Synthesis of isocyanide derived natural products with antibiotic activity

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Synthesis of isocyanide derived natural products with antibiotic activity

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Abstract

This thesis concerns itself with developing methodology for the synthesis of vinyl-isocyanide natural products and evaluating the biological properties of their common aglycone’s vinyl-isocyanide warhead.

Firstly, methodology for the total synthesis of isocyanide containing natural products isolated from terrestrial and marine sources is reviewed, describing the different strategies that have been employed for their synthesis as well as any associated biological activity.

The development of a novel isocyanide derived phosphonate ester and its use in an $E$-selective Horner-Wadsworth-Emmons protocol for the synthesis of the aglycone, phenol vinyl-isocyanide, from $p$-hydroxybenzaldehyde is then described. It was found that increasing the steric bulk of phosphonate ester groups increased the $E$-selectivity of homologation from 7 : 3 to 9 : 1. Total selectivity was subsequently achieved when temporarily reducing the acidity of the substrates through phenol silylation, although these species typically began to isomerise upon desilylation and isolated samples of phenol vinyl-isocyanide typically possessed $>95$ d.e. Phenol vinyl-isocyanide is then demonstrated as a potent melogenesis and bacterial inhibitor with low toxicity, leading us to develop some antibiotic structure activity relationships.

Having synthesised the aglycone, we intended to use this in the total synthesis of Rhabduscin and Byelyankacin. Being an unstable species, methodology was then developed for the synthesis of these rhamnopyranosides using $p$-hydroxybenzaldehyde for the glycosylation step. The previously developed Horner-Wadsworth-Emmons reagent was then used to achieve the first synthesis of Byelyankacin. Work is on-going towards completing the synthesis of a suitable rhamnopyranoside aldehyde for the synthesis of Rhabduscin using the same synthetic operations reported for Byelyankacin.

Finally, we report the synthesis of Paerucumarin, an isocyanide derived di-hydroxy coumarin species that has a related biosynthesis to Byelyankacin, Rhabduscin and their common glycone. This synthesis proceeds via the structurally related natural product, Pseudoverdin.
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Abbreviations

AFA  acetic formic anhydride
approx.  approximately
b.p.  boiling point
Bn  benzyl
br.  broad
Bu  Butyl
CDMT  2-Chloro-4,6-bis[3-(perfluorohexyl)propyloxy]-1,3,5-triazine
CPBA  chloroperbenzoic acid
CSA  camphor sulfonic acid
d  doublet
DBU  1,8-Diazabicyclo[5.4.0]undec-7-ene
dd  doublet of doublets
ddd  doublet of doublets of doublets
delta  chemical shift
DIBAL  diisobutylaluminum hydride
DIEA  N,N-Diisopropylethylamine
DIPEA  diisopropyl amine
DMAP  dimethyl aminopyridine
DMAP  dimethylaminopyridine
DMF  N,N-dimethylformamide
DMP  Dess-Martin periodinane
DMSO  dimethyl sulfoxide
DMT  dimethoxytriphenylmethyl
e.g.  for example
EC50  half maximal effective concentration
equiv.  equivalent
Et  ethyl
Fig.  figure
g  gram
h.  hour
HMPA  hexamethylphosphoramide
HWE  Horner-Wadsworth-Emmons
Hz  Hertz
i  iso
IBX  2-Iodoxybenzoic acid
IC50  half maximal inhibitory concentration
inc  including
IR  infra red
IUPAC  international union of pure and applied chemistry
J  coupling constant
KHMDs  potassium hexamethyldisilazane
LDA  lithium diisopropylamine
LHMDs  lithium hexamethyldisilazane
Ltd.  limited
m  meta
M  Mega
m  multiplet
m.p.  melting point
Me  methyl
mg  milligram
MIC  minimum inhibitory concentration
min.  minute
mL  millilitre
mM  millimolar
MOM  methoxymethyl
MRSA  methicillin resistant staphylococcus aureus
MS  Mass Spec
Ms  Mesyl
NBS  N-bromosuccinamide
NCS  isothiocyanate
ng  nano gram
NHMDS  sodium hexamethyldisilazane
NIS  N-iodosuccinimide
NMR  nuclear magnetic resonance
o  ortho
p  para
PCC  pyridinium chlorochromate
PDC  Pyridinium dichromate
Ph  phenyl
PhosMIC  methyl isocyanophosphonate
ppm  parts per million
Pr  propyl
Py.  pyridine
q  quartet
quant.  quantitative (yield)
r.t.  room temperature
SAR  structure activity relationships
t  triplet
t  tertiary
TBAF  tetra-butyl ammoniumfluoride
TBS  tertbutyl(dimethyl)silyl
Tf  triflate
TFA  trifluoroacetic acid
THF  tetrahydrofuran
TLC  thin layer chromatography
TMS  trimethyl silane
Tol.  toluene
Ts  tosyl
μg  micro gram
μM  micromolar
1 Literature Review – Methodology for the synthesis of isocyanide containing natural products (covering up to end of 2014)

1.1 Introduction

Naturally occurring isocyanides (or isonitriles) represent a diverse range of secondary metabolites that have been isolated from a select number of marine (Figure 1), terrestrial and freshwater (Figure 2) organisms, including bacteria, fungi, blue-green algae, plants, marine sponges and nudibranches. These naturally occurring isocyanides have been shown to exhibit an impressive range of biological activity, with different classes of natural product known to exhibit antibiotic, antifungal, antiviral, anticancer, herbicidal, antifouling and antimalarial activity.

Figure 1  Range of marine organisms from which isocyanide containing natural products have been isolated: A Sponge - Axinella cannabina; B Sponge - Acanthella sp.; C Sponge - Hymeniacidon amphilecta; D Nudibranch - Phylldia varicase sp.
Figure 2 Range of terrestrial and freshwater bacteria and fungi responsible for the production of isocyanide containing natural products: A Cyanobacteria - *Hapalosiphon fontinalis*; B Cyanobacteria - *Fischerella sp*; C Fungi - *Aspergillus sp*.; D Fungi - *Trichoderma hamatum*.

Figure 3 Representative range of isocyanide containing natural products that have been shown to exhibit potentially useful biological and medicinal activity.
Methodology for the synthesis of isocyanide containing natural products

The structures of this fascinating class of natural product range from simple acyclic isocyanides through to complex polycyclic isocyanides that contain multiple stereocenters, many of which exhibit useful biological and medicinal properties (Figure 3). A number of these isocyanide natural products are highly toxic and are often produced as a defence mechanism by the host organism to deter predators. They normally emit a ‘foul’ and ‘penetrating’ odour, which often affords the first clue that a natural product containing an isocyanide group has been isolated. The existence of a strong stretching absorption band in the infrared spectra at around 2100 cm\(^{-1}\) is also highly diagnostic for the presence of an isocyanide group.\(^3\) Isocyanides are isomeric to their corresponding nitriles, containing an unusually stable trivalent mono-coordinated carbon atom, whose structure is best represented as a zwitterionic species that can potentially display nucleophilic, electrophilic and carbene-like character (Figure 4).\(^4\) Consequently, it is unsurprising that many isocyanide containing natural products are relatively unstable, with their isocyanide functionalities having the capacity to react via a wide range of nucleophilic, electrophilic or oxidative pathways in biological systems.

\[
R\text{-N}\equiv\text{C} \quad \equiv \quad R\text{-N}\equiv\text{C}^- \quad \equiv \quad R\text{-NC}
\]

Figure 4 Structural representations of the isocyanide functional group.

Since the discovery of the first natural isocyanide xanthocillin 1 in 1957,\(^5\) a growing number of naturally occurring isocyanide containing natural products have been isolated from fungal, bacterial and marine organisms. The biosynthetic origin of the isocyanide group in a number of these natural products has been investigated, with their biogenesis found to be dependent on whether they originate from marine or terrestrial organisms. Marine isocyanides, derived from either sesquiterpenoid or diterpene skeletons, are generally considered to be formed from reaction of inorganic cyanide with reactive cationic or epoxy terpenoid intermediates (Figure 5),\(^6,7\) whilst terrestrial isocyanides are derived from functionalisation of amino acid precursors.\(^2,7\) For example, elegant isotopic labelling studies (\(^{13}\)C and \(^{14}\)C) have demonstrated that inorganic cyanide is incorporated into terpenoid isocyanides originating from a range of different sponges,\(^8-11\) as shown for the proposed biosynthetic pathways leading to axisonitrile-3 2 (Figure 6),\(^12,13\) and the amphilectane isocyanides 9 and 10 (Figure 7).\(^6,14\) Current evidence suggests that these terpenoid isocyanides are formed as direct metabolic products of the sponges themselves, rather than being produced by symbiotic cyanobacteria,\(^6\) with these reactive metabolites thought to be produced for their antifeedant, antifouling and/or toxic properties. Isocyanides have also been shown to be present in some species of nudibranch,
however these isocyanides are thought to accumulate by dietary transfer from sponge feeding.\textsuperscript{15}
Methodology for the synthesis of isocyanide containing natural products

Figure 7 Proposed biosynthetic pathways leading to tricyclic and tetracyclic amphilectane diterpene isocyanides 9 and 10.6,14

Figure 8 Biosynthesis of L-tyrosine derived isocyanide natural products 12,13.16,17
Many terrestrial isocyanide natural products have been shown to be derived from selective functionalisation of the skeletons of α-amino acid precursors such as L-tyrosine or L-tryptophan, with the isocyanide functionalities of these natural products retaining the nitrogen atom of the parent amino acid (Figure 8). For example, Herbert and co-workers have shown that the nitrogen atoms of xanthocillin monomethyl ether 12 are derived from the amino groups of L-tyrosine 11 in Dichotomomyces cejpii, whilst Baldwin and co-workers have demonstrated that Trichoderma hamatum converts the p-hydroxybenzyl side chain of L-tyrosine into the carbocyclic ring and acid side chain of isonitrinic acid 13. The origin of the carbon atom of the isocyanide fragment in the majority of terrestrial natural products remains unknown, although isotope labelling studies in the terrestrial cyanophyte Hapalosiphon fontinalis suggested that the isocyanide carbon in hapalindole A isonitrile originated from a C2-donor originating from tetrahydrofolate metabolism. However, investigations by Clardy and Brady into bacterial metabolic pathways have shown that the carbon atom of indole isocyanide 17 originates from the C2-sugar atom of ribulose-5-phosphate 15. In this case, the enzyme IsnA was shown to be responsible for converting the amino group of L-tryptophan 14 into the isocyanide functionality of α-isocyano acid 16, with another enzyme IsnB then catalysing its decarboxylation to introduce the vinyl functionality of indole isocyanide 17 (Figure 9). A similar pathway has been proposed for the biosynthesis of paerucumarin 19 in Pseudomonas aeruginosa, with PvcA and PvcB being responsible for formation of isocyanide intermediate 18 that is then oxidatively transformed into the natural product via the action of PvcA and PvcD (Figure 9).

Alternatively, structurally complex polycyclic monoterpene indole alkaloid isocyanides are believed to originate from chloronium induced cyclisation of a common indole (Z)-vinyl-isocyanide intermediate 20 onto a terpene derived triene precursor 21. The isopropenyl fragment of 12-epi-hapalindole E isonitrile 23 then affords a carbocation intermediate that can undergo selective cyclisation onto different positions of its indole core to afford the respective skeletons of the hapalindoles, fischerindoles, welwitindolinones and ambiguine families of isocyanide natural products (Figure 10).
Methodology for the synthesis of isocyanide containing natural products

(a) antibiotic isocyanide 17 and (b) paerucumarin 19.

Figure 9 Biosynthetic origins of the carbon and nitrogen atoms found in the terrestrial vinyl isocyanides: (a) antibiotic isocyanide 17 and (b) paerucumarin 19.

Figure 10 Biosynthesis of L-tryptophan derived monoterpenoid indole alkaloid isocyanides 22-26.
The metabolic fate of most isocyanide natural products remains unknown, however Kobayashi and co-workers have demonstrated the presence of isocyanide hydratases (R-N≡C→R-NHCHO) in *Pseudomonas putida* N19, and *Athrobacter pascens F164*, and the occurrence of an N-substituted formamide deformylase (R-NHCHO→R-NH₂) in *Athrobacter pascens F164*, which gives some insight into the hydrolytic pathways used for their metabolism/degradation. Enzymatic catalysed isocyanide hydration in *Pseudomonas fluorescens* is thought to occur via nucleophilic attack of the thiol group of a cysteine functionality at the isocyanide carbon within its active site, followed by aspartic acid mediated hydrolysis of the resultant enzyme bound thioimidate intermediate by an incipient water molecule, followed by tautomerisation to afford a formamide product (Figure 11).

Many of these fascinating isocyanide containing natural products are only available in relatively small amounts from their natural sources, with the host organism often being difficult to obtain, or requiring specialised microbiological skills to culture. Because of this scarcity, significant efforts have been directed towards the development of methodology for their synthesis, with the ultimate aim of producing significant quantities of these natural products (and their structural analogues) so that their biological activities can be fully evaluated. However, the structural complexity and diversity of many of these naturally occurring isocyanides, coupled with difficulties in handling their relatively reactive isocyanide functionalities, means that their synthesis represents a significant challenge. A number of
reviews have been published describing the range of isocyanide containing natural products that have been isolated, categorising these natural products according to their origin, structure and associated biological activity. However, despite a recent review on marine terpenoids derived from inorganic cyanide, the last detailed review describing the synthesis of isocyanides was published in 1988, since which time there have been significant developments in the methodology available for their synthesis. Consequently, this review now describes in detail the different strategies that have been developed to construct the different skeletons of this fascinating class of natural product, describing how their reactive isocyanide functionalities were introduced in each case.

1.2 Syntheses of marine isocyanides derived from sesquiterpene biosynthetic pathways

Sponges from the genus Axinella, Acanthella, Halichondria and Coicalypta have been shown to produce a range of sesquiterpene isocyanides, with over 35 different marine derived C_{16} isocyanides having been reported to date, many of which show promising biological activities (Figure 12). These naturally occurring sesquiterpenoid isocyanides belong to eight different skeletal types comprised of one or two cyclic ring systems that normally contain a single isocyanide group. These isocyanides are often isolated in the presence of their corresponding isothiocyanate and N-formamide analogues, and have also been shown to accumulate in nudibranchs species. Total syntheses of a range of bisabolene, axane, spiroaxane, guaiane, pupukeanane, trachyopsane and cadinene derived isocyanides have been reported to date.

![Figure 12 Biologically active sesquiterpene derived isocyanide natural products](image-url)
1.1.1. Syntheses of bisabolene derived isocyanides

Ichikawa reported the first synthesis of \((\pm)-3\text{-isocyanothoecinellin} 36\) (Scheme 1),\(^{31}\) a bisabolene sesquiterpene natural product that was isolated from extraction of the nudibranch *Phyllidia sp.* and shown to have anti-fouling activity against barnacle larvae.\(^{32}\) For its synthesis, allyl alcohol 29 was treated with phosphorus tribromide, followed by nucleophilic displacement of the resultant bromide with sodium benzenesulfinate to afford allyl sulfone 30, which on treatment with triflic acid underwent a Ritter reaction with acetonitrile to generate a 1 : 1 mixture of acetamides 31 and 32. After chromatographic separation, isomer 31 underwent a Julia trans-olefination reaction with isobutyraldehyde to afford diene 33, which was reacted with \(\text{Et}_3\text{O}^+\text{BF}_4^-\) and the resultant imino ether hydrolysed *in situ* with aqueous acetic acid to produce an amine 34 that was *N*-formylated using acetic formic anhydride to generate formamide 35. Dehydration of formamide 35 with triflic anhydride in the presence of *N,N*-diisopropylethylamine (DIPEA) afforded \((\pm)-3\text{-isocyanothoecinellin} 36\) that was spectroscopically identical to a natural sample.\(^{31}\)

![Scheme 1 Ichikawa’s synthesis of \((\pm)-3\text{-isocyanothoecinellin} 36\).](image-url)
Zitano et al. have reported an alternative strategy employing silver cyanide as a nucleophile to intercept a tertiary cyclohexyl carbocation for the synthesis of (±)-3-isocyanotheonellin 36 and its geometric isomer 42 (Scheme 2). A 2 : 3 mixture of tertiary alcohols 38 and 39 were first prepared in five steps from commercially available 1,4-cyclohexanedione mono-ethylene ketal 37 involving: (i) reduction of the ketone functionality; (ii) O-tosylation of the resultant secondary hydroxyl group; (iii) reaction of the tosylate with the lithium anion of ethyl phenyl sulfone; (iv) acid catalysed acetal deprotection; (v) reaction of the resultant ketone group with MeLi. Treatment of the resultant mixture of epimeric alcohols 38 and 39 with trimethylsilyl cyanide (TMSCN) in the presence of AgBF₄ resulted in an Sₙ₁ reaction to give the desired isocyanides 40 and 41 in 92% yield, in an isomeric ratio of 3 : 7 in favour of the trans-isomer 41. These isomeric isocyanides were separated by chromatography and the minor isocyanide 40 treated with 4-methylpent-2-enal under Julia conditions to afford a 2 : 1 mixture of (±)-3-isocyanotheonellin (E,E)-36 and its geometric isomer (E,Z)-42, which could be separated by preparative reverse phase HPLC.

Scheme 2 Kitano’s synthesis of (±)-(E,E)-3-isocyanotheonellin 36.
1.1.2. Syntheses of the axisonitriles

The axisonitriles are a class of sesquiterpenoid isocyanides that were isolated from chromatographic fractionation of the organic extracts of the marine sponge *Axinella cannabina*, that are thought to be a key component of the sponge’s defence mechanism.\(^{13,34,35}\) Piers *et al.* reported the first synthesis of (±)-axisonitrile-1 \(^\text{55}\) in 1987 (Scheme 3),\(^{36,37}\) which commenced with the copper catalysed conjugate addition of vinyl Grignard reagent \(^\text{43}\) to cyclopenten-1-one \(^\text{44}\) to generate \(\omega\)-chloroketone \(^\text{45}\) (as a mixture of epimers), whose potassium enolate then underwent an intramolecular alkylation reaction to afford bicyclic ketone \(^\text{46}\) in 85% overall yield. Bicyclic ketone \(^\text{46}\) was subsequently converted into its silyl enol ether, \(\alpha\)-brominated and dehydrobrominated to produce enone \(^\text{47}\), which was treated with bis-silyl ketene acetal \(^\text{48}\) in the presence of a catalytic amount of \(\text{TiCl}_4\) under Mukaiyama conditions, resulting in an intermolecular conjugate addition reaction to afford an epimeric 2 : 3 mixture of \(\gamma\)-keto-acids \(^\text{49}\) and \(^\text{50}\). This mixture of ketones \(^\text{49}/^\text{50}\) was separated by chromatography and \(\gamma\)-keto-acid \(^\text{50}\) reduced under Wolf-Kishner conditions to afford carboxylic acid \(^\text{51}\) that was converted into its corresponding acyl azide before undergoing a thermal Curtius rearrangement in the presence of 2-trimethylsilyl-ethan-1-ol to afford carbamate \(^\text{52}\). This carbamate \(^\text{52}\) was then deprotected via treatment with tetrabutyl ammonium fluoride (TBAF) to afford amine \(^\text{53}\), which was \(N\)-formylated using acetic formic anhydride to give formamide \(^\text{54}\) that could be dehydrated using *para*-toluenesulphonylchloride (TsCl) in pyridine to afford (±)-axisonitrile-1 \(^\text{55}\).\(^{36,37}\)
Hart and Guevel also reported a synthesis of (±)-axisonitrile-1 \(55\) that commenced with conjugate addition of an unsaturated cuprate reagent \(57\) to \(\beta\)-ethoxy-\(\alpha\),\(\beta\)-unsaturated ester \(56\), with ethoxide elimination generating an \(\alpha\),\(\beta\)-unsaturated ester intermediate that underwent a further conjugate addition reaction with \(\text{Me}_2\text{CuLi}\) to install the quaternary centre of ketone \(58\) (Scheme 4).\(^{38}\) This ketone \(58\) was then protected as an acetal and its alkene bond ozonised to afford an aldehyde intermediate that was reacted with Horner-Wadsworth-Emmons reagent \(59\) to afford formamide \(60\). The bicyclic \([4.3.0]\) core of the natural product was then generated via acidic deprotection of acetal \(60\) to afford a ketone intermediate, which
was treated with pyrrolidine/acetic acid to generate an enamine intermediate that underwent a stereoselective intramolecular conjugate addition reaction to afford cis-fused bicyclic ester 61. Ester 61 was then transformed into bis-alkene-formamide 54 (via acid 62 and ketone 63) using a series of cerium catalysed methylation reactions using TMSCH₂MgCl as a nucleophile to introduce both of the exo-methylene fragments. Selective hydrogenation of the isopropylene alkene functionality of formamide 54 using Crabtree’s catalyst (Ir(cod)py(PyCy)₃PF₆) was then followed by dehydration of the formamide group with TsCl in pyridine to give (±)-axisonitrile-1 55.⁴⁸

In 2008, Sha and co-workers reported an alternative synthesis of (±)-axisonitrile-1 55 using a 6-exo-dig radical cyclisation approach to construct its bicyclic core (Scheme 5).³⁹ Conjugate addition of a cuprate derived from TMS-alkynyl Grignard reagent 65 to cyclopentenone 64 was followed by in situ trapping of the resulting enolate as a silyl enol ether, which was then treated with NaI and meta-chloroperbenzoic acid (mCPBA) to generate α-iodo ketone 66 (as a mixture of epimers). Irradiation of iodide 66 with light in the presence of hexabutylditin

Scheme 4 Hart’s synthesis of (±)-axisonitrile-1 55.⁴⁸
generated a radical species that underwent an intramolecular 6-endo-dig cyclisation reaction, which was followed by Bu$_3$SnH/AIBN mediated reduction to generate a mixture of bicyclic-[4.3.0]-vinyl-silanes (Z)/(E)-68, as well as the unwanted reduced monocyclic ketone 67. After purification by chromatography the mixture of (Z)/(E)-ketones 68 was treated with trifluoroacetic acid (TFA), which resulted in protodesilylation into ketone 69 that underwent stereoselective reduction with NaBH$_4$/CeCl$_3$ to afford endo-alcohol 70. Mesylation of alcohol 70 was followed by reaction with cyanide anion resulting in clean S$_2$2 inversion to afford nitrile 71, which was then treated with iPrMgBr to afford an imine intermediate that was reduced with Li/NH$_3$ to afford amine 53. (±)-Axisonitrile-1 55 was then generated via formylation of amine 53 with acetic formic anhydride, followed by dehydration of the resultant formamide using TsCl in pyridine. Honda and co-workers subsequently published an alternative synthesis of the advanced ketone intermediate 69, enabling them to claim a formal synthesis of (±)-Axisonitrile-1 55.  

Scheme 5 Sha’s synthesis of (±)-axisonitrile-1 55.
(+)-Axisonitrile-3 2 has been isolated from the sponge Axinella cannabina and shown to demonstrate useful antimalarial activity,\textsuperscript{13,41} that was subsequently shown to be due to coordination of its isocyanide fragment to iron in heme that helps avoid its sequestration into β-hematin, thus preventing the peroxidative and glutathione-mediated pathways that lead to heme destruction.\textsuperscript{42} For the first synthesis of non-natural (-)-axisonitrile-3 2 (Scheme 6),\textsuperscript{43} Caine and Deutsch transformed bicyclic ß-hydroxy-ketone 72 into dienol acetate 73 via treatment with acetic anhydride and a catalytic amount of sulphuric acid, which was then oxidised using mCPBA to afford hydroxy-enone 75 as a major diastereomer in 56% yield (as well as enone 74 in 42% yield). After chromatographic separation, the alcohol group of hydroxy-enone 75 was protected as its labile methoxyisopropylidene ether and a second alkene bond introduced via phenylselenylation of its ketone enolate followed by selenoxide elimination/methoxymethyl ether (MOM) deprotection to afford dienone 76. This dienone 76 was then irradiated as a dilute solution in dioxane which resulted in a remarkable photochemical skeletal rearrangement reaction (for mechanistic details see scheme 6)\textsuperscript{44} to yield a single tricyclodecenone product 78. The alkene bond of enone 78 was then hydrogenated, followed by a Wittig methenylation reaction to afford alkene 79. Treatment of alkene 79 with lithium and ethylamine resulted in reductive ring-opening of the cyclopropane with inversion of configuration at C\textsubscript{7} to afford the spirocyclic ring system of a bicyclic alcohol that was converted into tosylate 80 via treatment with TsCl in pyridine. Tosylate 80 was treated with KN\textsubscript{3} in the presence of 18-crown-6, resulting in clean S\textsubscript{N}2 inversion to afford an azide intermediate that was reduced with LiAlH\textsubscript{4} to afford amine 81. This amine 81 was then N-formylated with acetic formic anhydride to afford a formamide that was dehydrated with TsCl in pyridine to afford (-)-axisonitrile-3 2.\textsuperscript{43}
Methodology for the synthesis of isocyanide containing natural products

For their synthesis of natural (+)-axisonitrile 3 (Scheme 7), Kobayashi and co-workers used Evan’s syn-aldol methodology to assemble chiral aldehyde 82, which was reacted with the lithium enolate of cyclopentanone to afford an aldol product that was oxidised using trifluoroacetic anhydride (TFAA)/dimethyl sulfoxide (DMSO) conditions to give 1,3-diketone intermediate 83. Treatment of this diketone 83 with mild acid resulted in a C₇-enol intermediate that underwent an intramolecular O-alkylation/allylic substitution reaction to afford a cyclic enol ether whose keto group was reduced to afford an alcohol that was O-silyl protected to afford bicyclic enol-ether 84. The key spiro[4,5]decane skeleton of ketone 85 was then accessed in a stereoselective manner via thermal Claisen rearrangement of unsaturated bicyclic tetrahydropyran 84. Ketone 85 was then converted into oxime 88 in eight straightforward steps involving: (i) alkene hydrogenation; (ii) enolate methylation to afford ketone; (iii) ketone reduction; (iv) and (v) E₂-elimination of a mesylate; (vi) O-silyl deprotection.
Methodology for the synthesis of isocyanide containing natural products
to afford alcohol; (vii) alcohol oxidation; (viii) formation of \((E)\)-O-methyl-oxime 86. Stereoselective reduction of \(O\)-methyl oxime 86 with NaBH\(_3\)CN afforded methoxyamine 89 with good levels of stereosecontrol, which was then transformed into an \(N\)-methoxy-formamide 90 via treatment with acetic formic anhydride. Samarium iodide was then used for reductive \(N-O\) bond cleavage to afford an \(N-H\)-formamide intermediate that was dehydrated with TsCl in pyridine to afford (+)-axisonitrile-3 2.\(^{45}\)

Scheme 7 Kobayashi’s synthesis of (+)-axisonitrile-3 2.\(^{45}\)
Hart and co-workers have also published a synthesis of (±)-axisonitrile-4 101 (Scheme 8) employing a similar strategy to that reported for their synthesis of (±)-axisonitrile-1 55 (see Scheme 4).\textsuperscript{46} Peterson olefination of the lithium enolate of tert-butyl 3-methyl-2-(trimethylsilyl)-3-butenoate 92 with aldehyde 91 resulted in a 1:2 mixture of diene esters 93 and 94 that could be separated by chromatography. Acid catalysed acetal deprotection of acetal 94 afforded ketone 95 that underwent base-catalysed intramolecular conjugate addition reaction to afford the desired tetrasubstituted alkene 97 and the unwanted disubstituted alkenes 96 (as a mixture of epimers). Tetrasubstituted alkene 97 was purified by chromatography and then hydrolysed with TFA, followed by Wittig methylenation to afford acid 98. This acid 98 was then transformed into its corresponding formamide 100 using a reductive Curtius rearrangement protocol involving reaction of its carboxylate with diphenyl phosphorazidate (DPPA), followed by reduction of the resultant isocyanate 99 with LiEt\textsubscript{3}BH (Super-Hydride). Formamide 100 was then dehydrated into its corresponding isocyanide via treatment with TsCl and pyridine to afford (±)-axisonitrile-4 101.\textsuperscript{46}
Asaoka and co-workers reported an enantioselective synthesis of \((-\)-axonitrile-4 \textsuperscript{101}) starting with stereoselective conjugate addition of the cuprate of Grignard reagent \textsuperscript{104} to enone \textsuperscript{102} to afford the quaternary stereocentre of ketone \textsuperscript{104} (Scheme 9). The silyl fragment of enone \textsuperscript{104} was then eliminated via sequential treatment with CuCl\textsubscript{2} and TBAF to afford an enone intermediate that was then hydrogenated to afford ketone \textsuperscript{105}. Treatment of ketone \textsuperscript{105} with 6M HCl\textsubscript{(aq)} resulted in acetal deprotection to afford an aldehyde intermediate that underwent a spontaneous intramolecular aldol condensation reaction to afford bicyclic enone \textsuperscript{106}. This enone \textsuperscript{106} then underwent SnCl\textsubscript{4} catalysed intermolecular conjugate addition reaction with silyl ketene acetal \textsuperscript{107} to generate a 1 : 1.5 mixture of inseparable diastereomeric esters \textsuperscript{108} and \textsuperscript{109}. However, the esters \textsuperscript{111} could be selectively epimerised into their thermodynamically more stable isomer \textsuperscript{109} via simple treatment with potassium tert-butoxide. The resultant ester \textsuperscript{109} was then reacted with Ph\textsubscript{3}PC=CH\textsubscript{2} to afford an alkene whose ester group was hydrolysed under basic conditions to afford an acid intermediate. The dianion of this acid intermediate then underwent an aldol reaction with acetone, followed by base catalysed esterification with allyl bromide to generate a 5 : 3 mixture of tertiary alcohols \textsuperscript{110} and \textsuperscript{111}, which were dehydrated with POCl\textsubscript{3} to afford the terminal alkenes \textsuperscript{112} and \textsuperscript{113}. Treatment of the mixture of alkenes \textsuperscript{112} and \textsuperscript{113} with potassium tert-butoxide resulted in alkene isomerisation to afford the thermodynamically more stable tetrasubstituted alkene \textsuperscript{114}, which was transformed into isocyanate \textsuperscript{99} via palladium catalysed allyl deprotection, followed by Curtius rearrangement of a derived acyl azide intermediate. The resultant isocyanate \textsuperscript{99} was then reduced with LiEt\textsubscript{3}BH to afford a formamide intermediate that was dehydrated with TsCl in pyridine to generate \((-\)-axonitrile-4 \textsuperscript{101}).
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Scheme 9 Asaoka’s synthesis of (-)-axisonitrile-4 101.47
1.1.3. Syntheses of isocyano-4-cadinene, isocyanoisodauc-5-ene, isocyanopupukeanane and isocyanotrachyospane derived isocyanide natural products

Matsuda and co-workers described the first enantioselective synthesis of (+)-10-isocyano-4-cadinene 27 (Scheme 10), a marine sesquiterpene with antifouling activities against barnacles, which was isolated from the nudibranch Phyllidiidae. Dihydroxylation of the alkene functionality of Evans’ N-acyl-oxazolidin-2-one 115 resulted in spontaneous lactonisation of the resultant mixture of epimeric diols to afford a 1 : 1 mixture of 5-membered lactones whose primary alcohol groups were acetylated with acetic anhydride to afford a mixture of epimeric acetates 116. This mixture of acetates 116 was converted into a mixture of epimeric triols via global reduction using LiBH$_4$, followed by selective acetonide protection of their 1,2-diol fragments, followed by Swern oxidation of their remaining primary alcohol groups to afford a 3 : 2 mixture of epimeric aldehydes 117. Horner–Wadsworth–Emmons reaction of this mixture of epimeric aldehydes 117 with the lithium anion of diethyl 2-methyl-2-propenyl phosphonate 118 afforded a diene intermediate that was subjected to a one-pot deprotection of its acetonide group. Oxidative cleavage of the resultant diol with NaIO$_4$ afforded an aldehyde intermediate that was reduced with NaBH$_4$ into alcohol 119. This alcohol 119 was protected as its acetate, which was then reacted with methyl acrylate in the presence of MeAlCl$_2$, resulting in an intermolecular Diels-Alder reaction to afford cyclohexenyl acetate 120 as a mixture of four diastereomers. This mixture of diastereomeric acetates was equilibrated via treatment with NaOMe in methanol to afford an inseparable mixture of diastereomeric trans-esters, which could be separated via treatment with 1M HCl at 0 °C resulting in selective hydrolysis of the desired trans-ester to afford its corresponding trans-acid. After separation from the unreacted trans-ester 121, trans-acid 122 was then esterified via treatment with diazomethane, its primary alcohol group converted into an iodide, and its ester group reduced with disobutylaluminium hydride (DIBAL) to afford an alcohol that was then oxidised to its corresponding aldehyde 123 via treatment with Dess-Martin Periodinane (DMP). A samarium (II) iodide mediated Barbier cyclisation reaction was then employed to generate a primary radical species from the iodide functionality of 123 that underwent intramolecular cyclisation onto its aldehyde group to afford a cyclohexanol intermediate that was oxidised to its corresponding ketone 124 using DMP. Ketone 124 was then treated with the anion of para-toluenesulfonylmethyl isocyanide (TOSMIC), which resulted in formation of a 1 : 1 mixture of epimeric cyanides 125. Reduction of this mixture of cyanides 125 with DIBAL afforded a mixture of their corresponding aldehydes, whose potassium enolate was reacted with para-methoxyphenyl chloromethyl ether to afford an aldehyde intermediate containing a
quaternary α-stereocentre. The resultant aldehyde was reduced under Wolff-Kishner conditions to afford para-methoxybenzyl (PMB) ether 126, whose PMB ether group was cleaved oxidatively using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), followed by sequential Dess-Martin and Pinnick oxidation to afford acid 127. This acid 127 was then converted into (+)-10-isocyano-4-cadinene 27 via a three step protocol involving (i) Curtius rearrangement of an acyl azide intermediate; (ii) NaBH₄ reduction of the resultant isocyanate; (iii) POCl₃/Et₃N mediated dehydration of the resultant formamide.
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In 2003, Tu and co-workers utilised silver cyanide as a nucleophile to introduce the isocyanide functionality of the guaiane sesquiterpene (-)-7-epi-14-isocyano-isodauc-5-ene 137 (Scheme 11),\textsuperscript{50} which was first isolated from the methanol extract of the marine sponge *Acanthella acuta*.\textsuperscript{51} *α*-(−)-Santonin 128 was converted into epoxide 129 via a three step protocol involving selective 2,3-alkene hydrogenation, acetal protection/alkene bond migration [(5,6)-alkene $\rightarrow$ (6,7)-alkene] and stereoselective epoxidation.\textsuperscript{52} This epoxide 129 then underwent a ZnBr$_2$ mediated epoxide ring opening/ring expansion reaction to afford the new [5.3.0] ring system of lactone 130. Ketone 130 was then reduced with NaBH$_4$, followed by acid catalysed ketal deprotection and dehydration of the resultant hydroxyl group to afford the alkene bond of enone 131. Reductive cleavage of the lactone ring of enone 131 using Zn/AcOH was followed by acid catalysed alkene isomerisation to afford an enone whose acid group was esterified using diazomethane to afford ester 132. This ester 132 was reduced to give a diol whose primary alcohol functionality was tosylated and reduced with LiAlH$_4$ to give the isopropyl fragment of alcohol 133. Subsequent reductive deoxygenation (LiAlH$_4$/TiCl$_4$) of the allylic alcohol functionality of 133 resulted in formation of a pair of regioisomeric alkenes whose allylic methyl groups were oxidised with SeO$_2$ to afford a 1:1 mixture of enals 134 and 135. Regioisomer 134 was then purified by chromatography and reduced to afford allylic alcohol 136, whose alcohol functionality was converted into its corresponding allylic iodide via treatment with NaI in the presence of trimethylsilyl chloride (TMSCI). Finally, treatment of this iodide intermediate with silver cyanide resulted in nucleophilic displacement to afford the isocyanide functionality of (−)-7-epi-14-isocyano-isodauc-5-ene 137.\textsuperscript{50}
(-)-9-Isocyanopupukeanane 147 is an allomone produced by the sponge *Hymeniacidon sp.* that accumulates in the nudibranch *Phyllidia varicosa*, which was first isolated via extraction of the foul smelling mucus of these molluscs. It is believed to be used by both species as a defence secretion, with its synthesis in racemic form having first been reported by Corey et al. in 1979 (Scheme 12). In this synthesis, indan-1-one 138 was reacted with TosMIC to afford a mixture of diastereomeric C₁-homologated nitrile intermediates that were hydrolysed into their parent acids followed by esterification to afford their diastereomeric methyl esters 139/140, via sequential treatment with potassium hydroperoxide/hydroxide and CH₃N₂. Deprotonation of this mixture of esters 139/140 afforded an enolate that was alkylated with MeI to give α-methyl ester 142 (and 15% of its (cis)-epimer 141), which upon treatment with boron tribromide resulted in cleavage of both its methyl ester and methyl ether groups to give a phenolic acid. This acid was then hydrogenated with Nishimura’s catalyst (RhO/PtO) in the presence of AcOH-HClO₄ to afford lactone 143 as the major product. Lactone 143 was then
reduced with LiAlH₄ to afford a diol whose primary alcohol functionality was mono-tosylated, and whose secondary alcohol was oxidised with pyridinium chlorochromate (PCC) to generate the desired ketone 144. Treatment of ketone 144 with potassium tert-butoxide afforded an enolate that underwent intramolecular nucleophilic displacement of its tosyl group to give the spirocyclic core of ketone 145 as the major product. After separation, ketone 145 was then converted into an oxime via treatment with hydroxylamine, which was hydrogenated with Nishimura’s catalyst to afford amine 146. This amine 146 was then N-formylated with acetic formic anhydride to afford a formamide that was dehydrated via treatment with methanesulfonylchloride MsCl in pyridine to give the natural product (±)-9-isocyanopupukeanane 147.  

Scheme 12 Corey’s synthesis of (±)-9-isocyanopupukeanane 147.  

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A second synthesis of (+)-9-isocyanopupukeanane 147 was reported simultaneously by Yamamoto and Sham in 1979, with the construction of the core ring being achieved via an intramolecular Diels-Alder reaction of triene 151 (Scheme 13). Therefore, Claisen rearrangement of a vinyl ether, prepared in situ by treatment of alcohol 148 with mercuric acetate and vinyl ethoxid, afforded an aldehyde 149 which was reacted with vinyl magnesium bromide, followed by tetrahydropyran (THP) protection, to afford O-THP protected diene 150 as a mixture of stereoisomers. Treatment of this mixture of dienes 150 with CrO₃ resulted in allylic oxidation to afford a mixture of enone intermediates that were protected as their O-silyl enol ethers via treatment with LDA/TMSCl, which after purification by chromatography gave triene 151 as a single isomer. An intramolecular Diels-Alder cyclisation reaction was then initiated via heating triene 151 in benzene in the presence of acetic acid to afford the highly strained ring system of ketone 152. Ketone 152 was then reacted with ethylene glycol to afford a cyclic ketal, whose secondary alcohol functionality was oxidised (NCS, Me₂S, Et₃N) to afford ketone 153. Ketone 153 was then reacted with 2-propenyllithium to afford an alcohol whose ketal group was deprotected and its tertiary alcohol group mesylated/eliminated to afford the diene functionality of ketone 154. Ketone 154 was then globally hydrogenated using an iridium black catalyst to afford isopropyl ketone 145 as a single diastereomer, whose corresponding oxime was then reduced using a mixture of TiCl₃/DIBAL to afford an imine that was further reduced with DIBAL to afford amine 155 as a single diastereomer. Formylation of amine 155 using acetic formic anhydride, followed by dehydration of the resulting formamide using TsCl and pyridine then gave the isocyanide natural product (+)-9-isocyanopupukeanane 145. Both Corey’s and Yamamoto’s syntheses of (+)-9-isocyanopupukeanane 147 (schemes 12 and 13) proceed through 9-pupukeanone 145 as a late stage intermediate, which has since been targeted by a number of groups as a synthon for (+)-9-isocyanopupukeanane 147’s synthesis.
Ho and Jana have reported the synthesis of the structurally related (±)-9-isocyanoopupukeanane 7 using bicyclic tertiary alcohol 156 as starting material (Scheme 14), which was treated with a mixture of perchloric and acetic acid resulting in an acid-catalysed fragmentation reaction to afford an enone, whose alkene side-chain was epoxided using hydrogen peroxide-urea in acetic anhydride to afford a mixture of epoxide diastereomers 157. This mixture of epoxides 157 was globally reduced with LiAlH₄ to afford a mixture of diols that were bis-acetylated to afford a mixture of diacetates 158. Upon heating in a sealed tube, this mixture of diacetates 158 underwent a bis-elimination reaction to afford a triene intermediate in situ, which cyclised via an intramolecular Diels-Alder reaction to afford tricyclic olefin 159 that was then hydroborated/oxidised to afford a 1:5 mixture of...
regioisomeric alcohols 160 and 161. Alcohol 161 was purified by chromatography and then oxidised with pyridinium chlorochromate (PCC) to produce a ketone intermediate that was reacted with with iPrMgBr to afford tertiary alcohol 162. Alcohol 162 then underwent a Ritter reaction with TMSCN in the presence of sulfuric acid to afford a formamide intermediate that was dehydrated using TsCl in pyridine to afford (±)-9-isocyanonopupukeanane 7.60

Corey utilised his previously synthesised lactone 143 as an advanced starting material for the synthesis of (±)-2-isocyanonopupukeanane 171 (Scheme 15), reducing it to its corresponding diol 163 using LiAlH₄, followed by selective oxidation of its secondary alcohol functionality using NBS to generate δ-hydroxy ketone 164. This ketone 164 was oxidised with PCC to give aldehyde 165, which underwent a base catalysed intramolecular aldol reaction to afford tricyclic aldol 166 as the major product. Treatment of ketone 166 with ethanedithiol and catalytic BF₃·OEt₂ gave a thioketal intermediate that underwent desulphurisation on exposure to Raney nickel in ethanol to afford an alcohol intermediate that was oxidised to its
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corresponding ketone 167 using PCC. This ketone 167 then underwent reaction with excess hydroxylamine to afford an oxime 168 that was reduced with Nishimura’s catalyst 169 to afford a 1 : 1 mixture of epimeric amines that were converted into their corresponding formamides 170 and 171 via treatment with formyl acetic acid. This mixture of formamides was separated by chromatography, and formamide 171 then converted into (±)-2-isocyanopupukeanane 172 via treatment with MsCl in pyridine.61 A variety of synthetic strategies have since emerged for the synthesis of 2-pupukeanone 167, which was an advanced intermediate in Corey’s synthesis of 2-isocyanopupeanone 172.62–72

Scheme 15 Corey’s synthesis of (±)-2-isocyanopupukeanane 172.61

Ho and co-workers have also reported the synthesis of 2-isocyanolallopupeanane 184, a marine sponge sesquiterpene from Cioca-lypta sp.,53 which is closely related to Hymeniacidon sp. (Scheme 16).60,73 Base catalysed hydrolysis of ester 173 afforded a carboxylate intermediate that underwent a phase transfer catalysed dibromocarbene mediated cyclopropanation reaction to afford a dibromocyclopropane intermediate 174 that underwent a lactonisation/cyclopropyl ring-opening/bromide elimination reaction to afford tricyclic lactone 175. This lactone 175 was treated with MeMgI and copper iodide, resulting in an Sn2 substitution reaction to afford acid 176. Reduction of acid 176 with LiAlH4, followed by
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treatment of the resultant alcohol with TsCl/pyridine afforded tosylate 177, which upon reaction with NaCN gave a nitrile that was reduced with DIBAL to give aldehyde 178. Wittig methylenation of aldehyde 178 afforded a vinyl bromide intermediate that was metalated with tert-butyl lithium followed by treatment with AcN(OMe)Me to generate diene ketone 179. This ketone 179 was heated in toluene in a sealed tube at 225 °C to facilitate an intramolecular hetero-Diels-Alder reaction to afford tetracyclic enol ether 180. Ozonolysis of enol ether 180 resulted in ring cleavage to generate ketone 181, which was converted into formamide 183 over steps including: (i) thermal E2-elimination of its acetate group; (ii) hydrogenation of the resultant alkene bond; (iii) LiAlH4 reduction of its keto group; (iv) O-benzoylation of the resultant secondary alcohol group; (iv) thermal E2-elimination of the O-benzoate group. The alkene functionality of ketone 182 then underwent a Ritter reaction with NaCN in the presence of acetic acid to afford formamide 183, which was subsequently dehydrated using TsCl and pyridine to afford the natural product (±)-2-isocyanoallopupukeanane 185.60,73
Srikrishna et al. have also reported the synthesis of (−)-2-(isocyano)trachyopsane 192,⁴⁴ whose antipode was isolated from the nudibranch *Phyllidi varicosa* and shown to have reasonable antifouling activity.⁴⁷ For its total synthesis (Scheme 17),⁴⁴ the lithium enolate of (R)-(−)-carvone underwent a stereoselective intermolecular conjugate addition reaction to methyl methacrylate, with the resultant enolate intermediate ring closing on itself at its C₃-position to afford the bicyclo[2.2.2]octanecarboxylate skeleton of ester 185. This ester 185 was then transformed into its corresponding diazoketone 186 (COCl₂, CH₂N₂) that was used as a precursor to generate a highly reactive rhodium carbenoid species that underwent a regioselective intramolecular insertion reaction at its C₂-axial C-H bond to afford the tricyclic ring skeleton of diketone 187. Diketone 187 was then regio- and stereoselectively reduced using NaBH₄ to afford an alcohol intermediate whose alkene functionality was hydrogenated to afford the isopropyl fragment of alcohol 188. Heating alcohol 188 with camphor sulphonic acid (CSA) in benzene resulted in a highly regioselective biomimetic acid catalysed rearrangement of its neopukeane skeleton to afford the trachyospane skeleton of 189. The formamide functionality of 190 was then introduced *via* an acid catalysed Ritter reaction, with its keto group then being reduced out in two steps *via* formation of a thiketal that underwent desulfurisation with Raney nickel to afford formamide 191. Subsequent dehydration of formamide 191 with TsCl in pyridine resulted in formation of *ent*-2-(isocyano)trachyopsane 192.⁴⁴
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1.1.4. Syntheses of diterpene isocyanide natural products

Three different classes of naturally occurring C_{20} diterpenoid derived isocyanides isolated from marine sources have been synthesised that contain acyclic, kalihinane, and amphilectane skeletons respectively. (+)-Geranylinaloiisosocyanide 197 was the first marine diterpene isocyanide isolated in 1974 from the marine sponge *Halichondria sp.* that was shown to be active as an antibiotic against *S. aureus*. The first synthesis of (+)-geranylinaloiisosocyanide was reported by Ichikawa *et al.* (Scheme 18) who demonstrated that treatment of geranylgeraniol 192 with trichloroacetyl isocyanate followed by methoxide afforded carbamate 193. Treatment of carbamate 194 with triflic anhydride and DIPEA at -78 °C generated a cyanate that underwent a [3,3]-rearrangement reaction in situ to afford isocyanate 194. Treatment of this isocyanate 194 with triethylaluminium chloride resulted in

Scheme 17 Srikrishna’s synthesis of (-)-2-(isocyano)trachyopsane 192.
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formation of acetamide 195 that was hydrolysed via treatment with Et$_3$OBF$_4$ to give an imino ether intermediate that was hydrolysed with aqueous acetic acid to afford its corresponding amine. Treatment of this amine intermediate with acetic formic anhydride afforded formamide 196, which was dehydrated to generate the isocyanide functionality of (±)-geranyllinaloisocyanide 197 via treatment with Ph$_3$P, CBr$_4$, and Et$_3$N.$^{78}$

In 2011, Ichikawa et al. subsequently reported a stereoselective synthesis of (+)-geranyllinaloisocyanide 197 (Scheme 19), whereby the quaternary stereocentre of N-boc-amide 200 was installed via stereoselective [3,3] sigmatropic rearrangement of a cyanate intermediate 199 that was generated in situ via treatment of carbamate 198 with PPh$_3$, CBr$_4$ and Et$_3$N. Subsequent trapping of the isocyanate product with tert-butoxide to afford N-Boc-
amine 200. Conversion of N-Boc-amine 200 into oxazolidine 202 was then achieved over four steps involving: (i) O-TBDPS deprotection; (ii) N-O-acetal formation; (iii) alkene ozonolysis; (iv) Wittig reaction of the resultant aldehyde group with ylide 201. Hydrogenation of the alkene functionality of oxazolidine 202 was followed by DIBAL reduction and reaction of the resultant aldehyde with ylide 201 to afford enone 203. Reduction of ester 202 with DIBAL resulted in formation of an allylic alcohol that was converted into its corresponding mesylate and reacted with LiBr to afford its corresponding allylic bromide 204. Treatment of bromide 204 with an excess of the carbanion of sulfone 205 afforded a cross-coupled polyene sulfone, whose sulfone group was removed via treatment with LiEt₃BH / PdCl₂(dpdp) to afford polyene 206. Hydrolysis of the oxazolidine fragment of polyene 206 was followed by alcohol oxidation with DMP to afford an N-Boc-aldehyde that underwent a Wittig methylenation reaction, followed by N-Boc deprotection using trimethylsilyl trifluoromethanesulfonate (TMSOTf) in the presence of 2,6-lutidine to give polyene amine 207. This amine 207 was then treated with acetic formic anhydride to afford its corresponding formamidine that was dehydrated via reaction with PPh₃, CBr₄ and Et₃N to afford the isocyanide functionality of (+)-geranylinaloisocyanide 197. Comparison of the sign of the specific rotation of this synthetic material enabled the configuration of the quaternary stereocentre of the natural product 197 to be assigned as (S)- for the first time.77
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A range of tricyclic kalihinol isocyanides have been isolated from the sponge species *Acanthella sp.*, which contain either cis- or trans- fused decalin ring systems that are attached to either tetrahydrofuran, tetrahydropyran or dihydropyran rings. They are thought to be produced by sponges for their antifouling and cytotoxic activities, however they have also been shown to exhibit a range of other useful biological activity. For example, (+)-kalihinol A 3 exhibits antimalarial and antibiotic activity, kalihinol F 8 inhibits topoisomerase I in starfish, and (−)-kalihinol Y 208 inhibits the growth of *Bacillus subtilis*, *S. aureus*, *Candida albicans* and displays anthelmintic activity against *Nippostrongylus brasiliensis* (Figure 13).
Wood and co-workers have reported the synthesis of (±)-kalihinol C 223 (Scheme 20), whose cis-decalin core was assembled via an intramolecular Diels-Alder reaction of triene intermediate 214 that was assembled via sequential alkylation of the enolate of β-hydroxy ester 209 with alkyl bromide 210, followed by Horner-Wadsworth-Emmons homologation of aldehyde 212 with the anion of phosphonate 213. O-Silyl deprotection of triene 214 was followed by oxidation of the resultant alcohol with PCC to afford bicyclic ketone 215. This ketone 215 was then transformed into epoxy-aziridine 217 over five steps, involving: (i) stereoselective epoxidation with dimethyldioxirane (DMDO) to afford an α-epoxide; (ii) Wittig methylenation of the ketone functionality with concomitant epimerisation resulting in a trans-fused decalin ring; (iii) dissolving metal reduction of the O-benzyl protecting group; (iv) oxidation of the resultant alcohol to afford alkene 216; (v) stereoselective aziridination with PhI=NTs/Cu(OTf)$_2$ to afford epoxy-aziridine 217 as the major diastereomer. Addition of the lithium anion of ethyl propiolate to the ketone group of epoxy-aziridine 217 gave an alcohol intermediate whose alkyne functionality was fully hydrogenated to afford saturated ester 218. Reduction of ester 218 with DIBAL afforded a lactol intermediate that was reacted with Ph$_3$P=CMe$_2$ to afford the isopropylene fragment of alcohol 219. Seleno-etherification (PhSeCl/mCPBA) of the alkene bond of 219 resulted in formation of a tetrahydrofuran ring with modest preference (3:2) for the desired diastereomer 221 over its C$_{14}$-epimer 220. After purification by chromatography, treatment of aziridine 221 with LiEt$_3$BH resulted in formation of a tertiary N-tosyl-amino group, with reaction of its epoxy group with ammonium azide then affording an azido-alcohol group with good regio-control. Simultaneous reduction of both the N-Tosyl and azido groups of this intermediate was achieved under dissolving metal conditions (Na/NH$_3$) to afford a bis-amine 222 that was N-formylated using acetic formic anhydride and dehydrated using TsCl in pyridine to afford the bis-isocyanide functionality of (±)-kalihinol C 223.
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Scheme 20 Wood’s synthesis of (±)-kalihinol C 223.\textsuperscript{86}
Miyaoka recently reported the synthesis of (+)-kalihinol A \textit{3} (Scheme 21),\textsuperscript{81} starting from chiral epoxide \textit{224} that was converted into chloride \textit{225} via a series of six reactions involving: (i) regioselective epoxide ring opening using copper catalysed addition of BrMg(CH\textsubscript{2})\textsubscript{3}OBn; (ii) bis-acetylation of the resultant diol; (iii) \textit{O}-silyl deprotection of the allylic alcohol functionality; (iv) \textit{S}\textsubscript{n}2 allylic substitution of the hydroxyl group with chloride (using Cl\textsubscript{3}CCOCCl\textsubscript{3}/Ph\textsubscript{3}P); (v) reductive deprotection of both acetate protecting groups; (vi) monopivaloylation of the primary alcohol group. Treatment of the resultant alkene \textit{225} with iodonium di-sym-collidine perchlorate resulted in intramolecular iodoetherification to afford an iodotetrahydropyran \textit{226}, whose iodide group was removed via treatment with Bu\textsubscript{3}SnH and Et\textsubscript{3}B to afford tetrahydropyran \textit{227}. The benzyl protecting group of \textit{227} was removed via hydrogenolysis and the resulting free alcohol group oxidised to an aldehyde that was reacted with vinylimagnesium bromide and the resulting mixture of epimeric alcohols that were protected as their \textit{O}-TBDMS ethers \textit{228}. The pivaloyl protecting groups of \textit{228} were removed reductively via treatment with DIBAL, and the resultant epimeric primary alcohols oxidised to an aldehyde which was reacted with the anion of CH\textsubscript{2}=C(Me)CH\textsubscript{2}P(O)Ph\textsubscript{2} to afford the diene fragment of chloride \textit{229}. Triene \textit{229} was then \textit{O}-silyl deprotected/oxidised to afford an enone that underwent an endo-selective intramolecular Diels-Alder reaction to construct the \textit{cis}-decalin core of tricyclic ketone \textit{230}. Attempts to epoxidise the alkene functionality of \textit{230} with \textit{m}CPBA afforded poor selectivity for the desired \textit{\alpha}-epoxide, therefore an indirect five-step approach was devised for its synthesis. This involved: (i) reduction of the keto functionality of \textit{230} with NaBH\textsubscript{4}; (ii) \textit{O}-tert butylsilyl (TBS) protection of the resultant alcohol; (iii) stereoselective epoxidation of the alkene group from the least hindered face to afford the desired \textit{\alpha}-epoxide; (iv) \textit{O}-silyl deprotection; (v) oxidation of the alcohol group to generate epoxy-ketone \textit{231}. Epoxy ketone \textit{231} was then treated with sodium azide in dimethyl formamide (DMF) resulting in regioselective epoxide ring-opening to afford a mixture of \textit{trans}-decalin \textit{232} and \textit{cis}-decalin \textit{233}. \textit{Trans}-decalin \textit{233} was then treated with the anion of 2-(methylsulfonyl)benzothiazole to introduce the \textit{exo}-methylene fragment of azide \textit{234} that was diastereoselectively aziridinated using PhI=NTs/Cu(OTf)\textsubscript{2} to afford an aziridine. The azide and aziridine functionalities of this intermediate were then reduced using NaBH\textsubscript{4}/NiCl\textsubscript{2} and LiBH\textsubscript{3}Et\textsubscript{3} respectively, to afford mono-\textit{N}-tosylated \textit{bis}-amine \textit{235}. The \textit{N}-tosyl group of \textit{236} was then removed reductively \textit{via} treatment with lithium in naphthalene/THF, with both amino groups then being \textit{N}-formylated with acetic formic anhydride, and the resultant \textit{N}-formamide groups dehydrated (TsCl/pyridine) to afford the \textit{bis}-isocyanide functionalities of (+)-kalihinol A \textit{3}.\textsuperscript{81}
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Scheme 21 Miyaoka’s synthesis of (+)-kalihinol A 3.  

1. H₂, Pd/C, MeOH, r.t., 99%
2. DMP, CH₂Cl₂, r.t., 98%
3. VinylMgBr, THF, 0 °C, 99%
4. TBSCI, Imidazole, THF, r.t., 99%

Scheme 21 Miyaoka’s synthesis of (+)-kalihinol A 3.  

1. 1 Mixture of diastereomers

1. TBAL, r.t.
2. DMP, NaHCO₃, CH₂Cl₂, r.t., 30 min., 99%

1. 2-Mixture of diastereomers 232 : 233

1. Li, naphthalene, THF, -78 °C to r.t., 1 h.
2. AcOCHO, CH₂Cl₂, r.t., 4 h.
3. TMSN₃, Py, CH₂Cl₂, r.t., 12 h., 74% (3 steps)
Miyaoka et al. also reported the synthesis of (-)-kalihinol Y\textsubscript{208} in 2012 (Scheme 22),\textsuperscript{87} which has been shown to demonstrate antibacterial activity by inhibiting folate acid biosynthesis,\textsuperscript{79,88} as well as anthelminintic activity.\textsuperscript{84} Azide 234, prepared in the previous synthesis of Kalihinol A\textsubscript{3} (see scheme 21), was reduced into its corresponding amine using LiAlH\textsubscript{4}, N-formylated using acetic formic anhydride to afford formamide 236 and dehydrated \textit{via} treatment with TsCl and pyridine to afford (-)-kalihinol Y\textsubscript{208}.\textsuperscript{87}

\begin{center}
\begin{tikzpicture}
\node at (0,0) {234}
ode at (1,0) {236}node at (2,0) {(-)-Kalihinol Y\textsubscript{208}};

1. LiAlH\textsubscript{4}, THF, 0 °C, 2.5 h.; Na\textsubscript{2}SO\textsubscript{4}, 10H\textsubscript{2}O, r.t., 12 h.
2. AcOCHO, CH\textsubscript{2}Cl\textsubscript{2}, r.t., 8 h.
\end{tikzpicture}
\end{center}

Scheme 22 Miyaoka’s synthesis of (-)-kalihinol Y\textsubscript{208}.\textsuperscript{87}

The amphilectane derived isocyanides are a class of diterpenoid natural products derived from fused tricyclic or tetracyclic cyclohexyl skeletons that contain one or more quaternary isocyanide groups, that have been shown to exhibit antibiotic and antimalarial activity.\textsuperscript{89–91} (±)-8,15-Diisocyano-11 (20)-amphilectene 248, originally isolated by ethanolic extraction of a freeze dried sponge, is a fused tricyclic amphilectane diterpenoid that inhibits the growth of both \textit{S. aureus} and \textit{B. subtilis}.\textsuperscript{92} Its total synthesis has been reported by Piers and Llinas-Brunet (scheme 23),\textsuperscript{93,94} using a strategy which commenced with the conversion of ketone 237 into its corresponding enol triflate that then underwent an intramolecular Pd(0)-catalysed cross coupling reaction to afford the bicyclic diene ester 238. This ester 238 underwent a thermal intermolecular Diels-Alder reaction with propenal to introduce the third cyclohexenyl ring of aldehyde 239, which was obtained as a major diastereomeric product after base catalysed epimerisation. After purification by chromatography, aldehyde 239 was reduced with NaBH\textsubscript{4} to afford an alcohol that was then tosylated and reduced out using LiEt\textsubscript{3}BH to introduce the second methyl group of ester 240. Ester 240 was then treated with CrO\textsubscript{3}-3,5-dimethylpyrazole resulting in allylic oxidation to afford an enone that underwent conjugate reduction on treatment with Na/NH\textsubscript{3} to produce a ketone 241 with a new \textit{trans}-fused ring junction. This ketone 241 was then reacted with zinc dust, CH\textsubscript{2}Br\textsubscript{2} and TiCl\textsubscript{4} to introduce an \textit{exo}-methylene group, with O-silyl ether deprotection using TBAF affording an alcohol that was oxidised with Swern’s reagent to afford an aldehyde whose \textit{α}-stereocentre was epimerised \textit{via} treatment...
with sodium methoxide. The resultant major aldehyde diastereomer 243 was purified by chromatography and subjected to a Horner-Wadsworth-Emmons reaction with the potassium salt of trimethyl 2-phosphonopropionate 244 which gave a 7:2 mixture of geometric (E)/(Z) isomers that were separated by chromatography. Treatment of the major bis-ester 245 with sodium benzeneselenoate resulted in hydrolysis of both ester groups to afford a diacid intermediate whose enone functionality was then reduced via treatment with Li/NH₃. Treatment of the resultant saturated diacid with excess LDA, followed by α-alkylation of the resultant trianion with methyl iodide, introduced the geminal dimethyl group of diacid 246. Standard Curtius rearrangement methodology ((i) DPPA; (ii) 2-TMS-EtOH; (iii) TBAF) was then used to convert both diacid groups of 246 into the diamino functionalities of 247 which was then treated sequentially with acetic formic anhydride and Ph₃P, CCl₄ and Et₃N to afford the bis-isocyanide functionality of (±)-8,15-diisocyano-11-(20)-amphilectene 248. ⁹³,⁹⁴

Scheme 23 Piers’ synthesis of (±)-8,15-diisocyano-11-(20)-amphilectene 248. ⁹³,⁹⁴
Piers and Romero also reported the synthesis of (+)-8-isocyno-10,14-amphilectadiene 254 (Scheme 24),\(^9\) which was originally isolated from the marine sponge *Hymeniacidon amphilecta* and shown to inhibit the growth of *S. aureus* and *B. subtilis*.\(^8\)^\(^9\) The previously reported ketone 241 was reduced stereoselectively with Li(\(^n\)Bu)\(_3\)BH and the resulting hydroxyl group protected as its MOM ether, followed by O-silyl deprotection to afford an alcohol group that was oxidised with PCC to afford aldehyde 250. Epimerisation of the \(\alpha\)-stereocentre of aldehyde 250 with NaOMe was followed by Wittig reaction with Ph\(_3\)P=CMe, MOM protecting group removal and oxidation of the resultant secondary alcohol with PCC to afford ketone 251. Reaction of the lithium enolate of ketone 251 with phenyltrifluoromethanesulphonimide in HMPA afforded a vinyl triflate 252 that was treated with an excess of Me\(_2\)CuLi to give a diene. This diene was subsequently hydrolysed into its corresponding acid that was then converted into (+)-8-isocyno-10,14-amphilectadiene 254 over five steps (*via* amine 253) using the Curtius rearrangement methodology developed previously for the synthesis of (+)-8,15-diisocyno-11-(20)-amphilectacene 248 (see scheme 23).\(^9\)

![Scheme 24 Piers' synthesis of (+)-8-isocyno-10,14-amphilectadiene 254.](image-url)
Miyaoka and co-workers have reported the total synthesis of (±)-7-isocyanoamphilecta-11(20),15-diene 266 (Scheme 25), an antimalarial diterpenoid originally isolated from the tropical marine sponge Cymbastela hooperi. Lactone 255 was converted into bis-enone 256 in 15 steps, which was then treated with TBSOTf and DIPEA to regioselectively afford a silyl enol ether 257 that underwent an intramolecular endo-selective Diels-Alder reaction to afford the cis-decalin ring system of ketone 258. This ketone 258 was then treated with TBAF to afford a diketone that was epimerised via treatment with DIPEA to afford the trans-decalin framework of diketone 259. The trityl group of trans-decalin 259 was then removed via hydrogenation to give a primary alcohol that was oxidised with DMP to afford an aldehyde that underwent a Wittig homologation reaction with Ph₃P=CHCO₂Et to afford α,β-unsaturated ester 260. α,β-Unsaturated ester 260 was treated with pyrrolidine to afford an enamine that underwent a stereoselective intramolecular conjugate addition reaction to give the thermodynamically more stable all-trans tricyclic perhydrophenalene ring system of a diketone that was mono-olefinated with Ph₃P=CH₂ to afford enone 261. The sterically hindered ketone group of 261 was then methylenated via a Peterson olefination reaction using Me₃SiCH₂Li to give a mixture of carboxylic acid 262 and ketone 263, with acid 262 subsequently being recycled into ketone 263 via a two-step procedure involving Weinreb amide formation and reaction with MeMgBr. The least-hindered alkene functionality of ketone 263 was then aziridinated via treatment with PhI=NTs to afford N-tosyl-aziridine 264, whose ketone group was methylenated (Ph₃P=CH₂), its aziridine ring regioselectively reduced with LiBH₂Et₃ and its N-tosyl group removed using sodium naphthalenide in THF to afford amine 265. Amine 265 was then N-formylated via treatment with acetic formic anhydride and subsequently dehydrated using TsCl in the presence of pyridine to afford the isocyanide functionality of (±)-7-isocyanoamphilecta-11(20),15-diene 266.
Pronin and Shenvi have recently reported an elegant synthesis of (±)-7-isocyano-11(20),14-epi-amphilectadiene 9 (Scheme 26), which has good antimalarial activity towards chloroquine-resistant *P. falciparum*. They employed an elegant tandem Diels-Alder strategy to rapidly assemble its tricyclic core, employing a dilute solution of dienyl-ester 267 as a substrate for a Yb(OTf)$_3$ catalysed intermolecular Diels-Alder reaction with a Danishefsky’s diene-like dendralene that gave cross conjugated enone 268, which then underwent an intramolecular thermal Diels-Alder cycloaddition upon microwave heating in ortho-dichlorobenzene to afford
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the cis-fused enone 369. This tricycle 369 was treated with Lil and 2,6-lutidine, resulting in a Krapcho decarboxylation reaction that removed the bridgehead methyl ester to afford a tricyclic enedione with the correct trans-fused ring junction. Conjugate addition of the cuprate of Grignard reagent to the resultant enone in the presence of TMSCI afforded an intermediary silyl enol ether whose C7-ketone group was reacted with MeMgBr in situ to afford hydroxy-ketone 270 after work-up. Ketone 270 was then reacted with TMSCH2Li in the presence of CeCl3, resulting in nucleophilic addition of a TMSiCH2 group to the C4-ketone group, with subsequent exposure of the alcohol functionality to TFAA selectively affording monotrifluoroacetate 271. Ionisation of trifluoroacetate 271 using the weak oxophilic Lewis acid Sc(OTf)3 in the presence of 15 equivalents of TMSCN resulted in stereoselective nucleophilic displacement of the trifluoracetate group as well as elimination of the hydroxyl and silyl groups, to install the isocyanide and alkene functionalities of (±)-7-isocyano-11(20),14-epi-amphilectadiene 9.

Scheme 26 Shenvi’s synthesis of (±)-7-isocyano-11(20),14-epi-amphilectadiene 9.

In 1987, Corey and Magriotis reported the synthesis and configuration of 7,20-diisocyanoadociane 6 (scheme 27), a marine natural product isolated from Adocia sp., isolated from a freeze-dried sponge, which has been shown to have anti-malarial activity. For its synthesis, the enolate of chiral ester 272 underwent stereoselective addition to methyl
crotonate to afford a *bis*-ester intermediate (60% ee) whose methyl ester fragment was selectively reduced to afford an alcohol group that was protected as its O-TBS ether (7,20-Scheme 27). The menthyl ester fragment of 273 was then reduced with LiAlH₄ to afford an alcohol that was oxidised to its corresponding aldehyde using PDC, which then underwent a Wittig reaction with the anion of methylallyldiphenyl phosphonium bromide to afford the diene fragment of 274. Heating triene 274 in toluene at 150 °C resulted in a thermal intramolecular Diels-Alder reaction to afford *trans*-fused decalin 275 as a major diastereomer. The O-TBS group of 275 was deprotected and the resulting hydroxyl group oxidised to afford an aldehyde that was subjected to a Horner-Wadsworth-Emmons reaction with triethyl lithio-phosphono-(E)-crotonate 276 to give a triene ester intermediate that was reduced with LiAlH₄ to afford a primary alcohol that was then O-benzyl protected to give triene 277. Subsequent heating of triene 277 in toluene at 185 °C resulted in a second thermal intramolecular Diels-Alder reaction to assemble a tetracyclic ketal with a newly formed *cis*-fused ring junction. Treatment of this ketal with Pd/C and H₂ resulted in hydrogenation of its alkene bond and cleavage of its O-benzyl group, followed by oxidation of the resultant primary alcohol to afford aldehyde 278 as a major diastereomer. Reaction of aldehyde 278 with pyrrolidine in benzene in the presence of ρTSA afforded an enamine that was oxidatively cleaved via treatment with RuO₄ to afford a ketone group. The α-stereocentre of this ketone was then epimerised under thermodynamic control via treatment with NaOMe to give an all *trans*-ketone, whose enolate was methylated via treatment with LDA and excess MeI to afford ketone 279. The ketal group of ketone 279 was then hydrolysed and the resultant diketone 280 treated with excess MeLi to afford a *bis*-tert-diol that was acylated to afford its corresponding *bis*-trifluoracetate 281 via treatment with TFAA/pyridine. *Bis*-trifluoroacetate 281 was then treated with TMSCN and TiCl₄, directly affording a mixture of four *bis*-isocyanide diastereomers that were separated by chromatography, thus allowing the desired scalemic natural product (+)-7,20-diisocyanoadociane 6 (60% ee) to be isolated after chromatographic purification. More recently Miyaoka and co-workers have reported a multi-step synthesis of Corey’s diketone intermediate 280 which represents a formal synthesis of (+)-7,20-diisocyanoadociane 6. Additionally, Mander has published a formal synthesis of (+)-diisocyanoadociane 6 involving synthesis of diamine that had previously been shown as a viable intermediate for its synthesis by Garson and co-workers.
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Scheme 27 Corey’s synthesis of (+)-7,20-diisocyanoadociane 6.99
1.3 Syntheses of isocyanides derived from L-tyrosine or L-tryptophan

A range of isocyanide derived natural products derived from the \(\alpha\)-amino acids L-tyrosine and L-tryptophan have been isolated that may be classified into three categories: (i) Xanthocillins isolated from terrestrial and marine sources that contain a bis-vinyl isocyanide core; (ii) Terrestrial cyclopentyl isocyanides that contain dermadin or trichoviridin skeletons isolated from soil bacteria; (iii) Monoterpenoid indole alkaloid isocyanides such as the hapalindoles, fischerindoles, welwitindolinones and ambiguines that have been isolated from algae and cyanobacteria (Figure 14).  

![Chemical structures](image)

Figure 14 A representative range of L-tyrosine and L-indole derived isocyanide natural products.

1.1.5. Xanthocillin type isocyanides

Xanthocillin dimethyl ether \(\text{1}\), the first known natural isocyanide, was first isolated from \textit{Pencillium notatum} in 1957, with structural variants subsequently having been reported from a range of terrestrial and marine sources that have been shown to demonstrate a wide range of biological activity, including antibiotic activity, antiviral activity, antifungal activity, and as an agonist of thrombopoietin receptor (Figure 15).
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Figure 15 Biologically active xanthocillin derivatives 286-288.96,99,107,108

For their synthesis of Xanthocillin dimethyl ether 1 (Scheme 28),109 Hagedorn et al. generated the enolate of 4-methoxy-α-formylaminoacetophenone 289 using potassium tert-butoxide, which on reaction with iodine resulted in oxidative dimerisation to afford bis-formamide 290. Reduction of diketone 290 with NaBH₄ generated a 1,4-diol that was treated with POCl₃ to generate β,β’-dichlorodiisocyanide 291, which underwent bis-E1cB elimination on treatment with KOH and pyridine to afford xanthocillin dimethyl ether 1.109,110

Yamaguchi and Tatsuta have developed an efficient synthesis of xanthocillin dimethylether 1 (Scheme 29),104 involving hydrostannylation of propiolic acid 292 followed by an oxidative Baumgarten rearrangement to afford isocyanate 293 that was then reduced with LiBH₄ to afford formamide 294 via treatment with LiEt₃BH. Two equivalents of [(E)-vinyl]stannane 294 were then homocoupled using a modified Stille reaction that employed a mixed palladium(II)-copper(II) catalytic system to generate (Z,Z)-bis-formamide 295 in 62% yield. The final step of
the synthesis involved dehydration of \((Z,Z)\)-bis-formamide 295 using POCl₃ in pyridine to generate the bis-isocyanide functionality of xanthocillin dimethylether 1 in 59% yield.\(^{104}\)

![Scheme 29 Tatsuta’s synthesis of xanthocillin dimethylether 1.\(^{104}\)](image)

Xanthocillin 299 has also been shown to have agonist activity against thrombopoietin receptors in a human leukaemia cell line,\(^{106}\) with Tatsuta and co-worker preparing analogues (Scheme 30) via a modification of their original xanthocillin dimethyl ether 1 synthesis (See scheme 43) involving copper catalysed oxidative coupling of an O-TBS-protected vinyl-stannane 296 to afford bis-formamide 297. Generation of the bis-isocyanide functionality of 298 was achieved via treatment of bis-formamide 297 with triphosgene, followed by deprotection of the O-silyl groups of 298 with TBAF to afford xanthocillin 299.\(^{106}\)

![Scheme 30 Synthesis of xanthocillin 299.\(^{106}\)](image)
1.1.6. Syntheses of cyclopentanoid isocyanides

A series of fungal cyclopentyl derived antibiotics produced by the genus *Trichoderma hamatum* have been isolated from soil cultures,\(^{17,111–115}\) whose antibiotic activity effectively inhibit the growth of rumen bacteria.\(^{116}\) These naturally occurring cyclopentyl isocyanides are generally unstable and are difficult to isolate and handle, and are known to inhibit tyrosinase enzymes responsible for melanin biosynthesis as well as acting as inhibitors of multidrug transporters (Figure 16).

![Figure 16](image)

Figure 16 Biological activities of cyclopentyl isocyanide natural products 13, 282, 283 and 391.\(^{117–120}\)

The first synthesis of this family of natural products was carried out by Fukuyama and Yung (Scheme 31),\(^{115}\) who synthesised (±)-methyl 3-(3-isocyanato-6-oxabicyclo[3.1.0]hex-2-en-5-yl)-2-propenoate 305 as a stable analogue of its parent acid dermadin 282. Their synthesis started with treatment of C-tosyl-aza-diene 300 with the lithium enolate of methyl acetate to afford \(\alpha,\beta\)-unsaturated ester 301. Conjugate reduction of ester 301 with sodium cyanoborohydride, followed by \(N\)-formylation with formic acid/acetic anhydride in the presence of pyridine and epoxidation of its alkene functionality with \(m\)CPBA generated exo-epoxide 302. Potassium tert-butoxide was then used as a base to fragment its bicyclic system, with concomitant elimination of the epoxide group occurring to afford diene-ester 303. Subsequent *syn*-mono-epoxidation of diene ester 303 with \(m\)CPBA, was followed by \(O\)-mesylation and dehydration of the formamide functionality (\(\text{COCl}_2/\text{Et}_3\text{N}\)) to afford vinyl-isocyanide 304 in 89% yield. Dermadin methyl ester 305 was then produced in 53% yield via treatment of ester 304 with potassium tert-butoxide, resulting in E1cB elimination of the mesylate group to introduce its vinyl group.\(^{115}\)
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Scheme 31 Fukuyama’s synthesis of dermadin methyl ester (±)-305.

Baldwin et al. employed phosgene as an alternative formamide dehydrating agent in their low-yielding synthesis of the spirocyclic cyclopentyl isocyanide (±)-311 (Scheme 32), which commenced with treatment of cyclopentene 306 with peracetic acid to generate syn-epoxide 307 that was then dehydrated with COCl₂ to afford isocyanide 308. This isocyanide 308 underwent base promoted elimination of its epoxy group to afford vinyl-isocyanide 309 followed by oxidation of its allylic alcohol group with PCC to afford enone 310. The synthesis of the natural product 311 was completed via addition of (Z)-β-lithioacrylate to γ-ketoisocyanide 310, followed by ring closure using N,N’-dicyclohexylcarbodiimide (DCC) to facilitate lactonisation.
Baldwin and co-workers carried out the synthesis of another cyclopentanoid isocyanide antibiotic isonitrinic acid F 13 produced by the fungi genus *Trichoderma* (Scheme 33).

Formamide 312 was treated with *N*-bromosuccinimide (NBS) in the dark to afford bicyclic dihydrooxazole 313 that underwent Stille coupling with a β-stannyl acrylate 314 to afford α,β-unsaturated ester 315. Mild hydrolysis of the oxazole fragment of 316 using aqueous acetic acid was followed by treatment of the resultant formamides 316 (7:3 mixture in favour of (E)-isomer) with TsCl/Et₃N which gave a 3:1 mixture of O-tosyl-isocyanide 317 (50:50 mixture of (E)-/(Z)-isomers) and dienyl isocyanide 318. Treatment of this mixture of isocyanides with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) resulted in elimination/alkene isomerisation to afford a 4:1 mixture of diene 319 and its regioisomer 318 under thermodynamic control. Treatment of diene 319 with iodine in d⁶-benzene resulted in a 4:1 mixture of dienes 320 and 319 that could be separated by chromatography. Finally, subsequent hydrolysis of diene ester 320 with LiOH in THF gave isonitrinic acid F 13.

Scheme 33 Baldwin’s synthesis of isonitrinic acid F 13.
Baldwin also reported the total synthesis of isonitrin B \textsuperscript{329} (Scheme 34),\textsuperscript{123} another fungal cyclopentyl natural product isolated from the genus \textit{Trichoderma}. O-Silyl protected fulvene \textsuperscript{321} was treated with pTSA to afford 1-acetyl cyclopentadiene that then underwent a Diels-Alder reaction with a nitroso dienophile generated \textit{in situ} from the action of tetraethylammonium periodate on \textit{N}-hydroxycarbamate. The resultant bicyclic ketone \textsuperscript{322} was selectively reduced \textit{via} treatment with K-Selectride to afford an alcohol \textsuperscript{323} that was then \textit{O}-silyl protected. Subsequent treatment of the resultant bicyclic silyl ether with sodium amalgam in buffered methanol resulted in cleavage of its \textit{N-O} bond to afford cyclopentenyl carbamate \textsuperscript{324} that underwent a directed epoxidation reaction with \textit{mCPBA} to afford a \textit{syn}-epoxide. The Cbz-group of this epoxide was then removed \textit{via} hydrogenolysis to afford an amine intermediate that was reacted with tolylthiooxime and TsCl in the presence of propylene oxide and molecular sieves to afford tolylthiooxime \textsuperscript{325}. This tolylthiooxime \textsuperscript{325} was then treated with triphenylphosphine and acetic formic anhydride in the presence of propylene oxide, which resulted in insertion of triphenylphosphine into the \textit{N-STol} bond, followed by rearrangement and loss of triphenylphosphine oxide, to afford an amine \textsuperscript{326} that was then \textit{N}-formylated with formic acetic anhydride to give formamide \textsuperscript{327} in low yield. Dehydration of formamide \textsuperscript{327} was achieved \textit{via} treatment with triflic anhydride and DIPEA to afford vinyl isocyanide \textsuperscript{328}, with isonitrin B \textsuperscript{329} then being generated by desilylation of its hydroxyl groups using TBAF.\textsuperscript{123} Baldwin subsequently reported the use of triflic anhydride and DIPEA as a simple way of converting the vicinal diol fragment of isonitrin B \textsuperscript{329} into the second epoxide functionality of isonitrin A \textsuperscript{330}.\textsuperscript{124}

\begin{center}
\includegraphics[width=\textwidth]{sythesis.png}
\end{center}

\textit{synthesis continued overleaf}
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Scheme 34 Baldwin’s synthesis of (±)-isonitrin A 330 and (±)-isonitrin B 329.\textsuperscript{123, 124}

Baldwin \textit{et al.} have also described the use of dimethylidioxirane (DMDO) as a dehydrating agent for the conversion of an $N$-formamide group into an isocyanide group as part of their 1996 synthesis of (±)-trichoviridin 283 (Scheme 35),\textsuperscript{125} which is known to inhibit mushroom tyrosinase and melanin formation in larval haemolymph.\textsuperscript{126} Treatment of tolylthiooxime 330 with polymer bound Ph$_3$P and acetic formic anhydride in the presence of propylene oxide as an acid scavenger generated a mixture of $\alpha$-acetoxy-formamide 321 and vinyl-formamide 332. $\alpha$-Acetoxy formamide 331 was eliminated to give its vinylogous equivalent 332 via treatment with DBU, which was then dehydrated \textit{in situ} via sequential treatment with DMDO and triflic anhydride to afford vinyl-isocyanide 333 as the only isolable product in 50% yield. Isocyanide 333 was then protected as its dibromoimino derivative 334 via treatment with amberlyst A-26.
Br$_3^-$, allowing for methyl(trifluoromethyl)dioxirane to be used to introduce the second anti-epoxide ring of a bis-epoxide intermediate. Deprotection of the dibromoimino functionality of this bis-epoxide was then achieved via treatment with triethylphosphite to afford isocyanide 335 that was O-silyl deprotected with TBAF to give (±)-trichoviridin 283.$^{125}$

Scheme 35 Baldwin’s synthesis of (±)-trichoviridin 283.$^{125}$

Taber et al. subsequently reported an enantioselective synthesis of (−)-isonitrin B 329 (Scheme 36)$^{127}$ using chiral allylic alcohol 340 that was prepared in four steps from dibromo-diene 336 involving: (i) Sharpless asymmetric dihydroxylation reaction; (ii) bis-O-TBS protection to afford 337; (iii) lithiation of the dibromoalkene fragment to afford an alkynyl anion that underwent a non-selective aldol reaction with aldehyde 338; (iv) partial hydrogenation of the resultant alkyne to afford a cis-alkene. The resultant mixture of allylic diols 339/340 were separated by chromatography and alkene 340 subjected to a directed epoxidation reaction using mCPBA to afford a 3 : 1 mixture of epoxides 341 and 342 that were separated by chromatography.$^{127}$

Epoxide 341 was oxidised with PCC to afford a ketone 343 that was treated with the anion of (trimethylsilyl)diazomethane 344 (TMSDM) to afford an alkylidene carbene species 345 that underwent an intramolecular C-H insertion reaction to give cyclopentenyl epoxide 346. The primary silyloxy group of epoxide 346 was deprotected via treatment with TBAF and the resultant alcohol oxidised in a stepwise fashion to afford its corresponding acid 347 that was
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then treated with DPPA to give its corresponding acyl azide. Thermolysis of this acyl azide resulted in Curtius rearrangement to afford an isocyanate intermediate that was reduced with NaBH$_4$ to afford the desired formamide 348. Triflic anhydride in the presence of DIPEA was then used to facilitate dehydration of formamide 348 into its corresponding isocyanide, which upon HF/pyridine mediated O-silyl deprotection generated (-)-isonitrin B 329.$^{127}$

Scheme 36 Taber’s synthesis of (-)-isonitrin B 329.$^{127}$
1.1.7. Syntheses of monoterpenoid indole alkaloid isocyanides

More than 30 different chlorinated and non-chlorinated monoterpenoid indole isocyanides have been isolated from filamentous blue-green algae, where they have often been isolated in the presence of structurally related isothiocyanate analogues. This class of isocyanide natural product include the hapalindoles, fischerindoles, welwitindolinones and ambiguines, all of which contain an isocyanide group attached to $C_{11}$ of their respective polycyclic skeletons. A number of these naturally occurring isocyanides have been shown to exhibit useful biological activity (Figure 17), including antibacterial, antymycotic, antifungal, antialgal and insecticidal activity. The hapalindoles, isolated from the blue-green algae *Hapalosiphon fontinalis* (Ag.) Bornet (strigonemataceae), are indole alkaloids that Moore *et al.* originally isolated through culturing a terrestrial cyanophyte present in a soil sample.128,129 Moore and co-workers were also responsible for isolating the first ambiguines, fischerindoles and welwitindolinones from cultures of *Hapalosiphon welwitshii* and *Westiella intricata*.130,131

![Chemical structures of isocyanides](image)

**Figure 17** Monoterpenoid indole alkaloid isocyanides 23, 349–353.130,132–136

The first synthesis of hapalindole J isonitrile 349 was described by Natsume and co-workers in 1989 (Scheme 37).137,138 Their synthesis commenced with SnCl$_4$ catalysed coupling of a 5:2 mixture of sily-enol ethers 354/355 with a tertiary cationic species derived from alcohol 356, with the resultant mixture of structural isomers 357 and 358 then being treated with BF$_3$.OEt$_3$.
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to induce electrophilic cyclisation of the indole ring onto its keto group to afford alkene-indole 359 and the unwanted structural isomer 360. This alkene-indole 359 was then subjected to a free radical allylic bromination reaction using NBS and benzoyl peroxide, before being treated with sodium azide to afford an approximately 1 : 1 mixture of epimeric azides 361 and 362. Azide 362 was then reduced with LiAlH₄, accompanied by N-detosylation of its indole fragment and partial reduction of its alkene bond to afford a mixture of amines 363 and 364 that were then treated with acetic formic anhydride to afford an approximate 1 : 2 mixture of the unsaturated N-formamide 365 and saturated N-formamide 366. Formamide 366 was subsequently dehydrated to afford hapalindole J isonitrile 349 using POCl₃ in pyridine.¹³⁷,¹³⁸

Scheme 37 Natsume’s syntheses of (±)-hapalindole J isonitrile 347.¹³⁷,¹³⁸
Natsume also reported the synthesis of hapalindoles U isonitrile 374 from an intermediate 359 previously used in his synthesis of hapalindole J isonitrile 349 (Scheme 38).139 A 1 : 1 mixture of allylic alcohols 367 and 368 was generated via free radical allylic bromination of 359 with NBS followed by allylic hydroxylation using AgNO₃ in aqueous acetone. Separation and treatment of regioisomer 367 with H₂SO₄ in the presence of aqueous acetone resulted in its conversion into allylic alcohol 368. The alkene bond of 368 was then reduced with LiAlH₄, which also resulted in unwanted N-desosylation, therefore the N-tosyl group was reintroduced via treatment of the crude reaction product with TsCl and NaH. The resulting 10 : 3 mixture of N-tosyl alcohols 369 and 370 was separated and the minor cis-fused epimer 370 subjected to Swern oxidation, followed by epimerisation with Et₃N, Reductive animation of the resultant mixture of ketones with NH₄OAc/NaCNBH₃, followed by N-formylation with acetic formic anhydride in pyridine, resulted in a mixture of epimeric formamides 372 and 373, as well as a small amount of the unwanted epimeric alcohols 371. This complex mixture was separated to afford the desired N-tosyl formamide 373, whose N-tosyl group was removed via treatment with magnesium in methanol, followed by formamide dehydration using POCl₃ in pyridine to afford (±)-hapalindole U isonitrile 374.139

Scheme 38 Natsume’s synthesis of (±)-hapalindole U isonitrile 374.139
Natsume and co-workers also demonstrated that treatment of \( N \)-tosyl-formamide 375 with \( \text{Et}_3\text{SiH} \) in TFA resulted in reduction of its alkene bond to afford a mixture of reduced formamides 376 and 377 that could be separated via chromatography. The minor formamide 377 was then converted into (±)-hapalindole H isonitrile 378 using the same \( N \)-detosylation/formamide dehydration conditions described for (±)-hapalindole U isonitrile 378 (Scheme 39).\(^{139}\)

![Scheme 39 Natsume’s synthesis of (±)-hapalindole H isonitrile 378.\(^{139}\)](image)

In 2012 Rafferty and Williams employed a similar approach for their synthesis of (±)-hapalindole J isonitrile 349 (Scheme 40) via a route that also involved \( \text{SnCl}_4 \) mediated coupling of a silyl enol ether 380 with a cationic species derived from the tertiary alcohol fragment of \( N \)-silyl-indole 379 to give indole 381 as a mixture of diastereomers.\(^{140}\) Indole 381 was then cyclised via treatment with methanolic HCl with subsequent \( O \)-silyl-deprotection to generate the tetracyclic core of indole 382, which was oxidised into a ketone intermediate that underwent reductive amination to afford a 1 : 4 epimeric mixture of amines 383 and 384. After purification by chromatography, the \( cis \)-fused ring system of indole 385 was established via reduction of the cyclohexenyl bond of indole 385 with \( \text{LiAlH}_4 \), with the amino substituent of indole 384 then being \( N \)-formylated via treatment with formic acid, 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), a catalytic amount of \( N,N \)-dimethylaminopyridine (DMAP) and \( N \)-methylmorpholine (NMM) in \( \text{CH}_2\text{Cl}_2 \). The isocyanide functionality of (±)-hapalindole J isonitrile 349 was then introduced by dehydrating the formamide functionality of indole 386 using Burgess’ reagent 387 in benzene.\(^{140}\)
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Scheme 40 Williams’ synthesis of (±)-hapalindole J isonitrile 349.\textsuperscript{140}

The same paper also reported the synthesis of the trans-fused ring system of (±)-hapalindole U isonitrile 374 (Scheme 41) from the epimeric alcohol intermediate 474 that was oxidised using DMP to afford a ketone, whose indole nitrogen was N-tosylated to afford indole 389. The alkene bond of indole 389 was then reduced with LiAlH\textsubscript{4} with concomitant N-detosylation, to produce an alcohol (mixture of epimers) that was oxidised under Swern conditions to afford trans-fused ketones 390 and 391. The major diastereomer 391 was purified by chromatography and then transformed into (±)-hapalindole U isonitrile 374 (via amine 492) using the same sequence of synthetic steps used for completion of the synthesis of (±)-hapalindole U isonitrile 374.\textsuperscript{140}
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Scheme 41 Williams’ synthesis of (±)-hapalindole U isonitrile 374.140

Fukuyama and Chen reported the synthesis of (−)-hapalindole G isonitrile 25, which is an antibacterial, antifungal and antialgal isocyanide that was isolated from terrestrial alga Hapalosiphon fontinalis (scheme 42).141 (−)-Trans-carveol 393 was esterified with methyl(chloroformyl)acetate, followed by diazo transfer using para-AcNHC6H4SO2N3 to afford a diazomalonate 394 that underwent a copper catalysed intramolecular cyclopropanation reaction to afford tricyclic cyclopropyl ester 395. Heating cyclopropyl ester 395 with lithium chloride in the presence of camphor sulfonic acid (CSA) resulted in stereoselective ring opening of the cyclopropane ring by chloride anion, accompanied by decarboxymethylation to afford a lactone whose enolate was brominated (LDA, CBr4) to afford bromolactone 396. Bromolactone was reduced with DIBAL to afford a diol whose bromohydrin fragment underwent a Boord-like elimination reaction on treatment with zinc-copper couple to afford an alkene intermediate whose secondary alcohol group was then oxidised with Jones’ reagent to afford chloroketone 397. This chloroketone 397 then underwent a titanium mediated aldol reaction with 2-iodobenzaldehyde to give an epimeric mixture of hydroxy-ketones 398. This mixture of epimeric alcohols 398 were converted into their corresponding acetates that underwent DBU facilitated elimination reactions to afford an enone that was treated with TFA to initiate a Friedel-Crafts like cyclisation reaction that gave tricyclic iodo-ketone 399. The indole component was then constructed by a palladium facilitated carbylation reaction to afford an aryl carboxylic acid intermediate that was treated with DPPA to afford an acyl azide intermediate that underwent a Curtius rearrangement with allyl alcohol to afford allyl-carbamate 400. The Enone of 400 then underwent a conjugate addition reaction with
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LiCHSMe(SOMe), followed by hydrolysis with HgCl₂/perchloric acid to afford an aldehyde intermediate that condensed with the nitrogen atom of the carbamate group to afford the C₁-homologated N-alloc-indole 401. Stereoselective reduction of the keto group of indole 401 gave a β-alcohol that was O-mesylated and treated with lithium azide to afford α-azide 402. Reduction of azide 402 with sodium/mercury amalgam and subsequent formylation produced 403 that was then dehydrated using COCl₂ and Et₃N to afford hapalindole G isonitrile 25. ¹⁴¹

Scheme 42 Fukuyama’s synthesis of (−)-hapalindole G isonitrile 25. ¹⁴¹
Hapalindole A isonitrile 415 and Hapalindole K isonitrile 413 have been shown to inhibit green algae photosynthesis, with Hapalindole A isonitrile 415 being found to demonstrate both antialgal and antimyotic activity towards H. fontinalis. 131 For their racemic syntheses (Scheme 43), 142 Johnston and Chandra carried out a Friedel-Crafts acylation reaction of indole 404 with α-methyl tiglic acid to afford ketone 405 whose alkene fragment was cyclised onto its aryl ring via heating in molten AlCl3-NaCl to generate a tricyclic ketone that was N-tosylated to afford indole 406. Ketone 406 was then converted into its corresponding enol triflate that was treated with Zn(CN)2 and Pd(PPh3)4 to give an α,β-unsaturated nitrile that was reduced with DIBAL to afford an enal that was converted into its corresponding silyl enol ether 407 via treatment with TBSOTf and Et3N. Tetracyclic N-tosyl-indole 409 was then generated as a major diastereomer from Lewis acid mediated intermolecular Diels-Alder reaction of diene 407 with β-chloro-enone 408 in the presence of EtAlCl2. Reduction of ketone 409 with DIBAL was followed by treatment with triflic anhydride/pyridine resulting in elimination of the resultant triflate to afford an alkene intermediate that was O-silyl deprotected using TBAF to afford alcohol 410. A Ritter reaction involving treatment of the acetate of alcohol 410 with TMSCN was then used to generate formamide 411 as a precursor for the synthesis of both (±)-hapalindole K isonitrile 413 and hapalindole A isonitrile 415. For (±)-hapalindole K isonitrile 413, magnesium methoxide was used to effectively N-detosylate the indole ring of formamide 411, with the formamide functionality of indole 412 then being dehydrated by the action of COCl2/Et3N in 90% yield. 142 For (±)-hapalindole A isonitrile 415, LiAlH4 was used to reduce the cyclohexenyl functionality to afford a cis-fused ring and facilitate N-detosylation to afford indole 414, with dehydration of the resultant formamide with COCl2/Et3N once again affording its isocyanide functionality. 142 The authors also claimed a formal synthesis of (±)-hapalindole G isonitrile 25 142 involving reduction of the cyclohexenyl bond and N-detosylation of alcohol 410 with LiAlH4 to afford a cis-fused alcohol that was oxidised with DMP to afford ketone 416. The nitrogen atom of indole 416 was protected as an N-allyl carbamate, followed by treatment with Et3N which resulted in epimerisation to afford the trans-fused ring junction of ketone 417 that had previously been transformed into (-)-hapalindole G isonitrile 25 in five steps by Fukuyama and Chen. 141
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Scheme 43 Johnston’s syntheses of (±)-hapalindole G isonitrile 25, (±)-hapalindole K isonitrile 503, (±)-hapalindole A isonitrile 505.142
Li reported the synthesis of 12-epi-hapalindole Q isonitrile 422 via a bioinspired oxidative cyclisation strategy (Scheme 44).\textsuperscript{143} Coupling of aldehyde 418 with cis-boronic acid 419 provided amine 420, which was subsequently formylated using acetic formic anhydride and the indole portion of 420 de-sulfonated using solvated magnesium to afford diene 421. Upon exposure to Sc(OTf)\textsubscript{3} and DDQ, diene 421 underwent oxidative cyclisation to provide a cyclic formamide that was dehydrated using triphosgene and triethylamine base, providing racemic 12-epi-hapalindole Q isonitrile 422.\textsuperscript{143}

Scheme 44 Li’s synthesis 12-epi-hapalindole Q isonitrile 421.\textsuperscript{143}

Baran and Richter have reported an elegant ‘protecting group free’ syntheses of ent-(−)-12-epi-fischerindole G isonitrile 24 (Scheme 45) and (+)-12-epi-fischerindole I isonitrile 433 (scheme 46).\textsuperscript{22,144} Their synthesis of ent-12-epi-fischerindole G isonitrile 24 commenced with enolisation of (R)-carvone oxide 423, with lithium bis(trimethylsilyl)amide (LiHMDS) followed by regioselective addition of vinyl magnesium bromide to the α-position of its epoxide fragment. This gave an alcohol intermediate containing a quaternary stereocentre that was treated with N-chloro-succinimide (NCS)/Ph\textsubscript{3}P to afford β-chloroketone 424 with inversion of configuration. Copper catalysed coupling of the lithium enolate of chloroketone 424 with indole then gave an
indole-ketone 425 whose isopropenyl fragment was cyclised onto its aryl ring by heating with a clay catalyst (Montmorillonite K-10) in dichlorethane in a microwave to afford the tetracyclic core of indole 426. Reduction of ketone 426 with NaBH₄ from its α-face was followed by O-mesylation, displacement with LiN₃, and reduction of the resulting azide using sodium-mercury amalgam to afford amine 427. Amine 427 was then N-formylated with formic acid using the coupling agent 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) 428 and dehydrated using Burgess’ reagent 387 to afford ent-(−)-epi-fischerindole G isonitrile 24.²²,¹⁴⁴

Alternatively, for the synthesis of (+)-12-epi-fischerindole I isonitrile 433, ketone 429 was reductively aminated with NH₂OAc/NaBH₃CN to afford an amine that was N-formylated via treatment with formic acid and DMT-MM 430 (Scheme 46). Treatment of formamide 431 with the oxidant tert-butyl hyperchlorite and Et₃N, followed by deactivated silica gel (Et₃N), resulted in a cascade of chlorination/dehydrochlorination/tautomerisation reactions to give vinylformamide 432, which was dehydrated via treatment with Burgess’ reagent 387 to afford (+)-12-epi-fischerindole I isonitrile 433.²²,¹⁴⁴
Baran also employed this methodology to prepare (+)-welwitindolinone A isonitrile 438 from (+)-12-epi-fischerindole I isonitrile 434 (Scheme 47), which was treated with tert-butyl hyperchlorite and TFA, resulting in chlorination of the indole ring to afford a chloroimine intermediate 435. This chloroimine species 434 was intercepted by a water molecule to afford an unstable aminol 435 that eliminated an chloride anion to afford iminium species 436 that undergoes a 1,5-sigmatropic rearrangement reaction to afford both the amide and cyclobutyl ring functionalities of (+)-welwitindolinone A isonitrile 438 in 25% yield. Alternatively, treatment of (+)-12-epi-fischerindole I isonitrile 433 with the electrophilic fluorinating agent XeF₂ resulted in formation of welwitindolinone A isonitrile 438 (via fluorinated indole 437 through a similar rearrangement/ring contraction pathway in an improved 44% yield.
Baran has also reported another ‘protecting group free’ strategy for the synthesis of the marine isocyanides (-)-hapalindole U isonitrile 374 and (+)-ambiguine H isonitrile 447 (Scheme 48).\(^{145}\) The lithium enolate of the (S)-(+)‐carvone derived intermediate 439 was C3‐arylated with indole 440 in the presence of a Cu(II)‐2‐ethylhexanoate catalyst to generate indole‐ketone 441 as a single diastereomer in 50% yield. Slow addition of \([\text{Pd}(\text{P(ortho‐tol)})_3\text{OAc}])_2\) to a solution of bromo‐indole 441, NaOCHO, tetra‐n‐butylammonium bromide (TBAB) and \(\text{Et}_3\text{N}\) in DMF at 80 °C over five hours was successful in facilitating a palladium catalysed cross‐coupling reaction to afford the tetracyclic core of ketone 442. (-)-Hapalindole U isonitrile 374 was then prepared by reductive amination of ketone 442, followed by \(N\)-formylation of the resultant amine to generate a formamide (CDMT, HCO₂H) that was dehydrated using COCl₂ and \(\text{Et}_3\text{N}\). Installation of the tert‐prenyl unit of (+)-ambiguine H isonitrile 447 was achieved via reaction of (-)-hapalindole U isonitrile 374 with tert‐butyl hyperchlorite and prenyl 9‐BBN-H, which generated an imino‐borane species 443 that underwent a [3,3]‐rearrangement reaction that resulted in transfer of the tert‐prenyl group to its indole ring to afford chloroimidate 444. Exposure of chloroimidate 444 to light in the presence of \(\text{Et}_3\text{N}\) then resulted in a Norrish‐type radical fragmentation reaction to afford diradical species 445 that underwent intramolecular...
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radical hydrogen atom abstraction to afford a chloroimine 446 that eliminated chloride in situ to generate the isocyanide fragment of (+)-ambiguine H isonitrile 447.\(^{145}\)

Wood and co-workers have also reported the synthesis of (±)-welwitindolinone A isonitrile 348 (Scheme 49).\(^{146-148}\) Diene 448 underwent a highly stereo and regioselective [2+2] cycloaddition reaction with dimethyl ketene to afford a tricyclic ketone, which was reacted with Grignard reagent to afford the cyclobutanol fragment of phenyl triazene 449. Phenyl triazene 449 was then converted into oxazolidin-2-one 450 via a four step protocol involving: (i) reduction of the triazene with Raney nickel/H\(_2\) to afford an amino group; (ii) protection of the amino-alcohol group as an oxazolidin-2-one; (iii) acid catalysed acetonide hydrolysis; (iv) selective oxidation of the allylic alcohol functionality using Bu\(_2\)SnO and NBS. The alcohol group of 450 was then
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protected as its triisopropylsilyl (TIPS) group, and the resultant enone treated with LiHMDS (to protect the oxazolidin-2-one as its lithium amide), followed by conjugate reduction with L-Selectride and trapping of the resultant enolate with N-phenyltriflimide to afford an enol triflate intermediate. This enol triflate was then subjected to a Pd(0)-catalysed carboxylation reaction in the presence of MeOH to afford an ester that was reacted with excess MeMgBr/CeCl₃ to afford tertiary alcohol 451. Treatment of alcohol 451 with NaOCl/CeCl₃ resulted in formation of a chloronium intermediate that underwent a semi-pinacol rearrangement reaction to afford a single keto diastereomer 452. O-Silyl deprotection of ketone 452 was followed by stereoselective reduction of the ketone group to afford a diol 453 whose least-hindered alcohol group was selectively dehydrated via treatment with Martin’s sulfurane reagent [[Ph₂S(OC(CF₃)₂Ph)₂], with its remaining alcohol group then being oxidised to afford ketone 454. Ketone 454 was then reacted with (Boc)₂O in the presence of DMAP resulting in N-Boc protection of its oxazolidin-2-one group, followed by DBU mediated elimination of CO₂ from the oxazolidin-2-one ring to afford an N-Boc-aniline 455. Reaction of ketone 455 with O-methylhydroxylamine gave an oxime that was reduced with NaCNBH₃ to stereoselectively afford an N-OMe-amine that was treated with acetic formic anhydride to afford N-OMe-formamide 456. The N-O bond of 456 was reductively cleaved via treatment with SmI₂, with subsequent formic acid mediated N-Boc deprotection affording formamide 457. A mixture of phosgene/Et₃N was then employed to access both the isocyanide and isocyanate fragments of the cyclobutene intermediate 458, which cyclised on exposure to LiHMDS, thus generating the vinyl isocyanide and indan-2-one fragments of welwitindolinone A isonitrile 348.146–148
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Scheme 49 Wood’s synthesis of (±)-welwitindolinone A isonitrile 348.146–148

*N*-Methylwelwitindolinone D isonitrile 5 is known to demonstrate antifungal properties, and the potential to overcome P-glycoprotein-mediated drug resistance in human carcinoma cells. In 2011, Rawal and co-workers developed an approach for the synthesis of (±)-*N*-methylwelwitindolin-2-one D 5 that interestingly did not proceed via a formamide intermediate.149 In their synthesis (Scheme 50), trimethylsilyl triflate mediated alkylative coupling of silyl enol ether 459 with a carbocationic species derived from the tertiary alcohol fragment of *N*-methylindole 460 afforded ketone 461 as a single diastereomer. A palladium catalysed intramolecular enolate α-arylation cross coupling reaction was then used to
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construct the cyclic [4.3.1] scaffold of aldehyde 462. Aldehyde 462 was transformed into α-bromoketone 463 over three steps involving: (i) O-TBS deprotection; (ii) DMP oxidation; (iii) α-bromination of the keto group via treatment with potassium bis(trimethylsilyl)amide KHMDS and NBS. Treatment of indole 463 with DMDO and NaHCO₃ in DMSO resulted in stereoselective oxidation at the C₂- and C₃-positions of the indole ring, with concomitant intramolecular displacement of its bromide by a C₃-hydroxyl group to afford the cyclic ether bridge of a new tetrahydrofuran ring system. Treatment of the resultant aldehyde 464 with hydroxylamine afforded an oxime intermediate that was transformed into isothiocyanate 467 via treatment with NCS, Et₃N and the cyclic thiourea 465 in 65% yield. This reaction is thought to proceed via formation of a nitrile oxide species 466 in situ which then undergoes a [3+2] cycloaddition reaction with the cyclic thiourea 465 to afford an unstable 1,4,2-oxathiazoline species 466 that spontaneously decomposes to afford the isothiocyanate functionality of 467. Treatment of isothiocyanate 467 with oxazaphospholidine 468 resulted in desulphurisation to generate the isocyanide functionality of (±)-N-methylwelwitindolin-2-one D isonitrile 5 in 54% yield.¹⁴₉
Rawal and co-workers also reported the synthesis of \((\pm)-N\)-methylwelwitindolinone C isonitrile 22 using diketo-aldehyde 469 as a key intermediate that had been used previously in their synthesis of \(N\)-methylwelwitindolinone D isonitrile 5 (Scheme 51).\(^{150}\) Aldehyde 469 was selectively reduced using sodium trimethoxyborohydride to afford alcohol 470 whose ketone group was reacted with hydrazine to afford hydrazone 471, which was then treated with NCS to afford the vinyl chloride 472. Vinyl chloride 472 was then treated with the mild oxidant magnesium monoperoxyphthalate (MMPP) to afford oxindole 473, which was then oxidised with DMP to afford an aldehyde that was converted into \(N\)-methylwelwitindolinone C isonitrile 22 over three steps (via isothiocyanate 475) using conditions previously established for their synthesis of \((\pm)-N\)-methylwelwitindolinone D isonitrile 5.\(^{150}\)
A stereoselective synthesis of (-)-N-methylwelwitindolinone C isonitrile 22 has also been reported by Garg and co-workers (Scheme 52),\textsuperscript{151} starting from carvone derived ketone 476 that was transformed into bromoindole 478 over 3 three steps involving (i) methanolysis of the pivaloyl ester; (ii) iodine catalysed intermolecular alkylation of indole; (iii) O-TBS protection.\textsuperscript{152} Treatment of bromoindole 478 with sodium amide resulted in intramolecular cyclisation of its derived sodium enolate onto an indolyne fragment (via intermediate 479) to give a 2.5 : 1 mixture of ketone 481 and the seven-membered cyclic O-aryl ether 480. Ketone 481 was purified by chromatography and then converted into vinyl chloride 483 over five steps involving: (i) O-TBS deprotection; (ii) oxidation of the resultant alcohol with DMP; (iii) vinyl triflate formation from treatment of a ketone enolate with Comin’s reagent 482; (iv) palladium catalysed stannylation of the vinyl triflate; (v) copper catalysed chlorination of vinyl stannane.\textsuperscript{153} Treatment of vinyl chloride 483 with NBS resulted in C$_2$-bromination to afford a bromoindole that was then hydrolysed to afford indan-2-one 484. Reduction of the ketone functionality of indan-2-one 484 with LiEt$_3$B-D resulted in a deuterated alcohol that was then converted into carbamate 485 via sequential treatment with trichloroacetyl-isocyanate and potassium methoxide. This enabled an elegant deuterium kinetic isotope effect to be used to control the regioselectivity of an intramolecular nitrene C-H insertion reaction that was initiated by treatment of carbamate 485 with AgOTf, PhI(OAc)$_2$ and bathophenanthroline to afford oxazolidin-2-one 486 in 60% yield. The oxazolidin-2-one fragment of 487 was then hydrolysed with Ba(OH)$_2$ to afford an amine 487 whose secondary alcohol group was oxidised.
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into its corresponding ketone with DMP. Amine 487 was then N-formylated using acetic formic anhydride to afford formamide 488 that was subsequently dehydrated using Burgess’ reagent 387 to generate (-)-N-methylwelwitindolinone C isonitrile 22. Amine 487 could also be converted into N-methylwelwitindolinone C isonitrile 22 in lower yield via treatment with O,O-di(pyridinyl-2-yl)carbonothioate 489 and DMAP in dichloroethane to afford an isothiocyanate that was then desulphurised via treatment with oxazaphospholidine 490. Subsequent exposure of a solution of (-)-N-methylwelwitindolinone C isonitrile 22 and NaH in THF to air resulted in selective bridgehead oxidation to afford (-)-C₃-hydroxy-N-methylwelwitindolinone C isonitrile 352.¹⁵²
Garg was also able to achieve the enantiospecific synthesis of (+)-N-methylwelwitindolinone D isonitrile 5 by first converting previously synthesised ketone 481 into oxoindole 492 via a one pot-pot procedure that involved oxidation with NBS and hydrolysis using HCl (Scheme 53).\textsuperscript{154} The substrate had to be resilylated \textit{in situ} to afford oxoindole 492 which subsequently underwent deuteride reduction to give an alcohol that was then carbamoylated to form primary amide 493. Secondary amide 494 was afforded upon silver promoted nitrene insertion reaction of 493 with PhI(OAc)\textsubscript{2} and bathophenathroline, with subsequent hydrolysis/oxidation reactions yielding ketone 495. The ether functionality of 496 was realised upon exposure of ketone 495 to TABF in the open air, with the resulting furan species 496 being reduced to alcohol 497 using LiAlH\textsubscript{4}. Barium hydroxide facilitated hydrolysis afforded a diol, which was subsequently oxidised with IBX to afford \textit{bis}-ketone 498. The isocyanide functionality of 5 was generated upon formylation of amine 498 with formic acid and acetic anhydride, providing a formamide that was subsequently dehydrating using Burgess reagent 387 to afford (+)-N-methylwelwitindolinone D isonitrile 5.\textsuperscript{154}
1.4 Syntheses of miscellaneous isocyanide natural products

(E)-3-Indole vinyl-isocyanide 17 and (Z)-3-indole vinyl-isocyanide 20 are antibiotic metabolites that exhibit strong antimicrobial properties in low concentration against *Escherichia coli*, *Bacillus subtilis* and *Mucor muhei* TU 284. Hoppe and Schöllkopf reported the synthesis of these 3-indole vinyl-isocyanides via Horner-Wadsworth-Emmons reaction of the anion of diethyl isocyanomethylphosphonate 500 with 3-indole carboxaldehyde 499 using sodium bis(trimethylsilyl)amide (NaHMDS) as a base (Scheme 54). Under these conditions a 3 : 2 mixture of the vinylogous isocyanides (E)-17 and (Z)-20 isomers was produced that were separated by column chromatography.
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An isocyanide antibiotic indisocin 507 containing a 1-chlorovinylisocyanide moiety that was isolated from the actinomycete strain MG323-hF2 was synthesised via addition of vinyl magnesium bromide to isatin 501 to afford an alcohol that was then bis-acetylated to afford the acetate and acetamide fragments of indole 502 (Scheme 55). Selective methanolyis of the N-acetyl bond afforded an acetate 503 that was ozonised to afford aldehyde 504 that underwent Horner-Wadsworth-Emmons reaction with the anion of diethyl chloromethylisocyanophosphonate 505 to afford a 1 : 1 mixture of (Z)- and (E)- α,β-unsaturated-α-chloro-isocyanides 506 and 507 that could be separated via chromatography.

Isocyalexin A 511 is the first known isocyanide extracted from a plant, which was isolated after UV-irradiation of rutabaga root slices, followed by incubation in the dark, organic extraction and fractionation. Biological testing against four fungal root pathogens revealed antifungal activity against *A. brassicicola*, *L. maculans*, *R. solani*, *S. sclerotiorum*, suggesting that isocyalexin A 511 is produced as a phytoalexin to protect the plant against root pathogens. In 2012, Pedras and Yaya described the total synthesis of isocyalexin A 511 (Scheme 56), employing 3-nitroindole 508 as a starting material for a sequence of thallation-iodination-methoxylation reactions with good regiocontrol to install the C₄-methoxy group of indole 509.
This indole intermediate 509 was then hydrogenated to give an amine intermediate that was N-formylated with acetic formic anhydride to give formamide 510, with subsequent Et$_3$N mediated dehydration in the presence of POCls/Et$_3$N affording isocyalexin A 511 in 41% yield.\textsuperscript{157}

Factors 5 \textsuperscript{516} and 6 \textsuperscript{517} have been identified as two primary components of hazimycin metabolites produced by \textit{Pseudomonas sp.} SCC 1411, a bacterium isolated from soil that demonstrated \textit{in vitro} and \textit{in vivo} activity against Gram-positive and Gram-negative bacteria, and \textit{in vitro} activity against yeasts and dermatophytes. Wright and co-workers used POCls as a formamide dehydrating agent in their 1982 synthesis of hazimycin factor 5 (±)-516 and hazimycin factor 6 (meso)-517, which were shown to rapidly interconvert in the presence of base. For their synthesis (Scheme 57),\textsuperscript{158} N-formyl-L-tyrosine methyl ester 512 was oxidatively homocoupled using horse-radish peroxidase in low 6-10% yield to form the required \textit{bis}-aryl dimer 513, whose phenolic groups were then protected as their \textit{bis}-O-acetates 514. Standard POCls/Et$_3$N mediated formamide dehydration conditions were then used to efficiently generate the isocyanide fragments of 515. Tandem ammonolysis of the phenolic acetate groups and the two ester functionalities of 515 was achieved \textit{via} treatment with methanolic ammonia, with concomitant racemisation of both stereocentres occurring to generate a mixture of (±)-516 and (meso)-517 in a 1:1 ratio.\textsuperscript{158} Achab and Velay subsequently reported a formal synthesis of hazimycin based on palladium (0) catalysed coupling of an aryl iodide with an aryl stannane to afford \textit{bis}-formamide 514 in 56% yield (scheme 69).\textsuperscript{159}
Methodology for the synthesis of isocyanide containing natural products

Scheme 57 Wright's synthesis of hazimycin factor 5 (±)\textsuperscript{516} and hazimycin factor 6 (meso)\textsuperscript{517}.\textsuperscript{158, 159}

The sugar derived isocyanide antibiotic A32930A \textsuperscript{523} was prepared via nucleophilic displacement of the bis-O-benzylidene acetal protected mannitol bis-mesylate \textsuperscript{518} with two equivalents of the carboxylate anion of vinyl formamide \textsuperscript{519} to afford bis-formamide ester \textsuperscript{520} (Scheme 58). The benzylidene groups of bis-formamide \textsuperscript{520} were then globally deprotected via heating in formic acid, and the resultant tetrol performylated using acetic formic anhydride. The bis-formamide functionality of \textsuperscript{521} was then dehydrated using POCl\textsubscript{3}/Et\textsubscript{3}N to produce bis-isocyanide \textsuperscript{522}, whose remaining O-formyl groups were then hydrolysed under basic conditions (Na\textsubscript{2}CO\textsubscript{3}/NaHCO\textsubscript{3(aq)}) to afford the natural product bis-2-isocynano-3-methylcrotonoyl)-D-mannitol (A32930A) \textsuperscript{523}.\textsuperscript{160}
Gavagnin and co-workers have reported the synthesis of (-)-actisonitrile 529, a 1,3-propanediol ether based lipid derived isocyanide isolated from the nudibranch *Actinocyclus papillatus*, that demonstrated cytotoxicity against non-tumour H9c2 rat cardiac myoblast cells (IC$_{50}$ = 23 µM). For their synthesis of (-)-actisonitrile 529 (Scheme 59), chiral trityl-epoxide 524 was ring-opened using the alkoxide of hexadecanol to afford a secondary alcohol intermediate that was mesylated to generate ether 525. Reaction of ether (S)-525 with sodium azide resulted in nucleophilic substitution with clean inversion of configuration, followed by hydrogenation of the resultant azide to afford amine (R)-526, which was transformed into formamide 527 by treatment with ammonium formate. Acid catalysed removal of the trityl group of formamide 527 was followed by acetylation of the resultant alcohol group to afford a formamide 528 that was dehydrated via treatment with tosyl chloride in pyridine to afford (-)-actisonitrile 529.
A second synthesis of (−)-actisonitrile \( \text{529} \) was reported by Sugiyama et al. with oxazolidinone \( \text{530} \) being O-alkylated via treatment with 1-iodohexadecane and caesium hydroxide to afford ether \( \text{531} \) (Scheme 60).\(^{162}\) Ether \( \text{531} \) was then debenzylated via treatment with MsOH and anisole in nitromethane to afford \( \text{NH-oxazolidinone 532} \) that upon hydrolysis with LiOH afforded 1,2-amino-alcohol \( \text{533} \). 1,2-Amino-alcohol \( \text{533} \) was \( N \)-formylated using ethyl formate and the alcohol functionality of the resultant formamide acetylated with \( \text{Ac}_2\text{O/pyridine} \) to afford acetate \( \text{534} \), which upon exposure to \( \text{PPh}_3/\text{CBr}_4 \) was dehydrated to yield (−)-actisonitrile \( \text{529} \).\(^{162}\)
1.5 Conclusion

Since the discovery of xanthocillin as the first naturally occurring isocyanide, a diverse range of isocyanide containing natural products have been isolated from marine and terrestrial sources that exhibit a broad range of potent biological activities. The biological activities and structural complexity of many of these isocyanides has resulted in some impressive syntheses, however a significant number of biologically active isocyanide containing natural products remain to be synthesised (Figure 18).\textsuperscript{535-542} Compilation of this review has revealed that a finite number of strategies currently exist to install the reactive isocyanide group into structurally complex molecules (Figure 19 Common strategies for formamide dehydrationFigure 19), with a general preference for installing the reactive isocyanide group at a late stage of a synthetic protocol. By far, the most popular strategy employed for introducing the isocyanide functionality into a natural product target is by dehydration of its corresponding formamide, which is invariably generateded via $N$-formylation of its corresponding primary amine. However, a few noteworthy alternatives for the introduction of isocyanide group have been explored, based on the $N$-alkylation of nucleophilic cyanide species with cationic intermediates, desulfurisation of isothiocyanates and Horner-Wadsworth-Emmons reactions of $\alpha$-isocyano-containing phosphonates. From a synthetic perspective, the syntheses of many structurally challenging terpene derived isocyanide natural products are particularly noteworthy, with many ingeneous strategies having been developed for the construction of their complex polycyclic backbones in a stereoselective manner. In this respect, there is little doubt that the isocyanide class of natural products will continue to provide inspiration for the development of future methodology at the cutting edge of synthesis.
Methodology for the synthesis of isocyanide containing natural products

Figure 18 Biologically important isocyanides 535-542 that have yet to be synthesised. 163-168,170-172

![Chemical structures of biologically important isocyanides](image)

Figure 19 Common strategies for formamide dehydration.
2 The development of an E-selective Horner-Wadsworth-Emmons protocol for the synthesis of phenol-vinyl isocyanide

2.1 Insects as a source of Natural Products

A variety of organisms found in nature are capable of producing chemical structures (natural products) that exhibit potent biological activity. To date, a variety of blockbuster drugs have been developed from lead compounds produced by microorganisms found in plants and various members of the animal kingdom. However, natural products produced by microorganisms found within insects are currently an under-explored resource. Insects, which are found in nearly all environments, represent at least half of the known living organisms on this planet, making them the most diverse subset of the animal population. Insects emerged over 400 million years ago and this has facilitated the evolution of parasites (e.g. bacteria) that exclusively colonise insects as hosts. These highly evolved microorganisms express secondary metabolic pathways that produce toxins (insecticides) with targeted, pathogenic behaviour towards their host. The chemical structures (Figure 20) of these metabolites can be regarded as a potentially huge library of structurally diverse compounds for screening for pharmaceutical applications.

![Figure 20 Structures of insect natural products and their associated biological properties.](image)

Dolichodial *anticancer*  
Solenopains *ant defense*  
Antineplastic agents *cytotoxic*  
5-S-GAD *antibiotic*  
Pederin *defense*
Consequently, the remainder of this thesis will concern itself with the synthesis and biological evaluation of the vinyl-isocyanide containing secondary metabolites; phenol vinyl-isocyanide 542, Rhabduscin 543 and Byelyankacin 544, which are produced by the insect pathogens *Photorhabdus* and *Xenorhabdus* bacteria that exist in symbiosis with heterorhabdus nematodes (Figure 21).

![Figure 21 Isocyanide derived natural products from *Photorhabdus* and *Xenorhabdus* bacteria.](image)

### 2.2 Life cycle of heterorhabdus nematodes.

*Photorhabdus* and *Xenorhabdus* bacteria live within the *Heterorhabditis* nematode in a mutualistic relationship, where they exist in trilateral symbiosis to parasitize insect larvae. In the soil, juvenile nematodes host *Photorhabdus* and *Xenorhabdus* in their gut, carrying these organisms with them as they penetrate their larval insect prey directly, or enter through other natural orifices. Regurgitation by the nematodes then occurs, releasing between 50 to 200 bacterial cells into the host’s open blood system, with *Photorhabdus* and *Xenorhabdus* now switching to a pathogenic state, where they start to produce a series of secondary metabolites. These include phenol vinyl-isocyanide 542, Rhabduscin 543 and Byelyankacin 544 which combine to facilitate the host’s destruction by suppressing its immune system, facilitating bacterial reproduction by enabling the nematodes to feed and grow. Curiously, the bacteria is also known to continue producing these isocyanide derived natural products long after the host’s death, which may enable it to effectively defend and compete against other microbial competitors for the larval carcass. To complete the life cycle, the nematode then returns to its infective juvenile stage, and exits the insect carcass in search of new prey, whilst still carrying the *Photorhabdus* in its gut (Figure 22).
2.3 Inhibition of tyrosinases by Rhabduscin and Byelyankacin

The secondary metabolites Rhabduscin 543, Byelyankacin 544 and phenol vinyl-isocyanide 542 have been shown to exhibit low nanomolar inhibitory activity against mushroom tyrosinases and wax moth larvae phenol oxidase (Table 1).179

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Mushroom tyrosinase inhibition</th>
<th>Phenol oxidase inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhabduscin 543</td>
<td>15.1 x 10^{-9}</td>
<td>64.1 x 10^{-9}</td>
</tr>
<tr>
<td>Byelyankacin 544</td>
<td>37.1 x 10^{-9}</td>
<td>184.9 x 10^{-9}</td>
</tr>
<tr>
<td>Phenol-vinylisocyanide 542</td>
<td>7.9 x 10^{-9}</td>
<td>61.7 x 10^{-9}</td>
</tr>
</tbody>
</table>

It is known that insects employ tyrosinase enzymes as part of their innate immune response to invading pathogens to catalyse the oxidation of phenol intermediates for the formation of melanin polymer (melanogenesis). The role of the phenol oxidase is to convert tyrosine 11 into dopaquinone 545 that then acts as a reactive intermediate to generate 5,6-
dihydroxyindoles 546 and 547 that then polymerise to trap invading species in an impermeable coat of melanin, thus encasing the invading organism and suppressing its pathogenic actions (Scheme 61).¹⁸⁰

Scheme 61 Involvement of tyrosinase as a key enzyme in melanogenesis.

This knowledge enabled Clardy and co-workers to propose that Photorhabdus and Xenorhabdus deploy the vinyl-isocyanide metabolites 542, 543 and 544 as inhibitors of host tyrosinase oxidases that are responsible for melanin formation. This prevents the nematodes from being encased in melanin polymer, thus enabling them to continue to effectively parasitise their larval host.

Phenol oxidases are copper containing monooxygenases that are known to bind isocyanide ligands to form Cu(I)-complexes, and as a consequence it has been proposed that the isocyanide carbon of the metabolites were binding to copper in the active site of these tyrosinases as part of the inhibitory response.¹⁸¹ A similar metal binding event has been proposed to explain the bioactivity of the natural isocyanide kalihinol F 548 (a triisocyano derived terpene) towards zebra fish. In this case, it has been shown that binding of the isocyanide fragment to copper transport proteins present in developing zebra fish, results in noticeable defects in pigment formation, haematopoiesis, and neural development (Figure 23).¹⁸²
Development of an E-selective HWE protocol

Clardy and co-workers have provided further information on the biological mode of action of these vinyl-isocyanide natural products by using a *Xenorhabdus* knock out strain (isnAB disabled) to prepare a vinyl-azide analogue 549 probe *in vivo* (Figure 24). The azide handle of this analogue 550 was subjected to a copper catalysed click reaction with a biotin-alkyne substrate *in vivo* to afford a triazole adduct 551 containing a biotin fragment. Exposure of this adduct to a streptavidin labelled fluorophore resulted in strong complexation with the biotin fragment of complex 551 *in vivo*. This enabled fluorescence microscopy to be used to reveal that complex 552 was localised at the external cell walls of the bacteria, presented in a location where it could interact directly with the host’s bloodstream.

![Figure 23 Kalihinol F and its disruption of zebrafish growth via chelation to copper transporters.](image)

Figure 23 Kalihinol F and its disruption of zebrafish growth via chelation to copper transporters.

![Figure 24 Summary of Clardy’s study into cell wall localisation using fluorescent aglycone mimic 549.](image)

Figure 24 Summary of Clardy’s study into cell wall localisation using fluorescent aglycone mimic 549.
Given this evidence, a working hypothesis to explain the biological activity of these vinyl-isocyanide natural products may be proposed whereby the sugar fragment binds to the cell membrane, localising the isocyanide warhead outside of the bacterial cell-wall (Figure 25). This enables the isocyanide fragment to bind directly to the copper site of the host’s tyrosinase enzymes, thus preventing the host’s innate immune system from facilitating melanin formation. Examination of the tyrosinase inhibition results presented in Table 1 reveals that the ‘free’ phenol vinyl-isocyanide 542 exhibits the lowest IC₅₀ values, suggesting that the sugar fragments of Rhabduscin 543 and Byelyankacin 544 do not contribute significantly to their tyrosinase inhibitory activity. This suggests that tyrosinase inhibition in vivo may be associated with a hydrolytic glycosidic cleavage event that releases ‘free’ phenol vinyl-isocyanide 542 from membrane bound Rhabduscin 543 and Byelyankacin 544.

2.4 Antibacterial activity of structurally related vinyl isocyanides

Whilst there is now ample evidence that vinyl-isocyanide natural products can inhibit tyrosinases and suppress the host insect innate immune system, their continued production after the insect host has died led us to propose that they may exhibit a potential secondary antibiotic role. This antibiotic activity would enable the bacteria and nematode to effectively compete against other bacterial and fungal organisms for consumption of the insect carcass.
Development of an E-selective HWE protocol

This theory is not without evidence, since two separate studies have previously demonstrated the antibacterial properties of α,β-unsaturated isocyanides. Schöllkopf, in addition to preparing naturally occurring 3-indole vinyl-isocyanides 17 and isomeric antibiotic B371 20, prepared a small library of heterocyclic α,β-unsaturated isocyanides. These indole (553 and 554) and thiophene (555) based substrates were screened for their antibiotic activity using disc diffusion assays and shown to demonstrate antibiotic activity against E. coli, B. subtilis, and M. muhei (Figure 26).

Figure 26 Potent antibiotic heterocyclic isocyanides reported by Schöllkopf.

Following Schöllkopf’s pioneering work, a patent was filed in 1987 by a Japanese team investigating the synthesis of the α,β-unsaturated formamide containing natural product, Erbstatin 556. In addition to screening some formamide analogues, the inventors’ also prepared a range of α,β-unsaturated isocyanides (inc 557 and 558), some of which were reported to exhibit 100 fold more antibiotic activity than their corresponding formamides against S aureus and P. aeruginosa A3. (Figure 27).

Figure 27 Erbstatin 556 and reported isocyanide analogues with μM antibiotic activities against S aureus and P. aeruginosa A3.
2.5 Project Outline

Since these vinyl-isocyanide natural products are only produced by bacteria in very small amounts, the primary objective of this research program was to devise versatile synthetic methodology to prepare these vinyl-isocyanides for biological evaluation.

2.6 Biosynthesis of Rhabduscin and Byelyankacin

The biosynthetic origins of the isocyanide functionality of the common phenol vinyl isocyanide 542 fragment of these natural products involves the sequential action of two enzymes isnA and isnB on tyrosine 11 (Scheme 62). The first enzymatic step catalysed by isnA involves introduction of the α,β-unsaturated functionality and isocyanide group of intermediate 559. Labelling studies on the biosynthesis of the related indole isocyanide 17 indicate that the C1-unit of the isocyanide group of intermediate 559 is likely to originate from the C2 atom of ribulose-5-phosphate. IsnB then catalyses decarboxylation of intermediate 559 to afford the key aglycone 542. The sugar residues required for the construction of Rhabduscin 543 and Byelyankacin 544 are then coupled to the aglycone 542 unit through the action of glycosyltransferase (GT) enzymes (Scheme 62).

![Scheme 62 Biosynthesis of Rhabduscin 543 and Byelyankacin 544 (via aglycone 542).](image-url)
This led us to consider a bio-inspired retrosynthetic analysis of Rhabduscin 543 and Byelyankacin 544 involving coupling the aglycone 542 to an appropriate sugar fragment that was functionalised with an appropriate leaving group at its anomeric position (Scheme 63).

Consequently, the challenges encountered in developing a stereoselective synthesis of phenol vinyl-isocyanide (aglycone) 542 via Horner-Wadsworth-Emmons (HWE) reaction of p-hydroxybenzaldehyde 560 with the enolate of a suitable phosphonate derivative 500 will be discussed in this chapter. The synthesis of suitable sugar fragments and their coupling with aglycone 542 for the total synthesis of Rhabduscin 543 and Byelyankacin 544 will be discussed in chapter 4.

Scheme 63 Retrosynthesis of Byelyankacin 544, Rhabduscin 543 and phenol vinyl-isocyanide 542.

2.7 Use of Schöllkopf’s reagent for the synthesis of vinyl-isocyanide aglycone 542.

Schöllkopf and co-workers had already reported the use of isocyanide derived diethyl isocyanomethylphosphonate reagent (PhosMIC 500) for the synthesis of 3-indole vinyl isocyanide 17 (and antibiotic B371 20), via reaction of the phosphonate with carboxaldehyde 499 under typical Horner-Wadsworth-Emmons conditions (Scheme 64). However, their synthesis was not selective for the formation of E-α,β-unsaturated isocyanide products, with 17 produced as a 3 : 2 mixture with its Z- isomer 20, and subsequently separated by silica gel chromatography.
It was therefore envisaged that these conditions could potentially be applied to the synthesis of phenol vinyl-isocyanide 542, providing the desired aglycone for the synthesis of Byelyankacin 544 and Rhabduscin 543. Despite the commercial availability of diethyl isocyanomethylphosphonate 500, it was decided to develop an ‘in house’ synthesis of this reagent due its high cost and the limited quantities for sale (Scheme 65). In a repeat of a literature synthesis, aqueous dimethylamine 563, formamide 562 and aqueous formaldehyde 561 underwent a Mannich type condensation reaction to afford amino-formamide 564 that was then stirred with iodomethane at 0 °C to produce its corresponding quaternary amine salt 565. Having purified this salt by fractional crystallisation, an Arbuzov reaction was then carried out involving refluxing the amino-formamide 565 with triethylphosphite in ethanol to afford the desired phosphonate-formamide 566, a species that was fully characterised for the first time. Its structure was confirmed by the presence of formamide C-H peaks at 7.80 ppm and a methyl doublet of doublets at 3.50 ppm in the $^1H$ NMR spectrum, carbonyl peak at 162 ppm in the $^{13}C$ NMR spectrum, a carbonyl stretch in the infra-red spectra and a molecular ion at 218.0575.
Development of an E-selective HWE protocol

Owing to the number of different reagents available for the dehydration of formamides into their corresponding isocyanide, several of the more frequently used dehydrating agents were screened for the transformation of 566 into its corresponding Horner-Wadsworth-Emmons reagent (PhosMIC) 500 (Table 2). For each reaction, the appropriate dehydrating agent was added dropwise to formamide 566 and an excess of triethylamine in dichloromethane at -78 °C. These reactions were then allowed to warm up to room temperature overnight, with the reaction then being worked up and the crude product purified by flash column chromatography to afford the desired PhosMIC reagent. As can be seen from the results, the use of methanesulfonyl chloride gave the best yields, consistently affording PhosMIC 500 in around 50% isolated yield. This was not only an improvement on Schöllkopf’s POCl₃ dependent synthesis, the use of a milder dehydration agent also provided a less hazardous work-up. Evidence for PhosMIC’s isocyanide functionality was eluded by the presence of a characteristic N-C stretch at 2121 cm⁻¹ and a peak in the carbon NMR spectrum at 160.8 ppm.

Table 2 Screening of common dehydration reagents for synthesis of 500.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>POCl₃</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>TsCl</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>PPh₃/CBr₄</td>
<td>46</td>
</tr>
<tr>
<td>4</td>
<td>MsCl</td>
<td>51</td>
</tr>
</tbody>
</table>
This optimised methodology was then used to prepare PhosMIC 500 on a 5 gram scale, and its use was then trialled for the known synthesis of 3-indole vinyl-isocyanide 17 using conditions previously reported by Schöllkopf and co-workers (Scheme 61). Therefore, the phosphonate carbanion of 500 was generated by treating with 1.2 equivalents of NHMDS in THF at -78 °C, followed by addition of indole 3-carboxaldehyde 499 under anhydrous conditions, to give a 3:2 mixture of E/Z 3-indole vinyl-isocyanide 17/20 isomers. The poor selectivity of this HWE reaction was confirmed by analysis of the ¹H NMR spectrum of the crude reaction product which revealed well resolved resonances for their α-alkene protons at δ 5.7 ppm (Z, J = 8.6Hz) and δ 6.3 ppm (E, J = 14.2Hz) (Figure 28). However, the mixture of E and Z isomers could subsequently be separated by chromatography to afford each geometric isomer in its pure form.

Figure 28 Crude ¹H NMR spectrum of 3-indole vinyl-isocyanide 17 with alkene region expanded to show integration of E and Z isomer protons of 3:2 mixture of geometric isomers 17/20.
2.8 Synthesis of phenyl vinyl-isocyanide 542

Having established successful conditions that enabled PhosMIC reagent 500 to be used for the formation of the geometric isomers of 3-indole vinyl-isocyanide 17 our attention then turned to attempting to improve the E/Z selectivity of this reaction. Initially, we chose to investigate the E/Z selectivity of the HWE reaction between benzaldehyde 567 and PhosMIC 500 as substrates, with the aim of producing the E geometric isomer in >95 d.e. Starting with the conditions established for the synthesis of 3-indole vinyl-isocyanide 17, PhosMIC 500 was dissolved in THF, the resulting solution cooled to -78 °C and deprotonated using 1.2 equivalents of NHMDS. After stirring for 15 minutes benzaldehyde was added to the resultant ylide, and the whole was left stirring overnight. This afforded a 3 : 2 mixture of E/Z isomers of phenyl-vinyl isocyanides 568 and 569, however in this case this mixture of geometric isomers could not be separated by silica gel chromatography. Whilst there were no reports of E-selective HWE reactions using PhosMIC 500, a scan of the literature revealed a number of different strategies/approaches that had been used to improve the E-selectivity of analogous HWE reactions of phosphonate esters (such as 570) for the formation of \( \alpha,\beta \)-unsaturated esters (Figure 29). The majority of these reports included varying the nature of the base, solvent, and temperature used in the HWE reactions, whilst others made use of coordinating additives such as crown ethers or metal salts.

![Figure 29 Literature strategies reported to enhance the E selectivity of HWE reactions using acetate-phosphonate 570.](image)

Our first optimisation screen involved changing the nature of the alkali metal base used to generate the phosphonate carbanion required for HWE homologation. The use of n-BuLi as a
Development of an E-selective HWE protocol

Base for deprotonation of PhosMIC 500 was unsuccessful in improving E-selectivity, resulting in formation of a 1 : 1 mixture of E/Z geometric isomers (Table 3, Entry 2). The use of freshly prepared LDA as base increased the HWE reaction’s selectivity to 4 : 1 in favour of the E-isomer (Table 3, Entry 3), a selectivity that could not be improved upon by using one equivalent of 12-crown-4 to sequester the lithium counterion (Table 3, Entry 4). However, a major breakthrough occurred when LHMDS was used as a base for carbanion formation, giving the desired phenyl vinyl isocyanide as a 95 : 5 mixture of E/Z isomers in 30% isolated yield after chromatographic purification (Table 3, Entry 5). Once again, the addition of 12-crown-4 had no effect on the E/Z selectivity or yield of the HWE reaction when using LHMDS as a base (Table 3, Entry 6).

Table 3 Initial HWE base screen for phenyl-vinyl isocyanide 568

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Reaction Time</th>
<th>12-crown-4</th>
<th>E:Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaHMDS</td>
<td>16 h.</td>
<td></td>
<td>3 : 2</td>
</tr>
<tr>
<td>2</td>
<td>BuLi</td>
<td>16 h.</td>
<td></td>
<td>1 : 1</td>
</tr>
<tr>
<td>3</td>
<td>LDA</td>
<td>16 h.</td>
<td></td>
<td>4 : 1</td>
</tr>
<tr>
<td>4</td>
<td>LDA</td>
<td>16 h.</td>
<td>Yes</td>
<td>4 : 1</td>
</tr>
<tr>
<td>5</td>
<td>LiHMDS</td>
<td>2 h.</td>
<td></td>
<td>95 : 5</td>
</tr>
<tr>
<td>6</td>
<td>LiHMDS</td>
<td>2 h.</td>
<td>Yes</td>
<td>95 : 5</td>
</tr>
</tbody>
</table>

Whilst we had developed conditions for the preparation of phenyl-vinyl isocyanide 568, it soon became clear that this compound was a highly reactive and unstable molecule that was difficult to purify, handle and store. For example, neat phenyl vinyl-isocyanide was seen to polymerise when left standing on the bench for a few minutes, with complete decomposition of the product occurring during attempts to purify it by distillation. This susceptibility to polymerisation was also clearly observed when retrieving NMR samples that had been queued for analysis for an hour or more, with such samples being seen to contain increasing amounts of dark brown polymeric material over time (Figure 30). However, it was eventually discovered that samples of phenyl-vinyl isocyanide 568 could be stored at -18 °C.
in polar solvents such as chloroform, methanol or acetonitrile for up to 14 days without significant decomposition.

Figure 30 Degrading/polymerising NMR sample containing phenyl vinyl-isocyanide 568.

2.9 Synthesis of p-hydroxy phenyl vinyl-isocyanide 542

Having demonstrated that PhosMIC 500 could be used to prepare phenyl vinyl-isocyanide 568 with good levels of $E$ selectivity, our attention then turned to preparing the phenol vinyl-isocyanide (aglycone) 542. The previously optimised conditions employed for the HWE reaction of benzaldehyde 567 with the lithium anion of PhosMIC 500 were modified to employ 2.5 equivalents of LHMDS, due to the presence of the acidic phenolic proton (pKa 10) of the $p$-hydroxybenzaldehyde substrate. This HWE reaction gave the desired phenol vinyl-isocyanide 542 as a disappointing 7 : 3 mixture of its $E/Z$ (571) isomers (Scheme 66), that could not be separated by silica gel chromatography.

Scheme 66 Unselective synthesis of phenol vinyl-isocyanide 542.

At this stage, it was considered prudent to attempt to re-optimise the HWE conditions used to prepare phenol vinyl-isocyanide 542, and as a consequence, a range of bases were screened to try and identify more $E$-selective conditions for the synthesis of phenol vinyl-isocyanide 542. 2.1 equivalents of a range of bases (NHMDS, LHMDS, LDA, KHMDS, DBU,
DBN) were added to a solution of PhosMIC 500 in THF (with and without crown-ether) at -78 °C, to which a solution of p-hydroxybenzaldehyde 560 was then added. These experiments revealed that using, DBU, DBN or KHMDS as base gave no vinyl-isocyanide product (Table 4, Entries 1-3) and that NHMDS, LDA, gave poorer E/Z selectivities than LHMDS (Table 4, Entries 4-6). The use of LHMDS as base was optimal, affording a 7:3 ratio of the desired p-hydroxyphenyl vinyl-isocyanide 542 in 36% isolated yield. This time the presence of 15-crown-5 actually shut down the reaction, resulting in the formation of white precipitate upon addition of the aldehyde. (Table 4, Entry 7).

Table 4 Screen of bases commonly used for HWE reactions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>additive</th>
<th>% conversion</th>
<th>% selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DBN</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>DBU</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>KHMDS</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>NaHMDS</td>
<td>-</td>
<td>100</td>
<td>66:33</td>
</tr>
<tr>
<td>5</td>
<td>LDA</td>
<td>-</td>
<td>100</td>
<td>55:45</td>
</tr>
<tr>
<td>6</td>
<td>LiHMDS</td>
<td>-</td>
<td>100</td>
<td>70:30</td>
</tr>
<tr>
<td>7</td>
<td>LiHMDS</td>
<td>15-Crown-5</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

A quick solvent screen (dichloromethane, toluene, diethyl ether) revealed that only diethyl ether was successful in affording product in a slightly inferior 66:34 ratio. Carrying out the HWE reaction using an inverse addition protocol, involving dropwise addition of the lithium enolate of PhosMIC 500 to aldehyde 560 in THF at -78 °C did not result in improved E/Z selectivity. Certain HWE reactions of phosphonate esters are known to be reversible, a property that is often used to afford their more stable E alkene products under thermodynamic control. Consequently, it was decided to carry out the optimised HWE reaction of the enolate of PhosMIC 500 with p-hydroxyphenylbenzaldehyde 560 at various reaction temperatures (-78 °C, -40 °C, 0 °C, r.t. and 65 °C) to see whether the proportion of E-isomer formed would increase with increasing temperature. It was found that the E selectivity of this HWE reaction remained essentially unchanged over this temperature range.
Development of an E-selective HWE protocol

range, with lower isolated yields of vinyl isocyanide $542$ being obtained at higher temperatures.

![Mechanism of reversible HWE reactions](image)

Figure 31 Mechanism of reversible HWE reactions.

Subsequent tests on the protocol revealed that the optimum procedure in terms of selectivity/yield is to hold the reaction at -78 °C during carbanion formation and once addition of aldehyde is complete, bring the reaction rapidly to room temperature. Using this protocol phenol vinyl isocyanide was produced with 70% selectivity and isolated in 45% yield.

One reaction additive that did have an effect on the selectivity of this HWE methodology was LiCl, an effect that was originally reported by Masamune and Roush for the E-selective synthesis of involving triethyl phosphonoacetate (Scheme 67). $^{184}$ LiCl is added to the reaction so that it may coordinate to the phosphonate’s $\pi$-bonding oxygens, a process that serves to lower the pKa of the phosphonate’s methylene protons, enabling milder reaction conditions that favour the formation of reversible equilibria.

![Scheme 67 Masumune-Roush protocol for vinyl esters via Lithium coordination to phosphonates](image)

Scheme 67 Masumune-Roush protocol for vinyl esters via Lithium coordination to phosphonates.$^{184}$
Using the conditions reported by Masamune and Roush as inspiration, LiCl was mixed with PhosMIC 500 in THF at room temperature and left to coordinate for several minutes. The complex was then deprotonated using DBU base and after stirring for 15 minutes the p-hydroxybenzaldehyde 560 added (Scheme 68). After 16 h. TLC analysis of the crude reaction mixture highlighted that although a less polar unsaturated product had been formed, the starting aldehyde remained. Encouragingly, despite a low conversion (<15%) to the phenol vinyl-isocyanide 542, analysis of the crude $^1$H NMR confirmed that reaction had proceeded with 100% selectivity for the $E$-isomer. Close inspection of the reaction revealed that upon addition of LHMDS a white precipitate formed, and that maybe increasing the reaction temperature would improve the lithium-phosphonate’s solubility and drive the reaction to competition. Repeating the reaction at reflux and also with LHMDS base once again produced phenol vinyl-isocyanide solely as its $E$-isomer but failed to improve significantly upon the reaction’s abysmal conversion.

Scheme 68 Masumune-Roush conditions applied to synthesis of phenol vinyl-isocyanide 542.

Davies et al. reported the use of methyl Grignard as a base for the olefination of a variety $p$-substituted benzaldehydes involving ethyl phosphonoacetate 570, all of which had an $E$-selectivity greater than 99 : 1. Using his conditions, PhosMIC 500 was deprotonated using methyl magnesium chloride in THF and allowed to stir at room temperature for 15 min. before $p$-hydroxybenzaldehyde was added and the whole was refluxed overnight (Scheme 69). Analysis of the crude $^1$H NMR identified unreacted starting materials and a lack of conversion to the desired product. It was suggested that the lack of a second oxygen upon the phosphonate species had prevented magnesium chelation, and therefore the pKa of the phosphonate’s methylene protons had not been reduced, therefore preventing deprotonation by the Grignard reagent.
Development of an E-selective HWE protocol

Scheme 69 Davies conditions applied to synthesis of phenol vinyl-isocyanide 542.

The use a 1,2-dimethoxyethane (DME) as a coordinating solvent to encapsulate the base’s metal counter ions was reported by Heathcock & Thompson,\textsuperscript{186} again for olefinations involving ethyl phosphonoacetate 570. According to their protocol, the carbanion was given 15 min. at 0 °C to form using LHMDS in DME, with \( p \)-hydroxybenzaldehyde 560 added once the mixture had been warmed to room temperature (Scheme 70). Soon after the aldehyde’s addition, precipitation occurred and upon leaving the reaction stirring for 5 h. at room temperature no significant conversion to product had been observed, therefore the reaction mixture was heated at reflux overnight. Despite an increase of temperature, the precipitate remained and the reaction only reached 5% conversion to \( E \)-phenol vinyl-isocyanide 542. Refluxing a 50 : 50 mix of THF and DME to solubilise the precipitate resulted in full conversion to product, although phenol-vinyl isocyanide was formed as a 70 : 30 mixture of its \( E/Z \) isomers under these conditions.

Scheme 70 Trial of DME solvent for synthesis of phenol vinyl-isocyanide 542.

A number of examples have been reported where the reversible addition of iodine to alkene bonds has been used to isomerise mixtures of \( E/Z \) isomers to their thermodynamically more stable \( E \)-alkene isomers.\textsuperscript{187} Since we were keen to eliminate the need for chromatographic
separation, we next explored the potential isomerisation of phenol vinyl-isocyanide’s alkene functionality by dissolving the isomeric mixture of 542/571 in deuterated toluene and adding a small crystal of elemental iodine to the NMR tube (Scheme 71). The sample was monitored overnight by $^1$H NMR analysis, however no change in the ratio of the E and Z isomers was observed. Furthermore, attempts to catalyse E/Z isomerisation using reversible conjugate addition of diarylsulfide in THF to the alkene bonds of out vinyl-isocyanides also proved unsuccessful resulting in decomposition to afford multiple products.

Scheme 71 Attempts to isomerise a mixture of E/Z phenol vinyl-isocyanide 542.

Before seeking alternative reagents/routes it was proposed that the corresponding vinyl-formamide 572 may either proceed selectively or at least provide E/Z isomers that would be separable by chromatography. It was then assumed that once separated the vinyl-formamide 572 could then be dehydrated using the mesyl chloride conditions developed for the synthesis of PhosMIC (Scheme 72). Unfortunately it proved impossible to deprotonate the methyl protons of formido phosphonate 566 and despite conducting a small screen of strong bases (NaH, BuLi, LDA) no phenol-vinyl isocyanide 542 could be prepared via HWE homologation using the formido phosphonate 572. This is potentially why Erstabin 556 and structurally related vinyl-formamides were actually prepared via acid hydrolysis of their corresponding vinyl-isocyanides.188

Scheme 72 Failed HWE reaction using formido phosphonate 572.
Development of an E-selective HWE protocol

It should be noted that analysis of individual fractions eluted during numerous unsuccessful chromatographic attempts showed no significant change in the $E/Z$ ratio of the phenol vinyl-isocyanide 542 that was recovered. Significant success in separating mixtures of $E/Z$ alkenes of natural products has however been previously achieved by Mander using silica doped with silver nitrate as a stationary phase.\textsuperscript{189}

![Examples of natural products that have had their $E/Z$ isomers separated using Mander’s protocol.](image)

Consequently, silica gel TLC plates impregnated with 10% aqueous silver nitrate were prepared, and fractionation of the 3 : 2 mixture of $E/Z$ isomers was attempted, however its use as a stationary phase proved unsuccessful in affording any isomer enrichment. A similar lack of success was also observed when using silver impregnated silica for flash chromatography. A closer inspection of Mander’s review on using silver nitrate impregnated silica for separating mixtures of $E/Z$ geometric isomers revealed that the vast majority of successful separations that have been reported involve electron-rich alkenes. Therefore, it is likely that the unsuccessful chromatographic separation of our $E/Z$ isomers is a result of the presence of an electron poor alkene group that has insufficient electron-density to coordinate effectively to the silver atoms during the separation process.

As a last resort, phenol vinyl-isocyanide 542, as a 3 : 2 mixture of its $E/Z$ isomers was sent to Rob Field (John Inns Centre) for separation by reverse phase HPLC. After multiple attempts, the two isomers were separated using their analytical column (Figure 33), although the partial resolution of the two isomers highlights the difficulty in separating the phenol vinyl-isocyanide’s 542 isomers.
To investigate why improving the E-selectivity of the HWE reaction for the synthesis of phenol vinyl-isocyanide 542 had failed we decided to run some computational analysis. Using both the EDF2 and B3LYP density functionals with the 6-31G* basis for optimisations and vibrational frequencies and the 6-311+G, we calculated the free energy for both the optimised geometries of \( Z \)- and \( E \)-phenol vinyl-isocyanide 571/542 (Table 5).

**Table 5** Computational analysis of \( E \) and \( Z \) phenol vinyl-isocyanide 542

<table>
<thead>
<tr>
<th></th>
<th>EDF2</th>
<th>B3LYP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6-31G*</td>
<td>6-311+G**</td>
</tr>
<tr>
<td></td>
<td>5-31G*</td>
<td>6-311+G**</td>
</tr>
<tr>
<td>total energy/au</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis</td>
<td>-476.731438</td>
<td>-476.868500</td>
</tr>
<tr>
<td>trans</td>
<td>-476.731669</td>
<td>-476.868882</td>
</tr>
<tr>
<td>( \Delta E/kJ ) mol(^{-1} )</td>
<td>-0.61</td>
<td>-1.00</td>
</tr>
<tr>
<td>free energy correction/( \text{kJ mol}^{-1} )</td>
<td>cis</td>
<td>272.4957</td>
</tr>
<tr>
<td></td>
<td>trans</td>
<td>270.4026</td>
</tr>
<tr>
<td>( \Delta (G - E)/kJ ) mol(^{-1} )</td>
<td>-2.09</td>
<td>-2.07</td>
</tr>
<tr>
<td>( \Delta G/kJ ) mol(^{-1} )</td>
<td>-2.70</td>
<td>-3.09</td>
</tr>
<tr>
<td>equilibrium constant for cis to trans</td>
<td>2.97</td>
<td>3.48</td>
</tr>
<tr>
<td>mole fraction of trans at equilibrium</td>
<td>0.75</td>
<td>0.78</td>
</tr>
<tr>
<td>cis:trans ratio</td>
<td>1:3</td>
<td>1:3.5</td>
</tr>
</tbody>
</table>
The raw energy values from all 4 data sets were tabulated in atomic units (au) and these were subsequently converted into kJmol\(^{-1}\) enabling us to conclude that the Z-isomer has a free energy of 272 ± 1 kJmol\(^{-1}\), with the thermodynamically preferable E-isomer having a free energy of 270 ± 1 kJmol\(^{-1}\). By correcting for free-energy (zero-point energy, thermal energy and entropy) it was possible to confirm the actual difference in free energy between the two isomers as being in the region of 3 kJmol\(^{-1}\). This has been described as a negligible energy difference, and successfully evidences the challenges we are facing trying to establish conditions for a thermodynamic product that poses little to no energy preference to the kinetic product. Furthermore, by calculating the equilibrium constant for the interconversion between the Z and E forms, we can now state that at 25 °C an equilibrium mixture contains between 75% and 81% of the more stable trans isomer. The ratio is particularly interesting as it essentially rationalises the crude selectivities we are observing in our HWE homologations, and to a certain extent may explain why the percentage of Z isomer increases during lengthy storage. By thinking about mechanism of the HWE, and its associated energy profile, it seemed appropriate to account for this energy in terms of the steric clash that arises between the phosphonate ester groups and the alkene substituents during oxaphosphetane formation. It seemed logical that the highly strained four membered ring would actively create an equilibria whereby it would reverse through the starting materials to place the alkene substituents trans to one another (Figure 34).
Development of an E-selective HWE protocol

![Mechanism and energy level diagram for synthesis of Z- and E-phenol vinyl-isocyanide via HWE homologation.](image)

Figure 34 Mechanism and energy level diagram for synthesis of Z- and E-phenol vinyl-isocyanide via HWE homologation.

2.10 Development of novel, bulkier phosphonate.

From the above energy level diagram, which we theorised from the computational data, it was discussed that the use of more sterically crowded phosphonate ester might improve upon the HWE reaction’s selectivity. We envisaged that by substituting the phosphonate’s ethyl ether groups with larger iso-propyl ether groups, we would heavily disfavour the cis-oxaphosphetane as a result of increased steric bulk. This concept of phosphonate modification was not without precedent since Still and Gennari had developed a bis-trifluronated phosphonate version of the usual triethyl phosphonoacetate, whilst Ando has developed her own bisarylphosphonate species. Whilst both of these novel phosphonate esters had resulted in Z-selective H.W.E homologations, we were not deterred
Development of an E-selective HWE protocol

since both protocols had been for the synthesis of aryclates and we remained optimistic that this selectivity would not necessarily be the case for our non-chelating isocyanide substrate. To this end, the quaternary amine salt 565 used for the synthesis of the ethyl derivative (PhosMIC 500) was treated with triisopropyl phosphite and reacted under the previously used Arbuzov conditions to afford formamide 573, which upon dehydration with methanesulfonyl chloride in the presence of triethylamine base yielded the novel 1Pr-PhosMIC 574 (Scheme 73).

Pleasingly, when this bulkier phosphonate 574 was reacted with p-hydroxybenzaldehyde 560 under the optimal HWE conditions previously developed, an increase in E selectivity from 7:3 to 9:1 was observed (Scheme 74).

2.11 Protection of phenol functionality.

Despite extensive work targeted towards an E-selective synthesis of phenol vinyl isocyanide 542 via HWE reaction with PhosMICs 500/574 and p-hydroxybenzaldehyde 560 having been undertaken, it appeared that the acidity of the phenol group was still preventing the development of a truly E-selective HWE reaction for α,β-unsaturated isocyanides. To
investigate the validity of this hypothesis one final preparation of phenyl vinyl-isocyanide was carried out in the presence of pure phenol 575. To this end, a mixture of phenol and benzaldehyde was added to deprotonated \(^3\)Pr-PhosMIC 574 and allowed to stir under the optimum conditions reported in Table 4 (Scheme 75). Analysis of the crude \(^1\)H NMR spectrum proved to be most interesting, since the presence of phenol had indeed destroyed the selectivity of the HWE reaction, returning both isomers of phenyl vinyl isocyanide 542.

Scheme 75 Investigation of effect of phenol functionality upon HWE selectivity.

Returning to the reaction of \(p\)-hydroxybenzaldehyde 560 with PhosMIC 500 (c.f. Scheme 66) the effect of increasing equivalents of LHMDS base was scrutinised by carrying out reactions that contained 1.0, 1.5, 2.0, 2.5, 3.0 and 5.0 equivalents of LHMDS. These tests confirmed that a minimum of 2 equivalents of base were required for full conversion to phenol vinyl-isocyanide and that 2.5 equivalents were optimal. Alarmingly however, they also showed the inverse relationship between the equivalents of base and the selectivity of HWE reactions. To reduce the acidity of the substrate we sought to protect the phenol group as its silyl ether (TBS), methoxymethyl acetal (MOM) and acetate and then subject these aldehydes to the previously optimised HWE conditions using just 1.2 equivalents of LiHMDs base (Scheme 76). Since the \(\alpha,\beta\)-unsaturated isocyanide functionality had already proved to be highly sensitive, these protecting groups were chosen with consideration for their ability to be easily removed without the need for using strong acid. The silicon protecting group was installed by treating \(p\)-hydroxybenzaldehyde 560 with tert-butyldimethylsilylchloride and imidazole base. Initial attempts to purify the \(O\)-silyl benzaldehyde 576 by flash column chromatography were unsuccessful; however a kuglerhor distillation under reduced pressure proved efficient, affording \(O\)-silyl protected aldehyde 576 in near quantitative yield. Reflux of \(p\)-hydroxybenzaldehyde 560 with bromomethylmethyl ether in the presence of base afforded the \(O\)-MOM protected aldehyde 577, whilst 4-formylphenyl acetate 578 was accessed by mixing with acetyl chloride in the presence of \(Et_3N\) base. Pleasingly the \(O\)-sily
protected substrate 579 underwent HWE homologation with 99 : 1 selectivity for the desired E-isomer of O-silyl protected vinyl-isocyanide 579. A similar selectivity was observed when preparing O-MOM protected vinyl-isocyanide 580. Use of the O-acetate 578 failed to produce any of the desired vinyl-isocyanide product, and this was assumed to be a result of competing ketene-type decomposition of the acetate group to produce p-hydroxybenzaldehyde 560.

Scheme 76 Synthesis of O-protected benzaldehydes 576, 577, 578 and subsequent HWE reactions.

Having now isolated a sample of silyl-protected isocyanide 579, generation of the target phenol vinyl-isocyanide 542 by silyl ether removal was attempted using tetra butyl ammonium fluoride (TBAF), although this was without success (Scheme 77). Under these relatively acidic conditions degradation of the α,β-unsaturated isocyanide functionality was observed, and so milder, non-acidic conditions were sought. Pleasingly, when silyl-protected isocyanide 579 was dissolved in ethanol and treated with hydroxide, the resultant potassium ethoxide effectively deprotected the phenol to afford the target phenol vinyl-isocyanide 542 as a 95 : 5 mixture of its E/Z isomers (Scheme 77).
Development of an E-selective HWE protocol

Scheme 77 Synthesis of E-phenol vinyl-isocyanide 542 via desilylation.

2.12 Final conditions and synthesis of phenol-vinyl isocyanide (aglycone).

Despite now having a selective route to phenol vinyl-isocyanide 542, the low yield of this target aglycone from p-hydroxybenzaldehyde 560 was disappointing, with the HWE reaction being responsible for the greatest loss of mass during the two step sequence. Since monitoring of the HWE reaction by TLC and the subsequent analysis of the crude $^1$H NMR spectrum had shown full conversion of starting materials to product without any major impurities, the purification process was thought to be at fault. To this end, a route that no longer involved purification of silyl-protected isocyanide 579 was envisaged. Upon completion of the HWE reaction of aldehyde 560 with $^1$Pr-PhosMIC 574, the reaction mixture was worked-up as per protocol, THF removed in vacuo and the resultant oil quickly dissolved in ethanol and an excess of KOH added (Scheme 78). Within 1 h. the crude silyl-protected isocyanide 579 had been desilylated by the ethoxide and the resultant phenol vinyl-isocyanide purified by silica gel chromatography. Unfortunately, a slight loss of d.r. was observed for the isolated ‘free’ phenol vinyl-isocyanide, it being isolated as a 96 : 4 mixture of trans-/cis-isomers, with a sample left a room temperature slowly isomerising further over time.

Scheme 78 Final protocol for synthesis of E-phenol vinyl-isocyanide 542 (aglycone).
2.13 Conclusion

We sought to apply Schöllkopf’s diethy isocyanomethylphosphonate (PhosMIC 500) reagent for the synthesis of phenol vinyl-isocyanide 542, isolating this agylcone as its E-isomer upon silica gel chromatography. However, unlike the structurally related 3-indole vinyl-isocyanide 17, the 3 : 2 mixture of trans-/cis-isomers that arise from HWE homolagation were inseparable by chromatography, resulting in the need to develop an E-selective HWE protocol. Screening reagents, conditions and alternative procedures failed to increase the selectivity of the homologation reaction between PhosMIC 500 and p-hydroxybenaldehyde 560. We subsequently found that by increasing the size of the phosphonate ester groups from ethyl to isopropyl (PhosMIC 574) we were able to increase the E-selectivity of our HWE reaction from 7 : 3 to 9 : 1, with total selectivity being achieved by O-silyl protecting the aldehyde’s phenol group. This reduction in substrate acidity enabled us to use minimal LHMDS, and selectively access an E-vinyl-isocyanide intermediate that could be mildly desilylated to afford the desired phenol vinyl-isocyanide 542. Unfortunately there was a slight isomerisation during desilylation/isolation of the agylcone, it being isolated with a 95 : 5 d.r.

The subsequent chapters of this thesis detail our biological evaluation of the agylcone and its use in the synthesis of the two rhamnopyranose natural products, Byeyankacin 544 & Rhabduscin 543.
3 Biological evaluation of phenol vinyl-isocyanide

3.1 Melogenesis suppression

Previously (chapter 2) we introduced the vinyl-isocyanide derived secondary metabolites produced by the insect pathogens *Photorhabdus* and *Xenorhabdus* bacteria to suppress their prey’s immune response via inhibition of the tyrosinase oxidases responsible for melanin production (Figure 35).

![Chemical structures](image)

Clardy has already been able to deduce the biological mode of action for these vinyl-isocyanide derived natural products, successfully demonstrating 542, 543 and 544’s ability to inhibit mushroom tyrosinase and phenol oxidases (Table 1). During these studies it was noted that the free aglycone component (phenol vinyl-isocyanide 542) of both glycosides was significantly more potent than the glycosides. This lent itself to our working hypothesis that the sugar fragment serves to deliver the free phenol vinyl-isocyanide war head to the tyrosinase active site, and consequently we sought the E-selective synthesis of phenol vinyl-isocyanide 542 (and 3-indole vinyl-isocyanide 17) believing these to be the critical species for biological evaluation (chapter 2). Clardy’s studies had focussed on the inhibitory activity against tyrosinase and phenol oxidase enzymes that he selected for their relation to enzymes involved in melanogenesis (Section 2.3, Scheme 61). Through collaboration with Jean van den Elsen
(Department of Biochemistry, University of Bath) we were able to use his colony of *Manduca sexta* larvae (Figure 36) to further study the effects of phenol vinyl-isocyanide 542 and 3-indole vinyl-isocyanide 17 on melanin production.

Figure 36 *Manduca sexta* (tobocca hornworm) larvae (L) and pupa (R).

During pupation, the *Manduca sexta* larvae use melanin to form their chrysalis, a dark protective shell that protects them as they mature from caterpillar to moth. Since this melanin can be collected by draining the haemolymph from the *Manduca sexta*’s tail, we selected three larvae and injected one of them with phenol vinyl-isocyanide 542 and another with 3-indole vinyl-isocyanide 17. After feeding for one week, we studied the effects that the vinyl-isocyanides had had on melanogenesis, collecting the haemolymph of each caterpillar and exposing the pale blue solution to light. As expected the control sample (containing normal levels of melanin) instantly turned black (Figure 37). However, the samples collected from the larvae that had been dosed with the vinyl-isocyanides 542 and 17 remained clear, indicating that production of melanin by the two *Manduca sextas* had indeed been suppressed.

Figure 37 Vinyl-isocyanides ability to inhibit melanogenesis.

Impressively, the *Manduca sexta* survives being dosed with the vinyl-isocyanides 542 and 17, their apparent low toxicity proving that these isocyanide metabolites serve only to suppress the immune response, enabling the nematodes to kill the host.
3.2 Antibiotic Activity

The fact that *Photorhabdus* and *Xenorhabdus* continue to produce the vinyl-isocyanide metabolites (Figure 35) post host death made us keen to explore the possibility of a secondary antibiotic role that potentially enables the bacteria and nematode to effectively compete against other microorganisms. To this end, we screened phenol vinyl-isocyanide 542 against three clinically relevant strains of highly resistant *S. aureus* bacteria, testing their susceptibility to 542 by disk diffusion (Figure 38). For the disk diffusion, the dispersion disk was loaded via soaking in a 0.5 mg mL\(^{-1}\) solution of 542 in water : methanol (95 : 5), and then incubating the inoculated petri-dishes for 16 h.

![Figure 38 Disk diffusion assay for phenol vinyl-isocyanide inhibition of bacterial growth.](image)

The clear, zone of inhibition surrounding the dispersion disk clearly demonstrates the antibiotic nature of phenol vinyl-isocyanide 542, the three strains of bacteria all being susceptible. However, we understand that whilst disk diffusion assays provide a facile means of identifying bacterial susceptibility, the diameter of the clearance zone is dependent on a combination of the antibiotic’s strength and its rate of diffusion, and therefore these assays needed to be treated as a way of identifying hit compounds. Going forward we decided to use the disk diffusion assays to identify hit compounds, quantifying their antibiotic activity against highly resistant MRSA 252 through determination of their minimum inhibitory concentrations (MIC). In the case of phenol vinyl-isocyanide 542, the concentration required to kill 50% and 90% of the bacteria was 49.52 µg mL\(^{-1}\) (342 µM) and 90.81 µg mL\(^{-1}\) (626 µM) respectively. The results of these tests showing that the MIC values (molar) for our aglycone 542 are slightly higher than commercial antibiotic Erythromycin (MIC\(_{50}\) = 85.82 µg mL\(^{-1}\) (117 µM); MIC\(_{90}\) = 104.10 µg mL\(^{-1}\) (141 µM) (Figure 39).
Biological Evaluation of phenol vinyl-isocyanides

However, Erythromycin is a very large and difficult molecule to synthesise/isolate, a problem not shared by phenol vinyl-isocyanide 542 since it is easily prepared in 3 steps. Furthermore phenol vinyl-isocyanide's 542 small nature, water solubility and apparent low toxicity will all serve to promote this natural product as preferable drug candidate. To enable us to develop the best possible antibiotic candidate, further studies, including the synthesis of structurally related analogues was necessary. As vinyl-isocyanide antibiotics had been previously reported, we were keen to prepare these compounds for ourselves and compare their MIC$\text{so}_{50}$ and MIC$\text{so}_{90}$ values against MRSA 252 to those of phenol vinyl-isocyanide 542. Initially, we determined that the MIC values for the previously synthesised 3-indole vinyl-isocyanide 17 were poorer than those for phenol vinyl-isocyanide 542 (Figure 40).

![Figure 40](image_url) Antibiotic evaluation of phenol vinyl-isocyanide 542 vs 3-indole vinyl-isocyanide 17.

Schöllkopf’s SAR study had previously identified an improvement in antibiotic response when methylating the indole nitrogen of 17, brominating the phenyl ring or switching the indole component for a thiothene unit. Using our developed HWE protocol we synthesised the N-methyl 553, 5-bromo 554 and thiothene 555 derivatives, using 2.2 equivalents of base to ensure that the phosphonate ester reagent 574 remained deprotonated (Scheme 79).
Biological Evaluation of phenol vinyl-isocyanides

Scheme 79 Preparation and biological evaluation of previously reported ‘hit’ vinyl isocyanides.

The initial disk diffusion assays found that only the two indole derived species 553 and 554 inhibited bacterial growth. The methylation of the indole nitrogen (species 553) produced an antibiotic of similar potency to phenol vinyl-isocyanide 542, whilst bromination of the six membered ring produced a poorly antibiotic species 554. Since N-methyl derivative 553 had presented itself as a serious antibiotic candidate we decide to test the susceptibility of a non-resistant strain of bacteria, MSSA 476. The indole 553 species did not fare quite so well against this new strain of bacteria its MIC\(_{50}\) and MIC\(_{90}\) values of 133.51 and 144.93 µg mL\(^{-1}\) being significantly higher than the 34.19 and 100.24 µg mL\(^{-1}\) required by phenol vinyl-isocyanide 542. This, in our opinion, implies that the structure of phenol vinyl-isocyanide 542 would offer an antibiotic with a broader spectrum of activity, and consequently our efforts progressed into analysing the structure activity relationships (SAR) for our aglycone 542.
3.3 Structure Activity Relationships

In order to practically develop an antimicrobial agent with nanomolar inhibition, we carried out a small SAR study to evaluate the significance of each of phenol vinyl-isocyanide’s structural functionalities. Through the design and synthesis of a series analogues, each of which would be modified to remove or replace a key aspect of the molecule, we would be able to observe the affect that each portion of the molecule has on the antibiotic response. At first, we took advantage of the previously synthesised (Scheme 77) phenyl vinyl-isocyanide 568, with this removal of the hydroxyl group resulting in zero inhibition of bacterial growth. This was unsurprising since you have removed a polar functionality that is likely to be a key binding agent for the active site (Figure 41). We also screened two commercial, aliphatic isocyanides 582 and 583, both of which were biologically inert, serving to further highlight the requirement of more than just an isocyanide group.

![Figure 41 Biological evaluation of phenyl vinyl-isocyanide 568, tert-butyl isocyanide 582, and cyclohexy isocyanide 583.](image)

Next we sought to remove phenol vinyl-isocyanide’s ability to act as a hydrogen bond donor through alkylation of its OH. In reality, it was simpler to synthesise p-(methoxy)phenyl vinyl-isocyanide 585 from p-methoxybenzaldehyde 584 using our HWE conditions (Scheme 80). As expected, removing the hydrogen bond donating phenol also removed the antibiotic response, there being no clearance zone surrounding 585 in the disk diffusion assay.

![Scheme 80 Synthesis of methoxy(phenyl)vinyl-isocyanide 585.](image)
Having confirmed the importance of the phenol, and its potential to serve as a hydrogen bond donor, we decided to also validate the isocyanides’ importance by synthesising the isomeric nitrile species 587. This vinyl-nitrile was synthesised by reacting nitrile derived phosphonate ester 586 with \( p \)-hydroxybenzaldehyde 560 under our standard HWE conditions (Scheme 81).

As expected the MRSA 252 bacteria was not susceptible to phenol vinyl-nitrile 587, there being no clearance zone observable in the disk diffusion assay. Since the two molecules are isomeric, it would be reasonable to suggest a dependence on the isocyanide’s carbanion. We would assume that binding to an active site within the bacteria, may potentially be achieved via a similar copper binding interaction observed for phenol-oxidase enzymes.

Scheme 81 Synthesis of phenol vinyl-nitrile 587.

With nature’s choice of functional groups seemingly robust, we decided to complete our SAR studies with two modifications to phenol vinyl-isocyanide’s 542 core structure. First we sought to isolate the isocyanide functionality by ‘saturating’ the double bond and consequently deconjugating the isocyanide. Since the isocyanide would be vulnerable to reduction, treatment of phenol vinyl-isocyanide 542 with hydride or hydrogenation was not an option. Therefore, an approach via dehydration of the corresponding aliphatic formamide 590 was envisaged (Scheme 82). To this end, tyramine 588 was selectively \( N \)-formylated using acetic formic anhydride, with the resultant phenol ethyl-\( N \)-formamide 589 then being \( O \)-silylated upon subsequent exposure to TBSCI and imidazole. With the phenol protected, it was then possible to dehydrate formamide 590 into its corresponding isocyanide via treatment with methanesulfonylchloride and triethylamine base, with subsequent mixing with ethoxide removing the \( O \)-silyl protecting groups to afford phenol ethylisocyanide 591 (Scheme 82). By isolating the isocyanide, thus preventing donation of electron density from the electron rich aromatic ring, we again observed no antibiotic inhibition in the disk diffusion assay.
Biological Evaluation of phenol vinyl-isocyanides

The second alteration we wished to make to phenol vinyl-isocyanide was the removal of the ethyl linkage, therefore attaching the isocyanide functionality directly to the aromatic ring. Isocyano phenol 595 was synthesised in an analogous manner to phenol ethylisocyanide 591, starting with the selective N-formylation of amino-phenol 592 (Scheme 83). Treatment of amino-phenol 592 with acetic formic anhydride resulted in formido-phenol 593, with subsequently O-silylation by TBSCI and imidazole affording aryl formamide 594. Reaction of this formamide 594 with methanesulfonylchloride and triethylamine base resulted in its dehydration to the corresponding isocyanide. 4-isocyano phenol 595 was realised upon ethoxide mediated de-silylation, although this species began to decompose quickly at r.t. Owing to the conjugated nature of the isocyanide, MRSA 252 was found to be susceptible to 595 with a 26 mm clearance diameter being observed in the disk diffusion. Interestingly isocyano phenol was very potent, having low values for both its MIC$_{50}$ and MIC$_{90}$. Unfortunately this molecule was very unstable and difficult to handle, instantly polymerising in all solutions, and therefore deemed unsuitable for further investigation.
Biological Evaluation of phenol vinyl-isocyanides

The SAR confirmed that the natural structure produced by the Photorhabdus and Xenorhabdus bacteria is a highly effective compromise between potency and stability. In light of this, our future efforts would continue to focus around phenol derived vinyl-isocyanides.

3.4 Library of phenols

With the initial SAR results confirming the requirement of a conjugated phenol isocyanide, we decided to prepare a small library of E-phenolate vinyl-isocyanides and compare their biological response to the natural 4-phenol vinyl-isocyanide 542. In order to selectively prepare the E-isomers of ortho-, meta- and bis-phenol vinyl-isocyanides 602, 603 and 557 we began by O-silylating salicidaldehyde 596, 3-hydroxybenzaldehyde 597 and 3,4-dihydroxybenzaldehyde 598. Whilst the mono-phenol species both underwent the silylation (TBSCl/imidazole) reaction as before, the diol species required more than the standard 16 h. to reach completion by overcoming the steric crowding around the adjacent hydroxyls (Scheme 84). With all three of the silylated aldehydes 599, 600 and 601 isolated, our attention turned to their homologation. To this end, each of the prepared aldehydes was subjected to our optimised HWE protocol, affording the three vinyl-isocyanides 602, 603, 557 preferentially as their E-isomers upon de-silylation with ethoxide. Whilst the meta-phenol derivative 603 and diol 557 had comparative MIC\(_{50}\) values they did not perform as well for the MIC\(_{90}\), with the ortho-phenol 602 proving to be the poorest antibiotic out of the phenol regioisomers, potentially having a hydrogen bond donor too far away from the active sites acceptor.
Biological Evaluation of phenol vinyl-isocyanides

3.5 Isostere synthesis

Although encouraged by the low toxicity demonstrated by phenol vinyl-isocyanide 542 in the model system, we were inherently aware of the pharmacokinetic and toxicological problems associated with phenol derived drugs in vitro. These problems arise due to phenols being readily oxidised to quinones by the human body, and we therefore synthesised three bioisostere species (Scheme 85) that would successfully mimic the physiological and electronic properties of a phenol. As usual, the vinyl-isocyanide isosteres 607, 608 and 609 were synthesised from their parent carbonyl species 604, 605 and 606 using our optimised HWE protocol. All three of the isosteres retained phenol-vinyl isocyanide’s antibiotic response.
Biological Evaluation of phenol vinyl-isocyanides

3.6 Conclusions

Phenol vinyl-isocyanide 542 successfully inhibited melanogenesis in Manduca sexta larvae, confirming the immune suppressant action of these naturally occurring vinyl-isocyanides. Subsequent biological evaluation identified these isocyanides as promising antibiotic agents, demonstrating millimolar inhibition of a highly resistant strain of S. aureus bacteria. Initial comparison to previously reported antibiotic vinyl-isocyanides identified phenol vinyl-isocyanide as an equal (if not superior) antibiotic candidate. The SAR studies proved the requirement of the unsaturated isocyanide functionality and regiochemistry of the natural product’s phenol, which was subsequently replaced with pharmacokinetically and toxicologically preferable biosteres without reduction of antibiotic potency.
Phenol vinyl-isocyanide’s unique structure, low toxicity and antibiotic activity secured an additional Ph.D student (Liam Stephens) whose work to date was focussed around the synthesis of heteroaromatic vinyl-isocyanides such as 17 and 553. He will now focus on developing a library of phenolate vinyl-isocyanides (like 542), carrying out hit to lead optimisation to hopefully produce a vinyl-isocyanide derived antibiotic with nanomolar levels of inhibition.
4 Methodology for the Synthesis of Byelyankacin and Rhabduscin

4.1 Introduction

Previously the synthesis of $\alpha,\beta$-unsaturated isocyanides, via $E$-selective Horner-Wadsworth-Emmons homologation of their parent aldehydes was optimised (chapter 2), enabling the synthesis of $E$-phenol vinyl isocyanide 542 and a small library of antibiotic vinyl-isocyanides (chapter 3). With this in hand, our efforts now focused upon the synthesis of the two rhamnopyranoside natural products Byelyankacin 544 and Rhabduscin 543. For this, we intended to synthesise two sugar fragments tailored for glycosylation at their anomeric position, which upon stereoselective substitution with phenol vinyl-isocyanide 542 and subsequent removal of any protecting groups would yield Byelyankacin 544 and Rhabduscin 543 as single anomers (Scheme 42).

Scheme 42 Simplified representation of planned synthesis of natural products 543 & 544.

An important consideration for these sugar syntheses would be the choice of protecting group utilised to allow for selective modification of the anomeric substituents. To this end, the hydroxyl groups of the sugars would be protected as acetates since the mildly basic conditions required for acetate removal would be well tolerated by the sensitive vinyl-isocyanide functionality of both rhamnopyranosides 543 and 544. It was also intended to use neighbouring group participation of the C-2 acetate to control the stereochemistry of the glycosylation reactions, such that single anomers of each natural product would be formed.
4.2 Synthesis of Byelyankacin

Since Byelyankacin 544 can be derived from the commercially available sugar L-Rhamnose 610, its total synthesis would be tackled first, enabling the strategies and methodologies developed for its synthesis to be transferred onto the structurally similar, yet more complex Rhabduscin 543. For the synthesis of Byelyankacin 544, L-Rhamnose 610 was per-acetylated by dissolving in pyridine and treating with acetic anhydride and catalytic DMAP to afford per-acetylated Rhamnose 611 in near quantitative yield (Scheme 86). During this reaction, the DMAP activates the acetic anhydride via replacement of an O-acetate leaving fragment with a stronger, positively charged nitrogen derived leaving group, enabling nucleophilic attack by the deprotonated sugar hydroxyl groups to proceed quickly at low temperatures.

![Scheme 86 Per-acetylation of L-Rhamnose 610.](image)

Exploration of the literature presented a number of different glycosylation strategies for the aforementioned coupling of phenol vinyl-isocyanide 542 with a suitably derived sugar fragment. The simplest, in terms of fewest synthetic operations, appeared to be treatment of per-acetylated Rhamnose 610 with nucleophile 542 in the presence of a Lewis acid. The Lewis acid (LA) would effectively activate the anomic acetate group through coordination to the carbonyl, weakening the anomic C-O sigma bond. The ring oxygen’s lone pair is then able to kick in and eliminate the anomic acetate group, affording a sugar species containing an electrophilic carbonyl (Scheme 87). The neighbouring acetate group subsequently attacks this electrophilic carbonyl to form a furan-like ring that (in this case) effectively shields the anomic carbon’s β-face, forcing the incoming nucleophile to approach exclusively from the α-face.
Methodology for the synthesis of Byelyankacin and Rhabduscin

Scheme 87 Simplified representation of the mechanism involved with using a Lewis acid and neighbouring group participation effect to an α-glycosidic linkage.

The selectivity of these reactions would be easily determined through analysis of their $^1$H NMR spectra. Anomers are essentially diastereomers of one another and all protons would therefore experience different chemical environments depending on the orientation of the anomeric substituent. Ratios were easily determined through measuring the integrals of the two resultant peaks for the anomeric proton, with assignment of α versus β arising from investigation of the anomeric proton’s coupling constant (Figure 43).

According to the procedure described by Janetka et al., boron trifluoride diethyl etherate was added to a solution of per-acetylated Rhamnose 611 and phenol vinyl-isocyanide 542 in ice cold CH$_2$Cl$_2$ and left to stir for 36 h. at 45 °C (Scheme 88). Monitoring of the reaction indicated full consumption of starting materials after the allotted reaction time, and the formation of several new product spots. Analysis of the crude $^1$H NMR spectrum confirmed consumption of the starting material (loss of anomeric acetate peak), but also indicated destruction of the phenol vinyl-isocyanide peak (loss of alkene protons) without any clear product being identifiable amongst the many random peaks present in the spectrum. It was deemed likely that the Lewis acidic conditions required to activate the sugar was responsible for destruction of the vinyl-isocyanide, therefore preventing synthesis of the desired glycosyl vinyl-isocyanide 612.
Methodology for the synthesis of Byelyankacin and Rhabduscin

Since it appeared that the glycosylation conditions developed in the literature were not mild enough to be utilised with acid sensitive functionalities such as isocyanides, we decided to optimise conditions for the synthesis of the more stable glycosidic benzaldehyde fragment 612, which would then undergo HWE homologation and acetate deprotection to afford Byelyankacin 544 (Scheme 89).

To this end boron trifluoride diethyl etherate was added to a solution of per-acetylated Rhamnose 611 and p-hydroxybenzaldehyde 560 and left to stir for 36 h. at 45 °C. Under these conditions glycosylation proceeded without $\alpha$-selectivity, with subsequent flash column chromatography returning the mixture of anomers in very poor yield. In an attempt to rectify these problems with yield and selectivity, a brief screen of Lewis acids and conditions was undertaken (Table 6 entries 1-4). Changing the Lewis acid to TMS-triflate actually resulted in reduction of the $\alpha$-selectivity, whilst utilisation of aluminium trichloride also failed to increase selectivity and reduced the rate of reaction such that full consumption of starting material failed to occur within 36 h. Lastly, reducing the reaction temperature failed to achieve any conversion to the desired glycosyl aldehyde 613 (Table 6, entry 4), and it was decided to seek an alternative approach for this synthesis.
An alternative approach for glycosylation is to use a powerful trichloroacetimidate leaving group to activate the anomic position towards nucleophilic attack. The reaction is driven to completion by the formation of an energetically favourable amide-like product, with neighbouring group participation once again directing the approach of the incoming phenol from the bottom face (Figure 44).

For trichloroacetimidate synthesis, per-acetylated Rhamnose 611 was first selectively deprotected at its anomeric centre through reaction with benzylamine, followed by an acidic work-up to afford α-hydroxy tris-acetyl Rhamnose 614 (Scheme 90). Alcohol 614 was then reacted with trichloroacetonitrile, using caesium carbonate base, to yield trichloroacetimidate 615 that upon filtering through a short bed of silica gel was immediately reacted with p-hydroxybenzaldehyde 560 in the presence of TMS-triflate, affording Rhamnopyranoside 613 in 20% yield. Analysis of the 1H NMR spectrum for 613 found there to be only one set of peaks for all environments, suggesting a single anomer, with confident assignment as the α product owing to the anomeric proton at 5.55 ppm having the low coupling constant of 1.8 Hz.
Methodology for the synthesis of Byelyankacin and Rhabduscin

Despite having achieved the desired stereoselective synthesis of 613, the low yield of 20% was less than desirable, and therefore the alternative use of a thioether leaving group was explored. According to procedures outlined in the literature,194,195 thioether 616 was accessed as a mixture of anomers via treatment of per-acetylated Rhamnose 611 with thiophenol and boron trifluoride diethyl etherate in ice cold toluene (Scheme 91). The anomeric mixture of thioethers 616 then underwent stereoselective substitution with p-hydroxybenzaldehyde 560 when reacted with N-iodosuccinamide (NIS) and catalytic triflic acid, affording the desired rhamnopyranose 613 in 60% yield.

In this reaction, it is believed that the electrophilic iodine (from NIS) coordinates to the sulphur atom, activating the thiophenol leaving group to enable facile nucleophilic attack from the phenol oxygen via a similar mechanism to that of the Lewis acid activation strategy (Figure 45).
Methodology for the synthesis of Byelyankacin and Rhabduscin

Figure 45 Mechanism of NIS mediated activation of thioethers and subsequent glycosylation.

Having now achieved a highly selective method for synthesising glycosyl benzaldehyde 613, our attention turned to generating the α,β-unsaturated isocyanide functionality of Byelyankacin 544 via HWE homologation. Having previously encountered acetate instability during HWE homologation (Chapter 2), glycosyl aldehyde 613 was first treated with sodium methoxide in methanol to remove all of the acetate protecting groups, affording tris-hydroxy-aldehyde 617 in near quantitative yield (Scheme 92). Reaction of 617 with iPr-PhosMIC 574 under the optimised HWE conditions and 4.5 equivalents of LHMDS base resulted in complete conversion to Byelyankacin 544. The natural product was isolated by chromatography as 93 : 7 mixture of cis-/trans- isomers, and its NMR spectra found to be consistent to that report by Nakagawa and co-workers.\textsuperscript{196} Byelyankacin was subsequently screened for its antibiotic activity and found to have similar MIC\textsubscript{50} value to its aglycone (phenol vinyl-isocyanide) unit, possibly confirming our hypothesis about the sugar residue’s presence as a transport vector.

Scheme 92 Completed synthesis of byelyankacin 544.
4.3 Attempts to fix $E$ selectivity for sugar natural products

Despite the extensive optimisation work reported in chapter 2, the presence of additional hydroxyl groups upon glycosidic aldehyde 617 resulted in a slight reduction in $E$-selectivity during the HWE reaction responsible for the synthesis of Byelyankacin 544. Although ambitious, global $O$-silylation of Byleyankacin’s parent glycosidic aldehyde 617 was attempted using 5 equivalents of tert-butyl dimethyl silyl chloride and a large excess of imidazole base (Scheme 93). Analysis of this reaction by TLC and $^1$H NMR revealed a complex mixture of products, which were assumed to be a mixture of unreacted starting materials, plus the various regioisomers associated with a mixture of the various mono/bis/tris silylated products. Despite the use of highly forcing conditions, no further progress towards tris-silyl rhamnopyranoside 618 was made.

![Scheme 93 Attempted tris-silylation of sugar species.339](image)

Leaving this ill-fated global silylation behind, we envisioned that we could build upon our successful improvement in $E$-selectivity using $^3$Pr-PhosMIC 574 by designing and synthesising bulkier phosphonates substituted with either tert-butyl ethers or phenyl ethers. Upon accessing tri-tert-butyl phosphite via substitution of phosphorus trichloride with tert-butanol (Scheme 94), both the tri-tert-butyl and triphenyl phosphites were reacted with the usual amine salt 565 under Arbuzov conditions. However, the refluxing of tri-tert-butyl phosphite and triphenyl phosphite with the quaternary amine salt under Arbuzov conditions failed to afford either of the expected products (619 or 620) (Scheme 94). It appeared that the bulky nature of these two phosphites had rendered them unable to approach/substitute at the carbon centre adjacent the larger tertiary amine leaving group.
Methodology for the synthesis of Byelyankacin and Rhabduscin

Scheme 94 Failed Arbuzov reaction with larger trialky phosphites.

To overcome the steric limitations of the Arbuzov reliant protocol developed for the synthesis of the original two phosphonates 566 and 573, new routes to each of the required formamide precursors were developed. For the synthesis of 'Bu-PhosMIC 625 (Scheme 95), dibenzylamine 621 was mixed with p-formaldehyde 622 and the resulting slurry treated with the previously synthesised tri-tert-butyl phosphite. Refluxing this mixture provided access to the desired phosphonate 623. Hydrogenation of this dibenzyl protected amino-phosphonate 623 yielded amine 624 that was formylated upon treatment with acetic formic anhydride, generating phosphonate formamide 619 that in turn was dehydrated under the standard methanesulfonylchloride/triethylamine protocol to afford the desired 'Bu-PhosMIC 625.

Scheme 95 Synthesis of 'Bu-PhosMIC 625.
Meanwhile, for the synthesis of Ph-PhosMIC 629 (Scheme 96), a subtly different approach developed by Sierczky and co-workers was employed. To this end, benzyl carbamate 626 was mixed with \( p \)-formaldehyde 622 and triphenyl phosphite added to afford phosphonate-carbamate 627 that was subsequently converted to the corresponding amine salt 628 upon exposure to a solution of hydrogen bromide in acetic acid. The target Ph-PhosMIC 629 was achieved upon free-basing amine salt 628 using triethylamine and \textit{in situ} formylation of the resultant amine using acetic formic anhydride, the generated formamide 620 undergoing dehydration using methanesulfonyl chloride to afford Ph-PhosMIC 629.

Scheme 96 Synthesis of Ph-PhosMIC 629.

To investigate whether further increasing the steric bulk of the phosphonate ester would serve to improve \( E \)-selectivity in the HWE reaction, both of these novel PhosMICS (\(^t\)Bu 625 and Ph 629) were reacted with \( p \)-hydroxybenzaldehyde 560 under the optimal HWE conditions previously developed. For this test, each of the two phosphonates 625 and 629 were dissolved in anhydrous THF, cooled to -78 °C and deprotonated by LHMDS base. After stirring for 15 minutes, \( p \)-hydroxybenzaldehyde 560 was added, the solution warmed to r.t. and the whole left stirring under nitrogen for 16 h. (Scheme 97).

Scheme 97 Failed HWE reaction using novel bulkier phosphonates.
Methodology for the synthesis of Byelyankacin and Rhabduscin

It was highly disappointing that neither of these bulkier phosphonate reagents (625 and 629) underwent the desired HWE reaction intended to access phenol vinyl-isocyanide 542 with excellent levels of E-selectivity. Analysis of the crude $^1$H NMR of both reactions revealed a fairly clean mixture of the starting benzaldehyde 560 and phosphonate 625 or 629. Initially we were concerned that these phosphonate esters were too bulky to undergo deprotonation, and therefore a lack of carbanion formation had caused the reactions to fail. However, the nitrile version 630 of Ph-PhosMIC 630 had been shown to participate in HWE reactions using potassium tert-butoxide as a base (Scheme 98).\textsuperscript{198}

![Diagram](image)

Scheme 98 Zhang’s HWE homologation using diphenyl cyanophosphonate 630.\textsuperscript{198}

Given this precedent, we repeated the above test reactions (Scheme 97/Scheme 94) using potassium tert-butoxide in place of the LHMDS. Unfortunately this modification was not successful, with the crude $^1$H NMR spectra of these reactions again being consistent with those of the unreacted starting materials. Regrettably given the time limitations at this stage of the Ph.D, and the acquirement of a semi-preparative HPLC, it was decided to continue without further improvement to the HWE reaction’s selectivity.

### 4.4 Synthesis of Rhabduscin

Due to the lack of commercial availability of Rhabduscin’s required sugar residue 642, a synthesis to this key intermediate was developed from the commercially available methyl-\(\alpha\)-D-glucopyranose 633 involving: i) selective protection/deprotection of the hydroxyl groups C-4 and C-6 ii) selective elimination/substitution of C-6 and C-4 hydroxyl groups; (iii) reduction of azide/removal of O-benzly protecting groups (Scheme 99). The synthesis of Rhabduscin 543 would then be completed using the route/methodology conceived for Byelyankacin 544.
Methodology for the synthesis of Byelyankacin and Rhabduscin

Scheme 99 Planned synthetic route to Rhabduscin 543.

The first three steps in Rhabduscin’s 543 synthesis were to prepare di-\(O\)-benzyl protected sugar species 636 via a series of selective protections and deprotections of the hydroxyl groups, with an investigation of the literature suggesting the feasibility of a one-pot approach. To this end, commercially available methyl-\(\alpha\)-D-glucopyranose 633 was warmed to 30 °C and treated with benzaldehyde dimethyl acetal plus catalytic \(p\)-TsOH to facilitate selective acetal formation across the correctly orientated C-4 and C-6 hydroxyls (Scheme 100). This would then allow for the in situ di-benzylation of the remaining C-2 and C-3 hydroxyls via treatment with sodium hydride and benzylbromide, whilst subsequent exposure to aqueous acetic acid (post removal of organic solvent) was intended to effect the liberation of acetal protected C-4 and C-6 hydroxyls and afford di-\(O\)-benzyl protected sugar 636. In reality however, this approach failed to yield the desired product 636, with TLC analysis indicating full consumption of the starting materials and the formation of a complex mixture of products that could not be separated by chromatography.

Scheme 100 Failed one-pot synthesis of di-\(O\)-benzyl protected sugar 636.

In light of this failure, it was decided to approach the synthesis of 636 step-wise (Scheme 101) since this would enable proper isolation of the intermediate products, hopefully improving the efficiency of the operations. Acetal protection of the C-4 and C-6 hydroxyls in methyl-\(\alpha\)-D-glucopyranose 633 was again realised using benzaldehyde dimethyl acetal and 30 mol% of \(p\)-
TsOH (Scheme 101). Heating this mixture at 60 °C for 2 h. followed by neutralisation with triethylamine and aqueous workup afforded sugar acetal 634 that was easily purified on large scale by trituration with petroleum ether washings effectively removing excess benzaldehyde dimethyl acetal. The remaining two hydroxyls were then benzyl protected upon refluxing a suspension of potassium hydroxide, benzyl bromide and sugar acetal in toluene, affording relatively pure di-benzyl protected acetal 635 in excellent yield post aqueous work-up.

Attempts to remove the acetal protecting group of 635 using various acidic conditions failed to generate di-O-benzyl protected sugar 636 in any appreciable yield (Scheme 103). Increasing the stoichiometry/temperature of reaction had little effect. Refluxing of 635 with catalytic iodine and methanol according to the procedure first described by Sletten and Liotta (Scheme 102) did however prove successful, affording the required di-O-benzyl protected sugar 636 in 90% yield.\(^{199}\)

With the synthesis of the di-benzyl protected fragment 636 completed, work could then commence on the elimination/reduction operations required for the synthesis of sugar residue 642 (Scheme 103), which in turn would then enable the synthesis of Rhabduscin 543 via the methodology developed for the synthesis of Byelyankacin 544. Di-benzyl protected sugar 636 was regioselectively tosylated at its primary C-6 hydroxyl group using tosylchloride and triethylamine base, with the afforded O-tosylate 637 undergoing hydride reduction upon treatment with lithium aluminium hydride. This effectively yielded the required methyl functionality of 638 with modification of the hydride reduction’s work up such that the
reaction was quenched with aqueous ammonium chloride instead of water resulting in a 20% increase in reaction yield.

Scheme 103 Generation of the methyl group required for Rhabduscin.

To complete the synthesis of our desired pseudo starting material 642 the generation of the C-4 amine functionality and eventual removal of the remaining benzyl protecting groups had to be accomplished. To this end, secondary alcohol 638 was rapidly mesylated upon dissolving in ice cold pyridine and treating with methanesulfonylchloride to afford O-mesylate 639 (Scheme 104). Reaction of this leaving group containing species 639 with sodium azide, resulted in an S_N2 inversion about the C-4 centre thereby affording axial azido sugar species 640. The addition of crown ether was believed to increase the rate of this reaction by abstracting the sodium cation, therefore enhancing the nucleophilicity of the resulting aza-anion. It had been hoped that the amino-alcohol species 642 would simply be realised upon hydrogenation, with the presence of a palladium catalyst in a hydrogen atmosphere reducing the azide to the corresponding amine 641, whilst concomitantly de-benzylating the protected C-2 and C-3 hydroxyls. However complete reduction and deprotection of hydroxyls failed to occur under these conditions and it was suggested that whilst the initial azide reduction may have occurred rapidly, analysis of the ^1H NMR spectrum confirmed the presence of benzyl species 641 (Scheme 104).
Methodology for the synthesis of Byelyankacin and Rhabduscin

To establish more forcing reaction conditions, the azide 640 was placed in a bomb reactor and the hydrogen pressure increased from 1 to 5 atmospheres. However, the pressurised hydrogen atmosphere had a detrimental effect, failing to produce the desired reaction products, instead producing an uncharacterisable mixture of random products (Scheme 105). Providing additional kinetic energy to the reactants by repeating the atmospheric hydrogenations at the higher temperature of 40 °C had no effect.

The use of acid containing solvent mixtures did however prove worthwhile, and whilst the use of aqueous hydrochloric acid in methanol successfully reduced the azide 640 to amine 641, analysis of the $^1$H NMR spectrum confirmed that the benzyl groups had unfortunately remained attached (Scheme 106). Pleasingly, a mixture of dioxane and water (kept at pH 6) was found optimal for reduction and the concomitant de-benzylation of 640 to pseudo amino-alcohol starting material 642. TLC analysis confirmed the presence of a new, highly polar product that contained no aromatic or O-benzyl protons in its $^1$H NMR spectrum. Despite a very poor yield for this reaction, we were keen to achieve the synthesis of per-acetate derivative 644 since this would provide us with an intermediate that was directly comparable to the Byelyankacin synthesis. The small amount of crude di-hydroxy-amine 642 that we had
characterised was acetylated under the standard DMAP catalyzed conditions to afford amide \textbf{643} (Scheme 106), the $^1$H NMR spectrum of this species clearly showing the addition of two O-acetate CH$_3$ singlets at 2.11 and 2.08 ppm, plus an additional singlet at 2.00 ppm for the amide CH$_3$ group. This amide \textbf{643} was then further exposed to acetic anhydride, this time in the presence of sulphuric acid, to facilitate replacement of the methyl ether functionality with a third and final O-acetate. Although our sugar sample was becoming rapidly depleted, we were able to obtain a crude $^1$H NMR of this per-acetate \textbf{644}, the spectrum clearly showing replacement of the methoxy singlet at 3.39 ppm with a new O-acetate singlet at 1.95 ppm.

![Scheme 106 Successful hydrogenation protocol and subsequent per-acetylation.](image)

Unfortunately, no success was had at effectively scaling our hydrogenation protocol, and thus significant quantities of di-hydroxy amine \textbf{642} were never obtained. Regrettably, the deadline for this Ph.D was imminent, and therefore the synthesis of Rhabduscin \textbf{543} is yet to be completed. To realise this goal, we obviously need to improve our hydrogenation protocol such that we may access significant quantities of di-hydroxy amine \textbf{642}, and subsequently convert this to per-acetate \textbf{644}. Since this per-acetate provides a synthetic comparison to the synthesis of Byelyankacin \textbf{544} it was our intention to subject per-acetylated sugar \textbf{644} to the methodology that had been previously developed for the synthesis of Byelyankacin \textbf{544}. Reaction of this per-acetylated fragment \textbf{644} with thiophenol and boron trifluoride diethyl etherate would again be effective at functionalising the sugar fragment with a leaving group (Scheme 107). This reaction would afford thioether \textbf{645} that upon treatment with NIS, triflic acid and \textit{p}-hydroxybenzaldehyde \textbf{540} would selectively glycosylate \textbf{645} to yield aldehyde \textbf{646} as its $\beta$-anomer due to neighbouring group participation of the adjacent acetate. Selective hydrolysis of glycosyl benzaldehyde \textbf{646} with sodium methoxide would have resulted in
deprotection of the two O-acetates whilst leaving the more stable amide functionality untouched, yielding the final intermediate 647 in near quantitative yield. Finally, we intended to achieve Rhabduscin 543 upon reaction with LHMDS deprotonated 1Pr-PhosMIC 574 (Scheme 107).

![Scheme 107 Envisaged route to Rhabduscin 543.](image)

### 4.5 Conclusions

With the instability of phenol vinyl-isocyanide 542 preventing its use as a coupling partner for rhamnopyranoside synthesis, we instead developed a synthesis of glycosidic aldehydes that would subsequently undergo HWE homologation to afford Byelyankacin 544 and Rhabduscin 543. HWE reaction of the pyronaside-aldehyde 617 with our isopropyl phosphonate reagent effected Byelyankacin’s 544 synthesis with a 93 : 7 preference for the desired trans-isomer. For Rhamnose, synthesis of the starting amine-derived sugar 642 was required, it being our intention to subject this species to the synthetic operations previously used for Byelyankacin’s 544 synthesis. Whilst able to achieve the synthesis of this ‘starting sugar’ 642, the hydrogenation protocol used in the final step of its synthesis was un-reliable and low yielding, hampering our efforts towards Rhabduscin 543. It is hoped that we will have time in the near future to overcome this problem and then proceed on gram scale towards Rhabduscin 543, confident that the synthetic procedures used for Byelyankacin 544 will work equally as well for Rhabduscin’s synthesis.
5 Synthesis of Paerucumarin

5.1 Introduction

When researching the biosynthesis of vinyl-isocyanides Byelyankacin 544, Rhabduscin 543 and their aglycone (phenol vinyl-isocyanide 542), an additional ‘pseudo’ vinyl-isocyanide derived natural product Paerucumarin 19 (Figure 46) was brought to our attention. 3-indole vinyl-isocyanide and phenol vinyl-isocyanide 542 (and therefore Byelyankacin 544 and Rhabduscin 543) are biosynthesised by the action of isonitrile synthases (isnA/B) upon amino acids tryptophan 14 and tyrosine 11 (Figure 8). For this species, alternative Pvc gene clusters are expressed/replace isnA and isnB, transforming tyrosine 11 into a 3-isocyano-6,7-dihydroxycoumarin 19 natural product.20,200 For its biosynthesis, PvcA first acts as an isonitrile synthase, transforming the amine functionality of tyrosine 11 into its related isocyanide 648, which is then followed by PvcB promoted elimination to afford vinyl-isocyanide 559. Paerucumarin 19 is generated by PvcC/D oxidation of phenol 559 to diquinone 649 and its subsequent cyclisation/aromatisation.

![Figure 46 Biosynthesis of Paerucumarin 19.](image)

Based on the findings of the literature review, and indeed our previous syntheses of aliphatic isocyanides, it seemed highly likely that our synthesis would involve dehydration of Paerucumarin’s 19 parent formamide. We anticipated that since the formamide’s double bond would be part of the lactone ring, it would render this species stable enough to the harsh conditions required to effect its dehydration. Interestingly, this 3-formido-6,7-
dihydroxycoumarin is known as Pseudoverdin 700, a secondary metabolite of pPYP17 mutated \textit{Psuedomonas aeruginosa} bacteria usually responsible for the biosynthesis of the Pyoverdins 701 (Figure 47).\textsuperscript{201} This mutation produces Pseudoverdin 700 from dihydroxylation of tyrosine, with the installation of the C-2 phenol enabling facile ring closure through action upon the amino acid’s carboxyl group.\textsuperscript{202}

Since a synthesis of these natural products is yet to be reported, we were excited to develop an efficient synthesis of Paerucumarin 19 via Pseudoverdin 700. Owing to the structural difference of Paerucumarin 19 with respect to phenol vinyl-isocyanide 542 we anticipated these coumarin species to be biologically inert.

### 5.2 Synthesis of Pseudoverdin and Paerucumarin

When researching the synthesis of coumarin systems it became evident that the most popular strategy involved condensation of the corresponding salicylaldehyde with an appropriately functionalised methylene ester. Of particular interest was the method reported by Shen and co-workers, for their catalytic condensation of salicylaldehydes with ethyl isocynoacetate 702 provides a direct route to 3-formaldo-coumarins (Figure 48).\textsuperscript{203} For his mechanism, Shen implies that copper’s soft nature enables it to bind the isocyanide of the ester reagent, lowering the pKa of the methylene protons such that the pyridine may
deprotonate here. The resultant enolate then attacks the salicinaldehyde’s carbonyl functionality, with the generated hydroxyl anion then undergoing hydrogen exchange with the phenolic proton, an equilibrium process that serves to generate a nucleophilic oxygen for lactone formation. Post cyclisation, the remaining sp² hybridised alcohol group clips onto the isocyanide carbon, affording an oxazole-like intermediate that rearranges to yield both the alkene and formamide functionality of the coumarins (Figure 48).

With this in mind, it was our intention to condense 2,3,5-trihydroxybenzaldehyde 703 with ethyl isocyanoacetate 702, as per Shen’s protocol, and then dehydrate Psuedoverdin 700 with methane sulfonylchloride to afford the isocyanide functionality of Paerucumarin 19. At this time we were unsure as to whether the diol functionality would need to be O-silyl protected for the dehydration step, and this was something we intended to explore with a series of test reactions post-cyclisation. To start with, 2,3,5-trihydroxybenzaldehyde 702 was
Conduction of Paerucumarin

suspended in methanol, treated with copper iodide, pyridine and ethyl isocyanoacetate 702
and left to stir at 50 °C overnight (Scheme 108).

Scheme 108 – Attempted application of Meng’s protocol to 2,3,5-trihydroxybenzaldehyde 703.

Unfortunately no success was to be had using the highly expensive tris-hydroxybenzaldehyde 703, with multiple attempts at this reaction returning the starting aldehyde. We speculated that it was the presence of ‘surplus’ hydroxyls and their competitive deprotonation that was to blame for the failed cyclisation of this salicialdehyde. Furthermore, this may explain why there were no hydroxyl-coumarin examples published in Shen’s paper.203 To overcome this complication, we sought to find an alternative salicialdehyde with the C-4 and C-5 hydroxyls protected, leaving the C-2 hydroxyl free to participate in the condensation reaction without competition. Subsequent research brought benzodioxolane 706, and its synthesis from readily available sesamol 704, to our attention.204 Whilst the strategies for removing the methylene ether protecting group are limited to the use of highly acidic boron tribromide, we assumed that an aromatic formamide would be able to survive these unavoidably harsh conditions. Using the chemistry reported by Nichols and co-workers,204 the free-phenol functionality of sesamol 704 was first protected as its O-acetate via treatment with catalytic DMAP and acetic anhydride, using pyridine as a solvent and base. The resultant O-acetate 705 then underwent a Friedel-Crafts formylation, with concomitant acetate hydrolysis, through the action of tin tetrachloride and dichloromethyl methyl ether to afford the desired benzodioxolane functionalised salicaldehyde 706 (Scheme 109).

Scheme 109 Synthesis of suitable ‘protected’ salicaldehyde derivative 706.204
This benzodioxolane containing salicinaldehyde 706 was then subjected to Shen’s coumarin synthesis via condensation with ethyl isocyanoacetate 702 in the presence of catalytic copper iodide and pyridine base (Scheme 110). After stirring this combination for 7 h. in 50 °C methanol, the resultant pink precipitate was collected by filtration and characterised as the desired (and novel) 3-formido-coumarin 707. The four singlet proton peaks observed in 1H NMR spectrum of 707 were found to be comparable in shift to those recorded by Shen for the structurally similar 3-formido-6,7-dimethoxycoumarin. Definitive confirmation of its structure came from analysis of its mass spectrum and interpretation of infra-red stretches for the amide and lactone functionalities of 707.

The sensitivity/intolerance of isocyanides to harsh and/or acidic conditions made it necessary to carry out boron tribromide facilitated ether cleavage prior to dehydration of the formamide functionality, affording Pseudoverdin 700 in the process. The addition of excess boron tribromide to ether 707 successfully cleaved the methylene bis-ether functionality, as noted by loss of the CH₂ ether peak at 6.14 ppm in the 1H NMR spectrum (Scheme 111). Interestingly, we also noted the loss of the peaks at 10.12 and 8.59 ppm (formamide NH and CHO), suggesting that we hadn’t synthesised Pseudoverdin 700 as expected. Initially, it was assumed that the electron-deficient nature of the boron had served to concomitantly dehydrate the formamide by a similar mechanism to that of methanesulfonylchloride facilitated dehydration. However, we had not accidently synthesised Paerucumarin 19 because the infra-red spectrum for the isolated product did not contain the characteristic isocyanide stretch around 2100 cm⁻¹, instead we observed a nitrile stretch at 2251.71 cm⁻¹. Further evidence for the presence of a nitrile was found in the 13C NMR, with the 11th carbon not appearing as a broad quadrupolar shift around 160 ppm, but as a nitrile resonance at 110 ppm. The rearrangement of aromatic isocyanides to their corresponding nitrile has been reported at elevated temperatures. Therefore we believe that the harsh boron tribromide conditions have served to concomitantly cleave the ether group of 707 and dehydrate its formamide 700 functionality to initially yield Paerucumarin.
However, owing to the harsh reaction conditions, it appears that the isocyanide functionality has subsequently rearranged to the corresponding nitrile species \( 708 \). By reducing the reaction temperature to \(-78^\circ\text{C}\), we were able to isolate an inseparable mixture of nitrile-coumarin \( 708 \) and Paerucumarin \( 19 \), the IR of this reaction mixture clearly contains both the nitrile peak (2981.19 cm\(^{-1}\)) and isocyanide peak (2124.79 cm\(^{-1}\)).

\[
\text{Scheme 111 Problematic ether cleavages of formido-coumarin } 707. \\
\]

To circumnavigate this problem, it was decided to mask formamide \( 707 \) as its parent amine, and then attempt to cleave the ether group using boron tribromide, reformylating the amine if successful. To this end, formamide \( 707 \) underwent acidic hydrolysis using a 10:1 mixture of ethanol and aq. HCl, with the resultant amine \( 709 \) then being treated with boron tribromide. As a precaution, the amine was cooled to \(-78^\circ\text{C}\) for the deprotection and by monitoring the reaction we found the ether group to be effectively cleaved after 3 h., affording clean dihydroxy-amino-coumarin \( 710 \) in near quantitative yield over the two steps. The poor solubility of this dihydroxy-amino-coumarin \( 710 \) in solvents other than water or alcohol proved problematic for its formylation, with our usual approach using acetic formic anhydride in THF proving ineffective.
Pleasingly however, refluxing amine 710 in formic acid achieved its formylation, thus providing the first synthesis of Pseudoverdin 700. Finally, the formamide functionality of 700 was dehydrated using methanesulfonylchloride and pyridine, affording Paerucumarin 19 and an inseparable unknown impurity (Scheme 113). The spectra of Pseudoverdin 700 and crude $^1$H NMR spectrum of Paerucumarin 19 could be validated against those reported by the isolation teams.²⁰,²⁰¹

**Scheme 112 Progress towards Paerucumarin 19 via Pseudoverdin 700.**

5.3 **Conclusions**

Pseudoverdin 700 was synthesised from a dihydroxy-3-amino-coumarin species 707 that was accessed using an elegant literature condensation of salicaldehyde species 706 and ethyl isocyanateacetate 702. Furthermore, we demonstrated that Pseudoverdin may be dehydrated to Paerucumarin and work is on-going to cleanly isolate this isocyanide derived natural product via fractional crystalisation.
6 Experimental

6.1 General Considerations and Procedures

Commercially available solvents and reagents were obtained from Sigma-Aldrich Company Ltd., Fisher Scientific Ltd., Alfa Aesar or Fluorochem Ltd. and were used without further purification. ‘Petroleum ether’ refers to the fraction of petroleum ether boiling in the range of 40-60 °C. All reactions were performed in oven-dried apparatus, whilst anhydrous solvents were obtained from an Innovative Technology Inc. PS-400-7 solvent purification system. Phosphate buffer refers to the pH 7 buffer made by mixing dibasic sodium phosphate (61.5 mL, 0.5M) and monosodium phosphate (38.5 mL, 0.5M).

Analytical thin layer chromatography was performed using commercially available aluminium backed plates coated with Merck G/UV254 neutral silica. Plates were visualised under UV light (at 254 nm) or by staining with either iodine adsorbed onto silica or phosphomolybdic acid followed by heating. Flash chromatography was performed using chromatography grade silica, 60 Å particle size 35-70 microns from Fisher Scientific.

$^1$H NMR spectra were recorded at 500 MHz, 400 MHz 300 MHz or 250 MHz and $^{13}$C($^1$H) spectra were recorded at 125 MHz 100 MHz or 75 MHz on a Brüker Avance 500, 400 or 300 spectrometer respectively. Chemical shifts, $\delta$, are quoted in parts per million and are referenced to the residual solvent peak. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; dq doublet of quartets; m, multiplet; pent, pentet; td, triplet of doublets; ddd, doublet of doublet of doublets; app, apparent and br, broad. Coupling constants, $J$, are quoted to the nearest 0.1 Hz.

High resolution mass spectra were recorded on a Brüker Daltonics microTOF spectrometer with an electrospray source and external calibration. Masses were recorded in positive electrospray ionisation mode and were introduced by flow injection. Masses are accurate to 5 ppm and data was processed using Data Analysis software from Brüker Daltonics. Compounds
containing an isocyanide or trichloroacetimidate were unable to withstand ionisation, and therefore mass spectra were not obtained for these species.

Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer, using a Universal ATR accessory for sampling, with only selected absorbances quoted as $\nu$ in cm$^{-1}$. Optical rotations were recorded on an Optical Activity Ltd AA-10 automatic polarimeter with a path length of 1 dm; concentrations (c) are quoted in g/100 mL.

Purification/analysis of compounds by HPLC was performed on an Agilent technologies 1260 infinity semi-preparative HPLC using an eclipse XDB-C18 5 $\mu$m, using UV light (at 254 nm) for detection.

**General Procedure 1: Arbuzov Reactions**

The appropriate tri-alkyl phosphite (1.3 equiv.) is added to a suspension of $N$-((dimethylamino)methyl)formamide 565 (1.0 equiv.) in EtOH (10 mL g$^{-1}$) under a nitrogen atmosphere, and the whole refluxed (bath temp. 100 °C) for 8 h. The EtOH is subsequently removed in vacuo and the EtOAc soluble components of the resulting residue are collected after filtration as the crude product(s).

**General Procedure 2: Dehydration of formamides**

A dry flask purged with nitrogen is charged with the desired formamide (1.0 equiv.), anhydrous CH$_2$Cl$_2$ (10 mL g$^{-1}$) and Et$_3$N (9 equiv.). The whole is cooled to -78 °C and MsCl (3.0 equiv.) added dropwise. After stirring for 16 h., the reaction is quenched by the addition of aq. NaHCO$_3$, and extracted twice with CH$_2$Cl$_2$, the organics then being dried over MgSO$_4$ and concentrated (post filtration) to afford the crude reaction product(s).
Experimental

**General Procedure 3: O-silylation protocol**

The appropriate hydroxyl species (1 equiv.) and imidazole (1.1 equiv.) are dissolved in CH$_2$Cl$_2$ (10 mLg$^{-1}$) at r.t. and tert-butyl-di-methylsilylchloride (1.1 equiv.) added portion wise over 10 min. After stirring for 16 h., the reaction is quenched with H$_2$O and extracted thrice with CH$_2$Cl$_2$. The combined organics are washed with brine, dried over MgSO$_4$ and concentrated (post filtration) to afford the crude reaction product(s).

**General Procedure 4: N-formylation reactions**

The required amine (1.0 equiv.) is dissolved in THF (5 mLg$^{-1}$) and cooled to 0 °C in an ice bath. Acetic formic anhydride (1.1 equiv.) is then added dropwise and the whole is stirred for 1 to 2 h., before THF and excess acetic formic anhydride are removed by rotary evaporation to afford the crude product(s).

**General Procedure 5: O and N-Acetylation of sugars**

Over a period of 30 min., ice cold acetic anhydride (10 mLg$^{-1}$) is added dropwise to a mixture of the appropriate alcohol/amine (1.0 equiv.) and DMAP (10 mol%) dissolved in pyridine (20 mLg$^{-1}$), the whole then being stirred for 3 h. at 0 °C under nitrogen. The reaction mixture is then concentrated (post filtration) *in vacuo* and the evaporation residue dissolved in toluene (50 mLg$^{-1}$) and washed twice with aq. 1M HCl, once with water, once with brine and then dried over MgSO$_4$ and concentrated to afford the crude product(s).

**General Procedure 6: Synthesis of thioethers**

Boron trifluoro ethyl etherate (1equiv.) is added to a 0 °C solution of the appropriate sugar acetate (1 equiv.) and thiophenol (1 equiv.) dissolved in anhydrous toluene (5 mLg$^{-1}$). The reaction mixture is then warmed to r.t. and stirred for 2 h. before being cooled back to 0 °C.
and quenched with aq. 1M NaOH (5 mLg⁻¹). Following extraction of the aqueous phase with two portions of toluene, the combined organics were washed with brine, dried over MgSO₄ and concentrated (post filtration) to afford the crude product(s).

**General Procedure 7: Glycosidation reactions**

Triflic acid (0.25 equiv.) and N-iodosuccinamide (1.0 equiv.) are added in quick succession to a -40 °C solution of the appropriate sugar derived thioether (1.0 equiv.) and 4-hydroxybenzaldehyde (1 equiv.) dissolved in CH₂Cl₂ (5 mLg⁻¹) under a nitrogen atmosphere. After stirring at -40 °C for 16 h., Et₃N and CH₂Cl₂ are added and the whole mixture filtered through a pad of celite. The organic filtrate is then washed with aqueous Na₂S₂O₃ and brine, with the crude product(s) being afforded upon drying the organic phase over MgSO₄ and concentrating (post filtration) *in vacuo*.

**General Procedure 8: Hydrolosis of acetates**

NaOMe (0.5 M in MeOH) (0.25 equiv.) is added dropwise to the appropriate acetate (1 equiv.) dissolved in MeOH (5 mLg⁻¹) and stirred for 3 h. under nitrogen before being neutralised by pouring through a plug of Amberlite IR-120 resin and the resultant mother liquor evaporated to afford the crude product(s).

**Schöllkopf HWE Protocol**

Diethyl isocyanomethylphosphonate (1 equiv.) is stirred with NHMDS (1.1 equiv.) in THF (10 mLg⁻¹) and the mixture held under nitrogen at -78 °C for 15 min. Upon formation of the ylide, the appropriate aldehyde (1 equiv.) is added and the whole allowed to warm up to r.t. over 16 h. The reaction is quenched using phosphate buffer, partitioned with EtOAc and the organic portion dried over MgSO₄ and concentrated (post filtration) to afford the crude product(s).
Optimised HWE Procedure A: Synthesis of phenol derived isocyanides

Diisopropyl isocyanomethylphosphonate (1.2 equiv.) is stirred with LHMDS (1.2 equiv.) in THF (10 mL g⁻¹) and then held under nitrogen at -78 °C for 15 min. Upon formation of the ylide, the appropriate O-silyl protected hydroxybenzaldehyde (1 equiv.) is added and the whole immediately warmed up to room temperature before being left for 16 h. The reaction is quenched using phosphate buffer, diluted with EtOAc, dried over MgSO₄ and concentrated (post filtration). The resultant oil is then dissolved in ethanol (10 mL approx), KOH added (2 pellets) and stirred at room temperature for 2 h. before being concentrated, and the resultant oil partitioned between aq. NaHCO₃ and EtOAc. The organics are dried over MgSO₄ and concentrated (post filtration) to afford the crude phenol vinyl-isocyanide(s).

Optimised HWE Procedure B: Synthesis of heteroaromatic derived isocyanides

Diisopropyl isocyanomethylphosphonate (1.2 equiv.) is stirred with LHMDS (2.2 equiv.) in THF (10 mL g⁻¹) and then held under nitrogen at -78 °C for 15 min. Upon formation of the ylide, the appropriate heteroaromatic carbaldehyde (1 equiv.) is added and the whole immediately warmed to r.t. before being left for 16 h. The reaction is quenched using phosphate buffer, diluted with EtOAc before MgSO₄ is added and the whole concentrated (post filtration) to afford the crude heteroaromatic vinyl-isocyanide(s).

Optimised HWE Procedure C: Synthesis of non phenolate, non-heteroaromatic derived isocyanides

Diisopropyl isocyanomethylphosphonate (1.2 equiv.) is stirred with LHMDS (1.2 equiv.) in THF (10 mL g⁻¹) and then held under nitrogen at -78 °C for 15 min. Upon formation of the ylide, the appropriate benzaldehyde (1 equiv.) is added and the whole immediately warmed up to r.t. before being left for 16 h. The reaction is quenched using phosphate buffer, diluted with EtOAc before MgSO₄ is added and the whole concentrated (post filtration) to afford the crude product(s)
6.2 Compounds associated with the development of an \(E\)-selective Horner Wadsworth Emmons Reaction (chapter 2).

\(N\)\-((dimethylamino)methyl)formamide 564\textsuperscript{206}

\[
\begin{align*}
\text{H} & \quad \text{N} \\
\text{N} & \quad \text{O} \\
\text{H} & \quad \text{O}
\end{align*}
\]

Formaldehyde (37 wt.% in H\(_2\)O, 128 mL, 1.25 mol) was added to a 0 °C mixture of formamide (50 mL, 1.25 mol) and dimethylamine (40 wt.% in H\(_2\)O, 128 mL, 1.25 mol) and the resulting solution allowed to warm up to r.t. whilst stirring for 16 h. The reaction mixture was then extracted with CH\(_2\)Cl\(_2\) (3 x 75 mL), with the combined organics being dried over Na\(_2\)SO\(_4\) and the evaporation residue distilled (post filtration) to afford the desired compound as a colourless oil (65.2 g, 50% yield): b.p. 82 °C (2mmHg). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta_H = 8.20\) (1H, m, C\(\text{H}_2\text{O}\)), 7.11 (1H, br s, NH), 3.76 (2H, d, \(J = 5.1\) Hz, N\(\text{C}_2\text{H}_3\text{N}\)), 2.07 (6H, m, N\(\text{C}_3\text{H}_6\)); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta_C = 163.7, 63.4, 42.0\); IR (film, cm\(^{-1}\)):
\(\nu = 3314.23\) (N-H), 1672.90 (C=O); HRMS (ESI) calcd for C\(_4\)H\(_{11}\)N\(_2\)O [M+H]\(^+\): found m/z 103.0870, requires m/z 103.0871.

1-formamido-\(N,N,N\)-trimethylmethanaminium iodide 565\textsuperscript{206}

\[
\begin{align*}
\text{N} & \quad \text{H} \\
\text{N} & \quad \text{O} \\
\text{H} & \quad \text{I} \\
\text{O} & \quad \text{N}
\end{align*}
\]

\(N\)-((dimethylamino)methyl)formamide 564 (15.0 g, 0.147 mol) and Et\(_2\)O (200 mL) were cooled to 0 °C under Nitrogen, and iodomethane (22 mL, 0.442 mol) added. The whole was stirred for 2 h. before the Et\(_2\)O was removed in vacuo to produce a white solid that was recrystallised from hot ethanol to afford the title compound as a white powder (21.2 g, 59% yield): m.p. 149-150 °C. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta_H = 9.41\) (1H, br s, NH), 8.57 (0.2H, m, CHO (\(E\) rotamer)), 8.45 (0.8H, s, CHO (\(Z\) rotamer)), 5.00 (0.4H, d, \(J = 7.0\) Hz, N\(\text{C}_2\text{H}_2\text{N}\) (\(E\) rotamer)), 4.75 (1.6H, d, \(J =

158
Experimental

7.0 Hz, NCH₂N (Z rotamer)), 3.12 (9H, s, N(CH₃)₃); ¹³C NMR (75 MHz, CDCl₃): δC = 163.8, 54.1, 38.8 (m); IR (powder, cm⁻¹): ν = 1696.83 (C=O); HRMS (ESI) calculated for C₇H₁₃N₂O [M⁺]: found m/z 117.1041, requires m/z 117.1027

Diethyl(formamidomethyl)phosphonate 566

Triethylphosphite (17.9 mL, 0.103 mol) was added to N-((dimethylamino)methyl)formamide 565 (19.26 g, 0.079 mol) in EtOH (200 mL) as required by general procedure 1. The crude reaction products were purified by silica gel chromatography [CH₂Cl₂ : MeOH (95 : 5), Rf 0.15] to afford title compound as a colourless oil (14.0 g, 87% yield). ¹H NMR (300 MHz, CDCl₃): δH = 7.80 (1H, s, CHO), 7.42 (1H, br s, NH), 3.92 (4H, m, OCH₂CH₂), 3.50 (2H, dd, J = 12.4, 6.2 Hz, PCH₂), 1.11 (6H, t, J = 7.6 Hz, OCH₂CH₃); ¹³C NMR (75 MHz, CDCl₃): δC = 162.2, 62.5 (m), 32.8 (d, J = 157.8 Hz), 16.3 (m); ³¹P NMR (300 MHz, CDCl₃) δP = 22.8; IR (film, cm⁻¹): ν = 3476.97 (N-H), 1694.00 (C=O); HRMS (ESI) calculated for C₆H₁₄NNaO₂P [M+Na⁺]: found m/z 218.0575, requires m/z 218.0558.

Diethyl isocyanomethylphosphonate 500

Diethyl (formamidomethyl)phosphonate 566 (3.6 g, 0.020 mol) was dehydrated according to general procedure 2 when dissolved in CH₂Cl₂ (160 mL) and Et₃N (26 mL, 0.185 mol) and treated with MsCl (4.8 mL, 0.061 mol). The resulting foul smelling brown oil, was purified by silica gel chromatography [petroleum ether : EtOAc (50 : 50), Rf 0.28] to furnish title compound as a pale yellow oil (1.20 g, 32% yield). ¹H NMR (500 MHz, CDCl₃): δH = 4.24 (4H, m, OCH₂CH₂),
Experimental

3.76 (2H, d, J = 15.8 Hz, PCH$_2$), 1.38 (6H, m, OCH$_2$CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta_C = 160.8, 63.9, 37.6$ (d, $J = 156.7$ Hz), 16.3; $^{31}$P NMR (300 MHz, CDCl$_3$) $\delta_p = 14.1$; IR (film, cm$^{-1}$) $\nu = 2152$ (IC)

(E)-3-indole vinyl-isocyanide 17 (c.f. Anitbiotic B731 20) $^{155}$

Schöllkopf HWE Protocol.....

Diethyl isocyanomethylphosphonate 500 (244 µL, 1.38 mmol) was stirred with NHMDS (1.0M in THF) (1.5 mL, 1.52 mmol) in THF (10 mL) according to Schöllkopf HWE Protocol and indole-3-carbaldehyde 499 (200 mg, 1.38 mmol) added. The resultant foul smelling residue was purified by flash column chromatography [petroleum ether : EtOAc (50 : 50), R$_f$ 0.28] to afford the title compound as a pale yellow oil (65 mg, 28% yield).

Optimised method.....

The title compound was prepared according to optimised HWE procedure B using indole-3-carbaldehyde 499 (200 mg, 1.38 mmol), diisopropyl isocyanomethylphosphonate 574 (340 µL, 1.65 mmol) and LHMDS (1.0 M in THF) (3.30 mL, 3.31 mmol) in THF (10 mL). Purified by flash column chromatography [pentanes : EtOAc (50 : 50), R$_f$ 0.28] to furnish title compound as a pale yellow oil (125 mg, 57% yield). Selective for E-isomer.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta_H = 8.42$ (1H, br s, NH), 7.68 (1H, d, $J = 7.9$ Hz, ArH), 7.42 (1H, d, $J = 7.9$ Hz, ArH), 7.34 (1H, d, $J = 2.1$ Hz, ArH), 7.26 (2H, m, ArH), 7.13 (1H, d, $J = 14.4$ Hz, $\beta$ vinyl H),
Experimental

6.35 (1H, d, J = 14.4 Hz, α vinyl H); 13C NMR (75 MHz, CDCl₃): δC = 163.0, 136.9, 130.2, 126.3, 124.6, 123.4, 121.4, 119.9, 111.8, 111.1, 107.1.

(E)-(2-isocyanovinyl)benzene 568

![Chemical Structure Image]

The title compound was prepared according to optimised HWE procedure C using benzaldehyde (50 mg, 0.47 mmol), diethyl isocyanomethyolphosphonate 500 (100 µL, 0.56 mmol) and LHMDS (1.0 M in THF) (0.57 mL, 0.56 mmol) in THF (10 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20), Rf 0.90] to afford title compound as a black oil (33 mg, 35% yield). ¹H NMR (300 MHz, CDCl₃): δH = 7.36 (5H, m, ArH), 6.95 (1H, d, J = 14.3 Hz, (E) β vinyl H), 6.28 (1H, d, J = 14.3 Hz, (E) α vinyl H), 5.85; ¹³C NMR (75 MHz, CDCl₃): δC = 165.3, 136.7, 129.9, 129.3, 126.7; IR (film, cm⁻¹): ν = 2121 cm⁻¹.

Diisopropyl (formamidomethyl)phosphonate 573

![Chemical Structure Image]

Triisopropylphosphite (16.7, 0.067 mol) was added to N-((dimethylamino)methyl)formamide 565 (11.0 g, 0.045 mol) in EtOH (110 mL) as required by general procedure 1. The crude reaction products were purified by silica gel chromatography [pentanes : EtOAc (50 : 50), Rf 0.10] to afford the title compound as a colourless oil (8.50 g, 85% yield). ¹H NMR (300 MHz, CDCl₃): δH = 8.20 (1H, s, CHO), 6.91 (1H, br s, NH), 4.67 (2H, m, OCH₂), 3.65 (2H, dd, J = 12.8, 0.8 Hz, PCH₂), 1.30 (12H, m, CH₂); ³¹C NMR (75 MHz, CDCl₃): δC = 161.1, 71.7, 34.2 (d, J = 158.8 Hz), 24.0; ³¹P NMR (300 MHz, CDCl₃) δP = 21.1; IR (film, cm⁻¹): ν = 3267.99 (N-H), 1685.06 (C=O); HRMS (ESI) calculated for C₈H₁₆NNaO₄P [M+Na⁺]: found m/z 246.0899, requires m/z 246.0982.
Experimental

Diisopropyl (isocyanomethyl)phosphonate 574

Diisopropyl (formamidomethyl)phosphonate (2.33 g, 0.015 mol) 573 was dehydrated according to general procedure 2 when dissolved in CH₂Cl₂ (25 mL) and Et₃N (13 mL, 0.093 mol) and treated with MsCl (3.55 mL, 0.031 mol). The resulting foul smelling brown oil, was purified by silica gel chromatography [pentanes : EtOAc (50 : 50), Rᵣ 0.28] to furnish title compound as a pale yellow oil (1.57 g, 50% yield). ¹H NMR (300 MHz, CDCl₃): δ_H = 4.69 (2H, sept, J = 7.5 Hz, OC₃H) 3.64 (2H, d, J = 15.82 Hz, PC₂H₂), 1.27 (12H, d, J = 7.5 Hz, CH₃); ¹³C NMR (75 MHz, CDCl₃): δ_C = 160.0, 72.8, 38.2 (d, J = 156.9 Hz), 23.7; ³¹P NMR (300 MHz, CDCl₃) δ_P = 12.5; IR (film, cm⁻¹) ν = 2151.22 (IC).

4-((tert-butyldimethylsilyl)oxy)benzaldehyde 576²⁰⁸

The title compound was prepared according to general procedure 3 using p-hydroxybenzaldehyde 560 (5.00 g, 0.041 mol), imidazole (8.37 g, 0.123 mol) and TBSCl (7.35 g, 0.049 mol) in CH₂Cl₂ (200 mL). Distillation was used to purify the crude reaction products, furnishing the title compound as a colourless oil (7.21 g, 69% yield): b.p. 112°C (2mmHg). ¹H NMR (300 MHz, CDCl₃): δ_H = 9.86 (1H, s, CHO), 7.78 (2H, d, J = 8.5 Hz, ArH), 6.92 (2H, d, J = 8.5 Hz, ArH), 0.97 (9H, s, SiC(CH₃)₃), 0.23 (6H, s, SiC(CH₃)₂); ¹³C NMR (75 MHz, CDCl₃): δ_C = 190.9, 161.5, 131.9, 130.5, 120.5, 25.6, 18.3, 4.4; IR (film, cm⁻¹): ν = 1695.30 (C=O); HRMS (ESI) calculated for C₁₃H₂₀O₂SiNa [M+Na⁺]: found m/z 259.1145, requires m/z 259.1131.
Experimental

4-(methoxymethoxy)benzaldehyde 237

To p-hydroxybenzaldehyde (2.50 g, 0.021 mol) dissolved in anhydrous acetone (80 mL) was added K$_2$CO$_3$ (11.60 g, 0.084 mol). Bromomethyl methyl ether (2.12 mL, 0.026 mol) was added dropwise and the whole was refluxed for 2 h. The reaction mixture was cooled to r.t. and filtered. The filtrate was concentrated in vacuo and purified by silica gel column chromatography [petroleum ether : EtOAc (70 : 30), R$_f$ 0.56] to yield title compound as a colourless oil (3.02 g, 88% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$H = 9.88 (1H, s, CHO), 7.82 (2H, d, $J$ = 8.7 Hz, ArH), 7.13 (2H, d, $J$ = 8.7 Hz, ArH), 5.23 (2H, s, OCH$_2$O), 3.47 (3H, s, OCH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$C = 190.9, 161.2, 131.9, 130.7, 116.3, 94.1, 56.3; IR (film, cm$^{-1}$): $\nu$ = 1683.99 (C=O); HRMS (ESI) calcd for C$_9$H$_{10}$NaO$_3$ [M+Na]$^+$: found m/z 189.0527, requires m/z 189.0527

4-formylphenyl acetate 578

Acetyl chloride (2.89 g, 0.036 mol) was added dropwise to a mixture of p-hydroxybenzaldehyde 560 (3.00 g, 0.024 mol) and Et$_3$N (5.14 mL, 0.037 mol) dissolved in anhydrous THF (75 mL), the whole then being warmed to r.t. and stirred for 3 h. The reaction was worked up by quenching with aq. NH$_4$Cl and extraction with EtOAc (150 mL). The organics were washed with water (150 mL) and brine (150 mL) before being dried over MgSO$_4$ and concentrated to give a pale oil. Purification by silica gel column chromatography [petroleum ether : EtOAc (70 : 30), R$_f$ 0.56] yielded title compound as a colourless oil (3.50 g, 89% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$H = 9.92 (1H, s, CHO), 7.85 (2H, d, $J$ = 8.63 Hz, ArH), 7.21 (2H, d, $J$ =
Experimental

8.63 Hz, ArH), 2.26 (3H, s, OCH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C = 190.9, 168.7, 155.4, 134.0, 131.2, 122.38, 21.2$; IR (film, cm$^{-1}$): $\nu = 1760.10$ (C=O), 1696.76 (C=O); HRMS (ESI) calculated for C$_9$H$_8$O$_3$Na$^{[M+Na]^{+}}$: found m/z 187.0364, requires m/z 187.0371,

4-((tert-butyldimethylsilyl)oxy)phenyl vinyl-isocyanide 579

![TBSO](image)

The title compound was prepared according to general procedure C from LHMDS (1.0 M in THF) (1.80 mL, 0.0024 mol), diethyl isocyanomethylphosphonate 500 (0.30 mL, 0.0013 mol) and $p$-((tert-butyldimethylsilyl)oxy)benzaldehyde 576 (0.12 mL, 0.0012). The crude product was purified by kugelrhor distillation to give title compound as a brown oil (100 mg, 55%): b.p. 126 °C, 2mmHg). $^1$H NMR (300 MHz, CDCl$_3$): $\delta_H = 7.28$ (2H, d, $J = 8.7$ Hz, ArH), 6.88 (1H, d, $J = 14.4$ Hz, $\beta$ vinyl H), 6.81 (2H, d, $J = 8.7$ Hz, ArH), 6.16 (1H, d, $J = 14.4$ Hz, $\alpha$ vinyl H), 1.56 (9H, s, (CH$_3$)$_3$), 1.43 (6H, s, Si(CH$_3$)$_2$); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C = 157.4, 136.3, 128.1, 125.8, 120.6, 31.6, 25.6, 22.6, 18.2, 14.1, -4.3

4-(methoxymethoxy)phenyl vinyl-isocyanide 580

![MOMO](image)

Compound was originally prepared as a 3:2 mixture of its E/Z isomers using general procedure C with diethyl isocyanomethylphosphonate (PhosMIC) 500. Below we detail using $^1$Pr PhosMIC 574 for the E-selective synthesis of this compound, the product of this preparation was used for biological evaluation.
The title compound was prepared according to general procedure C from LHMDS (1.0 M in THF) (0.4 mL, 0.36 mmol), diisopropyl isocyanomethylphosphonate 574 (74 µL, 0.36 mmol) and 4-(methoxymethoxy)benzaldehyde 577 (0.12 mL, 0.0012) in THF (10 mL). Purification was achieved by silica gel chromatography [hexanes : EtOAc (70 : 30), Rf 0.34], affording the title compound as a black oil (28 mg, 49% yield). \( ^1 \)H NMR (300 MHz, CDCl\(_3\)): \( \delta_H = 7.20 \) (2H, d, \( J = 8.6 \) Hz, ArH), 6.94 (2H, d, \( J = 8.6 \) Hz, ArH), 6.81 (1H, d, \( J = 14.3 \) Hz \( \beta \) vinyl H), 6.09 (1H, d, \( J = 14.4 \) Hz, \( \alpha \) vinyl H), 5.10 (2H, s, OC\(_2\)H\(_2\)O), 3.39 (3H, s, OC\(_3\)H\(_3\)); \( ^{13} \)C NMR (75 MHz, CDCl\(_3\)): \( \delta_C = 158.5, 136.1, 128.1 \) (2C), 116.6 (2C), 94.2, 56.1; IR (film, cm\(^{-1}\)) \( \nu = 2929.29 \) (C-H aromatic), 2828.18 (C-H aromatic) 2119.28 (IC)

4-phenol vinyl-isocyanide 542\(^{20} \)

![4-phenol vinyl-isocyanide](image)

The title compound was prepared according to optimised HWE procedure A using 4-((tert-butyldimethylsilyl)oxy)benzaldehyde 576 (200 mg, 0.847 mmol), diisopropyl isocyanomethylphosphonate 574 (208 µL, 0.984 mmol) and LHMDS (1.0 M in THF) (1.0 mL, 1.05 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20), Rf 0.75]. \( ^1 \)H NMR (300 MHz, acetone-d\(_6\)): \( \delta_H = 7.47 \) (2H, d, \( J = 8.5 \) Hz, ArH), 7.08 (1H, d, \( J = 14.3 \) Hz \( \beta \) vinyl H), 6.92 (2H, d, \( J = 8.53 \), ArH), 6.63 (1H, d, \( J = 14.4 \) Hz, \( \alpha \) vinyl H); \( ^{13} \)C NMR (75 MHz, acetone-d\(_6\)): \( \delta_C = 165.8, 160.0, 137.3, 129.6, 125.2, 116.7, 109.4; \) IR (film, cm\(^{-1}\)) \( \nu = 3241.99 \) (O-H), 2925.49 (C-H aromatic), 2144.94 (N-C)

6.3 Biological evaluation of phenol vinyl-isocyanide (Chapter 3).

N-methyl-3-indole vinyl-isocyanide 553

Compound Prepared by Liam Stephens.
The title compound was prepared according to optimised HWE procedure C using N-methylindole-3-carbaldehyde (100 mg, 0.629 mmol), diisopropyl isocyanomethylphosphonate 574 (181 µL, 0.725 mmol) and LHMDS (1.0 M in THF) (0.8 mL, 0.81 mmol) in THF (10 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20), Rf 0.35], affording the title compound as a pale yellow oil (45mg, 39% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta_H = 7.75$-$7.25$ (5H, m, ArH), 7.17 (1H, d, $J = 14.3$ Hz, $\beta$ vinyl H), 6.35 (1H, d, $J = 14.3$ Hz, $\alpha$ vinyl H), 3.86 (3H, s, CH$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C = 162.9, 137.1, 130.7, 130.0, 125.2, 122.9, 121.1, 119.9, 110.0, 109.4, 160.2, 33.0$; IR (film, cm$^{-1}$) $\nu = 3051.80$ (C-H aromatic), 2929.57 (C-H aromatic), 2116.00 (IC)

(E)-4-bromo-3-(2-isocyanovinyl)-1H-indole 554

Compound Prepared by Liam Stephens.

The title compound was prepared according to optimised HWE procedure B using 4-bromo-1H-indole-3-carbaldehyde (100 mg, 0.448 mmol), diisopropyl isocyanomethylphosphonate 574 (110 µL, 0.538 mmol) and LHMDS (1.0 M in THF) (1.0 mL, 0.991 mmol) in THF (5 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20), Rf 0.51], affording the target compound as a pale brown oil (35 mg, 46% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta_H = 8.45$ (1H, br s, NH), 7.95 (1H, d, $J = 14.3$ Hz, $\beta$ vinyl H), 7.42 (1H, d, $J = 2.6$ Hz, ArH), 7.37 (1H, d, $J = 2.6$ Hz, ArH), 7.34 (1H, d, $J = 3.0$ Hz), 7.05 (1H, t, $J = 7.9$ Hz, ArH), 6.07 (1H, d, $J = 14.3$ Hz, $\alpha$ vinyl H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C = 132.0, 128.5, 126.8, 126.4, 124.1, 123.2, 120.9, 118.0, 114.4, 110.1$; IR (film, cm$^{-1}$) $\nu = 3658.2$ (C-H aromatic), 2979.4 (C-H aromatic), 2139.04 (IC)
Experimental

\((E)-(\text{isocyanovinyl})-2\text{-thiophene}\) 555

The title compound was prepared according to optimised HWE procedure B using thiophene-2-carboxaldehyde (100 mg, 0.820 mmol), diisopropyl isocyanomethylphosphonate 574 (200 µL, 0.984 mmol) and LHMDS (1.0 M in THF) (1.8 mL, 1.8 mmol) in THF (10 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (99 : 1), R\(_f\) 0.20], affording the title compound as a brown oil (54 mg, 52% yield). \(^1\)H NMR (300 MHz, acetone-\(d_6\)): \(\delta_H = 7.35\) (1H, s, Ar\(H_1\)), 7.17 (1H, s, Ar\(H_2\)), 7.08 (1H, s, Ar\(H_3\)), 7.12 (1H, d, \(J = 14.2\) Hz, \(\beta\) vinyl\(H_1\)), 6.22 (1H, d, \(J = 14.2\) Hz, \(\alpha\) vinyl\(H_1\)); \(^{13}\)C NMR (75 MHz, acetone-\(d_6\)) \(\delta_C = 160.7\) (IC), 129.8, 129.3, 128.0, 127.1, 116.4, 109.3; IR (film, cm\(^{-1}\)): \(\nu = 2976.09\) (C-H aromatic), 2857.55 (C-H aromatic), 2120.67 (IC).

\(4\)-(methoxy)phenyl vinyl-isocyanide 585

Compound Prepared by Liam Stephens.

The title compound was prepared according to optimised HWE procedure C using 4-methoxybenzaldehyde 584 (100 mg, 0.735 mmol), diisopropyl isocyanomethylphosphonate 574 (180 µL, 0.822 mmol) and LHMDS (1.0 M in THF) (0.8 mL, 0.81 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20), R\(_f\) 0.15], affording the title compound as a pale yellow oil (28mg, 24% yield). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta_H = 7.20\) (2H, d, \(J = 8.8\) Hz, Ar\(H_1\)), 6.85 (1H, d, \(J = 14.0\) Hz, \(\beta\) vinyl\(H_1\)), 6.80 (2H, d, \(J = 8.8\) Hz, Ar\(H_2\)), 6.10 (1H, d, \(J = 14.0\) Hz, \(\alpha\) vinyl\(H_1\)); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta_C = 160.6, 136.2, 131.5, 131.0, 128.2, 114.5, 114.1, 55.3\); IR (film, cm\(^{-1}\)): \(\nu = 2960.46\) (C-H aromatic), 2838.26 (C-H aromatic), 2118.44 (IC).
Experimental

Phenol vinyl-cyanide 587

Compound Prepared by Liam Stephens.

The title compound was prepared according to optimised HWE procedure A using 4-((tert-butyldimethylsilyl)oxy)benzaldehyde 560 (200 mg, 0.847 mmol), diethyl cyanomethylphosphonate 586 (208 µL, 0.984 mmol) and LHMDS (1.0 M in THF) (1.0 mL, 1.05 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude products was purified using silica gel chromatography [hexane : EtOAc (80 : 20), Rf 0.23], affording the title compound as a pale yellow oil (32 mg, 28% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta_H = 7.42$ (2H, d, $J = 8.8$ Hz, ArH), 7.40 (1H, d, $J = 16.6$ Hz, $\beta$ vinyl H), 6.80 (2H, d, $J = 8.8$ Hz, ArH), 5.95 (1H, d, $J = 16.6$ Hz, $\alpha$ vinyl H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C = 162.0, 152.3, 130.7, 127.0, 120.3, 117.0, 93.0$; IR (film, cm$^{-1}$): $\nu = 3279.81$ (O-H), 2220.42 (CΞN).

$N$-(4-hydroxyphenethyl)formamide 589$^{211}$

Tyramine 588 (200 mg, 1.45 mmol) was dissolved in THF (5 mL) and cooled to 0 °C and acetic formic anhydride (141 mg, 1.60 mmol) added dropwise. After stirring for 2 h., the reaction mixture was concentrated to yield crude target material as pale orange oil, which was used in the next step without further purification (240 mg, nr quant. yield). $^1$H NMR (300 MHz, DMSO-d$_6$): $\delta_H = 9.91$ (1H, s, CHO), 7.99 (2H, br s, NH + ArOH), 7.01 (2H, d, $J = 8.3$, ArH), 6.67 (2H, d, $J = 8.3$, ArH), 3.26 (2H, app q, $J = 6.8$ Hz, NHCH$_2$), 2.61 (2H, t, $J = 7.2$ Hz, NCH$_2$CH$_2$); $^{13}$C NMR (75 MHz, DMSO-d$_6$): $\delta_C = 161.3, 156.0, 129.8, 129.6, 115.4, 39.8, 34.5$; IR (powder, cm$^{-1}$): $\nu = 1648.55$ (C=O); HRMS (ESI) calculated for C$_9$H$_{13}$NO$_2$ [M+H]$^+$: found m/z 166.0869, requires m/z 166.0868.
The title compound was prepared according to general procedure 3 using N-4-hydroxyphenethyl)formamide 589 (0.89 g, 0.0054 mol), TBSCI (0.98 g, 0.0065 mol) and imidazole (1.10 g, 0.0162 mol) in CH₂Cl₂ (10 mL). The crude reaction residue was purified by silica gel chromatography [pentanes, Rf 0.61] to afford 590 as pale yellow oil (0.98 g, nr quant. yield). ¹H NMR (300 MHz, CDCl₃): δ_H = 8.04 (1H, s, CHO), 7.02 (2H, d, J = 8.6, ArH), 6.75 (2H, d, J = 8.6, ArH), 3.46 (2H, app q, J = 6.8 Hz, NHC₂H₂), 2.73 (2H, t, J = 6.8 Hz, NCH₂C₂H₂); 0.96 (9H, s, Si(CH₃)₃), 0.16 (6H, s, Si(CH₃)₂); ¹³C NMR (75 MHz, CDCl₃): δ_C = 161.4 154.2, 131.2, 129.6, 120.1, 39.4, 34.6, 25.7, 18.1, -4.4; IR (film, cm⁻¹): ν = 3289.88 (N-H), 2954.90 (C-H aromatic), 2929.82 (C-H aromatic), 2885.28 (C-H aromatic), 2857.88 (C-H aromatic), 1664.02 (C=O); HRMS (ESI) calculated for C₁₅H₂₆NO₂Si [M+H]^+: found m/z 280.1733, requires m/z 280.1732.

4-(2-isocyanophenyl)phenol 591

The title compound was prepared by first dehydrating N-(4-((tert-butyldimethylsilyloxy)phenethyl)formamide 590 (0.98 g, 0.0035 mol) according to general procedure 2, using MsCl (0.82 mL, 0.0107 mol) and Et₃N (4.48 mL, 0.0322 mol) in CH₂Cl₂ (15 mL) to yield the crude silyl protected phenol-isocyanide as a brown oil that was then dissolved in ethanol (15 mL) and treated with KOH (0.5 g approx.). After stirring for 2 h. at r.t., the crude reaction evaporation residue was partitioned between EtOAc and H₂O, the organics dried over MgSO₄ and then concentrated to give a pale brown oil that was purified by silica gel chromatography [pentanes : EtOAc (50:50), Rf 0.55] to afford 592 as pale yellow oil (0.42 g, 82% yield). ¹H NMR (300 MHz, DMSO-d₆): δ_H = 9.93 (1H, br s, OH), 7.07 (2H, d, J = 8.67 Hz, ArH),
Experimental

6.72 (2H, d, J = 8.67 Hz, ArH), 3.65 (2H, t, J = 6.78 Hz, NCH₂), 2.77 (2H, t, J = 6.78 Hz, NCHCH₂); ¹³C NMR (75 MHz, DMSO-d₆): δC = 165.5, 155.9 (IC), 130.1, 127.8, 115.5, 43.3, 43.3, 34.2; IR (film, cm⁻¹): ν = 2132.12 (IC)

*N-(4-Hydroxyphenyl)formamide 593*

![Chemical Structure](image)

The title compound was prepared according general procedure 4 by dissolving 4-aminophenol 592 (1.00 g, 0.0091 mol) in THF (10 mL) and reacting with acetic formic anhydride (0.89 g, 0.0101 mol). The resultant white solid was used in the next step without further purification (1.05 g, nr quant. yield). ¹H NMR (300 MHz, DMSO-d₆): δH = 9.90 (1H, br s, CHO), 9.26 (1H, br s, OH), 8.14 (1H, br s, NH), 8.49 (0.3H, d, J = 9.0 Hz, ArH (Z rotamer)), 7.36 (1.7H, d, J = 9.0 Hz, ArH (E rotamer)), 6.97 (0.3H, d, J = 9.0 Hz, ArH (Z rotamer)), 6.68 (1.7H, d, J = 9.0 Hz, ArH (E rotamer)); ¹³C NMR (75 MHz, DMSO-d₆): δC = 162.9 (Z rotamer), 159.2 (E rotamer), 154.5 (Z rotamer), 153.8 (E rotamer), 130.3 (E rotamer), 129.9 (Z rotamer), 121.1 (E rotamer), 120.5 (Z rotamer), 116.1 (Z rotamer), 115.5 (E rotamer); IR (film, cm⁻¹): ν = 3303.39 (N-H), 2978.48 (C-H aromatic), 2889.61 (C-H aromatic), 1657.48 (C=O); HRMS (ESI) calculated for C₇H₈NO₂ [M+H]⁺: found m/z 138.0568, requires m/z 138.0555.

*N-(4-((tert-butyldimethylsilyl)oxy)phenyl)formamide 594*

![Chemical Structure](image)

The title compound was prepared according to general procedure 3 using *N-(4-hydroxyphenyl)formamide 593* (585 mg, 4.27 mmol), TBSCl (768 mg, 768 mmol) and imidazole (800 mg, 12.81 mmol) in CH₂Cl₂ (15 mL). The crude reaction residue was purified by silica gel...
Experimental

chromatography [pentanes : EtOAc (80 : 20), Rf 0.15] to afford 594 as pale yellow oil (982 mg, 91% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta_H = 8.52\ (0.5H, d, J = 11.6\ Hz,\ CHO\ (rotamer)),\ 8.34\ (0.5H, d, J = 1.8\ Hz,\ CHO\ (rotamer)),\ 7.55\ (0.5H, br s, NH\ (rotamer)),\ 7.39\ (1H, d, J = 9.0\ Hz,\ ArH\ (rotamer)),\ 7.25\ (0.5H, br s, NH\ (rotamer)),\ 6.97\ (1H, d, J = 9.0\ Hz,\ ArH\ (rotamer)),\ 6.85\ (1H, d, J = 6.0\ Hz,\ ArH\ (rotamer)),\ 6.82\ (1H, d, J = 6.0\ Hz,\ ArH\ (rotamer)); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C = 162.8,\ 158.7,\ 121.5,\ 121.1,\ 120.5,\ 25.6,\ 18.2,\ -4.4;\ IR\ (film,\ cm^{-1}):\ \nu = 3268.98\ (N-H),\ 2958.75\ (C=H\ aromatic),\ 2930.62\ (C=H\ aromatic),\ 2859.05\ (C=H\ aromatic),\ 1672.20\ (C=O);\ HRMS\ (ESI)\ calculated\ for\ C$_{13}$H$_{21}$NO$_2$Si [M+H$^+$]:\ found\ m/z 252.1418,\ requires\ m/z 252.1419.

4-isocyanophenol 595

The title compound was prepared by first dehydrating $N$-[(tert-butyl(dimethyl)silyl)oxy]phenyl)formamide 594 (550 mg, 2.19 mmol) according to general procedure 2, using MsCl (0.50 mL, 0.0107 mol) and Et$_3$N (2.68 mL, 19.71 mmol) in CH$_2$Cl$_2$ (20 mL) to yield the crude O-silyl protected phenol-isocyanide as a brown oil that was then dissolved in ethanol (15 mL) and treated with KOH (0.5 g approx.). After stirring for 2 h. at r.t., the crude reaction evaporation residue was partitioned between EtOAc and H$_2$O, the organics dried over MgSO$_4$ and then concentrated to give a pale brown oil that was purified by silica gel chromatography [pentanes : EtOAc (80 : 20), Rf 0.45] to afford 595 as pale yellow oil (180 mg, 69% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta_H = 7.26\ (2H, d, J = 8.6\ Hz,\ ArH),\ 6.85\ (2H, d, J = 8.6\ Hz,\ ArH);\ ^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C = 160.4,\ 157.0,\ 128.0\ (2C),\ 116.2;\ IR\ (film,\ cm^{-1}):\ \nu = 3382.31\ (O-H),\ 2908.25\ (C=H\ aromatic),\ 2947.60\ (C=H\ aromatic),\ 1724.90\ (C=O).
**Experimental**

3-\((\text{tert-butyldimethylsilyl})\text{oxy}\)benzaldehyde 599\(^{212}\)

![Chemical Structure](attachment:structure1.png)

The title compound was prepared according to general procedure 2 using \(m\)-hydroxybenzaldehyde 596 (5.00 g, 0.041 mol), imidazole (3.10 g, 0.045 mol), TBSCI (7.35 g, 0.045 mol) in \(\text{CH}_2\text{Cl}_2\) (200 mL). Distillation was used to purify the crude reaction products, furnishing the title compound as a colourless oil (7.21 g, 69% yield): b.p. 112°C (2mmHg). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta \text{H} = 9.92 \ (1\text{H}, \text{s}, \text{CHO}), 7.45 \ (1\text{H}, \text{dt}, \ J = 7.6, 1.4 \text{ Hz, ArH}), 7.36 \ (1\text{H}, \text{t}, \ J = 7.8 \text{ Hz, ArH}), 7.31 \ (1\text{H}, \text{dd}, \ J = 2.5, 1.4 \text{ Hz, ArH}), 7.07 \ (1\text{H}, \text{ddd}, \ J = 7.8, 2.5, 1.4 \text{ Hz, ArH}), 0.97 \ (9\text{H}, \text{s, Si(CH}_3)_3\), 0.19 (6H, s, Si(CH}_3)_2\); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta \text{C} = 192.2, 156.4, 137.8, 130.1, 126.6, 23.6, 119.8, 25.6, -4.4\); IR (film, cm\(^{-1}\)): \(\nu = 1701.16 \ (\text{C=O})\); HRMS (ESI) calcd for \(\text{C}_{13}\text{H}_{20}\text{NaO}_2\text{Si}[\text{M+Na}]^+: \text{found m/z} 259.1113, \text{requires m/z} 259.1130.

2-\((\text{tert-butyldimethylsilyl})\text{oxy}\)benzaldehyde 600\(^{213}\)

![Chemical Structure](attachment:structure2.png)

The title compound was prepared according to general procedure 2 using 2-hydroxybenzaldehyde 597 (5.00 g, 0.041 mol), imidazole (3.10 g, 0.045 mol) and TBSCI (7.35 g, 0.045 mol) in \(\text{CH}_2\text{Cl}_2\) (200 mL). Distillation was used to purify the crude reaction products, furnishing the title compound as a colourless oil (7.21 g, 69% yield): b.p. 112°C (2mmHg). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta \text{H} = 10.44 \ (1\text{H}, \text{s, CHO}), 7.78 \ (1\text{H}, \text{d}, \ J = 7.8 \text{ Hz, ArH}), 7.41 \ (1\text{H}, \text{t}, \ J = 7.7 \text{ Hz, ArH}), 6.98 \ (1\text{H}, \text{t}, \ J = 7.8 \text{ Hz, ArH}), 6.85 \ (1\text{H}, \text{d}, \ J = 7.7 \text{ Hz, ArH}), 0.98 \ (9\text{H}, \text{s, Si(CH}_3)_3\), 0.24 (6H, s, Si(CH}_3)_2\); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta \text{C} = 189.9, 158.8, 135.6, 128.2, 127.4, 121.7, 121.0, 25.6, 18.3, -4.3\); IR (film, cm\(^{-1}\)): \(\nu = 1687.60 \ (\text{C=O})\); HRMS (ESI) calculated for \(\text{C}_{13}\text{H}_{20}\text{NaO}_2\text{Si}[\text{M+Na}]^+: \text{found m/z} 259.1213, \text{requires m/z} 259.1330.
Experimental

3,4-bis-((tert-butyldimethylsilyl)oxy)benzaldehyde 601

The title compound was prepared according to general procedure 2 using 3,4-dihydroxybenzaldehyde 598 (0.5 g, 3.62 mmol), imidazole (1.37 g, 21.72 mmol) and TBSCI (1.69 g, 10.86 mmol) in CH₂Cl₂ (200 mL). The crude products were purified by silica gel chromatography [pentanes : EtOAc (95 : 5), Rf 0.90], affording the title compound as colourless oil (1.13 g, 86% yield). ¹H NMR (300 MHz, CDCl₃): δH = 9.82 (1H, s, CHO), 7.38 (1H, d, J = 8.0 Hz, ArH), 7.36 (1H, s, ArH), 6.95 (1H, d, J = 8.2 Hz, ArH), 1.01 (9H, s, SiC(CH₃)₃), 1.00 (9H, s, SiC(CH₃)₂), 0.26 (6H, s, Si(CH₃)₂), 0.24 (6H, s, SiC(CH₃)_2); ¹³C NMR (75 MHz, CDCl₃): δC = 190.8, 153.3, 147.6, 130.7, 125.2, 120.8, 25.8, 18.4, -4.1; IR (film, cm⁻¹): ν = 2955.67 (C-H aromatic), 2930.85 (C-H aromatic), 2859.16 (C-H aromatic), 1694.53 (C=O); HRMS (ESI) calculated for C₁₉H₃₄NaO₃Si₂[M+Na⁺]: found m/z 389.1955, requires m/z 389.1944.

3-phenol vinyl-isocyanide 602

Compound prepared by Liam Stephens

The title compound was prepared according to optimised HWE procedure A using 3-((tert-butyldimethylsilyl)oxy)benzaldehyde 599 (200 mg, 0.847 mmol), diisopropyl isocyanomethylphosphonate 574 (208 µL, 0.984 mmol) and LHMDS (1.0 M in THF) (1.0 mL, 1.05 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20), Rf 0.63]. ¹H NMR (300 MHz, CDCl₃): δH = 7.30-7.20 (1H, m, ArH), 6.95 – 6.80 (3H, m, ArH), 6.90 (1H, d, J = 14.1 Hz, β
Experimental

vinyl H), 6.25 (1H, d, $J = 14.1$ Hz, $\alpha$ vinyl H); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C = 157.1, 134.4, 131.9, 130.5, 121.1, 117.2, 116.8, 79.6$; IR (film, cm$^{-1}$): $\nu = 3272.77$ (O-H), 2924.35 (C-H aromatic), 2853.09 (C-H aromatic), 2111.63 (IC)

This species rapidly isomerised to a mixture of E/Z isomers

2-phenol vinyl-isocyanide 603

![2-phenol vinyl-isocyanide](image)

The title compound was prepared according to optimised HWE procedure A using 2-((tert-butyldimethylsilyl)oxy)benzaldehyde 600 (200 mg, 0.847 mmol), diisopropyl isocyanomethyl phosphonate 574 (208 $\mu$L, 0.984 mmol) and LHMDS (1.0 M in THF) (1.0 mL, 1.05 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20), R$_f$ 0.20] to afford title compound as a pale yellow oil (21 mg, 25% yield). $^1$H NMR (300 MHz, acetone-d$_6$): $\delta_H = 7.58$ (1H, br d, $J = 7.3$ Hz, OH), 7.27 (1H, dd, $J = 7.7, 1.6$ Hz, ArH), 7.12-7.04 (1H, m, ArH), 6.99 (1H, d, $J = 14.1$ Hz, $\beta$ vinyl H), 6.85 (1H, d, $J = 8.5$ Hz, ArH), 6.77-6.66 (2H, m, ArH + $\alpha$ vinyl H); $^{13}$C NMR (75 MHz, acetone-d$_6$): $\delta_C = 157.1, 134.4, 131.9, 130.5, 121.1, 117.2, 116.8, 79.6$; IR (film, cm$^{-1}$): $\nu = 3361.05$ (O-H), 2976.09 (C-H aromatic), 2857.55 (C-H aromatic), 2116.04 (IC)

3,4-bis-phenol vinyl-isocyanide 557

![3,4-bis-phenol vinyl-isocyanide](image)
Experimental

The title compound was prepared according to optimised HWE procedure A using 3,4-bis-((tert-butyldimethylsilyl)oxy)benzaldehyde 601 (100 mg, 0.27 mmol), diisopropyl(isocyanomethyl)phosphonate 574 (66 µL, 0.984 mmol) and LHMDS (1.0 M in THF) (0.3 mL, 0.32 mmol) in THF (10 mL) and subsequently desilylated using ethoxide. The crude product was purified using silica gel chromatography [hexane : EtOAc (80 : 20), Rf 0.05] to afford title compound as a pale yellow oil (15 mg, 20% yield).

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\text{H} NMR (300 MHz, CD}_3\text{CN) } \delta \text{H} = 6.81-6.73 (2H, m, Ar H), 6.70-6.60 (2H, m, ArH + β vinyl H), 6.25 (1H, d, J = 14.32 Hz, α vinyl H); \text{C NMR (75 MHz, CD}_3\text{CN): } \delta \text{C} = 149.1, 147.2, 138.8, 126.5, 124.0, 121.4, 116.9, 114.5; \text{IR (film, cm}^{-1})\text{: } \nu = 3220.54 (\text{O-H}), 2982.63 (\text{C-H aromatic}), 2933.75 (\text{C-H aromatic}), 2119.57 (\text{IC})\].

(E)-(isocyanovinyl)-N-(4-formylphenyl) acetamide 607

The title compound was prepared according to optimised HWE procedure B using N-(4-formylphenyl)acetamide 604 (100 mg, 0.613 mmol), diisopropyl isocyanomethylphosphonate 574 (120 µL, 0.736 mmol) and LHMDS (1.0 M in THF) (3.00 mL, 3.03 mmol) in THF (5 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 50), Rf 0.20], affording the title compound as a brown oil (42 mg, 35% yield).

\[
\text{H NMR (300 MHz, CDCl}_3\text{): } \delta \text{H} = 7.54 (2H, d, J = 8.7 Hz, ArH), 7.31 (2H, d, J = 8.7 Hz, ArH), 6.90 (1H, d, J = 14.3 Hz, β vinyl H), 6.23 (1H, d, J = 14.3 Hz, α vinyl H), 2.20 (3H, s, CH\text{3}); \text{C NMR (75 MHz, CDCl}_3\text{): } \delta \text{C} = 168.5, 165.0, 139.8, 136.2, 128.6, 127.2, 119.7, 109.5, 24.9; \text{IR (film, cm}^{-1})\text{: } \nu = 3253.59 (\text{N-H}), 3183.68 (\text{C-H aromatic}), 3114.16 (\text{C-H aromatic}), 3071.34 (\text{C-H aromatic}), 2116.77 (\text{IC}), 1667.06 (\text{C=O})\].

(E)-(isocyanovinyl)-tert-butyl-4-formylphenyl 608
The title compound was prepared according to optimised HWE procedure B using tert-butyl-4-formylphenylcarbamate 605 (100 mg, 0.488 mmol), diisopropyl isocyanomethylphosphonate 574 (120 µL, 0.585 mmol) and LHMDS (1.0 M in THF) (1.0 mL, 1.0 mmol) in THF (5 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (95 : 5), Rf 0.30], affording the title compound as a brown oil (38 mg, 31% yield). $^1$H NMR (300 MHz, acetone-d$_6$): $\delta$H = 7.14 (2H, d, $J$ = 9.0 Hz, ArH), 7.04 (2H, d, $J$ = 9.0, ArH), 6.65 (1H, d, $J$ = 14.3 Hz, $\beta$ vinyl H), 5.97 (1H, d, $J$ = 14.3 Hz, $\alpha$ vinyl H), 1.29 (9H, s, (CH$_3$)$_3$); $^{13}$C NMR (75 MHz, acetone-d$_6$): $\delta$C = 160.7 (IC), 153.9, 142.3, 137.4, 128.7, 119.6, 112.5, 81.1, 29.2; IR (film, cm$^{-1}$): $\nu$ = 2976.09 (C-H aromatic), 2857.55 (C-H aromatic), 2123.08 (IC)

(E)-N-(4-(1-isocyanoprop-1-en-2-yl)phenyl)methanesulfonamide 609

The title compound was prepared according to optimised HWE procedure B using $N$-(4-acetylphenyl)-methanesulfonamide 604 (50 mg, 0.302 mmol), diisopropyl isocyanomethylphosphonate 574 (61 µL, 0.302 mmol) and LHMDS (1.0 M in THF) (0.6 mL, 0.55 mmol) in THF (5 mL). The crude product was purified using silica gel chromatography [hexanes : EtOAc (80 : 20), Rf 0.12 ], affording the title compound as a yellow oil (12 mg, 16% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$H = 7.33 (2H, d, $J$ = 9.0 Hz, ArH), 7.22 (2H, $J$ = 9.0 Hz, ArH), 5.99 (1H, s, vinyl CH), 3.01 (3H, s, SCH$_3$), 2.24 (3H, s, CH$_3$); $^{13}$C NMR (300 MHz, CDCl$_3$): $\delta$C = 142.9, 137.9, 128.9, 127.2, 120.2, 39.6, 16.8. IR (film, cm$^{-1}$): $\nu$ = 3249.99 (N-H) 3024.61 (C-H aromatic), 2983.74, (C-H aromatic), 2930.51 (C-H aromatic), 2114.18 (IC)
6.4 Compounds associated with the synthesis of Byelyankacin and Rhabduscin (Chapter 4).

Per-\text{-}O\text{-}acetyl\text{-}6\text{-}deoxy\text{-}\alpha\text{-}L\text{-}mannopyranose 611\textsuperscript{214}

![Chemical Structure Image]

The title compound was prepared according to general procedure 5 from L-Rhamnose 610 (3.00 g, 0.016 mol), DMAP (0.20 g, 10 mol%) and Ac\textsubscript{2}O (30 mL) in pyridine (30 mL). The title compound, a colourless glass, was used in the subsequent reactions without the need for purification (4.92 g, 89% yield). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta \text{H} = 6.03\) (1H, br d, \(J = 1.9\) Hz, CH-1), 5.32 (1H, dd, \(J = 10.1, 3.6\) Hz, CH-3), 5.26 (1H, m, CH-2), 5.14 (1H, t, \(J = 10.1\) Hz CH-4), 3.95 (1H, m, CH-5), 2.18 (3H, s, OAc), 2.17 (3H, s, OAc), 2.08 (3H, s, OAc), 2.02 (3H, s, OAc) 1.25 (3H, d, \(J = 6.2\) Hz, CH\textsubscript{3}); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta \text{C} = 170.1, 169.8, 169.8, 168.4, 90.6, 70.5, 68.7, 68.7, 68.6, 20.9, 20.8, 20.8, 20.7, 17.4\); IR (film, cm\textsuperscript{-1}): \(\nu = 1742.49\) (C=O); HRMS (ESI) calcd for C\textsubscript{14}H\textsubscript{20}NaO\textsubscript{9}[M+Na]\textsuperscript{+}: found m/z 355.1014, requires m/z 355.1005.

2,3,4-Tri-\text{-}O\text{-}acetyl\text{-}6\text{-}deoxy\text{-}\alpha\text{-}L\text{-}mannopyranose 614\textsuperscript{215}

![Chemical Structure Image]

Benzylamine (0.25 mL, 2.25 mmol) was added dropwise to a solution of Per-\text{-}O\text{-}acetyl\text{-}6\text{-}deoxy\text{-}\alpha\text{-}L\text{-}mannopyranose 611 (0.50 g, 1.51 mmol) in THF (10 mL), and left to stir under nitrogen for 16 h. After this time, aq. 1M HCl (5 mL) was added, and after stirring for an additional 30 min. the reaction was diluted further with additional aq. 1M HCl (25 mL) and extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 25 mL). The organics were combined, dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated to give the desired compound as an amorphous solid that was used in the next step without purification.
Experimental

(0.31 g, 70% yield). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta_H = 5.28\) (1H, dd, \(J = 10.1, 3.4\) Hz, CH-3), 5.18 (1H, app s, CH-1), 5.08 (1H, s, OH), 4.99 (1H, t, \(J = 10.1\) Hz, CH-4), 4.33 (1H, d, \(J = 4.1\) Hz, CH-2) 4.07 (1H, app m, CH-5), 2.09 (3H, s, OAc), 2.00 (3H, s, OAc), 1.93 (3H, s, OAc) 1.25 (3H, d, \(J = 6.2\) Hz, CH-3); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \(\delta_C = 170.4, 170.3, 170.2, 92.5, 91.9, 70.4, 68.6, 20.8, 20.7, 20.6, 17.4\); IR (powder, cm\(^{-1}\)): \(\nu = 3421.10\) (O-H), 1742.65 (C=O), 1721.27 (C=O); HRMS (ESI) calcd for C\(_{12}\)H\(_{18}\)NaO\(_8\)[M+Na]\(^+\): found m/z 313.0894, requires m/z 313.0870

\((2,3,4\text{-Tri-O-acetyl-}\alpha\text{-rhamnopyranosyloxy})\text{-trichloroacetimidate 615}\)

(2R,3R,4R,5S,6S)-2-hydroxy-6-methyltetrahydro-2H-pyran-3,4,5-triyl triacetate 614 (0.251 g, 0.86 mmol), trichloroacetonitrile (0.17 mL, 1.72 mmol) and CsCO\(_3\) (0.140 g, 0.43 mmol) were dissolved/suspended in CH\(_2\)Cl\(_2\) (10 mL) and stirred under nitrogen for 2.5 h. The reaction mixture was then filtered through a short pad of silica gel, which was subsequently washed with a mixed solution of petroleum ether : EtOAc (50 : 50) (200 mL). The combined filtrates were then concentrated in vacuo to give the title compound as a pale yellow oil (0.368 g, 99% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta_H = 8.72\) (1H, s, NH), 6.19 (1H, d, \(J = 2.0\) Hz, CH-1), 5.45 (1H, dd, \(J = 3.5, 2.0\) Hz, CH-2), 5.35 (1H, dd, \(J = 10.3, 3.5\) Hz, CH-3), 5.16 (1H, t, \(J = 10.3\) Hz, CH-4), 4.08 (1H, app m, CH-5), 2.18 (3H, s, OAc), 2.06 (3H, s, OAc), 2.00 (3H, s, OAc) 1.31, (3H, d, \(J = 6.2\) Hz, CH-3); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta_C = 169.9, 169.9, 169.8, 160.0, 94.6, 90.7, 70.3, 69.3, 68.8, 68.2, 20.8, 20.8, 20.7, 17.5\); IR (film, cm\(^{-1}\)): \(\nu = 1731.42\) (C=NH), 1712.27 (C=O); HRMS (ESI) calcd for C\(_{14}\)H\(_{18}\)Cl\(_3\)NaO\(_8\)[M+Na]\(^+\): found m/z 456.0007, requires m/z 455.9995
Experimental

4-(2,3,4-Tri-O-acetyl-α-rhamnopyranosyloxy)-benzaldehyde 613

First preparation (from trichloroacetimidate 615)

(2,3,4-Tri-O-acetyl-α-rhamnopyranosyloxy)-trichloroacetimidate 615 (0.36 g, 0.83 mmol) and 4-hydroxybenzaldehyde 560 (0.10 g, 0.83 mmol) was dissolved in CH₂Cl₂ (5 mL), and TMS.O Tf (0.15 mL, 0.83 mmol) added dropwise over 30 min. The resulting mixture was stirred for 48 h., before aq. NaHCO₃ was added and the whole stirred for an additional 5 min. Following extraction with CH₂Cl₂ (2 x 50 mL), the organics were dried over Na₂SO₄ and concentrated to afford a pale yellow oil. Purification was achieved by flash column chromatography [petroleum ether : EtOAc (50 : 50), Rf 0.33] to furnish title compound as a pale yellow oil (99 mg, 20% yield).

Second Preparation (from thioether 616 - next compound)

The title compound was prepared according to general procedure 7 from the addition of triflic acid (124 µL, 0.0014 mol) and N-iodosuccinamide (1.3 g, 0.0058 mol) to a solution of thiophenyl-2,3,4-Tri-O-acetyl-L-rhamnopyranoside 616 (2.25 g, 0.0058 mol) and 4-hydroxybenzaldehyde 560 (0.70 g, 0.0058) in CH₂Cl₂. The evaporation residue was purified by silica gel chromatography [petroleum ether : EtOAc (70 : 30), Rf 0.33] yielding a pale yellow oil (1.38 g, 60% yield). ¹H NMR (300 MHz, CDCl₃): δH = 9.90 (1H, s, CH), 7.85 (2H, d, J = 8.7 Hz, ArH), 7.19 (2H, d, J = 8.7 Hz, ArH), 5.55 (1H, d, J = 1.8 Hz, CH-1), 5.49 (1H, dd, J = 9.9, 3.5 Hz, CH-4), 5.43 (1H, m, CH-3), 5.39 (1H, t, J = 9.9 Hz, CH-2), 3.91 (1H, dq, CH-5), 2.19 (3H, s, OAc), 2.04 (3H, s, OAc), 2.02 (3H, s, OAc) 1.19 (3H, d, J = Hz, CH₃); ¹³C NMR (75 MHz, CDCl₃): δC = 190.7, 170.0, 160.4, 131.9, 131.4, 116.4, 95.3, 70.6, 69.3, 68.6, 67.6, 20.7, 17.4; IR (film, cm⁻¹): v =
2982.26 (C-H aromatic), 1746.54 (C=O ester), 1691 (C=O aldehyde); HRMS (ESI) calculated for C<sub>19</sub>H<sub>23</sub>O<sub>9</sub> [M+H]<sup>+</sup>: found m/z 395.1335, requires m/z 395.1342.

**Experimental**

The title compound was prepared according to general procedure 7 from the addition of boron trifluoro ethyl etherate (2.1 mL, 0.017 mol) to per-O-acetyl-6-deoxy-α-L-mannopyranose 611 (5.80 g, 0.017 mol) and thiophenol (1.68 mL, 0.017 mol) dissolved in toluene (30 mL). The crude evaporation residue was purified by silica gel chromatography [petroleum ether : EtOAc (70 : 30), Rf 0.30] to afford a pale yellow solid (5.12 g, 78 % yield).<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 7.40–7.49 (2H, m, ArH), 7.21–7.30 (3H, m, ArH), 5.49 (0.33H, d, J = 3.2 Hz, CH-2β), 5.44 (0.66H, d, J = 3.3 Hz, CH-2α) 5.35 (0.66H, app s, CH-1α) 5.28 (0.66H, dd, J = 10.2, 3.2 Hz, CH-3α), 5.11–5.03 (1H, app m, CH-4), 4.95 (0.33H, dd, J = 10.2, 3.3, CH-3β), 4.84 (0.33H, app s, CH-1β), 4.30 (0.66H, dq, J = 10.2, 6.0 Hz, CH-5α), 3.49 (0.33H, dq, J = 10.2, 6.0 Hz, CH-5β), 2.14 (1H, s, OAcβ), 2.08 (2H, s, OAcα), 2.01 (2H, s, OAcα), 1.98 (1H, s OAcβ), 1.95 (2H, s, OAcα), 1.92 (1H, s, OAcβ), 1.25 (1H, dd, J = 6.0 Hz, CH<sub>3</sub>), 1.18 (2H, dd, J = 6.0 Hz, CH<sub>3</sub>); 13C NMR (125 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 170.1, 170.0, 169.9, 133.2, 132.0, 131.8, 129.2, 129.1, 128.0, 127.8, 85.6, 85.3, 72.0, 71.8, 71.3, 71.1, 71.0, 70.2, 69.3, 67.7, 20.9, 20.8, 20.7, 20.6, 20.6, 17.7, 17.3; IR (powder, cm<sup>-1</sup>): ν = 2990.24 (C-H aromatic), 1742.27 (C=O); HRMS (ESI) calculated for C<sub>18</sub>H<sub>22</sub>NaO<sub>7</sub> [M+Na]<sup>+</sup>: found m/z 405.1016, requires m/z 405.0983

<sup>1</sup>-α-rhamnopyranosyloxy)-benzaldehyde 617<sup>254</sup>
The title compound was prepared according to general procedure 8 from a mixture of (2,3,4-tri-O-acetyl-α-rhamnopyranosyloxy)-benzaldehyde 613 (500 mg, 1.26 mmol) and NaOMe (0.5 M in MeOH) (0.6 mL, 0.315 mmol) in MeOH (10 mL). The crude was purified by silica gel chromatography [petroleum ether : acetone (60 : 40), Rf 0.55] to afford a beige solid (335 mg, quant. yield). 1H NMR (500 MHz, acetone-d6): δH = 9.93 (1H, s, CHO), 7.89 (2H, d, J = 8.8 Hz, ArH), 7.26 (2H, d, J = 8.8 Hz, ArH), 5.65 (1H, app s, CH-1), 4.31 (1H, app s, CH-2), 4.15 (1H, br s, OCH), 4.05 (2H, br s, 2x OCH), 3.86 (1H, d, J = 7.8 Hz, CH-3), 3.62 (1H, dq, J = 9.1, 5.9 Hz, CH-5), 3.51 (1H, t, J = 9.1 Hz, CH-4), 1.19 (3H, d, J = 5.9 Hz, CH3); 13C NMR (125 MHz, acetone-d6): δC = 206.8, 191.7, 162.6, 132.7, 132.4, 117.8, 99.5, 73.7, 72.5, 71.8, 71.0, 18.5 IR (powder, cm-1): ν = 3313.98 (O-H), 2935.43 (C-H aromatic), 1746.54 (C=O); HRMS (ESI) calculated for C13H16O6 [M+Na]+: found m/z 291.0823, requires m/z 291.0844.

4-(-α-Rhamnopyranosyloxy)-benzaldehyde 617 (100 mg, 0.37 mmol) was added to a -78 °C solution of diisopropyl isocyanomethylphosphonate 574 (77 µL, 0.37 mmol) and LHMDS (1.0M in THF) (1.67 mL, 1.67 mmol) according to a modified version of general procedure B (4.5 equiv. of base). The title compound was purified using silica gel chromatography [hexane : EtOAc ( 25 : 75 ), Rf 0.35], affording the natural product as pale brown oil (18 mg, 14% yield). 1H NMR (500 MHz, acetone-d6): δH = 7.40 (2H, d, J = 7.8 Hz, ArH), 7.06 (2H, d, J = 8.9 Hz, ArH), 6.99 (1H, d, J = 14.5 Hz, β vinyl H), 6.52 (1H, d, J = 14.5 Hz, α vinyl H), 5.52 (1H, m, CH-1), 4.32 (1H, s, OH), 4.13, (1H, s, OH), 4.04 (1H, s, OH), 3.98 (1H, m, CH-2), 3.82 (1H, m, CH-3), 3.57 (1H, m, CH-5), 3.45 (1H, m, CH-4), 1.20 (3H, app s, CH3); 13C NMR (125 MHz, acetone-d6): δC = 164.7 (IC), 159.0, 137.6, 129.5, 128.3, 117.8, 110.6, 99.7, 73.7, 72.2, 71.9, 70.8, 18.0; HRMS (ESI) calculated for C15H17NNaO5 [M+Na]+: found m/z 314.0983, requires m/z 314.1004; α[D]298 (MeOH) -105°
Experimental

Synthesis of Bulkier Phosphonates

Benzyl ((diphenoxyphosphoryl)methyl)carbamate 627

A mixture of benzylcarbamate 626 (10.00 g, 0.066 mol), acetic anhydride (9.5 mL, 0.099 mol) and paraformaldehyde 622 (1.9 g, 0.066 mol) was stirred in acetic acid for 3 h. at 70 °C under nitrogen. Triphenylphosphite (20.0 mL, 0.066 mol) was then added and the reaction temperature increased to 120 °C for 3 h. before the volatiles were removed by rotary evaporation (boiling water bath), and the resulting colourless residue purified by recrystallisation from MeOH, affording the title compound as colourless needles (15.20 g, 57% yield). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta_H = 7.45-7.07\) (15H, m, Ar\(\_H\)), 5.43 (1H, br s, NH), 5.14 (2H, s, CH\(_2\)Ar), 3.98 (2H, dd, \(J = 10.5, 6\) Hz, PCH\(_2\)); \(^13\)C NMR (75 MHz, CDCl\(_3\)): \(\delta_C = 149.9, 129.8, 128.5, 128.3, 125.5, 120.5, 67.4\); \(^31\)P NMR (300 MHz, CDCl\(_3\)): 16.3; IR (film cm\(^{-1}\)): \(\nu = 1720.15\) (C=O); HRMS (ESI) calculated for C\(_{21}\)H\(_{21}\)NO\(_5\)P [M+H]\(^+\): found m/z 398.1154, requires m/z 398.1157.

(Diphenoxyphosphoryl)methanaminium bromide 628

Benzyl ((diphenoxyphosphoryl)methyl)carbamate 627 (7.00 g, 0.017 mol) was dissolved in hydrogen bromide (33% w/w (45% w/v) soln. in acetic acid) (20 mL) and stirred at room temperature for 2 h. After this time, the reaction was concentrated to afford an orange oil, which when triturated with hot diethyl ether precipitated the desired hydro bromide salt as a beige solid (5.90 g, nr. quant. yield). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta_H = 8.81\) (3H, br s, NH\(_3\)), 7.50-7.18 (10H, m, Ar\(\_H\)), 3.88 (2H, d, \(J = 13.6\) Hz, PCH\(_2\)); \(^13\)C
Experimental

NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 149.6, 130.4, 126.2, 121.0, 33.8 (d, <i>J</i> = 157.5 Hz); <sup>31</sup>P NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>P</sub> = 15.1; HRMS (ESI) calculated for C<sub>13</sub>H<sub>15</sub>NO<sub>3</sub>P [M]<sup>+</sup>: found m/z 264.0774, requires m/z 264.0789.

**Diphenyl (formamidomethyl)phosphonate 620**

(Diphenoxypophosphoryl)methanaminium bromide 628 (5.10 g, 0.0147 mol) was dissolved in THF (25 mL), free-based using Et<sub>3</sub>N (4.00 mL, 0.0294 mol) and then formylated according to general procedure 4 using acetic formic anhydride (1.42 g, 0.0162). (NOTE: reaction mixture required filtration before removal of volatiles <i>in vacuo</i>). The crude products were purified by silica gel chromatography [pentanes : EtOAc (50 : 50), R<sub>f</sub> 0.10] to afford title compound as a white solid (4.92 g, near quant. yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ<sub>H</sub> = 8.05 (1H, s, CHO), 7.35-7.10 (11H, m, ArH + NH), 4.02 (2H, dd, <i>J</i> = 11.6, 5.6 Hz, PCH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> = 161.3, 149.8, 129.9, 125.8, 120.5, 33.6 (d, <i>J</i> = 160.7 Hz); <sup>31</sup>P (300 MHz, CDCl<sub>3</sub>): δ<sub>P</sub> = 16.3; IR (powder, cm<sup>-1</sup>): ν = 3290.59 (N-H), 2928.94 (C-H aromatic), 1669.87 (C=O); HRMS (ESI) calculated for C<sub>14</sub>H<sub>16</sub>N<sub>1</sub>O<sub>3</sub>P [M+H]<sup>+</sup>: found m/z 292.0732, requires m/z 292.0738.

**Diphenyl (isocyanomethyl)phosphonate 629**

(Diphenyl(formamidomethyl))phosphonate 620 (1.00 g, 0.0034 mol) was dehydrated according to general procedure 2 when dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and Et<sub>3</sub>N (4.20 mL, 0.030 mol) and treated with MsCl (0.77 mL, 0.010 mol). The resulting foul smelling brown oil, was purified by silica gel chromatography [pentanes : EtOAc (80 : 20), R<sub>f</sub> 0.28] to furnish title compound as a
pale orange oil (0.67 g, 72% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta_H = 7.45$-$7.20$ (10H, m, ArH), $\delta_H = 4.07$ (2H, d, $J = 15.4$ Hz, PCH$_2$); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C = 162.3, 149.9, 130.1, 126.1, 120.5, 40.4$ (d, $J = 159.7$ Hz); $^{31}$P (300 MHz, CDCl$_3$): $\delta_P = 7.16$; IR (film cm$^{-1}$): $\nu = 2135$ (IC)

Tri-tertbutylphosphite$^{217}$

Phosphorusrichloride (10.00 g, 0.075 mol) in diethyl ether (100 mL) is added dropwise onto a mixture of tert-butanol (96.23 mL, 0.225 mol) and Et$_3$N (61 mL, 0.500 mol) held at 0 °C under nitrogen. The whole is warmed up to r.t. and stirred for 24 h. before being diluted with diethyl ether (100 mL) and filtered. The colourless evaporation residue crystallised upon standing at 4 °C to afford the target material as colourless plates (11.2 g, 68% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta_H = 1.30$; $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta_C = 30.8$ (d, $J = 159.7$ Hz) 28.6; $^{31}$P (300 MHz, CDCl$_3$): $\delta_P = 141.0$

Di-tert-butyl (dibenzylamino)methylphosphonate 623

Paraformaldehyde 622 (0.110 g, 0.0039 mol) was added to dibenzylamine 621 (0.71 mL, 0.0035 mol) dissolved in MeCN (10 mL) and the whole refluxed (bath temp. 80 °C) under nitrogen for 1 h. The resulting suspension was then cooled to r.t. and P(O$^t$Bu)$_3$ (1.00 g, 0.0039 mol) added dropwise as a solution in MeCN (1 mL) and the whole allowed to stir for 16 h. before the volatiles were removed by rotary evaporation. The evaporation residue was dissolved in EtOAc, washed with 1M HCl, water and brine before being dried over MgSO$_4$ and
Experimental

Concentrated to yield a pale yellow oil that was purified by silica gel chromatography [pentanes : EtOAc (80 : 20), Rf 0.41] to furnish title compound as a colourless oil (1.24 g, 84% yield). $^1$H NMR (300 MHz, CDCl$_3$): δ$_H$ 7.45-7.17 (10H, m, ArH), 3.88 (2H, s, CH$_2$Ph), 2.75 (2H, d, J = 10.2 Hz, PCH$_2$), 1.47 (18H, s, O(CH$_3$)$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$): δ$_C$ 139.2, 129.0, 128.1, 126.9, 81.8 (d, J = 157.5 Hz), 59.0, 30.5, 29.9; $^{31}$P (300 MHz, CDCl$_3$): δ$_P$ 19.16; HRMS (ESI) calculated for C$_{23}$H$_{34}$NO$_3$P [M+H]$^+$: found m/z 404.2352, requires m/z 404.2354.

**Di-tert-butyl (aminomethyl)phosphonate 624**

![Di-tert-butyl (aminomethyl)phosphonate](image)

Di-tert-butyl ((dibenzylamino)methyl)phosphonate 623 (660 mg, 1.63 mmol) was dissolved in MeOH (10 mL), Pd/C (10% wt.) (174 mg, 10 mol%) added and the flask charged with H$_2$ (1 atm.) The whole was then stirred at r.t. for 16 h. before being filtered through celite and then concentrated to afford the desired amine as a pale brown residue which was used in the next step without further purification (390 mg, quant. yield). $^1$H NMR (300 MHz, DMSO-d$_6$): δ$_H$ 2.65 (2H, d, J = 9.8 Hz, CH$_2$), 1.40 (18H, s, O(CH$_3$)$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$): δ$_C$ 85.7, 35.2, 34.7

**Di-tert-butyl (formamidomethyl)phosphonate 619**

![Di-tert-butyl (formamidomethyl)phosphonate](image)

Di-tert-butyl (aminomethyl)phosphonate 624 (490 mg, 2.21 mmol) was formylated according to general procedure 4 using acetic formic anhydride (192 mg, 1.70 mmol) and THF (5 mL) to afford the title compound as a colourless oil that was used without further purification (402 mg, 73% yield). $^1$H NMR (300 MHz, CDCl$_3$): δ$_H$ = 8.11 (1H, s, CHO), 6.96 (1H, br s, NH), 3.63 (2H, dd, J = 12.8, 6.0 Hz, NCH$_2$), 1.49 (18H, d, J = 9.04 Hz, O(CH$_3$)$_3$).
Experimental

Di-tert-butyl (isocyanomethyl)phosphonate 625

Di-tert-butyl (formamidomethyl)phosphonate 619 (400 mg, 1.59 mmol) was dehydrated according to general procedure 2 when dissolved in CH$_2$Cl$_2$ (10 mL) and Et$_3$N (1.95 mL, 14.34 mmol) and treated with MsCl (0.36 mL, 4.780 mmol). The resulting foul smelling brown oil, was purified by silica gel chromatography [pentanes : EtOAc (80 : 20), R$_f$ 0.28] to furnish title compound as a pale orange oil (101 mg, 22% yield). $^1$H NMR (300 MHz, CDCl$_3$): $\delta$H 3.59 (2H, d, $J$ = 15.45 Hz, C$_2$H$_5$), 1.51 (18H, s, O(C$_3$H$_7$)$_3$); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$C 159.0, 84.6, 40.5 (d, $J$ = 158.0 Hz), 40.5, 30.2; IR (film cm$^{-1}$): $\nu$ = 2129 (N=C)

Synthesis of Rhabduscin

Methyl 4,6-O-benzylidene-D-glucopyranoside 634

Benzaldehyde dimethyl acetal (30 mL, 0.193 mol) was added to a solution of methyl-\(\alpha\)-D-glucopyranoside 633 (25 g, 0.129 mol) and pTSA (30 mol%) in anhydrous DMF (250 mL) and the whole stirred for 2 h. at 60 °C under N$_2$. The reaction was neutralised by addition of Et$_3$N, and the solvents removed via distillation under reduced pressure. The resulting oil was partitioned between chloroform and water, and the organic phase dried over MgSO$_4$ and concentrated (post filtration) to give a solid that was triturated with petroleum ether to afford the title compound as a white solid [petroleum ether: EtOAc (50 : 50), R$_f$ 0.01] (27.75 g, 76 % yield). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$H = 7.53-7.46 (2H, m, ArH), 7.40-7.34 (3H, m, ArH), 5.53 (1H,
Experimental

Methyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside 635

Benzyl bromide (44.2 mL, 0.372 mol) was added to a solution of Methyl 4,6-O-benzylidene-α-D-glucopyranoside 634 (15 g, 0.053 mol) and KOH (17.9 g, 0.319 mol) in anhydrous toluene. The resulting solution was refluxed under N₂ at 120 °C for 4 h., before being cooled to r.t. to allow for dilution with toluene and partition against water. The organic phase was dried over MgSO₄ and concentrated in vacuo to afford the desired compound as a yellow oil which was used in the subsequent reaction without purification (21.13 g, 86% yield). ¹H NMR (500 MHz, CDCl₃): δ_H = 7.69-7.61 (4H, t, J = 8.76 Hz, ArH), 7.56-7.29 (11H, m), 5.70 (1H, s, CHPh), 5.07 (1H, d, J = 11.3 Hz, OCH₂Ph), 5.00 (2H, dd, J = 12.3, 11.3 Hz, OCH₂Ph), 4.85 (1H, d, J = 12.3 Hz, OCH₂Ph), 4.75 (1H, d, J = 3.5 Hz, CH-1), 4.71 (1H, s, CH), 4.63 (1H, s, CH), 4.42 (1H, dd, J = 5.2, 4.5 Hz CH-3), 4.20 (1H, t, J = 9.3 Hz, CH₂”), 3.99 (1H, td, J = 10.0, 4.8 Hz, CH-5), 3.85 (1H, t, J = 10.6 Hz, CH₂”), 3.75 (1H, t, J = 9.4 Hz, CH-4), 3.71 (1H, dd, J = 10.6, 3.5 Hz, CH-2), 3.55 (3H, s, OCH₃); ¹³C NMR (125 MHz, CDCl₃): δ_C = 138.2, 127.6, 101.3, 99.2, 81.0, 79.2, 78.6, 75.3 73.8, 69.1, 62.3, 55.4; IR (film, cm⁻¹): ν = 3028.52 (C-H aromatic); HRMS (ESI) calculated for C₂₉H₃₃O₆ [M+H]⁺: found m/z 463.2093, requires m/z 463.2121
Iodine crystals (4.87 g, 0.0192 mol) were added to a solution of Methyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside 635 (24.0 g, 0.052 mol) in MeOH (500 mL) and refluxed under nitrogen at 80 °C for 16 h. The reaction mixture was allowed to cool to r.t. before aq. Na$_2$S$_2$O$_3$ was added and the solvents co-evaporated. The evaporation residue was partitioned between H$_2$O and EtOAc and the organic phase was dried over MgSO$_4$, concentrated under reduced pressure and purified using silica gel chromatography [petroleum ether : Et$_2$O (50 : 50), $R_f$ 0.09] to afford the title compound as a yellow oil (17.5 g, 90 % yield).

$^1$H NMR (500 MHz, CDCl$_3$): δ$_{H}$ = 7.40-7.28 (10H, m, Ar H), 5.02 (1H, d, $J$ = 11.41 Hz, OC$_2$H$_2$Ph), 4.74 (2H, dd, $J$ = 11.97, 11.41 Hz, OCH$_2$Ph), 4.66 (1H, d, $J$ = 11.97 Hz, OCH$_2$Ph), 4.60 (1H, d, $J$ = 3.62 Hz, CH-1), 3.82-3.71 (3H, m CH-3 & OCH$_3$), 3.61 (1H, dt, $J$ = 9.8, 4.1 Hz, CH-5), 3.52 (1H, t, $J$ = 9.6 Hz, CH-4), 3.49 (1H, dd, $J$ = 9.6, 3.6 Hz, CH-2), 3.38 (3H, s, OCH$_3$)$_3$; $^{13}$C NMR (125 MHz, CDCl$_3$): δ$_{C}$ = 138.7, 137.9, 128.5, 128.1, 128.0, 127.9, 127.9, 98.2, 81.2, 79.8, 73.1, 75.4, 70.71, 70.4, 62.4, 55.2; IR (film, cm$^{-1}$): $\nu$ = 3271.58 (O-H), 3060.49 (C-H aromatic); HRMS (ESI) calculated for C$_{21}$H$_{26}$NaO$_6$ [M+Na]$^+$: found m/z 397.1666, requires m/z 397.1622.

A solution of Methyl 2,3-di-O-benzyl-6-O-p-toluenesulfonyl-α-D-glucopyranoside 637 (15.0 g, 0.04 mol) in pyridine (180 mL) under nitrogen was cooled to -20 °C, and tosyl chloride (11.45 g, 0.06 mol) added and the resulting solution was left to warm to r.t. After stirring for 16 h., the reaction mixture was heated at 60 °C for 30 min and then returned to r.t. and partitioned between aq. NaHCO$_3$ and CH$_2$Cl$_2$. The organic phase was then dried using MgSO$_4$, concentrated under reduced pressure and purified using silica gel chromatography [petroleum ether: EtOAc (50 : 50), $R_f$ 0.45] to afford the title compound as a pale yellow oil that solidified upon standing to yield a white
Experimental

solid (15.56 g, 74% yield). $^1$H NMR (500 MHz, CDCl$_3$): $\delta_{\text{H}}$ = 7.86 (2H, d, $J = 8.34$ Hz, ArH tosyl), 7.37-7.27 (12H, m, 10 ArH benzyl & 2 ArH tosyl), 4.99 (1H, d, $J = 11.4$ Hz, OCH$_3$Ph), 4.7 (1H, d, $J = 11.8$ Hz, OCH$_3$Ph), 4.70 (1H, d, $J = 11.4$ Hz OCH$_3$Ph), 4.63 (1H, d, $J = 11.8$ Hz, OCH$_3$Ph), 4.56 (1H, d, $J = 3.6$ Hz CH-1), 4.23 (2H, d, $J = 3.6$ Hz, OCH$_2$ tosyl), 3.67-3.69 (2H, m, CH-3 & CH-5), 3.48-3.41 (2H, m, CH-2 & CH-4), 3.32 (3H, s, OCH$_3$), 2.43 (3H, s, SCH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta_{\text{C}}$ = 144.8, 138.6, 137.9, 132.9, 129.8, 128.6, 128.5, 128.1, 128.0, 127.9, 127.9, 98.1, 81.09, 79.5, 75.4, 73.2, 69.4, 55.3, 21.6; IR (film, cm$^{-1}$): $\nu$ = 3503.03 (O-H), 2911.08 (C-H aromatic); HRMS (ESI) calculated for C$_{28}$H$_{32}$NaO$_8$S [M+Na]$^+$: found m/z 551.1744, requires m/z 551.1710.

Methyl 2,3-Di-O-benzyl-6-deoxy-α-D-glucopyranoside 638

LiAlH$_4$ (0.65 g, 0.0170 mol) was added portion wise to a solution of Methyl 2,3-di-O-benzyl-6-O-p-toluenesulfonyl-α-D-glucopyranoside 367 (4.5 g, 0.0085 mol) in anhydrous THF. The resulting suspension was then refluxed (bath temp 80 °C) under nitrogen for 2 h. before being quenched by the careful addition of ice water. Once effervescence had ceased, the reaction liquor was filtered through celite (2 x 20 mL EtOAc washings), and the filtrate evaporation residue purified using silicic gel chromatography to afford the title compound as a colourless oil (2.57g, 85% yield). [petroleum ether: EtOAc (50 : 50), R$_f$ 0.60]; $^1$H NMR (500 MHz, CDCl$_3$): $\delta_{\text{H}}$ = 7.40-7.28 (10H, m, ArH), 5.04 (1H, d, $J = 11.3$ Hz, OCH$_3$Ph), 4.76 (1H, d, $J = 11.8$ Hz, OCH$_3$Ph), 4.68 (2H, t, $J = 12.4$ Hz, OCH$_3$Ph), 4.57 (1H, d, $J = 3.8$ Hz, CH-1), 3.73, (1H, t, $J = 9.4$ Hz, CH-3), 3.65 (1H, dq, $J = 9.0$, 6.2 Hz CH-5), 3.52 (1H, dd, $J = 9.51$, 3.5 Hz, CH-2), 3.38 (3H, s, OCH$_3$), 3.16 (1H, t, $J = 9.5$ Hz, CH-4), 1.21 (3H, d, $J = 6.2$ Hz, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta_{\text{C}}$ = 138.0, 138.0, 128.6, 128.5, 128.1, 128.01, 127.9, 127.9, 98.0, 81.3, 80.1, 75.3, 75.3, 73.0, 66.8, 55.1, 17.6; IR (film cm$^{-1}$): $\nu$ = 3362.69 (O-H), 3031.25 (C-H aromatic); HRMS (ESI) calculated for C$_{21}$H$_{32}$NaO$_8$ [M+Na]$^+$: found m/z 381.1708, requires m/z 381.1678.
Methyl 2,3-Di-O-benzyl-6-deoxy-4-O-methylsulfonyl-α-D-glucopyranoside 639

Methanesulfonylchloride (3.4 mL, 0.045 mol) was added to an ice cold solution of methyl 2,3-Di-O-benzyl-6-deoxy-α-D-glucopyranoside 638 (2.56 g, 0.007 mmol) and pyridine (5 mL) in CH₂Cl₂ (15 mL). The solution was allowed to warm up to r.t. before being partitioned between CH₂Cl₂ (50 mL) and aq. NaHCO₃, with the organics phase then being washed with brine and subsequently dried over MgSO₄. The evaporation residue from the organics was purified by silica gel chromatography [petroleum ether: EtOAc (50 : 50), Rf 0.70], affording the title compound as a white solid (2.64 g, 87 % yield).

1H NMR (500 MHz, CDCl₃): δH = 7.36-7.27 (10H, m, ArH), 5.09 (1H, d, J = 11.1 Hz, OCH₂Ph), 4.74 (1H, d, J = 11.8 Hz, OCH₂Ph), 4.65 (2H, dd, J=11.8, 11.1 Hz, OCH₂Ph), 4.55 (1H, d, J = 3.8 Hz, CH-1), 4.20 (1H, t, J = 9.3 Hz, CH-4), 3.97 (1H, t, J = 9.6 Hz, CH-3). 3.99-3.82 (1H, dq, J = 9.3, 6.0 CH-5), 3.58 (1H, dd, J = 9.62, 3.87 Hz, CH-2), 3.83 (3H, s, OCH₃), 2.79 (3H, s, SCH₃), 1.33 (3H, d, J = 6.4 Hz, CH₃); 13C NMR (125 MHz, CDCl₃): δC = 138.2, 137.6, 128.6, 128.5, 128.2, 128.2, 127.8, 127.6, 97.4, 83.2, 80.5, 75.4, 73.3, 65.3, 55.4, 38.8, 17.59; IR (powder, cm⁻¹): ν = 2979.79 (C-H aromatic); HRMS (ESI) calculated for C₂₂H₂₈O₇NaO₇S [M+Na]⁺: found m/z 459.1454, requires m/z 459.1453.

Methyl-4-azido-2,3-di-O-benzyl-4,6-dideoxy-α-D-galactopyranoside 640

NaN₃ was added portion wise to a solution of Methyl 2,3-Di-O-benzyl-6-deoxy-4-O-methylsulfonyl-α-D-glucopyranoside 639 (2.00 g, 4.6 mmol), 15-crown-5 (0.318 mL, 1.61 mmol) in DMF (15 mL). The whole was stirred under nitrogen at 120 °C for 4 h. before being cooled to r.t. and the DMF removed in vacuo. The title compound was obtained as a yellow oil following purification using silica gel chromatography [petroleum ether: EtOAc (50 : 50), Rf
Experimental

0.71] (1.01 g, 57 % yield). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta_{HH} = 7.46-7.27\) (10H, m ArH), 4.86 (2H, app. t, \(J = 11.3\) Hz, OCH\(_2\)Ph), 4.77 (1H, d, \(J = 11.9\) Hz, OCH\(_2\)Ph), 4.68 (1H, d, \(J = 11.9\) Hz, OCH\(_2\)Ph), 4.60 (1H, d, \(J = 3.6\) Hz, CH-1), 4.06 (1H, dd, \(J = 9.88, 3.2\) Hz, CH-3), 3.95- 3.85 (2H, m, CH-4 \& CH-5), 3.71 (1H, d, \(J = 3.6\) Hz CH-2), 3.36 (3H, s, OCH\(_3\)), 3.15- 3.05 (3H, m, CH-6), 3.05 (1H, d, \(J = 3.6\) Hz C-1), 3.36 (3H, s, OCH\(_3\)); \(^13\)C NMR (125 MHz, CDCl\(_3\)): \(\delta_C = 138.3, 138.1, 128.3, 128.0, 127.9, 98.5, 75.0, 72.6, 64.0, 55.3, 53.5, 16.6\); IR (film, cm\(^{-1}\)): \(\nu = 2903.87\) (C-H aromatic), 2107.81 (N=N=N); HRMS (ESI) calculated for C\(_{21}\)H\(_{25}\)N\(_3\)O\(_4\) [M+H]\(^+\): found m/z 406.1744, requires m/z 406.1743.

4-amino-2,3-di-O-benzyl-\(\alpha\)-D-mannopyranose 641

Methyl-4-azido-2,3-di-O-benzyl-4,6-dideoxy-\(\alpha\)-D-galactopyranoside 640 (0.5 g, 0.0013 mol) was dissolved in a mixture of mixture of MeOH and aq. 1M HCl (1 : 1) (20 mL total) and Pd/C added (180 mg, 0.00013 mol). The resultant suspension was then stirred under an atmosphere of hydrogen for 24 h., before being filtered through celite and concentrated to afford the target compound as a pale yellow oil (crude). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta_{HH} = 7.42-7.25\) (10H, m, ArH), 4.84-4.67 (4H, app. m, OCH\(_2\)Ph), 4.65 (1H, d, \(J = 3.8\) Hz, CH-1), 4.02 (1H, q, \(J = 6.6\) Hz, CH-5), 3.92 (1H, dd, \(J = 10.9, 4.1\) Hz, CH-3), 3.84 (1H, dd, \(J = 10.9, 3.8\) Hz, CH-2), 3.43 (1H, app br s, CH-4), 3.38 (3H, s, OCH\(_3\)), 1.31 (3H, d, \(J = 6.6\) Hz, CH\(_3\)); \(^13\)C NMR (125 MHz, CDCl\(_3\)): \(\delta_C = 138.4, 138.1, 128.3, 128.0, 127.9, 98.5, 75.0, 72.6, 64.0, 55.3, 53.5, 16.6\); HRMS (ESI) calculated for C\(_{21}\)H\(_{29}\)NO\(_4\) [M+H]\(^+\): found m/z 358.2019, requires m/z 358.2018

4-amino-2,3-dideoxy-\(\alpha\)-D-mannopyranose 642
Experimental

Methyl-4-azido-2,3-di-O-benzyl-4,6-dideoxy-α-D-galactopyranoside 640 (0.5 g, 0.0013 mol) was dissolved in a mixture of mixture of dioxane and water (1 : 1) (20 mL total) and Pd/C added (180 mg, 0.00013 mol). The resultant suspension was then stirred under an atmosphere of hydrogen for 24 h., before being filtered through celite and concentrated to afford the target compound as a pale yellow oil (30 mg - crude). 1H NMR (500 MHz, D₂O): δ_H = 4.79 (1H, d, J = 4.4 Hz, CH-1), 4.13 (1H, q, J = 6.5 Hz, CH-5), 3.93 (1H, dd, J = 10.5, 4.4 Hz, CH-2), 3.77 (1H, dd, J = 10.5, 4.0 Hz, CH-3), 3.39 (3H, s, OCH₃), 3.15 (1H, d, J = 4.0 Hz, CH-4), 1.23 (3H, d, J = 6.5 Hz, CH₃); 13C NMR (125 MHz, D₂O): δ_C = 99.7, 69.5, 67.9, 65.8, 55.6, 55.2, 16.4; HRMS (ESI) calculated for C₇H₁₅NO₄ [M+H]^+: found m/z 178.1092, requires m/z 178.1079.

4-N-acetyl-2,3-O-acetyl-α-D-mannopyranose 643

The title compound was prepared according to general procedure 5 from 4-amino-2,3-dideoxy-α-D-mannopyranose 642 (50 mg, 0.31 mmol), DMAP (4 mg, 10 mol%) and Ac₂O (1 mL) in pyridine (5 mL). The title compound, a colourless glass, was used in the subsequent reactions without isolation. (30 mg approx.). 1H NMR (300 MHz, CDCl₃): δ_H = 5.58 (1H, d, J = 8.7 Hz, CH-1), 5.32-5.23 (1H, m, CH), 4.95-4.87 (1H, m, CH), 4.56-4.49 (1H, m, CH), 4.23 (1H, qd, J = 6.8, 1.5 Hz, CH-5), 3.39 (3H, s, OCH₃), 2.11 (3H, s, OAc), 2.08 (3H, s, OAc), 2.00 (3H, s, NAc), 1.16 (3H, d, J = 6.8 Hz).

4-N-acetyl-1,2,3-O-acetyl-α-D-mannose 644
Acetic anhydride (50 µL) and H₂SO₄ (4 µL, 0.045 mmol) were added to a solution of 4-N-acetyl-2,3-O-acetyl-α-D-mannopyranose 643 (30 mg, 0.099 mmol) in CH₂Cl₂ (5 mL). The whole was allowed to stir at r.t. overnight before being quenched with aq. NaHCO₃ and extracted with further CH₂Cl₂. The combined organics were dried over MgSO₄ and the concentrated to afford the title compound as a dirty oil. (no yield) ¹H NMR (300 MHz, CDCl₃): δ_H = 6.23 (1H, d, J = 3.8 Hz, CH-1), 5.82 (1H, d, J = 9.4 Hz, CH-3), 5.20 (1H, dd, J = 7.6, 3.8 Hz, CH-2), 4.56-4.28 (1H, m, CH-4), 4.29 (1H, qd, J = 6.4, 1.5 Hz, CH-5), 2.08 (3H, s, OAc), 2.01 (3H, s, OAc), 1.95 (3H, s, OAc), 1.94 (3H, s, NAc), 1.10 (3H, d, J = 6.8 Hz).

6.5 Compounds associated with the synthesis of Paerucumarin (Chapter 5)

5-hydroxybenzo[1,3]dioxole acetate 705²⁰⁴

![5-hydroxybenzo[1,3]dioxole acetate](image)

The title compound was prepared according to general procedure 5 from sesamol 704 (5.00 g, 0.036 mol), DMAP (0.40 g, 10 mol%) and Ac₂O (10 mL) in pyridine (30 mL). The title compound, a colourless oil, was used in the subsequent reactions without the need for purification (5.78 g, 89% yield). ¹H NMR (300 MHz, CDCl₃): δ_H = 6.63 (1H, d, J = 8.3 Hz, ArH), 6.48 (1H, d, J = 2.3 Hz, ArH), 6.39 (1H, dd, J = 8.3, 2.3 Hz), 5.79 (2H, s, OCH₂O), 2.10 (3H, s, OAc); ¹³C NMR (75 MHz, CDCl₃): δ_C = 169.7, 148.0, 145.3, 145.0, 113.9, 107.9, 103.7, 101.7, 20.8; IR (film, cm⁻¹): ν = 2903.69 (C-H aromatic), 1757.08 (C=O).

6-Hydroxybenzo[1,3]dioxole-5-carboxaldehyde 706²⁰⁴

![6-Hydroxybenzo[1,3]dioxole-5-carboxaldehyde](image)
5-hydroxybenzo[1,3]dioxole acetate 705 (5.68 g, 0.031 mol) was dissolved in CH$_2$Cl$_2$ (100 mL) and cooled to 0 °C before SnCl$_4$ (9.77 mL, 0.063 mol) and then Cl$_2$CHOCH$_3$ (3.42 mL, 0.037 mol) were added sequentially through a dropping funnel. After two hours of stirring the reaction mixture was poured over ice and the resultant organic phase washed with aq. 2M HCl (3x 20 mL) and H$_2$O (2x 20 mL) before being dried over MgSO$_4$ and concentrated (post filtration) to afford a crude solid. Purification was achieved upon trituration with cold MeOH to yield the desired product as a white powder (4.21 g, 81% yield): m.p. 123-125 °C (reference: 125-126 °C); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$H = 9.62 (1H, s, CH$_2$O), 6.86 (1H, s, ArH), 6.47 (1H, s, ArH), 6.02 (2H, s, OC$_2$H$_2$O); $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$C = 193.7, 161.5, 141.3, 113.6, 109.3, 102.1, 98.3; IR (film, cm$^{-1}$): $\nu = 2983.00$ (C-H aromatic), 1641.49 (C=O).

$N$-(6-oxo-6H-[1,3]dioxolo[4,5-g]chromen-7-yl)formamide 707

\[ \text{CuI (22 mg, 0.12 mmol) was added to a stirring mixture of 6-hydroxybenzo[1,3]dioxole-5-carboxaldehyde 706 (200 mg, 1.20 mmol), isocyanooacetate (0.135 mL, 1.20 mmol) and pyridine (0.1 mL, 1.20 mmol) in MeOH (5 mL) at 50 °C. After stirring for 10 h., the reaction was cooled to 0 °C and the precipitated product collected by filtration. Further washing with cold MeOH (2 mL) afforded the title compound as a pale pink solid (222 mg, 58% yield). } \]

$^1$H NMR (300 MHz, DMSO-d$_6$): $\delta$H = 10.12 (1H, br s, NH), 8.59 (1H, s, CHO), 8.37 (1H, s, C=CH), 7.30 (1H, s, ArH), 7.13 (1H, s, ArH), 6.14 (2H, s, OCH$_2$O); $^{13}$C NMR (75 MHz, DMSO): $\delta$C = 161.5, 157.83, 149.6, 146.6, 125.1, 121.8, 113.3, 105.6, 102.7, 98.0; IR (film, cm$^{-1}$): $\nu = 3335.82$ (N-H), 3044.73 (C-H aromatic), 2915.49 (C-H aromatic), 1713.41 (C=O ester) 1676.65 (C=O formamide); HRMS (ESI) calculated for C$_{11}$H$_8$NO$_5$ [M+H]$^+$: found m/z 234.0394, requires m/z 234.0402.
Experimental

7-amino-6H-[1,3]dioxolo[4,5-g]chromen-6-one 709

\[
\text{\begin{center}
\includegraphics[scale=0.5]{figure1.png}
\end{center}}
\]

\[N\{6-oxo-6H-[1,3]dioxolo[4,5-g]chromen-7-yl\}formamide 707\] (200 mg, 0.862 mmol) was refluxed in a 10 mL mixture of MeOH and 33% HCl (8 : 2) for 1 h. After this time, the reaction mixture was cooled and neutralised to pH 8 using \(\text{K}_2\text{CO}_3\) filtered and then concentrated to afford the target compound as a bright red solid, which did not need purification (174 mg, nr quant. yield). \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta H = 7.23 (1\text{H}, \text{s, ArH}), 7.12 (1\text{H}, \text{s, ArH}), 7.05 (1\text{H}, \text{s, ArH}), 6.08 (2\text{H}, \text{s, CH}_2), 5.58 (2\text{H}, \text{br s, NH}_2)\); \(^13\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta C = 158.7, 148.3, 146.0, 145.0, 126.0, 119.8, 114.2, 104.4, 102.4, 98.0\); IR (film, cm\(^{-1}\)): \(\nu = 3435.79 \text{ (N-H)}, 1718.01 \text{ (C=O)}\); HRMS (ESI) calculated for \(C_{10}H_{7}NO_4\) [M+H]: found m/z 206.0440, requires m/z 206.0453.

3-amino-6,7-dihydroxy-2H-chromen-2-one 710

\[
\text{\begin{center}
\includegraphics[scale=0.5]{figure2.png}
\end{center}}
\]

\(\text{BBr}_3\) (150 mg, 0.731 mmol) was added to a suspension of 7-amino-6H-[1,3]dioxolo[4,5-g]chromen-6-one 709 in \(\text{CH}_2\text{Cl}_2\) (10 mL) held at -78 °C under an \(\text{N}_2\) atmosphere. After stirring for 4 h the reaction was quenched with methanol and the target compound, a red solid, was collected by Buchner filtration and needed no further purification (141 mg, nr quant. yield). \(^1\)H NMR (250 MHz, DMSO-\(d_6\)): \(\delta H = 7.52 (1\text{H}, \text{s, ArH}), 6.99 (1\text{H}, \text{s, ArH}), 6.81 (1\text{H}, \text{s, ArH}), 6.09 (2\text{H}, \text{br s, NH}_2)\); \(^13\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta C = 161.47, 159.67, 148.92, 146.62, 143.06, 132.66, 115.33, 112.39, 105.73\); IR (film, cm\(^{-1}\)): \(\nu = 3705.36 \text{ (O-H)}, 3505.36 \text{ (N-H)}, 1711.91 \text{ (C=O)}\); HRMS (ESI) calculated for \(C_{9}H_{8}NO_4\) [M+H]: found m/z 194.0451, requires m/z 194.0453.
Experimental

**Pseudoverdin 700**

3-amino-6,7-dihydroxy-2H-chromen-2-one 710 (120 mg, 0.622 mmol) was formylated by refluxing in formic acid (20 mL) for 4 h., the title compound being collected as a beige solid upon Buchner filtration (141 mg, 96% yield). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta_H = 10.0$ (1H, s, NH), 8.45 (1H, s, C=CH), 8.35 (1H, s, CHO), 6.95 (1H, s, ArH), 6.75 (1H, s, ArH); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta_C = 161.0$, 157.8, 148.0, 144.5, 143.5, 125.3, 120.5, 111.9, 110.7, 102.6; HRMS (ESI) calculated for C$_{10}$H$_7$NO$_5$ [M+H]$^+$: found m/z 220.0235, requires m/z 220.0245

### 6.6 Biological Procedures/Investigations

**Identifying hit compounds using disk diffusion assays**

The majority of the susceptibility testing of bacteria using disk diffusion were carried out by Liam Stephens, whilst phenol vinyl-isocyanide 542 and Byelyankacin 544 were investigated under the direction of Diana Alves.

A cotton-bud that had been dipped in a pre-prepared inoculum of bacteria was swabbed/streaked over the entire surface of a Mueller-Hinton agar petri-dish, with subsequent rotations of the disk (60 °) ensuring even coverage. Meanwhile, dispense disks are soaked for 15 min. in a 1mg ml$^{-1}$ solution of the antibiotic dissolved in a 9 : 1 mixture of water and methanol. Once the compound has been loaded onto the disk, the disk is carefully placed on the surface of the agar and the dishes incubated at 37 °C for 16 hours in ambient air. After this time the diameter of the ‘clear’ bacteria-free zone was measured using a ruler.
Quantifying hit compounds using MIC

All antibiotic minimum inhibitory concentrations (MIC) of the antibiotic compounds were determined by Liam Stephens.

96-well microplates, each containing a volume of 200 μl with 1 : 2 dilution of the antibiotic (64 to 0.0625 μg/ml range) were inoculated with an initial standard inoculum of a 105 cfu/ml of an overnight culture. A 0.5 McFarland scale was used for inoculum standardisation. Incubation at 37 °C with shaking (90 rpm) followed for 18 hours. The MIC for each isolate was scored by direct visualisation. A negative control containing only broth was added and the experiments were run in triplicate.

6.7 Computation calculations using Spartan

The conformational energies of phenol vinyl-isocyanide 542 was run using Spartan’14 student edition (trial version) under the direction of Prof. Ian Williams.

The geometry of each isomer was fully optimised to a planar structure in an energy minimum with all real vibrational frequencies. Free energy corrections at 298.15 K and 1 atm are calculated using ideal gas, rigid-rotor and harmonic oscillator approximations and include zero-point energy and thermal partition-function contributions. Both the EDF2 and B3LYP density functionals have been employed with the 6-31G* basis for optimisations and vibrational frequencies and the6-311+G** basis set for geometries only. Most of the energy difference between the isomers came from the free-energy correction (zero-point energy, thermal energy and entropy) rather than from their intrinsic potential energies. At 25 °C an equilibrium mixture contains between 75% and 81% of the more stable trans isomer.
7 References

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