.23 mmol, 4.0 mol%) in MeCN (10 mL). The reaction mixture was heated to 90 °C and stirred for 16 h. The solvent was removed *in vacuo* and the resulting crude residue purified by flash column chromatography (1% EtOAc/petroleum ether 40-60 → 2% EtOAc/petroleum ether 40-60) yielding the title compound (0.19 g, 0.034 mmol, 35 %) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 – 8.52 (1H, m, ArC**H**), 7.92 (1H, d, *J* 8.5, ArC**H**), 7.86 – 7.82 (2H, m, ArC**H**), 7.63 – 7.61 (2H, m ArC**H**), 3.99 (3H, s, OC**H$_3$**), 0.37 (9H, s, Si(C**H$_3$**)$_3$); *m/z* (ES$^+$) 283.3 (M+H$^+$, 100%). Data are in accordance with the literature.[69]

1-Ethynyl-2-naphthoic acid (2.4)



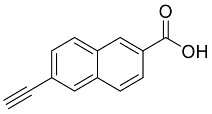
According to the modified procedure of Wright *et al.*:[69] a solution of 1 M NaOH (2 mL) was added dropwise to a stirred solution of methyl 1-((trimethylsilyl)ethynyl)-2-naphthoate **2.3** (0.12 g, 0.42 mmol, 1.0 equiv.) in 2:1 EtOH:CH_2Cl_2 (4 mL) and then the reaction mixture was stirred at rt for 16 h. After this time, the reaction was quenched by dropwise addition of 2 M HCl (3 mL) until precipitate formed. The organics were extracted with EtOAc (3 × 8 mL), washed with brine (10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* yielding the title compound as a brown solid (0.08 g, 0.41 mmol, 97%) which required no further purification. ^1H NMR (400 MHz, acetone-*d₆*) δ 8.51 – 8.46 (m, 1H, ArC**H**), 7.98 – 7.92 (m, 2H, 2 × ArC**H**), 7.89 (d, *J* = 8.5, 1H, ArC**H**), 7.65 – 7.60 (m, 2H, 2 × ArC**H**), 4.33 (s, 1H, CC**H**); *m/z* (ESI$^-$) 195 (M-H$^+$, 100%). Data are in accordance with the literature.[69]

Methyl 6-((trimethylsilyl)ethynyl)-2-naphthoate (2.6)



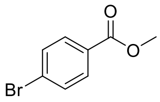
According to the modified procedure of Wright *et al.*:[69] bis(triphenylphosphine)palladium(II) chloride (0.040 g, 0.096 mmol, 5.0 mol%) was added to a solution of methyl 6-bromo-2-naphthoate **2.5** (0.50 g, 1.9 mmol, 1.0 equiv.), ethynyltrimethylsilane (0.55 mL, 3.8 mmol, 2.0 equiv.), triethylamine (0.51 mL, 3.8 mmol, 2.0 equiv.), and CuI (0.020 g, 0.2 mmol, 10 mol%) in MeCN (10 mL). The reaction mixture was heated to 90 °C and stirred for 16 h. The reaction mixture was cooled to rt and the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (1% EtOAc/petroleum ether 40-60→ 2% EtOAc/petroleum ether 40-60) giving the title compound (0.51 g, 1.8 mmol, 94%) as an off white solid. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.57 – 8.55 (1H, m, Ar**H**), 8.06 (1H, dd, *J* = 8.5, 1.5, Ar**H**), 8.03 – 8.00 (1H, m, Ar**H**), 7.87 (1H, d, *J* = 8.5, Ar**H**), 7.84 – 7.80 (1H, m, Ar**H**), 7.56 (1H, dd, *J* = 8.5, 1.5, Ar**H**), 3.98 (3H, s, C**H$_3$**), 0.29 (9H, s, Si(C**H$_3$**)$_3$); ^{13}C NMR (101 MHz, CDCl$_3$) δ 167.1 (**C**=O), 135.0 (**C**CO$_2$Me), 132.0 (Ar**C**), 131.7 (Ar**C**H), 130.8 (Ar**C**H), 129.4 (Ar**C**H), 129.3 (Ar**C**H), 128.1 (Ar**C**), 128.0 (Ar**C**H), 126.0 (Ar**C**H), 123.0 (**C**C≡C), 104.9 (**C**≡CSi), 96.3 (C≡**C**Si), 52.4 (**C**H$_3$), 0.0 (Si(**C**H$_3$)$_3$); LRMS *m/z* (ESI$^+$) 283 (M+H$^+$, 100%). Data are in accordance with the literature.[191]

6-Ethynyl-2-naphthoic acid (2.7)



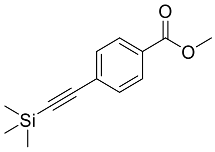
According to the procedure of Wright *et al*.:[69] a solution of 1 M NaOH (4 mL) was added dropwise to a stirred solution of methyl 6-((trimethylsilyl)ethynyl)-2-naphthoate **2.6** (0.20 g, 0.71 mmol, 1.0 equiv.) in 2:1 EtOH:CH$_2$Cl$_2$ (8 mL). The reaction mixture was stirred at rt for 16 h before quenching by dropwise addition of HCl (6 mL) until the title compound precipitates. The aqueous layer was extracted with EtOAc (3 × 15 mL) and the organic layers combined, washed with brine (20 mL), dried (MgSO$_4$), filtered and the solvent removed *in vacuo* yielding the title compound as an off white solid (0.12 g, 0.68 mmol, 96%) which required no further purification. ^{1}H NMR (400 MHz, acetone-*d$_6$*) δ 8.67 (1H, dt, *J* = 1.5, 0.5, ArC**H**), 8.18 (1H, d, *J* = 1.5, ArC**H**), 8.17 – 8.10 (2H, m, 2 × ArC**H**), 8.04 (1H, dq, *J* = 8.5, 0.5, ArC**H**), 7.65 (1H, dd, *J* = 8.5, 1.5, ArC**H**), 3.86 (1H, s, CC**H**); LRMS *m/z* (ESI$^-$) 195 (M-H$^+$, 100%). Data are in accordance with the literature.[191]

Methyl 4-bromobenzoate (2.10)



According to the procedure of Galán *et al.*:[192] Sulfuric acid (5 drops) was added to a stirred solution of 4-bromobenzoic acid **2.9** (1.0 g, 5.0 mmol, 1.0 equiv.) in MeOH (13 mL) and the reaction mixture was heated to reflux for 18 h. After this time, the reaction mixture was cooled to rt and the solvent was removed *in vacuo*. The resulting crude residue was resuspended in EtOAc (15 mL) and washed with sat. $NaHCO_3$ (20 mL), H_2O (2 × 20 mL), dried ($MgSO_4$), filtered and then the solvent removed *in vacuo* to give the title compound (1.1 g, 5.0 mmol, 99%) as a white solid which required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.79 (m, 2H, 2 × ArC**H**), 7.54 – 7.47 (m, 2H, 2 ×ArC**H**), 3.84 (s, 3H, $CO_2C\mathbf{H_3}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4 (**C**=O), 131.7 (Ar**C**H), 131.1 (Ar**C**H), 129.1 (Ar**C**), 128.0 (Ar**C**), 52.3 ($CO_2\mathbf{C}H_3$). Data are in accordance with the literature.[193]

Methyl 4-((trimethylsilyl)ethynyl)benzoate (2.11)

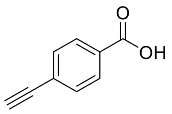


According to the modified procedure of Wright *et al.*: bis(triphenylphosphine)palladium(II) chloride (0.040 g, 0.096 mmol, 2.0 mol%) was added to a solution of methyl 4-bromobenzoate **2.10** (0.41 g, 1.9 mmol, 1.0 equiv.), ethynyltrimethylsilane (0.55 mL, 3.8 mmol, 2.0 equiv.), triethylamine (0.51 mL, 3.8 mmol, 2.0 equiv.), and copper(I) iodide (0.020 g, 0.23 mmol, 4.0 mol%) in MeCN (10 mL). The reaction mixture was heated to 90 °C and stirred for 16 h. The solvent was removed *in vacuo* and the resulting crude residue purified by flash column chromatography (1% EtOAc/petroleum ether 40-60 → 2% EtOAc/petroleum ether 40-60) giving the title compound (0.19 g, 0.034 mmol, 93 %) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 – 7.92 (m, 2H, 2 × Ar**H**), 7.55 – 7.47 (m, 2H, 2 × Ar**H**), 3.91 (s, 3H, $CO_2C\mathbf{H_3}$), 0.26 (s, 9H, $Si(C\mathbf{H_3})_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4 ($\mathbf{C}O_2CH_3$), 131.8 (Ar**C**H), 129.6 (Ar**C**), 129.3 (Ar**C**H), 127.7 (Ar**C**), 104.0 (**C**≡CSi), 97.6 (C≡**C**Si), 52.1 ($\mathbf{C}O_2CH_3$), -0.2 ($Si(\mathbf{C}H_3)_3$; LRMS *m/z* (ESI$^+$) 233.1 (M+H$^+$, 100%) Data are in accordance with the literature.[194]

OR

According to the modified procedure of Wright *et al.*:[69] Tetrakis(triphenylphosphine)palladium(0) (0.058 g, 0.050 mmol, 2.5 mol%) was added to a solution of methyl 4-bromobenzoate **2.10** (0.43 g, 2.0 mmol, 1.0 equiv.), ethynyltrimethylsilane (0.56 mL, 4.0 mmol, 2.0 equiv.), copper(I) iodide (0.019 g, 0.10 mmol, 5.0 mol%) and triethylamine (0.55 mL, 4.0 mmol, 2.0 equiv.) in N_2 sparged (30 mins) THF (20 mL) and the reaction vessel was sealed and heated to 60 °C for 18 h. After this time, the reaction mixture was cooled to rt and then filtered through Celite™ before removal of solvent *in vacuo*. The resulting crude residue was further purified by flash column chromatography (1% EtOAc/ petroleum ether 40-60 → 2% EtOAc/ petroleum ether 40-60) to give the title compound (0.42 g, 2.0 mmol, 99%) as an off white solid. Data as above.

4-Ethynylbenzoic acid (2.12)

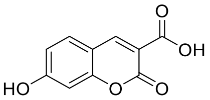


According to the modified procedure of Wright *et al.*:[69] lithium hydroxide hydrate (0.32 g, 7.5 mmol, 5.0 equiv.) was added to a stirred solution of 4-((trimethylsilyl)ethynyl)benzoic acid **2.11** (0.35 g, 1.5 mmol, 1.0 equiv.) in THF (10 mL) and H_2O (5 mL) and the reaction mixture was vigorously stirred at rt for 18 h. After this time, the THF was removed *in vacuo* and the remaining aqueous layer was acidified by addition of pH 1 sulfate buffer. The aqueous phase was extracted with EtOAc (3 × 10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give the title compound (0.22 g, 1.0 mmol, 67%) as a bronze coloured solid which required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 6.5 Hz, 2H, 2 × Ar**H**), 7.47 – 7.36 (m, 2H, 2 × Ar**H**), 3.14 (s, 1H, C≡C**H**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7 (**C**=O), 131.8 (Ar**C**H), 131.0 (Ar**C**), 129.5 (Ar**C**H), 126.3 (Ar**C**), 82.8 (C≡CH), 80.0 (C≡CH); LRMS *m/z* (ESI^-) 145.1 ($M-H^+$, 100%). Data are in accordance with the literature.[195]

OR

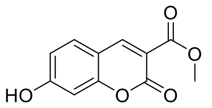
According to the modified procedure of Wright *et al.*:[69] a solution of 1 M NaOH (2 mL) was added dropwise to a stirred solution of methyl 4-((triisopropylsilyl)ethynyl)benzoate (0.14 g, 0.61 mmol, 1.0 equiv.) in 2:1 EtOH:CH_2Cl_2 (6 mL) and then the reaction mixture was stirred at rt for 16 h. After this time, the reaction was quenched by dropwise addition of 2 M HCl (3 mL) until precipitate formed. The organics were extracted with EtOAc (3 × 8 mL), washed with brine (10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* yielding the title compound as a brown solid (0.088 g, 0.60 mmol, 98%) which required no further purification. Data as above.

7-Hydroxy-2-oxo-2*H*-chromene-3-carboxylic acid (2.14)



According to the procedure of Yang *et al.*:[196] 2,2-dimethyl-1,3-dioxane-4,6-dione **2.13** (0.17 g, 1.2 mmol, 1.3 equiv.) was added to a stirred solution of 2,4-dihydroxybenzaldehyde (0.14 g, 1.0 mmol, 1.0 equiv.) in H_2O and the reaction mixture was stirred at reflux for 18 h. After this time, the reaction mixture was cooled to rt and the solid filtered and recrystalised (MeOH) to give the title compound (0.066 g, 0.32 mmol, 32%) as a pale orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.77 (s, 1H, COO**H**), 11.13 (s, 1H, O**H**), 8.67 (d, *J* = 0.5 Hz, 1H, ArC**H**), 7.74 (d, *J* = 8.5 Hz, 1H, ArC**H**), 6.84 (dd, *J* = 8.5, 2.5 Hz, 1H, ArC**H**), 6.73 (s, 1H, ArC**H**); ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.7 (**C**=O), 164.4 (**C**=O), 158.0 (**C**OH), 157.5 (Ar**C**), 149.8 (Ar**C**H), 132.5 (Ar**C**H), 114.5 (Ar**C**), 113.0 (Ar**C**), 111.1 (Ar**C**H), 102.3 (Ar**C**H); LRMS *m/z* (ESI$^-$) 205.3 (M-H$^+$). Data are in accordance with literature.[196]

Methyl 7-hydroxy-2-oxo-*2H*-chromene-3-carboxylate (2.15)

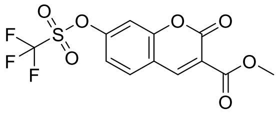


According to procedure of Abumelha *et al.*:[197] piperidine (5 drops) was added to a stirred solution of 2,4-dihydroxybenzaldehyde **2.13** (1.0 g, 7.2 mmol, 1.0 equiv.) and dimethyl malonate (1.3 mL, 11 mmol, 1.5 equiv.) in MeOH (5 mL) and then the reaction mixture was heated to 60 °C for 48 h. The reaction mixture was cooled to 0 °C and the solid was filtered off and washed with ice cold MeOH (3 × 10 mL) to give the title compound as a pale yellow solid (1.4 g, 9.6 mmol, 87%) which required no further purification; ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.67 (s, 1H, C**H**), 7.63 (d, *J* = 8.7 Hz, 1H, C**H**), 6.85 (dd, *J* = 8.6, 2.3 Hz, 1H, C**H**), 6.73 (d, *J* = 2.2 Hz, 1H, C**H**), 3.88 (s, 3H, C**H$_3$**); LRMS *m/z* (ESI$^+$) 221.0 (M+H$^+$, 100%). Data are in accordance with the literature.[197]

OR

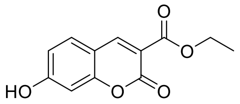
According to the procedure of Galán *et al.*:[192] Sulfuric acid (5 drops) was added to a stirred solution of 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylic acid **2.14** (0.10 g, 0.10 mmol, 1.0 equiv.) in MeOH (2 mL) and the reaction mixture was heated to reflux for 18 h. After this time, the reaction mixture was cooled to rt and the solvent was removed *in vacuo*. The resulting crude residue was resuspended in EtOAc (15 mL) and washed with sat. $NaHCO_3$ (20 mL), H_2O (2 × 20 mL), dried ($MgSO_4$), filtered and then the solvent removed *in vacuo* to give the title compound (0.11 g, 5.0 mmol, 99%) as a pale yellow solid which required no further purification. Data as above.

Methyl 2-oxo-7-(((trifluoromethyl)sulfonyl)oxy)-*2H*-chromene-3-carboxylate (2.16)



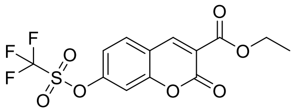
According to procedure of Vasiljeva *et al.*:[198] triflic anhydride (0.54 mL, 3.2 mmol, 1.2 equiv.) was added to a stirred solution of methyl 7-hydroxy-2-oxo-2H-chromene-3-carboxylate **2.15** (0.59 g, 2.7 mmol, 1.0 equiv.) and pyridine (0.56 mL, 3.2 mmol, 1.2 equiv.) in CH_2Cl_2 at 0 °C and the reaction mixture was stirred for 1 h. After this time, the reaction was quenched by addition of H_2O (20 mL) and organics were extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) to give the title compound as a yellow solid (0.55 g, 1.6 mmol, 60%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, *J* = 0.5 Hz, 1H, ArC**H**), 7.73 (d, *J* = 8.5 Hz, 1H, ArC**H**), 7.31 (d, *J* = 2.5 Hz, 1H, ArC**H**), 7.28 (dd, *J* = 8.5, 2.5 Hz, 1H, ArC**H**), 3.97 (s, 3H, C$\mathbf{H_3}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.1 (**C**=O), 155.7 (Ar**C**H), 155.4 (Ar**C**H), 152.5 (**C**=O), 147.6 (**C**OSO_2CF_3), 131.3 (Ar**C**H), 131.3 (Ar**C**H), 119.0 (Ar**C**), 118.3 (Ar**C**), 117.7 (Ar**C**), 115.2 (q, *J* = 366.0, $COSO_2$**C**F_3) 110.5 (Ar**C**H), 53.2 (**C**H_3). Data are in accordance with the literature.[198]

Ethyl 7-hydroxy-2-oxo-*2H*-chromene-3-carboxylate (2.17)



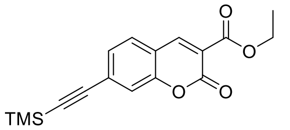
According to procedure of Vieira *et al.*:[199] piperidine (5 drops) was added to a stirred suspension of 2,4-dihydroxybenzaldehyde **2.13** (0.10 g, 0.72 mmol, 1.0 equiv.) in diethyl malonate (0.23 g, 1.4 mmol, 2.0 equiv.) and the reaction mixture was stirred at rt for 18 h. The reaction mixture was acidified by addition of 2 M HCl (2 mL) until a precipitate formed. The solid was filtered and washed with Et_2O (3 × 10 mL) to give a crude residue that was further purified by flash column chromatography (70% EtOAc/CH_2Cl_2) giving the title compound as a pale yellow solid (0.090 g, 0.74 mmol, 53 %); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.08 (s, 1H, O**H**), 8.67 (s, 1H, C**H**CCO$_2$), 7.75 (d, *J* = 8.5 Hz, 1H, C**H**CCH), 6.84 (dd, *J* = 8.5, 2.5 Hz, 1H, CHC**H**COH), 6.72 (d, *J* = 2.0 Hz, 1H, HOCC**H**), 4.26 (q, *J* = 7.0 Hz, 2H, C**H$_2$**), 1.29 (t, *J* = 7.0 Hz, 3H, C**H$_3$**); LRMS *m/z* (ESI$^+$) 257.0 (M+Na$^+$, 100%). Data are in accordance with the literature.[199]

Ethyl 2-oxo-7-(((trifluoromethyl)sulfonyl)oxy)-*2H*-chromene-3-carboxylate (2.18)



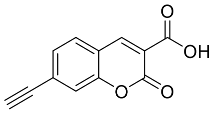
According to procedure of Starčević *et al.*:[200] triflic anhydride (0.74 mL, 4.2 mmol, 1.2 equiv.) was added dropwise to a stirred solution of ethyl 7-hydroxy-2-oxo-*2H*-chromene-3-carboxylate **2.17** (0.83 g, 3.5 mmol, 1.0 equiv.) and pyridine (0.33 mL, 4.2 mmol, 1.2 equiv.) in CH_2Cl_2 (25 mL) cooled to 0 °C and the reaction mixture was stirred for 0.5 h. The reaction mixture was quenched by addition of H_2O (30 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), the organic layers were combined, dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60) to give the title compound (0.52 g, 1.4 mmol, 41%) as a yellow solid; ^1H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H, C**H**CCO_2), 7.72 (d, *J* = 8.5 Hz, 1H, OCCHC**H**), 7.32 – 7.29 (m, 1H, OCC**H**CH), 7.28 (d, *J* = 6.0 Hz, 1H, OCC**H**), 4.43 (q, *J* = 7.0 Hz, 2H, C**H₂**), 1.42 (t, *J* = 7.0 Hz, 3H, CH_2); LRMS *m/z* (ESI^+) 367.3 ($M+H^+$, 100%). Data are in accordance with the literature.[200]

Ethyl 2-oxo-7-((trimethylsilyl)ethynyl)-*2H*-chromene-3-carboxylate (2.19)



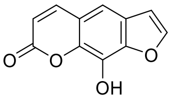
According to the modified procedure of Wright *et al.*:[69] bis(triphenylphosphine)palladium(II) chloride (0.038 g, 0.055 mmol, 5.0 mol%) was added to a solution of ethyl 2-oxo-7-(((trifluoromethyl)sulfonyl)oxy)-2*H*-chromene-3-carboxylate **2.18** (0.40 g, 1.1 mmol, 1.0 equiv.), ethynyltrimethylsilane (0.31 mL, 2.2 mmol, 2.0 equiv.), triethylamine (0.29 mL, 2.2 mmol, 2.0 equiv.), and copper(I) iodide (0.023 g, 0.12 mmol, 10 mol%) in MeCN (10 mL) The reaction mixture was heated to 90 °C and stirred for 16 h. After this time, the solvent was removed *in vacuo* and the resulting crude residue purified by flash column chromatography (10% EtOAc/petroleum ether 40-60 → 20% EtOAc/petroleum ether 40-60) yielding the title compound (0.39 g, 1.0 mmol, 95%) as a yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H, ArC**H**), 7.52 (d, *J* = 8.0 Hz, 1H, ArC**H**), 7.42 – 7.33 (m, 2H, 2 × ArC**H**), 4.41 (q, *J* = 7.0 Hz, 2H, C**H₂**), 1.41 (t, *J* = 7.0 Hz, 3H, C**H₃**), 0.28 (s, 9H, Si(C**H₃**)₃); *m/z* (ESI^+) 315 ($M+H^+$, 100%). Data are in accordance with the literature.[69]

7-Ethynyl-2-oxo-*2H*-chromene-3-carboxylic acid (2.20)



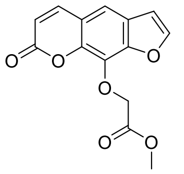
According to the modified procedure of Wright *et al.*:[69] a solution of 1 M NaOH (4 mL) was added dropwise to a stirred solution of ethyl 2-oxo-7-((trimethylsilyl)ethynyl)-2*H*-chromene-3-carboxylate **2.19** (0.20 g, 0.63 mmol, 1.0 equiv.) in THF (8 mL). The reaction mixture was stirred at rt for 16 h. before quenching by dropwise addition of 2 M HCl (6 mL) to precipitate the title compound. The aqueous layer was extracted with EtOAc (3 × 15 mL) and the organic layers were combined, washed with brine (20 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* yielding the title compound as a yellow solid (0.10 g, 0.47 mmol, 75%) which required no further purification. ^{1}H NMR (400 MHz, CD_2Cl_2) δ 12.01 (s, 1H, COO**H**), 8.90 (d, *J* = 1.0 Hz, 1H, ArC**H**), 7.75 (d, *J* = 8.0 Hz, 1H, ArC**H**), 7.59 (s, 1H, C**H**CCOOH), 7.55 (dd, *J* = 8.0, 1.0 Hz, 1H, ArC**H**), 3.51 (s, 1H, CCHCHCC≡C**H**); *m/z* (ES$^-$) 213.3 (M-H$^+$, 100%). Data are in accordance with the literature.[69]

9-Hydroxy-*7H*-furo[3,2-*g*]chromen-7-one (2.22)



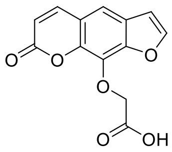
According to procedure of Shen *et al.*:[201] boron tribromide (0.19 mL, 2.0 mmol, 2.0 equiv.) was added to a vigorously stirred solution of 9-methoxy-*7H*-furo[3,2-*g*]chromen-7-one **2.21** (0.22 g, 1.0 mmol, 1.0 equiv.) in CH_2Cl_2 (9 mL) at 0 °C, the reaction mixture was warmed to rt and stirred for 16 h. After this time, the reaction mixture was cooled to 0 °C and quenched by slow addition of saturated Na_2CO_3 (10 mL), the organics were extracted with EtOAc (3 × 15 mL). The organic layers were dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give the title compound as a white solid (0.17 g, 0.85 mmol, 85%) which required no further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (1H, s, O**H**), 8.12 (1H, d, *J* = 9.5, C**H**), 8.07 (1H, dd, *J* = 2.0, 1.0, ArC**H**), 7.45 (1H, d, *J* = 1.5, ArC**H**), 7.04 (1H, dd, *J* = 2.0, 0.5, ArC**H**), 6.40 (1H, d, *J* = 9.5, C**H**); LRMS *m/z* (ESI$^-$) 200 (M−H$^+$, 100%). Data are in accordance with the literature.[201]

Methyl 2-((7-oxo-*7H*-furo[3,2-*g*]chromen-9-yl)oxy)acetate (2.23)



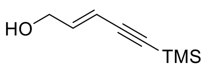
According to procedure of Wright *et al.*:[69] bromomethyl acetate (0.10 mL, 1.02 mmol, 1.2 equiv.) was added dropwise to a stirred solution of 9-hydroxy-*7H*-furo[3,2-*g*]chromen-7-one **2.21** (0.17 g, 0.85 mmol, 1.0 equiv.) and potassium carbonate (0.35 g, 2.55 mmol, 3.0 equiv.) in DMF (5 mL), the reaction mixture was stirred at rt for 16 h. The solvent was removed under a stream of N_2 and the resulting crude residue was redissolved in EtOAc (10 mL) and washed with H_2O (3 × 20 mL). The organic phase was dried ($MgSO_4$), filtered, and solvent removed *in vacuo* yielding the title compound as a white solid (0.20 g, 0.73 mmol, 86%) which required no further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 9.5 Hz, 1H, O=CC**H**), 7.71 (d, *J* = 2.0 Hz, 1H, ArC**H**), 7.41 (s, 1H, ArC**H**), 6.84 (d, *J* = 2.0 Hz, 1H, ArC**H**), 6.40 (d, *J* = 9.5 Hz, 1H, C**H**), 5.17 (s, 2H, C**H₂**), 3.82 (s, 3H, C**H₃**); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.3 (**C**=O), 160.1 (**C**=O), 147.2 (Ar**C**), 146.8 (Ar**C**H), 144.3 (**C**H), 142.7 (Ar**C**), 130.8 (Ar**C**), 126.1 (Ar**C**), 116.6 (Ar**C**), 114.9 (**C**H), 113.6 (Ar**C**H), 106.8 (Ar**C**H), 69.0 (**C**H$_2$), 52.3 (**C**H$_3$); LRMS *m/z* (ESI$^+$) 275 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 275.0550, $C_{14}H_{11}O_6$ (M+H$^+$) requires 275.0550. Data are in accordance with the literature.[69]

2-((7-Oxo-*7H*-furo[3,2-g]chromen-9-yl)oxy)acetic acid (2.24)



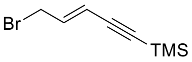
According to procedure of Wright *et al.*:[69] Lithium hydroxide (1 M in H_2O, 1.2 mL, 1.2 mmol, 2.0 equiv.) was added to a solution of methyl 2-((7-oxo-*7H*-furo[3,2-*g*]chromen-9-yl)oxy)acetate **2.23** (0.17 g, 0.63 mmol) in THF (3 mL) and the reaction mixture stirred at rt for 16 h. After this time, the THF was removed *in vacuo* and the aqueous phase acidified with pH 1 sulfate buffer (5 mL). The organics were extracted with EtOAc (3 × 20 mL) and washed with brine (30 mL), dried ($MgSO_4$), filtered, and solvent removed *in vacuo* giving the title compound as an off white solid (0.14 g, 0.54 mmol, 85%) which required no further purification. ^{1}H NMR (400 MHz, DMSO-*d₆*) 8.16 (d, *J* = 9.5 Hz, 1H, C**H**), 8.12 (d, *J* = 2.0 Hz, 1H, ArC**H**), 7.66 (s, 1H, ArC**H**), 7.10 (d, *J* = 2.0 Hz, 1H, ArC**H**), 6.46 (d, *J* = 9.5 Hz, 1H, C**H**), 5.13 (s, 2H, C**H₂**); LRMS *m/z* (ESI$^-$) 259 (M−H$^+$, 100%). Data are in accordance with the literature.[69]

(E)-5-(Trimethylsilyl)pent-2-en-4-yn-1-ol (2.26)



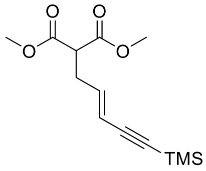
According to procedure of Nicolau *et al.*:[202] *n*-BuLi (1.5 M in hexanes, 9.1 mL, 13 mmol, 2.1 equiv.) was added dropwise to a stirred solution of *(E)*-pent-2-en-4-yn-1-ol **2.25** (0.52 mL, 6.1 mmol, 1.0 equiv.) in THF (10 mL) at −78 °C, the reaction mixture was stirred for 0.5 h then trimethylsilyl chloride (1.7 mL, 13 mmol, 2.10 equiv.) was added dropwise. The reaction mixture was slowly warmed to rt for 2 h before cooling to 0 °C and adding 2 M HCl (10 mL) and stirring 1 h. The reaction mixture was diluted with H_2O (10 mL) and extracted with Et_2O (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated *in* *vacuo* to give a crude residue which was further purified by column chromatography (20% EtOAc/ petroleum ether 40-60) giving the title compound as a colourless oil (0.29 g, 1.8 mmol, 30%); ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.31 (1H, dt, *J* = 16.0, 5.0 Hz, (CC**H**), 5.81 – 5.71 (1H, m, C**H**CH$_2$), 4.25 – 4.17 (2H, m, C**H$_2$**), 0.19 (9H, s, (C**H$_3$**)$_3$); ^{13}C NMR (101 MHz, CDCl$_3$) δ 143.0 (**C**HCH$_2$), 110.5 (**C**HC), 103.1 (**C**≡CSi), 95.4 (C≡**C**Si), 63.0 (**C**H$_2$), 0.00 ((**C**H$_3$)$_3$); LRMS *m/z* (ES$^+$) 155.3 (M+H$^+$, 100%). Data are in accordance with the literature.[202]

***(E)*-(5-Bromopent-3-en-1-yn-1-yl)trimethylsilane (2.27)**



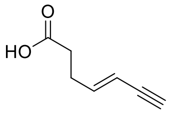
According to procedure of Robinson *et al.*:[203] *N*-bromosuccinimide (0.40 g, 2.3 mmol, 1.2 equiv.) and triphenylphosphine (0.59 g, 2.3 mmol, 1.2 equiv.) were added in one portion to *(E)*-5-(trimethylsilyl)pent-2-en-4-yn-1-ol **2.26** (0.29 g, 1.9 mmol, 1.0 equiv.) in CH$_2$Cl$_2$ (20 mL) at −30 °C and the reaction mixture was stirred for 0.5 h then warmed to rt. The solvent was then removed *in vacuo* giving a crude residue which was further purified by flash column chromatography (2% EtOAc/ petroleum ether 40-60) giving the title compound as a pale yellow oil (0.35 g, 1.65 mmol, 87%). ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.31 (dt, *J* = 15.5, 8.0 Hz, 1H, CH$_2$C**H**), 5.75 (dt, *J* = 15.5, 1.0 Hz, 1H, CC**H**), 3.97 (dd, *J* = 8.0, 1.0 Hz, 2H, C**H**$_2$), 0.19 (s, 9H, (C**H**$_3$)$_3$); ^{13}C NMR (101 MHz, CDCl$_3$) δ 138.9 (**C**HCH$_2$), 114.3 (**C**HC), 101.9 (**C**≡CSi), 97.6 (C≡**C**Si), 31.4 (**C**H$_2$) , -0.27 ((**C**H$_3$)$_3$); LRMS *m/z* (ESI$^+$) 216.9 (M+H$^+$, 50%) 218.9 (M+H$^+$, 50%). Data are in accordance with the literature.[203]

Dimethyl *(E)*-2-(5-(trimethylsilyl)pent-2-en-4-yn-1-yl)malonate (2.28)



Dimethyl malonate (0.16 mL, 1.4 mmol, 3.0 equiv.) was added dropwise to a stirred suspension of sodium hydride (60 % in mineral oil, 0.056 g, 1.40 mmol, 3.0 equiv.) in a mixture of DMF/THF (2:1, 6 mL/3 mL) at 0 °C. The reaction mixture was warmed to room temperature over an hour and then *(E)*-(5-bromopent-3-en-1-yn-1-yl)trimethylsilane **2.27** (0.10 g, 0.46 mmol, 1.0 equiv.) was added in THF (1 mL) along with KI (0.025 g, 0.25 mmol, 0.30 equiv.) and the reaction mixture was stirred for 16 h at room temperature. The reaction mixture was quenched by slow addition of sat. NH_4Cl solution (8 mL) and then diluted with petroleum ether 40-60 (6 mL), the layers were separated, and the aqueous layer extracted with petroleum ether 40-60 (3 × 6 mL). The organic layers were combined, dried ($MgSO_4$), filtered and solvent removed *in vacuo* giving a crude residue which was further purified by flash column chromatography (5% EtOAc/ petroleum ether 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound as a pale yellow oil (0.090 g, 0.34 mmol, 73%); R_f 0.55 (20% EtOAc/ petroleum ether 40-60); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.11 (dt, *J* = 15.5, 7.0 Hz, 1H, $CH_2C\mathbf{H}$), 5.60 (d, *J* = 15.5 Hz, 1H, $C\mathbf{H}C$), 3.75 (s, 6H, 2 × $C\mathbf{H_3}$), 3.43 (t, *J* = 7.5 Hz, 1H, $C\mathbf{H}$), 2.69 (td, *J* = 7.5, 1.5 Hz, 2H, $C\mathbf{H_2}$), 0.17 (s, 9H, $(C\mathbf{H_3})_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.8 ($\mathbf{C}O_2Me$), 140.1 ($CH_2\mathbf{C}H$), 112.9 ($\mathbf{C}HC$), 103.0 ($\mathbf{C}{\equiv}CSi$), 94.5 ($C{\equiv}\mathbf{C}Si$), 52.7 ($\mathbf{C}H$), 51.0 ($CO_2\mathbf{C}H_3$), 32.0 ($\mathbf{C}H_2$), -0.18 ($(\mathbf{C}H_3)_3$); LRMS *m/z* (ESI^+) 291.1 ($M+Na^+$, 100%); HRMS *m/z* (ESI^+): found 291.1021, $C_{13}H_{20}O_4NaSi$ ($M+Na^+$) requires 291.1023.

(*E*)-Hept-4-en-6-ynoic acid (2.29)



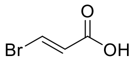
According to the modified procedure of Wright *et al.*:[69] lithium chloride (0.04 g, 0.76 mmol, 1.0 equiv.) and H_2O (5 drops) were added to a stirred solution of dimethyl (*E*)-2-(5-(trimethylsilyl)pent-2-en-4-yn-1-yl)malonate **2.28** (0.20 g, 0.76 mmol, 1.0 equiv.) in DMF (4 mL). The reaction mixture was heated to 150 °C and stirred for 4 h. The reaction mixture was cooled to rt, diluted with H_2O (10 mL) and extracted with Et_2O (3 × 15 mL). The organic layers were combined and washed with brine (5 × 20 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the crude decarboxylated product. The crude product was immediately dissolved in THF (5 mL) and then lithium hydroxide (1M in H_2O) was added and the reaction and then stirred for 16 h. The THF was removed *in vacuo* and 2 M HCl (10 mL) was added to precipitate the product which was extracted with EtOAc (3 × 15 mL). The organic layers were combined and dried over $MgSO_4$, filtered, and solvent removed *in vacuo* to yield the title compound (0.0075 g, 0.061 mmol, 8%) as a white solid which required no further purification. ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.39 – 6.10 (m, 1H, C**H**CH_2), 5.63 – 5.44 (m, 1H, CC**H**), 2.82 (m, 1H, **H**C≡C) 2.76 – 2.33 (m, 4H, CHC**H₂**, C**H₂**CO_2H); LRMS *m/z* (ESI^-) 123.0 ($M-H^+$); HRMS *m/z* (ESI^-): found 123.0441, $C_7H_7O_2$ ($M-H^+$) requires 123.0452. Data are in accordance with literature.[69]

OR

Lithium hydroxide monohydrate (0.047 g, 1.1 mmol, 5.0 equiv.) was added to a stirred solution of methyl (*E*)-hept-4-en-6-ynoate **2.40** (0.030 g, 0.22 mmol, 1.0 equiv.) in a mixture of THF and H_2O (2:1, 2 mL total) and the reaction mixture was stirred vigorously for 18 h at rt.

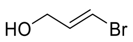
After this time, the reaction was judged to be complete by TLC analysis and the THF was removed *in vacuo*, pH 1 sulfate buffer (3 mL) was added and the organics were extracted with EtOAc (3 × 5 mL). The organic phase was dried ($MgSO_4$), filtered, and then removed *in vacuo* to give the title compound (0.023 g, 0.19 mmol, 85%) as an off-white solid that required no further purification. Data as above.

(*E*)-3-Bromoacrylic acid (2.31)



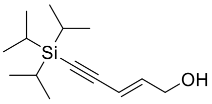
According to procedure of Zeng *et al*.:[79] hydrobromic acid (48% in H_2O, 4.0 mL) was added to a flask containing propiolic acid **2.30** (0.88 mL, 14 mmol, 1.0 equiv.) and the reaction mixture was heated to 95 °C and stirred for 2 h. After this time, the reaction mixture was cooled to rt, and the precipitate filtered off and washed with ice cold water (3 × 2 mL). The resulting solid was dried thoroughly under high vacuum to give the title compound (1.4 g, 9.1 mmol, 65%) as a white solid that required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 14.0 Hz, 1H, BrC**H**), 6.54 (d, *J* = 14.0 Hz, 1H, C**H**CO_2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.7 (**C**O_2H), 129.8 (Br**C**), 128.01 (**C**HCO_2H). Data are in accordance with the literature.[79]

(*E*)-3-Bromoprop-2-en-1-ol (2.32)



According to procedure of Zeng *et al*.:[79] (*E*)-3-bromoacrylic acid **2.31** (1.4 g, 9.3 mmol, 1.0 equiv.) was added portionwise to a stirred solution of lithium aluminium hydride solution (4 M in Et_2O, 4.7 mL, 19 mmol, 2.0 equiv.) at 0 °C and the reaction mixture was then stirred for 2 h. After this time, the reaction mixture was quenched at 0 °C by the successive addition of H_2O (0.4 mL), 15% NaOH solution (0.8 mL) and H_2O (0.9 mL). The ice bath was removed, and the reaction mixture stirred for 30 mins at rt before dilution with Et_2O (10 mL). The reaction mixture was filtered through Celite™, dried ($MgSO_4$) and the solvent removed *in vacuo* to give the title compound (0.69 g, 5.1 mmol, 55%) as a yellow oil which required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.58 – 6.11 (m, 2H, BrC**H**, C**H**CH_2), 4.18 – 4.04 (m, 2H, C**H₂**), 1.87 – 1.65 (m, 1H, O**H**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.5 (Br**C**), 107.8 (**C**HCH_2), 63.0 (**C**H_2). Data are in accordance with the literature.[79]

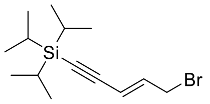
(*E*)-5-(Triisopropylsilyl)pent-2-en-4-yn-1-ol (2.33)



According to procedure of Cho *et al.*:[81] 2,6-di-*tert*-butyl-4-methylphenol (0.057 g, 0.26 mmol, 0.10 equiv.), CuI (0.049 g, 0.26 mmol, 10 mol%) and tetrakis(triphenylphosphine)palladium(0) (0.15 g, 0.13 mmol, 5.0 mol%) were added successively to an N_2 sparged (20 mins) solution of (*E*)-3-bromoprop-2-en-1-ol **2.32** (0.35 g, 2.6 mmol, 1.0 equiv.) and (triisopropylsilyl)acetylene (0.69 mL, 3.1 mmol, 1.2 equiv.) in diisopropylamine (8.2 mL) and the reaction mixture was stirred at rt for 2 h. After this time, the reaction was quenched by adding sat. NH_4Cl solution (10 mL) and the organics were extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (20 mL), dried ($MgSO_4$), filtered and solvent removed *in vacuo*. The resulting crude residue was further purified by flash column chromatography (5% EtOAc/ petroleum ether 40-60 → 15% EtOAc/ petroleum ether 40-60) to give the title compound (0.42 g, 1.8 mmol, 70%) as a yellow oil. R_f 0.39 (15% EtOAc/ petroleum ether 40-60; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (dt, *J* = 16.0, 5.0 Hz, 1H, CH_2C**H**), 5.80 (dt, *J* = 16.0, 2.0 Hz, 1H, C**H**C), 4.21 (dd, *J* = 5.0, 2.0 Hz, 2H, C**H₂**), 1.47 – 1.40 (m, 1H, O**H**), 1.08 (m, 21H, Si(C**H**(CH_3)$_2$)$_3$, Si(CH(C**H₃**)$_2$)$_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.5 (CH_2**C**H), 110.9 (**C**HC), 104.8 (**C**≡CSi), 91.7

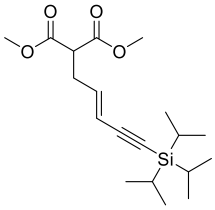
(C≡**C**Si), 63.0 (**C**H_2), 18.6 (Si(CH(**C**H_3)$_2$)$_3$), 11.3 (Si(**C**H(CH_3)$_2$)$_3$). Data are in accordance with the literature.[81]

(*E*)-(5-Bromopent-3-en-1-yn-1-yl)triisopropylsilane (2.34)



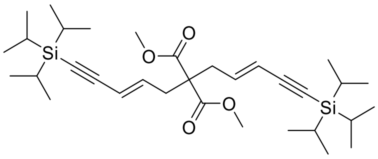
According to procedure of Kurauchi *et al*.:[204] *N*-bromosuccinimide (0.38 g, 2.1 mmol, 1.2 equiv.) and triphenylphosphine (0.56 g, 2.1 mmol, 1.2 equiv.) were added to a stirred solution of (*E*)-5-(triisopropylsilyl)pent-2-en-4-yn-1-ol **2.33** (0.42 g, 1.8 mmol, 1.0 equiv.) in CH_2Cl_2 (20 mL) cooled to -30 °C and the reaction mixture was stirred for 0.5 h before warming to rt. After this time, the reaction solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (petroleum ether 40-60) to give the title compound (0.45 g, 1.5 mmol, 84%) as a pale yellow oil. R_f 0.68 (petroleum ether 40-60); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.23 (dt, *J* = 15.5, 8.0 Hz, 1H, CH_2C**H**), 5.73 (dt, *J* = 15.5, 1.0 Hz, 1H, C**H**C), 3.92 (dd, *J* = 8.0, 1.0 Hz, 2H, C**H₂**), 1.10 – 0.99 (m, 21H, m, Si(C**H**(CH_3)$_2$)$_3$, Si(CH(C**H₃**)$_2$)$_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.5 (**C**HC), 114.8 (CH_2**C**H), 103.8 (**C**≡CSi), 94.2 (C≡**C**Si), 31.6 (**C**H_2), 18.6 (Si(CH(**C**H_3)$_2$)$_3$), 11.2 (Si(**C**H(CH_3)$_2$)$_3$). Data are in accordance with the literature.[204]

Dimethyl (*E*)-2-(5-(triisopropylsilyl)pent-2-en-4-yn-1-yl)malonate (2.36)



Dimethyl malonate (0.52 mL, 4.5 mmol, 3.0 equiv.) was added dropwise to a stirred solution of sodium hydride (60% in mineral oil, 0.18 g, 4.5 mmol, 3.0 equiv.) in DMF (4 mL) at 0 °C and the reaction mixture was stirred for 10 mins. After this time, (*E*)-(5-bromopent-3-en-1-yn-1-yl)triisopropylsilane **2.34** (0.45 g, 1.5 mmol, 1.0 equiv.) in THF (4 mL) was added dropwise followed by potassium iodide (0.12 g, 0.75 mmol, 0.50 equiv.) and the reaction mixture was warmed to rt and stirred for 18 h. The reaction mixture was quenched by addition of sat aq. NH$_4$Cl (20 mL) and the organics extracted with petroleum ether 40-60 (3 × 10 mL), dried (MgSO$_4$), filtered and the solvent removed *in vacuo*. The resulting crude residue was further purified by flash column chromatography (petroleum ether 40-60 → 4% EtOAc/ petroleum ether 40-60) to give the title compound as colourless crystalline solid (0.45 g, 1.3 mmol, 85%). R$_f$ 0.27 (5% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.07 (dt, *J* = 16.0, 7.5 Hz, 1H, CH$_2$C**H**), 5.61 (dt, *J* = 16.0, 1.5 Hz, 1H, C**H**C), 3.73 (s, 6H, 2 × C**H$_3$**), 3.43 (t, *J* = 7.5 Hz, 1H, C**H**), 2.67 (td, *J* = 7.5, 1.5 Hz, 2H, C**H$_2$**), 1.04 (d, *J* = 2.5 Hz, 21H, Si(C**H**(CH$_3$)$_2$)$_3$, Si(CH(C**H$_3$**)$_2$)$_3$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 169.0 (C=O), 139.6 (CH$_2$**C**H), 113.3 (**C**HC), 104.9 (**C**≡CSi), 90.6 (C≡**C**Si), 52.7 (O**C**H$_3$), 51.1 (**C**H, 32.1 (**C**H$_2$), 18.6 (Si(CH(**C**H$_3$)$_2$)$_3$), 11.3 (Si(**C**H(CH$_3$)$_2$)$_3$); LRMS *m/z* (ESI$^+$) 353.2 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 353.2144, C$_{19}$H$_{33}$O$_4$Si (M+H$^+$) requires 353.2143.

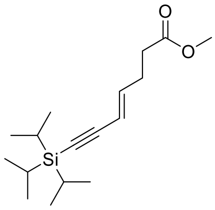
Dimethyl 2,2-bis((*E*)-5-(triisopropylsilyl)pent-2-en-4-yn-1-yl)malonate (2.37)



Isolated as a side-product of the above procedure as a white solid (0.13 g, 0.22 mmol, 15%). R$_f$ 0.47 (5% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.98 (dt, *J* = 15.5, 7.5 Hz, 2H, CH$_2$C**H**), 5.63 (dt, *J* = 15.5, 1.0 Hz, 2H, C**H**C), 3.77 (s, 6H, 2 × C**H$_3$**), 2.71 (dd, *J* = 7.5, 1.5 Hz, 4H, C**H$_2$**CH), 1.09 – 0.99 (m, 42H, (2 × Si(C**H**(CH$_3$)$_2$)$_3$)$_2$, 2 × Si(CH(C**H$_3$**)$_2$)$_3$)$_2$); ^{13}C NMR

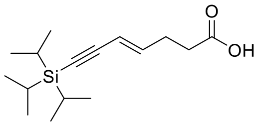
(126 MHz, $CDCl_3$) δ 170.7 (**C**=O), 137.7 (CH_2**C**H), 114.6 (**C**HC), 104.8 (**C**≡CSi), 90.8 (C≡**C**Si), 57.6 (**C**), 52.7 (O**C**H_3), 36.6 (**C**H_2CH), 18.6 (CSi(CH(**C**H_3)$_2$)$_3$)$_2$), 11.3 (Si(**C**H(CH_3)$_2$)$_3$)$_2$); LRMS *m/z* (ESI^+) 595.4 ($M+Na^+$, 100%); HRMS *m/z* (ESI^+): found 573.3788, $C_{33}H_{57}O_4Si_2$ ($M+H^+$) requires 573.3790.

Methyl (*E*)-7-(triisopropylsilyl)hept-4-en-6-ynoate (2.38)



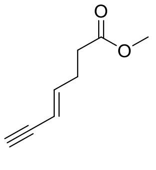
Lithium chloride (0.12 g, 2.7 mmol, 5.0 equiv.) was added to a stirred solution of dimethyl (*E*)-2-(5-(triisopropylsilyl)pent-2-en-4-yn-1-yl)malonate **2.36** (0.19 g, 0.53 mmol, 1.0 equiv.) in DMF (10 mL) and H_2O (0.10 mL) and the reaction mixture was heated to 150 °C for 4 h. After this time, the reaction mixture was cooled to rt and then quenched with H_2O (20 mL). The organics were extracted with Et_2O (3 × 10 mL) and the combined organic layers were washed with brine (5 × 20 mL), dried ($MgSO_4$), and the solvent removed *in vacuo* to give the title compound as an orange oil (0.16 g, 0.53 mmol, 100%) which required no further purification. R_f 0.71 (5% EtOAc/ petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 6.24 – 6.15 (m, 1H, CH_2C**H**), 5.64 – 5.56 (m, 1H, C**H**C≡CSi), 3.71 (s, 3H, CO_2C**H**$_3$), 2.51 – 2.41 (m, 4H, C**H**$_2$$CO_2CH_3$, C**H**$_2$CH), 1.13 – 1.04 (m, 21H, Si(C**H**(CH_3)$_2$)$_3$, Si(CH(C**H**$_3$)$_2$)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1 (**C**=O), 142.8 (CH_2**C**H), 111.3 (**C**HC≡CSi), 105.3 (**C**≡CSi), 89.8 (C≡**C**Si), 51.7 (CO_2**C**H_3), 33.0 (**C**$H_2CO_2CH_3$), 28.1 (**C**H_2CH), 18.6 (Si(CH(**C**H_3)$_2$)$_3$), 11.29 (Si(**C**H(CH_3)$_2$)$_3$); LRMS *m/z* (ESI^+) 295.1 ($M+H^+$, 100%); HRMS *m/z* (ESI^+): found 295.2090, $C_{17}H_{31}O_2Si$ ($M+H^+$) requires 295.2088.

(*E*)-7-(Triisopropylsilyl)hept-4-en-6-ynoic acid (2.39)



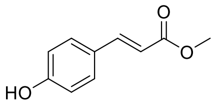
Lithium hydroxide monohydrate (0.017 g, 0.40 mmol, 5.0 equiv.) was added to a stirred solution of methyl (*E*)-7-(triisopropylsilyl)hept-4-en-6-ynoate **2.38** (0.024 g, 0.080 mmol, 1.0 equiv.) in a mixture of THF and H_2O (2:1, 1 mL total) and the reaction mixture was stirred vigorously for 18 h at rt. After this time, the reaction was judged to be complete by TLC analysis and the THF was removed *in vacuo*, pH 1 sulfate buffer (3 mL) was added and the organics were extracted with EtOAc (3 × 5 mL). The organic phase was dried ($MgSO_4$), filtered, and then removed *in vacuo* to give the title compound (0.018 g, 0.064 mmol, 80%) as an off-white solid that required no further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.18 (dt, *J* = 16.0, 6.5 Hz, 1H, (C**H**CH_2), 5.60 (dt, *J* = 16.0, 1.5 Hz, 1H, CC**H**), 2.55 – 2.38 (m, 4H, CHC**H₂**, (C**H₂**CO_2H), 1.11 – 1.02 (m, 21H, ((C**H₃**)$_2$CH)$_3$Si, ((CH_3)$_2$C**H**)$_3$Si); ^{1}H NMR (126 MHz, $CDCl_3$) δ 178.4 (**C**=O), 142.4 (C**C**H), 111.6 (**C**HCH_2), 105.2 (SiC≡**C**), 90.0 (Si**C**≡C), 32.9 (**C**H$_2CO_2$H), 27.8 (CH**C**H$_2$), 18.6 (((**C**H$_3$)$_2$CH)$_3$Si), 11.3 (((CH_3)$_2$**C**H)$_3$Si); LRMS *m/z* (ES$^-$) 279.1 (M-H$^+$, 100%); HRMS *m/z* (ES$^-$): found 279.1787, $C_{16}H_{27}O_2Si$ (M-H$^+$) requires 279.1775.

Methyl (*E*)-hept-4-en-6-ynoate (2.40)



Tetrabutylammonium fluoride solution (1 M in THF, 0.82 mL, 0.82 mmol, 1.8 equiv.) was added to a stirred solution of methyl (*E*)-7-(triisopropylsilyl)hept-4-en-6-ynoate **2.38** (0.14 g, 0.47 mmol, 1.0 equiv.) and glacial acetic acid (0.050 mL, 0.91 mmol, 2.0 equiv.) in THF (4.7 mL) at 0 °C and the reaction mixture was warmed to rt and stirred for 24 h. After this time, the reaction mixture was diluted by addition of H_2O (10 mL) and then the organics were extracted with EtOAc (3 × 20 mL), then the combined organic layers were dried ($MgSO_4$), filtered, and the solvent removed *in vacuo*. The resulting crude residue was further purified by flash column chromatography (petroleum ether 40-60 → 2.5% EtOAc/ petroleum ether 40-60) to give the title compound (0.039 g, 0.28 mmol, 60%) as a pale yellow oil. R_f 0.25 (2.5% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.30 – 6.20 (m, 1H, C**H**CH_2), 5.54 (dq, *J* = 16.0, 1.5 Hz, 1H, CC**H**), 3.70 (s, 3H, $CO_2C\mathbf{H_3}$), 2.83 (d, *J* = 2.0 Hz, 1H, **H**C≡C), 2.51 – 2.40 (m, 4H, $CH\mathbf{CH_2}$, $\mathbf{CH_2}CO_2CH_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.9 (**C**=O), 144.0 (**C**$CHCH_2$), 109.9 (C**C**H), 82.0 (HC≡**C**), 76.5 (H**C**≡C), 51.7 (**C**H_3), 32.9 (**C**$H_2CO_2CH_3$), 28.1 ($CH\mathbf{C}H_2$); LRMS *m/z* (ESI$^+$) 139.0 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 139.0754, $C_8H_{11}O_2$ (M+H$^+$) requires 139.0754.

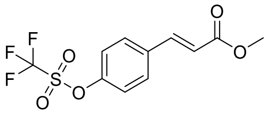
Methyl *(E)*-3-(4-hydroxyphenyl)acrylate (2.49_1)



According to the modified procedure of Pirrung *et al.*:[205] sulfuric acid (5 drops) was added to a stirred solution of *(E)*-3-(4-hydroxyphenyl)acrylic acid **2.43** (1.0 g, 6.1 mmol, 1.0 equiv.) in MeOH (4 mL) and the reaction mixture was heated to reflux for 18 h. The reaction mixture cooled and then solvent removed *in vacuo*, and the crude residue was redissolved in EtOAc (20 mL). The organic layer was then washed with sat. $NaHCO_3$ solution (2 × 20 mL), brine (20 mL), dried ($MgSO_4$), filtered, and solvent removed *in vacuo* to give the title compound as a pale yellow oil (1.1 g, 6.0 mmol, 98%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 16.0 Hz, 1H, C**H**), 7.36 (d, *J* = 8.5 Hz, 2H, 2 × ArC**H**), 6.78 (d, *J* = 8.5 Hz, 2H 2 × ArC**H**), 6.23 (d, *J* = 16.0 Hz, 1H, C**H**CO_2CH_3), 3.73 (s, 3H, C$\mathbf{H_3}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.7 (**C**=O), 144.6 (**C**H), 130.0

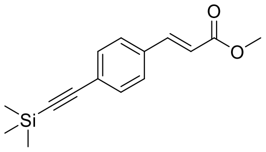
(Ar**C**H), 128.1 (**C**OH), 127.2 (Ar**C**CH), 115.8 (Ar**C**H), 115.2 (**C**HCO$_2$CH$_3$), 51.7 (**C**H$_3$); LRMS *m/z* (ESI$^-$) 177.0 (M-H$^+$, 100%). Data are in accordance with the literature.[205]

Methyl *(E)*-3-(4-(((trifluoromethyl)sulfonyl)oxy)phenyl)acrylate (2.49)



According to procedure of Lee *et al.*:[206] triflic anhydride (1.2 mL, 7.2 mmol, 1.2 equiv.) was added dropwise to a solution of methyl *(E)*-3-(4-hydroxyphenyl)acrylate **2.49_1** (1.1 g, 6.0 mmol, 1.0 equiv.) and pyridine (1.1 mL, 13 mmol, 2.2 equiv.) in CH$_2$Cl$_2$ (20 mL) at 0 °C and then the reaction mixture was stirred for 1 h. The reaction mixture was quenched by addition of H$_2$O (20 mL) and the layers were separated. The aqueous layer was extracted with CH$_2$Cl$_2$ (3 × 20 mL) and the combined organic layers were dried (MgSO$_4$), filtered, and the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60) to give the title compound as a white solid (1.6 g, 5.1 mmol, 85%); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.67 (d, *J* = 16.0 Hz, 1H, C**H**), 7.60 (d, *J* = 8.5 Hz, 2H, 2 × ArC**H**), 7.30 (d, *J* = 8.5 Hz, 2H, 2 × ArC**H**), 6.44 (d, *J* = 16.0 Hz, 1H, C**H**CO$_2$CH$_3$), 3.82 (s, 3H, C**H$_3$**); ^{13}C NMR (101 MHz, CDCl$_3$) δ 166.7 (**C**=O), 150.3 (Ar**C**O), 142.4 (**C**H), 134.7 (Ar**C**CH), 129.7 (Ar**C**H), 121.9 (Ar**C**H), 119.9 (**C**HCO$_2$CH$_3$), 118.8 (q, *J* = 318.0 Hz, **C**F$_3$), 51.86 (**C**H$_3$). Data are in accordance with the literature.[206]

Methyl *(E)*-3-(4-((trimethylsilyl)ethynyl)phenyl)acrylate (2.50)

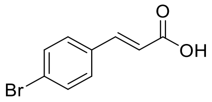


According to the modified procedure of Chisholm *et al.*:[207] bis(triphenylphosphine)palladium(II) chloride (0.0090 g, 0.013 mmol, 2.0 mol%) was added to a solution of methyl *(E)*-3-(4-(((trifluoromethyl)sulfonyl)oxy)phenyl)acrylate **2.50** (0.20 g, 0.65 mmol, 1.0 equiv.), ethynyltrimethylsilane (0.18 mL, 1.3 mmol, 2.0 equiv.), triethylamine (0.17 mL, 1.3 mmol, 2.0 equiv.), and copper(I) iodide (0.0045 g, 0.078 mmol, 4.0 mol%) in N_2 sparged MeCN (10 mL). The reaction mixture was heated to 90 °C and stirred for 16 h. After this time, the solvent was removed *in vacuo* and the resulting crude residue was further purified by flash column chromatography (5% EtOAc/ petroleum ether 40-60→ 20% EtOAc/ petroleum ether 40-60) giving the title compound as a white solid (0.093 g, 0.36 mmol, 55%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 16.0 Hz, 1H, C**H**), 7.49 – 7.42 (m, 4H, 4 × Ar**C**H), 6.43 (d, *J* = 16.0 Hz, 1H, C**H**CO_2CH_3), 3.81 (s, 3H, C**H$_3$**), 0.26 (s, 9H, Si(C**H$_3$**)$_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2 (**C**=O), 143.8 (**C**H), 134.3 (Ar**C**), 132.4 (Ar**C**H), 127.8 (Ar**C**H), 125.0 (Ar**C**), 118.5 (**C**HCO_2CH_3), 104.4 (**C**≡CSi), 96.7 (C≡**C**Si), 51.7 (**C**H$_3$), -0.15 (Si(**C**H$_3$)$_3$) LRMS *m/z* (ESI$^+$) 281.1 (M+Na$^+$, 100%). Data are in accordance with the literature.[207]

OR

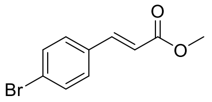
According to the modified procedure of Chisholm *et al.*:[207] bis(triphenylphosphine)palladium(II) chloride (0.014 g, 0.020 mmol, 2.0 mol%) was added to a solution of methyl *(E)*-3-(4-bromophenyl)acrylate **2.53** (0.24 g, 1.0 mmol, 1.0 equiv.), ethynyltrimethylsilane (0.28 mL, 2.0 mmol, 2.0 equiv.), triethylamine (0.28 mL, 2.0 mmol, 2.0 equiv.), and copper(I) iodide (0.0076 g, 0.040 mmol, 4.0 mol%) in N_2 sparged MeCN (10 mL). The reaction mixture was heated to 90 °C and stirred for 16 h. After this time, the solvent was removed *in vacuo* and the resulting crude residue was further purified by flash column chromatography (5% EtOAc/ petroleum ether 40-60→ 20% EtOAc/ petroleum ether 40-60) giving the title compound (0.13 g, 0.52 mmol, 52%) as a white solid. Data as above.

(*E*)-3-(4-Bromophenyl)acrylic acid (2.52)



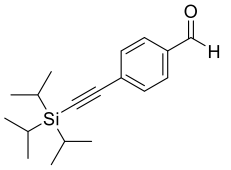
According to procedure of Upare *et al*.:[208] piperidine (0.050 mL, 0.51 mmol, 0.1 equiv.) was added to a stirred solution of 4-bromobenzaldehyde **2.51** (0.93 g, 5.0 mmol, 1.0 equiv.) and propanedioic acid (1.2 g, 11 mmol, 2.2 equiv.) in pyridine (2.5 mL) and the reaction mixture was heated to reflux for 2 h. After this time, the reaction mixture was cooled to rt and poured slowly over ice cold 2 M HCl (40 mL). The precipitate that was formed was filtered off and dried *in vacuo* to give the title compound (0.85 g, 3.8 mmol, 75%) as a white solid which required no further purification. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.47 (s, 1H, COO**H**), 7.68 – 7.63 (m, 2H, 2 × ArC**H**), 7.63 – 7.59 (m, 2H, 2 × ArC**H**), 7.56 (d, *J* = 16.0 Hz, 1H, C**H**COOH), 6.56 (d, *J* = 16.0 Hz, 1H, C**H**); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 167.4 (**C**OOH), 142.6 (**C**H), 133.5 (Ar**C**), 131.9 (Ar**C**H), 130.2 (ArC**H**), 123.5 (Ar**C**Br), 120.1 (**C**HCOOH). Data are in accordance with the literature.[208]

Methyl (*E*)-3-(4-bromophenyl)acrylate (2.53)



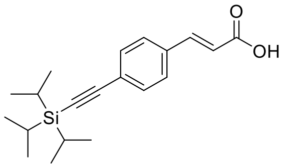
According to the modified procedure of Stone *et al.*:[209] sulfuric acid (5 drops) was added to a stirred solution of (*E*)-3-(4-bromophenyl)acrylic acid **2.52** (0.45 g, 2.0 mmol, 1.0 equiv.) in MeOH (5 mL) and the reaction mixture was heated to reflux for 18 h. After this time, the reaction mixture was cooled to rt and the solvent removed *in vacuo* and then redissolved in EtOAc (15 mL). The organic layer was washed with sat. $NaHCO_3$ (10 mL), H_2O (2 × 10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo* to give the title compound (0.43 g, 1.8 mmol, 90%) as a white solid which required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 16.0 Hz, 1H, C**H**), 7.52 (d, *J* = 8.5 Hz, 2H, 2 × Ar**H**), 7.38 (d, *J* = 8.5 Hz, 2H, 2 × Ar**H**), 6.42 (d, *J* = 16.0 Hz, 1H, C**H**CO_2CH_3), 3.80 (s, 3H, C**H₃**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1 (**C**=O), 143.4 (**C**H), 133.3 (Ar**C**CH), 132.1 (Ar**C**H), 129.4 (Ar**C**H), 124.5 (Ar**C**Br), 118.5 (**C**HCO_2CH_3), 51.7 (C**H₃**). Data are in accordance with the literature.[209]

4-((Triisopropylsilyl)ethynyl)benzaldehyde (2.54)



According to the modified procedure of Charvet *et al.*:[210] tetrakis(triphenylphosphine)palladium (0.054 g, 0.050 mmol, 5.0 mol%) was added to a solution containing 4-bromobenzaldehyde **2.51** (0.19 g, 1.0 mmol, 1.0 equiv.), (triisopropylsilyl)acetylene (0.34 mL, 1.5 mmol, 1.5 equiv.), copper(I) iodide (0.019 g, 0.010 mmol, 10 mol%) and diisopropylamine (0.14 mL, 1.0 mmol, 1.0 equiv.) in toluene (2 mL, freeze pump thaw degassed, three cycles). The reaction vessel was sealed and then stirred for 4 h at rt, TLC analysis at this stage confirmed full consumption of starting material so the reaction mixture was diluted with CH_2Cl_2 (10 mL) and then washed with H_2O. The organic layer was dried ($MgSO_4$) and the solvent removed *in vacuo* giving a crude residue which was further purified by flash column chromatography (pentane → 20% CH_2Cl_2/ pentane) giving the title compound (0.26 g, 0.91 mmol, 91%) as a pale yellow oil. ^1H NMR (400 MHz, $CDCl_3$) δ 10.00 (s, 1H, CO**H**), 7.86 – 7.78 (m, 2H, 2 × ArC**H**), 7.66 – 7.56 (m, 2H, 2 × ArC**H**), 1.14 (m, 21H, Si(C**H**(CH_3)$_2$)$_3$, Si(CH(C**H$_3$**)$_2$)$_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.4 (**C**=O), 135.5 (Ar**C**), 132.6 (ArCH), 129.7 (Ar**C**), 129.5 (Ar**C**H), 105.9 (**C**≡CSi), 95.8 (C≡**C**Si), 18.6 (Si(CH(**C**H_3)$_2$)$_3$), 11.3 (Si(**C**H(CH_3)$_2$)$_3$). Data are in agreement with the literature.[210]

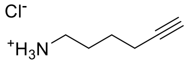
(*E*)-3-(4-((Triisopropylsilyl)ethynyl)phenyl)acrylic acid (2.55)



Piperidine (1 drop) was added to a stirred solution of 4-((triisopropylsilyl)ethynyl)benzaldehyde **2.54** (0.057 g, 0.20 mmol, 1.0 equiv.) and propanedioic acid (0.046 g, 0.44 mmol, 2.2 equiv.) in pyridine (1 mL) and the reaction was heated to reflux for 2 h. After this time, the reaction was judged to be complete by TLC and consequently cooled to rt. pH 1 sulfate buffer (5 mL) was added to the reaction mixture and then organics were extracted with EtOAc (3 × 5 mL), the organic layer was dried ($MgSO_4$), and the solvent removed *in vacuo* to give the title compound (0.062 g, 0.19 mmol, 95%) as a pale yellow solid which required no further purification. R_f 0.0 (20% EtOAc/ petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 16.0 Hz, 1H, C**H**), 7.49 (s, 4H, 4 × Ar**H**), 6.44 (d, *J* =

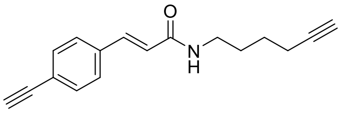
16.0 Hz, 1H, C**H**COOH), 1.17 – 1.08 (m, 21H, Si(C**H**(CH$_3$)$_2$)$_3$, Si(CH(C**H$_3$**)$_2$)$_3$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 171.7 (**C**=O), 146.1 (**C**H), 133.7 (Ar**C**), 132.5 (Ar**C**H), 128.1 (Ar**C**H), 125.9 (Ar**C**), 117.7 (**C**HCOOH), 106.4 (**C**≡CSi), 93.7 (C≡**C**Si), 18.6 (Si(CH(**C**H$_3$)$_2$)$_3$), 11.2 (Si(**C**H(CH$_3$)$_2$)$_3$); LRMS *m/z* (ESI$^-$) 327.1 (M-H$^+$, 100%); HRMS *m/z* (ESI$^-$): found 327.1787, $C_{20}H_{27}O_2Si$ (M-H$^+$) requires 327.1786.

Hex-5-yn-1-amine hydrochloride (2.58)



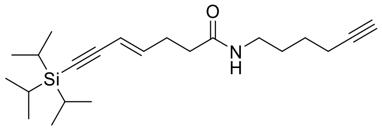
According to procedure of Altman *et al.*:[211] triphenylphosphine (27 g, 100 mmol, 1.0 equiv.) and phthalimide (15 g, 100 mmol, 1.0 equiv.) were added to a stirred solution of hex-5-yn-1-ol **2.56** (10 g, 100 mmol, 1.0 equiv.) and diisopropyl azodicarboxylate (20 mL, 102 mmol, 1.0 equiv.) in THF (64 mL) degassed with N_2 for 0.5 h and cooled to 0 °C. The ice bath was removed and the solution was allowed to stir for 18 h at room temperature. Hydrazine hydrate (2.0 g, 8.0 mL, 61 mmol, 0.6 equiv.) was added and the reaction mixture heated to reflux for 6 h. The reaction mixture was cooled to rt then conc. HCl (20 mL) was added portionwise. The reaction mixture was heated under reflux for 2 h and then allowed to cool to rt and stirred for 18 h. The white precipitate was filtered off and the THF was removed completely *in vacuo* then CH_2Cl_2 (300 mL) and water (400 mL) were added to the resulting solid. The layers were separated, and the aqueous phase further extracted with CH_2Cl_2 (3 x 250 mL). The aqueous phase was dried thoroughly *in vacuo* to give the title compound as an off white solid (9.9 g, 73 mmol, 73%) which was used without further purification. ^{1}H NMR (400 MHz, methanol-*d$_4$*) δ 2.90 (t, *J* = 7.5, 2H, NC**H**$_2$), 2.25 – 2.16 (m, 3H, C**H**, C**H$_2$**C≡CH), 1.82 – 1.65 (m, 2H, NCH$_2$C**H$_2$**), 1.54 (m, 2H, C**H$_2$**CH$_2$C); ^{13}C NMR (101 MHz, methanol-*d$_4$*) δ 84.1 (**C**H), 70.4 (CH$_2$**C**), 40.3 (N**C**H$_2$), 27.6 (**C**H$_2$C), 26.4 (NCH$_2$**C**H$_2$) , 18.6 (**C**H$_2$CH$_2$C). Data are in accordance with literature.[211]

***(E)*-3-(4-ethynylphenyl)-*N*-(hex-5-yn-1-yl)acrylamide (2.59)**



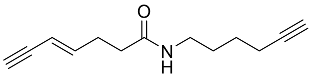
1-Hydroxybenzotriazole hydrate (0.052 g, 0.40 mmol, 1.5 equiv.) and *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (0.077 g, 0.40 mmol, 1.5 equiv.) was added to a stirred solution of *(E)*-3-(4-ethynylphenyl)acrylic acid (0.046 g, 0.27 mmol, 1.0 equiv.), hex-5-yn-1-amine (0.036 g, 0.27 mmol, 1.0 equiv.) and triethylamine (0.077 mL, 0.54 mmol, 2.0 equiv.)in CH_2Cl_2 (1.5 mL) and the reaction mixture was heated to 40 °C for 18 h. The reaction mixture was cooled to rt and the solvent removed *in vacuo* giving a crude residue which was further purified by flash column chromatography (40% EtOAc/ petroleum ether 40-60) to give the title compound as a white solid (0.026 g, 0.097 mmol, 36%). R_f 0.26 (40% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (d, *J* = 15.5 Hz, 1H, C**H**), 7.45 (q, *J* = 8.0 Hz, 4H, 4 × ArC**H**), 6.39 (d, *J* = 15.5 Hz, 1H, C**H**CO_2), 5.83 (t, *J* = 6.0 Hz, 1H, N**H**), 3.43 – 3.38 (m, 2H, C**H**$_2$), 3.16 (s, 1H, C≡C**H**), 2.24 (td, *J* = 7.0, 2.5 Hz, 2H, C**H$_2$**), 2.04 (s, 1H, C≡C**H**) 1.71 (dq, *J* = 12.0, 8.0, 7.0 Hz, 2H, C**H$_2$**), 1.59 (p, *J* = 7.0 Hz, 2H, C**H$_2$**); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.6 (**C**=O), 140.0 (**C**H), 135.2 (Ar**C**), 132.5 (Ar**C**H), 127.6 (Ar**C**H), 123.3 (Ar**C**), 121.7 (**C**HCO_2), 84.0 (ArC≡**C**H), 83.3 (C≡**C**H), 78.9 (Ar**C**≡CH), 68.8 (**C**≡CH), 39.3 (**C**H_2), 28.7 (**C**H_2), 25.7 (**C**H_2), 18.1 (**C**H_2); LRMS *m/z* (ESI$^+$) 252.2 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 252.1385, $C_{17}H_{18}NO$ (M+H$^+$) requires 252.1383.

(*E*)-*N*-(hex-5-yn-1-yl)-7-(triisopropylsilyl)hept-4-en-6-ynamide (2.60)



1-Hydroxybenzotriazole hydrate (0.013 g, 0.090 mmol, 1.5 equiv.) and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (0.019 g, 0.090 mmol, 1.5 equiv.) were added successively to a stirred solution of (*E*)-7-(triisopropylsilyl)hept-4-en-6-ynoic acid **2.39** (0.018 g, 0.060 mmol, 1.0 equiv.), hex-5-yn-1-amine hydrochloride (0.012 g, 0.090 mmol, 1.5 equiv.) and triethylamine (0.020 mL, 0.14 mmol, 2.0 equiv.) in CH_2Cl_2 (1 mL) and the reaction mixture was stirred for 18 h at rt. After this time, the reaction solvent was removed *in vacuo* and the resulting crude residue was further purified by flash column chromatography (CH_2Cl_2 → 3% acetone/CH_2Cl_2) giving the title compound (0.010 g, 0.027 mmol, 47%) as a colourless oil. R_f 0.62 (5% acetone/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.17 (dt, *J* = 16.0, 7.0 Hz, 1H, (CC**H**), 5.58 (dt, *J* = 16.0, 1.5 Hz, 1H, (CHCH$_2$), 5.49 (s, br, 1H, (CON**H**), 3.32 – 3.24 (m, 2H, NHC**H$_2$**), 2.45 (dtd, *J* = 8.0, 7.0, 1.5 Hz, 2H, C**H$_2$**), 2.30 – 2.20 (m, 4H, (CHC**H$_2$**, C**H$_2$**C), 1.96 (t, *J* = 2.7 Hz, 1H, CH), 1.68 – 1.50 (m, 4H, NHCH$_2$C**H$_2$**, C**H$_2$**CH$_2$C), 1.14 – 1.01 (m, 21H, ((CH$_3$)$_2$C**H**)$_3$Si, ((C**H$_3$**)$_2$CH)$_3$Si); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.7 (**C**=O), 143.2 (**C**HCH$_2$), 111.3 (SiC≡C**C**H), 105.4 (SiC≡**C**), 89.8 (Si**C**≡C), 84.0 (**C**H), 68.8 (CH$_2$**C**), 39.0 (NH**C**H$_2$), 35.6 (**C**H$_2$C), 28.90 (**C**H$_2$CONH), 28.7 (NHCH$_2$**C**H$_2$), 25.6 (**C**H$_2$CH$_2$C), 18.6 (CH**C**H$_2$), 18.1 (((**C**H$_3$)$_2$CH)$_3$Si), 11.3 (((CH$_3$)$_2$**C**H)$_3$Si); LRMS *m/z* (ESI$^+$) 382.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 360.2717, $C_{22}H_{38}ONSi$ (M+H$^+$) requires 360.2717.

(*E*)-*N*-(hex-5-yn-1-yl)hept-4-en-6-ynamide (1.64)

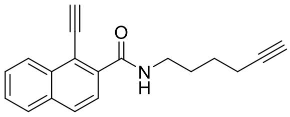


Acetic acid (0.0040 mL, 0.054 mmol, 1.9 equiv.) and TBAF (1 M in THF, 0.049 mL, 0.049 mmol, 1.8 equiv.) were added to a stirred solution of (*E*)-*N*-(hex-5-yn-1-yl)-7-(triisopropylsilyl)hept-4-en-6-ynamide **2.60** (0.010 g, 0.028 mmol, 1.0 equiv.) in THF (0.5 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the reaction solvent was removed *in vacuo* and the resulting crude residue was purified by flash column chromatography (5% acetone/CH_2Cl_2) to give the title compound (0.0020 g, 0.0098 mmol, 35%) as a colourless oil. R_f 0.5 (5% acetone/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.22 (dt, *J* = 16.0, 7.0 Hz, 1H, $C\mathbf{H}CH_2$), 5.52 (dq, *J* = 16.0, 2.0 Hz, 1H, $CC\mathbf{H}$), 5.45 (s, 1H, $N\mathbf{H}$), 3.32 – 3.24 (m, 2H, $NHC\mathbf{H_2}$), 2.80 (d, *J* = 2.0 Hz, 1H, $\mathbf{H}C{\equiv}C$), 2.46 (qd, *J* = 7.5, 1.5 Hz, 2H, $CHC\mathbf{H_2}CH_2CONH$), 2.27 – 2.20 (m, 4H, $C\mathbf{H_2}CONH$, $C\mathbf{H_2}C{\equiv}CH$), 1.96 (t, *J* = 2.5 Hz, 1H, $CH_2C{\equiv}C\mathbf{H}$), 1.60 (s, 4H, $NHCH_2C\mathbf{H_2}$, $C\mathbf{H_2}CH_2C$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.5 ($\mathbf{C}{=}O$), 144.4 ($\mathbf{C}HCH_2$), 109.9 ($C\mathbf{C}H$), 84.0 ($CH_2\mathbf{C}{\equiv}CH$), 82.0 ($HC{\equiv}\mathbf{C}CH$), 76.4 ($H\mathbf{C}{\equiv}CCH$), 68.8 ($CH_2C{\equiv}\mathbf{C}H$), 39.0 ($NH\mathbf{C}H_2$), 35.5 ($\mathbf{C}H_2CONH$), 28.9 ($CH\mathbf{C}H_2$), 28.7 ($NHCH_2\mathbf{C}H_2$), 25.7 ($\mathbf{C}H_2CH_2C$), 18.10 ($\mathbf{C}H_2C$); LRMS *m/z* (ESI$^+$) 204.2 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 204.1385, $C_{13}H_{18}ON$ (M+H$^+$) requires 204.1383. Data are in accordance with the literature.[69]

OR

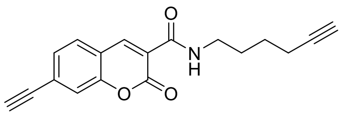
Propylphosphonic anhydride solution (≥50 wt. % in ethyl acetate, 0.18 mL, 0.60 mmol, 1.4 equiv.) was added to a stirred solution of *(E)*-hept-4-en-6-ynoic acid **2.29** (0.050 g, 0.40 mmol, 1.0 equiv.), hex-5-yn-1-amine (0.060 g, 0.48 mmol, 1.2 equiv.), and *N*,*N*-diisopropylethylamine (0.27 mL, 1.6 mmol, 3.0 equiv.) in CH_2Cl_2 (3 mL) and the reaction mixture was stirred at rt for 18 h. The reaction solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (1% MeOH/CH_2Cl_2) giving the title compound as a white solid (0.030 g, 0.16 mmol, 40%). Data as above.

1-Ethynyl-*N*-(hex-5-yn-1-yl)-2-naphthamide (1.60)



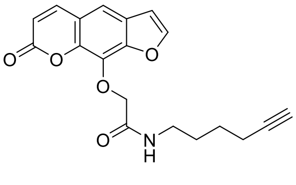
According to the modified procedure of Wright *et al.*:[69] propylphosphonic anhydride solution (≥50 wt. % in ethyl acetate, 0.12 mL, 0.36 mmol, 1.4 equiv.) was added to a stirred solution 1-ethynyl-2-naphthoic acid **2.4** (0.050 g, 0.26 mmol, 1.0 equiv.), hex-5-yn-1-amine (0.040 g, 0.31 mmol, 1.2 equiv.), and DIPEA (0.18 mL, 1.0 mmol, 3.0 equiv.) in CH_2Cl_2 (6 mL) at room temperature. The reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by column chromatography (20% EtOAc/ petrol 40-60 → 30% EtOAc/ petrol 40-60) yielding the title compound as a white solid (0.030 g, 0.052 mmol, 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (ddt, *J* = 8.5, 1.5, 1.0 Hz, 1H, ArC**H**), 7.99 – 7.83 (m, 3H, 3 × ArC**H**), 7.68 – 7.53 (m, 2H, 2 × ArC**H**), 7.21 (s, 1H, CON**H**), 3.93 (s, 1H, C**H**), 3.58 (td, *J* = 7.0, 5.5 Hz, 2H, C**H**$_2$), 2.29 (td, *J* = 7.0, 2.5 Hz, 2H, C**H**$_2$), 1.98 (t, *J* = 2.5 Hz, 1H, C**H**), 1.89 – 1.75 (m, 2H, C**H**$_2$), 1.74 – 1.67 (m, 2H, C**H**$_2$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1 (**C**=O), 136.4 (**C**CONH), 133.7 (Ar**C**), 133.3 (Ar**C**), 129.6 (Ar**C**H), 128.3 (Ar**C**H), 127.7 (Ar**C**H), 127.7 (Ar**C**H), 126.9 (Ar**C**H), 125.6 (Ar**C**H), 116.1 (**C**C≡CH), 89.1 (CC≡**C**H), 84.1 (C≡**C**H), 80.0 (C**C**≡CH), 68.8 (C≡**C**H), 39.7 (**C**H$_2$), 28.5 (**C**H$_2$), 25.9 (**C**H$_2$), 18.2 (**C**H$_2$); LRMS *m/z* (ES$^+$) 276.4 (M+H$^+$, 100%). Data are in accordance with the literature.[69]

7-Ethynyl-*N*-(hex-5-yn-1-yl)-2-oxo-2*H*-chromene-3-carboxamide (1.62)



According to the modified procedure of Wright *et al*.:[69] propylphosphonic anhydride solution (≥50 wt. % in ethyl acetate, 0.10 mL, 0.35 mmol, 1.4 mmol) was added to a stirred solution of 7-ethynyl-2-oxo-2*H*-chromene-3-carboxylic acid **2.20** (0.050 g, 0.23 mmol, 1.0 equiv.), hex-5-yn-1-amine (0.040 g, 0.28 mmol, 1.2 equiv.), and *N*,*N*-diisopropylethylamine (0.10 mL, 0.69 mmol, 3.0 equiv.) in CH_2Cl_2 (3 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* and the resulting crude residue further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) giving the title compound as a white solid (0.050 g, 0.16 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) 8.90 (d, *J* =0.5 Hz, 1H, ArC**H**), 8.80 (s, 1H, ArC**H**), 7.66 (d, *J* = 8.0 Hz, 1H, ArC**H**), 7.48 (dd, *J* = 8.0 Hz, 1.5, ArC**H**), 3.52 (td, *J* = 7.0, 6.0 Hz, 2H, C**H**$_2$), 3.38 (s, 1H, C**H**), 2.28 (td, *J* = 7.0, 2.5 Hz, 2H, C**H**$_2$), 1.99 (t, *J* = 2.5 Hz, 1H, C**H**), 1.85 – 1.73 (m, 2H, C**H$_2$**), 1.72 – 1.61 (m, 2H, C**H**$_2$); LRMS *m/z* (ESI$^+$) 294.3 (M+H$^+$, 100%). Data are in accordance with the literature.[69]

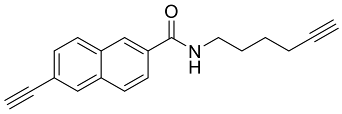
N-(Hex-5-yn-1-yl)-2-((7-oxo-*7H*-furo[3,2-*g*]chromen-9-yl)oxy)acetamide (1.63)



Propylphosphonic anhydride solution (≥50 wt. % in ethyl acetate, 0.15 mL, 0.52 mmol, 1.4 equiv.) was added to a stirred solution of 2-((7-oxo-*7H*-furo[3,2-*g*]chromen-9-

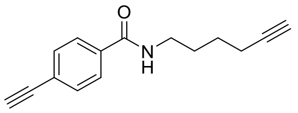
yl)oxy)acetic acid **2.24** (0.10 g, 0.38 mmol, 1.0 equiv.), hex-5-yn-1-amine (0.050 g, 0.46 mmol, 1.2 equiv.), and DIPEA (0.50 mL, 1.0 mmol, 3.0 equiv.) in CH_2Cl_2 (6 mL) at room temperature. The reaction mixture was stirred overnight at rt. After this time, the reaction mixture was concentrated *in vacuo* to give a crude residue which was further purified by flash column chromatography (1% MeOH/CH_2Cl_2 → 2.5% MeOH/CH_2Cl_2) to give the title compound as a white solid (0.08 g, 0.25 mmol, 65%). ^{1}H NMR (400 MHz, Acetone-*d$_6$*) δ 8.05 (d, *J* = 10.0 Hz, 1H, OCOCHC**H**), 7.98 (d, *J* = 2.0 Hz, 1H, OCHC**H**), 7.66 (s, 1H, ArC**H**), 7.64 (s, br, 1H, N**H**), 7.03 (d, *J* = 2.0 Hz, 1H, OC**H**CH), 6.38 (d, *J* = 10.0 Hz, 1H, OCOC**H**CH), 4.87 (s, 2H, OC**H₂**), 3.37 (q, *J* = 7.0 Hz, 2H, NHC**H₂**), 2.31 (t, *J* = 2.5 Hz, 1H, C**H**), 2.21 (td, *J* = 7.0, 2.5 Hz, 2H, C**H₂**C), 1.75 – 1.63 (m, 2H, NHCH$_2$C**H₂**), 1.61 – 1.49 (m, 2H, C**H₂**CH$_2$C); ^{13}C NMR (101 MHz, Acetone-*d$_6$*) δ 168.3 (**C**=O), 160.2 (**C**=O), 148.5 (OCH**C**H), 148.3 (Ar**C**O), 145.6 (OCOCH**C**H), 144.1 (Ar**C**), 131.7 (Ar**C**), 127.2 (Ar**C**), 117.7 (Ar**C**), 115.7 (Ar**C**H), 115.4 (OCO**C**HCH), 108.0 (O**C**HCH), 84.8 (**C**≡CH), 73.1 (O**C**H$_2$), 70.2 (C≡C**H**), 39.0 (NH**C**H$_2$), 29.5 (NHCH$_2$**C**H$_2$), 26.6 (**C**H$_2$CH$_2$C), 18.5 (**C**H$_2$C); LRMS *m/z* (ESI$^+$) 340.2 (M+H$^+$, 100%). Data are in accordance with the literature.[69]

6-Ethynyl-*N*-(hex-5-yn-1-yl)-2-naphthamide (1.59)



According to the modified procedure of Wright *et al.*:[69] propylphosphonic anhydride solution (≥50 wt. % in ethyl acetate, 0.23 mL, 0.77 mmol, 1.3 equiv.) was added to a stirred solution 6-ethynyl-2-naphthoic acid **2.7** (0.10 g, 0.51 mmol, 1.0 equiv.), hex-5-yn-1-amine (0.080 g, 0.61 mmol, 1.1 equiv.), and DIPEA (0.25 mL, 1.5 mmol, 3.0 equiv.) in CH_2Cl_2 (6 mL) at room temperature. The reaction mixture was stirred for 18 h at rt. After this time the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/petroleum ether 40-60 → 30% EtOAc/petroleum ether 40-60) to give the title compound (0.060 g, 0.22 mmol, 43%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, *J* = 1.5 Hz, 1H, Ar**H**), 8.02 (d, *J* = 1.5 Hz, 1H, Ar**H**), 7.87 – 7.81 (m, 3H, 3 × Ar**H**), 7.56 (dd, *J* = 8.5, 1.5 Hz, 1H, Ar**H**), 6.41 (t, br, *J* = 5.5 Hz, 1H, N**H**), 3.54 (td, *J* = 7.0, 5.5 Hz, 2H, NHC**H₂**), 3.20 (s, 1H, ArC≡C**H**), 2.27 (td, *J* = 7.0, 2.5 Hz, 2H, C**H₂**C), 1.98 (t, *J* = 2.5 Hz, 1H, CH_2C≡C**H**), 1.79 (tt, *J* = 7.5, 6.5 Hz, 2H, NHCH$_2$C**H₂**), 1.70 – 1.60 (m, 2H, C**H₂**CH$_2$C); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.3 (**C**=O), 134.1 (Ar**C**), 132.8 (Ar**C**), 132.2 (Ar**C**), 132.0 (Ar**C**H), 129.5 (Ar**C**H), 129 (Ar**C**H), 128.3 (Ar**C**H), 127.1 (Ar**C**H), 124.4 (Ar**C**H), 121.3 (Ar**C**), 84.1 (Ar**C**≡CH), 83.6 (CH$_2$**C**≡CH), 78.6 (ArC≡**C**H), 68.9 (CH$_2$C≡**C**H), 39.7 (NH**C**H$_2$), 28.7 NHCH$_2$**C**H$_2$, 25.8 (**C**H$_2$CH$_2$C), 18.2 (**C**H$_2$C); LRMS *m/z* (ESI$^+$) 276.4 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 276.1383, $C_{19}H_{18}ON$ (M+H$^+$) requires 276.1383. Data are in accordance with the literature.[69]

4-Ethynyl-*N*-(hex-5-yn-1-yl)benzamide (1.61)

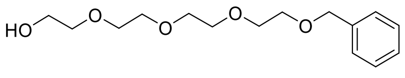


TBAF (1 M in THF, 0.35 mL, 0.35 mmol, 3.5 equiv.) was added to a stirred solution of 4-((triisopropylsilyl)ethynyl)-*N*-(6-(trimethylsilyl)hex-5-yn-1-yl)benzamide **2.12** (0.045 g, 0.10 mmol, 1.0 equiv.) and AcOH (0.022 mL, 0.39 mmol, 3.9 equiv.) in THF (1 mL) at 0 °C and the reaction mixture was stirred for 1 h. The reaction mixture was then warmed to rt and stirred a further 4 h before quenching with H_2O. The layers were separated and the aqueous layer extracted with EtOAc (3 × 5 mL), the combined organic layers were then dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 25% EtOAc/ petroleum ether 40-60) to give the title compound (0.015 g, 0.067 mmol, 67%) as a white solid. R_f 0.26 (20% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 – 7.71 (m, 2H, 2 × ArC**H**), 7.58 – 7.52 (m, 2H, 2 × ArC**H**), 6.31 (d, *J* = 6.0 Hz, 1H, N**H**), 3.50 (td, *J* = 7.0, 5.5 Hz, 2H, NHC**H₂**), 3.21 (s, 1H, ArC≡C**H**), 2.28 (td, *J* = 7.0, 2.5 Hz, 2H, C**H₂**C), 2.00 (t, *J* = 2.5 Hz, 1H, CH_2C≡C**H**), 1.82 – 1.72 (m, 2H, NHCH₂C**H₂**), 1.69 – 1.59 (m, 2H, C**H₂**C); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8 (**C**=O), 134.7 (Ar**C**), 132.3 (Ar**C**H), 126.9 (Ar**C**H), 125.3 (Ar**C**), 84.0 (CH₂**C**), 82.8 (Ar**C**≡CH), 79.5 (ArC≡**C**H), 68.9 (CH₂C≡**C**H), 39.6 (NH**C**H₂), 28.6 (NHCH₂**C**H₂), 25.7 (**C**H₂CH₂C), 18.1 (**C**H₂C); LRMS *m/z* (ESI$^+$) 248.0 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 226.1227, $C_{15}H_{16}ON$ (M+H$^+$) requires 226.1226. Data are in accordance with the literature.[69]

OR

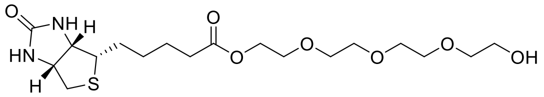
Propylphosphonic anhydride solution (50% in EtOAc, 0.25 mL, 1.0 mmol, 1.4 equiv.) was added to a stirred solution of 4-ethynylbenzoic acid (0.10 g, 0.68 mmol, 1.0 equiv.), hex-5-yn-1-amine hydrochloride (0.11 g, 0.82 mmol, 1.2 equiv.) and *N*,*N*-diisopropylethylamine (0.38 mL, 2.4 mmol, 3.0 equiv.) in CH_2Cl_2 (6 mL) and the reaction mixture was stirred for 1h. After this time, the solvent removed *in vacuo* and the resulting crude residue was purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) giving the title compound (0.096 g, 0.43 mmol, 63%) as a white solid. Data as above.

1-Phenyl-2,5,8,11-tetraoxatridecan-13-ol (2.65)



According to procedure of Bequignat *et al.*:[212] tetraethylene glycol **2.67** (0.86 mL, 5.0 mmol, 1.7 equiv.) was added portionwise to a stirred suspension of sodium hydride (60% in mineral oil, 0.21 g, 5.3 mmol, 1.8 equiv.) in THF (25 mL) at 0 °C and the reaction mixture was then left for 30 mins to come to rt with stirring. After this time, benzyl bromide (0.36 mL, 3.0 mmol, 1.0 equiv.) was added and the reaction mixture stirred for a further 4 h. The reaction mixture was quenched by the addition of sat. brine (30 mL) and the layers were separated. The aqueous layer was further extracted with CH_2Cl_2 (3 x 30 mL) and then the organic layers were combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (CH_2Cl_2 → 5% $MeOH/CH_2Cl_2$) giving the title compound (0.73 g, 2.6 mmol, 85%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 5H, 5 × ArC**H**), 4.57 (s, 2H, C$\mathbf{H_2}$$C_6H_5$), 3.79 – 3.56 (m, 16H, 7 × C$\mathbf{H_2}$O, C$\mathbf{H_2}$OH), 2.38 (s, 1H, CH_2O**H**); LRMS *m/z* (ESI$^+$) 307.2 (M+Na$^+$, 100%). Data are in accordance with the literature.[212]

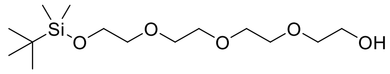
2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]204midazole-4-yl)pentanoate (2.62)



According to the modified procedure of Vinciguerra *et al.*:[213] pentafluorophenyl trifluoroacetate (0.038 mL, 0.22 mmol, 1.4 equiv.) was added to a stirred solution of d-biotin (0.040 g, 0.16 mmol, 1.0 equiv.) and triethylamine (0.11 mL, 0.80 mmol, 5.0 equiv.) in DMF (1.6 mL) and the reaction mixture was stirred at rt for 1 h. After this time, tetraethylene glycol (0.28 mL, 1.6 mmol, 10 equiv.) was added and the reaction mixture was heated to 60 °C and stirred for 18 h. The reaction mixture was cooled to rt and the solvent removed in vacuo to give a crude residue which was further purified by reverse phase flash column chromatography (5% $MeCN/H_2O$ → 40% $MeCN/H_2O$) and then freeze dried overnight to give the title compound (0.062 g, 0.14 mmol, 90%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$)

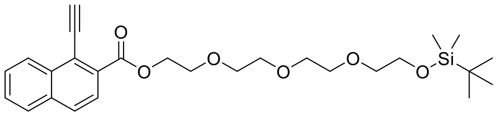
δ 5.42 (s, 1H, N**H**CONH), 4.81 (s, 1H, NHCON**H**), 4.55 – 4.48 (m, 1H, NHC**H**CHH'S), 4.36 – 4.28 (m, 1H, NHC**H**CH), 4.25 (q, *J* = 4.5 Hz, 2H, C**H₂**OH), 3.77 – 3.59 (m, 14H, 7 × C**H₂**O), 3.16 (ddd, *J* = 8.5, 6.5, 4.5 Hz, 1H, NHCHC**H**), 2.93 (dd, *J* = 13.0, 5.0 Hz, 1H, C**H**H'S), 2.74 (d, *J* = 12.5 Hz, 1H, CH**H'**S), 2.38 (t, *J* = 7.5 Hz, 2H, C**H₂**CO₂), 1.80 – 1.59 (m, 4H, CHC**H₂**, C**H₂**CH₂CO₂), 1.54 – 1.40 (m, 2H, CHCH₂C**H₂**); LRMS *m/z* (ESI$^+$) 421.1 (M+H$^+$, 100%). Data are in accordance with the literature.[213]

2,2,3,3-Tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-ol (2.69)



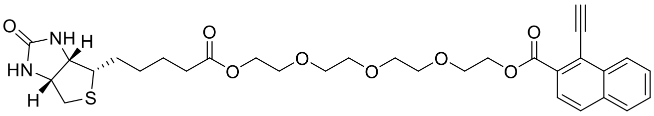
According to procedure of Qian *et al.*:[214] tetraethylene glycol **2.67** (3.0 g, 16 mmol, 1.0 equiv.) was added to stirred suspension of NaH (60% in mineral oil, 0.62 g, 16 mmol, 1.0 equiv.) in THF (110 mL) at rt and then the reaction mixture was stirred for 0.5 h. *tert*-Butyldimethylsilyl chloride (2.5 mL, 17 mmol, 1.1 equiv.) was added and the resultant milky suspension was vigorously stirred for 1 h. The reaction mixture was poured onto Et_2O (300 mL) and then washed with sat. K_2CO_3 solution (200 mL) and brine (200 mL), the organic layer was dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 70 % EtOAc/ petroleum ether 40 – 60) to give the product as a colourless oil (3.9 g, 13 mmol, 81%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.79 – 3.74 (m, 2H, C**H₂**OH), 3.74 – 3.59 (m, 12H, 6 × C**H₂**O), 3.56 (t, *J* = 5.5 Hz, 2H, C**H₂**OSi), 0.89 (s, 9H, C(C**H₃**)₃), 0.07 (s, 6H, Si(**CH₃**)₂); ^{13}C NMR (101 MHz, $CDCl_3$) δ 72.6 (**C**H₂OH), 72.5 (**C**H₂O), 70.7 (**C**H₂O), 70.6 (**C**H₂O), 70.3 (**C**H₂O), 62.7 (**C**H₂O), 61.7 (**C**H₂OSi), 25.9 (C(**C**H₃)₃), 18.3 (**C**(CH₃)₃), -5.33 (Si(**C**H₃)₂); LRMS *m/z* (ESI$^+$) 331.2 (M+Na$^+$). One carbon resonance is not resolved in the region corresponding to the PEG chain. Data are in accordance with the literature.[214]

2,2,3,3-Tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl 1-ethynyl-2-naphthoate (2.70)



N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (0.015 g, 0.077 mmol, 1.5 equiv.) was added to a stirred solution of 1-ethynyl-2-naphthoic acid **2.4** (0.010 g, 0.051 mmol), 2,2,3,3-tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-ol (0.057 g, 0.051 mmol, 1.0 equiv.), and 4-(dimethylamino)pyridine (0.0094 g, 0.077 mmol, 1.5 equiv.) in CH_2Cl_2 (1 mL) and the reaction mixture was heated to 40 °C for 18 h. After this time, the reaction mixture was cooled to rt and the solvent was removed *in vacuo* to give a crude residue which was further purified by column chromatography (50% EtOAc/ petroleum ether 40-60) to give the title compound (0.012 g, 0.026 mmol, 47%) as a colourless viscous oil. R_f 0.29 (50% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.59 (dd, *J* = 8.0, 1.5 Hz, 1H, ArC**H**), 7.98 (d, *J* = 8.5 Hz, 1H, ArC**H**), 7.89 – 7.85 (m, 2H, 2 × ArC**H**), 7.66 – 7.59 (m, 2H, 2 × ArC**H**), 4.66 – 4.46 (m, 2H, C**H₂**O), 3.94 (s, 1H, C**H**), 3.89 (m, 2H, C**H₂**O), 3.77 – 3.71 (m, 4H, 2 × C**H₂**O), 3.69 (m, 2H, C**H₂**O), 3.65 (s, 4H, 2 × C**H₂**O), 3.54 (m, 2H, C**H₂**O), 0.88 (s, 9H, C(C**H₃**)₃), 0.05 (s, 6H, Si(C**H₃**)₂); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6 (**C**=O), 134.4 (**C**CO₂), 133.8 (Ar**C**), 131.4 (Ar**C**), 128.71 (Ar**C**H), 128.3 (Ar**C**H), 128.1 (Ar**C**H), 127.7 (Ar**C**H), 127.6 (Ar**C**H), 125.7 (Ar**C**H), 121.4 (Ar**C**H), 89.07 (**C**≡CH), 79.5 (C≡**C**H), 72.7 (**C**H₂O), 70.7 (**C**H₂O), 70.7 (**C**H₂O), 70.7 (**C**H₂O), 69.1 (**C**H₂O), 69.0 (**C**H₂O), 64.9 (**C**H₂O), 64.6 (**C**H₂O), 62.7 (**C**H₂O), 25.9 (C(**C**H₃)₃), 18.4 (**C**(CH₃)₃), -5.2 (Si(**C**H₃)₂); LRMS *m/z* (ESI$^+$) 509.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): Found 487.2507, $C_{27}H_{39}O_6SiNa$ (M+H$^+$) requires 487.2510.

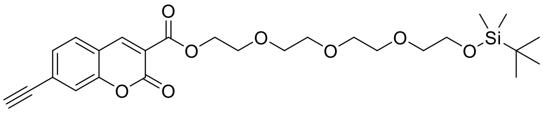
13-Oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-*d*]imidazol-4-yl)-3,6,9,12-tetraoxaheptadecyl 1-ethynyl-2-naphthoate (2.71)



Acetic acid (0.014 mL, 0.24 mmol, 1.5 equiv.) and tetrabutylammonium fluoride (1 M in THF, 0.19 mL, 0.19 mmol, 1.2 equiv.) were added to a stirred solution of 2,2,3,3-Tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl 1-ethynyl-2-naphthoate **2.70** (0.080 g, 0.16 mmol) in THF (1.5 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* and then the crude reaction mixture was passed through a short plug of silica (5% MeOH/ CH_2Cl_2) to give the desilylated alcohol (0.040 g, 0.11 mmol, 69%) whose identity was confirmed by LRMS analysis. LRMS *m/z* (ES^+) 395.2 ($M+Na^+$, 100%). The alcohol was immediately dissolved in CH_2Cl_2 (1 mL) and then d-biotin (0.027 g, 0.11 mmol, 1.0 equiv.), 4-(dimethylamino)pyridine (0.021 g, 0.17 mmol, 1.5 equiv.) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.033 g, 0.17 mmol, 1.5 equiv.) were added. The reaction mixture was heated to 40 °C for 18 h. After this time, the reaction mixture was allowed to cool to rt and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% MeOH/ CH_2Cl_2) to give the title compound as a white solid (0.060 g, 0.10 mmol, 94%). R_f 0.41 (5% MeOH/ CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 8.61 – 8.52 (m, 1H, ArC**H**), 7.96 (d, *J* = 8.5 Hz, 1H, ArC**H**), 7.92 – 7.82 (m, 2H, 2 × ArC**H**), 7.67 – 7.57 (m, 2H, 2 × ArC**H**), 5.86 (s, 1H, N**H**), 5.25 (s, 1H, N**H**), 4.55 (dd, *J* = 5.5, 4.0 Hz, 2H, C**H₂**O), 4.46 (dd, *J* = 8.0, 5.0 Hz, 1H, CONHC**H**CH), 4.25 (ddt, *J* = 10.0, 6.5, 3.0 Hz, 1H, C**H**CHH'S), 4.20 (q, *J* = 4.5 Hz, 2H, C**H₂**O), 3.96 (s, 1H, C≡C**H**), 3.91 – 3.84 (m, 2H, C**H₂**O), 3.74 – 3.60 (m, 10H, 5 × C**H₂**O), 3.09 (ddd, *J* = 8.5, 6.5, 4.5 Hz, 1H, CONHCHC**H**), 2.86 (dd, *J* =

13.0, 5.0 Hz, 1H, CH**H'**S), 2.71 (d, *J* = 13.0 Hz, 1H, C**H**H'S), 2.34 (t, *J* = 7.5 Hz, 2H, C**H₂**), 1.72 – 1.57 (m, 4H, 2 × C**H₂**), 1.47 – 1.34 (m, 2H, C**H₂**); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7 (**C**=O), 166.6 (**C**=O), 163.5 (**C**=O), 134.4 (**C**CO_2), 133.8 (Ar**C**), 131.4 (Ar**C**), 128.7 (Ar**C**H), 128.3 (Ar**C**H), 128.2 (Ar**C**H), 127.7 (Ar**C**H), 127.5 (Ar**C**H), 125.7 (Ar**C**H), 121.4 (Ar**C**), 89.2 (**C**≡CH), 79.5 (C≡**C**H), 70.7 (**C**H_2O), 70.7 (**C**H_2O), 70.6 (**C**H_2O), 70.5(**C**H_2O), 69.2 (**C**H_2O), 69.1 (**C**H_2O), 64.6 (**C**H_2O), 63.4 (**C**H_2O), 61.9 (**C**HCHH'), 60.1 (CONH**C**HCH), 55.5 (**C**HH'), 40.5 (CONHCH**C**H), 33.8 (**C**H_2), 28.3 (**C**H_2), 28.2 (**C**H_2), 24.7 (**C**H_2); LRMS *m/z* (ESI$^+$) 621.2 (M+Na$^+$).

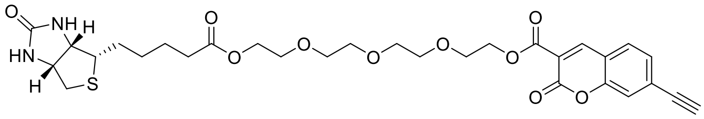
2,2,3,3-Tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl-7-ethynyl-2-oxo-*2H*-chromene-3-carboxylate (2.72)



N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.14 g, 0.71 mmol, 1.5 equiv.) was added to a stirred solution of 7-ethynyl-2-oxo-*2H*-chromene-3-carboxylic acid **2.20** (0.10 g, 0.47 mmol, 1.0 equiv.), 2,2,3,3-tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-ol (0.15 g, 0.47 mmol, 1.0 equiv.), and 4-(dimethylamino)pyridine (0.087 g, 0.71 mmol, 1.5 equiv.) in CH_2Cl_2 (5 mL) and the reaction mixture was heated to 40 °C for 18 h. After this time, the reaction mixture was cooled to rt and the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (50% EtOAc/ petroleum ether 40-60) to give the title compound as a pale yellow viscous oil (0.066 g, 0.13 mmol, 28%); R_f 0.30 (50% EtOAc/ petroleum ether 40-60); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 (d, *J* = 0.5 Hz, 1H, ArC**H**), 7.57 (d, *J* = 8.0 Hz, 1H, ArC**H**), 7.45 – 7.44 (m, 1H, ArC**H**), 7.41 (dd, *J* = 8.0, 1.5 Hz, 1H, ArC**H**), 4.53 – 4.46 (m, 2H, C**H₂**O), 3.88 – 3.80 (m, 2H, C**H₂**O), 3.75 (m, 2H, C**H₂**O), 3.72 – 3.67 (m, 2H, C**H₂**O), 3.65 (s, 4H, 2 × C**H₂**O), 3.54 (t, *J* = 5.5 Hz, 2H, C**H₂**O), 3.36

(s, 1H, C≡C**H**), 0.88 (s, 9H, $Si(CH_3)_2C(\mathbf{CH_3})_3$), 0.05 (s, 6H, $Si(\mathbf{CH_3})_2C(CH_3)_3$); LRMS *m/z* ($ES^+$) 527.7 ($M+Na^+$).

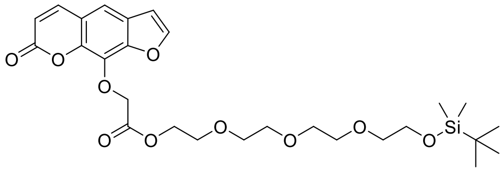
13-Oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-*d*]imidazol-4-yl)-3,6,9,12-tetraoxaheptadecyl 7-ethynyl-2-oxo-*2H*-chromene-3-carboxylate (2.73)



Acetic acid (0.0087 mL, 0.15 mmol, 1.5 equiv.) and tetrabutylammonium fluoride (1 M in THF, 0.12 mL, 0.12 mmol, 1.2 equiv.) were added to a stirred solution of 2,2,3,3-tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl 7-ethynyl-2-oxo-*2H*-chromene-3-carboxylate **2.72** (0.050 g, 0.10 mmol) in THF (1 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* and then the crude reaction mixture was passed through a short silica plug (5% MeOH/ CH_2Cl_2) to give the desilylated alcohol (0.038 g, 0.095 mmol, 96%) as a yellow oil whose identity was confirmed by LRMS. LRMS *m/z* (ES^+) 391.4 ($M+H^+$). The alcohol (0.035 g, 0.090 mmol, 1.0 equiv.) was dissolved in CH_2Cl_2 (1 mL) and then D-biotin (0.022 g, 0.09 mmol, 1.0 equiv.), 4-(dimethylamino)pyridine (0.017 g, 0.14 mmol, 1.5 equiv.) and *N*-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (1.5 equiv.) were added. The reaction mixture was heated to 40 °C for 18 h. After this time, the reaction mixture was allowed to cool to rt and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% MeOH/ CH_2Cl_2) to give the title compound (0.042 g, 0.068 mmol, 76%) as a yellow gummy solid. R_f 0.20 (5% MeOH/ CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 8.53 (s, 1H, ArC**H**), 7.58 (d, *J* = 8.0 Hz, 1H, ArC**H**), 7.43 (s, 1H, ArC**H**), 7.40 (dd, *J* = 8.0, 1.5 Hz, 1H, ArC**H**), 5.70 (s, 1H, N**H**), 5.13 (s, 1H, N**H**), 4.49 (td, *J* = 6.0, 5.0, 3.5 Hz, 3H, C**H₂**O, CONHC**H**CH), 4.34 – 4.28 (m, 1H, C**H**CHH'S), 4.21 (q, *J* = 4.5

Hz, 2H, C**H₂O**), 3.87 – 3.80 (m, 2H, C**H₂**O), 3.74 – 3.61 (m, 10H, 5 × C**H₂**O), 3.37 (s, 1H, C≡C**H**), 3.14 (ddd, *J* = 8.5, 6.5, 4.5 Hz, 1H, CONHCHC**H**), 2.90 (dd, *J* = 13.0, 5.0 Hz, 1H, C**H**H'S), 2.73 (d, *J* = 13.0 Hz, 1H, CH**H'**S), 2.35 (t, *J* = 7.5 Hz, 2H, C**H₂**), 1.75 – 1.60 (m, 4H, 2 × C**H₂**), 1.43 (tdd, *J* = 14.5, 8.5, 6.0 Hz, 2H, C**H₂**); ^{13}C NMR (126 MHz, CDCl$_3$) δ 173.6 (**C**=O), 163.3 (**C**=O), 162.7 (**C**=O), 156.2 (**C**=O), 154.8 (**C**-O), 148.0 (Ar**C**H), 129.5 (Ar**C**H), 128.4 (Ar**C**H), 128.3 (**C**CO$_2$), 120.1 (Ar**C**H), 118.2 (Ar**C**), 118.1 (**C**C≡CH), 82.3 (C**C**≡CH), 81.9 (CC≡**C**H), 70.6 (**C**H$_2$O), 70.6 (**C**H$_2$O), 70.5 (**C**H$_2$O), 69.2 (**C**H$_2$O), 68.9 (**C**H$_2$O), 65.0 (**C**H$_2$O), 63.4 (**C**H$_2$O), 61.9 (**C**HCHH'), 60.1 (CONH**C**HCH), 55.4 (CONHCH**C**H), 40.6 (**C**HH'S), 33.7 (**C**H$_2$), 28.3 (**C**H$_2$), 28.2 (**C**H$_2$), 24.7 (**C**H$_2$); LRMS *m/z* (ESI$^+$) 639.2 (M+Na$^+$, 100%).

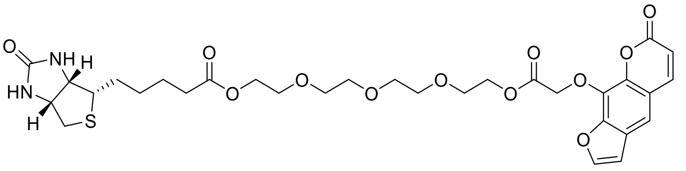
2,2,3,3-Tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl 2-((7-oxo-*7H*-furo[3,2-*g*]chromen-9-yl)oxy)acetate (2.74)



N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.11 g, 0.57 mmol, 1.5 equiv.) was added to a stirred solution of 2-((7-oxo-*7H*-furo[3,2-*g*]chromen-9-yl)oxy)acetic acid **2.24** (0.10 g, 0.38 mmol, 1.0 equiv.), 4-(dimethylamino)pyridine (0.070 g, 0.57 mmol, 1.5 equiv.) in CH$_2$Cl$_2$ (6 mL) and the reaction mixture was heated to 40 °C for 18 h. After this time, the reaction mixture was cooled to rt and the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (50% EtOAc/ petroleum ether 40-60) to give the title compound (0.091 g, 0.17 mmol, 43%) as a colourless viscous oil. R$_f$ 0.30 (50% EtOAc/ petroleum ether 40-60); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.76 (d, *J* = 9.5 Hz, 1H, C**H**), 7.70 (d, *J* = 2.0 Hz, 1H, ArC**H**), 7.38 (s, 1H, ArC**H**), 6.81 (d,

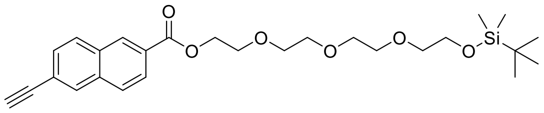
J = 2.0 Hz, 1H, ArC**H**), 6.37 (d, J = 9.5 Hz, 1H, C**H**), 5.18 (s, 2H, OC**H₂**), 4.38 – 4.30 (m, 2H, CO_2CH_2), 3.76 (t, J = 5.5 Hz, 2H, CH_2OSi), 3.73 – 3.68 (m, 2H, $CH_2CH_2CO_2$), 3.68 – 3.59 (m, 8H, 4 × CH_2O), 3.55 (t, J = 5.5 Hz, 2H, CH_2CH_2OSi), 0.88 (s, 9H, $C(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_2$); LRMS *m/z* (ESI$^+$) 573.2 (M+Na$^+$).

14-Oxo-18-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-d]imidazol-4-yl)-3,6,9,12,13-pentaoxaoctadecyl 2-((7-oxo-*7H*-furo(3,2-*g*)chromen-9-yl)oxy)acetate (2.75)



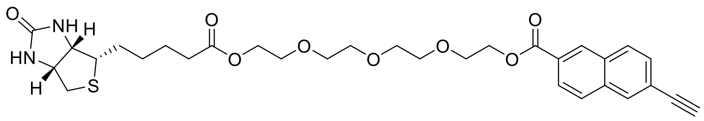
Acetic acid (0.014 mL, 0.24 mmol, 1.5 equiv.) and tetrabutylammonium fluoride (1 M in THF, 0.19 mL, 0.19 mmol, 1.2 equiv.) were added to a stirred solution of 2,2,3,3-Tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl 2-((7-oxo-*7H*-furo[3,2-*g*]chromen-9-yl)oxy)acetate **2.74** (0.090 g, 0.16 mmol, 1.0 equiv.) in THF (2 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* and then the crude reaction mixture was passed through a short silica plug (5% MeOH/ CH_2Cl_2) to give the desilylated alcohol (0.068 g, 0.16 mmol, 98%) as a colourless oil whose identity was confirmed by LCMS analysis. LRMS *m/z* (ESI$^+$) 459.2 (M+Na$^+$). The alcohol (0.034 g, 0.078 mmol) was dissolved in CH_2Cl_2 (1 mL) and D-biotin (0.019 g, 0.078 mmol, 1.0 equiv.), 4-(dimethylamino)pyridine (0.015 g, 0.12 mmol, 1.5 equiv.) and *N*-(3-imethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (0.023 g, 0.12 mmol, 1.5 equiv.) was added. the reaction mixture was heated to 40 °C for 18 h. After this time, the reaction mixture was allowed to cool to rt and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% MeOH/ CH_2Cl_2) to give the title compound (0.026 g, 0.039 mmol, 50%) as a white gummy solid. R$_f$ 0.27 (5% MeOH/ CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 9.5 Hz, 1H, C**H**), 7.72 (d, *J* = 2.0 Hz, 1H, ArC**H**), 7.40 (s, 1H, ArC**H**), 6.83 (d, *J* = 2.0 Hz, 1H, ArC**H**), 6.39 (d, *J* = 9.5 Hz, 1H, C**H**), 5.81 (s, 1H, N**H**), 5.26 (s, 1H, N**H**), 5.20 (s, 2H, OC**H₂**), 4.54 – 4.49 (m, 1H, C**H**CHH'S), 4.38 – 4.34 (m, 2H, CO₂C**H₂**), 4.32 (dd, *J* = 8.0, 4.5 Hz, 1H, CONHC**H**CH), 4.23 (td, *J* = 4.5, 3.5 Hz, 2H, C**H₂**O), 3.75 – 3.60 (m, 12H, 6 × C**H₂**O), 3.16 (ddd, *J* = 8.5, 6.5, 4.5 Hz, 1H, CONHCHC**H**), 2.92 (dd, *J* = 13.0, 5.0 Hz, 1H, C**H**H'S), 2.75 (d, *J* = 13.0 Hz, 1H, CH**H'**S), 2.37 (t, *J* = 7.5 Hz, 2H, C**H₂**CO₂), 1.79 – 1.59 (m, 4H, 2 × C**H₂**), 1.49 – 1.42 (m, 2H, C**H₂**); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.6 (**C**=O), 168.8 (**C**=O), 163.4 (**C**=O), 160.0 (**C**=O), 147.0 (**C**-O), 146.8 (Ar**C**H), 144.3 (**C**H), 142.5 (**C**-OCH₂), 132.4 (Ar**C**) 130.7 (Ar**C**), 128.7 (Ar**C**), 116.4 (ArCH), 114.7 (**C**H), 113.4 (Ar**C**H), 106.7 (Ar**C**H), 70.5 (**C**H₂O), 70.5 (**C**H₂O), 70.4 (**C**H₂O), 69.0 (**C**H₂O), 68.8 (**C**H₂O), 68.8 (**C**H₂O), 64.2 (**C**H₂O), 63.3 (**C**H₂O), 61.8 (CONH**C**HCH), 60.0 (**C**HCHH'S), 55.4 (CONHCH**C**H), 40.4 (**C**HH'S), 38.6 (**C**H₂), 33.7 (**C**H₂), 28.1 (**C**H₂), 24.6 (**C**H₂); LRMS *m/z* (ESI$^+$) 685.7 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): Found 663.2217, $C_{31}H_{39}N_2O_{12}S$ (M+H$^+$) requires 663.2218.

2,2,3,3-Tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl 6-ethynyl-2-naphthoate (2.76)



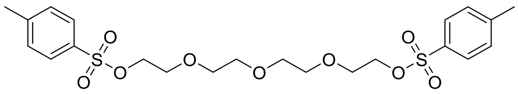
N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.15 g, 0.77 mmol, 1.5 equiv.) was added to a stirred solution of 6-ethynyl-2-naphthoic acid **2.7** (0.10 g, 0.51 mmol, 1.0 equiv.) 2,2,3,3-tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-ol (0.15 g, 0.51 mmol, 1.0 equiv.), and 4-(dimethylamino)pyridine (0.094 g, 0.77 mmol, 1.5 equiv.) in CH_2Cl_2 (5 mL) and the reaction mixture was heated to 40 °C for 18 h. After this time, the reaction mixture was cooled to rt and the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (1% EtOAc/petroleum ether 40-60 → 2% EtOAc/petroleum ether 40-60) to give the title compound (0.17 g, 0.35 mmol, 69%) as a colourless viscous oil. R$_f$ 0.28 (1% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.59 (s, 1H, ArC**H**), 8.09 (ddd, *J* = 8.5, 4.0, 1.5 Hz, 1H, ArC**H**), 8.05 (d, *J* = 4.0 Hz, 1H, ArC**H**), 7.90 (d, *J* = 8.5 Hz, 1H, ArC**H**), 7.84 (d, *J* = 8.5 Hz, 1H, ArC**H**), 7.58 (dd, *J* = 8.5, 1.5 Hz, 1H, ArC**H**), 4.54 (dd, *J* = 5.5, 4.0 Hz, 2H, C$\mathbf{H_2}$O), 3.90 – 3.86 (m, 2H, C$\mathbf{H_2}$O), 3.74 (q, *J* = 5.0, 4.0 Hz, 4H, 2 × C$\mathbf{H_2}$O), 3.69 (dd, *J* = 6.0, 4.0 Hz, 2H, C$\mathbf{H_2}$O), 3.65 (q, *J* = 2.0 Hz, 4H, 2 × C$\mathbf{H_2}$O), 3.53 (t, *J* = 5.5 Hz, 2H, C$\mathbf{H_2}$O), 0.88 (s, 9H, C(C$\mathbf{H_3}$)$_3$), 0.05 (s, 6H, Si(C$\mathbf{H_3}$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4 (**C**=O), 134.9 (Ar**C**), 132.1 (Ar**C**), 132.0 (Ar**C**H), 130.9 (Ar**C**H), 129.4 (ArCH), 129.4 (Ar**C**H), 128.26 (Ar**C**), 128.0 (Ar**C**H), 126.1 (Ar**C**H), 121.9 (Ar**C**), 83.5 (**C**≡CH), 78.8 (C≡**C**H), 72.7 (**C**H$_2$O), 70.7 (**C**H$_2$O), 70.7 (**C**H$_2$O), 69.3 (**C**H$_2$O), 64.4 (**C**H$_2$O), 62.7 (**C**H$_2$O), 25.9 (C(**C**H$_3$)$_3$), 18.4 (**C**(CH$_3$)$_3$), -5.3 (Si(**C**H$_3$)$_2$); LRMS *m/z* (ESI$^+$) 509.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): Found 487.2507, $C_{27}H_{39}O_6SiNa$ (M+H$^+$) requires 487.2510. Overlapping peaks in the PEG region account for missing ^{13}C resonances.

13-Oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-d]imidazol-4-yl)-3,6,9,12-tetraoxaheptadecyl 6-ethynyl-2-naphthoate (2.77)



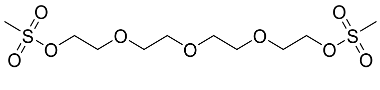
Acetic acid (0.030 mL, 0.53 mmol, 1.5 equiv.) and tetrabutylammonium fluoride (1 M in THF, 0.42 mL, 0.42 mmol, 1.2 equiv.) were added to a stirred solution of 2,2,3,3-tetramethyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl 6-ethynyl-2-naphthoate **2.76** (0.17 g, 0.35 mmol, 1.0 equiv.) in THF (3 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* and then the crude reaction mixture was passed through a short silica plug (5% MeOH/ CH_2Cl_2) to give desilylated alcohol (0.092 g, 0.25 mmol, 71%) as a colourless oil whose identity was confirmed by LRMS. LRMS *m/z* (ESI$^+$) 373.4 (M+H$^+$, 100%). The desilylated alcohol (0.070 g, 0.19 mmol, 1,0 equiv.) was immediately dissolved in CH_2Cl_2 (2 mL) and then D-biotin (0.046 g, 0.19 mmol, 1.0 equiv.), 4-(dimethylamino)pyridine (0.035 g, 0.29 mmol, 1.5 equiv.) and *N*-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (0.056 g, 0.29 mmol, 1.5 equiv.) were added. The reaction mixture was heated to 40 °C for 18 h. After this time, the reaction mixture was allowed to cool to rt and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (1% MeOH/ CH_2Cl_2 → 5% MeOH/ CH_2Cl_2) to give the title compound (0.072 g, 0.12 mmol, 64%) as a white gummy solid. R$_f$ 0.38 (5% MeOH/ CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.57 (d, *J* = 1.5 Hz, 1H, ArC**H**), 8.10 – 8.05 (m, 1H, ArC**H**), 8.02 (s, 1H, C**H**), 7.88 (d, *J* = 8.5 Hz, 1H, ArC**H**), 7.82 (d, *J* = 8.5 Hz, 1H, ArC**H**), 7.56 (dd, *J* = 8.5, 1.5 Hz, 1H, ArC**H**), 5.96 (s, 1H, N**H**), 5.40 (s, 1H, N**H**), 4.54 – 4.50 (m, 2H, C**H$_2$**O), 4.46 (dd, *J* = 8.0, 5.0 Hz, 1H, CONHC**H**CH), 4.28 – 4.23 (m, 1H, C**H**CHH’S), 4.18 (q, *J* = 4.5 Hz, 2H, C**H$_2$**O), 3.86 (dd, *J* = 5.5, 4.0 Hz, 2H, C**H$_2$**O), 3.76 – 3.56 (m, 10H, 5 × C**H$_2$**O), 3.22 (s, 1H, C≡C**H**), 3.09 (ddd, *J* = 8.5, 6.5, 4.5 Hz, 1H, CONHCHC**H**), 2.86 (dd, *J* = 13.0, 5.0 Hz, 1H, CH**H’**S), 2.71 (d, *J* = 13.0 Hz, 1H, C**H**H’S), 2.32 (t, *J* = 7.5 Hz, 2H, C**H$_2$**), 1.79 – 1.56 (m, 4H, 2 × C**H$_2$**), 1.45 – 1.31 (m, 2H, C**H$_2$**); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7 (**C**=O), 166.4 (**C**=O), 163.6 (**C**=O), 134.9 (Ar**C**), 132.0 (Ar**C**), 132.0 (Ar**C**H), 130.9 (Ar**C**H), 129.4 (Ar**C**H), 129.4 (Ar**C**H), 128.2 (Ar**C**), 128.0 (Ar**C**H), 126.1 (Ar**C**H), 121.9 (Ar**C**), 83.5 (**C**≡CH), 78.9 (C≡**C**H), 70.7 (**C**H$_2$O), 70.6 (**C**H$_2$O), 69.2 (**C**H$_2$O), 69.1 (**C**H$_2$O), 64.4 (**C**H$_2$O), 63.4 (**C**H$_2$O), 61.9 (CONH**C**HCH), 60.1 (**C**HCHH’S), 55.5 (CONHCH**C**H), 40.5 (**C**HH’S), 33.8 (**C**H$_2$), 28.3 (**C**H$_2$), 28.2 (**C**H$_2$), 24.7 (**C**H$_2$), 20.1 (**C**H$_2$); LRMS *m/z* (ESI$^+$) 598.2 (M+H$^+$, 100%). Overlapping peaks in the PEG region account for missing ^{13}C resonances.

((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) (2.78)



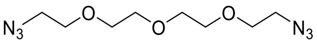
According to procedure of Bouzide *et al*:[215] to a stirred solution of tetraethylene glycol **2.67** (0.10 g, 0.51 mmol, 1.0 equiv.) and *p*-toluenesulfonyl chloride (0.29 g, 1.5 mmol, 3.0 equiv.) in CH_2Cl_2 (5.0 mL) at 0 °C was added powdered potassium hydroxide (0.23 g, 4.1 mmol, 8.0 equiv.). The reaction mixture was warmed to rt and stirred for 4 h, after this time, the reaction mixture was diluted with CH_2Cl_2 (5 mL) and poured into H_2O (20 mL). The organics were extracted with CH_2Cl_2 (2 x 10 mL) and the combined organic layers were washed with brine (20 mL), dried ($MgSO_4$), filtered and solvent removed *in vacuo* giving a crude residue that was further purified by flash column chromatography (60 % EtOAc/ petroleum ether 40-60) to give the title compound as a colourless oil (0.16 g, 0.32 mmol, 62%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.72 (m, 4H, 4 × ArC**H**), 7.41 – 7.27 (m, 4H, 4 × ArC**H**), 4.23 – 4.04 (m, 4H, 2 × C$\mathbf{H_2}$O), 3.75 – 3.63 (m, 4H, 2 × C$\mathbf{H_2}$O), 3.62 – 3.47 (m, 8H, 4 × C$\mathbf{H_2}$O), 2.44 (s, 6H, 2 × C$\mathbf{H_3}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.8 (**C**SO_2), 133.0 (**C**CH_3), 129.8 (Ar**C**H), 128.0 (Ar**C**H), 70.8 (O**C**H_2), 70.6 (O**C**H_2), 69.3 (O**C**H_2), 68.7 (O**C**H_2); LRMS *m/z* (ESI$^+$) 503.2 (M+H$^+$, 100%). Data are in accordance with the literature.[215]

((Oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl) dimethanesulfonate (2.79)



According to procedure of Wu *et al.*:[216] methanesulfonyl chloride (2.8 mL, 35 mmol, 3.4 equiv.) was added dropwise to a stirred solution of tetraethylene glycol **2.67** (2.0 g, 10 mmol, 1.0 equiv.) and triethylamine (6.5 mL, 49 mmol, 4.8 equiv.) in CH_2Cl_2 (10 mL) at 0 °C, the reaction mixture was then warmed to rt and stirred for 18 h. The reaction mixture was quenched by adding H_2O and the layers were separated, the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) and the organic layers were combined, washed with H_2O (2 × 20 mL), brine (3 × 20 mL), dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give the title compound as a colourless oil (3.3 g, 9.1 mmol, 91%) which required no further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.42 – 4.33 (m, 4H, 2 × C**H_2**OS), 3.80 – 3.73 (m, 4H, 2 × C**H_2**CH_2OS), 3.70 – 3.60 (m, 8H, 4 × C**H_2**O), 3.07 (s, 6H, 2 × C**H_3**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 70.7 (**C**H_2OS), 70.5 (**C**H_2CH_2OS), 69.2 (**C**H_2O), 69.0 (**C**H_2O), 37.7 (**C**H_3); LRMS *m/z* (ES$^+$) 351.1 (M+H$^+$, 100%). Data are in accordance with the literature.[216]

1-Azido-2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethane (2.80)

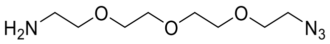


According to the modified procedure of Harrison *et al.*:[217]

Method one: sodium azide (0.035 g, 0.55 mmol, 2.2 equiv.) was added to a stirred solution of ((oxybis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl) bis(4-methylbenzenesulfonate) **2.78** (0.13 g, 0.25 mmol, 1.00 equiv.) in H_2O, the reaction mixture was heated to reflux and stirred for 24 h. The reaction mixture was cooled to rt, diluted with sat. brine solution (10 mL) and then the organics extracted with Et_2O (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and then the solvent was removed *in vacuo* to give the title compound as a colourless oil (0.061 g, 0.24 mmol, 99%) which was used without further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.76 – 3.61 (m, 12H, 6 × C**H_2**O), 3.39 (t, *J* = 5.0 Hz, 4H, 2 × C**H_2**N_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 70.7 (**C**H_2O), 70.0 (**C**$H_2CH_2N_3$), 50.6 (**C**H_2N_3). Data are in accordance with the literature.[217]

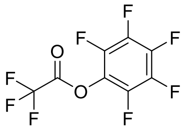
Method two: sodium azide (2.4 g, 38 mmol, 4.0 equiv.) was added to a stirred solution of ((oxybis{ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl) dimethanesulfonate **2.79** (3.3 g, 9.4 mmol, 1.0 equiv.) in EtOH/DMF (4:1, 20 mL/5 mL) and the reaction mixture was heated to reflux and stirred for 6 h. The reaction mixture was cooled to rt, poured onto a mixture of EtOAc/H_2O (1:1, 20 mL/20 mL) and the layers were separated. The organic layer was washed with H_2O (2 × 20 mL), brine (20 mL), dried ($MgSO_4$), filtered and solvent removed *in vacuo* to yield the title compound as a colourless oil (2.0 g, 9.3 mmol, 98%) which was used without further purification. Data as above.

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-amine (2.66)



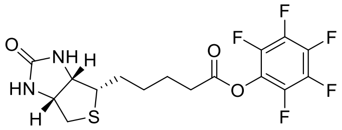
According to procedure of Okoth *et al.*:[85] triphenylphosphine (0.11 g, 0.42 mmol, 1.0 equiv.) in Et_2O (2 mL) was added dropwise to a stirred solution of 1-azido-2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethane **2.80** (0.10 g, 0.42 mmol, 1.0 equiv.) in 2 M HCl (3 mL) and the reaction mixture was stirred at rt for 18 h. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL) to remove residual triphenylphosphine oxide. Powdered KOH was added to the aqueous layer until pH 12 was reached and then the product was extracted with CH_2Cl_2 (4 × 15 mL), the organic layers were combined, dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give the title compound as a pale yellow oil (0.092 g, 99%) which required no further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 – 3.59 (m, 12H, C**H₂**O), 3.50 (t, *J* = 5.0 Hz, 2H, C**H₂**N_3), 3.39 (t, *J* = 5.0 Hz, 2H, N**H₂**), 2.89 – 2.83 (m, 2H, C**H₂**NH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 73.4 (**C**H_2O), 70.7 (**C**H_2O), 70.6 (**C**H_2O), 70.6 (**C**H_2O), 70.2 (**C**H_2O), 70.0 (**C**H_2O), 50.6 (**C**H_2N_3), 41.7 (**C**H_2NH_2). Data are in accordance with the literature.[85]

Perfluorophenyl 2,2,2-trifluoroacetate (2.81)



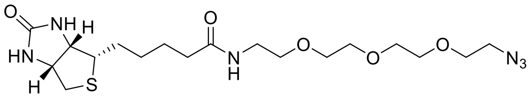
According to procedure of Green *et al.*:[218] trifluoroacetic anhydride (7.1 mL, 41 mmol, 1.5 equiv.) was added to a flask containing pentafluorophenol (5.2 g, 27 mmol, 1.0 equiv.) and the reaction mixture was heated to 40 °C and stirred for 18 h. After this time, distillation was carried out at atmospheric pressure to give the title compound (6.1 g, 21 mmol, 76%) as a colourless oil. b.p. 125 °C (lit. 122 – 125 °C). Data are in accordance with the literature.[218]

Perfluorophenyl 5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-d]218midazole-4-yl)pentanoate (2.64)



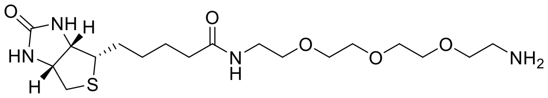
According to procedure of Wurm *et al.*:[219] pentafluorophenyl trifluoroacetate **2.81** (0.24 mL, 1.4 mmol, 1.4 equiv.) was added dropwise to a stirred solution of D-biotin (0.24 g, 1.0 mmol, 1.0 equiv.) and triethylamine (0.24 mL, 1.8 mmol, 1.8 equiv.) in DMF (10 mL) and the reaction mixture was stirred at rt for 1 h. The solvent was removed under a stream of N_2 and the crude residue was triturated with Et_2O to give the title compound as a white solid (0.37 g, 1.2 mmol, 89%) which required no further purification; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.46 (s, 1H, N**H**), 6.38 (s, 1H N**H**), 4.31 (dd, *J* = 7.5, 5.0 Hz, 1H, CONHC**H**CH), 4.21 – 4.10 (m, 1H, C**H**CHH'S), 3.12 (dt, *J* = 8.0, 5.0 Hz, 1H, CONHCHC**H**), 2.89 – 2.82 (m, 1H, CH**H'**S), 2.78 (t, *J* = 7.5 Hz, 2H, C**H$_2$**), 2.58 (d, *J* = 12.5 Hz, 1H, C**H**H'S), 1.78 – 1.59 (m, 4H, 2 × C**H$_2$**), 1.58 – 1.35 (m, 2H, C**H$_2$**); LRMS *m/z* (ESI$^+$) 433.0 (M+Na$^+$, 100%). Data are in accordance with the literature.[219]

***N*-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-d]imidazol-4-yl)pentanamide (2.82)**



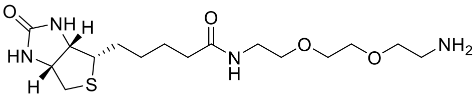
According to procedure of Tantama *et al.*:[220] 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine **2.66** (0.044 g, 0.20 mmol, 2.0 equiv.), and triethylamine (0.020 mL, 0.15 mmol, 1.5 equiv.) were added to a stirred solution of perfluorophenyl 5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate **2.64** (0.041 g, 0.10 mmol, 1.0 equiv.) in DMF (1 mL) and the reaction mixture was heated to 40 °C and stirred for 18 h. The solvent was removed under a stream of N_2 and then the crude residue was triturated with Et_2O to give the title compound as a white solid (0.035 g, 0.080 mmol, 80%) which required no further purification; R_f 0.28 (10% MeOH/ CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (t, *J* = 5.5 Hz, 1H, N**H**), 6.80 (s, 1H, N**H**), 5.85 (s, 1H, N**H**), 4.49 (dd, *J* = 8.0, 5.0 Hz, 1H, CONHC**H**CH), 4.29 (ddd, *J* = 8.0, 4.5, 1.5 Hz, 1H, C**H**CHH'S), 3.69 – 3.59 (m, 10H, 5 × C**H₂**O), 3.55 (t, *J* = 5.0 Hz, 2H, C**H₂**O), 3.47 – 3.32 (m, 4H, 2 × C**H₂**N₃, NHC**H₂**), 3.12 (td, *J* = 7.5, 4.5 Hz, 1H, CONHCHC**H**), 2.88 (dd, *J* = 13.0, 5.0 Hz, 1H, CH**H'**S), 2.73 (d, *J* = 13.0 Hz, 1H, C**H**H'S), 2.21 (t, *J* = 7.5 Hz, 2H, C**H₂**), 1.77 – 1.59 (m, 4H, 2 × C**H₂**), 1.41 (m, 2H, C**H₂**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3 (**C**=O), 164.2 (**C**=O), 70.5 (**C**H₂O), 70.3 (**C**H₂O), 70.0 (**C**H₂O), 69.9 (**C**H₂O), 69.9 (**C**H₂O), 61.6 (CONH**C**HCH), 60.1 (**C**HCHH'S), 55.6 (CONHCH**C**H), 50.6 (**C**H₂N), 40.4 (**C**HH'S), 39.0 (**C**H₂N), 35.9 (**C**H₂), 28.2 (**C**H₂), 28.0 (**C**H₂), 25.6 (**C**H₂); LRMS *m/z* (ESI$^+$) 467.2 (M+Na$^+$, 100%). One carbon resonance is not resolved in the region corresponding to the PEG chain. Data are in accordance with the literature.[220]

***N*-(2-(2-(2-(2-Aminoethoxy)ethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-*d*]220midazole-4-yl)pentanamide (2.63)**



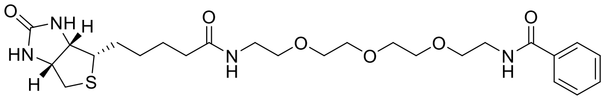
According to procedure of Fusz *et al.*:[221] palladium on carbon (0.0050 g, 0.020 mmol, 0.15 equiv.) was added to a stirred solution of *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide **2.82** (0.035 g, 0.080 mmol, 1.0 equiv.) in EtOH (1 mL) under an atmosphere of H_2 and the reaction mixture was stirred at rt for 18 h. After this time, the reaction mixture was passed through a plug of Celite™ (EtOH) and the solvent removed *in vacuo* to give the title compound as an orange hygroscopic solid (0.030 g, 0.072 mmol, 90%) which required no further purification. R_f 0.05 (10% MeOH/ CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 5.5 Hz, 1H, N**H**), 6.76 (s, 1H, N**H**), 5.97 (s, 1H, N**H**), 4.47 (dd, *J* = 8.0, 4.5 Hz, 1H, CONHC**H**CH), 4.28 (dd, *J* = 8.0, 4.5 Hz, 1H, C**H**CHH'S), 3.68 – 3.49 (m, 12H, 6 × C**H₂**O), 3.39 (p, *J* = 5.0 Hz, 2H, C**H₂**O), 3.19 (s, 2H, N**H₂**), 3.11 (td, *J* = 7.5, 4.5 Hz, 1H, CONHCHC**H**), 2.92 – 2.83 (m, 3H, C**H₂**NH₂, CH**H'**S), 2.72 (d, *J* = 13.0 Hz, 1H, C**H**H'S), 2.21 (t, *J* = 7.0 Hz, 2H, C**H₂**), 1.76 – 1.57 (m, 4H, 2 × C**H₂**), 1.41 (m, 2H, C**H₂**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.5 (**C**=O), 164.2 (**C**=O), 72.1 (**C**H₂NH₂), 70.4 (**C**H₂O), 70.4 (**C**H₂O), 70.1 (**C**H₂O), 70.0 (**C**H₂O), 70.0 (**C**H₂O), 61.8 (CONH**C**HCH), 60.2 (**C**HCHH'S), 55.8 (CONHCH**C**H), 41.2 (**C**HH'S), 40.6 (**C**H₂NH), 35.8 (**C**H₂), 28.2 (**C**H₂), 28.1 (**C**H₂), 25.6 (**C**H₂); LRMS *m/z* (ESI$^+$) 419.2 (M+H$^+$, 100%). One carbon resonance is not resolved in the region corresponding to the PEG chain. Data are in accordance with the literature.[221]

***N*-(2-(2-(2-Aminoethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (2.83)**



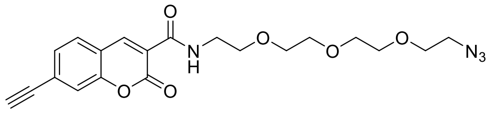
According to the modified procedure of Reizelman *et al.*:[222] perfluorophenyl 5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoate **2.64** (0.041 g, 0.10 mmol, 1.0 equiv.) was added to a stirred solution of 2-(2-(2-aminoethoxy)ethoxy)ethanamine (0.14 mL, 1.0 mmol, 10 equiv.) and triethylamine (0.070 g, 0.50 mmol, 5.0 equiv.) in DMF (1 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* to give a crude residue that was further purified by reverse phase flash column chromatography (5% MeCN/H_2O → 40% MeCN/H_2O) and then freeze dried overnight to give the title compound (0.020 g, 0.053 mmol, 53%) as a pale yellow oil. ^1H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 2H, $CH_2N\mathbf{H_2}$), 8.02 – 7.88 (m, 1H, CON**H**), 6.46 (s, 1H, N**H**CONH), 6.39 (s, 1H, NHCON**H**), 4.31 (dd, *J* = 7.5, 5.0 Hz, 1H, C**H**CHH'S), 4.13 (ddd, *J* = 8.0, 4.5, 2.0 Hz, 1H, NHC**H**CH), 3.53 (m, 4H, 2 × $C\mathbf{H_2}O$), 3.40 (t, *J* = 6.0 Hz, 2H, $NHC\mathbf{H_2}$), 3.19 (q, *J* = 5.5 Hz, 2H, $C\mathbf{H_2}NH_2$), 3.10 (ddd, *J* = 8.5, 6.0, 4.5 Hz, 1H, NHCHC**H**), 2.91 – 2.78 (m, 5H, 2 × $C\mathbf{H_2}O$, CHC**H**H'S), 2.58 (d, *J* = 12.5 Hz, 1H, CH**H'**S), 2.07 (t, *J* = 7.5 Hz, 2H, $C\mathbf{H_2}CONH$), 1.68 – 1.38 (m, 4H, $C\mathbf{H_2}CH_2CONH$, $CHC\mathbf{H_2}CH_2$), 1.30 (tdd, *J* = 15.0, 10.5, 6.5 Hz, 2H, $CHCH_2C\mathbf{H_2}$); LRMS *m/z* (ESI$^+$) 375.0 (M+H$^+$, 100%). Data are in accordance with literature.[222]

***N*-(13-Oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-*d*]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)benzamide (2.84)**



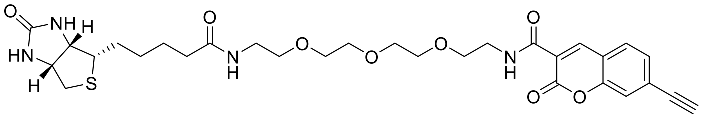
Pentafluorophenyl trifluoroacetate **2.81** (0.15 mL, 0.87 mmol, 1.3 equiv.) was added dropwise to a stirred solution of benzoic acid (0.082 g, 0.67 mmol, 1.0 equiv.) and triethylamine (0.10 mL, 0.80 mmol, 1.2 equiv.) in CH_2Cl_2 (7 mL) and the reaction mixture was stirred at rt for 1 h. After this time *N*-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide **2.63** (0.28 g, 0.67 mmol, 1.0 equiv.) was added and the reaction mixture was stirred at rt for 2 h. The solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (1% MeOH/ CH_2Cl_2 → 10% MeOH/ CH_2Cl_2) to give the title compound (0.13 g, 0.25 mmol, 38%) as a white solid. R_f 0.40 (10% MeOH/ CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.79 (m, 2H, 2 × ArC**H**), 7.52 – 7.46 (m, 1H, ArC**H**), 7.45 – 7.39 (m, 2H, 2 × ArC**H**), 7.19 (s, 1H, N**H**), 6.67 (t, *J* = 5.5 Hz, 1H, N**H**), 6.43 (s, 1H, N**H**), 5.37 (s, 1H, N**H**), 4.54 – 4.41 (m, 1H, CONHC**H**CH), 4.26 (ddd, *J* = 8.0, 4.5, 1.5 Hz, 1H, C**H**CHH'S), 3.76 – 3.55 (m, 12H, 6 × C**H₂**O), 3.51 (t, *J* = 5.0 Hz, 2H, C**H₂**NH), 3.39 (td, *J* = 5.5, 4.0 Hz, 2H, C**H₂**NH), 3.11 (qd, *J* = 7.5, 5.0 Hz, 1H, CONHCHC**H**), 2.87 (dd, *J* = 13.0, 5.0 Hz, 1H, CH**H'**S), 2.71 (d, *J* = 13.0 Hz, 1H, C**H**H'S), 2.18 (t, *J* = 7.5 Hz, 2H, C**H₂**), 1.79 – 1.55 (m, 4H, 2 × C**H₂**), 1.41 (dt, *J* = 9.0, 7.5 Hz, 2H, C**H₂**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2 (**C**=O), 167.5 (**C**=O), 163.8 (**C**=O), 134.4 (Ar**C**), 131.4 (Ar**C**H), 128.4 (Ar**C**H), 127.1 (Ar**C**H), 70.32 (**C**H₂O), 70.3 (**C**H₂O), 70.1 (**C**H₂O), 70.0 (**C**H₂O), 69.9 (**C**H₂O), 69.9 (**C**H₂O), 61.7 (CONH**C**HCH), 60.1 (**C**HCHH'S), 55.4 (CONHCH**C**H), 40.4 (**C**HH'S), 39.7 (**C**H₂NH), 39.0 (**C**H₂NH), 35.8 (C**H₂**), 28.1 (C**H₂**), 28.0 (C**H₂**), 25.5 (C**H₂**); LRMS *m/z* (ESI⁺) 545.2 (M+Na⁺, 100%).

***N*-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-7-ethynyl-2-oxo-*2H*-chromene-3-carboxamide (2.85)**



Pentafluorophenyl trifluoroacetate **2.81** (0.11 mL, 0.62 mmol, 1.3 equiv.) was added dropwise to a solution of 7-ethynyl-2-oxo-*2H*-chromene-3-carboxylic acid **2.20** (0.10 g, 0.48 mmol, 1.0 equiv.) and triethylamine (0.080 mL, 0.58 mmol, 1.2 equiv.) in CH_2Cl_2 (5 mL) and the reaction mixture was stirred for 1 h. The solvent was removed *in vacuo* to give the crude pentafluorophenyl ester which was redissolved in CH_2Cl_2 (5 mL) and then 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine (0.11 g, 0.48 mmol, 1.0 equiv.) and triethylamine (0.080 mL, 0.58 mmol, 1.2 equiv.) were added and the reaction mixture was stirred at rt for 18 h. The reaction solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (1% MeOH/ CH_2Cl_2 → 10% MeOH/ CH_2Cl_2) giving the title compound (0.075 g, 0.18 mmol, 38%) as a pale yellow oil. R_f 0.32 (1% MeOH/ CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H, N**H**), 8.86 (d, *J* = 0.7 Hz, 1H, ArC**H**), 7.64 (d, *J* = 8.0 Hz, 1H, ArC**H**), 7.52 – 7.40 (m, 2H, 2 × ArC**H**), 3.69 (m, 14H, 7 × C$\mathbf{H_2}$O), 3.39 (t, *J* = 5.0 Hz, 2H, C$\mathbf{H_2}$O), 3.36 (s, 1H, C≡C**H**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.5 (**C**=O), 160.8 (**C**=O), 154.0 (**C**-O), 147.5 (**C**CO_2), 142.0 (Ar**C**), 129.7 (Ar**C**H), 128.8 (Ar**C**H), 128.0 (Ar**C**H), 120.0 (Ar**C**H), 118.8 (**C**C≡CH), 82.2 (C≡**C**H), 81.9 (**C**≡CH), 70.7 (**C**H_2O), 70.7 (**C**H_2O), 70.7 (**C**H_2O), 70.6 (**C**H_2O), 70.0 (**C**H_2O), 69.5 (**C**H_2O), 50.7 (**C**H_2NH), 39.8 (**C**H_2NH); LRMS *m/z* (ESI$^+$) 437.2 (M+Na$^+$, 100%).

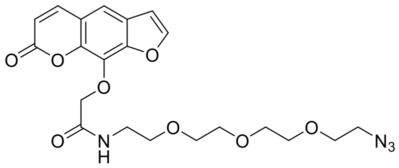
7-Ethynyl-2-oxo-*N*-(13-oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-*d*]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-*2H*-chromene-3-carboxamide (2.86)



Triphenylphosphine (0.032 g, 0.12 mmol, 1.1 equiv.) was added to a stirred solution of *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-7-ethynyl-2-oxo-*2H*-chromene-3-carboxamide **2.85** (0.081 g, 0.11 mmol, 1.0 equiv.) in THF (1 mL) and the reaction mixture was stirred for 1 h at rt. After this time, the reaction solvent was removed *in vacuo* to give the crude amine that was redissolved in CH_2Cl_2 (1 mL) and then perfluorophenyl 5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-d]imidazol-4-yl)pentanoate **2.64** (0.045 g, 0.11 mmol, 1.0 equiv.) and triethylamine (0.030 mL, 0.22 mmol, 2.0 equiv.) were added successively. The reaction mixture was stirred for 18 h at rt and then the solvent was removed to give a crude residue which was further purified by flash column chromatography (1% MeOH/ CH_2Cl_2 → 10% MeOH/ CH_2Cl_2) giving the title compound as a pale yellow gummy solid (0.027 g, 0.044 mmol, 40%); R_f 0.34 (10% MeOH/ CH_2Cl_2); ^{1}H NMR (700 MHz, Chloroform-*d*) δ 8.98 (s, 1H, CON**H**), 8.87 (s, 1H, C**H**), 7.65 (d, *J* = 8.0 Hz, 1H, ArC**H**), 7.47 (d, *J* = 1.5 Hz, 1H, ArC**H**), 7.44 (dd, *J* = 8.0, 1.5 Hz, 1H, ArC**H**), 6.77 (t, *J* = 5.5 Hz, 1H, CON**H**), 6.58 (s, 1H, HNCON**H**), 5.63 (s, 1H, **H**NCONH), 4.49 (s, 1H, CONHC**H**CH), 4.31 (s, 1H, C**H**CHH'S), 3.70 – 3.59 (m, 12H, 6 × OC**H**$_2$), 3.56 (t, *J* = 5.0 Hz, 2H, OC**H**$_2$), 3.42 (s, 2H, OC**H**$_2$), 3.37 (s, 1H, C≡C**H**), 3.12 (s, 1H, NHCHCHC**H**), 2.88 (dd, *J* = 13.0, 5.0 Hz, 1H, CHC**H**H'S), 2.73 (d, *J* = 13.0 Hz, 1H, CH**H'**S), 2.21 (t, *J* = 7.5 Hz, 2H, C**H**$_2$CONH), 1.77 – 1.60 (m, 4H, 2 × C**H**$_2$), 1.41 (s, 2H, C**H**$_2$); ^{13}C NMR (176 MHz, $CDCl_3$) δ 206.1 (**C**=O), 173.3 (**C**=O), 160.9 (**C**=O), 154.0 (**C**=O), 147.5 (**C**H), 129.7 (Ar**C**H), 128.8 (Ar**C**H), 127.9 (Ar**C**), 119.9 (Ar**C**H), 118.8 (Ar**C**), 118.8 (Ar**C**), 82.3 (C≡**C**H), 81.9 (**C**≡CH), 70.6 (O**C**H$_2$), 70.6

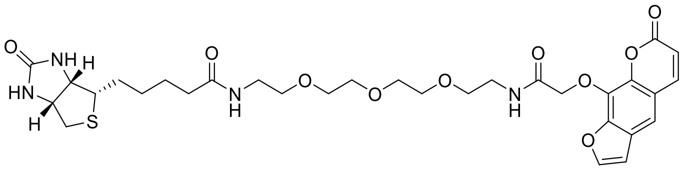
(O**C**H$_2$), 70.5 (O**C**H$_2$), 70.2 (O**C**H$_2$), 70.0 (O**C**H$_2$), 69.5 (O**C**H$_2$), 61.8 (NH**C**HCH), 60.3 (HN**C**HCHH'S), 55.6 (HNCH**C**H), 40.6 (CH**C**HH'S), 39.7 (CONH**C**H$_2$), 39.2 (CONH**C**H$_2$), 36.0 (**C**H$_2$CONH), 28.2 (**C**H$_2$), 28.1 (**C**H$_2$), 25.6 (**C**H$_2$); LRMS *m/z* (ESI$^+$) 637.2 (M+Na$^+$); HRMS *m/z* (ESI$^+$): found 637.2298, $C_{30}H_{38}N_4O_8SNa$ (M+Na$^+$) requires 637.2303. One aromatic quaternary carbon resonance is missing/overlapping in the ^{13}C NMR spectrum.

***N*-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-2-((7-oxo-7*H*-furo[3,2-*g*]chromen-9-yl)oxy)acetamide (2.87)**



Pentafluorophenyl trifluoroacetate **2.81** (0.13 mL, 0.65 mmol, 1.3 equiv.) was added dropwise to a solution of 2-((7-oxo-7*H*-furo[3,2-*g*]chromen-9-yl)oxy)acetic acid **2.24** (0.13 g, 0.50 mmol, 1.0 equiv.), 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine (0.12 g, 0.55 mmol, 1.1 equiv.) and triethylamine (0.075 mL, 0.65 mmol, 1.2 equiv.) in CH_2Cl_2 (5.0 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the reaction solvent was removed *in* vacuo and then the crude residue was further purified by flash column chromatography (CH_2Cl_2 → 2.5% MeOH/ CH_2Cl_2) to give the title compound (0.12 g, 0.26 mmol, 52%) as an off-white solid. R$_f$ 0.35 (5% MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.78 (d, *J* = 9.5 Hz, 1H, C**H**), 7.72 (d, *J* = 2.5 Hz, 1H, ArC**H**), 7.57 – 7.50 (m, 1H, N**H**), 7.43 (s, 1H, ArC**H**), 6.85 (d, *J* = 2.5 Hz, 1H, ArC**H**), 6.39 (d, *J* = 9.5 Hz, 1H, C**H**), 4.95 (s, 2H, COC**H$_2$**), 3.72 – 3.53 (m, 14H, 7 × C**H$_2$**O), 3.35 (t, *J* = 5.0 Hz, 2H, C**H$_2$**N$_3$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 168.2 (**C**=O), 159.8 (**C**=O), 147.3 (Ar**C**), 147.1 (Ar**C**H), 144.2 (**C**H), 142.9 (Ar**C**), 130.8 (Ar**C**O), 126.2 (Ar**C**), 116.5 (Ar**C**), 115.0 (**C**H), 114.3 (Ar**C**H), 106.9 (Ar**C**H), 72.3 (CO**C**H$_2$), 70.7 (**C**H$_2$O), 70.6 (**C**H$_2$O), 70.6 (**C**H$_2$O), 70.4 (**C**H$_2$O), 70.0 (CH_2O), 69.6 (CH_2O), 50.7 (CH_2N_3), 39.0 ($CONHCH_2$); LRMS *m/z* (ESI^+) 483.2 ($M+Na^+$, 100%); HRMS *m/z* (ESI^+): found 483.1485, $C_{21}H_{24}O_8N_4Na$ ($M+Na^+$) requires 483.1486.

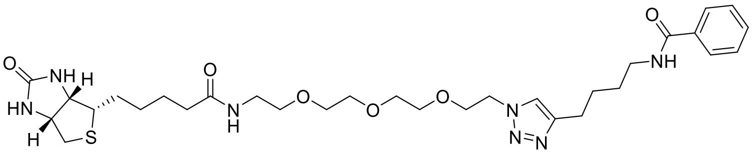
_N_-(2-Oxo-1-((7-oxo-7H-furo[3,2-g]chromen-9-yl)oxy)-6,9,12-trioxa-3-azatetradecan-14-yl)-5-((3a_S_,4_S_,6a_R_)-2-oxohexahydro-_1H_-thieno[3,4-d]imidazol-4-yl)pentanamide (2.88)



Triphenylphosphine (0.029 g, 0.11 mmol, 1.1 equiv.) was added to a stirred solution of *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-2-((7-oxo-7*H*-furo[3,2-*g*]chromen-9-yl)oxy)acetamide **2.87** (0.046 g, 0.10 mmol, 1.0 equiv.) in THF (1 mL) and the reaction mixture was stirred for 1 h at rt. After this time, the reaction solvent was removed *in vacuo* to give the crude amine that was redissolved in CH_2Cl_2 (1 mL) and then perfluorophenyl 5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-d]imidazol-4-yl)pentanoate **2.64** (0.041 g, 0.10 mmol, 1.0 equiv.) and triethylamine (0.027 mL, 0.2 mmol, 2.0 equiv.) were added successively. The reaction mixture was stirred for 18 h at rt and then the solvent was removed to give a crude residue which was further purified by flash column chromatography (1% MeOH/ CH_2Cl_2 → 10% MeOH/ CH_2Cl_2) giving the title compound (0.053 g, 0.080 mmol, 80%) as an off-white gummy solid. R_f 0.33 (10% MeOH/ CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 9.5 Hz, 1H, ArC**H**), 7.71 (d, *J* = 2.0 Hz, 1H, ArC**H**), 7.64 – 7.52 (m, 1H, N**H**), 7.43 (s, 1H, ArC**H**), 6.84 (dd, *J* = 2.5, 1.0 Hz, 1H, ArC**H**), 6.80 (t, *J* = 5.5 Hz, 1H, N**H**), 6.39 (s, 1H, N**H**), 6.36 (d, *J* = 9.5 Hz, 1H, C**H**), 5.59 (s, 1H, N**H**), 4.92 (d, *J* = 1.5 Hz, 2H, COC$\mathbf{H_2}$), 4.50 – 4.41 (m, 1H, CONHC**H**CH), 4.27 (ddd, *J* = 8.0, 4.5, 1.5 Hz, 1H, C**H**CHH'S), 3.69 – 3.46 (m, 14H, 6 × C$\mathbf{H_2}$O, C$\mathbf{H_2}$NH), 3.37 (td, *J* = 5.5, 4.0 Hz, 2H, C$\mathbf{H_2}$NH), 2.86 (dd, *J* = 13.0, 5.0 Hz, 1H, CH**H'**S), 2.71 (d, *J*

= 13.0 Hz, 1H, C**H**H'S), 2.18 (t, *J* = 7.5 Hz, 2H, C**H₂**), 1.75 – 1.55 (m, 4H, 2 × C**H₂**), 1.45 – 1.36 (m, 2H, C**H₂**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3 (**C**=O), 168.3 (**C**=O), 163.9 (**C**=O), 159.9 (**C**=O), 147.3 (**C**-O), 147.1 (Ar**C**H), 144.4 (**C**H), 142.8 (Ar**C**) , 130.7 (Ar**C**), 126.3 (**C**-O), 116.5 (Ar**C**), 114.9 (**C**H), 114.4 (Ar**C**H), 107.0 (Ar**C**H), 72.2 (CO**C**H_2), 70.4 (**C**H_2O), 70.4 (**C**H_2O), 70.3 (**C**H_2O), 70.1 (**C**H_2O), 69.9 (**C**H_2O), 69.6 (**C**H_2O), 61.7 (CONH**C**HCH), 60.2 (**C**HCHH'S), 55.5 (CONHCH**C**H), 40.5 (**C**HH'S), 39.1 (**C**H_2NH), 38.9 (**C**H_2NH) 35.9 (**C**H_2), 28.2 (**C**H_2), 28.1 (**C**H_2), 25.6 (**C**H_2); LRMS *m/z* (ESI^+) 683.2 ($M+Na^+$, 100%); HRMS *m/z* (ESI^+): found 661.2532, $C_{31}H_{41}N_4O_{10}S$ ($M+H^+$) requires 661.2538.

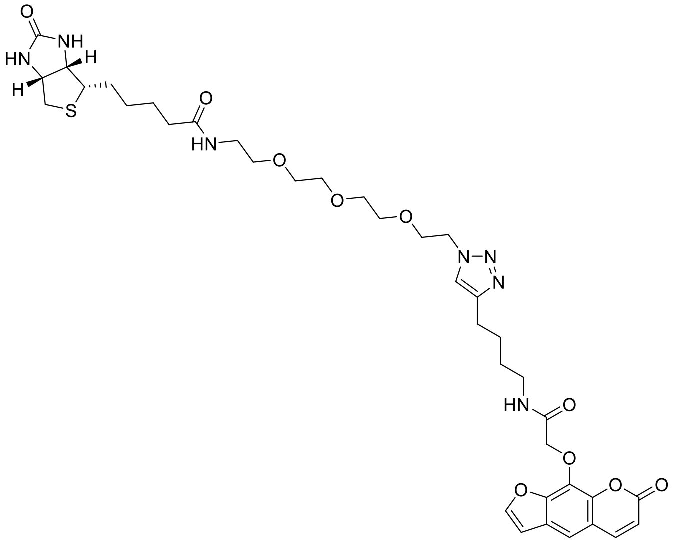
***N*-(4-(1-(13-oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-*d*]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-*1H*-1,2,3-triazol-4-yl)butyl)benzamide (2.9)**



Copper sulfate hydrate (0.0050 g, 0.020 mmol, 0.10 equiv.) and sodium-l-ascorbate (0.0040 g, 0.020 mmol, 0.10 equiv.) were added to a stirred solution of *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-*1H*-thieno[3,4-d]imidazol-4-yl)pentanamide **2.82** (0.090 g, 0.20 mmol, 1.0 equiv.) and *N*-(hex-5-yn-1-yl)benzamide (0.078 g, 0.24 mmol, 1.2 equiv.) in a mixture of DMF/H_2O (5:1, 3.1 mL/ 0.6 mL) and the reaction mixture was stirred at rt for 48 h. The solvent was removed under a stream of N_2 and then the crude residue was further purified by flash column chromatography (5% MeOH/ CH_2Cl_2 → 10% MeOH/ CH_2Cl_2) to give the title compound as a white solid (0.13 g, 0.20 mmol, 99%). R_f 0.36 (10% MeOH/ CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 – 7.78 (m, 2H, 2 × ArC**H**), 7.51 (s, 1H, ArC**H**), 7.49 – 7.45 (m, 1H, ArC**H**), 7.41 (t, *J* = 7.5 Hz, 2H, 2 × ArC**H**), 6.86 (t, *J* = 5.5 Hz, 1H, N**H**), 6.73 (t, *J* = 5.5 Hz, 1H, N**H**), 6.16 (s, 1H, N**H**), 5.16 (s, 1H, N**H**), 4.51

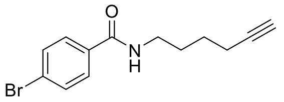
(t, *J* = 5.0 Hz, 2H, C**H**$_2$N), 4.47 (dd, *J* = 8.0, 5.0 Hz, 1H, CONHC**H**CH), 4.29 (dd, *J* = 8.0, 4.5 Hz, 1H, C**H**CHH'S), 3.87 (t, *J* = 5.0 Hz, 2H, C**H**$_2$), 3.65 – 3.52 (m, 12H, 6 × C**H**$_2$O), 3.52 (t, *J* = 5.0 Hz, 2H, C**H**$_2$NH), 3.47 (q, *J* = 6.5 Hz, 2H, C**H**$_2$), 3.40 (q, *J* = 5.5 Hz, 2H, C**H**$_2$), 3.12 (td, *J* = 7.5, 4.5 Hz, 1H, CONHCHC**H**), 2.88 (dd, *J* = 13.0, 5.0 Hz, 1H, CH**H'**S), 2.76 (t, *J* = 7.5 Hz, 2H, C**H**$_2$), 2.70 (d, *J* = 13.0 Hz, 1H, C**H**H'S), 2.22 – 2.12 (m, 2H, C**H**$_2$), 1.77 – 1.57 (m, 4H, C**H**$_2$), 1.45 – 1.37 (m, 2H, C**H**$_2$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 173.3 (**C**=O), 167.6 (**C**=O), 163.6 (Ar**C**H), 147.6 (**C**=O), 134.7 (**C**CO$_2$), 131.3 (Ar**C**H), 128.5 (Ar**C**H), 127.0 (Ar**C**H), 122.1 (Ar**C**H), 70.5 (**C**H$_2$O), 70.4 (**C**H$_2$O), 70.4 (**C**H$_2$O), 70.1 (**C**H$_2$O), 69.9 (**C**H$_2$O), 69.6 (**C**H$_2$O), 61.8 (CONH**C**HCH), 60.1 (**C**HCHH'S), 55.5 (CONHCH**C**H), 50.1 (**C**H$_2$N), 40.5 (**C**HH'S), 39.8 (**C**H$_2$), 39.2 (**C**H$_2$), 35.8 (**C**H$_2$), 28.9 (**C**H$_2$), 28.1 (**C**H$_2$), 28.1 (**C**H$_2$), 26.8 (**C**H$_2$), 25.5 (**C**H$_2$), 25.1 (**C**H$_2$); LRMS *m/z* (ESI$^+$) 646.4 (M+H$^+$, 100%); HRMS: found 646.3372, $C_{31}H_{48}N_7O_6S$ (M+H$^+$) requires 646.3381.

N-(2-(2-(2-(2-(4-(4-(2-((7-Oxo-7*H*-furo[3,2-*g*]chromen-9-yl)oxy)acetamido)butyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)ethyl)-5-((3a*R*,4*R*,6a*S*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (2.91)



Copper sulfate pentahydrate (0.0025 g, 0.0010 mmol, 10 mol%) and sodium l-ascorbate (0.0020 g, 0.0010 mmol, 10 mol%) were added successively to a stirred solution of *N*-(hex-5-yn-1-yl)-2-((7-oxo-7*H*-furo[3,2-*g*]chromen-9-yl)oxy)acetamide **1.63** (0.041 g, 0.12 mmol, 1.2 equiv.) and *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide **2.82** (0.045 g, 0.10 mmol, 1.0 equiv.) in a mixture of DMF/H_2O (5:1, 1.85 mL) and the reaction mixture was stirred at rt for 48 h. After this time, the reaction solvent was removed under a stream of N_2 and the resulting crude residue was further purified by flash column chromatography (CH_2Cl_2 → 10% MeOH/CH_2Cl_2) to give the title compound as a white foamy solid (0.060 g, 0.077 mmol, 77%). R_f 0.24 (10% MeOH/CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, *J* = 9.5 Hz, 1H, C**H**), 7.75 (d, *J* = 2.0 Hz, 1H, ArC**H**), 7.53 (s, 1H, CH_2CC**H**), 7.47 (s, 1H, ArC**H**), 7.44 (t, *J* = 6.0 Hz, 1H, N**H**), 6.87 (d, *J* = 2.0 Hz, 1H, ArC**H**), 6.83 (t, *J* = 5.5 Hz, 1H, N**H**), 6.46 (s, 1H, N**H**), 6.40 (d, *J* = 9.5 Hz, 1H, C**H**), 5.59 (s, 1H, N**H**), 4.94 (s, 2H, COC**H₂**), 4.55 – 4.48 (m, 3H, C**H₂**CCH, CONHC**H**CH), 4.31 (ddd, *J* = 8.0, 4.5, 1.5 Hz, 1H, C**H**CHH'S), 3.89 (t, *J* = 5.0 Hz, 2H, C**H₂**O), 3.67 – 3.53 (m, 10H, 5 × C**H₂**O), 3.44 (q, *J* = 6.5, 6.0 Hz, 4H, C**H₂**NH, C**H₂**N), 3.14 (td, *J* = 7.5, 4.5 Hz, 1H, CONHCHC**H**), 2.90 (dd, *J* = 13.0, 5.0 Hz, 1H, C**H**H'S), 2.80 – 2.71 (m, 3H, CH**H'**S, NHC**H₂**), 2.26 – 2.17 (m, 6H, NHCH₂C**H₂**, C**H₂**CH₂C, C**H₂**CONH), 1.80 – 1.59 (m, 4H, C**H₂**CH₂C, CHC**H₂**CH₂), 1.43 (p, *J* = 8.5, 8.0 Hz, 2H, CHCH₂C**H₂**); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4 (**C**=O), 168.2 (**C**=O), 163.9 (**C**=O), 159.9 (**C**=O), 147.6 (Ar**C**H), 147.4 (Ar**C**), 147.1 (Ar**C**), 144.4 (Ar**C**H), 142.9 (Ar**C**), 130.8 (Ar**C**), 126.3 (Ar**C**), 122.1 (Ar**C**H), 116.5 (Ar**C**), 114.9 (Ar**C**H), 114.6 (Ar**C**H), 107.0 (Ar**C**H), 72.4 (CO**C**H₂), 70.5 (**C**H₂O), 70.5 (**C**H₂O), 70.4 (**C**H₂O), 70.1 (**C**H₂O), 69.9 (**C**H₂O), 69.6 (**C**H₂O), 61.8 (CONH**C**HCH), 60.2 (**C**HCHH'S), 55.6 (CONHCH**C**H), 50.1 (**C**H₂C), 40.5 (**C**HH'S), 39.1 (**C**H₂NH), 38.9 (**C**H₂N), 31.0 (NH**C**H₂), 29.0 (**C**H₂CH₂C), 28.2 (NHCH₂**C**H₂), 28.1 (CH₂**C**H₂CO), 26.8 (CH**C**H₂CH₂), 25.6 (**C**H₂CH₂CO), 25.3 (CHCH₂**C**H₂); LRMS *m/z* (ESI$^+$) 784.4 (M+H$^+$); HRMS *m/z* (ESI$^+$): found 784.3324, $C_{37}H_{50}O_{10}N_7S_1$ (M+H$^+$) requires 784.3334.

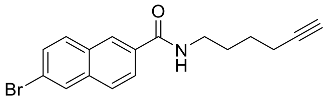
4-Bromo-*N*-(hex-5-yn-1-yl)benzamide (2.92)



N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.72 g, 3.8 mmol, 1.5 equiv.) was added to a stirred solution of 4-bromobenzoic acid (0.50 g, 2.5 mmol, 1.0 equiv.), hex-5-yn-1-amine hydrochloride **2.58** (0.37 g, 2.8 mmol, 1.1 equiv.) and triethylamine (0.52 mL, 3.8 mmol, 1.5 equiv.) in CH_2Cl_2 (25 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the reaction was quenched by adding H_2O (25 mL) and the organics were extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give the crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 30% EtOAc/

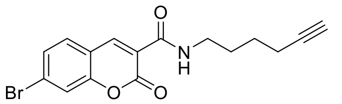
petroleum ether 40-60) to give the title compound (0.36 g, 1.3 mmol, 52%) as a white solid. R_f 0.26 (20% EtOAc/ Petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 – 7.59 (m, 2H, 2 × ArC**H**), 7.58 – 7.50 (m, 2H, 2 × ArC**H**), 6.38 – 6.29 (m, br, 1H, N**H**), 3.45 (td, *J* = 7.0, 6.0 Hz, 2H, NHC**H₂**), 2.24 (td, *J* = 7.0, 2.5 Hz, 2H, $NHCH_2C\mathbf{H_2}$), 1.97 (t, *J* = 2.5 Hz, 1H, C**H**), 1.78 – 1.68 (m, 2H, C**H₂**C), 1.66 – 1.54 (m, 2H, $C\mathbf{H_2}CH_2C$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6 (**C**=O), 133.5 (Ar**C**), 131.8 (Ar**C**H), 128.5 (Ar**C**H), 126.0 (Ar**C**Br), 84.0 ($CH_2\mathbf{C}$), 68.9 (**C**H), 39.6 ($NH\mathbf{C}H_2$), 28.6 ($\mathbf{C}H_2C$), 25.7 ($\mathbf{C}H_2CH_2C$), 18.1 ($NHCH_2\mathbf{C}H_2$); LRMS *m/z* (ESI$^+$) 280.0 (M+H$^+$); HRMS *m/z* (ESI$^+$): found 280.0333, $C_{13}H_{15}ONBr$ (M+H$^+$) requires 280.0332.

6-Bromo-*N*-(hex-5-yn-1-yl)-2-naphthamide (2.93)



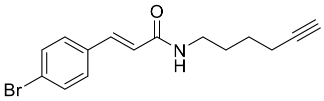
N-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.11 g, 0.75 mmol, 1.5 equiv.) was added to a stirred solution of 6-bromo-2-naphthoic acid (0.13 g, 0.5 mmol, 1.0 equiv.), hex-5-yn-1-amine hydrochloride **2.58** (0.073 g, 0.55 mmol, 1.1 equiv.), triethylamine (0.15 mL, 1.1 mmol, 2.2 equiv.) and 1-hydroxybenzotriazole hydrate (0.10 g, 0.75 mmol, 1.5 equiv.) in CH_2Cl_2 (5.0 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the reaction mixture was diluted with H_2O and the organics extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and the solvent remove *in vacuo*. The resulting crude residue was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) to give the title compound (0.10 g, 0.31 mmol, 61%) as a white solid. R_f 0.53 (30% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, *J* = 2.0 Hz, 1H, ArC**H**), 8.03 (d, *J* = 2.0 Hz, 1H, ArC**H**), 7.83 (dd, *J* = 8.5, 2.0 Hz, 1H, ArC**H**), 7.80 – 7.75 (m, 2H, 2 × ArC**H**), 7.60 (dd, *J* = 8.5, 2.0 Hz, 1H, ArC**H**), 6.36 (t, *J* = 5.5 Hz, 1H, N**H**), 3.54 (td, *J* = 7.0, 5.5 Hz, 2H, NHC**H₂**), 2.28 (td, *J* = 7.0, 2.5 Hz, 2H, C**H₂**C), 1.98 (t, *J* = 2.5 Hz, 1H, C**H**), 1.87 – 1.75 (m, 2H, NHCH$_2$C**H₂**), 1.69 – 1.64 (m, 2H, C**H₂**CH$_2$C); ^{13}C NMR (126 MHz, CDCl$_3$) δ 167.2 (**C**=O), 135.6 (Ar**C**), 132.4 (Ar**C**), 131.1 (Ar**C**), 130.4 (Ar**C**H), 130.3 (Ar**C**H), 129.9 (Ar**C**H), 127.6 (Ar**C**H), 127.2 (Ar**C**H), 124.6 (Ar**C**H), 121.9 (Ar**C**), 84.1 (CH$_2$**C**), 68.9 (**C**H), 39.7 (NH**C**H$_2$), 28.7 (NHCH$_2$**C**H$_2$), 25.8 (**C**H$_2$CH$_2$C), 18.2 (**C**H$_2$C); LRMS *m/z* (ESI$^+$) 330.0 (M+H$^+$, 50%), 332.0 (M+H$^+$, 50%); HRMS *m/z* (ESI$^+$): found 330.0488, $C_{17}H_{17}ON^{79}Br$ (M+H$^+$) requires 330.0488.

7-Bromo-*N*-(hex-5-yn-1-yl)-2-oxo-2*H*-chromene-3-carboxamide (2.94)



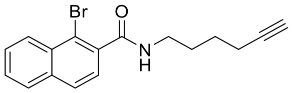
Piperidine (5 drops) was added to a stirred solution of 4-bromo-2-hydroxybenzaldehyde (0.071 g, 0.34 mmol, 1.0 equiv.) and ethyl 3-(hex-5-yn-1-ylamino)-3-oxopropanoate (0.081 g, 0.41 mmol, 1.2 equiv.) in EtOH (3.4 mL) and the reaction mixture was heated to 60 °C and stirred for 48 h. After this time the reaction was cooled to rt and the solvent removed *in vacuo* giving a crude residue that was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40/60 → 30% EtOAc/ petroleum ether 40/60) to give the title compound (0.050 g, 0.14 mmol, 42%) as an off white solid. R*f* 0.47 (30% EtOAc/ petroleum ether 40/60); ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.84 (s, 1H, ArC**H**), 8.72 (t, *J* = 6.0 Hz, 1H, N**H**), 7.58 (d, *J* = 1.5 Hz, 1H, ArC**H**), 7.54 (d, *J* = 8.0 Hz, 1H, ArC**H**), 7.50 (dd, *J* = 8.0, 1.5 Hz, 1H, ArC**H**), 3.47 (td, *J* = 7.0, 6.0 Hz, 2H, NHC**H₂**), 2.24 (td, *J* = 7.0, 2.5 Hz, 2H, C**H₂**C), 1.96 (t, *J* = 2.5 Hz, 1H, C**H**), 1.80 – 1.70 (m, 2H, NHCH$_2$C**H₂**), 1.62 (m, 2H, C**H₂**CH$_2$C); ^{13}C NMR (126 MHz, CDCl$_3$) δ 161.1 (**C**=O), 160.8 (**C**=O), 154.4 (Ar**C**), 147.5 (Ar**C**H), 130.6 (Ar**C**H), 128.9 (Ar**C**H), 128.4 (Ar**C**), 120.0 (Ar**C**H), 118.7 (Ar**C**), 117.5 (Ar**C**), 83.9 (CH$_2$**C**), 68.8 (**C**H), 39.4 (NH**C**H$_2$), 28.5 (NHCH$_2$**C**H$_2$), 25.8 (**C**H$_2$CH$_2$C), 18.1 ($\mathbf{C}H_2C$); LRMS *m/z* (ESI$^+$) 370.0 (M+Na$^+$, 50%), 372.1 (M+Na$^+$, 50%); HRMS *m/z* (ESI$^+$): found 370.0051, $C_{16}H_{14}O_3N^{79}BrNa$ (M+Na$^+$) requires 370.0049.

(*E*)-3-(4-Bromophenyl)-*N*-(hex-5-yn-1-yl)acrylamide (2.95)



N-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.29 g, 1.5 mmol, 1.5 equiv.) was added to a stirred solution of (*E*)-3-(4-bromophenyl)acrylic acid (0.23 g, 1.0 mmol, 1.0 equiv.), hex-5-yn-1-amine hydrochloride **2.58** (0.15 g, 1.1 mmol, 1.1 equiv.), triethylamine (0.30 mL, 2.2 mmol, 2.2 equiv.) and 1-hydroxybenzotriazole hydrate (0.20 g, 1.5 mmol, 1.5 equiv.) in CH_2Cl_2 (10 mL) and the reaction mixture was stirred at rt for 18 h. The reaction mixture was diluted with H_2O and the layers were separated, the aqueous layer was then further extracted with EtOAc (2 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and the solvent remove *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/petroleum ether 40-60 → 40% EtOAc/petroleum ether 40-60) to give the title compound (0.11 g, 0.37 mmol, 37%) as a white solid. R_f 0.17 (30% EtOAc/petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, *J* = 15.5 Hz, 1H, C**H**CHCO), 7.50 – 7.44 (m, 2H, ArC**H**), 7.37 – 7.30 (m, 2H, ArC**H**), 6.38 (d, *J* = 15.5 Hz, 1H, CHC**H**CO), 5.83 (t, *J* = 6.0 Hz, 1H, N**H**), 3.41 (td, *J* = 7.0, 6.0 Hz, 2H, CONHC$\mathbf{H_2}$), 2.24 (td, *J* = 7.0 Hz, 2.5, 2H, C$\mathbf{H_2}$C), 1.96 (t, *J* = 2.5 Hz, 1H, C**H**), 1.75 – 1.63 (m, 2H, $NHCH_2C\mathbf{H_2}$), 1.59 (m, 2H, C$\mathbf{H_2}$$CH_2C$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.6 (**C**=O), 139.7 (**C**HCHCO), 133.8 (Ar**C**), 132.0 (Ar**C**H), 129.2 (Ar**C**H), 123.8 (Ar**C**), 121.3 (CH**C**HCO), 84.0 ($CH_2\mathbf{C}$), 68.8 (**C**H), 39.3 (NH**C**H_2), 28.7 ($NHCH_2\mathbf{C}H_2$), 25.7 ($\mathbf{C}H_2CH_2C$), 18.1 ($\mathbf{C}H_2C$); LRMS *m/z* (ESI$^+$) 306.2 (M+H$^+$, 50%), 308.0 (M+H$^+$, 50%); HRMS *m/z* (ESI$^+$): found 306.0489, $C_{15}H_{17}ON^{79}Br$ (M+H$^+$) requires 306.0488.

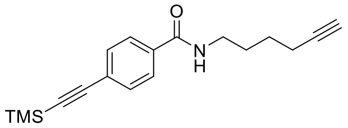
1-Bromo-*N*-(hex-5-yn-1-yl)-2-naphthamide (2.96)



N-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.11 g, 0.75 mmol, 1.5 equiv.) was added to a stirred solution of 1-bromo-2-naphthoic acid (0.13 g, 0.5 mmol, 1.0 equiv.), hex-5-yn-1-amine hydrochloride **2.58** (0.073 g, 0.55 mmol, 1.1 equiv.), triethylamine (0.15 mL, 1.1 mmol, 2.2 equiv.) and 1-hydroxybenzotriazole hydrate (0.10 g, 0.75 mmol, 1.5 equiv.) in CH_2Cl_2 (5.0 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the reaction mixture was diluted with H_2O and the organics were extracted with CH_2Cl_2 (3 × 10 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and the solvent remove *in vacuo*. The resulting crude residue was then further purified by flash column chromatography (10% EtOAc/petroleum ether 40/60 → 30% EtOAc/petroleum ether 40-60) to give the title compound (0.10 g, 0.31 mmol, 62%) as a white solid. R$_f$ 0.52 (30% EtOAc/petroleum ether 40/60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (dd, *J* = 8.5, 1.0 Hz, 1H, ArC**H**), 7.84 (m, 2H, 2 × ArC**H**), 7.66 (ddd, *J* = 8.5, 7.0, 1.5 Hz, 1H, ArC**H**), 7.60 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H, ArC**H**), 7.50 (d, *J* = 8.5 Hz, 1H, ArC**H**), 6.08 (s, br, 1H, N**H**), 3.56 (td, *J* = 7.0, 6.0 Hz, 2H, NHC**H₂**), 2.30 (td, *J* = 7.0, 2.5 Hz, 2H, C**H₂**C), 1.99 (t, *J* = 2.5 Hz, 1H, C**H**), 1.82 (tt, *J* = 7.5, 6.5 Hz, 2H, NHCH₂C**H₂**), 1.74 – 1.65 (m, 2H, C**H₂**CH₂); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.7 (**C**=O), 136.5 (Ar**C**), 134.6 (Ar**C**), 131.9 (Ar**C**), 128.3 (Ar**C**H), 128.3 (Ar**C**H), 128.2 (Ar**C**H), 127.9 (Ar**C**H), 127.6 (Ar**C**H), 125.2 (Ar**C**H), 119.7 (Ar**C**), 84.0 (CH₂**C**), 68.8 (**C**H), 39.6 (NH**C**H₂), 28.5

($NHCH_2$**C**H_2), 25.8 (**C**H_2CH_2C), 18.1 (**C**H_2C); LRMS *m/z* (ESI^+) 330.0 ($M+H^+$, 50%), 332.0 ($M+H^+$, 50%); HRMS *m/z* (ESI^+): found 330.0489, $C_{17}H_{17}ON^{79}Br$ ($M+H^+$) requires 330.0488.

***N*-(Hex-5-yn-1-yl)-4-((trimethylsilyl)ethynyl)benzamide (2.97)**

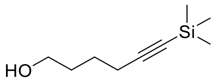


Bis(triphenylphosphine)palladium(II) dichloride (0.018 g, 0.025 mmol, 5.0 mol%) was added to an N_2 sparged, stirred solution of 4-bromo-*N*-(hex-5-yn-1-yl)benzamide **2.92** (0.14 g, 0.50 mmol, 1.0 equiv.), ethynyltrimethylsilane (0.14 mL, 1.0 mmol, 2.0 equiv.), triethylamine (0.14 mL, 1.0 mmol, 1.0 equiv.) and copper(I) iodide (0.0050 g, 0.025 mmol, 5.0 mol%) in MeCN (5 mL) and the reaction vessel was sealed and heated to 60 °C for 18 h. After this time, the reaction mixture was cooled to rt and then filtered through a pad of celite to remove fine particle impurities, the filtrate was concentrated *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) giving the title compound (0.097 g, 0.33 mmol, 66%) as a yellow oil. R_f 0.41 (20% EtOAc/petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.0 Hz, 2H, ArC**H**), 7.55 – 7.49 (m, 2H, ArC**H**), 6.32 (t, *J* = 6.0 Hz, 1H, N**H**), 3.49 (q, *J* = 6.5 Hz, 2H, NHC**H₂**), 2.28 (td, *J* = 7.0, 2.5 Hz, 2H, C**H₂**C), 2.00 (t, *J* = 2.5 Hz, 1H, C**H**), 1.77 (p, *J* = 7.0 Hz, 2H, $NHCH_2C$**H₂**), 1.68 – 1.57 (m, 2H, C**H₂**CH_2C), 0.28 (s, 9H, Si(C**H₃**)₃); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8 (**C**=O), 134.2 (Ar**C**CO_2), 132.1 (Ar**C**H), 126.8 (Ar**C**H), 126.3 (Ar**C**C), 104.0 (**C**Si), 96.9 (ArC**C**), 84.0 (CH_2**C**), 68.9 (**C**H), 39.6 (NH**C**H_2), 28.7 (**C**H_2C), 25.8 ($NHCH_2$**C**H_2), 18.1 (**C**H_2CH_2C), -0.12 (Si(**C**H_3)₃); LRMS *m/z* (ESI^+) 298.5 ($M+H^+$, 100%); HRMS *m/z* (ESI^+): found 298.1623, $C_{18}H_{24}ONSi$ ($M+H^+$) requires 298.1622.

OR

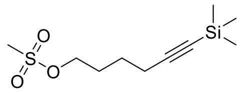
Tetrakis(triphenylphosphine)palladium(0) (0.014 g, 0.013 mmol, 2.5 mol%) was added to a stirred solution of 4-bromo-*N*-(hex-5-yn-1-yl)benzamide **2.92** (0.14 g, 0.50 mmol, 1.0 equiv.), ethynyltrimethylsilane (0.14 mL, 1.0 mmol, 2.0 equiv.), triethylamine (0.14 mL, 1.0 mmol, 1.0 equiv.) and copper(I) iodide (0.0050 g, 0.025 mmol, 5.0 mol%) in freeze pump thaw degassed THF (three cycles, 5 mL) and the reaction vessel was sealed and heated to 60 °C for 18 h. After this time, the reaction mixture was cooled to rt, filtered through celite to remove fine particle impurities and the filtrate concentrated *in vacuo.* The resulting crude residue was further purified by flash column chromatography (5% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) giving the title compound (0.10 g, 0.35 mmol, 70%) as a yellow oil. Data as above.

6-(Trimethylsilyl)hex-5-yn-1-ol (2.99)



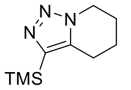
According to procedure of Peixoto *et al.*:[223] 4-(dimethylamino)pyridine (0.12 g, 1.0 mmol, 0.20 equiv.) was added to a stirred solution of hex-5-yn-1-ol **2.56** (0.50 g, 5.1 mmol, 1.0 equiv.) and the reaction mixture was cooled to -78 °C. *n*-BuLi (2.5 M in hexanes, 4.5 mL, 11 mmol, 2.2 equiv.) was added dropwise and the reaction mixture was stirred for 1 h. Chlorotrimethylsilane (2.3 mL, 18 mmol, 3.6 equiv.) was added and the reaction mixture was warmed to rt and then stirred a further 2 h. After this time, the reaction mixture was quenched by addition of 1 M HCl (5 mL) and stirred for 30 mins before diluting with EtOAc (20 mL). The layers were separated, and the aqueous layer was further extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with sat. $NaHCO_3$ (20 mL), brine (20mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The resulting crude residue was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 20% EtOAc/ petroleum ether 40-60) to give the title compound (0.78 g, 4.6 mmol, 90%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (t, *J* = 6.0 Hz, 2H, HOC**H₂**), 2.27 (t, *J* = 7.0 Hz, 2H, C**H₂**C), 1.73 – 1.54 (m, 4H, $HOCH_2C$**H₂**, C**H₂**CH_2C), 1.40 (s, br, 1H, O**H**), 0.14 (s, 9H, Si(C**H₃**)₃); ^{13}C NMR (101 MHz, $CDCl_3$) δ 107.1 (CH_2**C**), 84.8 (**C**Si), 62.3 (HO**C**H_2), 31.8 (**C**H_2C), 24.8 ($HOCH_2$**C**H_2), 19.6 (**C**H_2CH_2C), 0.10 (Si(**C**H_3)₃); LRMS *m/z* (ESI$^+$) 171.2 (M+H$^+$, 100%). Data are in accordance with the literature.[223]

6-(Trimethylsilyl)hex-5-yn-1-yl methanesulfonate (2.100)



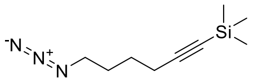
According to procedure of Egger *et al.*:[224] triethylamine (1.5 mL, 11 mmol, 2.4 equiv.) was added to a stirred solution of 6-(trimethylsilyl)hex-5-yn-1-ol **2.99** (0.78 g, 4.6 mmol, 1.0 equiv.) in CH_2Cl_2 (5 mL) at 0 °C. Methanesulfonyl chloride (0.61 mL, 7.8 mmol, 1.7 equiv.) was added dropwise and the reaction mixture stirred for 1 h. After this time, the reaction was quenched by slow addition of H_2O (10 mL) and then allowed to stir for 15 mins. The layers were separated and the organic layer was washed with H_2O (5 mL), brine (5 mL), dried ($MgSO_4$), filtered and then the solvent removed *in vacuo* giving the title compound (1.1 g, 4.6 mmol, 100%) as a yellow oil which required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.26 (t, *J* = 6.5 Hz, 2H, OC**H₂**), 3.01 (s, 3H, C**H₃**S), 2.29 (t, *J* = 7.0 Hz, 2H, C**H₂**C), 1.93 – 1.81 (m, 2H, OCH_2C**H₂**), 1.70 – 1.58 (m, 2H, C**H₂**CH_2C), 0.14 (s, 9H, Si(C**H₃**)₃); ^{13}C NMR (101 MHz, $CDCl_3$) δ 106.0 (**C**Si), 85.4 (CH_2**C**), 69.4 (O**C**H_2), 37.3 (**C**H_3S), 28.0 (**C**H_2C), 24.3 (OCH_2**C**H_2), 19.15 (**C**H_2CH_2C), 0.0 (Si(**C**H_3)₃). Data are in accordance with the literature.[224]

3-(Trimethylsilyl)-4,5,6,7-tetrahydro-(1,2,3)triazolo[1,5-*a*]pyridine (2.101)



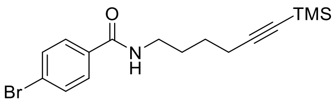
Sodium azide (0.60 g, 9.2 mmol, 2.0 equiv.) was added to a stirred solution of 6-(trimethylsilyl)hex-5-yn-1-yl methanesulfonate **2.100** (1.1 g, 4.6 mmol, 1.0 equiv.) in EtOH (15 mL) and DMF (2.3 mL) and the reaction mixture was heated to 80 °C for 18 h. After this time, the reaction mixture was cooled to rt and the poured onto a 1:1 mixture of EtOAc/H_2O (20 mL) and the organics extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (4 × 20 mL), dried ($MgSO_4$), filtered and then solvent removed *in vacuo* to give the title compound (0.90 g, 4.6 mmol, 100%) as a yellow oil which required no further purification. R_f 0.10 (20% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.40 (t, *J* = 6.0 Hz, 2H, NC**H₂**), 2.87 (t, *J* = 6.5 Hz, 2H, C**H₂**CC), 2.10 – 2.02 (m, 2H, C**H₂**CH_2CC), 1.97 – 1.89 (m, 2H, NCH_2C**H₂**), 0.34 (s, 9H, Si(C**H₃**)₃); ^{13}C NMR (126 MHz, $CDCl_3$) δ 141.5 (CH_2C**C**), 138.9 (CH_2**C**C), 46.0 (N**C**H_2), 22.7 (**C**H_2CC), 21.4 (NCH_2**C**H_2), 20.5 (**C**H_2CH_2CC), -1.0 (Si(**C**H_3)$_3$); LRMS *m/z* (ESI$^+$) 196.0 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 196.1260, $C_9H_{18}N_3Si$ (M+H$^+$) requires 196.1265.

(6-Azidohex-1-yn-1-yl)trimethylsilane (2.102)



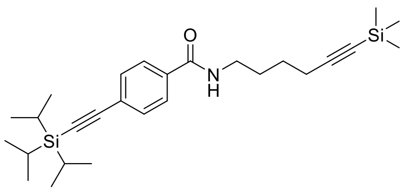
According to procedure of Egger *et al.*:[224] sodium azide (0.34 g, 5.2 mmol, 1.2 equiv.) was added to a stirred solution of 6-(trimethylsilyl)hex-5-yn-1-yl methanesulfonate **2.100** (1.1 g, 4.3 mmol, 1.0 equiv.) in DMF (10 mL) and the reaction mixture was stirred at 65 °C for 15 mins. After this time, the reaction mixture was cooled and H_2O (15 mL) added. The organics were extracted with Et$_2$O (3 × 30 mL)and then the organic layers combined and washed thoroughly with water (2 × 20 mL) and brine (20 mL). The organics were dried (MgSO$_4$), filtered, and the solvent removed *in vacuo* to give the title compound as a pale yellow oil (0.79 g, 4.1 mmol, 94%) which required no further purification. ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.30 (t, *J* = 7.0 Hz, 2H, N$_3$C**H$_2$**), 2.27 (t, *J* = 7.0 Hz, 2H, C**H$_2$**C), 1.80 – 1.66 (m, 2H, N$_3$CH$_2$C**H$_2$**), 1.66 – 1.51 (m, 2H, C**H$_2$**CH$_2$C), 0.15 (s, 9H, Si(C**H$_3$**)$_3$); ^{13}C NMR (101 MHz, CDCl$_3$) δ 106.3 (CH$_2$**C**), 85.1 (**C**Si), 50.9 (N$_3$**C**H$_2$), 27.8 (N$_3$CH$_2$**C**H$_2$), 25.5 (**C**H$_2$CH$_2$C), 19.3 (**C**H$_2$C), 0.0 (Si(**C**H$_3$)$_3$); LRMS *m/z* (ESI$^+$) 196.0 (M+H$^+$, 100%). Data are in accordance with the literature.[224]

4-Bromo-*N*-(6-(trimethylsilyl)hex-5-yn-1-yl)benzamide (2.103)



Triphenylphosphine (0.87 g, 3.3 mmol, 1.2 equiv.) and H$_2$O (0.5 mL) were added to a stirred solution of (6-azidohex-1-yn-1-yl)trimethylsilane **2.102** (0.54 g, 2.8 mmol, 1.0 equiv.) in THF (30 mL) and the reaction mixture was heated to 60 °C for 4.5 h. After this time, the solvent was removed and then the crude mixture redissolved in CH$_2$Cl$_2$ (25 mL). 4-Bromobenzoic acid (0.50 g, 2.5 mmol, 0.9 equiv.), triethylamine (0.76 mL, 5.5 mmol, 2.0 equiv.), 1-hydroxybenzotriazole hydrate (0.51 g, 3.8 mmol, 1.3 equiv.) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1.1 g, 3.8 mmol, 1.3 equiv.) were added successively and then the reaction mixture stirred at rt for 18 h. The reaction mixture was diluted with H$_2$O (30 mL) and the layers separated, the aqueous layer was then further extracted with CH$_2$Cl$_2$ (3 × 20 mL). The combined organic layers were dried (MgSO$_4$), filtered and solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% EtOAc/petroleum ether 40-60 → 20% EtOAc/petroleum ether 40-60) to give the title compound (0.47 g, 1.3 mmol, 54%) as a white solid. R*f* 0.12 (10% EtOAc/petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 – 7.63 (m, 2H, 2 × Ar**H**), 7.61 – 7.56 (m, 2H, 2 × Ar**H**), 6.24 (t, *J* = 6.0 Hz, 1H, N**H**), 3.50 (td, *J* = 7.0, 6.0 Hz, 2H, NHC**H₂**), 2.32 (t, *J* = 7.0 Hz, 2H, C**H₂**C), 1.80 – 1.71 (m, 2H, $NHCH_2C$**H₂**), 1.68 – 1.59 (m, 2H, C**H₂**CH_2C), 0.17 (s, 9H, Si(C**H₃**)₃); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6 (**C**=O), 133.6 (Ar**C**), 131.8 (Ar**C**H), 128.5 (Ar**C**H), 126.0 (Ar**C**), 106.8 (CH_2**C**), 85.2 (**C**$Si(CH_3)_3$), 39.6 (NH**C**H_2), 28.6 (**C**H_2C), 25.9 ($NHCH_2$**C**H_2), 19.6 (**C**H_2CH_2C), 0.2 (Si(**C**H_3)₃); LRMS *m/z* (ESI$^+$) 352.0 (M+H$^+$, 50%); HRMS *m/z* (ESI$^+$): found 352.0729, $C_{16}H_{23}ON^{79}BrSi$ (M+H$^+$) requires 352.0727.

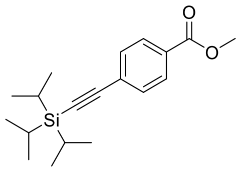
4-((Triisopropylsilyl)ethynyl)-*N*-(6-(trimethylsilyl)hex-5-yn-1-yl)benzamide (2.104)



Tetrakis(triphenylphosphine)palladium(0) (0.014 g, 0.013 mmol, 2.5 mol%) was added to a stirred solution of 4-bromo-*N*-(6-(trimethylsilyl)hex-5-yn-1-yl)benzamide **2.103** (0.18 g, 0.50 mmol, 1.0 equiv.), (triisopropylsilyl)acetylene (0.22 mL, 1.0 mmol, 2.0 equiv.), CuI (0.0050 g, 0.025 mmol, 5.0 mol%) and triethylamine (0.14 mL, 1.0 mmol, 2.0 equiv.) in freeze pump thaw degassed THF (three cycles, 5 mL) and the reaction vessel was sealed and heated to 60 °C for 18 h. After this time, the reaction mixture was cooled to rt, filtered through celite to remove fine solid particles and the solvent removed *in vacuo*. The resulting crude residue was further purified by flash column chromatography (5% EtOAc/ petroleum ether 40-60 → 15% EtOAc/ petroleum ether 40-60) to give the title compound (0.23 g, 0.50 mmol, 100%) as a pale-yellow gummy solid. R*f* 0.25 (10% EtOAc/ petroleum ether 40/60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 – 7.69 (m, 2H, 2 × Ar**H**), 7.57 – 7.52 (m, 2H, 2 × Ar**H**), 6.19 (t, *J* = 5.5 Hz, 1H, N**H**), 3.52 (td, *J* =

7.0, 5.5 Hz, 2H, NHC**H$_2$**), 2.32 (t, *J* = 7.0 Hz, 2H, C**H$_2$**C), 1.76 (m, NHCH$_2$C**H$_2$**), 1.68 – 1.62 (m, 2H, C**H$_2$**CH$_2$C), 1.16 (m, 21H, Si(C**H**(CH$_3$)$_2$)$_3$, Si(CH(C**H$_3$**)$_2$)$_3$), 0.17 (s, 9H, Si(C**H$_3$**)$_3$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 166.7 (**C**=O), 134.0 (Ar**C**), 132.2 (Ar**C**H), 126.7 (Ar**C**H, Ar**C**), 106.9 (CH$_2$**C**), 106.1 (**C**≡CSi(CH(CH$_3$)$_2$)$_3$), 93.5 (**C**Si(CH(CH$_3$)$_2$)$_3$), 85.1 (**C**Si(CH$_3$)$_3$), 39.6 (NH**C**H$_2$), 28.7 (NHCH$_2$**C**H$_2$), 25.9 (**C**H$_2$CH$_2$C), 19.6 (**C**H$_2$C), 18.7 Si(CH(**C**H$_3$)$_2$)$_3$, 11.3 Si(**C**H(CH$_3$)$_2$)$_3$, 0.2 (Si(**C**H$_3$)$_3$); LRMS *m/z* (ESI$^+$) 454.2 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 495.2954, $C_{27}H_{44}ONSi_2$ (M+H$^+$) requires 454.2955.

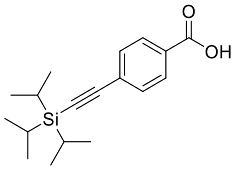
Methyl 4-((triisopropylsilyl)ethynyl)benzoate (2.105)



According to the modified procedure of Wright *et al.*:[69] tetrakis(triphenylphosphine)palladium(0) (0.015 g, 0.013 mmol, 2.5 mol%) was added to a stirred solution of methyl 4-bromobenzoate **2.10** (0.11 g, 0.50 mmol, 1.0 equiv.), (triisopropylsilyl)acetylene (0.22 mL, 1.0 mmol, 2.0 equiv.), CuI (0.0050 g, 0.025 mmol, 5.0 mol%) and triethylamine (0.14 mL, 1.0 mmol, 2.0 equiv.) in freeze pump thaw degassed THF (three cycles, 5 mL) and the reaction vessel was sealed and heated to 60 °C for 18 h. After this time, the reaction mixture was cooled to rt, filtered through a plug of celite to remove fine particles and the filtrate concentrated *in vacuo*. The resultant crude residue was further purified by flash column chromatography (1% Et$_2$O/ petroleum ether 40/60 → 2.5% Et$_2$O/ petroleum ether 40/60) to give the title compound (0.14 g, 0.45 mmol, 89%) as a colourless oil. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.96 – 7.88 (m, 2H, 2 × ArC**H**), 7.49 – 7.41 (m, 2H, 2 × ArC**H**), 3.85 (s, 3H, C**H$_3$**), 1.06 (m, 21H, 3 × SiCH(C**H$_3$**)$_2$, 3 × SiC**H**(CH$_3$)$_2$); ^{13}C NMR (101 MHz, CDCl$_3$) δ

166.6 (**C**=O), 131.9 (2 × Ar**C**H), 129.5 (2 × Ar**C**H), 129.4 (Ar**C**), 128.2 (Ar**C**), 106.1 (**C**CSi), 94.4 (C**C**Si), 52.2 (**C**H_3), 18.6 (Si(CH(**C**H_3)$_2$)$_3$), 11.3 (Si(**C**H(CH_3)$_2$)$_3$); LRMS *m/z* (ESI$^+$) 317.2 (M+H$^+$, 100%). Data are in accordance with the literature.[225]

4-((Triisopropylsilyl)ethynyl)benzoic acid (2.106)

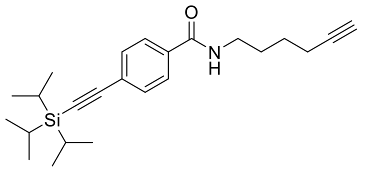


According to the procedure of Wright *et al.*:[69] lithium hydroxide (0.084 g, 2.0 mmol, 5.0 equiv.) was added to a stirred solution of methyl 4-((triisopropylsilyl)ethynyl)benzoate **2.105** (0.13 g, 0.41 mmol, 1.0 equiv.) in THF/H_2O (2.7 mL/ 1.4 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the THF was removed *in vacuo* and the aqueous phase was acidified with pH 1 sulfate buffer. The organics were extracted with EtOAc (3 × 10 mL) and the organic phase was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give the title compound (0.12 g, 0.40 mmol, 99%) as a colourless oil which required no further purification. ^{1}H NMR (400 MHz, Acetone-*d*$_6$) δ 8.07 – 7.99 (m, 2H, 2 × Ar**H**), 7.66 – 7.59 (m, 2H, 2 × Ar**H**), 1.16 (m, 21H, Si(CH(C**H**$_3$)$_2$)$_3$, Si(C**H**(CH_3)$_2$)$_3$); ^{13}C NMR (101 MHz, Acetone-*d*$_6$) δ 166.0 (**C**=O), 131.9 (Ar**C**H), 130.3 (Ar**C**), 129.7 (Ar**C**H), 127.6 (Ar**C**), 106.4 (**C**CSi), 93.3 (C**C**Si), 18.1 (Si(CH(**C**H_3)$_2$)$_3$), 11.1 (Si(**C**H(CH_3)$_2$)$_3$); LRMS *m/z* (ESI$^-$) 301.1 (M-H$^+$, 100%). Data are in accordance with the literature.[226]

OR

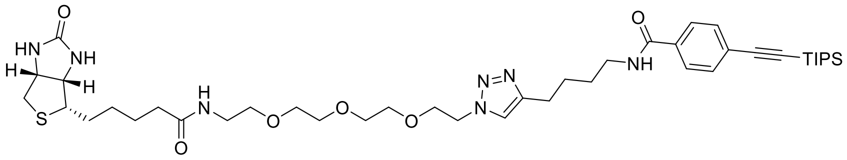
4-Bromobenzoic acid (0.20 g, 1.0 mmol, 1.0 equiv.), (triisopropylsilyl)acetylene (0.27 mL, 0.12 mmol, 1.2 equiv.), bis(triphenylphosphine)palladium(II) dichloride (0.070 g, 0.10 mmol, 10 mol%) and copper(I) iodide (0.038 g, 0.20 mmol, 20 mol%) were added to a microwave vial flushed with N_2. DMF (2.5 mL) and diisopropylamine (0.70 mL, 5.0 mmol, 5.0 equiv.) were added successively and then the vial was capped and heated to 140 °C in the microwave for 25 mins. After this time, the reaction mixture was filtered through a celite plug and then the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/hexane → 40% EtOAc/hexane) to give the title compound (0.25 g, 0.83 mmol, 83%) as a colourless oil which crystalised upon standing. Data as above.

_N_-(Hex-5-yn-1-yl)-4-((triisopropylsilyl)ethynyl)benzamide (2.107)



1-Hydroxybenzotriazole hydrate (0.065 g, 0.48 mmol, 1.5 equiv.) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.092 g, 0.48 mmol, 1.5 equiv.) were added successively to a stirred solution of 4-((triisopropylsilyl)ethynyl)benzoic acid **2.106** (0.097 g, 0.32 mmol, 1.0 equiv.), hex-5-yn-1-amine hydrochloride (0.047 g, 0.35 mmol, 1.1 equiv.) and triethylamine (0.097 mL, 0.70 mmol, 2.2 equiv.) in CH_2Cl_2 (3.2 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the reaction mixture was diluted with H_2O (5 mL) and the organics were extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and solvent removed *in vacuo*. The resulting crude residue was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 20% EtOAc/ petroleum ether 40-60) to give the title compound (0.090 g, 0.24 mmol, 74%) as a colourless oil. R_f 0.23 (15% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 – 7.67 (m, 2H, 2 × Ar**H**), 7.53 – 7.48 (m, 2H, 2 × Ar**H**), 6.26 (t, *J* = 6.0 Hz, 1H, N**H**), 3.47 (td, *J* = 7.0, 5.5 Hz, 2H, NHC**H₂**), 2.25 (td, *J* = 7.0, 2.5 Hz, 2H, C**H₂**C), 1.97 (t, *J* = 2.5 Hz, 1H, C**H**), 1.80 – 1.70 (m, 2H, NHCH₂C**H₂**), 1.66 – 1.57 (m, 2H, C**H₂**CH₂C), 1.13 (m, 21H, Si(C**H**(CH₃)₂)₃, Si(CH(C**H₃**)₂)₃); ^{13}C NMR (126 MHz, CDCl₃) δ 166.8 (**C**=O), 134.0 (Ar**C**), 132.2 (ArCH), 126.7 (Ar**C**H), 126.7 (Ar**C**), 106.1 (**C**CSi), 93.5 (C**C**Si), 84.0 (CH₂**C**), 68.9 (**C**H), 39.6 (NH**C**H₂), 28.7 (NHCH₂**C**H₂), 25.8 (**C**H₂CH₂C), 18.7 (Si(CH(**C**H₃)₂)₃), 18.1 (**C**H₂C), 11.3 (Si(**C**H(CH₃)₂)₃); LRMS *m/z* (ESI$^+$) 382.2 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 382.2558, $C_{24}H_{36}ONSi$ (M+H$^+$) requires 382.2561.

***N*-(4-(1-(13-oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-1*H*-1,2,3-triazol-4-yl)butyl)-4-((triisopropylsilyl)ethynyl)benzamide (2.108)**

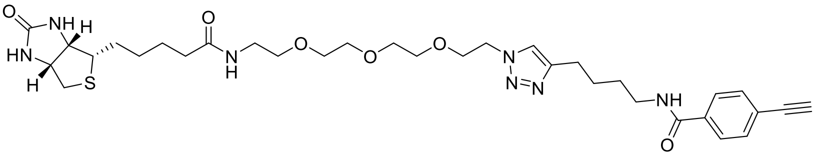


Copper(I) bromide (0.0021 g, 0.015 mmol, 0.15 equiv.) and triethylamine (0.013 mL, 0.10 mmol, 1.0 equiv.) were added to a stirred solution of 4-((triisopropylsilyl)ethynyl)-*N*-(6-(trimethylsilyl)hex-5-yn-1-yl)benzamide **2.104** (0.043 g, 0.12 mmol, 1.2 equiv.) and *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (0.045 g, 0.10 mmol, 1.0 equiv.) in DMF (2 mL) and H₂O (2 drops) and the reaction mixture was heated to 100 °C for 18 h. After this time, the reaction mixture was cooled to rt and the solvent removed under a stream of N₂. The resulting crude residue was then purified by flash column chromatography (CH₂Cl₂ → 10% MeOH/CH₂Cl₂) to give the title compound as a colourless oil (0.040 g, 0.048 mmol, 60%). R*f* 0.45 (10% MeOH/CH₂Cl₂); ^{1}H NMR (500 MHz, CDCl₃) δ 7.83 – 7.77 (m, 2H, 2 × ArC**H**), 7.56 – 7.49 (m, 3H, 2 × ArC**H**, ArC), 7.10 (t, *J* = 5.5 Hz, 1H, N**H**), 6.79 (t, *J* = 5.5 Hz, 1H, N**H**), 6.53 (s, 1H, CON**H**), 5.53 (s, 1H, CON**H**), 4.52 (t, *J* = 5.0 Hz, 2H, C$\mathbf{H_2}$CCHN), 4.49 (dd, *J* = 8.0, 5.0 Hz, 1H, NHC**H**CH), 4.30 (dd, *J* = 8.0, 4.5 Hz, 1H, C**H**CHH'S), 3.88 (t, *J* = 5.0 Hz, 2H, NC$\mathbf{H_2}$CH$_2$O), 3.69 – 3.52 (m, 12H, 6 × C$\mathbf{H_2}$O), 3.48 (q, *J* = 6.5 Hz, 2H, NHC$\mathbf{H_2}$CH$_2$O), 3.42 (qd, *J* = 5.0, 2.0 Hz, 2H, NHC$\mathbf{H_2}$CH$_2$), 3.13 (tq, *J* = 7.5, 4.5, 3.5 Hz, 1H, CONHCHC**H**), 2.89 (dq, *J* = 10.0, 5.0 Hz, 1H, CHC**H**H'S), 2.81 – 2.70 (m, 3H, NHCHCH**H'**S, C$\mathbf{H_2}$CONH), 2.27 – 2.15 (m, 2H, NHCH$_2$C$\mathbf{H_2}$), 1.85 – 1.59 (m, 6H, CHC$\mathbf{H_2}$, C$\mathbf{H_2}$CH$_2$CONH, C$\mathbf{H_2}$CH$_2$CCHN), 1.44 (m, 2H, CHCH$_2$C$\mathbf{H_2}$), 1.14 (d, *J* = 2.0 Hz, 21H, Si(CH(C$\mathbf{H_3}$)$_2$)$_3$, Si(C**H**(CH$_3$)$_2$)$_3$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 173.4 (**C**=O), 166.8 (**C**=O), 164.0 (**C**=O), 148.0 (Ar**C**), 134.1 (Ar**C**), 132.0 (Ar**C**H), 127.0 (Ar**C**H), 126.5 (Ar**C**), 122.3 (Ar**C**H), 106.2 (**C**CSi), 93.3 (C**C**Si), 70.5 (**C**H$_2$NH), 70.4 (**C**H$_2$O), 70.1 (**C**H$_2$O), 69.9 (**C**H$_2$O), 69.5 (**C**H$_2$O), 61.8 (CONH**C**HCH), 60.2 (**C**HCHH'S), 55.6 CONHCH**C**H), 50.1 (**C**H$_2$CCHN), 40.5 (CH**C**HH'S), 39.9 (NH**C**H$_2$), 39.1 (NH**C**H$_2$), 35.9 (**C**H$_2$CCHN), 28.8 (C$\mathbf{H_2}$CONH) , 28.2 (CONHCH$_2$**C**H$_2$), 28.1 (CHC$\mathbf{H_2}$), 26.8 (**C**H$_2$CH$_2$CONH), 25.6 (**C**H$_2$CH$_2$CCHN), 25.1 (CHCH$_2$**C**H$_2$), 18.7 (Si(CH(**C**H$_3$)$_2$)$_3$), 11.3 (Si(**C**H(CH$_3$)$_2$)$_3$); LRMS *m/z* (ESI$^+$) 826.2 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 826.4710, $C_{42}H_{68}O_6N_7SSi$ (M+H$^+$) requires 826.4716.

OR

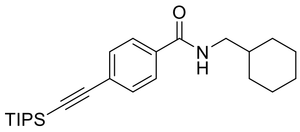
Copper sulfate hydrate (0.0015 g, 0.060 mmol, 0.10 equiv.) and sodium-L-ascorbate (0.012) g, 0.060 mmol, 0.10 equiv.) were added to a stirred solution of *N*-(hex-5-yn-1-yl)-4-((triisopropylsilyl)ethynyl)benzamide **2.107** (0.031 g, 0.070 mmol, 1.2 equiv.) and *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (0.026 g, 0.060 mmol, 1.0 equiv in a mixture of DMF/H$_2$O (5:1, 0.5 mL/ 0.1 mL) and the reaction mixture was stirred at rt for 48 h. The solvent was removed under a stream of N$_2$ to give a crude residue which was then purified by flash column chromatography (CH_2Cl_2 → 10% MeOH/CH_2Cl_2) to give the title compound as a colourless oil (0.030 g, 0.036 mmol, 48%). Data as above.

4-Ethynyl-*N*-(4-(1-(13-oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-1*H*-1,2,3-triazol-4-yl)butyl)benzamide (2.109)



Potassium fluoride (0.082 g, 1.4 mmol, 15 equiv.) was added to a stirred solution of *N*-(4-(1-(13-oxo-17-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-1*H*-1,2,3-triazol-4-yl)butyl)-4-((triisopropylsilyl)ethynyl)benzamide **2.108** (0.078 g, 0.090 mmol, 1.0 equiv.) in MeOH (1 mL) and the reaction mixture was stirred at rt for 336 h. After this time, the reaction solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (CH_2Cl_2 → 10% MeOH/ CH_2Cl_2) to give the title compound (0.026 g, 0.039 mmol, 43%) as a pale orange viscous oil. R$_f$ 0.37 (10% MeOH/ CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 – 7.78 (m, 2H, 2 × Ar**H**), 7.56 – 7.47 (m, 3H, 2 × Ar**H**, Ar**H**), 7.25 (t, *J* = 5.5 Hz, 1H, N**H**), 6.84 (t, *J* = 5.5 Hz, 1H, N**H**), 6.58 (s, br, 1H, N**H**), 5.66 (s, 1H, N**H**), 4.52 (t, *J* = 5.0 Hz, 2H, NC**H$_2$**), 4.48 (dd, *J* = 8.0, 5.0 Hz, 1H, NHC**H**CH), 4.28 (dd, *J* = 8.0, 4.5 Hz, 1H, C**H**CHH'S), 3.88 (t, *J* = 5.0 Hz, 2H, NHC**H$_2$**), 3.61 – 3.51 (m, 12H, 6 × C**H$_2$**O), 3.47 (q, *J* = 6.5 Hz, 2H, NHC**H$_2$**CH$_2$), 3.41 (tt, *J* = 8.0, 3.5 Hz, 2H, NHCH$_2$C**H$_2$**), 3.21 (s, 1H, CC**H**), 3.12 (td, *J* = 7.5, 4.5 Hz, 1H, NHCHC**H**), 2.88 (dd, *J* = 13.0, 5.0 Hz, 1H, CHC**H**H'S), 2.81 – 2.70 (m, 3H, CHCH**H'**S, C**H$_2$**CH$_2$CCHN), 1.79 (dq, *J* = 12.0, 7.0 Hz, 2H, C**H$_2$**CCHN), 1.74 – 1.58 (m, 6H, HC**H$_2$**, C**H$_2$**CH$_2$CONH, C**H$_2$**CONH), 1.41 (h, *J* = 8.0, 7.5 Hz, 2H, CHCH$_2$C**H$_2$**); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4 (C=O), 166.8 (C=O), 164.0 (**C**=O), 147.6 (CH$_2$**C**CHN), 134.8 (Ar**C**), 132.1 (Ar**C**H), 127.2 (Ar**C**H), 125.0 (Ar**C**), 122.2 (CH_2**C**CHN), 82.9 (**C**≡CH), 79.4 (C≡**C**H), 70.5 (**C**H_2O), 70.4 (**C**H_2O), 70.1 (**C**H_2O), 69.9 (**C**H_2O), 69.5 (NH**C**H_2), 61.8 (NH**C**HCH), 60.2 (**C**HCHH'S), 55.7 (NHCH**C**H), 50.1 (N**C**H_2), 40.5 (CH**C**HH'S), 39.9 (NH**C**H_2), 39.1 (NHCH_2**C**H_2), 28.8 **C**H_2CONH, 28.2 (CH**C**H_2, 28.1 **C**H_2CH_2CONH, 26.7 (**C**H_2CCHN), 25.6 (CHCH_2**C**H_2), 25.1 (NHCH_2CH_2**C**H_2); LRMS *m/z* (ESI^+) 670.0 ($M+H^+$, 100%); HRMS *m/z* (ESI^+): found 670.3377, $C_{33}H_{47}O_6N_7S$ ($M+H^+$) requires 670.3381.

***N*-(Cyclohexylmethyl)-4-((triisopropylsilyl)ethynyl)benzamide (2.111)**

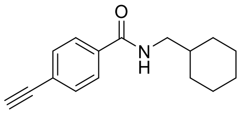


Propylphosphonic anhydride solution (≥50% wt. % in EtOAc, 0.53 mL, 0.89 mmol, 1.1 equiv.) was added to a stirred solution of 4-((triisopropylsilyl)ethynyl)benzoic acid (0.25 g, 0.81 mmol, 1.0 equiv.), cyclohexylmethylamine (0.16 mL, 1.2 mmol, 1.5 equiv.) and triethylamine (0.22 mL, 1.6 mmol, 2.0 equiv.) in CH_2Cl_2 (8 mL) and the reaction mixture was stirred at rt for 16 h. After this time, the solvent was removed *in vacuo* and then the crude residue redissolved in EtOAc (10 mL) and washed successively with H_2O (10 mL), sat. $NaHCO_3$ (10 mL) and sat. NH_4Cl (10 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (hexane → 15% EtOAc/hexane) to give the title compound (0.28 g, 0.69 mmol, 85%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.66 (m, 2H, 2 × ArC**H**), 7.55 – 7.48 (m, 2H, 2 × ArC**H**), 6.12 (s, 1H, CON**H**), 3.30 (t, *J* = 6.5 Hz, 2H, NHC**H₂**), 1.84 – 1.71 (m, 4H, C**H₂**CHC**H₂**), 1.68 (m, 1H, CH_2C**H**CH_2), 1.33 – 1.15 (m, 4H, C**H₂**CH_2C**H₂**), 1.13 (m, 21H, Si(CH(C**H₃**)$_2$)$_3$, Si(C**H**(CH_3)$_2$)$_3$), 1.00 (q, *J* = 10.5 Hz, 2H, CH_2C**H₂**CH_2); LRMS *m/z* (ESI^+) 398.2 ($M+H^+$, 100%).

OR

4-Bromobenzoic acid (0.20 g, 1.0 mmol, 1.0 equiv.), (triisopropylsilyl)acetylene (0.27 mL, 0.12 mmol, 1.2 equiv.), bis(triphenylphosphine)palladium(II) dichloride (0.070 g, 0.10 mmol, 10 mol%) and copper(I) iodide (0.038 g, 0.20 mmol, 20 mol%) were added to a microwave vial flushed with N_2. DMF (2.5 mL) and diisopropylamine (0.70 mL, 5.0 mmol, 5.0 equiv.) were added successively and then the vial was capped and heated to 140 °C in the microwave for 25 mins. After this time, the reaction mixture was filtered through an ISOLUTE® silica-TMT cartridge and the solvent removed *in vacuo*. The crude residue was then redissolved in CH_2Cl_2 (10 mL) and cyclohexylmethylamine (0.20 mL, 1.5 mmol, 1.5 equiv.), triethylamine (0.28 mL, 2.0 mmol, 2.0 equiv.) and propylphosphonic anhydride solution (≥50% wt. % in EtOAc, 0.66 mL, 0.89 mmol, 1.1 equiv.) added. The reaction mixture was stirred at rt for 16 h. After this time, the solvent was removed *in vacuo* and then the crude residue redissolved in EtOAc (10 mL) and washed successively with H_2O (10 mL), sat. $NaHCO_3$ (10 mL) and sat. NH_4Cl (10 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (hexane → 15% EtOAc/hexane) to give the title compound (0.26 g, 0.65 mmol, 85%) as an off-white solid. Data as above.

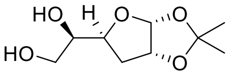
***N*-(Cyclohexylmethyl)-4-ethynylbenzamide (2.112)**



Tetrabutylammonium fluoride on silica gel (~1.5 mmol/g, 0.041 g, 0.28 mmol, 1.1 equiv.) was added to a stirred solution of *N*-(cyclohexylmethyl)-4-((triisopropylsilyl)ethynyl)benzamide **2.111** (0.10 g, 0.25 mmol, 1.0 equiv.) in THF (2.5 mL) and the reaction mixture was stirred at rt for 2 h. After this time, the reaction mixture was filtered to remove the solid supported tetrabutylammonium fluoride and the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (hexane → 25% EtOAc/hexane) to give the title compound (0.058 g, 0.24 mmol, 96%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 – 7.68 (m, 2H, 2 × ArC**H**), 7.58 – 7.51 (m, 2H, 2 × ArC**H**), 6.13 (s, 1H, CONH), 3.30 (t, *J* = 6.5 Hz, 2H, NHC**H₂**), 3.19 (s, 1H, CC**H**), 1.76 (m, , 4H, C**H₂**CHCH₂, CH₂CHC**H₂**), 1.68 (m, 1H, CH₂C**H**CH₂), 1.32 – 1.13 (m, 4H, C**H₂**CH₂CH₂, CH₂CH₂C**H₂**), 1.00 (m, 2H, CH₂C**H₂**CH₂); LRMS *m/z* (ESI$^+$) 242.4 (M+H$^+$, 100%).

8.3. Experimental Procedures for Chapter 4

(*R*)-1-([3a*R*,5*S*,6a*R*]-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)ethane-1,2-diol (3.118)



According to the procedure of Chan et al.:[102]

Step one: pyridine (9.8 mL, 120 mmol, 2.2 equiv.) was added to diacetone-d-glucose **3.94** (15 g, 58 mmol, 1.0 equiv.) in CH_2Cl_2 (70 mL) at 0°C, followed by slow addition of triflic anhydride (12 mL, 70 mmol, 1.2 equiv.) and the reaction mixture was stirred at rt for 1 h. After this time, the reaction was quenched by addition of ice water (50 mL) and the organics were extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and solvent removed *in vacuo* to give the crude triflate which was used directly in the next step.

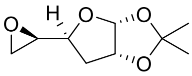
Step two: the crude triflate was redissolved in toluene (350 mL). Tetrabutylammonium borohydride (37 g, 145 mmol, 2.5 equiv.) was added and the reaction mixture was stirred at

80 °C for 7 h. The reaction mixture was then cooled to rt and water (300 mL) added; the organics were extracted with toluene (3 × 200 mL), combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude deoxysugar which was used directly in the next step.

Alternative workup for step two: The reaction mixture was cooled to rt and quenched *via* the addition of water (100 mL), the quenched reaction mixture was stirred vigorously until effervescence ceased and then then the toluene removed *in vacuo*. The aqueous phase was further diluted with sat. NH_4Cl solution (150 mL) and water (100 mL) and then extracted with Et_2O (4 × 250 mL). The organic phase was dried (Na_2SO_4), and the solvent removed *in vacuo* to give the crude deoxysugar which was used directly in the next step.

Step three: the crude deoxysugar was dissolved in 75% acetic acid (140 mL acetic acid/ 40 mL H_2O) and stirred for 18 h. The solvent was removed by azeotropic distillation with toluene to give a crude residue which was further purified by column chromatography (30% acetone/petroleum ether 40-60 → 50% acetone/petroleum ether 40-60) to yield the title compound (8.5 g, 42 mmol, 72% over three steps) as a colourless oil. δ_H (400 MHz, $CDCl_3$) δ 5.80 (d, *J* = 3.5 Hz, 1H, OC**H**O), 4.75 (t, *J* = 4.0 Hz, 1H, OC**H**CHH’), 4.23 (dt, *J* = 11.0, 4.5 Hz, 1H, CHH’C**H**), 3.93 (dt, *J* = 7.5, 4.0 Hz, 1H, C**H**OH), 3.72 (ddd, *J* = 10.5, 6.5, 3.5 Hz, 1H, C**H**H’OH), 3.60 (ddd, *J* = 11.0, 6.5, 4.0 Hz, 1H, CH**H’**OH), 2.40 (d, *J* = 4.0 Hz, 1H, O**H**), 2.11 – 2.00 (m, 2H, O**H**, OCHC**H**H’), 1.85 (ddd, *J* = 13.5, 10.5, 5.0 Hz, 1H, OCHCH**H’**C), 1.51 (s, 3H, C**H₃**), 1.32 (s, 3H, C**H₃**); δ_C (101 MHz, $CDCl_3$) δ 111.3 (**C**(CH_3)$_2$), 105.1 (O**C**HO), 80.5 (**C**HCHH’), 78.6 (CHH’**C**H), 71.9 (**C**HOH), 63.4 (**C**HH’OH), 33.6 (**C**HH’CH), 26.7 (**C**H_3), 26.1 (**C**H_3). The data are in accordance with the literature.[102]

(3a*R*,5*S*,6a*R*)-2,2-dimethyl-5-(*[R]*-oxiran-2-yl)tetrahydrofuro[2,3-*d*][1,3]dioxole (3.117)



According to the procedure of Chan et al.:[102] triphenylphosphine (7.8 g, 30 mmol, 1.2 equiv.) and diisopropyl azodicarboxylate (5.6 mL, 25 mmol, 1.0 equiv.) were added to a stirred solution of (*R*)-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)ethane-1,2-diol **3.118** (5.0 g, 25 mmol, 1.0 equiv.) in toluene (150 mL) and the reaction mixture was heated to reflux for 18h. After this time, the solvent was removed *in vacuo* giving a syrup that was diluted with ether (10 mL) and sonicated to precipitate the triphenylphosphine oxide by-product which was filtered off and the solid washed with ice-cold diethyl ether (3 × 10 mL). The filtrate was then concentrated *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% acetone/petroleum ether 40-60) to give the title compound (2.4 g, 13 mmol, 52%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84 (d, *J* = 3.5 Hz, 1H, OC**H**O), 4.74 (dd, *J* = 4.5, 4.0 Hz, 1H, OC**H**CHH'), 4.20 (dt, *J* = 10.5, 4.5 Hz, 1H, CHH'C**H**), 3.14 (td, *J* = 4.0, 2.5 Hz, 1H, C**H**OCHO), 2.82 (dd, *J* = 5.0, 4.0 Hz, 1H, C**H**H'O), 2.60 (dd, *J* = 5.0, 2.5 Hz, 1H, CH**H'**O), 2.06 (dd, *J* = 13.5, 4.5 Hz, 1H, C**H**H'CH), 1.75 – 1.72 (m, 1H, CH**H'**CH), 1.50 (s, 3H, C**H₃**), 1.32 (s, 3H, C**H₃**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 111.3 (**C**(CH_3)$_2$), 105.5 (O**C**HO), 80.3 (O**C**HCHH'), 77.9 (CHH'**C**H), 45.1 (**C**HH'O), 34.3 (OCH**C**HO), 26.8 (**C**HH'CH), 26.1 (**C**H₃), 22.0 (**C**H₃). LRMS *m/z* (ESI^{+}) 209.1 (M+Na^{+}, 100%). Data are in accordance with the literature.[102]

OR

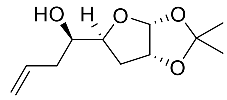
According to the modified procedure of Tsunoda et al.:[148] (triphenylphosphoranylidene)acetonitrile (0.15 g, 0.5 mmol, 2.0 equiv.) was added to a solution of ®-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)ethane-1,2-diol (0.051 g, 0.25 mmol, 1.0 equiv.) in toluene (2.5 mL) in a flame dried microwave vial. The reaction vial was sealed and then the reaction heated to 150 °C for 22 h. After this time, the reaction mixture was cooled to rt and then the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (30% EtOAc/petroleum ether 40-60) to give the title compound (0.017 g, 0.093 mmol, 37%) as a colourless oil. Data as above.

OR

Triphenylphosphine (12 g, 46 mmol, 1.1 equiv.) and di-(4-chlorobenzyl)azodicarboxylate (17 g, 46 mmol, 1.1 equiv.) were added to a stirred solution ®(*R*)-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)ethane-1,2-diol (8.6 g, 42 mmol, 1.0 equiv.) in toluene (250 mL) and the reaction mixture was heated to reflux for 18h. After this time, the reaction mixture was cooled to rt and then further to 0 °C in order to precipitate the reduced di-(4-chlorobenzyl)azodicarboxylate by-product which was then filtered off and washed with ice cold toluene (2 × 40 mL). The filtrate solvent was removed *in vacuo* and then redissolved in Et_2O (50 mL). After sonication; the triphenylphosphine oxide by-product precipitated, was removed by filtration and washed with ice-cold Et_2O (3 × 10 mL). The solvent was then removed *in vacuo* and the resulting crude residue purified by flash column chromatography (5% acetone/petroleum ethe 40-60 → 15% acetone/ petroleum ether 40-60) to give the title compound (5.5 g, 30 mmol, 72%) as a colourless oil. Data as above.

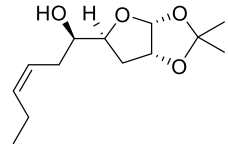
(R)-1-([3a***R***,5***S***,6a***R***]-2,2-dimethyltetrahydrofuro[2,3-***d***][1,3]dioxol-5-yl)but-3-en-1-ol

(3.116)



According to the procedure of Chan et al.:[102] vinyl magnesium bromide (1.0 M in THF, 37 mL, 37 mmol, 2.0 equiv.) was added dropwise to CuI (0.37 g, 1.9 mmol, 0.10 equiv.) in THF (60 mL) at −20 °C. (3a*R*,5*S*,6a*R*)-2,2-dimethyl-5-(*[R]*-oxiran-2-yl)tetrahydrofuro[2,3-*d*][1,3]dioxole **3.117** (3.6 g, 20 mmol, 1.0 equiv.) was added dropwise in THF (9 mL) and the reaction mixture was stirred for at −20 °C 1 h. After this time, the reaction mixture was quenched with sat. NH_4Cl (50 mL) and then extracted with EtOAc (3 × 50 mL). The organic layers were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the crude residue which was further purified by flash column chromatography (30% EtOAc/petroleum ether 40-60) to give the title compound as a colourless oil (3.3 g, 16 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 – 5.76 (m, 2H, CH$_2$=C**H**, OC**H**O), 5.19 – 5.08 (m, 2H, C$\mathbf{H_2}$), 4.74 (t, *J* = 4.0 Hz, 1H, OC**H**CHH'), 4.20 (dt, *J* = 10.5, 4.0 Hz, 1H, CHH'C**H**), 3.95 (td, *J* = 8.5, 6.0, 3.0 Hz, 1H, C**H**OH), 2.24 – 2.16 (m, 2H, C$\mathbf{H_2}$CHOH), 2.07 – 1.95 (m, 2H, O**H**, CH**H'**CH), 1.88 (ddd, *J* = 13.5, 10.5, 4.5 Hz, 1H, C**H**H'CH), 1.50 (s, 3H, C$\mathbf{H_3}$), 1.32 (s, 3H, C$\mathbf{H_3}$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.0 (CH$_2$=**C**H), 117.8 (**C**H$_2$), 111.2 (**C**(CH$_3$)$_2$), 105.2 (O**C**HO), 80.6 (O**C**HCHH'), 80.5 (CHH'**C**H), 69.9 (**C**HOH), 37.3 (**C**H$_2$CHOH), 31.8 (**C**HH'CH), 26.8 (**C**H$_3$), 26.1 (**C**H$_3$). LRMS *m/z* (ESI$^+$) 237.1 (M+Na$^+$, 100%). Data are in accordance with the literature.

(R,Z)-1-([3a*R*,5*S*,6a*R*]-2,2-Dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-en-1-ol (3.114)



According to the modified procedure of Richardson et al.:[227] sodium borohydride (0.18 g, 4.6 mmol, 20 mol%) was added to a stirred solution of $Ni(OAc)_2{\cdot}4H_2O$ (0.82 g, 4.6 mmol, 20 mol%) in EtOH (110 mL) and the flask was purged with H_2. Ethylenediamine (0.61 mL, 9.2 mmol, 40 mol%) was added and then the reaction mixture was sparged with H_2 for 20 mins. After this time, (1*R*)-1-((3a*R*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-yn-1-ol **5.1** (5.4 g, 23 mmol, 1.0 equiv.) was added in EtOH (20 mL) and sparging was continued for a further 20 mins before leaving the reaction mixture to stir for a further 18 h under an atmosphere of H_2. The H_2 atmosphere was purged and then solvent removed *in vacuo* to give a crude residue that was passed through a silica plug (30% EtOAc/petroleum ether 40-60) to give the title compound 5.4 g, 23 mmol, 99%) as a colourless oil. R_f 0.23 (30% EtOAc/petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.82 (d, *J* = 3.5 Hz, 1H, OC**H**O), 5.54 (dtt, *J* = 11.0, 7.0, 1.5 Hz, 1H, CH_3CH_2C**H**), 5.38 (dtt, *J* = 10.5, 7.0, 1.5 Hz, 1H, CH=C**H**CH_2), 4.75 (t, *J* = 4.0 Hz, 1H, OC**H**CH'CHO), 4.22 (ddd, *J* = 10.5, 4.5, 3.5 Hz, 1H, CH'C**H**), 3.92 (ddd, *J* = 8.0, 6.0, 3.5 Hz, 1H, C**H**OH), 2.27 – 2.13 (m, 2H, C**H₂**CHOH), 2.06 (pd, *J* = 7.5, 1.5 Hz, 2H, CH_3C**H₂**), 2.01 – 1.87 (m, 3H, C**H**H'CH, CH**H'**CH, O**H**), 1.51 (s, 3H, C**H₃**), 1.32 (s, 3H, C**H₃**), 0.97 (t, *J* = 7.5 Hz, 3H, C**H₃**CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.9 (CH_3CH_2**C**H), 123.7 (**C**HCH_2), 111.2 (**C**(CH_3)$_2$), 105.3 (O**C**HO), 80.6 (O**C**HCHH'CH), 80.5 (CHH'**C**H), 70.5 (**C**HOH), 31.8 (**C**HH'CH), 30.7 (**C**H_2CHOH), 26.8 (**C**H_3), 26.18 (**C**H_3), 20.7 (CH_3**C**H_2), 14.2 (**C**H_3CH_2); LRMS *m/z*

(ESI$^+$) 265.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 265.1412, $C_{13}H_{22}O_4Na$ (M+Na$^+$) requires 265.1410; $[\alpha]_D^{25}$= -14.4 (c = 0.088, $CHCl_3$); IR (ν_{max}): 3450, 2981, 1374.

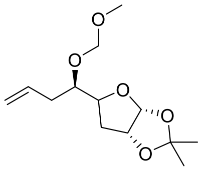
OR

2,6-lutidine (0.11 mL, 0.94 mmol, 2.0 equiv.), osmium tetroxide (2.5% in ^{t}BuOH, 0.02 mL), and sodium metaperiodate (0.40 g, 1.88 mmol, 4.0 equiv.)) were added to a stirred solution of *(R)*-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-en-1-ol **3.116** (0.10 g, 0.47 mmol, 1.0 equiv.) in a mixture of dioxane and water (3:1, 3 mL/ 1 mL) and the reaction mixture was stirred for 2h then diluted with H_2O (10 mL), extracted with DCM (3 × 10 mL), the organics were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude aldehyde which was redissolved in THF (2.5 mL). Meanwhile, LiHMDS (1M in THF, 1.3 mL, 1.3 mmol, 1.5 equiv.) was added dropwise to a solution of ethyltriphenylphosphonium bromide (0.36 g, 0.94 mmol, 2.0 equiv.) in THF (5 mL) at 0 °C and the reaction mixture was stirred for 30 minutes. The reaction mixture was cooled to −78 °C and then crude aldehyde was added in THF (2.5 mL) dropwise. The reaction mixture was stirred 1 h. at -78 °C then warmed to 40 °C for 4 h. before quenching with sat. NH_4Cl (10 mL). The organics were extracted with EtOAc (3 × 15 mL), the organics were combined, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product which was further purified by column chromatography (30% EtOAc/ petroleum ether 40-60) to give the title compound as a colourless oil (0.080 g, 70%) and as an inseparable mixture of alkene stereoisomers (2:1 *Z*/*E*). Data for (*Z*)-alkene as above.

OR

According to the modified procedure of Knudsen et al.:[228] *t*-BuLi (1.7 M in pentane, 9.4 mL, 16 mmol, 8.0 equiv.) was added to a stirred solution of (*Z*)-1-bromobut-1-ene **4.20** (1.1 g, 8.0 mmol, 4.0 equiv.) in Et_2O (15 mL) at -78 °C and the reaction mixture was stirred for 10 mins at this temperature before being warmed to rt and stirred for a further 1 h. After this time, the reaction mixture was cooled to -20 °C and CuI (0.038 g, 0.20 mmol, 10 mol%) was added followed by (3a*R*,5*S*,6a*R*)-2,2-dimethyl-5-(*[R]*-oxiran-2-yl)tetrahydrofuro[2,3-*d*][1,3]dioxole **3.117** (0.37 g, 2.0 mmol, 1.0 equiv.) and the reaction mixture was stirred at this temperature for 1 h. The reaction was quenched *via* the slow addition of sat. NH_4Cl solution and then allowed to warm to rt. The organics were extracted with EtOAc (3 × 20 mL) and the combined organic phase dried ($MgSO_4$) and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) to give the title compound (0.47 g, 2.0 mmol, 95%) as a colourless oil. Data as above.

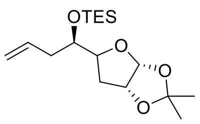
(3a*R*,6a*R*)-5-((*R*)-1-(Methoxymethoxy)but-3-en-1-yl)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxole (4.6)



According to the modified procedure of White et al.:[229] chloromethyl methyl ether (0.30 mL, 4.0 mmol, 2.0 equiv.) was added to a stirred solution of (1*R*)-1-((3a*R*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-en-1-ol **3.116** (0.43 g, 2.0 mmol, 1.0 equiv.) and *N*,*N*-diisopropylethylamine (0.70 mL, 4.0 mmol, 2.0 equiv.) in CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and then warmed to rt and stirred for a further 22 h. After this time, the reaction mixture was quenched by addition of H_2O (30 mL) and the organics extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried

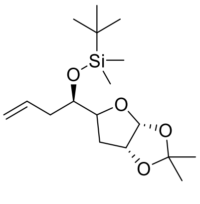
($MgSO_4$), filtered, and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 20% EtOAc/ petroleum ether 40-60) to give the title compound (0.41 g, 1.6 mmol, 78%) as a colourless oil. R$_f$ 0.50 (20% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.86 (ddt, *J* = 17.5, 10.5, 7.0 Hz, 1H, HH'C=C**H**), 5.81 (d, *J* = 3.5 Hz, 1H, OC**H**O), 5.14 (dq, *J* = 17.5, 1.5 Hz, 1H, **H**H'C=CH), 5.10 (ddt, *J* = 10.0, 2.0, 1.0 Hz, 1H, H**H'**C=CH), 4.75 (t, *J* = 4.5 Hz, 1H, CHH'CH), 4.74 (d, *J* = 6.5 Hz, 1H, C**H**H'OCH$_3$), 4.68 (d, *J* = 6.5 Hz, 1H, CH**H'**OCH$_3$), 4.26 (dt, *J* = 10.5, 4.0 Hz, 1H, OC**H**CHH'), 3.89 (td, *J* = 6.5, 4.0 Hz, 1H, C**H**O(CH$_2$OCH$_3$)), 3.39 (s, 3H, CHH'$_2$OC**H$_3$**), 2.38 – 2.23 (m, 2H, HH'C=CHC**H$_2$**), 2.10 – 2.02 (m, 1H, OCHC**H**H'), 1.90 (ddd, *J* = 13.5, 10.5, 5.0 Hz, 1H, OCHCH**H'**), 1.52 (s, 3H, C**H$_3$**), 1.34 (s, 3H, C**H$_3$**); ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.3 (HH'C=**C**H), 117.5 (HH'**C**=CH), 111.2 (**C**(CH$_3$)$_2$), 105.2 (O**C**HO), 96.7 (**C**H$_2$OCH$_3$), 80.5 (CHH'CH), 79.9 (O**C**HCHH'), 76.4 (**C**HO(CH$_2$OCH$_3$)), 55.7 (CH$_2$OC**H$_3$**), 36.8 (HH'C=CH**C**H$_2$), 33.1 (OCH**C**HH'), 26.8 (**C**H$_3$), 26.3 (**C**H$_3$). LRMS *m/z* (ESI$^+$) 281.1 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 281.1361, $C_{13}H_{22}O_5Na$ (M+Na$^+$) requires 281.1359; $[\alpha]_D^{25}$= -2.6 (c = 0.10, $CHCl_3$); IR (ν$_{max}$ cm^{-1}): 2935, 1642.

(((1*R*)-1-((3a*R*,6a*R*)-2,2-Dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-en-1-yl)oxy)triethylsilane (4.7)



According to the procedure of Xie et al.:[230] 2,6-lutidine (0.23 mL, 2.0 mmol, 2.0 equiv.) and triethylsilyl trifluoromethanesulfonate (0.34 mL, 1.5 mmol, 1.5 equiv.) were added to a stirred solution of *(R)*-1-([3a*R*,5*S*,6a*R*]-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-en-1-ol **3.116** (0.21 g, 1.0 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was warmed to rt and stirred for 1 h before being quenched by the addition of sat. $NaHCO_3$ (10 mL), the organics were extracted with CH_2Cl_2 (3 × 10 mL), dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give the crude product which was subjected to high vacuum in order to remove traces of solvent giving the title compound (0.33 g, 1.0 mmol, quant.) as a pale yellow oil which required no further purification. R_f 0.55 (5% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.81 (ddt, *J* = 17.5, 10.0, 7.0 Hz, 1H, H_2CC**H**), 5.75 (d, *J* = 3.5 Hz, 1H, OC**H**O), 5.10 – 5.02 (m, 2H, **H_2**CCH), 4.71 (t, *J* = 4.0 Hz, 1H, OC**H**CHH’), 4.17 (ddd, *J* = 10.0, 5.0, 3.0 Hz, 1H, CHH’C**H**), 3.96 (td, *J* = 6.5, 3.0 Hz, 1H, C**H**OSi), 2.22 – 2.14 (m, 2H, H_2CCHC**H_2**), 1.92 (m , 2H, OCHC**H**H’, OCHCH**H’**), 1.49 (s, 3H, C**H_3**), 1.31 (s, 3H, C**H_3**), 0.93 (t, *J* = 8.0 Hz, 9H, Si(CH_2C**H_3**)$_3$), 0.59 (q, *J* = 8.0 Hz, 6H, Si(C**H_2**CH_3)$_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 134.6 (H_2C**C**H), 117.1 (H_2**C**CH), 111.0 (**C**(CH_3)$_2$), 105.3 (O**C**HO), 80.8 (CHH’**C**H), 80.6 (O**C**HCHH’), 71.6 (**C**HOSi), 39.5 (H_2CCH**C**H_2), 32.1 (OCH**C**HH’), 26.9 (**C**H_3), 26.2 (**C**H_3), 6.9 (Si(CH_2**C**H_3)$_3$, 5.1 (Si(**C**H_2CH_3)$_3$); LRMS *m/z* (ESI^+) 329.2 ($M+H^+$, 100%); HRMS *m/z* (ESI^+): found 351.1964 ($M+Na^+$), $C_{17}H_{32}O_4Si$ ($M+Na^+$) requires 351.1962; $[\alpha]_D^{25}$= -9.5 (c = 0.1, $CHCl_3$); IR (ν_{max} cm^{-1}): 2954, 2877, 1644, 1458, 1372.

***tert*-Butyl(((1*R*)-1-((3a*R*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-en-1-yl)oxy)dimethylsilane (4.8)**



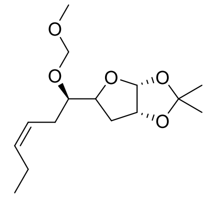
According to the modified procedure of Neuhaus et al.:[231] *tert*-butyldimethylsilyl chloride (0.36 g, 2.4 mmol, 1.2 equiv.) and imidazole (0.41 g, 6.0 mmol, 3.0 equiv.) were added to a stirred solution of (1*R*)-1-((3a*R*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-

en-1-ol **3.116** (0.43 g, 2.0 mmol, 1.0 equiv.) in CH_2Cl_2 (20 mL) at 0 °C and the reaction mixture was warmed to rt and then stirred for 22 h. After this time, the reaction was quenched by addition of H_2O (30 mL) and then the organics were extracted with CH_2Cl_2 (3 × 20 mL), the combined organic layers were dried ($MgSO_4$), filtered, and the solvent removed *in vacuo*. The resulting crude residue was further purified by flash column chromatography (petroleum ether 40-60 → 3% EtOAc/ petroleum ether 40-60) to give the title compound (0.31 g, 0.94 mmol, 47%) as a colourless oil. R_f 0.29 (5% Et_2O/ 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.83 (ddt, *J* = 17.5, 10.0, 7.0 Hz, 1H, $H_2CC\mathbf{H}$), 5.77 (d, *J* = 3.5 Hz, 1H, $OC\mathbf{H}O$), 5.12 – 5.04 (m, 2H, $\mathbf{H_2}C{=}CH$,), 4.73 (t, *J* = 4.0 Hz, 1H, $C\mathbf{H}CHH'$), 4.24 – 4.17 (m, 1H,$CHH'C\mathbf{H}$), 3.98 (td, *J* = 6.0 Hz, 3.0, 1H, $C\mathbf{H}OSi$), 2.20 (ddd, *J* = 7.0, 4.5, 1.5 Hz, 2H, $CHC\mathbf{H_2}$), 2.01 – 1.87 (m, 2H, $CHC\mathbf{H}H'$, $CHCH\mathbf{H'}$), 1.52 (s, 3H, $C\mathbf{H_3}$), 1.34 (s, 3H, $C\mathbf{H_3}$), 0.89 (s, 9H, $Si(CH_3)_2C(C\mathbf{H_3})_3$), 0.08 (s, 3H, $Si(C\mathbf{H_3})_2C(CH_3)_3$), 0.05 (s, 3H, $Si(C\mathbf{H_3})_2C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.7 ($H_2C\mathbf{C}H$), 117.2 ($H_2\mathbf{C}CH$), 111.0 ($\mathbf{C}(CH_3)_2$), 105.2 ($O\mathbf{C}HO$), 80.8 ($\mathbf{C}HCHH'$), 80.7 ($CHH'C\mathbf{H}$), 71.6 ($\mathbf{C}HOSi$), 39.5 ($H_2CCH\mathbf{C}H_2$), 32.1 ($CH\mathbf{C}HH'$), 26.9 ($C(\mathbf{C}H_3)_2$), 26.3 ($C(\mathbf{C}H_3)_2$), 25.9 ($Si(CH_3)_2C(\mathbf{C}H_3)_3$), 18.2 ($Si(CH_3)_2\mathbf{C}(CH_3)_3$), -4.3 ($Si(\mathbf{C}H_3)_2C(CH_3)_3$), -4.6 ($Si(\mathbf{C}H_3)_2C(CH_3)_3$). LRMS *m/z* (ESI$^+$) 351.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 351.1963, $C_{17}H_{32}O_4NaSi$ (M+Na$^+$) requires 351.1962; $[\alpha]_D^{25}$= -2.2 (c = 0.090, $CHCl_3$); IR (ν_{max} cm^{-1}) 2954, 2857, 1642.

OR

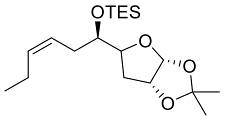
According to the procedure of Paterson et al.:[232] *tert*-butyldimethylsilyl trifluoromethanesulfonate (0.34 mL, 1.5 mmol, 1.5 equiv.) was added to a stirred solution of (1*R*)-1-((3a*R*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-en-1-ol **3.116** (0.21 mL, 1.0 mmol, 1.0 equiv.) and 2,6-lutidine (0.23 mL, 2.0 mmol, 2.0 equiv.) in CH_2Cl_2 (10 mL) at 0 °C and the reaction mixture was warmed to rt and then stirred for 1 h. After this time, the reaction mixture was quenched by addition of sat. $NaHCO_3$ solution (15 mL) and the organics were extracted with CH_2Cl_2, dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The resulting oil was dried thoroughly under high vacuum to give the title compound (0.32 g, 0.98 mmol, 98%) as a colourless oil which required no further purification. Data as above.

(3a*R*,6a*R*)-5-((*R*,*Z*)-1-(Methoxymethoxy)hex-3-en-1-yl)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxole (4.9)



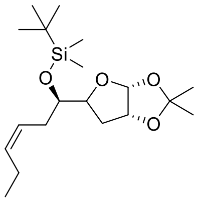
Sodium (meta)periodate (0.17 g, 0.80 mmol, 4.0 equiv.) and potassium osmate(VI) dihydrate (0.75 mg, 25 µmol, 1.0 mol%) were added to a stirred solution of (3a*R*,6a*R*)-5-((*R*)-1-(methoxymethoxy)but-3-en-1-yl)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxole **4.6** (0.052 g, 0.20 mmol, 1.0 equiv.) and 2,6-lutidine (0.047 mL, 0.40 mmol, 2.0 equiv.) in dioxane/ H_2O (2:1, 4 mL) and the reaction mixture was stirred at rt for 2 h. After this time, the reaction mixture was diluted with H_2O (4 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organics were dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give the crude aldehyde product which was further dried under high vacuum. Meanwhile, sodium bis(trimethylsilyl)amide solution (1 M in THF, 0.30 mL, 1.5 equiv.) was added to a stirred suspension of freshly dried (high vacuum) triphenyl(propyl)phosphonium bromide (0.12 g, 0.32 mmol, 1.6 equiv.) in THF (1.5 mL) at 0 °C and the reaction mixture was stirred for 15 mins. After this time, the reaction mixture was cooled to -78 °C and the crude aldehyde was added dropwise in THF (0.5 mL), the reaction mixture was slowly warmed to rt and stirred for a further 16 h. The reaction mixture was quenched by the addition of sat. NH_4Cl (4 mL) and the organics were extracted with EtOAc (3 × 10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The resulting crude residue was further purified by flash column chromatography (petroleum ether 40-60 → 10% EtOAc/ petroleum ether 40-60) giving the title compound (0.022 g, 0.076 mmol, 38%) as a colourless oil. R_f 0.27 (10% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.80 (d, *J* = 4.0 Hz, 1H, OC**H**O), 5.54 – 5.46 (m, 1H, CH_3CH_2C**H**CH), 5.46 – 5.36 (m, 1H, CH_3CH_2CHC**H**), 4.74 (dd, *J* = 5.0, 3.5 Hz, 1H, OC**H**CHH'), 4.72 (d, *J* = 6.5 Hz, 1H, C**H**H'OCH_3), 4.69 (d, *J* = 6.5 Hz, 1H, CH**H'**OCH_3), 4.25 (dt, *J* = 10.5, 4.0 Hz, 1H, CHH'C**H**), 3.85 (td, *J* = 6.5, 4.0 Hz, 1H, C**H**OCHH'O), 3.39 (s, 3H, OC**H$_3$**), 2.36 – 2.19 (m, 2H, CHCHC**H$_2$**), 2.12 – 2.00 (m, 3H, CH_3C**H$_2$**CHCH, OCHC**H**H'), 1.91 (ddd, *J* = 13.5, 10.5, 5.0 Hz, 1H, OCHCH**H'**), 1.51 (s, 3H, C**H$_3$**), 1.33 (s, 3H, C**H$_3$**), 0.98 (t, *J* = 7.5 Hz, 3H, C**H$_3$**CH_2CH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.1 (CH_3CH_2**C**HCH), 124.1 (CH_3CH_2CH**C**H), 111.1 (**C**$(CH_3)_2$), 105.3 (O**C**HO), 96.7 (CHO**C**HH'O), 80.5 (O**C**HCHH'), 79.9 (CHH'**C**H), 76.8 (**C**HOCHH'OC), 55.6 (OC**H$_3$**), 33.1 (**C**HH'CH), 30.0 (CHCH**C**H_2), 26.8 (**C**H_3), 26.3 (**C**H_3), 20.7 (CH_3**C**H_2CH), 14.1 (**C**H_3CH_2CH); LRMS *m/z* (ESI$^+$) 309.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 309.1673, $C_{15}H_{26}O_5Na$ (M+Na$^+$) requires 309.1673; $[\alpha]_D^{25}$= -4.5 (c = 0.10, $CHCl_3$); $[\alpha]_D^{25}$= -3.30 (c = 0.05, $CHCl_3$) IR (ν_{max} cm^{-1}): 2961, 1457.

(((1*R*,*Z*)-1-((3a*R*,6a*R*)-2,2-Dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-en-1-yl)oxy)triethylsilane (4.1)



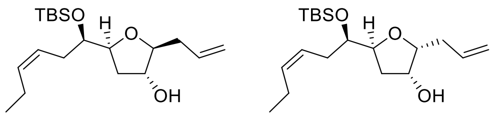
Sodium (meta)periodate (0.13 g, 0.60 mmol, 4.0 equiv.) and potassium osmate(VI) dihydrate (0.50 mg, 15 µmol, 1.0 mol%) were added to a stirred solution of (((1*R*)-1-((3a*R*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-en-1-yl)oxy)triethylsilane **4.7** (0.049 g, 0.15 mmol, 1.0 equiv.) and 2,6-lutidine (0.034 mL, 0.30 mmol, 2.0 equiv.) in dioxane/ H_2O (2:1, 3 mL) and the reaction mixture was stirred at rt for 2 h. After this time, the reaction mixture was diluted with H_2O (4 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organics were dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give the crude aldehyde product which was further dried under high vacuum. Meanwhile, sodium bis(trimethylsilyl)amide solution (1 M in THF, 0.23 mL, 0.23 mmol, 1.5 equiv.) was added to a stirred suspension of freshly dried (high vacuum) triphenyl(propyl)phosphonium bromide (0.092 g, 0.24 mmol, 1.6 equiv.) in THF (1 mL) at 0 °C and the reaction mixture was stirred for 15 mins. After this time, the reaction mixture was cooled to -78 °C and the crude aldehyde was added dropwise in THF (0.5 mL), the reaction mixture was slowly warmed to rt and stirred for a further 16 h. The reaction mixture was quenched by the addition of sat. NH_4Cl (4 mL) and the organics were extracted with EtOAc (3 × 10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The resulting crude residue was further purified by flash column chromatography (petroleum ether 40-60 → 5% EtOAc/ petroleum ether 40-60) giving the title compound (0.027 g, 0.076 mmol, 51%) as a colourless oil. R_f 0.49 (5% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.77 (d, *J* = 3.5 Hz, 1H, OC**H**O), 5.51 – 5.44 (m, 1H, $CH_3CH_2C\mathbf{H}CH$), 5.42 – 5.35 (m, 1H, $CH_3CH_2CHC\mathbf{H}$), 4.74 (dt, *J* = 5.0, 2.5 Hz, 1H, OC**H**O), 4.20 (td, *J* = 8.0, 7.5, 3.0 Hz, 1H, CHH'C**H**), 3.96 (td, *J* = 6.5, 3.0 Hz, 1H, C**H**OSi), 2.21 – 2.16 (m, 2H, $CHCHC\mathbf{H_2}$), 2.10 – 2.02 (m, 2H, $CH_3C\mathbf{H_2}CH$), 1.98 – 1.92 (m, 2H, OCHC**H**H', OCHCH**H'**), 1.52 (s, 3H, $C\mathbf{H_3}$), 1.34 (s, 3H, $C\mathbf{H_3}$), 0.97 (m, 12H, $C\mathbf{H_3}CH_2$, $Si(CH_2C\mathbf{H_3})_3$), 0.62 (q, *J* = 8.0 Hz, 6H, $Si(C\mathbf{H_2}CH_3)_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 133.6 ($CH_3CH_2\mathbf{C}HCH$, 124.5 ($CH_3CH_2CH\mathbf{C}H$), 111.0 ($\mathbf{C}(CH_3)_2$), 105.3 (O**C**HO), 80.8 (CHH'**C**H), 80.7 (O**C**HCHH'), 71.9 (**C**HOSi), 32.8 ($CHCH\mathbf{C}H_2$), 32.0 (OCH**C**HH'), 26.9 ($\mathbf{C}H_3$), 26.3 ($\mathbf{C}H_3$), 20.7 ($CH_3\mathbf{C}H_2$), 14.1 ($\mathbf{C}H_3CH_2$), 6.9 ($Si(CH_2\mathbf{C}H_3)_3$), 5.1 ($Si(\mathbf{C}H_2CH_3)_3$; LRMS *m/z* (ESI^+) 357.2 ($M+H^+$, 100%); HRMS *m/z* (ESI$^+$): found 379.2274, $C_{19}H_{36}O_4$ (M+Na$^+$) requires 379.2275; $[\alpha]_D^{25}$= -12.2 (c = 0.1, $CHCl_3$); IR (ν_{max}): 2956, 2877, 1458, 1373.

***tert*-Butyl(((1*R*,*Z*)-1-((3a*R*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-en-1-yl)oxy)dimethylsilane (4.11)**



Sodium (meta)periodate (0.17 g, 0.80 mmol, 4.0 equiv.) and potassium osmate(VI) dihydrate (0.75 mg, 25 µmol, 1.0 mol%) were added to a stirred solution of *tert*-butyl(((1*R*)-1-((3a*R*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-en-1-yl)oxy)dimethylsilane **4.8** (0.066 g, 0.20 mmol, 1.0 equiv.) and 2,6-lutidine (0.047 mL, 0.40 mmol, 2.0 equiv.) in dioxane/ H_2O (2:1, 4 mL) and the reaction mixture was stirred at rt for 2 h. After this time, the reaction mixture was diluted with H_2O (4 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organics were dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give the crude aldehyde product which was further dried under high vacuum. Meanwhile, sodium bis(trimethylsilyl)amide solution (1 M in THF, 0.30 mL, 1.5 equiv.) was added to a stirred suspension of freshly dried (high vacuum) triphenyl(propyl)phosphonium bromide (0.12 g, 0.32 mmol, 1.6 equiv.) in THF (1.5 mL) at 0 °C and the reaction mixture was stirred for 15 mins. After this time, the reaction mixture was cooled to -78 °C and the crude aldehyde was added dropwise in THF (0.5 mL), the reaction mixture was slowly warmed to rt and stirred for a further 16 h. The reaction mixture was quenched by the addition of sat. NH_4Cl (4 mL) and the organics were extracted with EtOAc (3 × 10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The resulting crude residue was further purified by flash column chromatography (petroleum ether 40-60 → 5% Et_2O/ petroleum ether 40-60) giving the title compound (0.041 g, 0.12 mmol, 58%) as a colourless oil. R_f 0.52 (5% EtOAc/ petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 5.77 (d, *J* = 3.5 Hz, 1H, OC**H**O), 5.52 – 5.43 (m, 1H, $CH_3CH_2C\mathbf{H}CH$), 5.43 – 5.33 (m, 1H, $CH_3CH_2CHC\mathbf{H}$), 4.74 (q, *J* = 3.0 Hz, 1H, OC**H**CHH'), 4.20 (m, 1H, CHH'C**H**), 3.96 (m, 1H, C**H**OSi), 2.24 – 2.14 (m, 2H, $CHCHC\mathbf{H_2}$), 2.11 – 1.99 (m, 2H, $CH_3C\mathbf{H_2}$), 1.99 – 1.91 (m, 2H, CHOC**H**H', CHOCH**H'**), 1.52 (s, 3H, $C\mathbf{H_3}$), 1.34 (s, 3H, $C\mathbf{H_3}$), 0.98 (t, *J* = 7.5 Hz, 3H, $C\mathbf{H_3}CH_2$), 0.89 (s, 9H, $Si(CH_3)_2C(C\mathbf{H_3})_3$), 0.11 (s, 3H, $Si(C\mathbf{H_3})_2C(CH_3)_3$), 0.02 (s, 3H, $Si(C\mathbf{H_3})_2C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 133.7 ($CH_3CH_2\mathbf{C}HCH$), 124.5 ($CH_3CH_2CH\mathbf{C}H$), 111.0 ($\mathbf{C}(CH_3)_2$), 105.2 (O**C**HO), 80.9 (OCHH'**C**H), 80.7 (OC**H**CHH'), 71.9 (**C**HOSi), 32.7 ($CHCH\mathbf{C}H_2$), 32.0 (CHO**C**HH'), 26.9 ($\mathbf{C}H_3$), 26.3 ($\mathbf{C}H_3$), 25.9 ($Si(CH_3)_2C(\mathbf{C}H_3)_3$), 20.7 ($CH_3\mathbf{C}H_2$), 18.2 ($Si(CH_3)_2\mathbf{C}(CH_3)_3$), 14.2 ($\mathbf{C}H_3CH_2$), -4.3 ($Si(\mathbf{C}H_3)_2C(CH_3)_3$), -4.6 ($Si(\mathbf{C}H_3)_2C(CH_3)_3$); LRMS *m/z* ($ESI^+$) 379.2 ($M+Na^+$, 100%); HRMS *m/z* ($ESI^+$): found 379.2275, $C_{19}H_{36}O_4NaSi$ ($M+Na^+$) requires 379.2275; $[\alpha]_D^{25}$= -1.50 (c = 0.060, $CHCl_3$); IR (ν_{max}): 2956, 2931, 2857.

(2*S*,3*R*)-2-Allyl-5-((*R*,*Z*)-1-((*tert*-butyldimethylsilyl)oxy)hex-3-en-1-yl)tetrahydrofuran-3-ol (4.12)



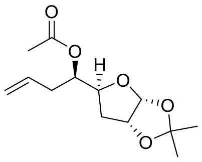
2 : 1

Allyltrimethylsilane (0.79 mL, 5.0 mmol, 5.0 equiv.) was added to a stirred solution of *tert*-Butyl(((1*R*,*Z*)-1-((3a*R*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-en-1-yl)oxy)dimethylsilane **4.11** (0.36 g, 1.0 mmol, 1.0 equiv.) in CH_2Cl_2 (8 mL) at 0 °C and the reaction mixture was stirred for 15 mins before addition of boron trifluoride diethyl etherate (48%, 0.43 mL, 3.0 mmol, 3.0 equiv.). The reaction mixture was then allowed to warm to rt and stirred for 18 h. After this time, the reaction mixture was quenched by the addition of sat. $NaHCO_3$ (10 mL) and the organics were extracted with CH_2Cl_2 (3 × 10 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/petroleum ether 40-60) to give the title compound (0.15 g, 0.45 mmol, 45%) as an inseparable mixture of epimers (2:1 desired:undesired) and as a colourless oil. R_f 0.35 (20% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.94 – 5.83 (m, 1H, $H_2CC\mathbf{H}$), 5.50 – 5.41 (m, 1H, $CH_3CH_2C\mathbf{H}CH$), 5.36 (dtd, *J* = 11.0, 7.5, 1.5 Hz, 1H, $CH_3CH_2CHC\mathbf{H}$), 5.21 – 5.12 (m, 1H, $\mathbf{H}H'CCH$), 5.09 (ddd, *J* = 10.5, 5.5, 3.5 Hz, 1H, $H\mathbf{H'}CCH$), 4.29 (t, *J* = 4.0 Hz, 1H, $CHH'C\mathbf{H}$, minor), 4.21 (ddt, *J* = 9.0, 5.5, 2.5 Hz, 1H, $CHH'C\mathbf{H}$, major), 4.04 (tt, *J* = 6.0, 3.5 Hz, 1H, $HOC\mathbf{H}CHH'$), 3.94 (ddd, *J* = 8.5, 5.5, 3.0 Hz, 1H, $C\mathbf{H}OSi$, major), 3.89 (td, *J* = 7.0, 3.0 Hz, 2H, $O\mathbf{H}$, $C\mathbf{H}OSi$, minor), 3.84 (ddd, *J* = 7.0, 5.5, 3.5 Hz, 1H, $H_2CCHCH_2C\mathbf{H}$, major), 3.69 (td, *J* = 7.0, 4.5 Hz, 1H, $H_2CCHCH_2C\mathbf{H}$, minor), 2.47 – 2.31 (m, 2H, $H_2CCHC\mathbf{H_2}$), 2.31 – 2.15 (m, 2H, $CH{=}CHC\mathbf{H_2}$), 2.14 – 2.01 (m, 2H, $CH_3C\mathbf{H_2}$), 1.90 – 1.83 (m, 1H, $HOCHC\mathbf{H}H'$), 1.74 (ddt, *J* = 12.0, 5.5, 2.5 Hz, 1H, $HOCHCH\mathbf{H'}$), 0.98 (td, *J* = 7.5, 2.0 Hz, 3H, $C\mathbf{H_3}CH_2$), 0.91 (dd, *J* = 6.0, 2.5 Hz, 9H, $Si(CH_3)_2C(C\mathbf{H_3})_3$), 0.09 (dd, *J* = 6.0, 3.5 Hz, 6H, $Si(C\mathbf{H_3})_2C(CH_3)_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 135.0 ($H_2C\mathbf{C}H$, major), 134.7 ($H_2C\mathbf{C}H$, minor), 133.6 ($CH_3CH_2\mathbf{C}HCH$, major), 133.6 ($CH_3CH_2\mathbf{C}HCH$, minor), 124.4 ($CH_3CH_2CH\mathbf{C}H$, minor), 124.3 ($CH_3CH_2CH\mathbf{C}H$, major), 117.2 ($H_2\mathbf{C}CH$, minor), 116.9 ($H_2\mathbf{C}CH$, major), 85.3 ($H_2CCHCH_2\mathbf{C}H$, minor), 82.0 ($\mathbf{C}HOSi$, major), 80.2 ($HO\mathbf{C}HCHH'CH$, major), 79.5 ($CHH'\mathbf{C}H$, major), 75.9 ($HO\mathbf{C}HCHH'$, minor), 73.6 ($HOCHCHH'\mathbf{C}H$, minor), 73.5 ($\mathbf{C}HOSi$, minor), 72.9 ($H_2CCHCH_2\mathbf{C}H$, major), 38.9 ($CHCH\mathbf{C}H_2$, minor), 34.7 ($HOCH\mathbf{C}HH'$, minor), 34.4 ($HOCH\mathbf{C}HH'$, major), 34.00 ($H_2CCH\mathbf{C}H_2$, major), 32.9 ($H_2CCH\mathbf{C}H_2$, minor), 32.6 ($CHCH\mathbf{C}H_2$, major), 25.9 ($Si(CH_3)_2C(\mathbf{C}H_3)_3$, minor), 25.9 ($Si(CH_3)_2C(\mathbf{C}H_3)_3$, major), 20.7 ($CH_3\mathbf{C}H_2$, minor), 20.7 ($CH_3\mathbf{C}H_2$, major), 18.1 ($Si(CH_3)_2\mathbf{C}(CH_3)_3$, minor), 18.1 ($Si(CH_3)_2\mathbf{C}(CH_3)_3$, major), 14.15 ($\mathbf{C}H_3CH_2$), -4.2 ($Si(\mathbf{C}H_3)_2C(CH_3)_3$, minor) -4.4 (Si($\mathbf{C}H_3$)$_2$C($CH_3$)$_3$, minor), -4.5 (Si($\mathbf{C}H_3$)$_2$C($CH_3$)$_3$, major), -4.5 (Si($\mathbf{C}H_3$)$_2$C($CH_3$)$_3$, major); LRMS *m/z* (ESI$^+$) 363.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 341.2508, $C_{19}H_{36}O_3Si$ (M+H$^+$) requires 341.2506; IR (ν_{max}): 3370, 3012, 2956, 2929, 2857, 1643, 1472, 1462, 1361.

OR

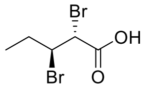
Tetrafluoroboric acid diethyl ether complex (1 M in CH_2Cl_2, 0.017 mL, 0.017 mmol, 0.5 equiv.) was added to a stirred solution of *tert*-butyl(((1*R*,*Z*)-1-((3a*R*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-en-1-yl)oxy)dimethylsilane (0.012 g, 0.033 mmol, 1.0 equiv.) and allyltrimethylsilane (0.042 mL, 0.26 mmol, 8.0 equiv.) in CH_2Cl_2 (0.1 mL) at rt, the reaction mixture was heated to reflux and stirred for 3 h. After this time, the reaction mixture was cooled to rt and then quenched by the addition of sat. $NaHCO_3$ (1 mL) and stirred vigorously for 15 mins. The organics were extracted with CH_2Cl_2 (3 × 2 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 20% EtOAc/ petroleum ether 40-60) to give the title compound (0.0050 g, 0.015 mmol, 45%) as a colourless oil and a 2:1 inseparable mixture of epimers.

(*R*)-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-en-1-yl acetate (4.13)



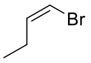
Acetic anhydride (0.14 mL, 1.5 mmol, 3.0 equiv.) and 4-(dimethylamino)pyridine (0.17 g, 1.5 mmol, 3.0 equiv.) were added to (*R*)-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-en-1-ol **3.116** (0.11 g, 0.50 mmol, 1.0 equiv.) in CH_2Cl_2 (5 mL) and the reaction mixture was stirred for 2 h. The reaction mixture was quenched with sat. $NaHCO_3$ (10 mL) and then extracted with CH_2Cl_2 (3 × 5 mL), the combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give a crude residue which was purified further by flash column chromatography (5% EtOAc/ petroleum ether 40-60 → 15% EtOAc/ petroleum ether 40-60) giving the title compound (0.13 g, 0.50 mmol, 93%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (d, *J* = 3.5 Hz, 1H, OC**H**O), 5.77 – 5.69 (m, 1H, H_2CC**H**), 5.15 – 5.01 (m, 3H, **H$_2$**CCH, C**H**OAc), 4.71 (dd, *J* = 4.5, 4.0 Hz, 1H, C**H**CHH’), 4.24 (dt, *J* = 10.0, 5.0 Hz, 1H, CHH’C**H**), 2.42 (dddt, *J* = 14.5, 6.5, 5.0, 1.5 Hz, 1H, H_2CCHC**H**H’), 2.38 – 2.30 (m, 1H, H_2CCHCH**H’**), 2.08 (dd, *J* = 9.5, 4.0 Hz, 1H, OCHC**H**H’), 2.04 (s, 3H, C**H$_3$**), 1.75 (ddd, *J* = 13.5, 10.5, 5.0 Hz, 1H, OCHCH**H’**), 1.50 (s, 3H, C**H$_3$**), 1.31 (s, 3H, C**H$_3$**); LRMS *m/z* (ESI$^+$) 279.1 (M+Na$^+$, 100%). Data are in accordance with the literature.[102]

(2R,3S)-2,3-Dibromopentanoic acid (4.19)



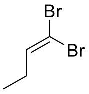
According to the procedure of Hickmann et al.:[233] bromine (4.3 mL, 83 mmol, 1.03 equiv.) was added to a stirred solution of *trans*-2-pentenoic acid **4.18** (8.0 mL, 80 mmol, 1.0 equiv.) in CH_2Cl_2 (170 mL) at 0 °C, the reaction mixture was then warmed to rt and stirred for 3 h. After this time, the reaction mixture was quenched by the addition of 10% (w/v) $NaHSO_3$ solution and the organics extracted with CH_2Cl_2 (3 × 50 mL). The organic layers were combined, dried ($MgSO_4$), filtered, and the reaction solvent removed *in vacuo* to give the crude product which was subjected to high vacuum in order to remove any traces of residual solvent, giving the title compound (20 g, 76 mmol, 95%) as a pale yellow oil which required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.96 (s, 1H, COO**H**), 4.38 (d, *J* = 11.0 Hz, 1H, C**H**BrCOOH), 4.29 (ddd, *J* = 11.0, 8.0, 2.5 Hz, 1H, CH_2C**H**Br), 2.31 – 2.17 (m, 1H, CH_3C**H**H'), 1.92 – 1.70 (m, 1H, CH_3CH**H'**), 1.04 (t, *J* = 7.0 Hz, 3H, C**H₃**); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.3 (**C**OOH), 53.3 (**C**HBrCOOH), 46.8 (CH_2**C**HBr), 28.3 (CH_3**C**H_2), 10.5 (**C**H_3CH_2). Data are in accordance with the literature.

(Z)-1-bromobut-1-ene (4.20)



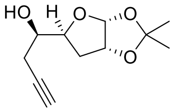
According to the procedure of Hickmann et al.:[233] (2*R*,3*S*)-2,3-dibromopentanoic acid **4.19** (20 g, 76 mmol, 1.0 equiv.) in DMF (10 mL) was added dropwise from a dropping funnel to a stirred suspension of sodium bicarbonate (11 g, 130 mmol, 1.7 equiv.) in DMF (30 ml) in a three-necked flask equipped with a short path distillation setup (shown below) stirred vigorously at 70 °C. Vacuum was applied (130 mbar) and the crude product was collected in the collection vessel cooled in a dry ice/ acetone bath. Upon completion of the distillation, the vacuum was released and the product was transferred to a separating funnel with flask rinsing using a minimum volume of pentane and then the crude product was washed with a minimum volume of H_2O, collected (bottom layer), dried ($MgSO_4$), and filtered into a pre-weighed flask (pentane rinse) to give the title compound (86% in pentane, 8.2 g, 53 mmol, 69% yield) which was used as a solution in pentane for future reactions. ^1H NMR (400 MHz, $CDCl_3$) δ 6.15 – 6.04 (m, 2H, C**H**CHBr, CHC**H**Br), 2.27 – 2.13 (m, 2H, CH_3C**H₂**), 1.02 (t, *J* = 7.5 Hz, 3H, C**H₃**CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.4 (**C**HCHBr), 107.0 (CH**C**HBr), 23.2 (CH_3**C**H_2), 12.7 (**C**H_3CH_2). Data are in accordance with the literature.[233]

1,1-dibromobut-1-ene (4.21)



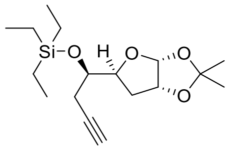
According to the procedure of Tang et al.:[162] propionaldehyde (0.72 g, 10 mmol, 1.0 equiv.) was added to a stirred solution of tetrabromomethane (3.3 g, 10 mmol, 1.0 equiv.) in CH_2Cl_2 (50 mL) in a three necked flask at 0 °C, triphenylphosphine (3.2 g, 20 mmol, 2.0 equiv.) was added portionwise over the course of 15 mins and then the reaction mixture was stirred a further 10 mins at 0 °C before being stirred at rt for 1 h. After this time, petroleum ether (50 mL) was added and the solution was stirred until solid crashed out, the solid was filtered and the above procedure repeated (4 ×) on the filtrate, reducing the petroleum ether each time (final volume ca. 5 mL). The crude product was then filtered through a short silica plug (petroleum ether) to give the title compound (1.2 g, 5.7 mmol, 57%) as a colourless, light sensitive oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.39 (t, *J* = 7.0 Hz, 1H, C**H**CBr_2), 2.11 (p, *J* = 7.5 Hz, 2H, CH_3C**H_2**), 1.03 (t, *J* = 7.5 Hz, 3H, C**H_3**CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.1 (**C**HCBr_2), 88.2 (CH**C**Br_2), 26.6 (CH_3**C**H_2), 12.3 (**C**H_3CH_2). Data are accordance with the literature.[162]

(*R*)-1-((3a*R*,5*S*,6a*R*)-2,2-Dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)but-3-yn-1-ol (4.23)



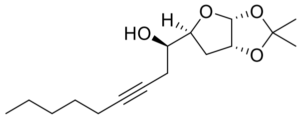
(3a*R*,5*S*,6a*R*)-2,2-Dimethyl-5-(*[R]*-oxiran-2-yl)tetrahydrofuro[2,3-*d*][1,3]dioxole **3.117** (0.19 g, 1.0 mmol, 1.0 equiv.) was added in THF (1.3 mL) to a stirred solution of lithium acetylide, ethylenediamine complex (90%, 0.10 g, 1.0 mmol, 1.0 equiv.) in THF/DMSO (1.3 mL/ 2.5 mL) cooled to 0 °C and the reaction mixture was allowed to come to rt over a period of 14 h. After this time, the reaction mixture was quenched by addition of sat. NH_4Cl (15 mL) and the organics extracted with EtOAc (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60 → 40% EtOAc/ petroleum ether 40-60) to give the title compound (0.19 g, 0.91 mmol, 91%) as a colourless oil. R_f 0.28 (30% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.84 (d, *J* = 3.5 Hz, 1H, OC**H**O), 4.79 (t, *J* = 4.0 Hz, 1H, OC**H**CHH'), 4.34 (dt, *J* = 10.5, 4.5 Hz, 1H, CHH'C**H**), 4.03 (td, *J* = 6.5, 4.5 Hz, 1H, C**H**OH), 2.53 – 2.39 (m, 2H, C**H$_2$**CCH), 2.12 (dd, *J* = 13.5, 4.5 Hz, 1H, OCHC**H**H'), 2.10 – 2.07 (m, 1H, CC**H**), 1.89 (ddd, *J* = 13.5, 10.5, 5.0 Hz, 1H, OCHCH**H'**), 1.80 (s, 1H, O**H**), 1.55 (s, 3H, C**H$_3$**), 1.36 (s, 3H, C**H$_3$**); ^{13}C NMR (126 MHz, $CDCl_3$) δ 111.4 (**C**(CH_3)$_2$), 105.4 (O**C**HO), 80.6 (O**C**HCHH'), 79.8 (**C**CH), 79.8 (CHH'**C**H), 70.9 (C**C**H), 69.6 (**C**HOH), 32.6 (OCH**C**HH'), 26.8 (**C**H$_3$), 26.2 (**C**H$_3$), 23.3 (**C**H$_2$CCH); LRMS *m/z* (ESI$^+$) 235.1 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 235.0943, $C_{11}H_{16}O_4$ (M+Na$^+$) requires 235.0941; $[\alpha]_D^{25}$ = -14.4 (c = 0.09, $CHCl_3$); IR (ν_{max} cm^{-1}): 3443, 3284, 2987, 2937, 2121, 1636, 1432.

(((*R*)-1-((3a*R*,5S,6a*R*)-2,2-Dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-yn-1-yl)oxy)triethylsilane (4.25)



2,6-Lutidine (0.23 mL, 2.0 mmol, 2.0 equiv.) and triethylsilyl trifluoromethanesulfonate (0.34 mL, 1.5 mmol, 1.5 equiv.) were added to a stirred of (*R*)-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)but-3-yn-1-ol **4.23** (0.21 mL, 1.0 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) cooled to 0 °C. The reaction mixture was allowed to warm to rt and then stirred for 1 h before being quenched by addition of sat. $NaHCO_3$ (10 mL), the layers were separated and the organics further extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give the crude product which was dried thoroughly under high vacuum giving the title compound (0.33 g, 1.0 mmol, quant.) as a colourless oil which required no further purification. R_f 0.55 (5% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.76 (d, *J* = 3.5 Hz, 1H, OC**H**O), 4.72 (t, *J* = 4.0 Hz, 1H, OC**H**CHH'), 4.35 (ddd, *J* = 10.5, 4.5, 3.5 Hz, 1H, CHH'C**H**), 4.03 (td, *J* = 6.5, 3.5 Hz, 1H, C**H**OSi), 2.39 – 2.25 (m, 2H, HCCC$\mathbf{H_2}$), 2.02 (dd, *J* = 13.5, 4.5 Hz, 1H, OCHC**H**H'), 1.98 (t, *J* = 2.5 Hz, 1H, **H**CC), 1.89 (ddd, *J* = 13.5, 10.5, 5.0 Hz, 1H, OCHCH**H'**), 1.51 (s, 3H, C$\mathbf{H_3}$), 1.32 (s, 3H, C$\mathbf{H_3}$), 0.94 (t, *J* = 8.0 Hz, 9H, Si($CH_2C\mathbf{H_3}$)$_3$), 0.62 (q, *J* = 8.0 Hz, 6H, Si($CH_2C\mathbf{H_3}$)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 111.2 (**C**(CH_3)$_2$), 105.4 (O**C**HO), 80.7 (HC**C**), 80.7 (O**C**HCHH'), 80.2 (CHH'**C**H), 70.7 (**C**HOSi), 70.3 (H**C**C)$_3$, 32.3 (OCH**C**HH'), 26.9 (**C**H_3), 26.3 (**C**H_3), 24.9 (HCC**C**H_2), 6.9 (Si(CH_2**C**H_3)$_3$), 5.0 (Si(**C**H_2CH_3)$_3$); LRMS *m/z* (ESI$^+$) 349.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 349.1807, $C_{17}H_{30}O_4Si$ (M+Na$^+$) requires 349.1806; $[\alpha]_D^{25}$= -23.0 (c = 0.10, $CHCl_3$); IR (ν_{max} cm^{-1}): 2954, 2877, 1644, 1458, 1415.

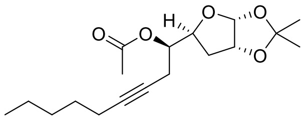
(*R*)-1-((3a*R*,5S,6a*R*)-2,2-Dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)non-3-yn-1-ol (4.29)



n-Butyllithium (2.5 M in hexanes, 0.56 mL, 1.35 mmol, 1.8 equiv.) was added to a stirred solution of 1-heptyne (0.18 mL, 1.35 mmol, 1.8 equiv.) in THF (5 mL) cooled to 0 °C and the reaction mixture was stirred for 1 h. After this time, the reaction mixture was cooled to -78 °C before successive addition of boron trifluoride diethyl etherate (48%, 0.14 mL, 0.56 mmol, 0.75 equiv.) and (3a*R*,5*S*,6a*R*)-2,2-dimethyl-5-(*[R]*-oxiran-2-yl)tetrahydrofuro[2,3-*d*][1,3]dioxole **3.117** (0.14 g, 0.75 mmol, 1.0 equiv.) in THF (2.5 mL) and the reaction mixture was stirred for a further 1 h. The reaction mixture was then quenched by the addition of sat. NH_4Cl (10 mL) and then extracted with EtOAc (3 × 10 mL) and the combined organic layers dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give the crude residue which was further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) to give the title compound (0.19 g, 0.66 mmol, 88%) as a colourless oil. R*f* 0.26 (30% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.84 (d, *J* = 3.5 Hz, 1H, OC**H**O), 4.78 (t, *J* = 4.0 Hz, 1H, OC**H**CHH'), 4.34 (dt, *J* = 10.5, 4.5 Hz, 1H, CHH'C**H**), 3.95 (td, *J* = 6.5, 4.5 Hz, 1H, C**H**OH), 2.42 (qdt, *J* = 16.5, 6.5, 2.5 Hz, 2H, $C\mathbf{H_2}CHOH$), 2.20 (s, 1H, O**H**), 2.17 (tt, *J* = 7.0, 2.5 Hz, 2H, $CH_2C\mathbf{H_2}C$), 2.11 (dd, *J* = 13.5, 4.5 Hz, 1H, OCHC**H**H'), 1.88 (ddd, *J* = 13.5, 10.5, 4.5 Hz, 1H, OCHCH**H'**), 1.54 (s, 3H, $C\mathbf{H_3}$), 1.50 (q, *J* = 7.0 Hz, 2H, $C\mathbf{H_2}CH_2C$), 1.41 – 1.25 (m, 7H, $CH_3C\mathbf{H_2}CH_2$, $CH_3CH_2C\mathbf{H_2}$, $C\mathbf{H_3}$), 0.92 (t, *J* = 7.0 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 111.3 ($\mathbf{C}(CH_3)_2$), 105.4 (O**C**HO), 83.4 ($CH_2\mathbf{C}C$), 80.6 (O**C**HCHH'), 79.9 (CHH'**C**H), 74.9 ($CH_2C\mathbf{C}$), 69.9 (**C**HOH), 32.7 (OCH**C**HH'), 31.1 ($CH_3CH_2\mathbf{C}H_2$), 28.6 ($\mathbf{C}H_2CH_2CC$), 26.8 ($\mathbf{C}H_3$), 26.2 ($\mathbf{C}H_3$), 23.7 ($\mathbf{C}H_2CHOH$), 22.2 ($CH_3\mathbf{C}H_2$), 18.7 ($CH_2\mathbf{C}H_2CC$), 14.0

($\mathbf{C}H_3CH_2$); LRMS *m/z* (ESI$^+$) 283.2 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 283.1901, $C_{16}H_{26}O_4$ (M+H$^+$) requires 283.1904; $[\alpha]_D^{25}$= -12.0 (c = 0.10, $CHCl_3$); IR (ν_{max} cm^{-1}): 3467, 2960, 2890, 1647, 1460.

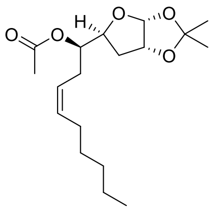
(*R*)-1-((3a*R*,5*S*,6a*R*)-2,2-Dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)non-3-yn-1-yl acetate (4.33)



4-Dimethylaminopyridine (0.24 g, 2.0 mmol, 3.0 equiv.) and acetic anhydride (0.19 mL, 2.0 mmol, 3.0 equiv.) were added to a stirred solution of (*R*)-1-((3a*R*,5S,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)non-3-yn-1-ol **4.29** (0.19 g, 0.66 mmol, 1.0 equiv.) in CH_2Cl_2 (7 mL) and the reaction mixture was stirred at rt for 2 h. After this time, the reaction mixture was quenched by the addition of sat. $NaHCO_3$ (10 mL) and the organics were extracted with CH_2Cl_2 (3 × 10 mL). The combined organics were dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give the crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 15% EtOAc/petroleum ether 40-60) to give the title compound (0.17 g, 0.54 mmol, 81%) as a pale yellow oil. R*f* 0.26 (15% EtOAc/petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.77 (d, *J* = 3.5 Hz, 1H, OC**H**O), 5.04 (q, *J* = 6.0 Hz, 1H, C**H**OCOCH$_3$), 4.71 (t, *J* = 4.0 Hz, 1H, OC**H**CHH'), 4.39 (dt, *J* = 10.0, 5.0 Hz, 1H, CHH'C**H**), 2.49 (dt, *J* = 6.0, 2.5 Hz, 2H, C**H$_2$**CHOCOCH$_3$), 2.14 – 2.07 (m, 3H, OCHC**H**H', C**H$_2$**C), 2.06 (s, 3H, OCOC**H$_3$**), 1.79 (ddd, *J* = 13.5, 10.5, 5.0 Hz, 1H, OCHCH**H'**), 1.49 (s, 3H, C**H$_3$**), 1.44 (h, *J* = 7.0 Hz, 2H, C**H$_2$**CH$_2$CC), 1.39 – 1.20 (m, 7H, C**H$_3$**, CH$_3$CH$_2$C**H$_2$**, CH$_3$C**H$_2$**), 0.88 (t, *J* = 7.0 Hz, 3H, C**H$_3$**CH$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.1 (**C**=O), 111.4 (**C**(CH$_3$)$_2$), 105.5 (O**C**HO), 82.7 (CH$_2$**C**C), 80.3 (O**C**HCHH'), 77.9 (CHH'**C**H), 74.5 (CH$_2$C**C**C), 72.0

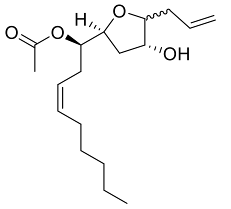
(**C**HOCOCH$_3$), 34.5 (OCH**C**HH'), 31.0 (CH$_3$CH$_2$**C**H$_2$), 28.5 (**C**H$_2$CH$_2$CC), 26.8 (**C**H$_3$), 26.3 (**C**H$_3$), 22.2 (CH$_3$**C**H$_2$), 21.6 (CC**C**H2CHOCO), 21.0 (OCO**C**H$_3$), 18.7 (CH$_2$**C**H$_2$CC), 14.0 (**C**H$_3$CH$_2$); LRMS *m/z* (ESI$^+$) 347.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 347.1887, $C_{18}H_{28}O_5$ (M+Na$^+$) requires 347.1887; $[\alpha]_D^{25}$= -1.9 (c = 0.12, CHCl$_3$); IR (v$_{max}$ cm^{-1}): 2934, 2860, 2160, 2029, 1746, 1434, 1373.

(*R*)-1-((3a*R*,5*S*,6a*R*)-2,2-Dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)non-3-en-1-yl acetate (4.34)



According to the procedure of Richardson et al.:[227] sodium borohydride (0.0041 g, 0.11 mmol, 20 mol%) was added to a stirred suspension of nickel(II) acetate tetrahydrate (0.027 g, 0.11 mmol, 20 mol%) in EtOH (3.5 mL) at rt under an atmosphere of H$_2$, The flask was then purged with H$_2$ before addition of ethylenediamine (0.014 mL, 0.22 mmol, 40 mol%) and the reaction mixture was sparged for 20 mins with H$_2$. After this time, (*R*)-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)non-3-yn-1-yl acetate **4.33** (0.17 g, 0.54 mmol, 1.0 equiv.) was added in EtOH (1.5 mL) followed by a further 20 mins of sparging with H$_2$, the rection mixture was then stirred under an atmosphere of H$_2$ at rt for 18 h. The reaction flask was purged with H$_2$ and then the solvent removed *in vacuo* to give a crude residue which was passed through a silica plug (30% EtOAc/ petroleum ether 40-60) to give the title compound (0.15 g, 0.44 mmol, 83%) as a pale yellow oil. R$_f$ 0.29 (15% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.79 (d, *J* = 3.5 Hz, 1H, OC**H**O), 5.49 (dtt, *J* = 10.5, 7.5, 1.0 Hz, 1H, CH$_2$CH$_2$C**H**CH), 5.32 (dtt, *J* = 10.5, 7.5, 1.5 Hz, 1H, CH$_2$CH$_2$CHC**H**), 5.05 (td, *J* = 6.5, 5.0 Hz, 1H, C**H**OCOCH$_3$), 4.74 – 4.69 (m, 1H, OC**H**CHH'), 4.25 (dt, *J* = 10.0, 5.0 Hz, 1H, CHH'C**H**), 2.36 (td, *J* = 7.0, 1.5 Hz, 2H, C**H$_2$**CHOCO), 2.11 – 1.98 (m, 6H, CH$_2$C**H$_2$**CHCH, OCHC**H**H', OCOC**H$_3$**), 1.77 (ddd, *J* = 13.5, 10.5, 5.0 Hz, 1H, OCHCH**H'**), 1.50 (s, 3H, C**H$_3$**), 1.38 – 1.22 (m, 9H, CH$_3$C**H$_2$**, CH$_3$CH$_2$C**H$_2$**, C**H$_2$**CH$_2$CHCH, C(C**H$_3$**)$_2$), 0.88 (t, *J* = 7.0 Hz, 3H, C**H$_3$**CH$_2$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 170.3 (**C**=O), 133.3 (CH$_2$CH$_2$C**H**CH), 123.5 (CH$_2$CH$_2$CHC**H**), 111.3 (**C**(CH$_3$)$_2$), 105.5 (O**C**HO), 80.2 (O**C**HCHH'), 78.5 (CHH'**C**H), 73.4 (**C**HOCOCH$_3$), 34.7 (OCH**C**HH'), 31.5 (**C**H$_2$CH$_2$CHCH), 29.2 (**C**H$_2$CHOCO), 29.2 (CH$_3$CH$_2$**C**H$_2$), 27.3 (CH$_2$**C**H$_2$CHCH), 26.8 (**C**H$_3$), 26.2 (**C**H$_3$), 22.6 (CH$_3$**C**H$_2$), 21.1 (OCO**C**H$_3$), 14.1 (**C**H$_3$CH$_2$); LRMS *m/z* (ESI$^+$) 349.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 349.1986, $C_{18}H_{30}O_5$ (M+Na$^+$) requires 349.1985; $[\alpha]_D^{25}$= -4.8 (c = 0.13, CHCl$_3$); IR (ν$_{max}$ cm^{-1}): 2927, 2160, 2028, 1717, 1570, 1470.

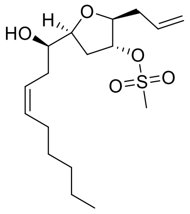
(1*R*,*Z*)-1-((2*S*,4*R*)-5-Allyl-4-hydroxytetrahydrofuran-2-yl)non-3-en-1-yl acetate (4.35)



Allyltrimethylsilane (0.63 mL, 4.0 mmol, 5.0 equiv.) was added to a stirred solution of (*R*)-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)non-3-en-1-yl acetate **4.34** (0.25 g, 0.79 mmol, 1.0 equiv.) in CH$_2$Cl$_2$ (8 mL) at 0 °C and the reaction mixture was stirred for 15 mins before addition of boron trifluoride diethyl etherate (48%, 0.34 mL, 2.4 mmol, 3.0 equiv.). The reaction mixture was then allowed to warm to rt and stirred for 18 h. After this time, the reaction mixture was quenched by the addition of sat. NaHCO$_3$ (10 mL) and the organics were extracted with CH$_2$Cl$_2$ (3 × 10 mL), dried (MgSO$_4$), filtered and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/petroleum ether 40-60) to give the title compound (0.25 g, 0.55

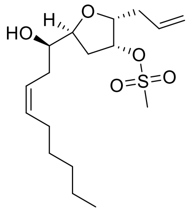
mmol, 70%) as an inseparable mixture of epimers (2:1 desired:undesired) and as a colourless oil. $R_{f\ (major)}$ 0.25 (20% EtOAc/ petroleum ether), $R_{f\ (minor)}$ 0.21 (20% EtOAc/ petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.90 – 5.75 (m, 1H, $H_2CC\mathbf{H}$), 5.48 (dtq, *J* = 11.0, 7.5, 1.5 Hz, 1H, $CH_2CH_2C\mathbf{H}CH$), 5.38 – 5.28 (m, 1H, $CH_2CH_2CHC\mathbf{H}$), 5.22 – 5.06 (m, 2H, $\mathbf{H_2}C{=}CH$), 5.02 (tt, *J* = 8, 5.5 Hz, 1H, $C\mathbf{H}OCOCH_3$), 4.28 (ddd, *J* = 9.0, 7.0, 5.5 Hz, 1H, $HOCHH'C\mathbf{H}$, minor), 4.19 (ddd, *J* = 9.0, 6.5, 5.0 Hz, 1H, $HOCHH'C\mathbf{H}$, major), 4.16 – 4.08 (m, 1H, $C\mathbf{H}OH$), 3.86 (ddd, *J* = 7.5, 6.5, 3.0 Hz, 1H, $H_2CCHCH_2C\mathbf{H}$, minor), 3.81 (td, *J* = 6.5, 3.0 Hz, 1H, $H_2CCHCH_2C\mathbf{H}$, major), 2.50 – 2.19 (m, 4H, $H_2CCHC\mathbf{H_2}$, $C\mathbf{H_2}CHOCO$), 2.08 – 1.97 (m, 6H, $CH_2C\mathbf{H_2}CHCH$, $OCOC\mathbf{H_3}$, $HOC\mathbf{H}H'$), 1.87 (ddd, *J* = 13.0, 6.5, 3.0 Hz, 1H, $HOCH\mathbf{H'}$), 1.38 – 1.23 (m, 6H, $CH_3C\mathbf{H_2}$, $CH_3CH_2C\mathbf{H_2}$, $C\mathbf{H_2}CH_2CHCH$), 0.92 – 0.83 (m, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4 ($\mathbf{C}{=}O$), 134.5 ($H_2C\mathbf{C}H$, minor), 134.0 ($H_2C\mathbf{C}H$, major), 133.2 ($CH_2CH_2\mathbf{C}HCH$, major), 133.1 ($CH_2CH_2\mathbf{C}HCH$, minor), 123.7 ($CH_2CH_2CH\mathbf{C}H$, major), 123.6 ($CH_2CH_2CH\mathbf{C}H$, minor), 117.6 ($H_2\mathbf{C}CH$, major), 117.2 ($H_2\mathbf{C}CH$, minor), 85.7 ($H_2CCHCH_2\mathbf{C}H$, major), 82.3 ($H_2CCHCH_2\mathbf{C}H$, minor), 78.5 ($CHH'\mathbf{C}H$, major), 77.7 ($CHH'\mathbf{C}H$, minor), 75.2 ($HO\mathbf{C}HCHH'CH$, major), 75.0 ($HO\mathbf{C}HCHH'CH$, minor), 74.2 ($\mathbf{C}HOCOCH_3$, major), 73.0 ($\mathbf{C}HOCOCH_3$), 38.5 ($H_2C{=}CH\mathbf{C}H_2$, major), 36.9 ($HOCH\mathbf{C}HH'CH$, minor), 35.9 ($HOCH\mathbf{C}HH'CH$, major), 33.7 ($H_2C{=}CH\mathbf{C}H_2$, minor), 31.5 ($\mathbf{C}H_2CH_2CHCH$), 30.95 ($\mathbf{C}H_2CH_2CHCH$, minor), 29.2 ($CH_3CH_2\mathbf{C}H_2$), 28.98 ($\mathbf{C}H_2CHOCO$), 27.3 ($CH_2\mathbf{C}H_2CHCH$, major), 27.3 ($CH_2\mathbf{C}H_2CHCH$, minor), 22.6 ($CH_3\mathbf{C}H_2$), 21.2 ($OCO\mathbf{C}H_3$, major), 21.2 ($OCO\mathbf{C}H_3$, minor), 14.08 ($\mathbf{C}H_3CH_2$); LRMS *m/z* (ESI$^+$) 333.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 333.2036, $C_{18}H_{30}O_4$ (M+Na$^+$) requires 333.2036; IR (ν_{max} cm^{-1}): 3422, 2960, 2930, 2858, 2159, 2027, 1742, 1642, 1437, 1373.

(2*S*,3*R*,5*S*)-2-Allyl-5-((*R*,*Z*)-1-hydroxynon-3-en-1-yl)tetrahydrofuran-3-yl methanesulfonate (4.36)



Triethylamine (0.084 mL, 0.61 mmol, 1.1 equiv.) and methanesulfonyl chloride (0.061 mL, 0.61 mmol, 1.1 equiv.) were added successively to a stirred solution of (1*R*,*Z*)-1-((2*S*,4*R*)-5-allyl-4-hydroxytetrahydrofuran-2-yl)non-3-en-1-yl acetate **4.35** and its epimer (0.17 g, 0.55 mmol, 1.0 equiv.) in CH_2Cl_2 (6 mL) at 0 °C. The reaction mixture was warmed to rt and stirred for 1 h before quenching by the addition of sat. $NaHCO_3$ (10 mL). The organics were extracted with CH_2Cl_2 (3 × 10 mL) and then dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to yield the crude mesylate which was subjected to high vacuum to remove traces of solvent. The crude mesylate was then redissolved in a mixture of MeOH/H_2O (7:1, 2.5 mL) and then potassium carbonate (0.15 g, 1.1 mmol, 2.0 equiv.) was added. The reaction mixture was stirred at rt for 2 h and then diluted with H_2O (10 mL). The organics were extracted with EtOAc (3 × 10 mL) and then the combined organic layers were dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) to give the title compound (0.14 g, 0.33 mmol, 60%) as a colourless oil and the epimeric product (see below) also as a colourless oil (total yield, 80%). R_f 0.18 (15% acetone/ petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 5.83 (ddt, *J* = 17.0, 10.5, 7.0 Hz, 1H, $H_2CC\mathbf{H}$), 5.62 – 5.53 (m, 1H, $CH_2CH_2C\mathbf{H}CH$), 5.47 – 5.37 (m, 1H, $CH_2CH_2CHC\mathbf{H}$), 5.24 – 5.13 (m, 2H, $\mathbf{H_2}CCH$), 4.97 (dt, *J* = 6.0, 2.0 Hz, 1H, C**H**OS), 4.24 – 4.09 (m, 3H, $H_2C{=}CHCH_2C\mathbf{H}$, CHH'C**H**, O**H**), 3.89 (ddd, *J* = 8.0, 5.5, 3.5 Hz, 1H, C**H**OH), 3.06 (s, 3H, $SO_2C\mathbf{H_3}$), 2.45 – 2.34 (m, 2H, $H_2CCHC\mathbf{H_2}$), 2.32 – 2.08 (m, 4H, SOCHCH**H’**, SOCHC**H**H’, $C\mathbf{H_2}CHOH$), 2.07 (d, *J* = 3.5 Hz, 2H, $CH_2C\mathbf{H_2}CHCH$), 1.43 – 1.26 (m, 6H, $C\mathbf{H_2}CH_2CHCH$, $CH_3CH_2C\mathbf{H_2}$, $CH_3C\mathbf{H_2}$), 0.91 (t, *J* = 7.0 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 133.5 ($CH_2CH_2\mathbf{C}HCH$), 133.0 ($H_2C\mathbf{C}H$), 124.1 ($CH_2CH_2CH\mathbf{C}H$), 118.7 ($H_2\mathbf{C}CH$), 83.7 (SO**C**HCHH’), 83.0 ($H_2CCHCH_2\mathbf{C}H$), 81.1 (CHH’**C**H), 70.7 (**C**HOH), 38.6 ($SO_2\mathbf{C}H_3$, 37.8 ($H_2CCH\mathbf{C}H_2$), 31.7 ($\mathbf{C}H_2CH_2CHCH$), 31.5 ($\mathbf{C}H_2CHOH$), 30.8 (SOCH**C**HH’CH), 29.2 ($CH_2\mathbf{C}H_2CHCH$), 27.4 ($\mathbf{C}H_2CH_2CHCH$), 22.6 ($CH_3\mathbf{C}H_2$), 14.1 ($\mathbf{C}H_3CH_2$); LRMS *m/z* (ESI$^+$) 369.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 369.1704, $C_{17}H_{30}O_5S$ (M+Na$^+$) requires 369.1706; $[\alpha]_D^{25}$= -12.0 (c = 0.1, $CHCl_3$); IR (ν_{max} cm^{-1}): 3398, 2929, 2859, 2116, 1643, 1436, 1357.

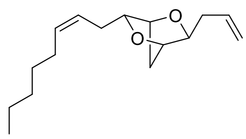
(2*R*,3*R*,5*S*)-2-Allyl-5-((*R*,*Z*)-1-hydroxynon-3-en-1-yl)tetrahydrofuran-3-yl methanesulfonate (4.37)



The title compound (0.038 g, 0.11 mmol, 20%) was isolated after column chromatography in the previously described reaction. R*f* 0.26 (30% EtOAc/petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.83 (ddt, *J* = 17.0, 10.5, 7.0 Hz, 1H, $H_2CC\mathbf{H}$), 5.61 – 5.52 (m, 1H, $CH_2CH_2C\mathbf{H}CH$), 5.43 – 5.34 (m, 1H, $CH_2CH_2CHC\mathbf{H}$), 5.24 – 5.20 (m, 1H, C**H**OS), 5.19 – 5.09 (m, 2H, $\mathbf{H_2}CCH$), 4.23 (ddd, *J* = 10.0, 6.5, 4.0 Hz, 1H, CHH’C**H**), 4.19 – 4.04 (m, 1H, $H_2C{=}CHCH_2C\mathbf{H}$), 3.88 (p, *J* = 5.5 Hz, 1H, C**H**OS), 3.05 (s, 3H, $SO_2C\mathbf{H_3}$), 2.51 – 2.38 (m, 2H, $H_2CCHC\mathbf{H_2}$), 2.38 – 2.26 (m, 2H, SOCHC**H**H’, SOCHCH**H’**), 2.19 – 2.15 (m, 3H, $C\mathbf{H_2}CHOH$, O**H**), 2.12 – 1.96 (m, 2H, $CH_2C\mathbf{H_2}CHCH$), 1.39 – 1.23 (m, 6H, $C\mathbf{H_2}CH_2CHCH$, $CH_3CH_2C\mathbf{H_2}$, $CH_3C\mathbf{H_2}$), 0.89 (t, *J* = 7.0 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 133.7 ($CH_2CH_2\mathbf{C}HCH$), 133.7 ($H_2C\mathbf{C}H$), 124.1

($CH_2CH_2CH\mathbf{C}H$), 117.8 ($H_2\mathbf{C}CH$), 82.1 ($\mathbf{C}HOS$), 81.4 ($H_2CCHCH_2\mathbf{C}H$), 80.0 ($CHH'\mathbf{C}H$), 71.9 ($\mathbf{C}HOH$), 38.7 ($SO_2\mathbf{C}H_3$), 34.0 ($H_2C{=}CH\mathbf{C}H_2$), 33.1 ($SOCH\mathbf{C}HH'$), 31.5 ($\mathbf{C}H_2CH_2CHCH$), 30.8 ($\mathbf{C}H_2CHOH$), 29.3 ($CH_3CH_2\mathbf{C}H_2$), 27.4 ($CH_2\mathbf{C}H_2CHCH$, 22.6 ($CH_3\mathbf{C}H_2$), 14.1 ($\mathbf{C}H_3CH_2$); LRMS *m/z* (ESI^+) 369.2 ($M+Na^+$, 100%); HRMS *m/z* (ESI^+): found 369.1707, $C_{17}H_{30}O_5S$ ($M+Na^+$) requires 369.1706; $[\alpha]_D^{25}$= -32.8 (c = 0.11, $CHCl_3$); IR (ν_{max} cm^{-1}): 3398, 2929, 2859, 2120, 1644, 1437, 1355.

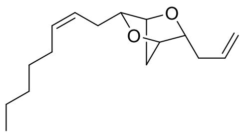
(3*S*,6*S*)-3-Allyl-6-((*Z*)-oct-2-en-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (4.38)



Sodium hydride (60% in mineral oil, 0.092 g, 2.3 mmol, 7.0 equiv.) was added portionwise to a stirred solution of (2*S*,3*R*,5*S*)-2-allyl-5-((*R*,*Z*)-1-hydroxynon-3-en-1-yl)tetrahydrofuran-3-yl methanesulfonate **4.36** (0.14 g, 0.33 mmol, 1.0 equiv.) in DMF (3.5 mL) at 0 °C and the reaction mixture was stirred for 20 mins at this temperature and then at rt for a further 18 h. After this time, the reaction was quenched by the addition of H_2O (8 mL) and the organics were extracted with petroleum ether 40-60 (4 × 5 mL), the combined organic phase was washed with a sat. brine solution (10 mL), dried (Na_2SO_4), filtered and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (5% EtOAc/petroleum ether 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound (0.055 g, 0.22 mmol, 67%) as a pale yellow oil. R_f 0.38 (10% EtOAc/petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 5.80 (ddt, *J* = 17.0, 10.0, 7.0 Hz, 1H, $H_2CC\mathbf{H}$), 5.48 (dtt, *J* = 11.0, 7.0, 1.5 Hz, 1H, $CH_2CH_2C\mathbf{H}CH$), 5.36 – 5.27 (m, 1H, $CH_2CH_2CHC\mathbf{H}$), 5.14 (dq, *J* = 17.0, 1.5 Hz, 1H, $\mathbf{H}H'CCH$), 5.07 (ddd, *J* = 10.0, 2.0, 1.0 Hz, 1H, $H\mathbf{H'}CCH$), 4.35 (d, *J* = 2.5 Hz, 1H, $H_2CCHCH_2CHC\mathbf{H}$), 4.25 (d, *J* = 2.5 Hz, 1H, $CHH'C\mathbf{H}$), 3.84 (dt, *J* = 8.5, 6.0 Hz, 2H, $H_2C{=}CHCH_2C\mathbf{H}$, $CHCHCH_2C\mathbf{H}$), 2.46 – 2.29 (m, 2H, $H_2CCHC\mathbf{H_2}$), 2.19 – 2.06 (m, 1H, $CHCHC\mathbf{H}H'CH$), 2.06 – 1.96

(m, 3H, $CHCHCH\mathbf{H'}CH$, $CH_2C\mathbf{H_2}CHCH$), 1.93 (dd, *J* = 10.0, 2.5 Hz, 1H, $C\mathbf{H}H'CH$), 1.86 (dd, *J* = 10.0, 2.5 Hz, 1H, $CH\mathbf{H'}CH$), 1.40 – 1.19 (m, 6H, $CH_3C\mathbf{H_2}$, $CH_3CH_2C\mathbf{H_2}$, $C\mathbf{H_2}CH_2CHCH$), 0.88 (t, *J* = 7.0 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.5 ($H_2C\mathbf{C}H$), 132.8 ($CH_2CH_2\mathbf{C}HCH$), 123.6 ($CH_2CH_2CH\mathbf{C}H$), 117.3 ($H_2\mathbf{C}CH$), 84.1 ($H_2CCHCH_2\mathbf{C}H$), 82.2 ($CHCHCH_2\mathbf{C}H$), 79.2 ($H_2CCHCH_2CH\mathbf{C}H$, 77.0 ($CHH'\mathbf{C}H$), 35.3 ($H_2CCH\mathbf{C}H_2$), 34.6 ($\mathbf{C}HH'CH$), 32.1 ($CHCH\mathbf{C}H_2CH$), 31.5 ($\mathbf{C}H_2CH_2CHCH$), 29.2 ($CH_3CH_2\mathbf{C}H_2$), 27.4 ($CH_2\mathbf{C}H_2CHCH$), 22.6 ($CH_3\mathbf{C}H_2$), 14.1 ($\mathbf{C}H_3CH_2$); LRMS *m/z* (ESI^+) 251.0 ($M+H^+$, 100%); HRMS *m/z* (ESI^+): found 251.2007, $C_{16}H_{27}O_2$ ($M+H^+$) requires 251.2006; $[\alpha]_D^{25}$= 79.6 (c = 0.1, $CHCl_3$); IR (ν_{max} cm^{-1}): 3002, 2958, 2926, 2858, 2117, 1642, 1456, 1365.

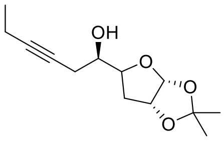
(3*R*,6*S*)-3-Allyl-6-((*Z*)-oct-2-en-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (4.39)



Sodium hydride (60% in mineral oil, 0.031 g, 0.77 mmol, 7.0 equiv.) was added portionwise to a stirred solution of (2*R*,3*R*,5*S*)-2-Allyl-5-((*R*,*Z*)-1-hydroxynon-3-en-1-yl)tetrahydrofuran-3-yl methanesulfonate **4.37** (0.038 g, 0.11 mmol, 1.0 equiv.) in DMF (1.5 mL) at 0 °C and the reaction mixture was stirred for 20 mins at this temperature and then at rt for a further 18 h. After this time, the reaction was quenched by the addition of H_2O (4 mL) and the organics were extracted with petroleum ether 40-60 (4 × 5 mL), the combined organic phase was washed with a sat. brine solution (10 mL), dried (Na_2SO_4), filtered and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (5% EtOAc/petroleum ether 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound (0.021 g, 0.084 mmol, 76%) as a pale yellow oil. R_f 0.44 (10% EtOAc/ petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 5.77 (ddt, *J* = 17.0, 10.5, 7.0 Hz, 1H, $H_2CC\mathbf{H}$), 5.56 – 5.46 (m, 1H, $CH_2CH_2C\mathbf{H}CH$), 5.38 – 5.28 (m, 1H, $CH_2CH_2CHC\mathbf{H}$), 5.14 – 5.06 (m, 2H, **H_2**CCH), 4.30 (dd, *J* = 5.5, 2.0 Hz, 2H, H_2CCHCH$_2$CHC**H**, CHH'C**H**), 3.91 (t, *J* = 7.0 Hz, 1H, H_2C=CHCH$_2$C**H**), 3.86 (dd, *J* = 8.0, 6.5 Hz, 1H, CHCHCH$_2$C**H**), 2.20 – 2.08 (m, 2H, H_2CCHC**H**H', CHCHC**H**H'), 2.07 – 1.96 (m, 4H, H_2CCHCH**H'**, CHCHCH**H'**, CH$_2$C**H$_2$**CHCH), 1.79 – 1.70 (m, 2H, C**H**H'CH, CH**H'**CH), 1.42 – 1.21 (m, 6H, CH$_3$C**H$_2$**, CH$_3$CH$_2$C**H$_2$**, C**H$_2$**CH$_2$CHCH), 0.90 (t, *J* = 7.0 Hz, 3H, C**H$_3$**CH$_2$); ^{13}C NMR (126 MHz, CDCl$_3$) δ 133.5 (H_2C**C**H), 132.8 (CH$_2$CH$_2$**C**HCH), 123.6 (CH$_2$CH$_2$CH**C**H), 117.3 (H_2**C**CH), 83.1 (H_2CCHCH$_2$**C**H), 82.5 (CHCHCH$_2$**C**H), 78.6 (H_2CCHCH$_2$CH**C**H), 78.5 (CHH'**C**H), 38.31 (H_2CCH**C**H$_2$), 31.9 (CHCH**C**H$_2$), 31.5 (**C**H$_2$CH$_2$CHCH), 30.7 (**C**HH'CH), 29.2 (CH$_3$CH$_2$**C**H$_2$), 27.4 (CH$_2$**C**H$_2$CHCH), 22.5 (CH$_3$**C**H$_2$), 14.1 (**C**H$_3$CH$_2$); LRMS *m/z* (ESI$^+$) 251.1 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 251.2010, $C_{16}H_{27}O_2$ (M+H$^+$) requires 251.2006; $[\alpha]_D^{25}$= 110.0 (c = 0.1, CHCl$_3$); IR (ν_{max} cm^{-1}): 2958, 2929, 2250, 1641, 1456.

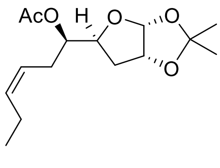
8.4. Experimental Procedures for Chapter 5

(1*R*)-1-((3a*R*,6a*R*)-2,2-Dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-yn-1-ol (5.1)



According to the modified procedure of Das et al.:[234] butyne (2.7 g, 50 mmol, 1.9 equiv.) was quickly charged to an ice-cooled flask from the vessel stored in the freezer and then cooled to -78 °C before slow addition of THF (110 ml). *n*-BuLi (2.5 M in hexanes, 26 mL, 50 mmol, 1.9 equiv.) was added dropwise and then the reaction mixture was warmed to 0 °C and stirred for 30 mins. After this time, the reaction mixture was cooled to -78 °C and boron trifluoride diethyl etherate (6.0 mL, 20 mmol, 0.75 equiv.) was added followed by (3a*R*,5*S*,6a*R*)-2,2-dimethyl-5-((*R*)-oxiran-2-yl)tetrahydrofuro[2,3-*d*][1,3]dioxole **3.117** (5.5 g, 26.3 mmol, 1.0 equiv.) in THF (20 mL) and the reaction mixture was stirred at this temperature for 1 h. The reaction mixture was quenched at -78 °C *via* addition of sat. NH$_4$Cl solution and then warmed to rt, the organics were extracted with EtOAc (3 × 75 mL) and then combined, dried ($MgSO_4$) and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) to give the title compound (5.4 g, 23 mmol, 86%) as a pale yellow oil that crystallises upon prolonged standing. R_f 0.39 (30% EtOAc/petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.81 (d, *J* = 3.5 Hz, 1H, OC**H**O), 4.75 (t, *J* = 4.5 Hz, 1H, OC**H**CHH'), 4.31 (dt, *J* = 10.0, 4.5 Hz, 1H, CHH'C**H**), 3.93 (td, *J* = 6.5, 4.5 Hz, 1H, CH_2C**H**OH), 2.45 – 2.32 (m, 2H, C**H_2**CHOH), 2.20 – 2.13 (m, 2H, CH_3C**H_2**), 2.08 (dd, *J* = 13.5, 4.5 Hz, 1H, C**H**H'CH), 1.85 (ddd, *J* = 13.5, 10.5, 5.0 Hz, 1H, CHOCH**H'**CH), 1.69 – 1.53 (m, 1H, br, O**H**), 1.52 (s, 3H, C**H_3**), 1.33 (s, 3H, C**H_3**), 1.12 (td, *J* = 7.5, 1.0 Hz, 3H, C**H_3**CH_2); ^{13}C NMR (151 MHz, $CDCl_3$) δ 111.3 (**C**$(CH_3)_2$), 105.4 (O**C**HO), 84.6 (CH_3CH_2**C**), 80.6 (O**C**HCHH'), 79.9 (CHH'**C**H), 74.4 (**C**CH_2CHOH), 69.9 (**C**HOH), 32.7 (OCH**C**HH'), 26.8 (**C**H_3), 26.2 (**C**H_3), 23.7 (C**C**H_2CHOH), 14.1 (**C**H_3CH_2), 12.4 (CH_3**C**H_2); LRMS *m/z* (ESI$^+$) 263.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 263.1253, $C_{13}H_{20}O_4Na$ (M+Na$^+$) requires 263.1254; $[\alpha]_D^{25}$= -30.0 (c = 0.14, $CHCl_3$); IR (ν_{max}): 3474, 2979, 2937, 2159.

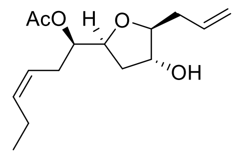
(*R*,*Z*)-1-((3a*R*,5*S*,6a*R*)-2,2-Dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-en-1-yl acetate (3.113)



Acetic anhydride (6.3 mL, 70 mmol, 3.0 equiv.) was added to (*R*,*Z*)-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-en-1-ol **3.114** (5.4 g, 22 mmol, 1.0 equiv.) and 4-(dimethylamino)pyridine (8.1 g, 70 mmol, 3.0 equiv.) in CH_2Cl_2 (110 mL) and the reaction mixture was stirred for 2 h. The reaction mixture was quenched with sat. $NaHCO_3$ (110 mL) and then extracted with CH_2Cl_2 (3 × 50 mL), the combined organic layers were dried

($MgSO_4$), filtered, and concentrated *in vacuo* to give a crude residue which was purified further by flash column chromatography (5% EtOAc/ petroleum ether 40-60 → 15% EtOAc/ petroleum ether 40-60) giving the title compound (5.9 g, 21 mmol, 93%) as a colourless oil. R_f 0.17 (10% EtOAc/petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.79 (d, *J* = 3.5 Hz, 1H, OC**H**O), 5.49 (dtt, *J* = 11.0, 7.5, 1.5 Hz, 1H, $CH_3CH_2C\mathbf{H}CH$), 5.30 (dtt, *J* = 10.5, 7.5, 1.5 Hz, 1H, $CH_3CH_2CHC\mathbf{H}$), 5.05 (dt, *J* = 7.0, 5.5 Hz, 1H, C**H**OCO), 4.75 – 4.69 (m, 1H, OC**H**CHH'), 4.25 (dt, *J* = 10.0, 5.0 Hz, 1H, CHH'C**H**O), 2.37 (ddt, *J* = 9.0, 7.5, 1.5 Hz, 2H, $C\mathbf{H_2}CHOCO$), 2.17 – 1.98 (m, 6H, $CH_3C\mathbf{H_2}CHCH$, $COC\mathbf{H_3}$, OCHC**H**H'), 1.77 (ddd, *J* = 13.5, 10.5, 5.0 Hz, 1H, OCHCH**H'**), 1.50 (s, 3H, $C\mathbf{H_3}$), 1.31 (s, 3H, $C\mathbf{H_3}$), 0.96 (t, *J* = 7.5 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.3 (**C**=O), 134.9 ($CH_3CH_2\mathbf{C}HCH$), 122.9 ($CH_3CH_2CH\mathbf{C}H$), 111.3 ($\mathbf{C}(CH_3)_2$), 105.5 (O**C**HO), 80.2 (O**C**HCHH'), 78.38 (CHH'**C**HO), 73.4 (**C**HOCO), 34.7 (OCH**C**HH'), 29.1 ($\mathbf{C}H_2CHOCO$), 26.8 ($\mathbf{C}H_3$), 26.2 ($\mathbf{C}H_3$), 21.1 ($O\mathbf{C}H_3$), 20.6 ($CH_3\mathbf{C}H_2$), 14.1 ($\mathbf{C}H_3CH_2$); LRMS *m/z* (ESI$^+$) 307.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 307.1516, $C_{15}H_{24}O_5Na$ (M+Na$^+$) requires 307.1516; $[\alpha]_D^{25}$= -15.7 (c = 0.14, $CHCl_3$); IR (ν_{max}): 2963, 1743, 1373.

***(R,Z)*-1-([2*S*,4*R*,5*S*]-5-Allyl-4-hydroxytetrahydrofuran-2-yl)hex-3-en-1-yl acetate (3.112)**



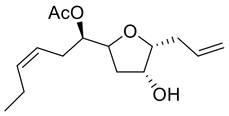
Tetrafluoroboric acid-diethyl ether complex (48% HBF_4, 1 M in CH_2Cl_2, 10 mL, 10 mmol, 0.50 equiv.) was added to a stirred solution of (*R*,*Z*)-1-((3a*R*,5*S*,6a*R*)-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-en-1-yl acetate **3.113** (6.0 g, 21 mmol, 1.0 equiv.) and allyltrimethylsilane (23 mL, 150 mmol, 7 equiv.) in CH_2Cl_2 (210 mL). The reaction mixture was stirred at rt for 15 mins and then heated to reflux for a further 4 h. After this time, the reaction mixture was cooled to rt and then quenched *via* the addition of sat.

$NaHCO_3$ solution (200 mL) and stirred vigorously until effervescence ceased. The layers were separated and the organics further extracted with CH_2Cl_2 (3 × 75 mL), the combined organic layer was then dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/petroleum ether 40-60 → 30% EtOAc/petroleum ether 40-60) giving the title compound (3.1 g, 12 mmol, 56%) as a colourless oil and as a >5:1 ratio of epimers. R_f 0.20 (30% EtOAc/petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.86 – 5.75 (m, 1H, $H_2CC\mathbf{H}$), 5.48 (dddt, *J* = 11.0, 9.0, 5.0, 1.5 Hz, 1H, $CH_3CH_2C\mathbf{H}CH$), 5.34 – 5.24 (m, 1H, $CH_3CH_2CHC\mathbf{H}$), 5.13 – 5.05 (m, 2H, $\mathbf{H_2}CCH$), 5.02 (dt, *J* = 7.5, 5.0 Hz, 1H, C**H**OCO), 4.19 (ddd, *J* = 9.0, 6.5, 5.0 Hz, 1H, CHH'C**H**), 4.11 (dt, *J* = 6.0, 3.0 Hz, 1H, C**H**OH), 3.80 (td, *J* = 6.5, 3.0 Hz, 1H, $H_2CCHCH_2C\mathbf{H}$), 2.42 – 2.24 (m, 3H, $H_2CCHC\mathbf{H}H'$, $C\mathbf{H_2}CHOCO$), 2.25 – 2.17 (m, 1H, $H_2CCHCH\mathbf{H'}$), 2.08 – 1.96 (m, 7H, O**H**, $COC\mathbf{H_3}$, $CH_3C\mathbf{H_2}$, HOCHC**H**H'), 1.87 (ddd, *J* = 13.0, 6.5, 3.0 Hz, 1H, HOCH**H'**), 0.95 (t, *J* = 7.5 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4 (**C**=O), 134.7 ($CH_3CH_2\mathbf{C}HCH$), 134.0 ($H_2C\mathbf{C}H$), 123.1 ($CH_3CH_2CH\mathbf{C}H$), 117.6 ($H_2\mathbf{C}CH$), 85.7 ($H_2CCHCH_2\mathbf{C}H$), 78.5 (CHH'**C**H), 75.2 (**C**HOH), 74.2 (**C**HOCO), 38.5 ($H_2CCH\mathbf{C}H_2$), 35.8 (HOCH**C**HH'), 28.9 ($\mathbf{C}H_2CHOCO$), 21.2 ($CO\mathbf{C}H_3$), 20.6 ($CH_3\mathbf{C}H_2$), 14.2 ($\mathbf{C}H_3CH_2$); LRMS *m/z* (ESI$^+$) 291.1 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 291.1566, $C_{15}H_{24}O_4Na$ (M+Na$^+$) requires 291.1567; IR (ν_{max}): 3439, 2963, 1742, 1642, 1437, 1373.

OR

Allyltrimethylsilane (0.65 mL, 5.7 mmol, 5.0 equiv.) and boron trifluoride etherate (0.31 mL, 1.92 mmol, 2.0 equiv.) were added to a solution of *(R,Z)*-1-([3a*R*,5*S*,6a*R*]-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)hex-3-en-1-yl acetate **3.113** (0.24 g, 0.85 mmol, 1.0 equiv.) in CH_2Cl_2 (15 mL) at 0 °C. The reaction was allowed to warm to room temperature and stirred for 18 h. then quenched with $NaHCO_3$ (15 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic layers were combined, dried ($MgSO_4$), filtered and concentrated *in vacuo* giving the crude product which was further purified with column chromatography (30% EtOAc/ petroleum ether 40-60 → 50% EtOAc/ petroleum ether 40-60) giving the title compound as an inseparable mixture of epimers (2:1) as a colourless oil (0.10 g, 41%). Data for major isomer as above, for minor see below.

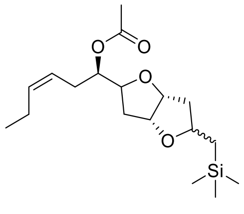
(1*R*,*Z*)-1-((4*R*,5*R*)-5-Allyl-4-hydroxytetrahydrofuran-2-yl)hex-3-en-1-yl acetate (5.2)



Tetrafluoroboric acid-diethyl ether complex (48% HBF_4, 0.26 mL, 0.78 mmol, 3.0 equiv.) was added to a stirred solution of (1*R*,*Z*)-1-((3a*R*,6a*R*)-5-(trimethylsilyl)hexahydrofuro[3,2-b]furan-2-yl)hex-3-en-1-yl acetate **5.3** (0.088 g, 0.26 mmol, 1.0 equiv.) in CH_2Cl_2 (2.6 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the reaction mixture was quenched *via* addition of sat. $NaHCO_3$ solution and stirred vigorously until effervescence ceased. The layers were then separated and the aqueous phase further extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layer was dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60 → 30% EtOAc/petroleum ether 40-60) to give the title compound (0.049 g, 0.18 mmol, 70%) as a colourless oil. R_f 0.26 (30% EtOAc/petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 5.84 (dddd, *J* = 17.5, 10.5, 7.5, 6.5 Hz, 1H, $H_2CC\mathbf{H}$), 5.48 (dtt, *J* = 11.0, 7.0, 1.5 Hz, 1H, $CH_3CH_2C\mathbf{H}CH$), 5.30 (dtt, *J* = 11.0, 7.5, 1.5 Hz, 1H, $CH_3CH_2CHC\mathbf{H}$), 5.17 (dq, *J* = 17.0, 1.5 Hz, 1H, $\mathbf{H}_2CCH$), 5.09 (dq, *J* = 10.0, 1.5 Hz, 1H, $\mathbf{H}_2CCH$), 5.01 (dt, *J* = 7.5, 5.5 Hz, 1H, C**H**OCO), 4.33 – 4.25 (m, 2H, C**H**OH, CHH’C**H**), 3.86 (ddd, *J* = 7.5, 6.5, 3.0 Hz, 1H,

$\mathrm{H_2C{=}CHCH_2C\mathbf{H}}$), 2.47 (dtt, *J* = 14.5, 6.5, 1.5 Hz, 1H, $\mathrm{H_2CCHC\mathbf{H}H'}$), 2.42 – 2.28 (m, 4H, $\mathrm{H_2CCHCH\mathbf{H'}}$, $\mathrm{O\mathbf{H}}$, $\mathrm{C\mathbf{H_2}CHOCO}$), 2.11 – 1.98 (m, 7H, $\mathrm{CH_3C\mathbf{H_2}}$, $\mathrm{HOCHC\mathbf{H}H'}$, $\mathrm{HOCHCH\mathbf{H'}}$, $\mathrm{OC\mathbf{H_3}}$), 0.96 (t, *J* = 7.5 Hz, 3H, $\mathrm{C\mathbf{H_3}CH_2}$); ^{13}C NMR (126 MHz, $\mathrm{CDCl_3}$) δ 170.5 ($\mathrm{\mathbf{C}{=}O}$), 134.7 ($\mathrm{CH_3CH_2\mathbf{C}HCH}$), 134.5 ($\mathrm{H_2C\mathbf{C}H}$), 123.1 ($\mathrm{CH_3CH_2CH\mathbf{C}H}$), 117.2 ($\mathrm{H_2\mathbf{C}CH}$), 82.3 ($\mathrm{H_2CCHCH_2\mathbf{C}H}$), 77.7 ($\mathrm{\mathbf{C}HOH}$), 74.9 ($\mathrm{\mathbf{C}HOCO}$), 73.0 ($\mathrm{CHH'\mathbf{C}H}$), 36.9 ($\mathrm{HOCH\mathbf{C}HH'}$), 33.7 ($\mathrm{H_2CCH\mathbf{C}H_2}$), 28.9 ($\mathrm{\mathbf{C}H_2CHOCO}$), 21.2 ($\mathrm{CO\mathbf{C}H_3}$), 20.60 ($\mathrm{CH_3\mathbf{C}H_2}$), 14.17 ($\mathrm{\mathbf{C}H_3CH_2}$); LRMS *m/z* (ESI$^+$) 291.1 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 291.1566, $\mathrm{C_{15}H_{24}O_4Na}$ (M+Na$^+$) requires 291.1567; $[\alpha]_D^{25}$= -47.6 (c = 0.042, $\mathrm{CHCl_3}$); IR (ν_{max}): 3439, 2963, 1742, 1642, 1437, 1373.

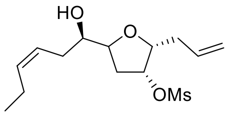
(1*R*,*Z*)-1-((3a*R*,6a*R*)-5-(Trimethylsilyl)hexahydrofuro[3,2-b]furan-2-yl)hex-3-en-1-yl acetate (5.3)



This compound was isolated as the major by-product of the Sakurai reaction conducted at 1.27 mmol in starting material under the tetrafluoroboric acid-diethyl ether complex conditions. Subjecting the solvent front from the initial purification to a second purification step by flash column chromatography (10% EtOAc/petroleum ether 40-60 → 15% EtOAc/petroleum ether 40-60) gave the title compound (0.093 g, 0.27 mmol, 21%) as a colourless oil and as an inseparable 1:1 mixture of epimers. R_f 0.36 (15% EtOAc/petroleum ether 40-60); ^{1}H NMR (500 MHz, $\mathrm{CDCl_3}$) δ 5.55 – 5.46 (m, 1H), 5.32 (dtdd, *J* = 10.5, 7.0, 5.0, 1.5 Hz, 1H), 5.07 (dt, *J* = 7.5, 5.0 Hz, 1H), 5.01 (dt, *J* = 7.5, 5.0 Hz, 1H), 4.71 (dt, *J* = 9.0, 4.5 Hz, 1H), 4.67 (t, *J* = 4.5 Hz, 1H), 4.37 (t, *J* = 5.0 Hz, 1H), 4.20 – 4.09 (m, 2H), 3.84 (tt, *J* = 9.5, 5.5 Hz, 1H), 2.43 – 2.26 (m, 3H), 2.23 – 2.13 (m, 1H), 2.12 – 2.03 (m, 7H), 2.01 – 1.86 (m, 1H), 1.57 – 1.42

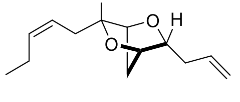
(m, 1H), 1.20 – 1.03 (m, 2H), 0.98 (t, *J* = 7.5 Hz, 3H), 0.96 – 0.87 (m, 1H), 0.79 (dd, *J* = 14.0, 9.0 Hz, 1H), 0.05 (d, *J* = 3.0 Hz, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.5, 170.5, 134.7, 134.7, 123.2, 123.1, 85.0, 84.8, 82.4, 82.4, 80.6, 78.2, 77.9, 77.6, 74.3, 73.8, 43.5, 42.5, 36.4, 34.7, 29.0, 28.8, 24.3, 23.9, 21.2, 21.2, 20.6, 14.2, -0.92, -0.93; LRMS *m/z* (ESI$^+$) 363.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 363.1964, $C_{18}H_{32}O_4SiNa$ (M+Na$^+$) requires 363.1962; IR (ν_{max}): 2956, 1742, 1371. Assignment of the NMR spectra was not possible due to complex overlapping resonances of the epimers, the compound was instead opened to the minor diastereoisomer **5.2** of the Sakurai product for characterisation.

(2*R*,3*R*)-2-Allyl-5-((*R*,*Z*)-1-hydroxyhex-3-en-1-yl)tetrahydrofuran-3-yl methanesulfonate (5.7)



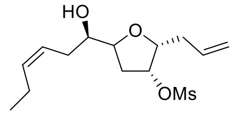
Triethylamine (0.28 mL, 0.2 mmol, 1.1 equiv.) and methanesulfonyl chloride (0.16 mL, 0.2 mmol, 1.1 equiv.) were added to a stirred solution of *(R,Z)*-1-((2*S*,4*R*,5*S*)-5-allyl-4-hydroxytetrahydrofuran-2-yl)hex-3-en-1-yl acetate **5.2** (0.048 g, 0.18 mmol, 1.0 equiv.) and in CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1 h. After this time, the reaction was quenched *via* the addition of sat. $NaHCO_3$ solution and the layers separated. The aqueous layer was further extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic layer was dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give the crude mesylate which was further dried under reduced pressure. The crude mesylate was redissolved in MeOH/H_2O (7:1, 2 mL) and K_2CO_3 (0.49 g, 0.36 mmol, 2.0 equiv.) was added, the reaction mixture was stirred at rt for 3 h. After this time, the reaction mixture was diluted with water (100 mL) and the organics were extracted with EtOAc (3 × 75 mL), the combined organic layer was dried ($MgSO_4$), filtered and the solvent removed to give a crude residue that was further purified by flash column chromatography (15% acetone/petroleum ether 40-60 → 25% acetone petroleum ether 40-60) to give the title compound (0.052 g, 0.17 mmol, 95%) as a pale yellow oil. R_f 0.11 (15% acetone/petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 5.86 (ddt, *J* = 17.0, 10.5, 7.0 Hz, 1H, $H_2CC\mathbf{H}$), 5.58 (dtt, *J* = 11.0, 7.0, 1.5 Hz, 1H, $CH_3CH_2C\mathbf{H}CH$), 5.38 (dtt, *J* = 10.5, 7.5, 1.5 Hz, 1H, $CH_3CH_2CHC\mathbf{H}$), 5.26 – 5.23 (m, 1H, C**H**OMs), 5.20 (dq, *J* = 17.0, 1.5 Hz, 1H, $\mathbf{H}_2CCH$), 5.14 (dq, *J* = 10.0, 1.5 Hz, 1H, $\mathbf{H}_2CCH$), 4.25 (ddd, *J* = 9.5, 6.5, 4.0 Hz, 1H, CHH'C**H**), 4.13 (td, *J* = 7.0, 3.0 Hz, 1H, $H_2C{=}CHCH_2C\mathbf{H}$), 3.90 (ddd, *J* = 7.5, 6.0, 3.5 Hz, 1H, C**H**OH), 3.08 (s, 3H, $SC\mathbf{H_3}$), 2.54 – 2.27 (m, 5H, O**H**, MsOCHC**H**H', MsOCHCH**H'**, $H_2CCHC\mathbf{H_2}$), 2.20 (tt, *J* = 6.0, 2.0 Hz, 2H, $C\mathbf{H_2}CHOH$), 2.08 (pd, *J* = 7.5, 1.5 Hz, 2H, $CH_3C\mathbf{H_2}$), 0.99 (t, *J* = 7.5 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 135.2 ($CH_3CH_2\mathbf{C}HCH$), 133.7 ($H_2C\mathbf{C}H$), 123.6 ($CH_3CH_2CH\mathbf{C}H$), 117.8 ($H_2\mathbf{C}CH$), 82.1 (**C**HOMs), 81.4 ($H_2C{=}CHCH_2\mathbf{C}H$), 80.0 (MsO**C**HCHH'), 71.8 (**C**HOH), 38.7 ($S\mathbf{C}H_3$), 34.0 (MsOCH**C**HH'), 33.1 ($H_2C{=}CH\mathbf{C}H_2$), 30.7 ($\mathbf{C}H_2CHOH$), 20.7 ($CH_3\mathbf{C}H_2$), 14.2 ($\mathbf{C}H_3CH_2$); LRMS *m/z* (ESI^+) 327.2 ($M+Na^+$, 100%); HRMS *m/z* (ESI^+): found 327.1237, $C_{14}H_{24}O_5SNa$ ($M+Na^+$) requires 327.1237; $[\alpha]_D^{25}$= -18.8 (c = 0.090, $CHCl_3$); IR (ν_{max}): 3538, 2964, 1642, 1437, 1355.

(3*R*,6*S*)-3-Allyl-6-((*Z*)-pent-2-en-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (5.8)



Sodium hydride (60% in mineral oil, 0.045 g, 1.1 mmol, 7.0 equiv.) was added portionwise to a stirred solution of (2*R*,3*R*)-2-allyl-5-((*R*,*Z*)-1-hydroxyhex-3-en-1-yl)tetrahydrofuran-3-yl methanesulfonate **5.7** (0.049 g, 0.16 mmol, 1.0 equiv.) in DMF (2 mL) at 0 °C and the reaction mixture was stirred for 20 mins at this temperature and then at rt for a further 18 h. After this time, the reaction was quenched *via* addition of H_2O (5 mL) and the organics were extracted with petroleum ether 40-60 (4 × 5 mL), the combined organic phase was washed with a sat. brine solution (15 mL), dried (Na_2SO_4), filtered and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (5% EtOAc/petroleum ether 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound (0.023 g, 0.11 mmol, 70%) as a pale yellow oil. R_f 0.43 (10% EtOAc/petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.74 (ddt, *J* = 17.0, 10.5, 7.0 Hz, 1H, $C\mathbf{H}CH_2$), 5.49 (dtt, *J* = 10.5, 7.5, 1.5 Hz, 1H, $CH_3CH_2C\mathbf{H}CH$), 5.29 (dtt, *J* = 10.5, 7.5, 1.5 Hz, 1H, $CH_3CH_2CHC\mathbf{H}CH_2$), 5.12 – 5.04 (m, 2H, $CHC\mathbf{H_2}$), 4.33 – 4.24 (m, 2H, $C\mathbf{H}CHCH_2CH{=}CH_2$, $CHCHCH_2CHC\mathbf{H}$), 3.89 (t, *J* = 7.0 Hz, 1H, $CHCHCH_2C\mathbf{H}$), 3.83 (dd, *J* = 8.0, 6.5 Hz, 1H, $C\mathbf{H}CH_2CHCH_2$), 2.11 (ddtd, *J* = 17.5, 9.0, 7.0, 1.5 Hz, 2H, $C\mathbf{H}H'CHCH_2$, $CH_3C\mathbf{H}H'CHCH$), 2.01 (dddd, *J* = 14.5, 8.5, 7.0, 1.0 Hz, 4H, $CH\mathbf{H'}CHCH_2$, $CHCHCH\mathbf{H'}$, $CH_3C\mathbf{H_2}$), 1.81 – 1.68 (m, 2H, $CHC\mathbf{H_2}CH$), 0.96 (t, *J* = 7.5 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 134.3 ($CH_3CH_2\mathbf{C}H{=}CH$), 133.5 ($\mathbf{C}H{=}CH_2$), 123.1 ($CH_3CH_2CH{=}\mathbf{C}H$), 117.3 ($CH{=}\mathbf{C}H_2$), 83.1 ($CH{=}CHCH_2\mathbf{C}H$), 82.5 ($\mathbf{C}HCH_2CH{=}CH_2$), 78.6 ($CH{=}CHCH_2CH\mathbf{C}H$), 78.5 ($\mathbf{C}HCHCH_2CH{=}CH_2$), 38.3 ($\mathbf{C}H_2CH{=}CH_2$), 31.8 ($CH{=}CH\mathbf{C}H_2$), 30.7 ($CH\mathbf{C}H_2CH$), 20.7 ($CH_3\mathbf{C}H_2$), 14.1 ($\mathbf{C}H_3CH_2$); LRMS *m/z* (ESI$^+$) 209.3 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$) found 209.1539, $C_{13}H_{21}O_2$ (M+H$^+$) requires 209.1536; $[\alpha]_D^{25}$= +127.7 (c = 0.13, $CHCl_3$); IR (ν_{max}): 2963, 1641, 1455, 1365.

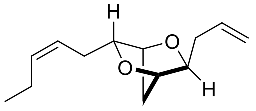
(2*R*,3*R*)-2-Allyl-5-((*R*,*Z*)-1-hydroxyhex-3-en-1-yl)tetrahydrofuran-3-yl methanesulfonate (3.111)



Triethylamine (0.28 mL, 0.2 mmol, 1.1 equiv.) and methanesulfonyl chloride (0.16 mL, 0.2 mmol, 1.1 equiv.) were added to a stirred solution of *(R,Z)*-1-((2*S*,4*R*,5*S*)-5-allyl-4-hydroxytetrahydrofuran-2-yl)hex-3-en-1-yl acetate **3.112** (0.048 g, 0.18 mmol, 1.0 equiv.) and in CH_2Cl_2 (2 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 1 h. After this time, the reaction was quenched *via* the addition of sat. $NaHCO_3$ solution and the layers separated. The aqueous layer was further extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic layer was dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give the crude mesylate which was further dried under reduced pressure. The crude mesylate was redissolved in MeOH/H_2O (7:1, 2 mL) and K_2CO_3 (0.49 g, 0.36 mmol, 2.0 equiv.) was added, the reaction mixture was stirred at rt for 3 h. After this time, the reaction mixture was diluted with water (100 mL) and the organics were extracted with EtOAc (3 × 75 mL), the combined organic layer was dried ($MgSO_4$), filtered and the solvent removed to give a crude residue that was further purified by flash column chromatography (15% acetone/petroleum ether 40-60 → 25% acetone petroleum ether 40-60) to give the title compound (0.052 g, 0.17 mmol, 95%) as a pale yellow oil. R_f 0.11 (15% acetone/petroleum ether 40-60); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.86 (ddt, *J* = 17.0, 10.5, 7.0 Hz, 1H, $H_2C=C\mathbf{H}$), 5.58 (dtt, *J* = 11.0, 7.0, 1.5 Hz, 1H, $CH_3CH_2C\mathbf{H}=CH$), 5.38 (dtt, *J* = 10.5, 7.5, 1.5 Hz, 1H, $CH_3CH_2CH=C\mathbf{H}$), 5.26 – 5.23 (m, 1H, C**H**OMs), 5.20 (dq, *J* = 17.0, 1.5 Hz, 1H, $\mathbf{H}_2C=CH$), 5.14 (dq, *J* = 10.0, 1.5 Hz, 1H, $\mathbf{H}_2C=CH$), 4.25 (ddd, *J* = 9.5, 6.5, 4.0 Hz, 1H, CHH'C**H**), 4.13 (td, *J* = 7.0, 3.0 Hz, 1H, $H_2C=CHCH_2C\mathbf{H}$), 3.90 (ddd,

J = 7.5, 6.0, 3.5 Hz, 1H, C**H**OH), 3.08 (s, 3H, SC**H₃**), 2.54 – 2.27 (m, 5H, O**H**, MsOCHC**H**H', MsOCHCH**H'**, H_2C=CHC**H₂**), 2.20 (tt, J = 6.0, 2.0 Hz, 2H, C**H₂**CHOH), 2.08 (pd, J = 7.5, 1.5 Hz, 2H, CH_3C**H₂**), 0.99 (t, J = 7.5 Hz, 3H, C**H₃**CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ 135.2 (CH_3CH_2**C**H=CH), 133.7 (H_2C=**C**H), 123.6 (CH_3CH_2CH=**C**H), 117.8 (H_2**C**=CH), 82.1 (**C**HOMs), 81.4 (H_2C=CHCH$_2$**C**H), 80.0 (CHH'**C**H), 71.8 (**C**HOH), 38.7 (S**C**H$_3$), 34.0 (MsOCH**C**HH'), 33.1 (H_2C=CH**C**H$_2$), 30.7 (**C**H$_2$CHOH), 20.7 (CH_3**C**H$_2$), 14.2 (**C**H$_3CH_2$); LRMS *m/z* (ESI$^+$) 327.2 (M+Na$^+$, 100%); HRMS *m/z* (ESI$^+$): found 327.1237, $C_{14}H_{24}O_5SNa$ (M+Na$^+$) requires 327.1237; $[\alpha]_D^{25}$= -18.8 (c = 0.090, $CHCl_3$); IR (ν_{max}): 3538, 2964, 1642, 1437, 1355.

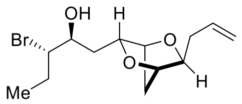
***(3S,6S)*-3-allyl-6-(*[Z]*-pent-2-en-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (3.101)**



Sodium hydride (60% in mineral oil, 2.8 g, 70 mmol, 5.0 equiv.) was added portionwise to a stirred solution of *(2S,3R,5S)*-2-allyl-5-((*R*,*Z*)-1-hydroxyhex-3-en-1-yl)tetrahydrofuran-3-yl methanesulfonate **3.111** (4.1 g, 14 mmol, 1.0 equiv.) in DMF (140 mL) at 0 °C and the reaction mixture was stirred for 20 mins at this temperature and then at rt for a further 18 h. After this time, the reaction was quenched *via* addition of H_2O (300 mL) and the organics were extracted with petroleum ether 40-60 (4 × 150 mL), the combined organic phase was washed with a sat. brine solution (200 mL), dried (Na_2SO_4), filtered and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (5% EtOAc/petroleum ether 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound (2.6 g, 13 mmol, 90%) as a pale yellow oil. R_f 0.36 (10% EtOAc/petroleum ether 40-60); ^1H NMR (500 MHz, $CDCl_3$) δ 5.80 (ddt, J = 17.0, 10.0, 7.0 Hz, 1H, C**H**=CH_2), 5.49 (dtt, J = 10.5, 7.5, 1.5 Hz, 1H, CH_3CH_2C**H**=CH), 5.29 (dtt, J = 10.5, 7.5, 1.5

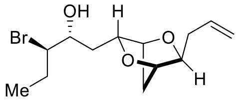
Hz, 1H, $CH_3CH_2CH{=}C\mathbf{H}CH_2$), 5.14 (dq, *J* = 17.0, 1.5 Hz, 1H, $CH{=}C\mathbf{H}_2$), 5.07 (ddt, *J* = 10.0, 2.0, 1.0 Hz, 1H, $CH{=}C\mathbf{H}_2$), 4.35 (d, *J* = 2.5 Hz, 1H, $CH{=}CHCH_2CHC\mathbf{H}$), 4.24 (d, *J* = 2.5 Hz, 1H, $C\mathbf{H}CHCH_2CH{=}CH_2$), 3.88 – 3.80 (m, 2H, $C\mathbf{H}CH_2CH{=}CH_2$, $CH{=}CHCH_2C\mathbf{H}$), 2.45 – 2.29 (m, 2H, $C\mathbf{H_2}CH{=}CH_2$), 2.17 – 2.08 (m, 1H, $CH{=}CHC\mathbf{H}H'$), 2.07 – 1.96 (m, 3H, $CH{=}CHCH\mathbf{H'}$, $CH_3C\mathbf{H_2}$), 1.93 (dd, *J* = 10.0, 2.5 Hz, 1H, $CHC\mathbf{H}_2CH$), 1.86 (dd, *J* = 10.0, 2.5 Hz, 1H, $CHC\mathbf{H}_2CH$), 0.96 (t, *J* = 7.5 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 134.5 ($CH_3CH_2\mathbf{C}H{=}CH$), 134.3 ($\mathbf{C}H{=}CH_2$), 123.1 ($CH_3CH_2CH{=}\mathbf{C}H$), 117.3 ($CH{=}\mathbf{C}H_2$), 84.1 ($CH{=}CHCH_2CH\mathbf{C}H$), 82.2 ($\mathbf{C}HCHCH_2CH{=}CH_2$), 79.2 ($CH{=}CHCH_2\mathbf{C}H$), 77.0 ($\mathbf{C}HCH_2CH{=}CH_2$), 35.3 ($\mathbf{C}H_2CH{=}CH_2$), 35.0 ($CH\mathbf{C}H_2CH$), 32.0 ($CH{=}CH\mathbf{C}H_2$), 20.7 ($CH_3\mathbf{C}H_2$), 14.1 ($C\mathbf{H_3}CH_2$); LRMS *m/z* ($ESI^+$) 209.2 ($M+H^+$, 100%); HRMS *m/z* ($ESI^+$): found 209.1537, $C_{13}H_{21}O_2$ ($M+H^+$) requires 209.1536; $[\alpha]_D^{25}$= 134.2 (c = 0.12, $CHCl_3$); IR (ν_{max}): 2963, 1640, 1453, 1367.

(2*S*,3*S*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-ol (5.13)



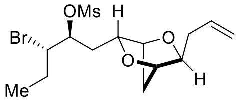
$NaHCO_3$ solution (0.5 M in H_2O, 1.55 mL) and *m*-chloroperoxybenzoic acid (0.089 g, 0.51 mmol, 1.07 equiv., do not exceed 1.1 equiv.) were added successively to a vigorously stirred solution of (3*S*,6*S*)-3-allyl-6-((*Z*)-pent-2-en-1-yl)-2,5-dioxabicyclo[2.2.1]heptane **3.101** (0.10 g, 0.48 mmol, 1.0 equiv.) in CH_2Cl_2 (4.8 mL) and the reaction mixture was stirred at rt for 2 h. After this time, the reaction mixture was diluted with CH_2Cl_2 (5 mL) and NaOH solution (1.0 M in H_2O, 5 mL) and the layers separated. The organic layer was washed with H_2O (5 mL) and then dried (Na_2SO_4), filtered and the solvent removed *in vacuo* to give a crude residue which was subjected to a quick column chromatography (15% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) to give a mixture of diastereotopic epoxides (0.086 g, 0.38 mmol, 80%) of the internal olefin (as judged by disappearance of ^{1}H NMR resonances corresponding to internal alkene peaks) which were immediately subjected to the next step without further characterisation. The epoxide mixture (0.086 g, 0.38 mmol, 1.0 equiv.) was dissolved in Et_2O (7.6 mL) and then magnesium bromide diethyl etherate (0.20 g, 0.76 mmol, 2.0 equiv.) was added and the reaction mixture stirred vigorously at rt for 2 h. After this time, the reaction mixture was diluted with Et_2O (5 mL) and washed with a half saturated (v/v) solution $NaHCO_3$ (20 mL) and then brine (20 mL). The organic layer was then dried (Na_2SO_4) and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% Et_2O/ CH_2Cl_2 → 10% Et_2O/ CH_2Cl_2) to give the title compound (0.031 g, 0.10 mmol, 27% from epoxide) as a colourless oil which was also accompanied by formation of the diastereomeric bromohydrin (0.072 g, 0.24 mmol, 62%, from epoxide, see below for data) giving a combined yield over two steps of 75%. Data for the title compound follows. R_f 0.42 (30% EtOAc/ CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.81 (ddt, *J* = 16.5, 9.5, 7.0 Hz, 1H, $CH_2{=}C\mathbf{H}$), 5.20 – 5.10 (m, 2H, $C\mathbf{H_2}{=}CH$), 4.41 (d, *J* = 2.5 Hz, 1H, $CH_2{=}CHCH_2CHC\mathbf{H}$), 4.28 (d, *J* = 2.5 Hz, 1H, $C\mathbf{H}CHCH_2CHOH$), 4.12 (ddd, *J* = 10.0, 4.0, 2.0 Hz, 1H, $C\mathbf{H}OH$), 3.95 (ddq, *J* = 9.5, 5.0, 2.5 Hz, 1H, $C\mathbf{H}Br$), 3.90 – 3.87 (m, 2H, $C\mathbf{H}CH_2CHOH$, $CH_2{=}CHCH_2C\mathbf{H}$), 3.23 (d, *J* = 4.0, Hz, 1H, $O\mathbf{H}$), 2.50 – 2.31 (m, 2H, $CH_2{=}CHC\mathbf{H_2}$), 2.08 – 1.86 (m, 4H, $C\mathbf{H_2}CH_3$, $HC\mathbf{H_2}CH$), 1.72 – 1.64 (m, 1H, $C\mathbf{H}H'CHOH$), 1.66 – 1.54 (m, 1H $CH\mathbf{H'}CHOH$), 1.10 (t, *J* = 7.5 Hz, 3H, $CH_2C\mathbf{H_3}$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 134.2 ($CH_2{=}\mathbf{C}H$), 117.5 ($\mathbf{C}H_2{=}CH$), 83.7 ($\mathbf{C}HOH$), 82.3 ($CH_2{=}CHCH_2\mathbf{C}H$), 79.8 ($CH_2{=}CHCH_2CH\mathbf{C}H$), 77.4 ($CHCH_2\mathbf{C}H$), 72.5 ($\mathbf{C}HCH_2CHOH$), 64.4 ($\mathbf{C}HBr$), 38.1 ($\mathbf{C}H_2CHOH$), 35.1 ($CH\mathbf{C}H_2CH$), 35.1 ($CH_2{=}CH\mathbf{C}H_2$), 28.2 ($\mathbf{C}H_2CH_3$), 12.6 ($CH_2\mathbf{C}H_3$); LRMS *m/z* (ESI$^+$) 345.0 (M+MeCN$^+$, 50%), 347.0 (M+MeCN$^+$, 50%); HRMS *m/z* (ESI$^+$): found 305.0748 and 307.0227, $C_{13}H_{22}{}^{79}BrO_3$ (M+H$^+$) requires 305.0747 and $C_{13}H_{22}{}^{81}BrO_3Na$ (M+H$^+$) requires 305.0726; $[\alpha]_D^{25}$= 55.7 (c = 0.070, $CHCl_3$); IR (ν_{max} cm^{-1}): 3432, 3077, 2977, 2879, 1653, 1457.

(2*R*,3*R*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-ol (3.109)



The title compound (0.072 g, 0.24 mmol, 62%, from epoxide) was isolated as a colourless oil as the second column fraction from the above procedure. R*f* 0.35 (30% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, CDCl$_3$) δ 5.82 (ddt, *J* = 17.5, 10.5, 7.0 Hz, 1H, CH$_2$=C**H**, 5.16 (dq, *J* = 17.5, 1.5 Hz, 1H, C**H**H'=CH), 5.09 (ddt, *J* = 10.0, 2.0, 1.0 Hz, 1H, CH**H'**=CH), 4.36 (dd, *J* = 2.0, 1.0 Hz, 2H, CH$_2$=CHCH$_2$CHC**H**, C**H**CHCH$_2$CHOH), 4.14 – 4.06 (m, 1H, C**H**CH$_2$CHOH), 3.99 (ddd, *J* = 8.5, 5.0, 3.5 Hz, 1H, C**H**Br), 3.87 (td, *J* = 7.0, 1.0 Hz, 1H, CH$_2$=CHCH$_2$C**H**), 3.76 (dddd, *J* = 9.5, 8.0, 3.5, 2.5 Hz, 1H, C**H**OH), 2.44 (dtt, *J* = 14.0, 7.0, 1.5 Hz, 1H, CH$_2$=CHC**H**H'), 2.36 (dtt, *J* = 14.0, 7.0, 1.5 Hz, 1H, CH$_2$=CHCH**H'**), 2.10 (d, *J* = 8.0 Hz, 1H, O**H**), 2.04 – 1.85 (m, 4H, C**H$_2$**CH$_3$, CHC**H$_2$**CH), 1.62 (ddd, *J* = 14.5, 9.5, 5.0 Hz, 1H, C**H**H'CHOH), 1.44 (ddd, *J* = 14.0, 8.5, 3.0 Hz, 1H, CH**H'**CHOH), 1.09 (t, *J* = 7.5 Hz, 3H, CH$_2$C**H$_3$**); ^{13}C NMR (151 MHz, CDCl$_3$) δ 134.4 (CH$_2$=**C**H), 117.3 (**C**H$_2$=CH), 82.22 (CH$_2$=CHCH$_2$**C**H), 81.5 (**C**HCH$_2$CHOH), 80.2 (CH$_2$=CHCH$_2$CH**C**H), 77.0 (**C**HCHCH$_2$CHOH), 70.2 (**C**HOH), 67.4 (**C**HBr), 40.0 (**C**H$_2$CHOH), 35.2 (CH$_2$=CH**C**H$_2$), 34.8 (CH**C**H$_2$CH), 29.0 (**C**H$_2$CH$_3$), 12.5 (CH$_2$**C**H$_3$); LRMS *m/z* (ESI$^+$) 345.0 (M+MeCN$^+$, 50%), 347.0 (M+MeCN$^+$, 50%); HRMS *m/z* (ESI$^+$): found 327.0568 and 329.0547, $C_{13}H_{21}{}^{79}BrO_3Na$ (M+Na$^+$) requires 327.0566 and $C_{13}H_{21}{}^{81}BrO_3Na$ (M+Na$^+$) requires 329.0546; $[\alpha]_D^{25}$= 70.7 (c = 0.14, CHCl$_3$); IR (ν_{max} cm^{-1}): 3427, 3077, 2968, 2879, 1642, 1455.

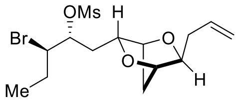
(2*S*,3*S*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl methanesulfonate (5.9)



Triethylamine (0.025 mL, 0.18 mmol, 1.8 equiv.) and methanesulfonyl chloride (0.012 mL, 0.15 mmol, 1.5 equiv.) were added successively to a stirred of solution of (2*S*,3*S*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-ol **5.13** (0.030 g, 0.10 mmol, 1.0 equiv.) in CH_2Cl_2 (1 mL) at 0 °C, the reaction mixture was then warmed to rt and stirred for 1 h. After this time, the reaction mixture was quenched *via* the addition of sat. $NaHCO_3$ solution and the layers separated. The aqueous layer was further extracted with CH_2Cl_2 (3 × 2 mL) and the combined organic layer was dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) to give the title compound (0.037 g, 0.98 mmol, 98%) as a colourless oil. R_f 0.42 (5% Et_2O/ CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ 5.80 (ddt, *J* = 17.0, 10.0, 7.0 Hz, 1H, $H_2C=C\mathbf{H}$), 5.19 – 5.07 (m, 2H, $\mathbf{H_2}C=CH$), 4.89 (td, *J* = 6.5, 3.5 Hz, 1H, $C\mathbf{H}OS$), 4.39 – 4.36 (m, 1H, $H_2C=CHCH_2CHC\mathbf{H}$), 4.31 (dd, *J* = 2.0, 1.0 Hz, 1H, $C\mathbf{H}CHCH_2CHOS$), 4.15 (dt, *J* = 10.0, 3.5 Hz, 1H, $C\mathbf{H}Br$), 3.96 (dd, *J* = 9.5, 4.5 Hz, 1H, $C\mathbf{H}CH_2CHOS$), 3.88 (td, *J* = 7.0, 1.0 Hz, 1H, $H_2C=CHCH_2C\mathbf{H}$), 3.12 (s, 3H, $SC\mathbf{H_3}$), 2.42 (dtt, *J* = 14.0, 7.0, 1.5 Hz, 1H, $H_2C=CHC\mathbf{H}H'$), 2.32 (dtt, *J* = 14.0, 7.0, 1.5 Hz, 1H, $H_2C=CHCH\mathbf{H'}$), 2.12 – 1.99 (m, 2H, $CHBrC\mathbf{H}H'CH_3$, $C\mathbf{H}H'CHOS$), 1.99 – 1.90 (m, 2H, $CHC\mathbf{H_2}CH$), 1.91 – 1.85 (m, 1H, $CHBrCH\mathbf{H'}CH_3$), 1.83 – 1.74 (m, 1H, $CH\mathbf{H'}CHOS$), 1.13 (t, *J* = 7.5 Hz, 3H, $CH_2C\mathbf{H_3}$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 134.2 ($H_2C=\mathbf{C}H$), 117.4 ($H_2\mathbf{C}=CH$), 82.2 ($H_2C=CHCH_2\mathbf{C}H$), 80.5 ($\mathbf{C}HCH_2CHOS$), 80.0 ($\mathbf{C}HOSO_2$), 79.7 ($\mathbf{C}HCHCH_2CHOS$), 77.2 ($H_2C=CHCH_2CH\mathbf{C}H$), 57.5 ($\mathbf{C}HBr$), 38.8 ($S\mathbf{C}H_3$), 36.4

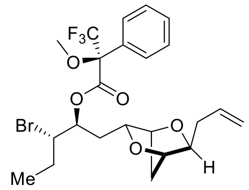
(**C**H_2CHOS), 35.2 (H_2C=CH**C**H_2), 34.9 (CH**C**H_2CH), 27.9 (**C**$H_2$$CH_3$), 12.4 ($CH_2$**C**$H_3$); LRMS *m/z* ($ESI^+$) not found (compound unstable); HRMS *m/z* ($APCI^+$): found 383.0522 and 385.0499, $C_{14}H_{24}O_5{}^{79}BrS$ ($M+H^+$) requires 383.0522 and $C_{14}H_{24}O_5{}^{81}BrS$ ($M+H^+$) requires 385.0501; ; $[\alpha]_D^{25}$= 150.0 (c = 0.04, $CHCl_3$); IR (ν_{max} cm^{-1}): 2941, 2158, 2033, 1644.

(2*R*,3*R*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl methanesulfonate (3.102)



Triethylamine (0.017 mL, 0.12 mmol, 1.2 equiv.) and methanesulfonyl chloride (0.0085 mL, 0.11 mmol, 1.1 equiv.) were added successively to a stirred of solution of (2*R*,3*R*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-ol **3.109** (0.030 g, 0.10 mmol, 1.0 equiv.) in CH_2Cl_2 (1 mL) at 0 °C, the reaction mixture was then warmed to rt and stirred for 1 h. After this time, the reaction mixture was quenched *via* the addition of sat. $NaHCO_3$ solution and the layers separated. The aqueous layer was further extracted with CH_2Cl_2 (3 × 2 mL) and the combined organic layer was dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60 → 30% EtOAc/ petroleum ether 40-60) to give the title compound (0.035 g, 0.92 mmol, 92%) as a colourless oil. R_f 0.40 (30% EtOAc/ petroleum ether 40-60); ^1H NMR (600 MHz, $CDCl_3$) δ 5.79 (ddt, *J* = 17.5, 10.5, 7.0, Hz, 1H, H_2C=C**H**), 5.16 – 5.08 (m, 2H, **H_2**C=CH), 4.98 (dt, *J* = 10.0, 3.0 Hz, 1H, C**H**OS), 4.44 (d, *J* = 2.5 Hz, 1H, H_2C=CHCH$_2$CHC**H**), 4.30 (d, *J* = 2.0 Hz, 1H, C**H**CHCH$_2$CHOS), 4.21 (dt, *J* = 11.0, 3.0 Hz, 1H, C**H**Br), 3.96 (dd, *J* = 10.5, 3.5 Hz, 1H, C**H**CH$_2$CHOS), 3.90 (t, *J* = 7.5 Hz, 1H, H_2C=CHCH$_2$C**H**), 3.16 (s, 3H, SC**H$_3$**), 2.44 – 2.35 (m, 1H, H_2C=CHC**H**H’), 2.30 – 2.22 (m, 1H, H_2C=CHCH**H’**), 2.07 (dtdd, *J* = 11.0, 7.5, 5.5, 3.5 Hz, 1H, C**H**H'CH$_3$), 1.96 (dd, *J* = 10.5, 2.5 Hz, 1H, H$_2$C=CHCH$_2$CHCHC**H**H'), 1.92 (dd, *J* = 10.5, 2.5 Hz, 1H, H$_2$C=CHCH$_2$CHCHCH**H'**), 1.85 (ddd, *J* = 14.0, 10.0, 3.5 Hz, 1H, C**H**H'CHOS), 1.81 – 1.68 (m, 2H, CH**H'**CHOS, CHBrCH**H'**CH$_3$), 1.14 – 1.08 (m, 3H, CH$_2$C**H$_3$**); ^{13}C NMR (151 MHz, CDCl$_3$) δ 134.0 (H$_2$C=**C**H), 117.6 (H$_2$**C**=CH), 82.2 (H$_2$C=CHCH$_2$**C**H), 79.8 (**C**HOS), 79.8 (**C**HCHCH$_2$CHOS), 79.4 (H$_2$C=CHCH$_2$CH**C**H), 77.4 (**C**HCH$_2$CHOS), 57.7 (**C**HBr), 38.1 (S**C**H$_3$), 35.4 (H$_2$C=CH**C**H$_2$), 35.0 (CH**C**H$_2$CH), 33.9 (**C**H$_2$CHOS), 26.3 (**C**H$_2$CH$_3$), 12.6 (CH$_2$**C**H$_3$); LRMS *m/z* (ESI$^+$) not found (compound unstable); HRMS *m/z* (APCI$^+$): found 383.0524 and 385.0504, $C_{14}H_{24}O_5{}^{79}BrS$ (M+H$^+$) requires 383.0522 and $C_{14}H_{24}O_5{}^{81}BrS$ (M+H$^+$) requires 385.0501; ; $[\alpha]_D^{25}$= 117.1 (c = 0.03, CHCl$_3$); IR (ν$_{max}$ cm^{-1}): 2935, 2160, 2032, 1642.

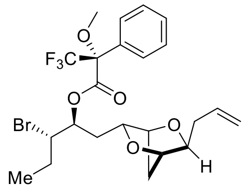
(2*S*,3*S*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (5.14)



According to the procedure of Hoye et al.:[176] *N,N'*-dicyclohexylcarbodiimide (0.011 g, 0.050 mmol, 2.5 equiv.) and 4-(dimethylamino)pyridine (0.0061 g, 0.050 mmol, 2.5 equiv.) were added successively to a stirred solution of (2*S*,3*S*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-ol **5.13** (0.061 g, 0.020 mmol, 1.0 equiv) and (*S*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (0.012 g, 0.050 mmol, 2.5 equiv.) in CH$_2$Cl$_2$ (0.5 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 20% EtOAc/ petroleum ether 40-60) to give the title compound (0.0040 g, 0.076 mmol, 38%) as a colourless oil. R$_f$ 0.29 (20%

EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.57 (dd, *J* = 7.0, 3.0 Hz, 2H, 2 × Ar**H**), 7.41 (m, 3H, 3 × Ar**H**), 5.77 (ddt, *J* = 17.0, 10.5, 7.0 Hz, 1H, H$_2$C=C**H**), 5.30 (td, *J* = 6.0, 3.5 Hz, 1H, C**H**O(MTPA)), 5.15 – 5.05 (m, 2H, **H$_2$**C=CH), 4.29 – 4.25 (m, 1H, H$_2$C=CHCH$_2$CHC**H**), 4.22 (d, *J* = 2.0 Hz, 1H, C**H**CHCH$_2$CHO(MTPA)), 4.06 (dt, *J* = 9.5, 4.0 Hz, 1H, C**H**Br), 3.84 – 3.80 (m, 1H, H$_2$C=CHCH$_2$C**H**), 3.78 (dd, *J* = 8.5, 6.0 Hz, 1H, C**H**CH$_2$CHO(MTPA)), 3.57 (s, 3H, OC**H$_3$**), 2.34 (dt, *J* = 14.0, 7.0 Hz, 1H, H$_2$C=CHC**H**H’), 2.24 (dt, *J* = 14.0, 7.0 Hz, 1H, H$_2$C=CHCH**H’**), 1.92 – 1.72 (m, 5H, H$_2$C=CHCH$_2$CHCHC**H$_2$**, C**H$_2$**CH$_3$, C**H**H’CHO(MTPA)), 1.67 (ddd, *J* = 14.5, 8.5, 7.0 Hz, 1H, CH**H’**CHO(MTPA)), 1.06 (t, *J* = 7.5 Hz, 3H, CH$_2$C**H$_3$**); ^{13}C NMR (151 MHz, CDCl$_3$) δ 165.9 (**C**=O), 134.3 (H$_2$C=**C**H), 131.6 (Ar**C**), 129.7 (Ar**C**H), 128.5 (Ar**C**H), 127.5 (Ar**C**H), 123.0 (q, *J* = 289.5 Hz, **C**F$_3$), 117.4 (H$_2$**C**=CH), 84.7 (q, *J* = 28.0 Hz, **C**(OCH$_3$)), 82.2 (H$_2$C=CHCH$_2$**C**H), 80.2 (**C**HCHCH$_2$CHO(MTPA)), 79.5 (**C**HCH$_2$CHO(MTPA)), 76.9 (H$_2$C=CHCH$_2$CH**C**H), 74.9 (**C**HO(MTPA)), 57.3 (**C**HBr), 55.7 (O**C**H$_3$), 35.4 (**C**H$_2$CHO(MTPA)), 35.2 (H$_2$C=CH**C**H$_2$), 34.7 (CH**C**H$_2$CH), 28.2 (**C**H$_2$CH$_3$), 12.4 (CH$_2$**C**H$_3$); LRMS *m/z* (ESI$^+$) 543.0 (M+Na$^+$, 50%) and 545.0 (M+Na$^+$, 50%); HRMS *m/z* (ESI$^+$): found 543.0964 and 545.0945, $C_{23}H_{28}O_5{}^{79}BrF_3Na$ (M+Na$^+$) requires 543.0964 and $C_{23}H_{28}O_5{}^{81}BrF_3Na$ (M+Na$^+$) requires 545.0946; $[\alpha]_D^{25}$= 5.0 (c = 0.08, CHCl$_3$); IR (ν$_{max}$ cm^{-1}): 2933, 2159, 1976, 1747, 1644, 1452.

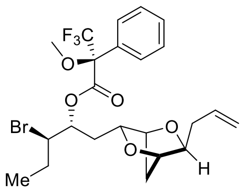
(2S,3S)-1-((3S,6S)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl (2R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (5.15)



According to the procedure of Hoye et al.:[176] *N*,*N*′-dicyclohexylcarbodiimide (0.011 g, 0.060 mmol, 2.5 equiv.) and 4-(dimethylamino)pyridine (0.0061 g, 0.060 mmol, 2.5 equiv.)

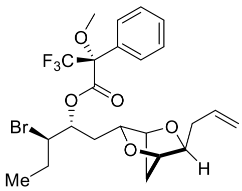
were added successively to a stirred solution of (2*S*,3*S*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-ol **5.13** (0.073 g, 0.020 mmol, 1.0 equiv.) and (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (0.012 g, 0.060 mmol, 2.5 equiv.) in CH_2Cl_2 (0.5 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 20% EtOAc/ petroleum ether 40-60) to give the title compound (0.010 g, 0.017 mmol, 87%) as a colourless oil. R_f 0.33 (20% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 – 7.55 (m, 2H, 2 × Ar**H**), 7.47 – 7.39 (m, 3H, 3 × Ar**H**), 5.86 – 5.76 (m, 1H, H_2C=C**H**), 5.29 (td, *J* = 6.5, 3.5 Hz, 1H, C**H**O(MTPA)), 5.19 – 5.08 (m, 2H, **H₂**C=CH), 4.35 (d, *J* = 2.0 Hz, 1H, $H_2C=CHCH_2CH$C**H**), 4.31 (t, *J* = 1.5 Hz, 1H, C**H**$CHCH_2CHO$(MTPA)), 4.04 (ddt, *J* = 9.5, 4.5, 2.0 Hz, 1H, C**H**Br), 3.95 – 3.83 (m, 2H, $H_2C=CHCH_2C$**H**, C**H**CH_2CHO(MTPA)), 3.59 (s, 3H, OC**H₃**), 2.40 (tdd, *J* = 8.5, 5.0, 1.5 Hz, 1H, H_2C=CHC**H**H'), 2.35 – 2.26 (m, 1H, H_2C=CHCH**H'**), 1.99 – 1.90 (m, 3H, CHC**H₂**CH, C**H**H'CHO(MTPA)), 1.80 – 1.62 (m, 3H, C**H₂**CH_3, H**H'**CHO(MTPA)), 0.99 (t, *J* = 7.5, 2.0 Hz, 3H, CH_2C**H₃**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.9 (**C**=O), 134.2 (H_2C=**C**H), 131.8 (Ar**C**), 129.7 (Ar**C**H), 128.5 (Ar**C**H), 127.4 (Ar**C**H), 123.3 (q, *J* = 288.5 Hz, **C**F_3), 117.4 (H_2**C**=CH), 84.55 (q, *J* = 28.0 Hz, **C**(OCH_3)), 82.3 ($H_2C=CHCH_2$**C**H), 80.5 (**C**HCH_2CHO(MTPA)), 79.5 (**C**H$CHCH_2CHO$(MTPA)), 77.1 ($H_2C=CHCH_2CH$**C**H), 74.9 (**C**HO(MTPA)), 56.8 (**C**HBr), 55.6 (O**C**H_3), 35.2 (H_2C=CH**C**H_2), 35.1 (**C**H_2CHO(MTPA)), 34.8 (CH**C**H_2CH), 27.7 (**C**H_2CH_3), 12.4 (CH_2**C**H_3); LRMS *m/z* (ESI$^+$) 543.0 (M+Na$^+$, 50%) and 545.0 (M+Na$^+$, 50%); HRMS *m/z* (ESI$^+$): found 543.0963 and 545.0942, $C_{23}H_{28}O_5{}^{79}BrF_3Na$ (M+Na$^+$) requires 543.0964 and $C_{23}H_{28}O_5{}^{81}BrF_3Na$ (M+Na$^+$) requires 545.0946; $[\alpha]_D^{25}$= 56.7 (c = 0.03, $CHCl_3$); IR (ν_{max} cm^{-1}): 2940, 2159, 2021, 1977, 1748, 1645, 1453.

(2*R*,3*R*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (5.16)



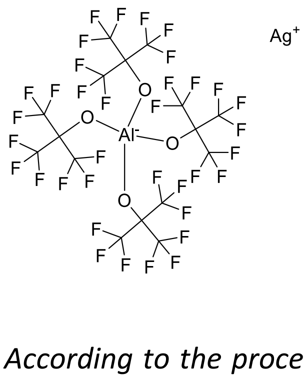
According to the procedure of Hoye et al.:[176] *N*,*N*′-dicyclohexylcarbodiimide (0.013 g, 0.060 mmol, 2.5 equiv.) and 4-(dimethylamino)pyridine (0.0073 g, 0.060 mmol, 2.5 equiv.) were added successively to a stirred solution of (2*R*,3*R*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-ol **3.109** (0.073 g, 0.024 mmol, 1.0 equiv.) and (*S*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (0.014 g, 0.060 mmol, 2.5 equiv.) in CH_2Cl_2 (0.5 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 20% EtOAc/ petroleum ether 40-60) to give the title compound (0.0040 g, 0.059 mmol, 99%) as a colourless oil. R_f 0.28 (20% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (dd, *J* = 6.5, 3.0 Hz, 2H, 2 × Ar**H**), 7.41 (m, 3H, 3 × Ar**H**), 5.79 (ddt, *J* = 17.5, 10.5, 7.0, 1.0 Hz, 1H, $H_2C=C\mathbf{H}$), 5.40 – 5.32 (m, 1H, C**H**O(MTPA)), 5.15 – 5.05 (m, 2H, $\mathbf{H_2}C=CH$), 4.37 (d, *J* = 2.0 Hz, 1H, $H_2C=CHCH_2CHC\mathbf{H}$), 4.21 – 4.18 (m, 1H, $H_2C=CHCH_2CHCHCH_2C\mathbf{H}$), 4.09 – 4.01 (m, 1H, C**H**Br), 3.88 – 3.79 (m, 2H, $H_2C=CHCH_2C\mathbf{H}$, C**H**CH_2CHO(MTPA)), 3.56 (s, 3H, $OC\mathbf{H_3}$), 2.35 (dtd, *J* = 14.0, 7.0, 1.5 Hz, 1H, $H_2C=CHC\mathbf{H}H'$), 2.28 (dtd, *J* = 14.0, 7.0, 1.5 Hz, 1H, $H_2C=CHCH\mathbf{H'}$), 1.93 – 1.84 (m, 2H, $CHC\mathbf{H_2}CH$), 1.82 – 1.70 (m, 2H, $C\mathbf{H_2}$CHO(MTPA)), 1.66 (dqdd, *J* = 14.5, 7.0, 4.0, 1.0 Hz, 1H, $CHBrC\mathbf{H}H'CH_3$), 1.62 – 1.52 (m, 1H, $CHBrCH\mathbf{H'}CH_3$), 0.97 (t, *J* = 7.0, 1.0 Hz, 3H, $CH_2C\mathbf{H_3}$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.0 (**C**=O), 134.3 ($H_2C=\mathbf{C}H$), 131.8 (Ar**C**), 129.8 (Ar**C**H), 128.5 (Ar**C**H), 127.4 (Ar**C**H), 123.27 (q, J = 288.5 Hz, **C**F_3), 117.4 (H_2**C**=CH), 84.67 (q, J = 30.0 Hz, **C**(OCH_3)), 82.3 (H_2C=$CHCH_2$**C**H), 79.9 (**C**HCH_2CHO(MTPA)), 79.8 (H_2C=$CHCH_2CHCHCH_2$**C**H), 77.2 (H_2C=$CHCH_2CH$**C**H), 74.5 (**C**HO(MTPA)), 57.3 (**C**HBr), 55.6 (O**C**H_3), 35.2 (H_2C=CH**C**H_2), 34.9 (CH**C**H_2CH), 34.7 (**C**H_2CHO(MTPA), 27.3 (**C**H_2CH_3), 12.5 (CH_2**C**H_3); LRMS *m/z* (ESI^+) 543.0 ($M+Na^+$, 50%) and 545.0 ($M+Na^+$, 50%); HRMS *m/z* (ESI^+): found 543.0962 and 545.0942, $C_{23}H_{28}O_5{}^{79}BrF_3Na$ ($M+Na^+$) requires 543.0964 and $C_{23}H_{28}O_5{}^{81}BrF_3Na$ ($M+Na^+$) requires 545.0946; $[\alpha]_D^{25}$= 11.3 (c = 0.15, $CHCl_3$); IR (ν_{max} cm^{-1}): 2972, 2360, 2159, 1749, 1642, 1452.

(2*R*,3*R*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (5.17)



According to the procedure of Hoye et al.:[176] *N*,*N'*-dicyclohexylcarbodiimide (0.013 g, 0.060 mmol, 2.5 equiv.) and 4-(dimethylamino)pyridine (0.0073 g, 0.060 mmol, 2.5 equiv.) were added successively to a stirred solution of (2*R*,3*R*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-ol **3.109** (0.073 g, 0.024 mmol, 1.0 equiv.) and (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (0.014 g, 0.060 mmol, 2.5 equiv.) in CH_2Cl_2 (0.5 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 20% EtOAc/ petroleum ether 40-60) to give the title compound (0.0098 g, 0.059 mmol, 86%) as a colourless oil. R*f* 0.22 (20% EtOAc/ petroleum ether 40-60); ^1H NMR (600 MHz, $CDCl_3$) δ 7.62 – 7.53 (m, 2H, 2 × Ar**H**), 7.41 (m, 3H, 3 × Ar**H**), 5.78 (ddt, J = 17.0, 10.5, 7.0 Hz, 1H, H_2C=C**H**), 5.42 (dt, J = 9.5, 3.0 Hz, 1H, C**H**O(MTPA)), 5.12 (dq, *J* = 17.0, 1.5 Hz, 1H, **H**H’C=CH), 5.08 (dd, *J* = 10.5, 2.0 Hz, 1H, H**H’**C=CH), 4.37 – 4.34 (m, 1H, H_2C=CHCH_2CHC**H**), 4.15 (d, *J* = 2.0 Hz, 1H, H_2C=CHCH_2CHCHCH_2C**H**), 4.06 – 3.98 (m, 1H, C**H**Br), 3.83 – 3.79 (m, 1H, H_2C=CHCH_2C**H**), 3.71 (dd, *J* = 10.5, 3.5 Hz, 1H, C**H**CH_2CHO(MTPA)), 3.57 (s, 3H, OC**H₃**), 2.32 (dt, *J* = 14.0, 7.0 Hz, 1H, H_2C=CHC**H**H’), 2.25 (dt, *J* = 14.0, 7.0 Hz, 1H, H_2C=CHCH**H’**), 1.93 – 1.76 (m, 4H, CHC**H₂**CH, CHBrC**H**H’CH_3, C**H**H’CHO(MTPA)), 1.70 (ddq, *J* = 14.5, 10.5, 7.5 Hz, 1H, CHBrCH**H’**CH_3), 1.66 – 1.56 (m, 1H, CH**H’**CHO(MTPA)), 1.04 (t, *J* = 7.0 Hz, 3H, CH_2C**H₃**); ^{13}C NMR (151 MHz, CDCl_3) δ 166.0 (**C**=O), 134.3 (H_2C=**C**H), 131.7 (Ar**C**), 129.8 (Ar**C**H), 128.6 (Ar**C**H), 127.4 (Ar**C**H), 123.3 (q, *J* = 288.5 Hz, **C**F_3), 117.4 (H_2**C**=CH), 84.68 (q, *J* = 28.0 Hz, **C**(OCH_3)), 82.2 (H_2C=CHCH_2**C**H), 79.8 (**C**HCH_2CHO(MTPA)), 79.7 (H_2C=CHCH_2CHCHCH_2**C**H), 77.1 (H_2C=CHCH_2CH**C**H), 74.2 (**C**HO(MTPA)), 58.4 (**C**HBr), 55.6 (O**C**H_3), 35.7 (**C**H_2CHO(MTPA)), 35.2 (H_2C=CH**C**H_2), 34.8 (CH**C**H_2CH), 28.2 (**C**H_2CH_3), 12.6 (CH_2**C**H_3); LRMS *m/z* (ESI$^+$) 543.0 (M+Na$^+$, 50%) and 545.0 (M+Na$^+$, 50%); HRMS *m/z* (ESI$^+$): found 543.0961 and 545.0942, $C_{23}H_{28}O_5{}^{79}BrF_3Na$ (M+Na$^+$) requires 543.0964 and $C_{23}H_{28}O_5{}^{81}BrF_3Na$ (M+Na$^+$) requires 545.0944; $[\alpha]_D^{25}$= 51.4 (c = 0.070, CHCl_3); IR (ν_{max} cm^{-1}): 2970, 2361, 2118, 1748, 1642, 1452.

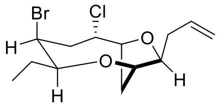
Silver (I) tetrakis((1,1,1,3,3,3-hexafluoro-2-(trifluoromethyl)propan-2-yl)oxy)aluminate (3.100)



According to the procedure of Krossing.:[138] nonafluoro-*tert*-butyl alcohol (4.0 mL, 29 mmol, 4.1 equiv.) was added dropwise to freshly dried (high vacuum) lithium aluminium hydride

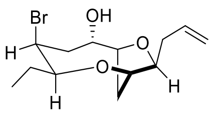
(4 M in Et_2O, 1.8 mL, 7.0 mmol, 1.0 equiv.) redissolved in toluene (35 mL) at 0 °C. After the addition was complete, the reaction mixture was sonicated and then heated to 80 °C for 18 h. The reaction mixture was cooled to -20 °C and left for 0.5 h, then the toluene was decanted, and the residue dried under high vacuum at 80 °C for 2 h. to give a white solid. The solid was cooled to rt and then redissolved in CH_2Cl_2 (35 mL) before addition of silver (I) fluoride (1.8 g, 7.0 mmol, 1.0 equiv.), the reaction mixture was then heated to 40 °C and stirred for 18 h with exclusion of light. The reaction mixture was cooled to rt and filtered through a plug of celite, then the solvent was removed *in vacuo* to give the title compound as a light brown solid which required no further purification; LRMS *m/z* (ES^-) 966.9 ($M\text{-}Ag^+$). Data are in accordance with the literature.[138]

(3*S*,4*R*,6*S*,9*S*)-9-Allyl-4-bromo-6-chloro-3-ethyl-2,8-dioxabicyclo[5.2.1]decane (5.16)



According to the modified procedure of Chan et al.:[117] titanium tetrachloride solution (0.5 M in CH_2Cl_2, 0.11 mL, 0.055 mmol, 2.0 equiv.) and silver (I) tetrakis((1,1,1,3,3,3-hexafluoro-2-(trifluoromethyl)propan-2-yl)oxy)aluminate **3.94** (0.092 g, 0.83 mmol, 3.1 equiv.) in CH_2Cl_2 (1.0 mL) were added successively to a rapidly stirred solution of (2*R*,3*R*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl methanesulfonate **3.102** (0.010 g, 0.028 mmol, 1.0 equiv.) in CH_2Cl_2 (3.0 mL) at -40 °C and the reaction mixture was stirred for 2 h whilst maintaining the bath temperature. After this time, the reaction mixture was cooled to -78 °C and TBACl (0.040 g, 0.14 mmol, 5.0 equiv.) was added, the reaction mixture was stirred for a further 1 h before being quenched *via* the addition of $NaHCO_3$ (10 mL). The reaction mixture was warmed to rt and then the organics were extracted with CH_2Cl_2 (3 × 5 mL), dried ($MgSO_4$), and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (5% EtOAc/ petroleum ether 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound (0.0040 g, 0.014 mmol, 50%). R_f 0.17 (5% EtOAc/petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.85 (ddt, *J* = 17.0, 10.0, 7.0 Hz, 1H, CH_2=C**H**), 5.18 (dq, *J* = 17.0, 1.5 Hz, 1H, C**H**H'=CH), 5.08 (dd, *J* = 10.0, 1.5 Hz, 1H, CH**H'**=CH), 4.49 – 4.44 (m, 1H, CH_2CH C**H**CHCl), 4.43 – 4.37 (m, 1H, C**H**Cl), 4.38 – 4.31 (m, 1H, C**H**Br), 4.19 – 4.13 (m, 1H, CH_2=CHCH$_2$CHOC**H**), 3.89 (td, *J* = 7.4, 2.4 Hz, 1H, CH_2=CHCH$_2$**C**H), 3.41 (s, 1H, C**H**CH$_2$CH$_3$), 2.83 – 2.71 (m, 1H, C**H**H'CHBr), 2.64 (dd, *J* = 12.5, 5.5 Hz, 1H, CHC**H**H'CH), 2.58 (dt, *J* = 14.0, 7.0 Hz, 1H, CH_2=CHC**H**H'), 2.54 – 2.49 (m, 1H, CH_2=CHCH**H'**), 2.49 – 2.43 (m, 1H, CH**H'**CHBr), 1.96 – 1.86 (m, 1H, CHCH**H'**CH), 1.84 – 1.75 (m, 1H, CHC**H**H'CH$_3$), 1.54 (s, 1H, CHCH**H'**CH$_3$), 0.94 (t, *J* = 7.5 Hz, 3H, C**H$_3$**); ^{13}C data not assigned due to excessive broadness and several absent peaks; LRMS *m/z* (ESI$^+$) 323.0 (M+H$^+$, 50%), 325.0 (M+H$^+$, 50%); HRMS *m/z* (ESI$^+$): found 323.0409 and 325.0386, $C_{13}H_{21}{}^{79}Br^{35}ClO_2$ (M+H$^+$) requires 323.0408 and $C_{13}H_{21}{}^{81}Br^{35}ClO_2$ (M+H$^+$) requires 325.0385; $[\alpha]_D^{25}$= + 21.0 (c = 0.1, $CHCl_3$); IR (ν_{max} cm^{-1}): 3077, 2925, 2875, 1646.

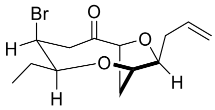
(3*S*,4*R*,6*S*,9*S*)-9-Allyl-4-bromo-3-ethyl-2,8-dioxabicyclo[5.2.1]decan-6-ol (3.104)



According to the modified procedure of Chan et al.:[117] titanium tetrachloride solution (0.5 M in CH_2Cl_2, 0.13 mL, 0.066 mmol, 2.0 equiv.) and silver (I) tetrakis((1,1,1,3,3,3-hexafluoro-2-(trifluoromethyl)propan-2-yl)oxy)aluminate **3.94** (0.11 g, 0.10 mmol, 3.1 equiv.) in CH_2Cl_2 (1.0 mL) were added successively to a rapidly stirred solution of (2*R*,3*R*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl methanesulfonate **3.102** (0.012 g, 0.033 mmol, 1.0 equiv.) in CH_2Cl_2 (3.0 mL) at -40 °C and the reaction mixture was stirred for 2 h whilst maintaining the bath temperature. After this time, the reaction mixture was cooled to -78 °C and silver benzoate (0.025 g, 0.11 mmol, 3.3 equiv.) was added, the reaction mixture was stirred for a further 1 h before being quenched *via* the addition of $NaHCO_3$ (10 mL) and excess TBAI. The reaction mixture was warmed to rt and then the organics were extracted with CH_2Cl_2 (3 × 5 mL), dried ($MgSO_4$), and the solvent removed *in vacuo* to give a crude residue that was passed through a plug of silica (Et_2O). The crude benzoate ester was redissolved in MeOH (1.0 mL) and then K_2CO_3 (0.023 g, 0.17 mmol, 5.0 equiv.) was added and the reaction mixture stirred at rt for 2 h. After this time, the reaction mixture was diluted with H_2O (5 mL) and organics extracted with EtOAc (3 × 5 mL). The organic layer was dried ($MgSO_4$), and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (15% acetone/ petroleum ether 40-60 → 25% acetone/ petroleum ether 40-60) to give the title compound (0.0015 g, 0.0049 mmol, 15%) as a colourless oil. R_f 0.19 (25% acetone/ petroleum ether 40/60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.88 (ddt, *J* = 17.5, 10.0, 7.0 Hz, 1H, H_2C=C**H**), 5.19 (dt, *J* = 17.5, 1.5 Hz, 1H, **H**H'C=CH), 5.09 (ddd, *J* = 10.0, 2.0, 1.0 Hz, 1H, H**H'**C=CH), 4.40 – 4.33 (m, 2H, C**H**Br, C**H**OH), 4.27 (dd, *J* = 8.0, 3.5 Hz, 1H, C**H**CHOH), 4.11 (t, *J* = 2.5 Hz, 1H, H_2C=CHCH_2CHC**H**), 3.87 (td, *J* = 7.5, 2.5 Hz, 1H, H_2C=CHCH_2C**H**), 3.33 – 3.28 (m, 1H, C**H**CH_2CH_3), 2.61 – 2.53 (m, 1H, H_2C=CHC**H**H'), 2.49 (q, *J* = 7.0 Hz, 1H, H_2C=CHCH**H'**), 2.44 (d, *J* = 14.5 Hz, 1H, H_2C=CHCH_2CHCHC**H**H'), 2.29 (ddd, *J* = 16.0, 9.0, 3.0 Hz, 1H, C**H**H'CHBr), 2.19 (d, *J* = 15.5 Hz, 1H, CH**H'**CHBr), 2.14 – 2.02 (m, 1H, O**H**), 1.92 – 1.78 (m, 2H, CHCH**H'**CH, C**H**H'CH_3), 1.70 – 1.47 (m, 1H, CH**H'**CH_3), 0.96 (t, *J* = 7.5 Hz, 3H, CH_2C**H₃**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 134.9 (H_2C=**C**H), 117.1 (H_2**C**=CH), 84.2 (**C**HCHOH), 83.2 (H_2C=CHCH_2**C**H), 78.2 (**C**HCH_2CH_3), 76.3 (**C**HOH), 76.1 (H_2C=CHCH_2CH**C**H), 58.2 (**C**HBr), 42.4 (**C**H_2CHBr), 34.4 (H_2C=CH**C**H_2), 33.0 (CH**C**H_2CH), 29.9 (**C**H_2CH_3), 10.3 (CH_2**C**H_3); LRMS *m/z* (ESI$^+$)

305.8 (M+H$^+$, 50%), 307.8 (M+H$^+$, 50%); HRMS *m/z* (ESI$^+$): found 327.0567 and 329.0547, $C_{13}H_{21}{}^{79}BrO_3Na$ (M+Na$^+$) requires 327.0566 and $C_{13}H_{21}{}^{81}BrO_3Na$ (M+Na$^+$) requires 329.0546; $[\alpha]_D^{25}$= +40.0 (c = 0.060, $CHCl_3$); IR (ν_{max} cm^{-1}): 3409, 2924, 2855, 2158, 2018, 1653.

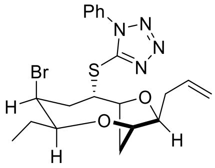
(3*S*,4*R*,9*S*)-9-Allyl-4-bromo-3-ethyl-2,8-dioxabicyclo[5.2.1]decan-6-one (3.107)



Sodium bicarbonate (0.036 g, 0.43 mmol, 10 equiv.) and Dess-Martin periodinane (0.054 g, 3.0 equiv.) were added successively to a stirred solution of (3*S*,4*R*,6*S*,9*S*)-9-allyl-4-bromo-3-ethyl-2,8-dioxabicyclo[5.2.1]decan-6-ol **3.104** (0.013 g, 0.043 mmol, 1.0 equiv.) in CH_2Cl_2 (0.5 mL) and the reaction mixture was stirred at rt for 1.5 h. After this time, the solvent was removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (CH_2Cl_2 → 5% Et_2O/ CH_2Cl_2) to give the title compound (0.0090 g, 0.030 mmol, 70%) as a colourless oil. R_f 0.18 (5% Et_2O/ CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 5.87 (ddt, *J*=17.0, 10.0, 7.0, 1H, $H_2C=C\mathbf{H}$), 5.21 (dq, *J*=17.0, 1.5, 1H, **H**H'C=CH), 5.13 – 5.07 (m, 1H, H**H'**C=CH), 4.36 (ddd, *J*=8.0, 5.5 2.5, 1H, C**H**Br), 4.33 – 4.27 (m, 2H, $H_2C=CHCH_2CHC\mathbf{H}_3$, C**H**$COCH_2$), 4.00 (td, *J*=7.0, 2.0, 1H, $H_2C=CHCH_2C\mathbf{H}$), 3.64 (dd, *J*=11.5, 8.0, 1H, C**H**H'CHBr), 3.32 (ddd, *J*=7.5, 4.5, 2.5, 1H, CHBrC**H**), 2.76 (dd, *J*=11.5, 5.5, 1H, COCH**H'**), 2.69 – 2.58 (m, 3H, $H_2C=CHC\mathbf{H_2}$, CHC**H**H'CH), 2.18 (ddd, *J*=15.0, 9.0, 2.5, 1H, CHCH**H'**CH), 1.88 – 1.77 (m, 1H, CHC**H**H'CH_3), 1.54 (dtd, *J*=14.5, 7.5, 4.5, 1H, CHCH**H'**CH_3), 0.93 (t, *J*=7.5, 3H, $CH_2C\mathbf{H_3}$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 211.8 (**C**=O), 134.4 ($H_2C=\mathbf{C}H$), 117.5 ($H_2\mathbf{C}=CH$), 85.6 ($H_2C=CHCH_2\mathbf{C}H$), 81.0 (**C**HCO), 76.4 ($H_2CCHCH_2CH\mathbf{C}H$), 76.2 (CHBr**C**H), 56.9 (**C**HBr), 43.5 (CO**C**H_2), 34.7 (CH**C**H_2CH), 33.7 ($H_2C=CH\mathbf{C}H_2$), 29.3 (**C**H_2CH_3), 10.4 ($CH_2\mathbf{C}H_3$); LRMS *m/z* (ESI$^+$) 303.0 (M+H$^+$, 50%), 305.0 (M+H$^+$, 50%); HRMS *m/z* (ESI$^+$): found 303.0592 and 305.0571, $C_{13}H_{20}{}^{79}BrO_3$ (M+H$^+$) requires

303.0590 and $C_{13}H_{20}{}^{81}BrO_3$ (M+H$^+$) requires 305.0570; $[\alpha]_D^{25}$= +101.0 (c = 0.10, $CHCl_3$); IR (ν_{max} cm^{-1}): 2966, 2928, 1705, 1641.

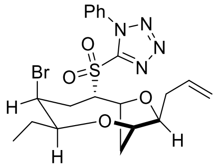
5-(((3*S*,4*R*,6*S*,9*S*)-9-Allyl-4-bromo-3-ethyl-2,8-dioxabicyclo[5.2.1]decan-6-yl)thio)-1-phenyl-1*H*-tetrazole (5.27)



Titanium tetrachloride solution (0.5 M in CH_2Cl_2, 0.30 mL, 0.15 mmol, 2.0 equiv.) and silver (I) tetrakis((1,1,1,3,3,3-hexafluoro-2-(trifluoromethyl)propan-2-yl)oxy)aluminate **3.94** (0.25 g, 0.23 mmol, 3.1 equiv.) in CH_2Cl_2 (1.0 mL) were added successively to a rapidly stirred solution of (2*R*,3*R*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl methanesulfonate **3.102** (0.028 g, 0.074 mmol, 1.0 equiv.) in CH_2Cl_2 (7.5 mL) at -40 °C and the reaction mixture was stirred for 2 h whilst maintaining the bath temperature. After this time, the reaction mixture was cooled to -78 °C and 1-phenyl-1*H*-tetrazole-5-thiol (0.13 g, 0.74 mmol, 10 equiv.) was added and the reaction mixture was stirred for a further 1 h before being quenched *via* the addition of $NaHCO_3$ (10 mL). The reaction mixture was warmed to rt and then the organics were extracted with CH_2Cl_2 (3 × 5 mL), dried ($MgSO_4$), and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (20% EtOAc/ petroleum ether 40-60 → 30% EtOAc/petroleum ether 40-60) to give the title compound (0.019 g, 0.035 mmol, 48%) as a colourless oil. R_f 0.31 (30% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.64 – 7.51 (m, 5H, 5 × Ar**H**), 5.86 (ddt, *J* = 17.5, 10.0, 7.0 Hz, 1H, H_2C=C**H**), 5.19 (dp, *J* = 17.5, 1.5 Hz, 1H, **H**H'C=CH), 5.13 – 5.06 (m, 1H, H**H'**C=CH), 4.77 (dd, *J* = 7.5, 5.5 Hz, 1H, C**H**CHS), 4.35 (tq, *J* = 3.0, 1.5 Hz, 1H, C**H**Br), 4.18 (td, *J*

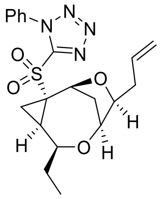
= 2.5, 1.5 Hz, 1H, H$_2$C=CHCH$_2$CHC**H**), 4.04 – 3.94 (m, 1H, C**H**S), 3.90 (tq, *J* = 6.5, 2.5, 2.0 Hz, 1H, H$_2$C=CHCH$_2$C**H**), 3.44 – 3.38 (m, 1H, C**H**CH$_2$CH$_3$), 2.70 (d, *J* = 14.5 Hz, 1H, C**H**H'CHBr), 2.61 – 2.53 (m, 3H, CH**H'**CHBr, CHC**H**H'CH, H$_2$C=CHC**H**H'), 2.48 (dt, *J* = 13.5, 7.0 Hz, 1H, H$_2$C=CHCH**H'**), 2.04 – 1.98 (m, 1H, CHCH**H'**CH), 1.85 (dddd, *J* = 14.0, 8.5, 7.5, 1.5 Hz, 1H, CHC**H**H'CH$_3$), 1.60 – 1.49 (m, 1H, CHCH**H'**CH$_3$), 0.97 (t, *J* = 7.5, Hz, 3H, CH$_2$C**H$_3$**); ^{13}C NMR (151 MHz, CDCl$_3$) δ 153.1 (Ar**C**), 134.6 (H$_2$C=**C**H), 133.7 (Ar**C**), 130.1 (Ar**C**H), 129.7 (Ar**C**H), 123.9 (Ar**C**H), 117.3 (H$_2$**C**=CH), 83.9 (H$_2$C=CHCH$_2$**C**H), 82.2 (**C**HCHS), 81.3 (**C**HS), 78.9 (**C**HCH$_2$CH$_3$), 76.2 (H$_2$C=CHCH$_2$CH**C**H), 59.5 (**C**HBr), 34.5 (CH**C**H$_2$CH), 34.2 (**C**H$_2$CHBr), 34.0 (H$_2$C=CH**C**H$_2$), 29.8 (**C**H$_2$CH$_3$), 10.2 (CH$_2$**C**H$_3$); LRMS *m/z* (ESI$^+$) 465.1 (M+H$^+$, 50%), 467.1 (M+H$^+$, 50%); HRMS *m/z* (ESI$^+$): found 465.0955 and 467.0933, $C_{20}H_{26}{}^{79}BrN_4O_2S$ (M+H$^+$) requires 465.0954 and $C_{20}H_{26}{}^{81}BrN_4O_2S$ (M+H$^+$) requires 457.0934; $[\alpha]_D^{25}$= 29.0 (c = 0.1, CHCl$_3$); IR (ν$_{max}$ cm^{-1}): 2970, 2916, 1659.

5-(((3*S*,4*R*,6*S*,9*S*)-9-Allyl-4-bromo-3-ethyl-2,8-dioxabicyclo[5.2.1]decan-6-yl)sulfonyl)-1-phenyl-1*H*-tetrazole (5.20)



Ammonium molybdate tetrahydrate (0.013 g, 0.011 mmol, 0.3 equiv.) in aqueous hydrogen peroxide solution (30%, 66 uL) was added to a stirred solution of 5-(((3*S*,4*R*,6*S*,9*S*)-9-allyl-4-bromo-3-ethyl-2,8-dioxabicyclo[5.2.1]decan-6-yl)thio)-1-phenyl-1*H*-tetrazole **5.27** (0.016 g, 0.035 mmol, 1.0 equiv.) in EtOH (0.5 mL) at 0 °C. The reaction mixture was then warmed to rt and stirred for 18 h. After this time, the reaction mixture was diluted with EtOAc and H$_2$O and the layers separated, the aqueous layer was further extracted EtOAc (3 × 1 mL) and then the combined organic layers washed with sat. Na$_2$S$_2$O$_3$ solution, dried (MgSO$_4$) and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (20% acetone/ petroleum ether 40-60 → 30% acetone/ petroleum ether 40-60) to give the title compound (0.011 g, 0.021 mmol, 65%) as a white foamy solid. R$_f$ 0.18 (20% acetone/ petroleum ether 40-60); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.71 – 7.66 (m, 2H, 2 × Ar**H**), 7.64 – 7.56 (m, 3H, 3 × Ar**H**), 5.72 (ddt, *J* = 17.0, 10.0, 7.0 Hz, 1H, H$_2$C=C**H**), 5.11 (dq, *J* = 17.0, 1.5 Hz, 1H, **H**H'C=CH), 5.06 (ddt, *J* = 10.5, 2.0, 1.0 Hz, 1H, H**H'**C=CH), 4.90 (dd, *J* = 8.0, 5.0 Hz, 1H, C**H**CHS), 4.34 (dt, *J* = 4.0, 2.0 Hz, 1H, C**H**Br), 4.08 (td, *J* = 2.5, 1.0 Hz, 1H, H$_2$C=CHCH$_2$CHC**H**), 3.95 – 3.88 (m, 1H, C**H**S), 3.76 (ddd, *J* = 8.5, 6.5, 2.5 Hz, 1H, H$_2$C=CHCH$_2$C**H**), 3.30 (ddd, *J* = 8.5, 4.5, 1.5 Hz, 1H, C**H**CH$_2$CH$_3$), 2.76 – 2.70 (m, 1H, C**H**H'CHBr), 2.47 (d, *J* = 14.7 Hz, 1H, CHC**H**H'CH), 2.38 (dddt, *J* = 13.5, 8.5, 7.5, 1.0 Hz, 1H, H$_2$C=CHC**H**H'), 2.31 (m, 2H, H$_2$C=CHCH**H'**, CH**H'**CHBr), 2.01 (ddd, *J* = 14.5, 8.0, 2.0 Hz, 1H, CHCH**H'**CH), 1.78 (ddq, *J* = 14.5, 8.5, 7.5 Hz, 1H, CHC**H**H'CH$_3$), 1.54 – 1.43 (m, 1H, CHCH**H'**CH$_3$), 0.92 (t, *J* = 7.5 Hz, 3H, CH$_2$C**H$_3$**); ^{13}C NMR (151 MHz, CDCl$_3$) δ 152.4 (Ar**C**), 133.9 (H$_2$C=**C**HCH$_2$), 133.0 (Ar**C**), 131.5 (Ar**C**H), 129.5 (ArC**H**), 126.1 (ArC**H**), 117.7 (H$_2$**C**=CH), 83.8 (H$_2$CCHCH$_2$**C**H), 78.8 (**C**HCH$_2$CH$_3$), 75.7 (H$_2$CCHCH$_2$CH**C**H), 73.7 (**C**HCHS), 72.4 (**C**HS), 56.4 (**C**HBr), 33.9 (H$_2$C=CH**C**H$_2$), 33.4 (CH**C**H$_2$CH), 32.4 (**C**H$_2$CHBr), 29.5 (**C**H$_2$CH$_3$), 10.0 (**C**H$_3$); LRMS *m/z* (ESI$^+$) 497.1 (M+H$^+$, 50%), 499.1 (M+H$^+$, 50%); HRMS *m/z* (ESI$^+$): found 497.0852 and 499.0832, $C_{20}H_{26}{}^{79}BrN_4O_4S$ (M+H$^+$) requires 497.0853 and $C_{20}H_{26}{}^{81}BrN_4O_4S$ (M+H$^+$) requires 499.0832; $[\alpha]_D^{25}$= 54.1 (c = 0.1, CHCl$_3$); IR (ν$_{max}$ cm^{-1}): 2980, 2918, 1595, 1497.

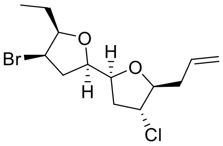
5-(((1*S*,2*S*,4*R*,5*S*,7*S*,8*S*)-8-Allyl-5-ethyl-6,9-dioxatricyclo[5.2.1.0^{2,4}]decan-2-yl)sulfonyl)-1-phenyl-1*H*-tetrazole (5.28)



NaHMDS solution (0.1 M in THF, 0.10 mL, 0.10 mmol, 1.1 equiv.) was added dropwise to a stirred solution of 5-(((3*S*,4*R*,6*S*,9*S*)-9-allyl-4-bromo-3-ethyl-2,8-dioxabicyclo[5.2.1]decan-6-yl)sulfonyl)-1-phenyl-1*H*-tetrazole **5.20** (0.0045 g, 0.091 mmol, 1.0 equiv.) in THF (1 mL) at -78 °C. The reaction mixture was gradually warmed to rt and stirred a further 20 mins at this temperature before being quenched *via* the addition of sat. NH_4Cl solution (1 mL). The organics were extracted with EtOAc (3 × 1 mL) and then the combined organic layers dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (20% acetone/ petroleum ether 40-60 → 30% acetone/ petroleum ether 40-60) to give the title compound (0.0010 g, 0.0024 mmol, 26%) as a colourless oil. R$_f$ 0.40 (30% acetone/ petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 – 7.56 (m, 5H, 5 × Ar**H**), 5.80 (ddt, *J* = 17.0, 10.0, 7.0 Hz, 1H, $H_2C=C\mathbf{H}$), 5.20 – 5.11 (m, 2H, $\mathbf{H_2}C=CH$), 5.10 – 5.04 (m, 1H, C**H**CS), 4.20 – 4.14 (m, 2H, $H_2C=CHCH_2C\mathbf{H}$, $CH_3CH_2C\mathbf{H}$), 3.67 (ddd, *J* = 8.0, 6.5, 1.5 Hz, 1H, $H_2C=CHCH_2CHC\mathbf{H}$), 3.02 (d, *J* = 14.0 Hz, 1H, $H_2C=CHC\mathbf{H}H'$), 2.57 – 2.41 (m, 2H, $C\mathbf{H_2}CHCS$), 2.18 – 2.11 (m, 1H, $CH_3CH_2CHC\mathbf{H}$), 2.06 (ddd, *J* = 14.0, 8.0, 2.5 Hz, 1H, $H_2C=CHCH\mathbf{H'}$), 1.89 (dd, *J* = 8.0, 5.5 Hz, 1H, $CH_3CH_2CHCHC\mathbf{H}H'$), 1.57 (dd, *J* = 7.5, 6.0 Hz, 2H, $CH_3C\mathbf{H_2}$), 1.46 (dd, *J* = 10.5, 5.5 Hz, 1H, $CH_3CH_2CHCHCH\mathbf{H'}$), 0.97 (t, *J* = 7.5 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.7 (Ar**C**), 134.2 ($H_2C=\mathbf{C}H$), 133.1 (Ar**C**), 131.5 (Ar**C**H), 129.6 (Ar**C**H), 125.7 (Ar**C**H), 117.5 ($H_2\mathbf{C}=CH$), 86.2 ($H_2C=CHCH_2CH\mathbf{C}H$), 75.8 ($H_2C=CHCH_2\mathbf{C}H$), 73.7 (**C**HCS), 70.0 (CH_3CH_2**C**H), 48.9 (**C**S), 35.5 (H_2C=CH**C**H_2), 33.3 (CH_3CH_2CH**C**HCH_2), 32.9 (**C**H_2CHCS), 29.4 (CH_3**C**H_2), 10.8 (CH_3CH_2CHCH**C**H_2), 10.0 (**C**H_3CH_2); LRMS *m/z* (ESI^+) 417.2 ($M+H^+$, 100%); HRMS *m/z* (ESI^+): found 417.1591, $C_{20}H_{25}N_4O_4S$ ($M+H^+$) requires 417.1591; $[\alpha]_D^{25}$= 64.5 (c = 0.1, $CHCl_3$); IR (ν_{max} cm^{-1}): 2970, 2916, 1642.

8.5. Experimental Procedures for Chapter 6

(2*S*,2'*R*,4*R*,4'*R*,5*S*,5'*R*)-5-Allyl-4'-bromo-4-chloro-5'-ethyloctahydro-2,2'-bifuran (6.2)



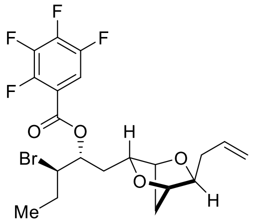
Titanium tetrachloride solution (0.5 M in CH_2Cl_2, 0.10 mL, 0.052 mmol, 2.0 equiv.) and silver (I) tetrakis((1,1,1,3,3,3-hexafluoro-2-(trifluoromethyl)propan-2-yl)oxy)aluminate **3.94** (0.092 g, 0.081 mmol, 3.1 equiv.) in CH_2Cl_2 (1.0 mL) were added successively to a rapidly stirred solution of (2*S*,3*S*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl methanesulfonate **5.9** (0.010 g, 0.026 mmol, 1.0 equiv.) in CH_2Cl_2 (2.5 mL) at -40 °C and the reaction mixture was stirred for 2 h whilst maintaining the bath temperature. After this time, the reaction mixture was cooled to -78 °C and TBACl (0.036 g, 0.13 mmol, 5.0 equiv.) was added, the reaction mixture was stirred for a further 1 h before being quenched *via* the addition of $NaHCO_3$ (10 mL). The reaction mixture was warmed to rt and then the organics were extracted with CH_2Cl_2 (3 × 5 mL), dried ($MgSO_4$), and the solvent removed *in vacuo* to give a crude residue that was further purified by flash column chromatography (5% EtOAc/ petroleum ether 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound (0.0040 g, 0.012 mmol, 48%) as a colourless oil. R_f 0.64 (20% EtOAc/ petroleum ether 40-60); ^1H NMR (600 MHz, $CDCl_3$) δ 5.83 (ddt, *J*=17.5, 10.5, 7.0, 1H, H_2C=C**H**), 5.19 – 5.04 (m, 2H, **H$_2$**C=CH), 4.44 (ddd, *J*=7.0, 3.5, 2.0, 1H, C**H**Br), 4.29 (q, *J*=6.5, 1H, CHClCH_2C**H**O), 4.09 – 3.99

(m, 2H, $\mathrm{C\mathbf{H}Cl}$, $\mathrm{H_2C{=}CHCH_2C\mathbf{H}}$), 3.90 (dt, *J*=9.0, 6.0, 1H, $\mathrm{CHBrCH_2C\mathbf{H}O}$), 3.48 (td, *J*=6.5, 3.5, 1H, $\mathrm{CH_3CH_2C\mathbf{H}}$), 2.79 (ddd, *J*=15.5, 9.0, 7.0, 1H, $\mathrm{CHBrC\mathbf{H}H'}$), 2.43 – 2.36 (m, 3H, $\mathrm{CHBrCH\mathbf{H'}}$, $\mathrm{CHClC\mathbf{H}H'C}$, $\mathrm{H_2C{=}CHC\mathbf{H}H'}$), 2.36 – 2.30 (m, 1H, $\mathrm{H_2C{=}CHCH\mathbf{H'}}$), 2.27 (ddd, *J*=13.5, 6.5, 4.5, 1H, $\mathrm{CHClCH\mathbf{H'}}$), 1.80 (dt, *J*=14.0, 7.0, 1H, $\mathrm{CH_3C\mathbf{H}H'}$), 1.68 (tt, *J*=14.0, 7.5, 1H, $\mathrm{CH_3CH\mathbf{H'}}$), 0.95 (t, *J*=7.5, 3H, $\mathrm{C\mathbf{H_3}CH_2}$); ^{13}C NMR (151 MHz, $\mathrm{CDCl_3}$) δ 133.7 ($\mathrm{H_2C{=}\mathbf{C}H}$), 118.0 ($\mathrm{H_2\mathbf{C}{=}CH}$), 86.5 ($\mathrm{H_2C{=}CHCH_2\mathbf{C}H}$), 84.0 ($\mathrm{CH_3CH_2\mathbf{C}H}$), 79.9 ($\mathrm{CHClCH_2\mathbf{C}HO}$), 79.7 ($\mathrm{CHBrCH_2\mathbf{C}HO}$), 59.3 ($\mathrm{\mathbf{C}HCl}$), 53.1 ($\mathrm{\mathbf{C}HBr}$), 40.1 ($\mathrm{CHBr\mathbf{C}H_2}$), 38.4 ($\mathrm{CHCl\mathbf{C}H_2}$), 38.0 ($\mathrm{H_2C{=}CH\mathbf{C}H_2}$), 26.3 ($\mathrm{CH_3\mathbf{C}H_2}$), 10.4 ($\mathrm{\mathbf{C}H_3CH_2}$); LRMS *m/z* ($\mathrm{ESI^+}$) 345.0 ($\mathrm{M+H^+}$, 50%), 347.0 ($\mathrm{M+H^+}$, 50%); HRMS *m/z* ($\mathrm{ESI^+}$): found 345.0227 and 347.0204, $\mathrm{C_{13}H_{20}{}^{79}BrClO_2Na}$ ($\mathrm{M+Na^+}$) requires 345.0228 and $\mathrm{C_{13}H_{20}{}^{81}BrClO_2Na}$ ($\mathrm{M+Na^+}$) requires 347.0207; $[\alpha]_D^{25}$= -4.0 (c = 0.1, $\mathrm{CHCl_3}$); IR (ν_{max} $\mathrm{cm^{-1}}$): 2968, 2917, 2159, 2025, 1642.

OR

TBACl solution (0.1 M in HFIP, 0.20 mL, 0.020 mmol, 1.2 equiv.) was added to a stirred solution of (2*S*,3*S*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl methanesulfonate **5.9** (0.0065 g, 0.017 mmol, 1.0 equiv.) in HFIP (1.6 mL) and the reaction mixture was stirred at rt for 72 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% EtOAc/ petroleum 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound (0.0030 g, 0.0094 mmol, 55%). Data as above.

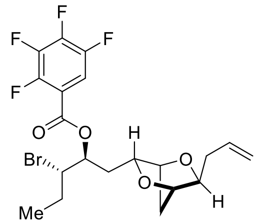
(2*R*,3*R*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl 2,3,4,5-tetrafluorobenzoate (6.10)



N,*N'*-Dicyclohexylcarbodiimide (0.033 g, 0.16 mmol, 2.5 equiv.) and 4-(dimethylamino)pyridine (0.020 g, 0.16 mmol, 2.5 equiv.) were added successively to a stirred solution of (2*R*,3*R*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-ol **3.109** (0.019 g, 0.063 mmol, 1.0 equiv.) and 2,3,4,5-tetrafluorobenzoic acid (0.031 g, 0.16 mmol, 2.5 equiv.) in CH_2Cl_2 (1 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 20% EtOAc/ petroleum ether 40-60) to give the title compound (0.026 g, 0.14 mmol, 85%) as a colourless oil. R_f 0.31 (30% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.66 (m, 1H, Ar**H**), 5.79 (ddt, *J* = 17.0, 10.0, 7.0 Hz, 1H, H_2C=C**H**), 5.44 (dt, *J* = 8.5, 3.5 Hz, 1H, C**H**OCO), 5.18 – 5.05 (m, 2H, **H_2**C=CH), 4.40 (d, *J* = 1.5 Hz, 1H, H_2CCHCH_2CHC**H**), 4.32 – 4.21 (m, 1H, C**H**$CHCH_2CHOCO$), 4.15 – 4.06 (m, 1H, C**H**Br), 3.92 – 3.82 (m, 2H, H_2CCHCH_2C**H**, C**H**CH_2CHOCO), 2.41 – 2.24 (m, 2H, H_2CCHC**H_2**), 2.01 – 1.88 (m, 4H, CHC**H_2**CH, C**H**H'CHOCO, C**H**H'CH_3), 1.86 – 1.71 (m, 2H, CH**H'**CHOCO, CH**H'**CH_3), 1.10 (t, *J* = 7.5 Hz, 3H, CH_2C**H_3**); ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.2 (**C**=O), 148.0 (dd, *J* = 269.0, 13.5 Hz, Ar**C**F), 146.7 (dd, *J* = 251.0, 13.5 Hz, Ar**C**F), 143.8 (dt, *J* = 262.0, 13.5 Hz, Ar**C**F), 141.8 (dt, *J* = 258.0, 13.5 Hz, Ar**C**F), 134.3 (H_2C=**C**H), 117.4 (H_2**C**=CH), 113.3 (dd, *J* = 20.5, 3.5 Hz, Ar**C**H), 82.3 ($H_2C=CHCH_2$**C**H), 80.5 (**C**HCH_2CHOCO), 79.9 (**C**$HCHCH_2CHOCO$), 77.1 ($H_2C=CHCH_2CH$**C**H), 73.9 (**C**HOCO), 59.1 (**C**HBr), 36.6 (**C**H_2CHOCO),

35.2 (H_2C=CH**C**H_2), 34.9 (CH**C**H_2CH), 28.5 (**C**H_2CH_3), 12.6 (CH_2**C**H_3); LRMS *m/z* (ESI$^+$) 481.1 (M+H$^+$, 50%), 483.1 (M+H$^+$, 50%); HRMS *m/z* (ESI$^+$): found 481.0633 and 483.0610, $C_{20}H_{22}{}^{79}BrF_4O_4$ (M+H$^+$) requires 481.0631 and $C_{20}H_{22}{}^{81}BrF_4O_4$ (M+H$^+$) requires 483.0613; $[\alpha]_D^{25}$= 96.8 (c = 0.1, $CHCl_3$); IR (ν_{max} cm^{-1}): 3007, 2924, 2880, 2249, 1727, 1628.

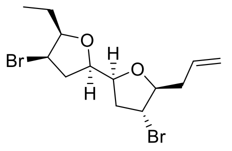
(2*S*,3*S*)-1-((3*S*,6*S*)-6-Allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl 2,3,4,5-tetrafluorobenzoate (6.11)



N,*N'*-Dicyclohexylcarbodiimide (0.017 g, 0.082 mmol, 2.5 equiv.) and 4-(dimethylamino)pyridine (0.010 g, 0.082 mmol, 2.5 equiv.) were added successively to a stirred solution of (2*S*,3*S*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-ol **5.13** (0.010 g, 0.032 mmol, 1.0 equiv.) and 2,3,4,5-tetrafluorobenzoic acid (0.016 g, 0.082 mmol, 2.5 equiv.) in CH_2Cl_2 (0.5 mL) and the reaction mixture was stirred at rt for 18 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (10% EtOAc/ petroleum ether 40-60 → 20% EtOAc/ petroleum ether 40-60) to give the title compound (0.014 g, 0.030 mmol, 94%) as a colourless oil. R*f* 0.28 (30% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.69 – 7.61 (m, 1H, Ar**H**), 5.79 (ddt, *J* = 17.0, 10.0, 7.0 Hz, 1H, H_2C=C**H**), 5.37 (td, *J* = 6.5, 3.0 Hz, 1H, C**H**OCOCAr)CHBrCH_2CH_3), 5.21 – 5.03 (m, 2H, **H$_2$**C=CH), 4.35 (d, *J* = 2.0 Hz, 1H, H_2C=CHCH_2CHC**H**), 4.30 (d, *J* = 1.5 Hz, 1H, C**H**CHCH_2CHOCO), 4.16 (ddd, *J* = 9.5, 4.0, 3.0 Hz, 1H, C**H**Br), 3.96 (dd, *J* = 9.0, 5.0 Hz, 1H, C**H**CH_2CHOCO), 3.89 – 3.83 (m, 1H, H_2C=CHCH_2C**H**), 2.44 – 2.27 (m, 2H, H_2C=CHC**H$_2$**), 2.01 – 1.91 (m, 4H, CHC**H$_2$**CH, C**H**H'CH_3, C**H**H'CHOCO), 1.91 – 1.77

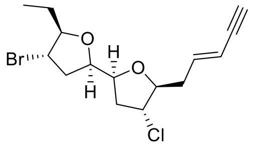
(m, 2H, CH**H'**CH$_3$, CH**H'**CHOCO), 1.11 (t, *J* = 7.5 Hz, 3H, CH$_2$C**H$_3$**); ^{13}C NMR (151 MHz, CDCl$_3$) δ 161.1 (**C**=O), 148.1 (dd, *J* = 264.0, 13.5 Hz, Ar**C**F), 146.5 (dd, *J* = 248.0, 13.5 Hz, Ar**C**F), 143.6 (dt, *J* = 264.0, 13.5 Hz, Ar**C**F), 141.3 (dt, *J* = 251.0, 13.5 Hz, Ar**C**F), 134.2 (H$_2$C=**C**H), 117.4 (H$_2$**C**=CH), 113.25 (dd, *J* = 26.0, 3.5 Hz, Ar**C**H) 82.2 (H$_2$C=CHCH$_2$**C**H), 80.6 (**C**HCH$_2$CHOCO), 79.7 (**C**HCHCH$_2$CHOCO), 77.1 (H$_2$C=CHCH$_2$CH**C**H), 74.3 (**C**HOCO), 57.9 (**C**HBr), 35.9 (**C**H$_2$CHOCO), 35.2 (H$_2$C=CH**C**H$_2$), 34.9 (CH**C**H$_2$CH), 28.4 (**C**H$_2$CH$_3$), 12.5 (CH$_2$**C**H$_3$); LRMS *m/z* (ESI$^+$) 503.0 (M+H$^+$, 50%), 505.0 (M+H$^+$, 50%); HRMS *m/z* (ESI$^+$): found 503.0451 and 505.0432, $C_{20}H_{21}{}^{79}BrF_4O_4Na$ (M+Na$^+$) requires 503.0452 and $C_{20}H_{21}{}^{81}BrF_4O_4Na$ (M+Na$^+$) requires 505.0432; $[\alpha]_D^{25}$= 36.0 (c = 0.14, CHCl$_3$); 3006, 2980, 2240, 1735, 1628.

(2*S*,2'*R*,4*R*,4'*R*,5*S*,5'*R*)-5-Allyl-4,4'-dibromo-5'-ethyloctahydro-2,2'-bifuran (6.18)



TBABr solution (0.1 M in HFIP, 0.11 mL, 0.011 mmol, 1.2 equiv.) was added to a stirred solution of (2*R*,3*R*)-1-((3*S*,6*S*)-6-allyl-2,5-dioxabicyclo[2.2.1]heptan-3-yl)-3-bromopentan-2-yl methanesulfonate **5.9** (0.0040 g, 0.010 mmol, 1.0 equiv.) in HFIP (0.9 mL) and the reaction mixture was stirred at rt for 72 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% EtOAc/ petroleum 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound (0.0020 g, 0.0035 mmol, 35%) as a colourless oil. R*f* 0.67 (20% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, CDCl$_3$) δ 5.85 (ddt, *J* = 17.0, 10.0, 7.0 Hz, 1H, H$_2$C=C**H**), 5.21 – 5.09 (m, 2H, **H$_2$**C=CH), 4.46 (ddd, *J* = 7.0, 3.5, 2.0 Hz, 1H, CH$_3$CH$_2$CHC**H**Br), 4.31 (q, *J* = 6.5 Hz, 1H, CHBrCH$_2$C**H**O), 4.17 (q, *J* = 6.0 Hz, 1H, H$_2$C=CHCH$_2$C**H**), 4.05 (dt, *J* = 7.5, 5.5 Hz, 1H, H$_2$C=CHCH$_2$CHC**H**Br), 3.93 (dt, *J* = 9.0, 6.0 Hz, 1H, CHBrCH$_2$C**H**O), 3.50 (td, *J* = 6.5, 3.5 Hz, 1H, CH$_3$CH$_2$C**H**), 2.81 (ddd, *J* = 15.5, 9.0, 7.0 Hz, 1H, $CH_3CH_2CHCHBrC\mathbf{H}H'$), 2.53 (dt, *J* = 14.0, 7.0 Hz, 1H, $H_2C{=}CHCH_2CHCHBrC\mathbf{H}H'$), 2.41 (m, 3H, $H_2C{=}CHC\mathbf{H}H'$, $H_2C{=}CHCH_2CHCHBrCH\mathbf{H'}$, $CH_3CH_2CHCHBrCH\mathbf{H'}$), 2.33 (dddt, *J* = 14.5, 7.5, 6.0, 1.5 Hz, 1H, $H_2C{=}CHCH\mathbf{H'}$), 1.81 (tt, *J* = 14.5, 7.0 Hz, 1H, $CH_3C\mathbf{H}H'$), 1.70 (ddd, *J* = 14.0, 7.5, 6.5 Hz, 1H, $CH_3CH\mathbf{H'}$), 0.98 (t, *J* = 7.5 Hz, 3H, $C\mathbf{H_3}CH_2$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 133.5 ($H_2C{=}\mathbf{C}H$), 117.9 ($H_2\mathbf{C}{=}CH$), 86.5 ($H_2C{=}CHCH_2\mathbf{C}H$), 83.9 ($CH_3CH_2\mathbf{C}H$), 80.0 ($CHBrCH_2\mathbf{C}HO$), 79.5 ($CHBrCH_2\mathbf{C}HO$), 53.0 ($CH_3CH_2CH\mathbf{C}HBr$), 48.5 ($H_2CC{=}HCH_2CH\mathbf{C}HBr$), 40.0 ($CH_3CH_2CHCHBr\mathbf{C}H_2$), 38.8 ($H_2CCHCH_2CHCHBr\mathbf{C}H_2$), 37.7 ($H_2C{=}CH\mathbf{C}H_2$), 26.1 ($CH_3\mathbf{C}H_2$), 10.3 ($\mathbf{C}H_3CH_2$); LRMS *m/z* ($ESI^+$) not found (sample not concentrated enough); HRMS *m/z* (ESI^+): found 390.9702, $C_{13}H_{20}{}^{79}Br^{81}BrO_2Na$ ($M+Na^+$) requires 390.9702; $[\alpha]_D^{25}$= -5.0 (c = 0.1, $CHCl_3$); IR (ν_{max} cm^{-1}): 2980, 1644.

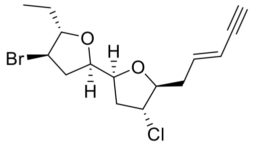
(2*R*,2'*S*,4*S*,4'*R*,5*R*,5'*S*)-4-Bromo-4'-chloro-5-ethyl-5'-((*E*)-pent-2-en-4-yn-1-yl)octahydro-2,2'-bifuran, laurendecumenyne B (3.9)



TBACl solution (0.1 M in HFIP, 0.024 mL, 0.0024 mmol, 1.2 equiv.) was added to a stirred solution of (2*R*,3*S*)-3-bromo-1-((3*S*,6*S*)-6-((*E*)-pent-2-en-4-yn-1-yl)-2,5-dioxabicyclo[2.2.1]heptan-3-yl)pentan-2-yl methanesulfonate **3.90** (0.00080 g, 0.0020 mmol, 1.0 equiv.) in HFIP (0.2 mL) and the reaction mixture was stirred at rt for 72 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% EtOAc/ petroleum 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound (0.00020 g, 0.00058 mmol, 29%). R_f 0.52 (10% EtOAc/ petroleum ether 40-60); ^1H NMR (600 MHz, $CDCl_3$) δ 6.23 (dt, *J* = 15.2, 7.2 Hz, 1H, $HC{\equiv}CCHC\mathbf{H}$), 5.59 – 5.55 (m, 1H, $HC{\equiv}CC\mathbf{H}$), 4.26 – 4.11 (m, 3H, $CHClCH_2C\mathbf{H}O$, $CHBrCH_2C\mathbf{H}O$, $CH_3CH_2C\mathbf{H}$), 4.04

– 3.97 (m, 2H, C**H**Br, C**H**CHCl), 3.96 – 3.93 (m, 1H, C**H**Cl), 2.83 (d, *J* = 2.5 Hz, 1H, **H**C≡C), 2.50 – 2.43 (m, 1H, HC≡CCHCHC**H**H’), 2.39 – 2.33 (m, 1H, HC≡CCHCHCH**H’**), 2.32 – 2.27 (m, 2H, CHBrC$\mathbf{H_2}$), 2.26 – 2.19 (m, 2H, HC≡CCHCHC$\mathbf{H_2}$), 1.72 – 1.65 (m, 1H, CH$_3$C**H**H’), 1.42 (dq, *J* = 17.0, 7.0 Hz, 1H, CH$_3$CH**H’**), 0.99 (t, *J* = 7.5 Hz, 3H, C$\mathbf{H_3}$CH$_2$); ^{13}C NMR (151 MHz, CDCl$_3$) δ 140.7 (HC≡CCHC**C**H), 111.9 (HC≡C**C**H), 88.7 (CH$_3$CH$_2$**C**H), 85.8 (**C**HCHCl), 81.9 (HC≡**C**), 79.5 (CHClCH$_2$**C**HO), 79.2 (CHBrCH$_2$**C**HO), 77.4 (H**C**≡C), 58.7 (**C**HCl), 48.8 (CH**C**HBr), 38.9 (CHBr**C**H$_2$), 38.0 (CHCl**C**H$_2$), 36.7 (HC≡CCHCH**C**H$_2$), 26.7 (CH$_3$**C**H$_2$), 10.0 (**C**H$_3$CH$_2$); LRMS *m/z* (ESI$^+$) not found (sample too weak); HRMS *m/z* (ESI$^+$) not found (sample too weak); $[\alpha]_D^{25}$ = -4.7 (c = 0.08, CHCl$_3$); IR (ν_{max} cm^{-1}): 2981, 2918, 1653, 1559.

(2*R*,2'*S*,4*R*,4'*R*,5*S*,5'*S*)-4-Bromo-4'-chloro-5-ethyl-5'-((*E*)-pent-2-en-4-yn-1-yl)octahydro-2,2'-bifuran, *ent*-notoryne (3.8)



TBACl solution (0.1 M in HFIP, 0.083 mL, 0.0083 mmol, 1.2 equiv.) was added to a stirred solution of (2*S*,3*R*)-3-bromo-1-((3*S*,6*S*)-6-((E)-pent-2-en-4-yn-1-yl)-2,5-dioxabicyclo[2.2.1]heptan-3-yl)pentan-2-yl methanesulfonate **3.91** (0.0028 g, 0.0070 mmol, 1.0 equiv.) in HFIP (0.2 mL) and the reaction mixture was stirred at rt for 72 h. After this time, the solvent was removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (5% EtOAc/ petroleum 40-60 → 10% EtOAc/ petroleum ether 40-60) to give the title compound (0.0013 g, 0.00045 mmol, 54%) as a colourless oil. R$_f$ 0.45 (20% EtOAc/ petroleum ether 40-60); ^{1}H NMR (600 MHz, CDCl$_3$) δ 6.24 (dt, *J* = 15.5, 7.5 Hz, 1H, HC≡CCHC**H**), 5.57 (dq, *J* = 16.0, 1.5 Hz, 1H, HC≡CC**H**), 4.22 (td, *J* = 7.0, 5.5 Hz, 1H, CHClCH$_2$C**H**O), 4.03 – 3.99 (m, 1H, C**H**CHCl), 3.98 – 3.95 (m, 1H, C**H**Cl), 3.91 (td, *J* = 7.5, 4.0 Hz, 1H, C**H**CHBr), 3.87 (dt, *J* = 9.0, 7.0 Hz, 1H, C**H**Br), 2.82 (d, *J* = 2.5 Hz, 1H, **H**C≡C), 2.66 (dt, *J* = 13.5, 7.0 Hz, 1H, CHBrC**H**H’), 2.53 – 2.41 (m, 1H, HC≡CCHCHC**H**H’), 2.41 – 2.33 (m, 1H, HC≡CCHCHCH**H’**), 2.32 – 2.20 (m, 2H, CHClC**H$_2$**), 2.14 (dt, *J* = 13.0, 8.5 Hz, 1H, CHBrCH**H’**), 1.75 (dqd, *J* = 15.0, 7.5, 4.0 Hz, 1H, CH$_3$C**H**H’), 1.49 (dq, *J* = 14.5, 7.5 Hz, 1H, CH$_3$CH**H’**), 1.00 (t, *J* = 7.5 Hz, 3H, C**H$_3$**CH$_2$); ^{13}C NMR (151 MHz, CDCl$_3$) δ 140.7 (HC≡CCHCH), 111.9 (HC≡C**C**H), 87.3 (CH$_3$CH$_2$**C**H), 85.7 (**C**HCHCl), 81.9 (HC≡**C**), 80.1 (HC≡CCHCHCH$_2$CHCHClCH$_2$**C**HO), 78.9 (CHBrCH$_2$**C**HO), 76.7 (H**C**≡C), 58.7 (**C**HCl), 47.3 (**C**HBr), 39.4 (CHBr**C**H$_2$), 37.9 (CHCl**C**H$_2$), 36.7 (HC≡CCHCH**C**H$_2$), 25.5 (CH$_3$**C**H$_2$), 10.0 (**C**H$_3$CH$_2$); LRMS *m/z* (ESI$^+$) not found (sample too weak); HRMS *m/z* (ESI$^+$): found 347.0410 and 349.0391, $C_{15}H_{21}{}^{79}BrClO_2$ and $C_{15}H_{21}{}^{81}BrClO_2$ (M+H$^+$) require 347.0408 and 349.0388; $[\alpha]_D^{25}$= +30.0 (c = 0.06, CHCl$_3$); IR (ν_{max} cm^{-1}): 2981, 2918, 2889, 1653, 1558.

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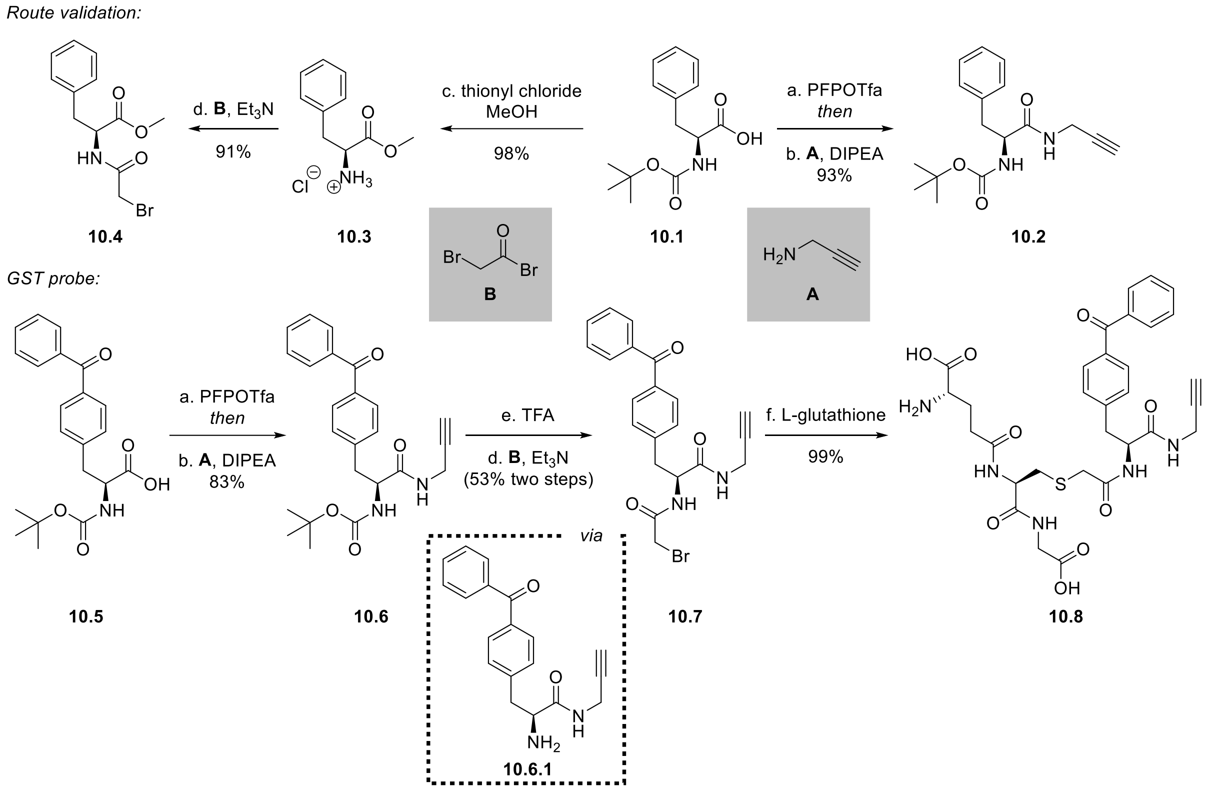
10. Appendix

10.1. Synthesis of Additional Probes

The following probes were also synthesised as part of stand-alone projects to support the van der Hoorn group, however, they warrant no discussion in the main body of the thesis. The first probe **10.8** was synthesised in order to enable further study of GSTs by Maria Font-Farre (Scheme 10.1.1).



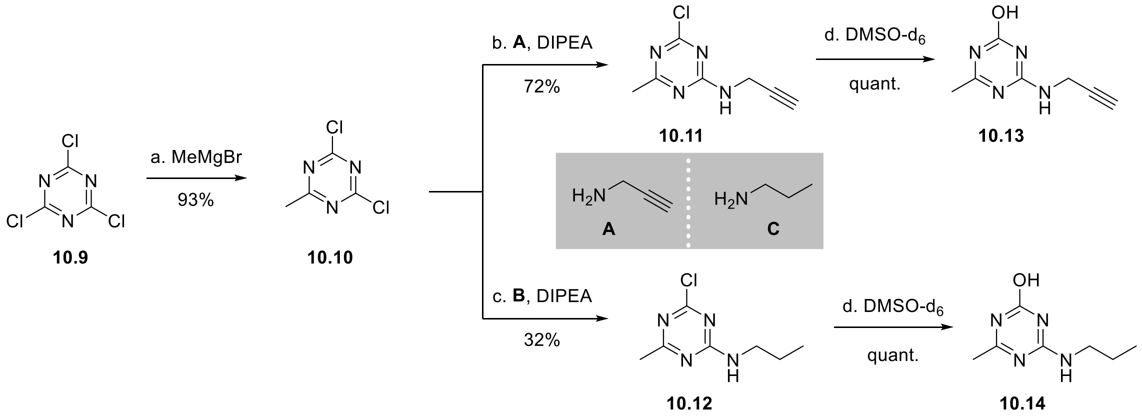
Scheme 10.1.1: Route validation and synthesis of GST probe **10.8**.

Route validation was conducted on (*tert*-butoxycarbonyl)-L-phenylalanine **10.1**, treatment of this with PFPOTfa and then subsequent addition of **A** gave **10.2** in excellent yield. Similarly formation of the methyl ester **10.3** could be achieved in excellent yield upon treatment of acid **10.1** with thionyl chloride, this was accompanied by concomitant deprotection of the

Boc-group. Treatment of amine **10.3** with **B** in the presence of triethylamine gave α-bromoamide **10.4**.

The route translated well for the synthesis of GST probe **10.8**, treatment of acid **10.5** under the established amide forming procedure gave amide **10.6** in good yield. The amide **10.6** could be Boc-deprotected with TFA and then the respective α-bromoamide **10.7** formed in 53% over the two steps. Stirring α-bromomamide **10.7** in the presence of L-glutathione in DMSO led to clean formation of the GST probe **10.8**.

In collaboration with Kyoko Morimoto, the following triazine probes **10.11** to **10.14** were synthesised in order to study ascorbate peroxidase (APX-1) enzymes in plants (Scheme 10.1.2).

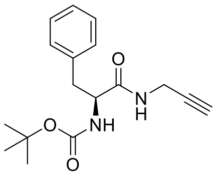


Scheme 10.1.2: Synthesis of triazine probes **10.11** to **10.14** from cyanuric chloride **10.9** for the study of APX-1 in plants.

Cyanuric chloride **10.9** was treated by methylmagnesium bromide to give triazine **10.10**. This triazine could be treated with either amine **A** or amine **C** to give the triazine probes **10.11** and **10.12** respectively. These probes could be readily hydrolysed by the water in d_6-DMSO in an NMR tube to give the probes **10.13** and **10.14**.

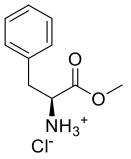
10.2. Experimental Procedures for Appendix

***tert*-Butyl *(S)*-(1-oxo-3-phenyl-1-(prop-2-yn-1-ylamino)propan-2-yl)carbamate (10.2)**



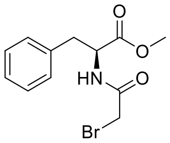
Pentafluorophenyl trifluoroacetate (0.22 mL, 1.3 mmol, 1.3 equiv.) was added dropwise to a stirred solution of (*tert*-butoxycarbonyl)-L-phenylalanine **10.1** (0.27 g, 1.0 mmol, 1.0 equiv.) and pyridine (0.097 mL, 1.2 mmol, 1.2 equiv.) in DMF (10 mL) and the reaction mixture was stirred at rt for 1 h. The reaction mixture was diluted with EtOAc (20 mL) and then washed with sat. $NaHCO_3$ solution (20 mL), and brine (3 × 30 mL). The organic layer was dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give the crude pentafluorophenyl ester which was passed through a short silica plug (EtOAc) to remove polar by- products. The crude product was redissolved in DMF (5 mL), propargyl amine (0.058 mL, 1.0 mmol, 1.0 equiv.) and DIPEA (0.16 mL, 1.1 mmol, 1.1 equiv.) was added to the stirred solution and then the reaction mixture was stirred at rt for 3 h. The reaction mixture was diluted with EtOAc (20 mL), washed with HCl (1 M, 20 mL), sat. $NaHCO_3$ (20 mL), and brine (3 × 20 mL). The organic layer was dried ($MgSO_4$), filtered, and solvent removed *in vacuo* to give the title compound as a yellow oil (0.25 g, 1.2 mmol, 93%) which required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3 (m, 3H, 3 × ArC**H**), 7.23 – 7.17 (m, 2H, 2 × ArC**H**), 6.11 (s, 1H, N**H**), 5.02 (s, 1H, N**H**), 4.33 (d, *J* = 9.0 Hz, 1H, C**H**$NHCO_2C(CH_3)_3$), 3.97 (m, 2H, NHC**H₂**C≡CH), 3.07 (d, *J* = 7.0 Hz, 2H, C**H₂**CHNHCO2C$(CH_3)_3$), 2.19 (q, *J* = 2.0, 1.0 Hz, 1H, $NHCH_2$C≡C**H**), 1.40 (s, 9H, $CHNHCO_2C$(C**H₃**)$_3$); LRMS *m/z* (ESI$^+$) 303.0 (M+H$^+$, 100%). Data are in accordance with the literature.[235]

Methyl L-phenylalaninate hydrochloride (10.3)



Thionyl chloride (0.38 mL, 5.2 mmol, 1.3 equiv.) was added dropwise to a stirred solution of (*tert*-butoxycarbonyl)-l-phenylalanine **10.1** (1.1 g, 4.0 mmol, 1.0 equiv.) in MeOH (10 mL) at 0 °C and then the reaction mixture was warmed to rt and stirred for 18h. The reaction solvent and volatile by-products were removed *in vacuo* to give the title compound as a green solid (0.84 g, 3.9 mmol, 98%) which required no further purification. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.36 – 7.25 (m, 3H, 3 × ArC**H**), 7.24 – 7.19 (m, 2H, 2 × ArC**H**), 4.28 (dd, *J* = 7.5, 6.0 Hz, 1H, CH_2**C****H**NH_3^+), 3.76 (s, 3H, CO_2C**H₃**), 3.25 – 3.08 (m, 2H, C**H₂**$CHNH_3^+$); ^{13}C NMR (101 MHz,Methanol-d_4) δ 170.5 (**C**=O), 135.3 (Ar**C**), 130.4 (ArCH), 130.2 (Ar**C**H), 129.0 (Ar**C**H), 55.2 (**C**HNH_3^+), 53.6 (CO_2**C**H_3), 37.4 (**C**$H_2CHNH_3^+$); LRMS *m/z* (ESI$^+$) 180.0 (M-Cl$^-$, 100%). Data are in accordance with the literature.[236]

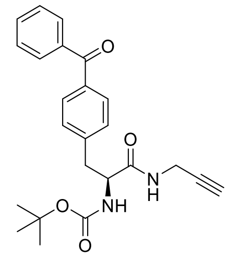
Methyl (2-bromoacetyl)-l-phenylalaninate (10.4)



Bromoacetyl bromide (0.048 mL, 0.55 mmol, 1.1 equiv.) was added dropwise to a stirred solution of methyl L-phenylalaninate hydrochloride **10.3** (0.11 g, 0.50 mmol, 1.0 equiv.) and triethylamine (0.15 mL, 1.1 mmol, 2.2 equiv.) in CH_2Cl_2 (5 mL) at 0 °C and the reaction mixture was warmed to rt and stirred for 4 h. After this time, the solvent was removed *in vacuo* and the resulting crude residue was further purified by flash column chromatography (40% EtOAc/ petroleum ether 40-60) to give the title compound as a white solid (0.14 g, 91%). ^{1}H NMR (400

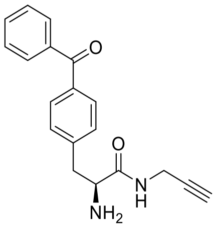
MHz, $CDCl_3$) δ 7.36 – 7.22 (m, 3H, 3 × ArC**H**), 7.16 – 7.10 (m, 2H, 2 × ArC**H**), 6.85 (d, *J* = 7.5 Hz, 1H, N**H**), 4.85 (dt, *J* = 8.0, 6.0 Hz, 1H, CH_2C**H**NHCO), 3.85 (d, *J* = 3.5 Hz, 2H, C**H₂**Br), 3.75 (s, 3H, CO_2C**H₃**), 3.15 (dd, *J* = 7.0, 6.0 Hz, 2H, C**H₂**CHNH); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.3 (**C**=O), 165.2 (**C**=O), 135.3 (**C**CH_2), 129.3 (Ar**C**H), 128.7 (ArCH), 127.4 (Ar**C**H), 53.7 (CH_2**C**HNH), 52.5 (CO_2**C**H_3), 37.7 (**C**H_2CHNH), 28.6 ($NHCO_2$**C**H_2Br); LRMS *m/z* (ESI^+) 322.0 ($M+Na^+$, 100%) 324.0 ($M+Na^+$). Data are in accordance with the literature.[237]

***tert*-Butyl *(S)*-(3-(4-benzoylphenyl)-1-oxo-1-(prop-2-yn-1-ylamino)propan-2-yl)carbamate (10.6)**



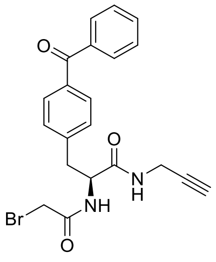
Pentafluorophenyl trifluoroacetate (0.22 mL, 1.3 mmol, 1.3 equiv.) was added dropwise to a stirred solution of (*tert*-butoxycarbonyl)-L-phenylalanine **10.5** (0.37 g, 1.0 mmol, 1.0 equiv.) and pyridine (0.097 mL, 1.2 mmol, 1.2 equiv.) in DMF (10 mL) and the reaction mixture was stirred at rt for 1 h. The reaction mixture was diluted with EtOAc (20 mL) and then washed with sat. $NaHCO_3$ solution (20 mL), and brine (3 × 30 mL), dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give the crude pentafluorophenyl ester which was passed through a short silica plug (EtOAc) to remove by- products. The crude product was redissolved in DMF (5 mL), Propargyl amine (0.058 mL, 1.0 mmol, 1.0 equiv.) and DIPEA (0.16 mL, 1.1 mmol, 1.1 equiv.) were added and then the reaction mixture was stirred at rt for 3 h. The reaction mixture was diluted with EtOAc (20 mL), washed with 1 M HCl (20 mL), sat. $NaHCO_3$ (20 mL), and brine (3 × 20 mL), dried ($MgSO_4$), filtered, and solvent removed *in vacuo* to give the title compound as a yellow oil (0.25 g, 0.83 mmol, 83%) which required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.71 (m, 4H, 4 × ArC**H**), 7.64 – 7.55 (m, 1H, ArC**H**), 7.52 – 7.44 (m, 2H, 2 × ArC**H**), 7.38 – 7.29 (m, 2H, ArC**H**), 6.15 (s, 1H, N**H**), 5.01 (s, 1H, C**H**), 4.39 (d, *J* = 8.0 Hz, 1H, C**H**$NHCO_2C(CH_3)_3$), 4.05 – 3.94 (m, 2H, NHC**H₂**C≡CH), 3.17 (qd, *J* = 14.0, 7.0 Hz, 2H, C**H₂**$CHNHCO_2C(CH_3)_3$), 2.21 (t, *J* = 2.5 Hz, 1H, $NHCH_2C$≡C**H**), 1.42 (s, 9H, $CHNHCO_2C$(C**H₃**)$_3$); LRMS *m/z* (ESI$^+$) 429.2 (M+Na$^+$, 100%). Data are in accordance with the literature.[9]

(S)-**2-Amino-3-(4-benzoylphenyl)-*N*-(prop-2-yn-1-yl)propenamide (10.6.1)**



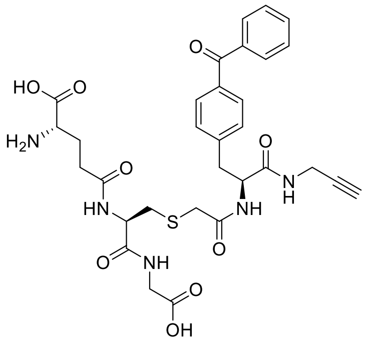
Trifluoroacetic acid (0.64 mL, 8.3 mmol, 10 equiv.) was added to a stirred solution of *tert*-Butyl *(S)*-(3-(4-benzoylphenyl)-1-oxo-1-(prop-2-yn-1-ylamino)propan-2-yl)carbamate **10.6** (0.25 g, 0.83 mmol, 1.0 equiv.) and the reaction mixture was stirred at rt for 18 h. Solvent was removed *in vacuo* to give the title compound as a yellow oil (0.20 g, 0.83 mmol, 100%) which required no further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.84 (t, *J* = 5.5 Hz, 1H, ArC**H**), 8.16 (d, *J* = 5.0 Hz, 2H, N**H**), 7.64 (ddt, *J* = 9.5, 6.5, 1.5 Hz, 4H, 4 × ArC**H**), 7.54 – 7.45 (m, 2H, 2 × ArC**H**), 7.39 – 7.31 (m, 2H, 2 × ArC**H**), 3.93 (d, *J* = 7.0 Hz, 1H, C**H**NH_2), 3.86 (dd, *J* = 5.5, 2.5 Hz, 2H, C**H₂**$CHNH_2$), 3.16 (t, *J* = 2.5 Hz, 1H, $NHCH_2C$≡C**H**), 3.12 – 2.94 (m, 2H, NHC**H₂**C≡CH); LRMS *m/z* (ESI$^+$) 307.0 (M+H$^+$, 100%). Data are in accordance with the literature.[9]

(S)-3-(4-benzoylphenyl)-2-(2-bromoacetamido)-*N*-(prop-2-yn-1-yl)propenamide (10.7)



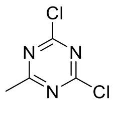
Bromoacetyl bromide (0.048 mL, 0.55 mmol, 1.1 equiv.) was added dropwise to a stirred solution of methyl *(S)*-2-amino-3-(4-benzoylphenyl)-*N*-(prop-2-yn-1-yl)propenamide **10.6.1** (0.20 g, 0.50 mmol, 1.0 equiv.) and triethylamine (0.15 mL, 1.1 mmol, 2.2 equiv.) in CH_2Cl_2 (5 mL) at 0 °C and the reaction mixture was warmed to rt and stirred for 4 h. After this time, the solvent was removed *in vacuo* and the resulting crude residue was further purified by flash column chromatography (CH_2Cl_2 → 5% acetone/ CH_2Cl_2) to give the title compound (0.10 g, 0.26 mmol, 54%) as a white solid. R_f 0.28 (5% acetone / CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 7.80 – 7.75 (m, 4H, 4 × ArC**H**), 7.63 – 7.56 (m, 1H, ArC**H**), 7.49 (dd, *J* = 8.5, 7.0 Hz, 2H, 2 × ArC**H**), 7.40 – 7.31 (m, 2H, 2 × ArC**H**), 7.06 – 7.02 (m, 1H, N**H**), 5.90 (d, *J* = 6.0 Hz, 1H, N**H**), 4.62 (td, *J* = 7.5, 6.5 Hz, 1H, $CH_2C\mathbf{H}NH$), 4.10 – 3.91 (m, 2H, $C\mathbf{H_2}CHNH$), 3.91 – 3.82 (m, 2H, $NHCO_2C\mathbf{H_2}Br$), 3.28 – 3.13 (m, 2H, $NHC\mathbf{H_2}C{\equiv}CH$), 2.22 (t, *J* = 2.5 Hz, 1H, $NHCH_2C{\equiv}C\mathbf{H}$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 196.2 (**C**=O), 169.3 (**C**=O), 165.7 (**C**=O), 140.7 (**C**CO), 137.5 (**C**CO), 136.7 ($\mathbf{C}CH_2$), 132.5 (Ar**C**H), 130.7 (Ar**C**H), 130.0 (Ar**C**H), 129.4 (Ar**C**H), 128.3 (Ar**C**H), 78.5 ($C{\equiv}\mathbf{C}H$), 72.16 ($\mathbf{C}{\equiv}CH$), 54.8 ($NH\mathbf{C}H_2Br$), 38.2 ($CH_2\mathbf{C}HNH$), 29.3 ($\mathbf{C}H_2CHNH$), 28.6 ($NH\mathbf{C}H_2C{\equiv}CH$); LRMS *m/z* ($ESI^+$) 449.0 ($M+Na^+$, 100%) 451.0 ($M+Na^+$, 100%); HRMS *m/z* ($ESI^+$): found 449.0470, $C_{21}H_{19}{}^{79}BrN_2O_3Na$ ($M+Na^+$) requires 449.0471; HRMS *m/z* (ESI^+): found 451.0450, $C_{21}H_{19}{}^{81}BrN_2O_3Na$ ($M+Na^+$) requires 451.0471.

***N^5*-*(R)*-3-((2-(((*S*)-3-(4-Benzoylphenyl)-1-oxo-1-(prop-2-yn-1-ylamino)propan-2-yl)amino)-2-oxoethyl)thio)-1-((carboxymethyl)amino)-1-oxopropan-2-yl)-l-glutamine (10.8)**



L-Glutathione reduced (0.031 g, 0.052 mmol, 1.1 equiv.) and potassium iodide (0.0040 g, 0.024 mmol, 0.50 equiv.) were added to a stirred solution of *(S)*-3-(4-benzoylphenyl)-2-(2-bromoacetamido)-*N*-(prop-2-yn-1-yl)propenamide **10.7** (0.020 g, 0.047 mmol, 1 equiv.) in DMSO (0.5 mL) and the reaction mixture was stirred at rt for 18 h. The reaction mixture was then purified by reverse- phase preparative HPLC (out of house) to give the title compound as a white solid (0.030 g, 0.046 mmol, 99%). ^{1}H NMR (400 MHz, D_2O) δ 7.67 (m, 5H, 5 × ArC**H**), 7.50 (m, 2H, 2 × ArC**H**), 7.36 (d, 2H, 2 × ArC**H**), 4.61 (t, *J* = 8.0 Hz, 1H, $H_2NC\mathbf{H}CH_2$), 4.38 (t, *J* = 9.0, Hz, 1H, $C\mathbf{H}CH_2S$), 3.85 (m, 5H, $NHC\mathbf{H_2}COOH$, $ArC\mathbf{H_2}CH$, $NHC\mathbf{H}CONH$), 3.20 (m, 2H, $NHC\mathbf{H_2}C{\equiv}CH$), 3.04 (dd, *J* = 13.5, 9.0 Hz, 2H, $CH_2C\mathbf{H_2}CONH$), 2.74 – 2.53 (m, 2H, $SC\mathbf{H_2}CONH$), 2.49 (d, *J* = 2.5 Hz, 1H, $NHCH_2C{\equiv}C\mathbf{H}$), 2.43 (t, *J* = 7.5 Hz, 2H, $SC\mathbf{H_2}CH$), 2.07 (d, J = 7.0 Hz, 2H, $CH_2C\mathbf{H_2}CHNH_2$); LRMS (ESI$^-$) 652.2 (M-H$^+$) . Data in accordance with the literature.[9]

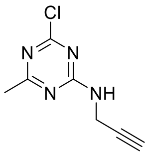
2,4-Dichloro-6-methyl-1,3,5-triazine (10.10)



Methylmagnesium bromide (3 M in Et_2O, 8.2 mL, 25 mmol, 1.5 equiv.) was added dropwise to a stirred solution of cyanuric chloride 1**0.9** (3.0 g, 16 mmol, 1.0 equiv.) in CH_2Cl_2 (20 mL) at

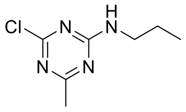
0 °C, the reaction mixture was warmed to rt and stirred for 3 h. After this time, the reaction mixture cooled to 0 °C and quenched by addition of sat. NH_4Cl solution (30 mL). The layers were separated and the organics were extracted with CH_2Cl_2 (3 × 40 mL) and then was washed with brine (2 × 50 mL), dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give the title compound (2.5 g, 15 mmol, 93%) as a yellow solid that required no further purification. ^1H NMR (400 MHz, $CDCl_3$) 2.64 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 178.6 (CCl), 171.0 (CCH_3), 25.4 (CH_3). Data are in accordance with the literature.[238]

4-Chloro-6-methyl-*N*-(prop-2-yn-1-yl)-1,3,5-triazin-2-amine (10.11)



Propargyl amine (0.20 mL, 3.0 mmol, 1.0 equiv.) and *N*,*N*-diisopropylethylamine (0.50 mL, 3.0 mmol, 1.0 equiv.) dissolved in THF (5 mL) were added dropwise to a stirred solution of 2,4-dichloro-6-methyl-1,3,5-triazine **10.10** (0.50 g, 3.0 mmol, 1.0 equiv.) in THF (2.5 mL) at -20 °C, the reaction mixture was warmed to rt and stirred for 3 h. After this time, the reaction solvent was removed *in vacuo* and the crude residue dissolved in EtOAc (10 mL) which was washed with H_2O (3 × 10 mL) and brine (20 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* giving a crude residue which was further purified by flash column chromatography (1% MeOH/ CH_2Cl_2 → 2% MeOH/ CH_2Cl_2) giving the title compound (0.39 g, 2.2 mmol, 72%) as an off white solid existing as a 1:1 mixture of rotamers/ tautomers. ^1H NMR (500 MHz, $CDCl_3$) δ 5.79 (s, br, 1H, NH), 5.77 (s, br, 1H, NH’), 4.29 (dd, *J* = 5.5, 2.5 Hz, 2H, CH_2), 4.27 (dd, *J* = 5.5, 2.5 Hz, 2H, CH'_2), 2.48 (s , 3H, CH_3), 2.43 (s , 3H, CH'_3), 2.29 (t, *J* = 2.5 Hz, 1H, C≡CH), 2.29 (t, *J* = 2.5 Hz, 1H, C≡CH’); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.8 (CCH_3), 177.9 (CCH_3), 170.9 (CCl), 170.2 (CCl), 165.7 (CNH), 165.6 (CNH), 78.8 (C≡CH), 78.6 (C≡CH), 72.6 (C≡CH), 72.4 (C≡CH), 31.1 (CH_2), 31.1 (CH_2), 25.7 (CH_3), 25.3 (CH_3); LRMS *m/z* (ESI^+) 183.0 ($M+H^+$, 75%), 185.0 ($M+H^+$, 25%); HRMS *m/z* (ESI^+): Found 183.04334, $C_7H_8N_4{}^{35}Cl$ ($M+H^+$) requires 183.04333; HRMS *m/z* (ESI^+): Found 185.04042, $C_7H_8N_4{}^{37}Cl$ ($M+H^+$) requires 185.04025.

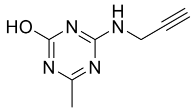
4-Chloro-6-methyl-*N*-propyl-1,3,5-triazin-2-amine (10.12)



Propylamine (0.049 mL, 0.50 mmol, 1.0 equiv.) was added dropwise to a stirred solution of 2,4-dichloro-6-methyl-1,3,5-triazine **10.10** (0.082 g, 0.50 mmol, 1.0 equiv.) and *N*,*N*-diisopropylethylamine (0.077 mL, 0.60 mmol, 1.2 equiv.) in CH_2Cl_2 (5 mL) at 0 °C and the reaction mixture was stirred for 3 h. The reaction was quenched by addition of H_2O (10 mL) and the organics extracted with CH_2Cl_2 (3 × 10 mL). The combined organics were dried ($MgSO_4$), filtered, and the solvent removed *in vacuo* to give a crude residue which was further purified by flash column chromatography (CH_2Cl_2 → 2.5% MeOH/ CH_2Cl_2) to give the title compound as a colourless oil (0.030 g, 0.16 mmol, 32%) which crystallised upon standing and exists as a pair of rotamers. R_f 0.23 (5% MeOH/CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 6.09 (s, 1H, $NHCH_2CH_2CH_3$), 5.86 (s, 1H, $NH'CH_2CH_2CH_3$), 3.46 – 3.36 (m, 4H, $NHCH_2CH_2CH_3$, $NHCH_2'CH_2CH_3$), 2.43 (s, 3H, CH_3), 2.37 (s, 3H, CH_3'), 1.61 (h, *J* = 7.5 Hz, 2H, $NHCH_2CH_2CH_3$), 1.61 (h, *J* = 7.5 Hz, 2H, $NHCH_2CH_2'CH_3$), 0.95 (t, *J* = 7.5 Hz, 3H, $NHCH_2CH_2CH_3$), 0.94 (t, *J* = 7.5 Hz, 3H, $NHCH_2CH_2CH_3'$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.3 ($NCCH_3$), 177.0 ($NC'CH_3$), 170.6 (NCCl), 169.5 (NC'Cl), 165.9 ($NCNHCH_2CH_2CH_3$), 165.8 ($NC'NHCH_2CH_2CH_3$), 42.8 ($NCNHCH_2CH_2CH_3$), 42.8 ($NCNHC'H_2CH_2CH_3$), 25.5 (CH_3), 25.1 (CH_3), 22.5 ($NCNHCH_2CH_2CH_3$),

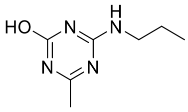
22.5 (NCNHCH$_2$C’H$_2$CH$_3$), 11.2 (NCNHCH$_2$CH$_2$CH$_3$); LRMS *m/z* (ESI$^+$) 187.0 (M+H$^+$, 75%), 189.0 (M+H$^+$, 25%); HRMS *m/z* (ESI$^+$): found 187.0746, $C_7H_{12}N_4{}^{35}Cl$ (M+H$^+$) requires 187.0745.

4-Methyl-6-(prop-2-yn-1-ylamino)-1,3,5-triazin-2-ol (10.13)



DMSO-*d*$_6$ (0.40 mL) was added to 4-chloro-6-methyl-*N*-(prop-2-yn-1-yl)-1,3,5-triazin-2-amine **10.11** (0.010 g, 0.055 mmol) in an NMR tube and the reaction mixture was heated to 130 °C for 18 h. After this time the reaction mixture was analysed by ^{1}H NMR spectroscopy to confirm the reaction had gone to completion. Characterisation was carried out directly on the sample which was then used in labelling experiments after suitable dilution. The compound exists as two non-interconvertible conformational isomers by NMR spectroscopic analysis. ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 9.46 (s, 1H, NHCH$_2$C≡CH), 8.84 (s, 1H, NH’CH$_2$C≡CH), 4.29 (d, *J* = 2.5 Hz, 2H, NHCH$_2$C≡CH), 4.07 (dd, *J* = 6.0, 2.5 Hz, 2H, NHCH$_2$’C≡CH), 3.35 (t, *J* = 2.5 Hz, 1H, NHCH$_2$C≡CH), 3.20 (t, *J* = 2.5 Hz, 1H, NHCH$_2$C≡CH’), 2.36 (s, 3H, CH$_3$), 2.19 (s, 3H, CH$_3$’); ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 172.1 (ArCCH$_3$), 168.0 (ArC’CH$_3$), 163.3 (ArCNHCH$_2$C≡CH), 159.3 (ArC’NHCH$_2$C≡CH), 153.9 (ArCOH), 148.4 (ArC’OH), 80.4 (NHCH$_2$C≡CH), 79.4 (NHCH$_2$C≡CH’), 75.4 (NHCH$_2$C≡CH), 74.2 (NHCH$_2$C≡CH’), 31.2 (NHCH$_2$C≡CH), 30.4 (NHCH$_2$’C≡CH), 22.3 (CH$_3$), 21.1 (C’H$_3$); LRMS *m/z* (ESI$^+$) 165.0 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 165.0773, $C_7H_9ON_4$ (M+H$^+$) requires 165.0771.

4-Methyl-6-(propylamino)-1,3,5-triazin-2-ol (10.14)



DMSO-*d*$_6$ (0.40 mL) was added to 4-chloro-6-methyl-*N*-propyl-1,3,5-triazin-2-amine **10.12** (0.015 g, 0.089 mmol) in an NMR tube and the reaction mixture was heated to 130 °C for 18 h. After this time the reaction mixture was analysed by ^{1}H NMR spectroscopy to confirm the reaction had gone to completion. Characterisation was carried out directly on the sample which was then used in labelling experiments after suitable dilution. The compound exists as two non-interconvertible conformational isomers by NMR spectroscopic analysis. ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 9.67 (s, 1H, $NHCH_2CH_2CH_3$), 9.62 (t, *J* = 6.0 Hz, 1H, $NH'CH_2CH_2CH_3$), 3.47 – 3.39 (m, 2H, $NHCH_2CH_2CH_3$), 3.31 – 3.23 (m, 2H, $NHCH_2'CH_2CH_3$), 2.35 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 1.61 – 1.47 (m, 4H, $NHCH_2CH_2CH_3$, $NHCH_2CH_2'CH_3$), 0.95 – 0.85 (m, 6H, $NHCH_2CH_2CH_3$, $NHCH_2CH_2CH_3'$); ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 171.9 ($ArCCH_3$), 168.7 ($ArC'CH_3$), 159.5 ($ArCNHCH_2CH_2CH_3$), 158.3 ($ArC'NHCH_2CH_2CH_3$), 150.3 (ArCOH), 147.2 (ArC'OH), 43.3 ($NHCH_2CH_2CH_3$), 43.1 ($NHC'H_2CH_2CH_3$), 22.4 ($NHCH_2CH_2CH_3$), 22.3 ($NHCH_2C'H_2CH_3$), 21.9 (CH_3), 21.5 ($C'H_3$), 11.5 ($NHCH_2CH_2CH_3$), 11.5 ($NHCH_2CH_2C'H_3$), LRMS *m/z* (ESI$^+$) 169.2 (M+H$^+$, 100%); HRMS *m/z* (ESI$^+$): found 169.1084, $C_7H_{13}ON_4$ (M+H$^+$) requires 169.1084.