

P450–Mediated Azepane Natural Product Synthesis



A thesis submitted to the Board of the Faculty of the
Physical Sciences in partial fulfilment of the requirements
for the degree of Master of Science (by Research) of the
University of Oxford.

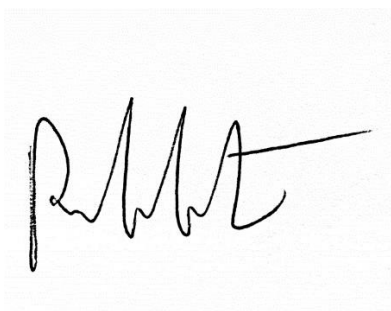
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Lincoln College
Trinity Term 2023

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Declaration

The work described in this thesis was carried out in the Chemistry Research Laboratory and the Inorganic Chemistry Laboratory, University of Oxford, from October 2021 until September 2023, under the supervision of Professor Jeremy Robertson. All the work is my own unless otherwise stated and has not been submitted previously for any other degree at this or any other university.

A handwritten signature in black ink, appearing to read 'VR', with a long horizontal stroke extending to the right.

Virgile Rouffeteau
September 2023

Abstract

Chapter 1 introduces methods and applications for C–H activation, cytochrome P450 enzymes and their uses in synthesis, and the relevant families of compounds in which the present work takes place.

Chapter 2 describes the strategy and the attempts of building the natural product balanol and analogues via C–H amination from enzymatically made substrate, along with new bicyclic lactones synthesised via C–H activation.

Chapter 3 presents progress in the synthesis of the carbon skeleton of the natural product cephalotaxine via an intramolecular Schmidt reaction, along with a significantly improved literature method for assembling the cephalotaxine core.

Chapter 4 explores the P450-catalysed oxidations of the previously made cephalotaxine core structure, to access new analogues of this natural product and to draw a P450-reactivity profile of this molecule.

Acknowledgements

First, I want to thank my supervisor Professor Jeremy Robertson for his patience, kindness and infinite chemistry knowledge. You helped me become a better research student and I am thankful for your guidance and what you've helped me achieve throughout this project.

I also want to thank Professor Luet Wong for welcoming me in his lab and helping make the most of my short time there.

My thanks would not be complete without mentioning all the kind people I met in both labs. Thank you, Alethea, for being there when I started and was lost in the lab, Mary and Jiarui for helping me when I couldn't complete my syntheses and was losing hope, Yi Ni for always being so joyful and energetic, Vicky for giving so much of your time for my project, always lifting me up, I owe you many of my results. I will not forget our time together.

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Kimberly, I cannot thank you enough for everything you did for me, and I would not be here without you. You brought smiles on my face when I couldn't, and I cannot wait to explore the world and our life together.

Abbreviations

% e.e.	Enantiomeric excess
1°	Primary
2°	Secondary
3°	Tertiary
Ac	Acetyl
AIBN	Azobisisobutyronitrile
ATP	Adenosine triphosphate
B ₂ Pin ₂	Bis(pinacolato)diboron
Bn	Benzyl
Boc	<i>tert</i> -Butyloxycarbonyl
Cbz	Benzyloxycarbonyl
CDI	Carbonyldiimidazole
c-MET	Hepatocyte growth factor receptor
COD	1,5-Cyclooctadiene
COSY	¹ H- ¹ H Correlation Spectroscopy
Δ	Heat
DBA	Tris(dibenzylideneacetone)
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	Dicyclohexylcarbodiimide
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone

DEAD	Diethyl azodicarboxylate
DEPAA	Diethylphosphonoacetic Acid
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DME	1,2-Dimethoxyethane
DMF	Dimethylformamide
DMP	Dess–Martin Periodinane
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dppf	1,1'-Bis(diphenylphosphino)ferrocene
EI	Electron Ionisation
Et	Ethyl
EtOAc	Ethyl acetate
FDA	Food and Drug Administration
GC	Gas chromatography
GDH	Glucose dehydrogenase
HMPA	Hexamethylphosphoramide
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
IPA	Isopropanol
IPTG	Isopropyl β -D-1-thiogalactopyranoside
KHMDS	Potassium bis(trimethylsilyl)amide
LC	Liquid chromatography
LDA	Lithium diisopropylamide
LHMDS	Lithium bis(trimethylsilyl)amide
LRMS	Low resolution mass spectrometry

M	Molar
<i>m</i> -CPBA	<i>meta</i> -Chloroperoxybenzoic acid
Me ₄ Phen	3,4,7,8-Tetramethyl-1,10-phenanthroline
Ms	Mesyl
MS	Mass spectrometry
n.d.	Not detected
NADP ⁺	Nicotinamide adenine dinucleotide phosphate
NBA	<i>N</i> -Bromoacetamide
NBS	<i>N</i> -Bromosuccinimide
NIS	<i>N</i> -Iodosuccinimide
NMR	Nuclear Magnetic Resonance
Oct	Octanoate
<i>o</i> -Tol	<i>ortho</i> -Tolyl
pA	Surface area
<i>p</i> -ABSA	<i>para</i> -Acetamidobenzenesulfonyl azide
PG	Protecting Group
PPA	Polyphosphoric acid
PTFE	Polytetrafluoroethylene
<i>p</i> -TsOH	<i>para</i> -Toluenesulfonic acid
Pyr, Py	pyridine
Quant.	Quantitative yield
R	Unspecified organic group
rpm	Revolutions per minute
RT	Room Temperature
R _t	Retention time (GC)
T ₃ P	Propylphosphonic anhydride

TBAF	Tetra- <i>n</i> -butylammonium fluoride
TBDPS	<i>tert</i> -Butyldiphenylsilyl
TBS	<i>tert</i> -Butyldimethylsilyl
<i>t</i> -Bu	<i>tert</i> -Butyl
TCCA	Trichloroisocyanuric acid
TCE	Trichloroethylene
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMS	Trimethylsilyl
Tpa	Tris(2-pyridylmethyl)amine
tRNA	Transfer ribonucleic acid
Ts	Tosyl (<i>para</i> -toluenesulfonyl)

Amino Acid Codes

Amino acid	Three letter code	One letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Variants' Name Code

Code	Mutations
GV	A74G/F87V
G-Q	A74V/F87(-)/L188Q
K19	H171LQ307H/N319Y
KSK19	F87A/H171L/Q307H/N319Y
RK	R47L/Y51F/F87A/H171L/Q307H/N319Y
KU3	N238H/I259V/A276T
RL YF	R47L/Y51F
R19	R47L/Y51F/H171L/Q307H/N319Y
RKA	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328I
RP	R47L/Y51F/I401P
RT2	R47L/Y51F/A191T/N239H/I259V/A276T/L353I
RK	R47L/Y51F/F87A/H171L/Q307H/N319Y

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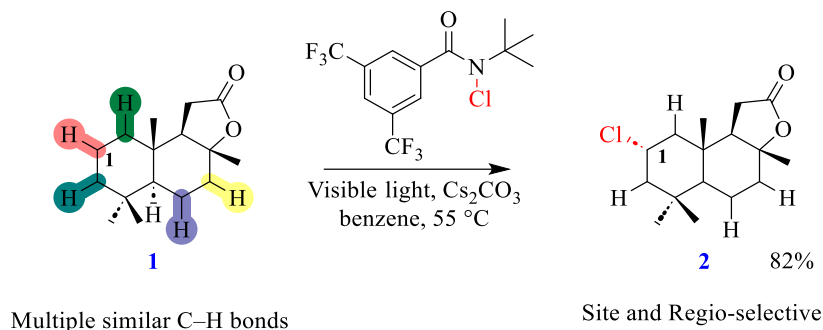
Chapter 1 – Introduction

“It is like a voyage of discovery into unknown lands, seeking not for new territory but for new knowledge. It should appeal to those with a good sense of adventure.” Frederick Sanger, 10th December 1980.

C–H activation

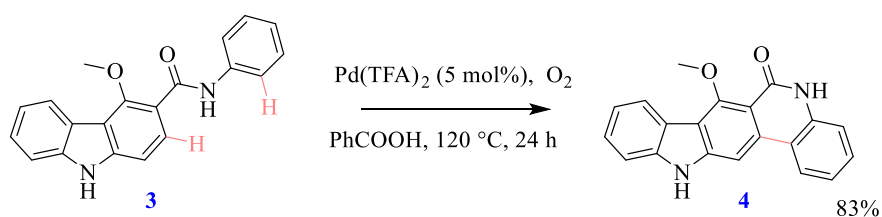
In the complex and intricate world of organic molecules, ranging from pharmaceuticals to fragrances to smart polymers, there is one near-constant: the Carbon-Hydrogen bond. Almost all organic compounds possess this feature. The fundamentals of organic chemistry reside in this hydrocarbon link. For the better part of the last century, the primary goal of organic research was directed towards functional groups, how to interchange them, and how to interact with them with high precision and, most important, high selectivity. This approach allowed for complex transformations and molecules to be achieved, but restricted the modifications to certain areas of the compound of interest. When removed from the vicinity of functional groups, the C–H bond is non-polar, and thus lacks reactivity, which explains the historical functional group approach to synthesis. In the past two decades, focus has shifted towards the goal of manipulating specific C–H bonds, in the presence of other C–H bonds and far from functional groups. Indeed, being able to modify a single hydrocarbon bond allows a great deal of flexibility in syntheses, whether in fragment building or in total synthesis. These types of chemical transformation (which multiply possible disconnections and allow new intermediates) facilitate the synthesis of new and pre-existing compounds.¹ A fitting example is the addition of a reactive handle (i.e., a functional group) into a previously unreactive region of a certain compound. The chlorination of sclareolide **1** (a common fragrance molecule) at C1 is key to a short synthesis of the intermediate **2** *en route* to the potent cytotoxin chlorolissoclimide,² even though C1 and its surroundings are devoid of any functional groups and present multiple similar C–H bonds

(**Scheme 1**). The base (Cs_2CO_3) promotes the amidyl radical, rather than the chlorine (promoted by oxygen or acid traces),³ and the former attacks the least hindered secondary C–H available in the substrate, thus the C1 position.⁴



Scheme 1 C–H activation in synthesis.

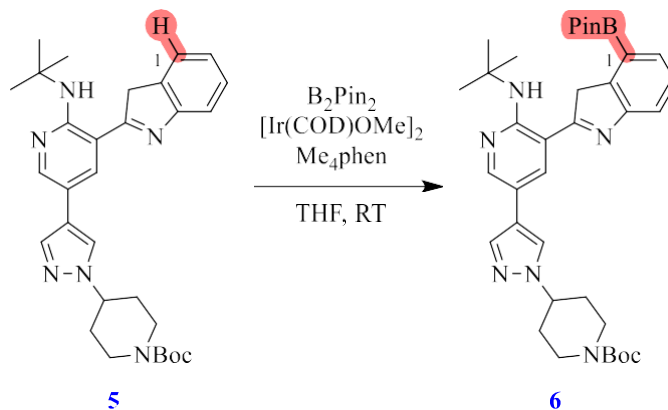
Within modern target synthesis, C–H activation can be more than just another step in the route, and is often the key or strategic step. Nagarajan's synthesis⁵ of the antimalarial calothrixins is built on a C–H activation step to create the core pentacycle **4** of this family of compounds (**Scheme 2**). A palladium catalyst in molten benzoic acid under oxygen achieved C–C bond formation by activating the two targeted $\text{C}(\text{sp}^2)\text{--H}$ bonds, leading to lactam **4**.



Scheme 2 C–H activation towards calothrixins.

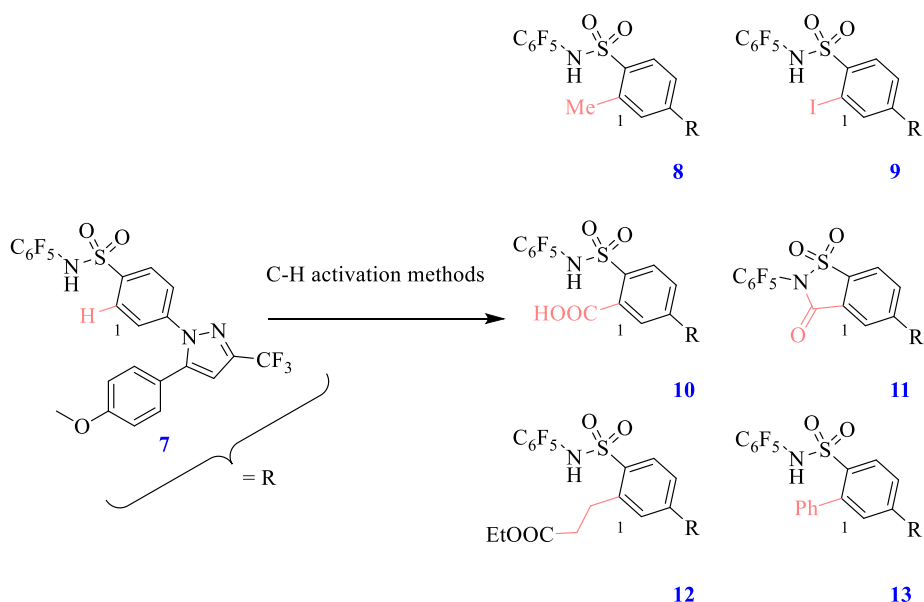
Another application where C–H activations have strategic value is in late-stage diversification or functionalisation. In the context of medicinal chemistry, this approach enables subtle changes and fine tuning of the target compound to enhance its pharmacokinetics,^{1, 6} allowing more efficient structure-activity studies without the need of additional syntheses. **Scheme 3** shows such an application; by modifying the penultimate product **5** before accessing the kinase inhibitor⁷ (the product being the deprotected analogue), Hartwig and Larsen were

able to afford several new products, from borane **6** after a successful C–H activation, all exhibiting high bioactivity,⁷ shortening structure-activity studies.



Scheme 3 C–H activation for late stage diversification of a c-MET kinase inhibitor.

To increase its activity as a COX2* inhibitor, FDA-approved celecoxib **7** was submitted to late-stage functionalisation at C1 to create six new potent bioactive compounds (**Scheme 4**, compounds **8** to **13**) in just a few additional steps.⁸



Scheme 4 C–H activation towards drug exploration.

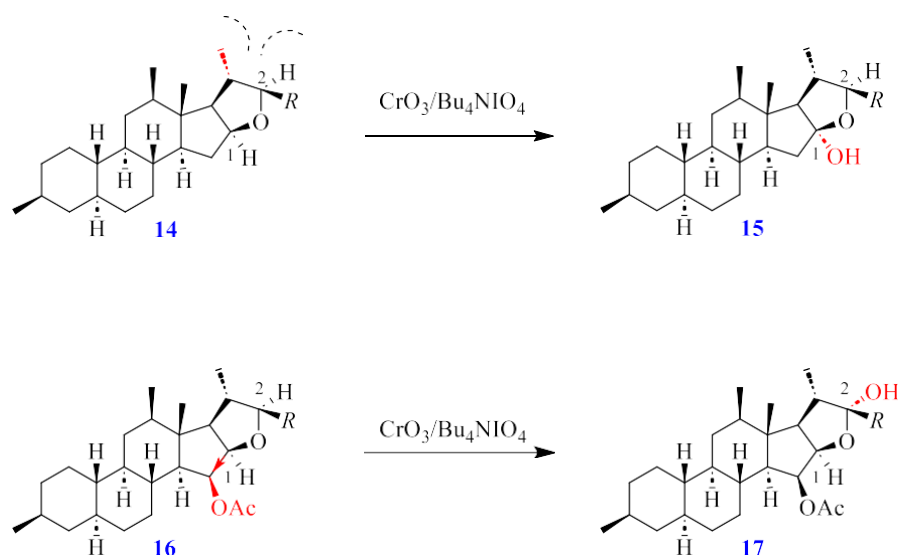
* Cyclooxygenase-2 (COX2) is an enzyme involved in inflammation.

Bond Differentiation

The examples above illustrate that these C–H transforming reactions can be potent tools for synthesis; however, chemists need them to also be precise. Often, the target bond is not alone in the molecule, and several nearby bonds might be similar to the targeted one in their polarity or steric environment. Thus, several criteria must be balanced to better predict the bonds' and reagents' behaviours and to achieve high selectivity.⁹

- **The type of bonds**, balancing the hyperconjugative bond-weakening effect and steric hindrance of the bonds. Generally, tertiary bonds are more reactive than secondary bonds, which are more reactive than primary bonds, with the exception of extreme crowding. This is due to the delocalisation of electrons from neighbouring bonds into the (C–H) σ^* , which increases the reactivity of the C–H bond (in cases where this bond is the nominal nucleophile).
- **Electron delocalisation** from the (C–H) σ into the adjacent σ^* and π^* orbitals.
- **Inductive effects**, when connected electron-withdrawing groups create subtle polarity differences between C–H bonds, even with remote groups.

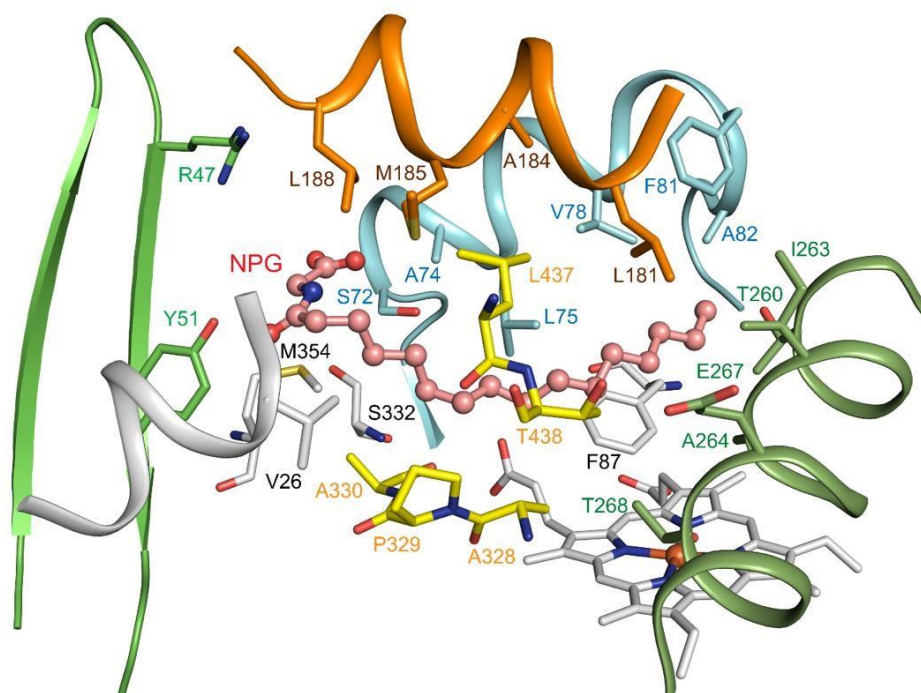
An example of the balance between these factors is in Fuchs' oxidation of the steroidal substrate shown in **Scheme 5**.¹⁰ Here, where the difference lies in steric hindrance (compound **14**, R being an alkane chain with an oxygenated group four carbons away), the less hindered bond is activated (C1 site); however, when a close electron-withdrawing group (in compound **16**, the EWG is acetoxy) deactivates the C–H bond by reducing its electron density, which renders it less reactive towards the electrophilic reagent, the activated C–H bond changes to the C2 site.¹⁰



Scheme 5 Bond differentiation in steroids.

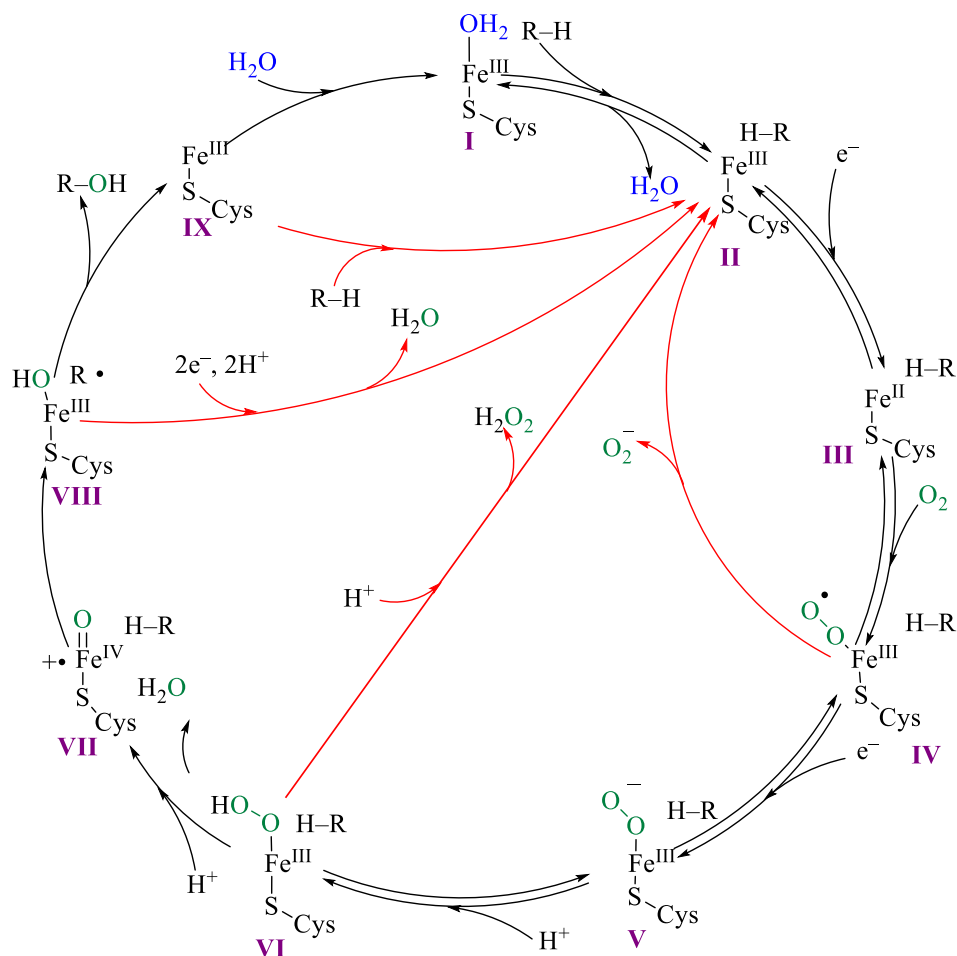
Cytochrome P450 Enzymes

The cytochrome P450 (CYP) superfamily of heme-containing enzymes¹¹ are primary monooxygenases (wherein dioxygen acts as the oxidant), oxidising fatty acids in their active site (as seen in **Scheme 6**, a wild type of P450 found in *Bacillus megaterium* with its natural substrate in its active site). They were first observed in 1958 by Klingenberg¹² who bubbled CO into rat liver microsomes, resulting in a coloured species with a spectrum presenting a maximum absorbance at 450 nm (hence their name, “Pigment-450”). Six years later, Omura and Sato¹³ deduced its belonging to the class of type-*b* hemoproteins (with a protoporphyrin IX ring structure). Then, four years after that, in 1968, Gunsalus¹⁴ identified these enzymes in the bacterium *Pseudomonas putida*.



Scheme 6 P450_{BM3} with its natural substrate at the active site with several residues.

P450s are evident in numerous and diverse organisms, but their non-implication in the primary “living” functions, such as assimilating energy or breathing,¹² made their role a mystery. While they are not responsible for primary tasks, they are key to the synthesis of complex organic molecules, such as steroids in mammals and herbicides in plants. They are also responsible for processing xenobiotics, breaking down these foreign organic compounds by oxidising their carbon skeleton, rendering them treatable by the liver and kidneys. Their evolutionary origins may lie in the surge in oxygen concentration that occurred during the Great Oxidation Event¹⁵ as they mainly catalyse oxidations¹⁶ of C–H bonds (in particular hydroxylation) using molecular dioxygen via the catalytic cycle summarised in **Scheme 7**. The focus in this thesis is on CYP102A1 – P450_{BM3} – the third P450 discovered by Fulco¹⁷ from *Bacillus megaterium* a few years after Klingenberg. This enzyme catalyses the hydroxylation of fatty acids with only NADPH and oxygen,^{17,18} making it catalytically self-sufficient and, therefore, of great research interest.



Scheme 7 P450 catalytic cycle.

The resting configuration of the enzyme is state **I**, with water as an axial ligand. Upon migration of the organic substrate ($R-H$) into the active site, water is expelled and replaced by the substrate. This change affects the ligand symmetry surrounding the iron atom, resulting in a transition to a high-spin electronic configuration, increasing the ionic radius, which modifies the active site leading to state **II**.

With the substrate in place, the iron is readily reduced by electron-transfer to form the complex **III**.

Molecular oxygen is then trapped by this Fe^{II} complex, forming radical **IV**. This intermediate can lose superoxide ion, reverting to state **II**.

Reduction of **IV** produces anion **V**, this step usually being rate-determining in wild-type

enzymes.

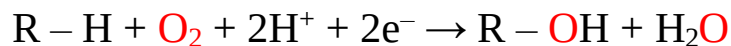
Species **V** is protonated leading to hydroperoxy intermediate **VI**. The side-reaction of loss of hydrogen peroxide can also regenerate state **II**.

Protonation and elimination of water leads to the iron-oxo (ferryl) intermediate **VII**.

This key species is thought to be responsible for the defining reaction with the bound substrate, abstracting a hydrogen atom, creating state **VIII** and organic radical R[•]. Intermediate **VII** can also revert to state **II** by reduction with release of water.

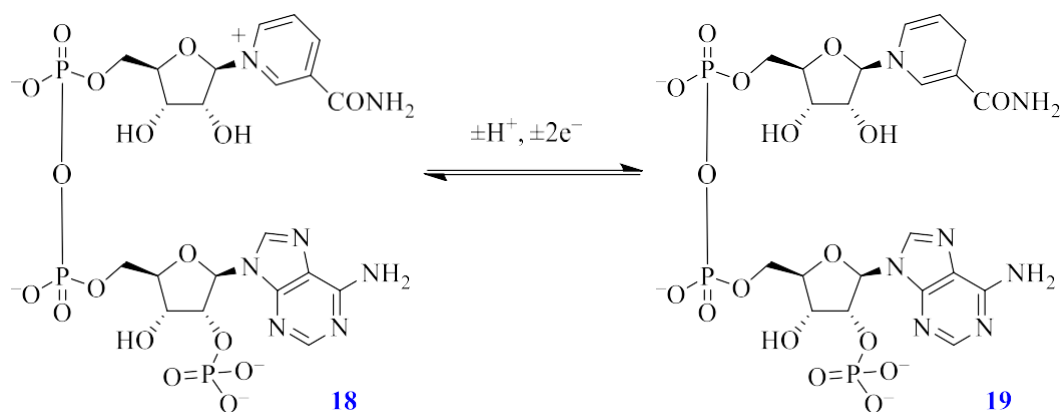
The new organic radical then reacts rapidly with the newly-formed iron-hydroxyl group in a “rebound” manner, departing from the active site as an alcohol leaving behind an Fe^{III} species, state **IX**. This quickly comes back to its resting state **I** with the addition of water, or into state **II** if another substrate molecule is present in the active site.

The overall catalytic cycle can be elegantly summarised as:



As external monooxygenases, P450s are unable to produce the required electrons themselves, and so require cofactors, in this case NADPH and NADP⁺ (**Scheme 8**) which shuttle, overall, a molecule of hydrogen from an added reductant, see below.

In most experiments, due to the high cost of NADP⁺, it is added as a catalyst (0.1% for example), being reformed by the action of GDH (glucose dehydrogenase, an enzyme) fuelled by glucose. GDH catalyses the transformation of glucose into gluconolactone, with an overall transfer of hydrogen into the cycle, carried by NADP⁺/NADPH. The gluconolactone is subsequently hydrolysed to gluconic acid in the mildly basic reaction medium, driving the forward reaction (**Scheme 9**).



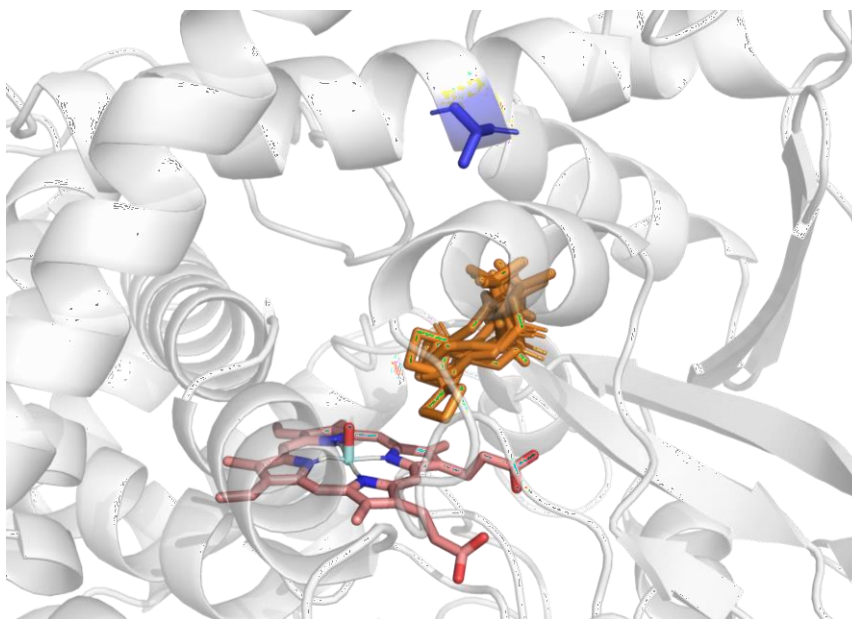
Scheme 8 NADP⁺/NADPH equilibrium.

Engineering P450

Even with valuable great activity, the reactivity of natural P450s (i.e., wild type enzymes) can be difficult to control, and the hydroxylation of complex substrates does not always take place at the desired site;¹⁹ however, the 3D rendition of the enzyme (**Scheme 6**) shows the key to influencing this: once in the active site, only those C–H bonds which approach the ferryl oxygen can be hydroxylated. Thus, modifying the active site to steer the substrate into different positions should result in different metabolites. The question then arises: is it possible to modify the active site, and, if so, how?

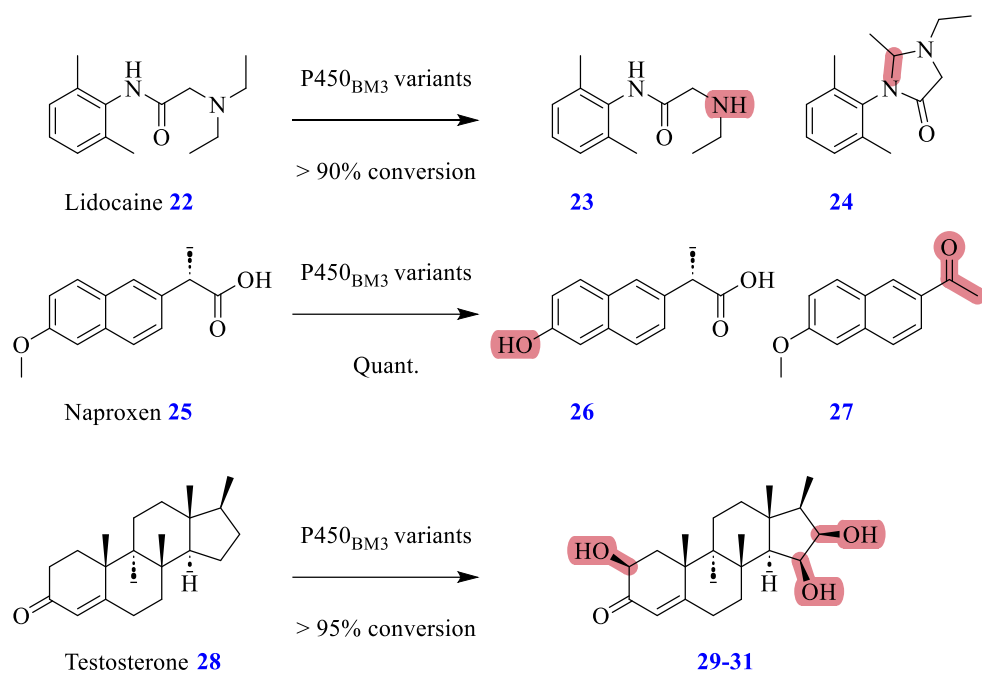
As enzymes, P450s comprise a chain of amino-acids, produced according to the instructions provided by the genes held within the bacterial DNA. Thus, at a high level, mutating this DNA will result in a different combination of amino-acids, modifying the active site. To circumvent this randomness, techniques have been developed, such as site-directed mutagenesis (SDM) and docking technologies.¹⁸ SDM can act at any residue, and remote mutations (relative to the active site) may influence the outcome by allosteric regulation, whereas docking-guided mutagenesis focuses on the active site, directly influencing its geometry and spatial structure.²⁰ As an example, the first reported hydroxylations of non-natural substrates 1,1,2,2-tetrachloroethane²¹ and 2-phenylpropanal²² with mutated P450 targeted alanine-328, threonine-268, and phenylalanine-14. A shorthand summary of mutations at a specific residue is presented as wild-type residue | position | new residue. Accordingly, replacing a phenylalanine at position 87 with an alanine is coded F87A; replacing serine-264 by isoleucine is coded S264I, and so on. In the Wong group's early P450 engineering studies, to accommodate bulkier substrates, such as polyaromatic hydrocarbons, two residues close to the iron centre were replaced with smaller ones (F87A and A264G) to create more space, and two more (R47L and Y51F) at the mouth of the access channel were introduced to cancel a competitive binding site.²³

Subsequently, libraries of engineered P450s were created by this approach, and the accepted substrates became more and more diverse.^{24–26} Leaving the domain of linear alkanes, drugs, such as naproxen, lidocaine and even testosterone, were subjected to the action of P450s mutants.^{27,28} Following iterative rounds of screening, docking (typical simulations **Scheme 10**), and mutagenesis – to identify and produce effective variants – these transformations can surpass 90% conversion, making them powerful catalysts for synthesis, even with catalyst loading of 0.01 mol%.²⁰

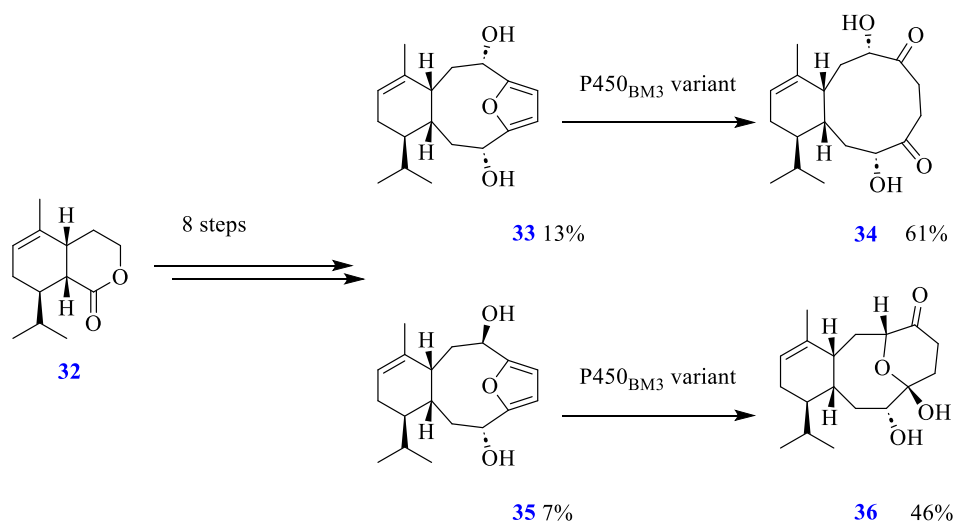


Scheme 10 Example of docking simulations by Atkinson.²⁰
Orange structure: productive poses of a specific substrate within a specific P450_{BM3} variant.

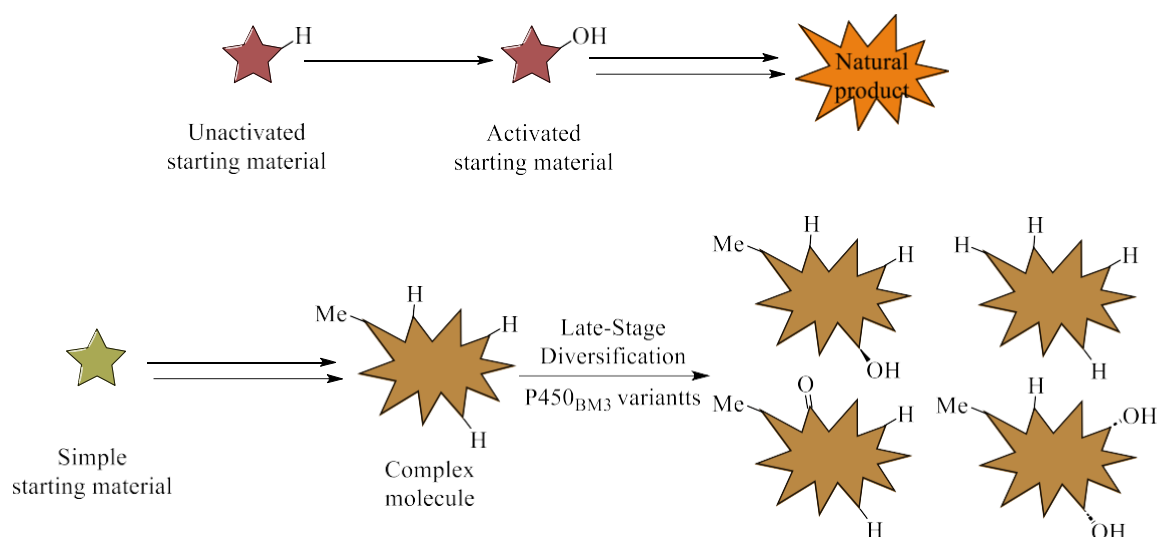
As visible in **Scheme 11**, P450-mediated hydroxylation, combined with secondary processes, can create multiple functional groups. Thus, it was shown that such reactions could lead to dealkylation (with lidocaine **22**) and elimination (with naproxen **25**, of CO₂). In general, by installing hydroxyl groups, elimination, carbonyl formation, ring-opening and closing, and diol formation (etc.) can be triggered.^{16,29} By harnessing these abilities – i.e., by choosing the correct sequence of amino acids – the synthesis of complex natural products (**Scheme 12**) and drug targets can be made more accessible,^{30,31} including those of industrial relevance.^{32,33} With the abilities and opportunities that engineered P450 variants provide, new ways to approach complex synthesis open up. This idea, either as a starting point or a method for late-stage diversification, is central to the work proposed at the outset of this project and discussed in this thesis (**Scheme 13**).



Scheme 11 P450 activity on pharmaceuticals.



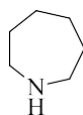
Scheme 12 Synthesis and P450 hydroxylation of eleuthoside intermediates.



Scheme 13 P450 uses in synthesis.

Azepane Rings

The general term azepane is applied to the class of saturated seven-membered heterocycles comprising six carbons and one nitrogen, the parent member of this class being azepane itself (**Scheme 14**).

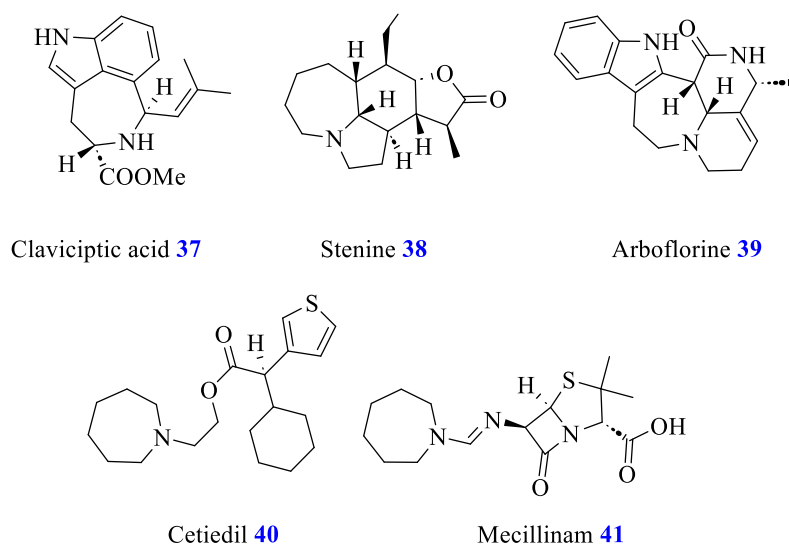


Scheme 14 The azepane motif.

This flexible ring is found within numerous natural products, all by definition alkaloids, such as clavicipitic acid **37**, stanine **38**, arboflorine **39**, etc (**Scheme 15**).³⁴

They are also part of many pharmaceuticals,³⁵ and compounds with substituted azepanes demonstrate a wide array of bioactivity – including glycosidase inhibition, anti-diabetes, anti-cancer, anti-viral, and antihistamine activity, against Alzheimer's disease, as serotonin receptor agonists³⁶ – and occur in new potent drugs, some of which are already FDA-approved: Cetiedil **40**, a vasodilator, or Mecillinam **41**, an antidiabetic (**Scheme 15**).³⁴ From the synthetic chemist's perspective, the azepane ring is not usually a starting point, due to either the low reactivity of

the bare azepane or the prohibitive cost of pre-functionalised azepane-containing compounds. Thus, strategies for preparing such molecules usually include an azepane-forming step, such as ring-closing metathesis, ring-expansion, halo-cyclisation, intramolecular reductive amination.³⁷ The epitome of this issue can be found in the multiple synthetic pathways leading to balanol **42** (**Scheme 16**), as discussed in the following section.



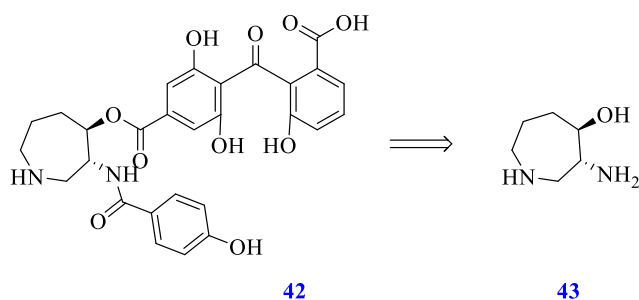
Scheme 15 Azepane-containing natural products and drugs.

Balanol

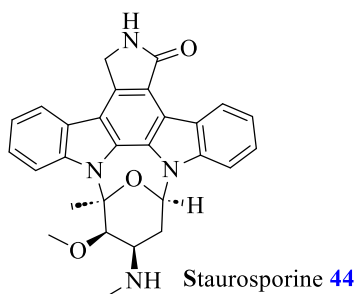
Isolation and Bioactivity

Balanol (**42**, **Scheme 16**) was isolated in 1993 by the Jiang team,³⁸ from the endoparasitic fungus *Verticillium balanoides*. This species grows on *Pinus palustris*, more widely known as the longleaf pine, native to the southeastern United States. Balanol was first identified in a search for new protein kinase C (PKC) inhibitors since this kinase, along with PKA, plays a key role in cell growth, differentiation, and overall activity, with their overexpression being associated with signal transduction diseases, such as cancer and central nervous system disorders.³⁹ Balanol was found to inhibit these proteins at nanomolar concentrations by preventing them from using ATP.⁴⁰ Balanol is a congener of ATP (in this

case the relationship lies in their similar activity), and its inhibitory activity is of a similar order to that of the bis-indole staurosporine⁴¹ (**44**, **Scheme 17**), itself isolated in 1977⁴² and synthesised in 1995.⁴³

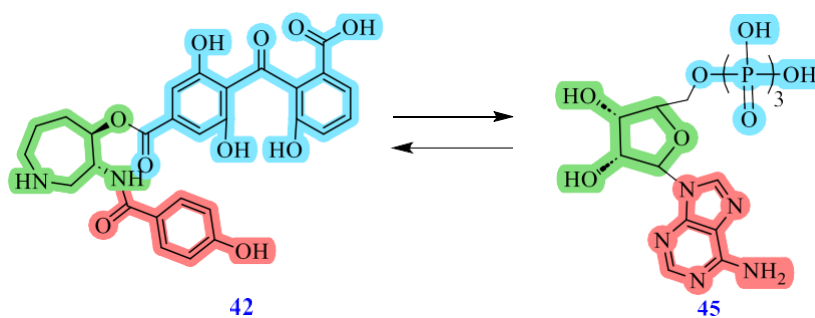


Scheme 16 Balanol and its key 1,2-aminoalcohol azepane core **43**.



Scheme 17 Protein kinase inhibitor staurosporine.

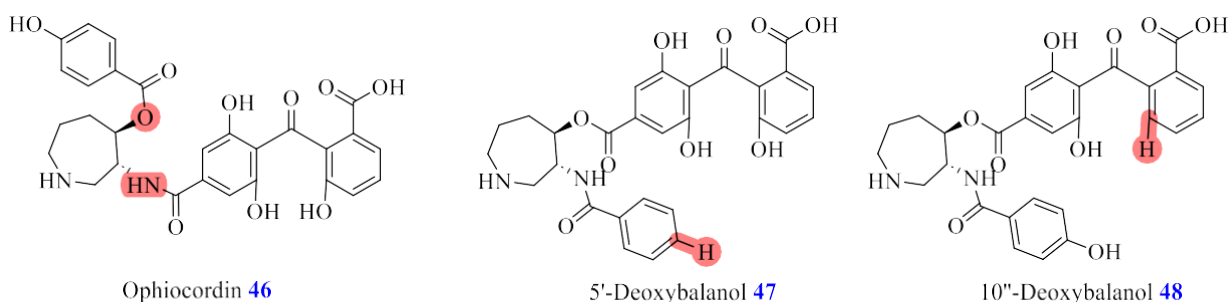
The *p*-hydroxybenzamide moiety of balanol is proposed to correspond to ATP's adenine, the azepane to its ribose, and finally the complex benzophenone subsituent to its triphosphate (**Scheme 18**).⁴⁰ Each moiety is able to form the same bonds as their ATP counterpart does with the kinases.⁴⁰ The conformational mobility of the azepane ring and the benzophenone moiety is key to its selectivity, allowing it to fold into many protein microenvironments.⁴⁴



Scheme 18 Balanol–AITP resemblances.

Analogue

Several balanol analogues have been identified and prepared since the original report of its isolation and identification. For example, ophiocordin **46** (Scheme 19) is a balanol regioisomer in which the nitrogen and oxygen acyl substituents are swapped; this secondary metabolite was found in the fungus *Cordyceps ophioglossoides* and shown to exhibit antifungal activity.⁴⁴ Balanol is slightly more active towards PKC than PKA [binding constants (nM) towards PKA/PKC: 4.7/5.3], but its 5'-deoxy variant **47** switches selectivity strongly towards PKA (3.5/69), and its 10''-deoxy counterpart **48** even more so (3.9/640).⁴⁵



Scheme 19 Balanol analogues.

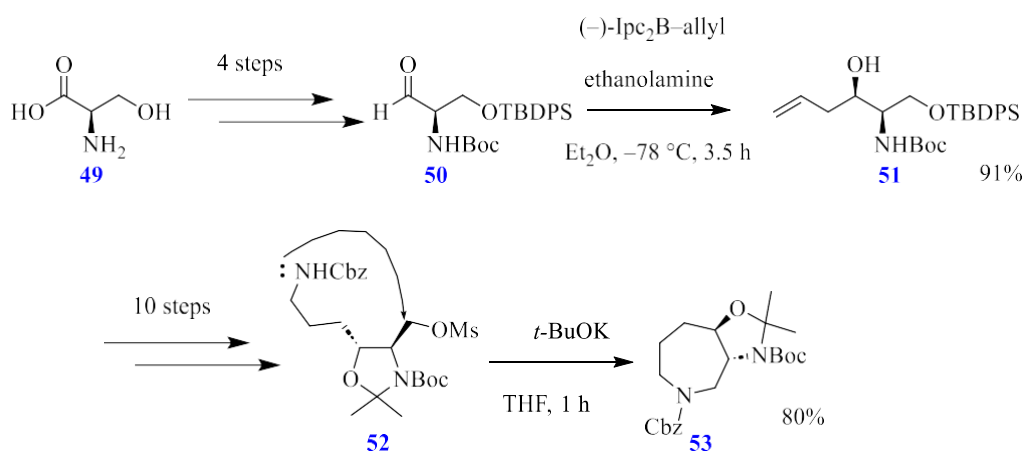
Previous Syntheses

Owing to its high activity and selectivity, the synthesis of balanol soon became, and remains to this day, a hot topic, with the first total synthesis, by Nicolaou, being published the year following the report of its isolation and structure.⁴⁶ Nicolaou's retrosynthetic analysis

highlighted the key problem within the balanol synthesis: how to afford the core hexahydroazepine **43**, the azepane amino-alcohol? Their strategy, outlined below in **Scheme 20**, revolved around a 7-*exo-tet* cyclisation, leading to the key intermediate **53** in 16 steps and 36% overall yield (32 steps and 4.3% yield for the total synthesis). Since then, many other groups have attempted shorter routes with new disconnections. Focusing solely on the azepane core structure, it requires an average of 8.6 steps to be formed (over the 32 published attempts).⁴⁷ The principal routes to this key azepane are detailed in **Schemes 20–25**, and illustrate the different main approaches: nucleophilic substitution (**A**), reductive amination (**B**), ring-closing metathesis (**C**), radical cyclisation (**D**), and ring expansion (**E**), with representative examples chosen from the literature.

A, Nicolaou et al. (1994)

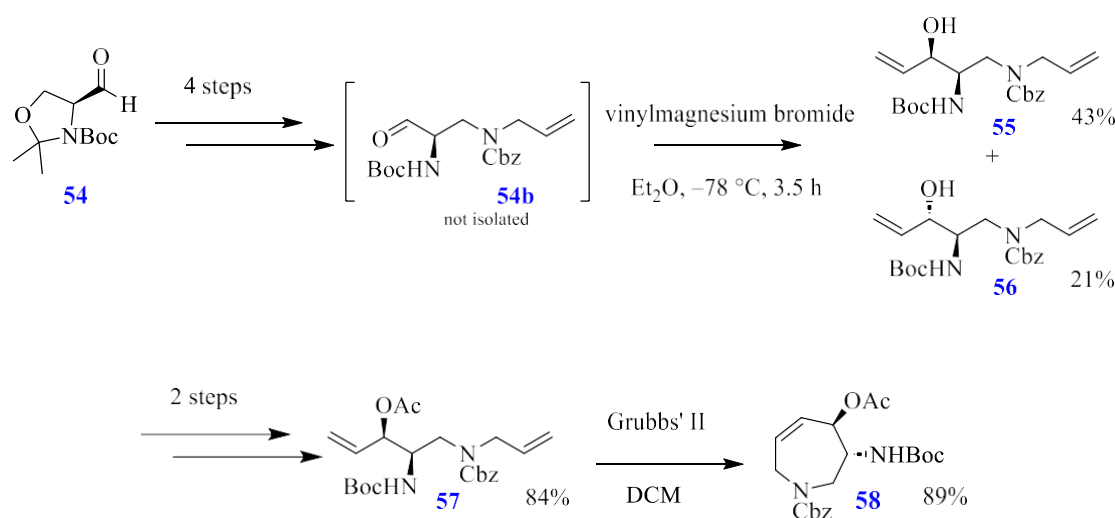
The Nicolaou strategy⁴⁶ relies on an efficient nucleophilic substitution, a 7-*exo-tet* cyclisation of **52**, between a Cbz-protected amine and a mesylate. In this route, the stereochemical configuration of the 1,2-amino-alcohol is set a few steps earlier, first by the chiral pool starting material, D-serine **49**, then by a stereoselective allylation of aldehyde **50** by Brown's diisopinocampheylborane reagent to afford the desired 1,2-*syn*-configured intermediate **51** (**Scheme 20**).



Scheme 20 Summary of Nicolaou's route to the balanol azepane core.

B, Chattopadhyay et al. (2013)

Another approach,⁴⁸ reported by Chattopadhyay in 2013 (**Scheme 21**), put to use ring-closing metathesis, developed by Villemin in 1980,⁴⁹ and later popularised with the discovery of stable well-defined metathesis catalysts by Grubbs, Schrock, Chauvin, and their groups.⁵⁰ Starting from another chiral pool starting material, Garner's aldehyde **54**, addition of vinylmagnesium bromide to derived aldehyde **54b** gave a 2:1 mixture of *syn* and *anti* isomers (**55/56**). The undesired *anti* isomer **56** was converted into its *syn* isomer **55** via a Mitsunobu-type inversion. With the chiral diene obtained and protected, ring-closing metathesis with the second-generation Grubbs' catalyst afforded the unsaturated azepane **58**. Subsequent deprotection, functionalisation, and hydrogenation gave the target amide **67**, see below.

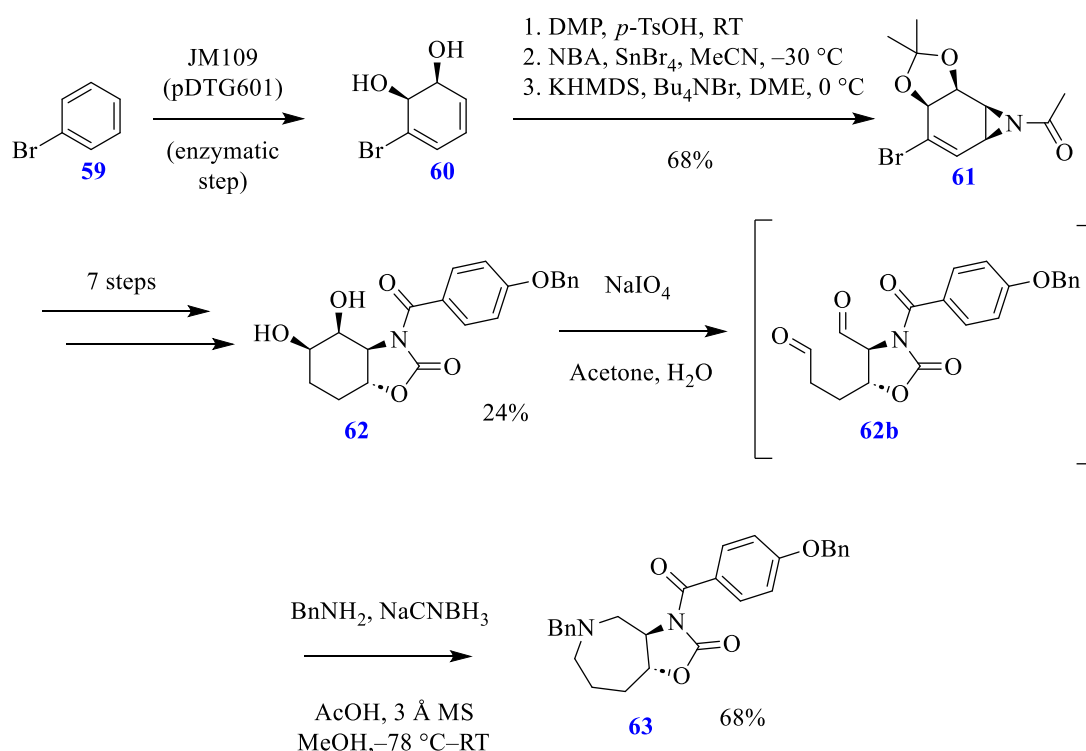


Scheme 21 Summary of Chattopadhyay's route to balanol azepane core.

C, Hudlicky et al. (2008)

The route developed by Hudlicky in 2008⁵¹ formed the heterocycle via sequential oxidative diol cleavage and reductive amination of the resulting dialdehyde (**Scheme 22**). The route started from diol **60**, itself obtained from bromobenzene **59** by enzymatic dihydroxylation.^{52,53} The β -configuration of the diol (as the acetonide) directs initial bromonium

formation to the bicyclic molecule's *exo* face, requiring the nitrogen nucleophile to attack from above (~~the more hindered endo face~~) which, after ejecting the bromide under basic conditions, leads to the observed configuration in the product, **61**. This aziridine was then opened with inversion by acetic acid, with substitution occurring preferentially at the activated allylic position. The released diol in intermediate **62** was then cleaved by NaIO₄ to give dialdehyde **62b**, which was then directly treated with benzylamine to form the azepane core **63** after reduction *in situ*.

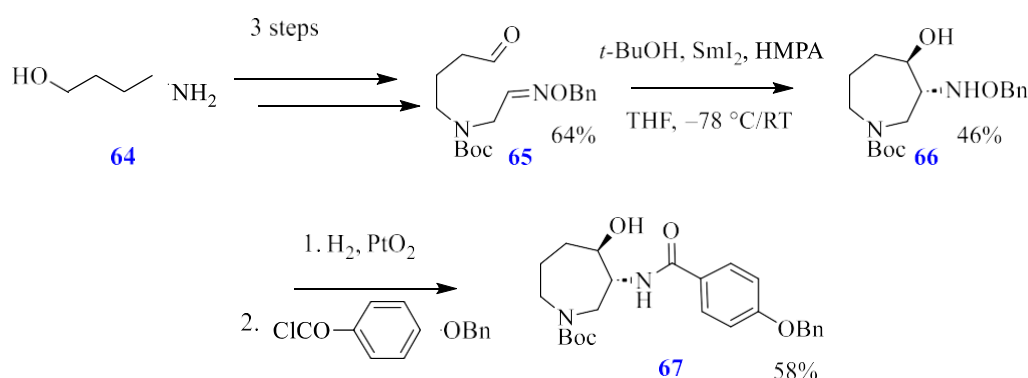


Scheme 22 Summary of Hudlicky's route to balanol azepane core.

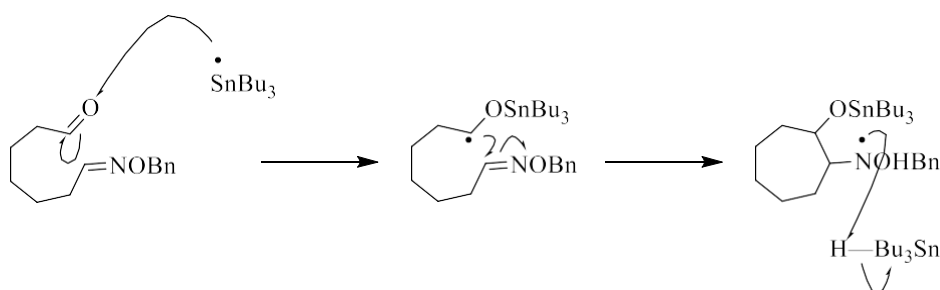
D, Naito et al. (1998)

The route reported by Naito in 1998, differentiating itself from others as a racemic route, started with 4-aminobutanol **64**,⁵⁴ from which the derived aldehyde-aldoxime **65** was to be cyclised under homolytic conditions (**Scheme 23**). Initial trials involved the well-known couple Bu₃SnH and AIBN, following the outline mechanism presented in **Scheme 24** ; however, this gave mediocre yields at best and low selectivity for the *trans* diastereomer **66** ($\leq 1:3$). Instead,

the action of SmI_2 in *tert*-butanol and HMPA,^{55–58} added to increase the reagent's reactivity, yielded the target 1,2-aminoalcohol **66** in 46% yield, with only 7% of the *cis* isomer. This *anti* stereoselectivity was proposed to arise from a combination of electronic (minimising dipolar repulsion) and steric (minimising torsional interactions) factors in the transition state as the $\text{Sm}^{\text{III}}(\text{HMPA})_n$ -chelated ketyl radical approaches the aldoxime π -system. The resulting hydroxylamine group in **66** was then hydrogenated and *N*-acylated to yield the target amide **67**.



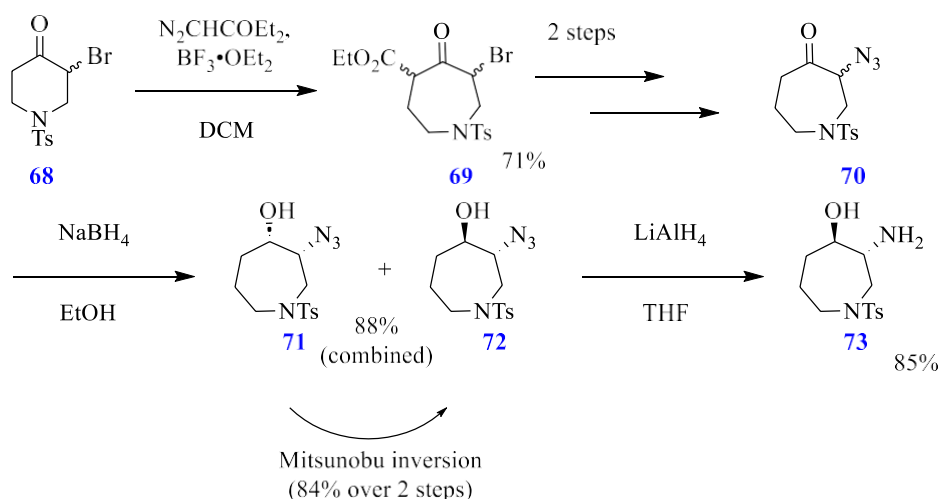
Scheme 23 Summary of Naito's route to balanol's azepane core.



Scheme 24 Proposed mechanism of the initial radical cyclisation.

E, Warner *et al.* (1995)

Warner developed a fifth method in 1995,⁵⁹ starting with the ring expansion of bromopiperidinone **68** (**Scheme 25**) by Lewis acid-mediated addition of ethyl diazoacetate. The bromide was then displaced by azide and the ketone reduced, resulting in both *cis* and *trans* diastereomers **71** and **72**, respectively. Mitsunobu inversion of the *cis* alcohol **71** quickly corrected this lack of stereoselectivity, and the racemic azepane core **73** was completed.

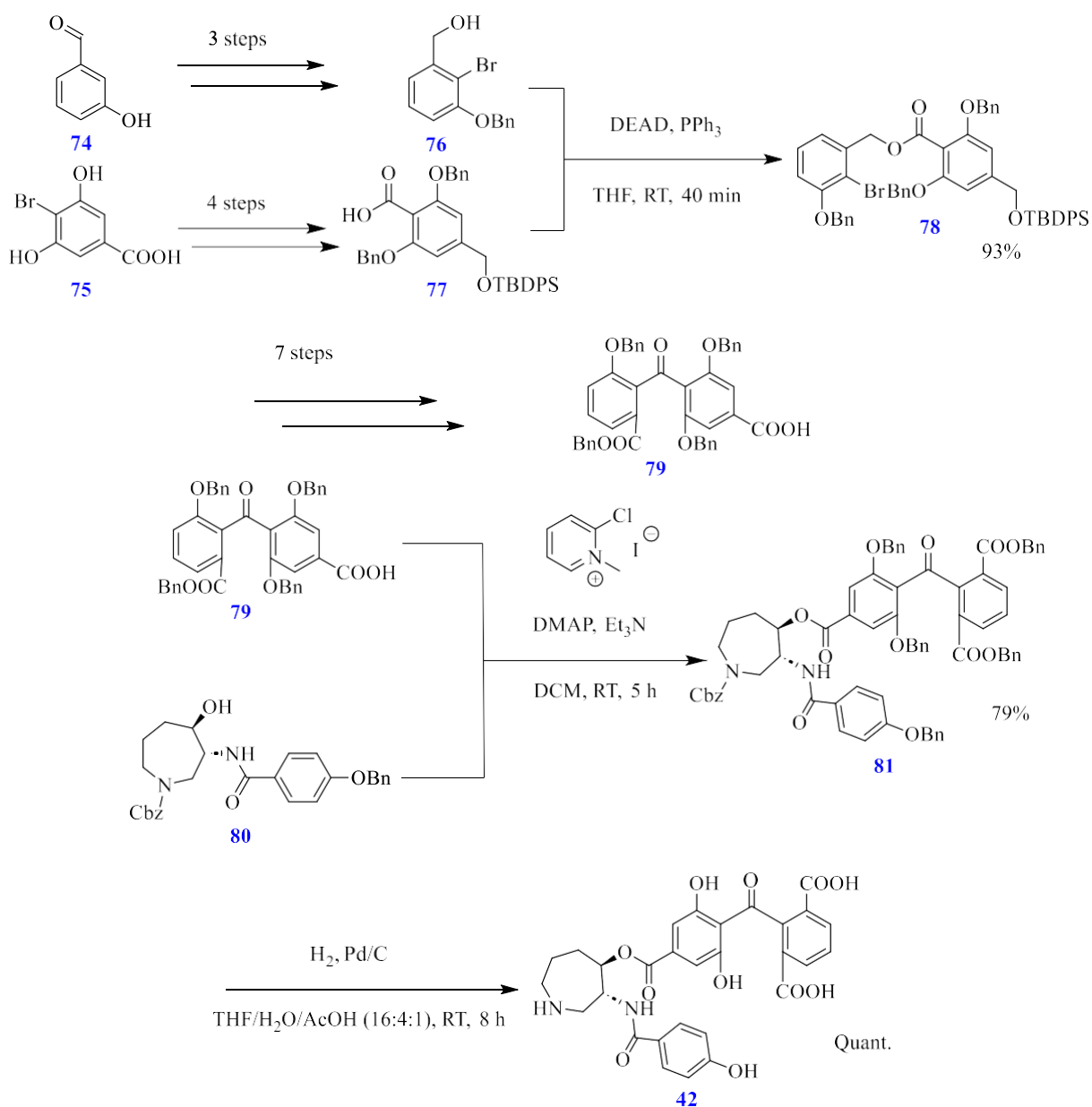


Scheme 25 Summary of Warner's route to balanol azepane core.

Completion of the total synthesis

With the azepane core available, the remaining steps required to complete the total synthesis were straightforward, with no general divergence from the Nicolaou route.⁴⁶ In the various syntheses, the amide acyl substituent is always the first moiety to be installed, often achieved prior to forming the core. The benzophenone part requires more steps, originating from hydroxybenzaldehyde **74** and α -resorcylic acid derivative **75**, combined after appropriate elaboration. From the ester **78**, further modifications including acyl transfer led to the carboxylic acid component of the ester in balanol. Final Mukaiyama esterification and global deprotection afford the natural product **42** (**Scheme 26**).

All these routes have in common the functionalisation of the molecule before the azepane ring is created; this project aimed to propose a new paradigm in the balanol synthesis: forming the 1,2-aminoalcohol functionality on a bare azepane template. Efforts towards realising this strategy are detailed in **Chapter 2**.

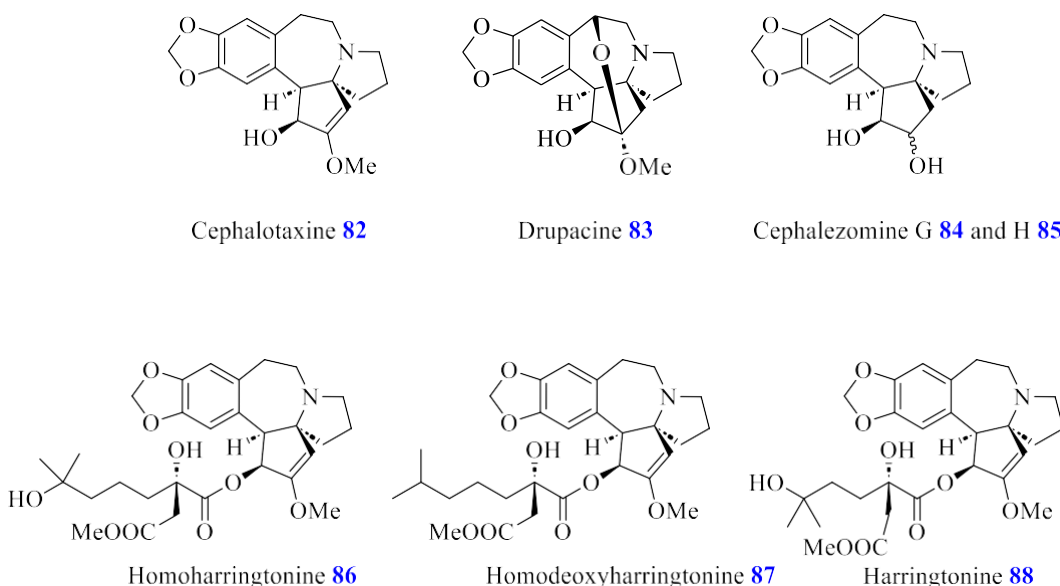


Scheme 26 Usual strategy for the synthesis of balanol's branches.

Cephalotaxine

History

Cephalotaxine **82** (**Scheme 27**) is the first member of a class of alkaloids with promising bioactivity extracted from the genus *Cephalotaxus*, the plum yew family endemic to Asia, which is commonly used in traditional medicine.⁶⁰ The alkaloid was isolated in 1963 by the Paudler team,⁶¹ fully identified by Powell in 1969,⁶² and first synthesised in 1972 by Weinreb and Auerbach.⁶³ To date, about 70 molecules of this class have been reported.^{60, 64}

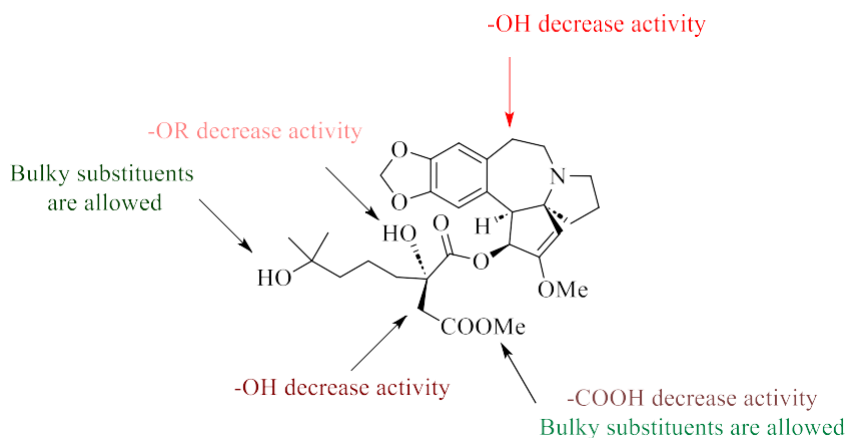


Scheme 27 Compounds of the cephalotaxine family.

Bioactivity

These alkaloids are mainly known to be anti-leukemic.⁶⁵ One of them, homoharringtonine **86** (HHT), is FDA-approved⁶⁶ and commercialised under the name omacetaxine mepesuccinate. To be noted is the reported lack of any bioactivity of cephalotaxine **82** itself, whereas its ester, the above-mentioned HHT, is recommended for the treatment of chronic myeloid leukaemia resistant to tyrosine kinase.⁶⁷ Thus, the ester side chain is key to its bioactivity, and indeed, many studies on this alkaloid class's bioactivity have focused on

modifying the side chain to optimise its behaviour *in vivo*.⁶⁸ The key structure-activity features of HHT are summarised in **Scheme 28**.⁶⁹



Scheme 28 Structure-activity summary of homoharringtonine **86**.

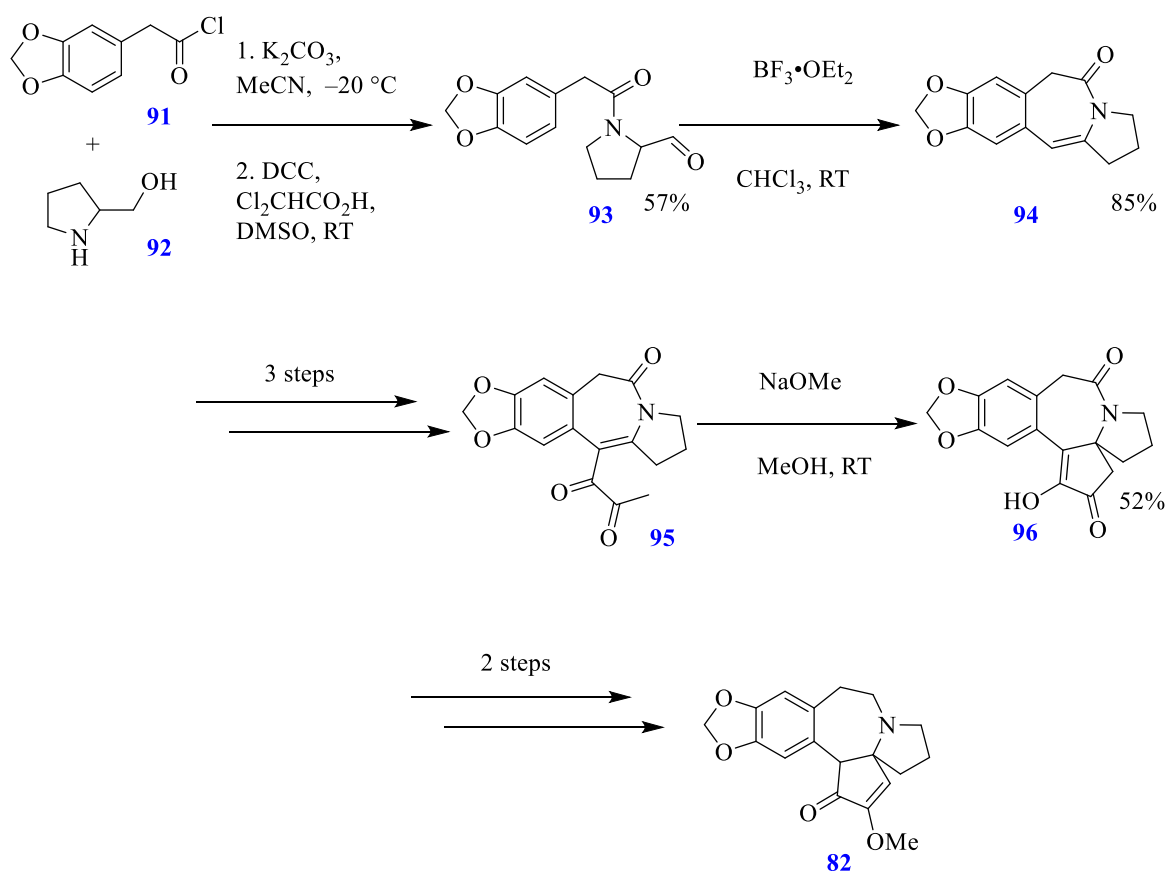
These studies led to a broad understanding of its mechanism of action as an inhibitor of protein translation through binding to the ribosomal A-site, preventing protein synthesis at the first step. In particular, HHT binds to the site via its ester side chain, while the bulky pentacycle blocks any interaction between the ribosome and tRNAs, effectively killing the targeted cell (explanatory Schemes: page 355 of reference 70).

Previous Syntheses

Since their identification, this class of molecules has been at the centre of the synthetic chemistry community's interest, and the number of syntheses bloomed, with now more than 100 routes to various analogues of the cephalotaxine class (over 150 results on Web of Science with the key words "cephalotaxine synthesis", and over 70 for "cephalotaxine total synthesis").^{64,73} Many different strategies have been devised over the years but since the cephalotaxine carbon skeleton is always the objective or the main key intermediate, attention will be on cephalotaxine itself.

Weinreb et al. (1972)

The first synthesis,⁶³ completed by Weinreb and Auerbach in 1972, can be summarised in two key steps (**Scheme 29**). First, azepane **94** was formed by intramolecular Friedel–Crafts alkylation and dehydration within amide **93**. Second, the cyclopentane ring was closed via formal intramolecular Michael addition, then rearrangement and methylation produced the alkaloid **82**.

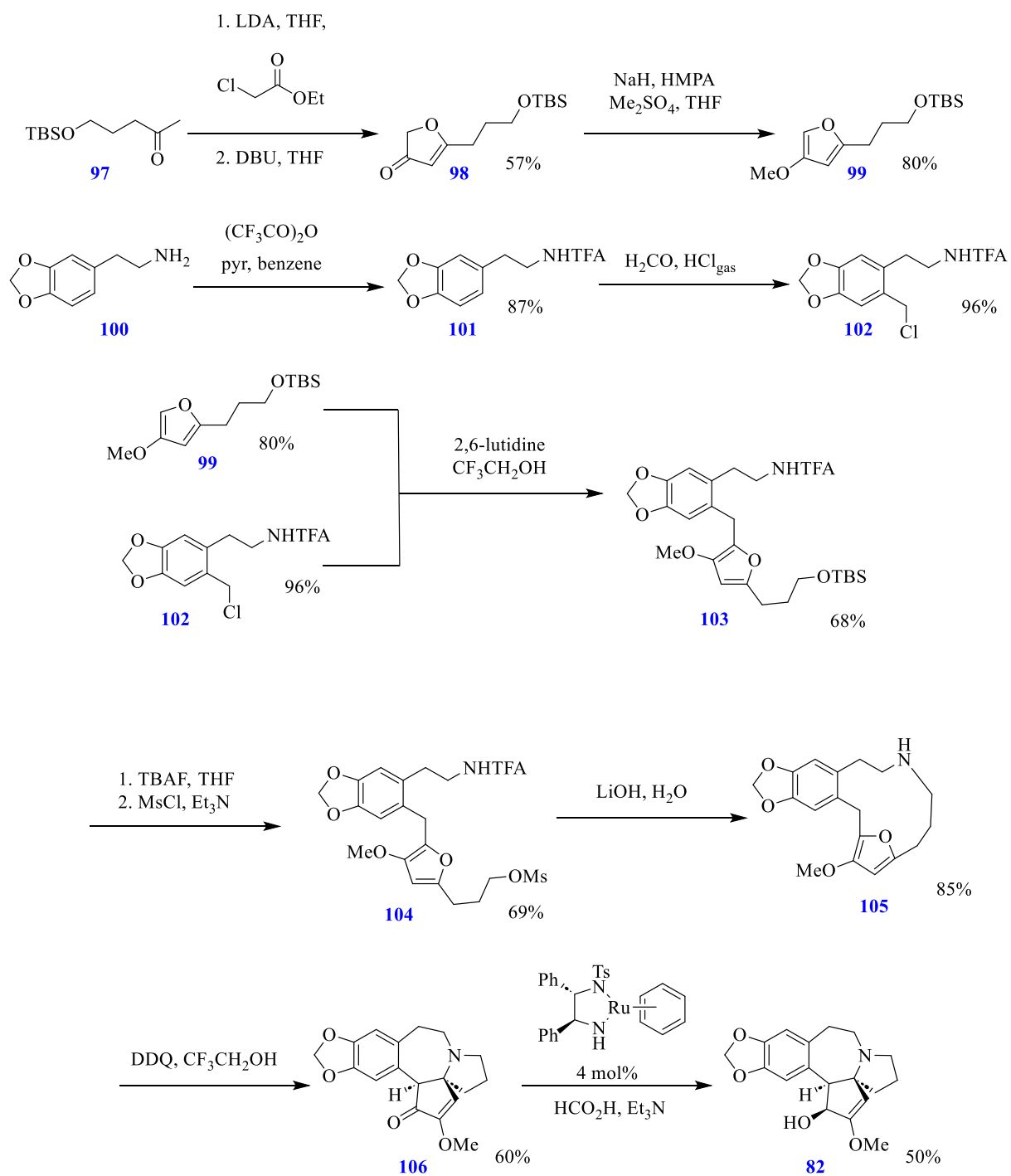


Scheme 29 Summary of Weinreb's route towards cephalotaxine **82**.

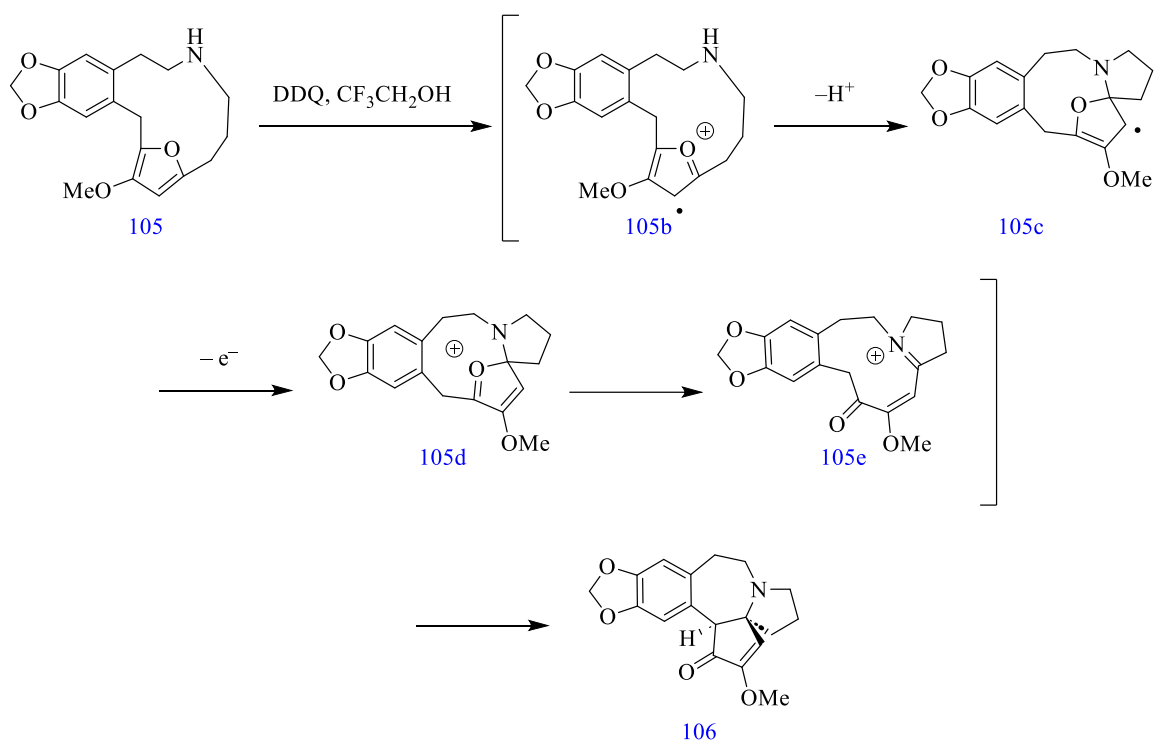
Rather than detailing every reported strategy, the discussion below focuses on three additional approaches: the most efficient one to date,⁷⁴ and the two devised by Mori⁷⁵ and Hanaoka,⁷⁶ as they present two intermediates, namely Mori's **110** and Hanaoka's **120**, commonly-used key intermediates among other routes.⁶⁰

To date, the shortest route – at just nine steps – was developed by Beaudry in 2019⁷⁴ using a furan oxidation-transannular Mannich cyclisation (**Scheme 30**). The route started with the synthesis of furanone **98** from commercially available ketone **97** via a Claisen-type condensation and *O*-cyclisation. Furan **99** was readily prepared then alkylation with chloride **102** (from amine **100** in two steps) gave trisubstituted furan **103**. Cyclisation was achieved after activation of the primary alcohol, and macrocycle **105** was converted into (–)-cephalotaxinone **106** by the action of DDQ in trifluoroethanol, via a radical rearrangement and furan ring-opening. The latter compound was then converted into the desired (–)-cephalotaxine **82** by Noyori reduction, with 97% e.e.⁷⁷ The unreacted (+)-enantiomer of **106** was racemized into (±)-**106** following the Mori/Stoltz conditions.⁷⁴

The mechanism proposed for the crucial transannular reaction linking **105** and **106** is shown in **Scheme 31** and involves the single-electron oxidation of macrocycle **105** into radical cation **105b**. Transannular trapping of this radical cation by the tethered amine affords **105c** which, after a second oxidation, releases cation **105d**, rapidly transformed into cation **105e**. The latter finally undergoes Mannich cyclisation to produce the desired ketone **106**.



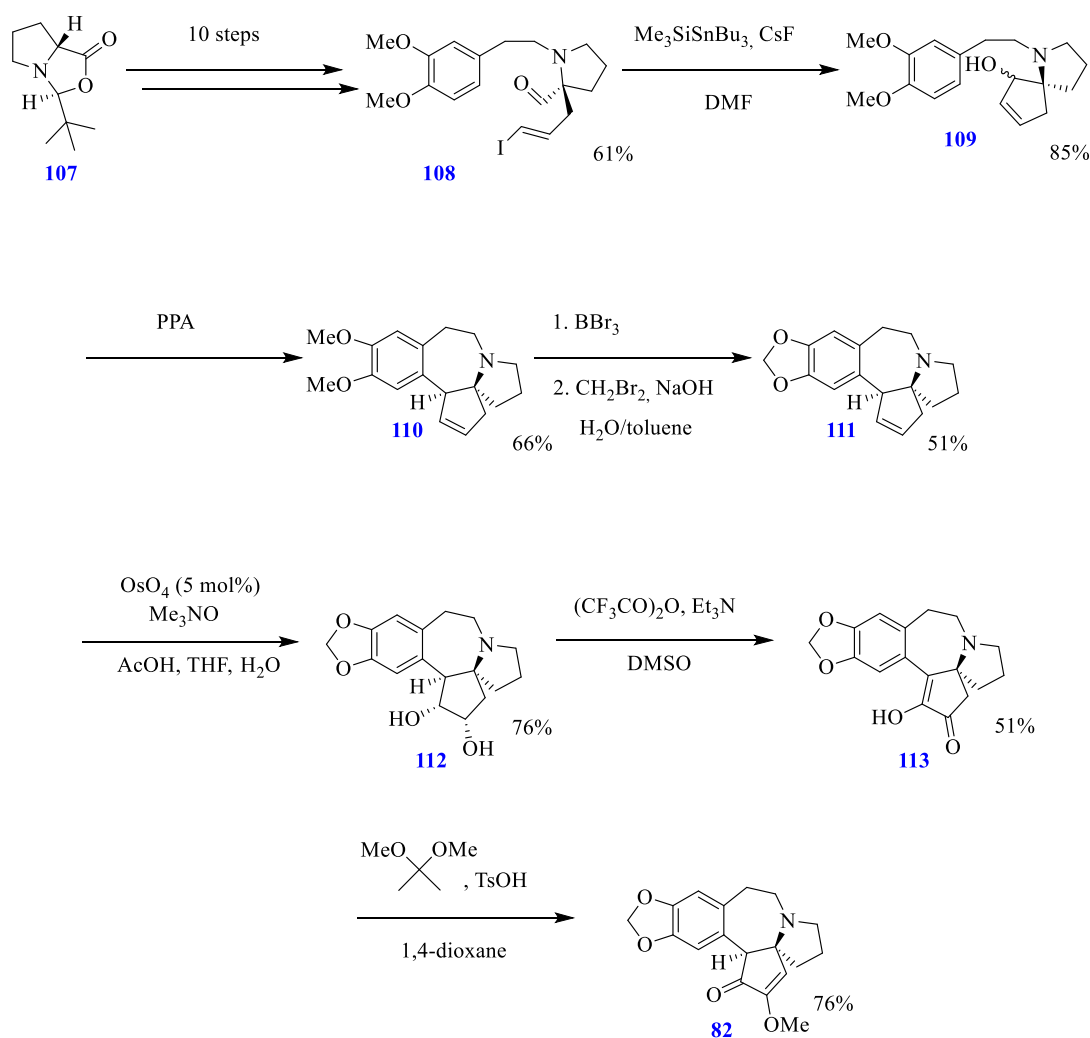
Scheme 30 Beaudry's strategy towards cephalotaxine.



Scheme 31 Proposed mechanism for the furan-opening step.

Mori et al. (1995)

Earlier, Mori had reported the synthesis of intermediate **110** (**Scheme 32**) while working towards several members of the cephalotaxine family.⁷⁵ Since that report, this alkene is routinely the target of other routes,⁶⁰ as only five steps are required from this intermediate to give cephalotaxine. Mori's intermediate was prepared via two cyclisations, the first by stannyl anion generation from precursor **108**, readily prepared in ten high-yielding steps (61% overall). The cyclisation was achieved in high yield, especially as the stannylated side-products (isolated in 3% yield) were easily converted into the desired alcohol **109** with TBAF. The second cyclisation was achieved by polyphosphoric acid to afford the key intermediate **110**, transformed into cephalotaxine **82** in 15% yield over five steps.

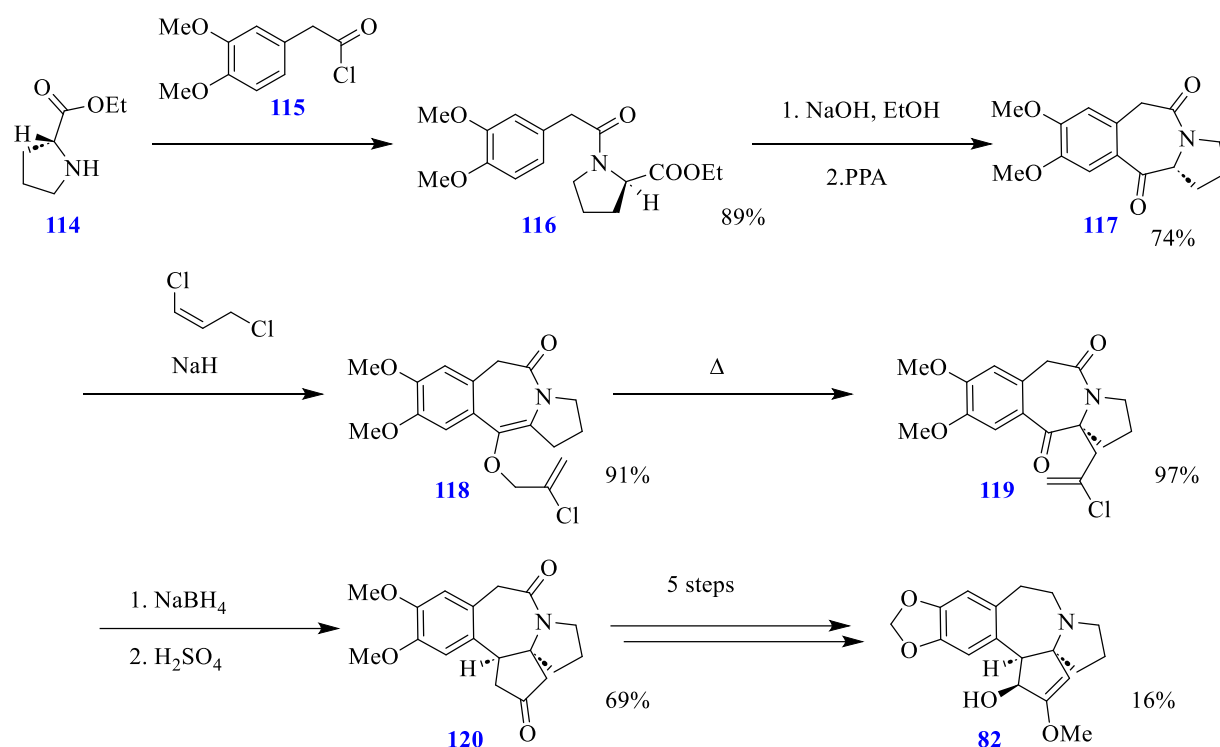


Scheme 32 Mori's strategy towards cephalotaxine.

Hanaoka et al. (1986)

In 1986, Hanaoka⁷⁶ reported an original route towards cephalotaxine using a Claisen rearrangement and a cationic cyclisation, via key intermediate **120**, known thereafter as Hanaoka's intermediate. The route (**Scheme 33**), as several before and since, started with proline, using the already present absolute configuration to build upon. Acylation of ethyl proline **114** with acid chloride **115** gave amide **116**, which was cyclised after ester hydrolysis using polyphosphoric acid to create the azepane ring in **117**. *O*-alkylation of the ketone and Claisen rearrangement, then further modifications, afforded the tetracycle **120**, readily

converted into the desired dioxole pentacycle in two steps. This intermediate (not shown) then required three further steps in order to introduce the required functionality in the cyclopentyl ring of cephalotaxine **82**.



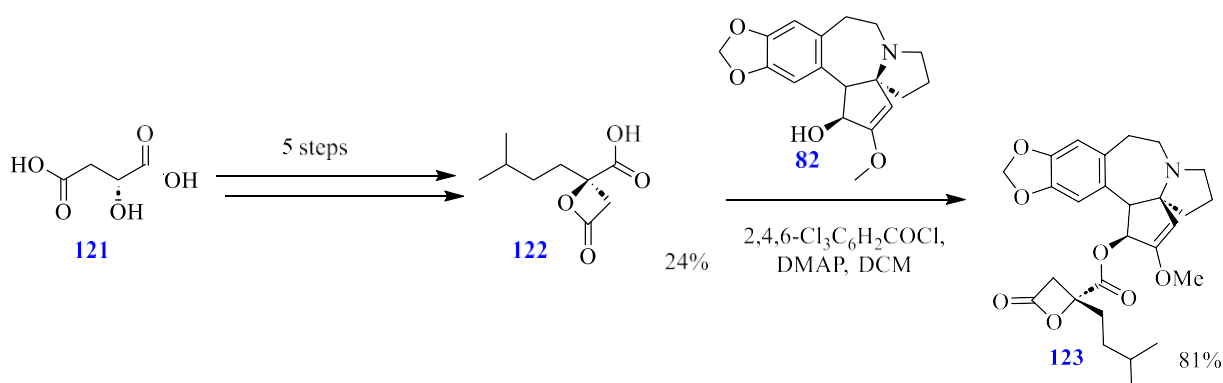
Scheme 33 Hanaoka's strategy towards cephalotaxine.

Branching the side chain, Gin et al. (2008)

To illustrate a final synthetic point about this family, the spotlight is directed to the numerous branched compounds produced by Gin via an elegant strain-accelerated acylation (**Scheme 34**).⁷⁸ In Gin's route to the cephalotaxine core, a previously unreported [3,3]-sigmatropic rearrangement of *N*-vinyl-2-aryl aziridines, also a strain-release type of reaction, was used to create the azepane ring.

The free alcohol group in cephalotaxine provided the obvious chemical handle to attach the side chain via esterification; however, direct esterification with the requisite carboxylic acids was prevented by steric hindrance of both the pentacycle **82** and the acid's chain. To circumvent

this issue, strained butyrolactone **122** was used. The authors proposed that the angle compression within the four-membered ring created a corresponding exocyclic angle expansion, reducing this steric stress. Moreover, the authors conjectured that the angle strain added two more positive effects: it increases the s-character of the exocyclic bonds, potentially increasing the electrophilicity of the carboxyl; and, by increasing the p-character of the endocyclic bonds (as the s/p ratio is fixed per carbon), it could increase the stability of a possible acylium like intermediate via vicinal p-delocalisation. This last effect could, of course, also reduce the electrophilicity of the carboxyl for the same reason. Thankfully, the overall positive effect of this strain led to successful esterifications, with a wide panel of modified acid components and ring-opening solvolysis expanding the range of known cephalotaxine esters leading to among the most potent bioactive variants of this alkaloid class.⁶⁸



Scheme 34 Gin's strategy towards cephalotaxine **82** and its ester **123**.

These routes, and the many others not described here, all included appropriate functionality in the cyclopentyl ring of the cephalotaxine core either as part of or followed by ring-forming reactions. This project again aspired to reverse the established paradigm, here using engineered P450_{BM3} variants to achieve both late-stage functionalisation and late-stage diversification as a strategy for the synthesis of cephalotaxine itself and a variety of ester analogues. This will be the focus of discussion in **Chapter 3** and **Chapter 4**.

Chapter 2 – Balanol

Project Aims

Cyclic amines are ubiquitous in many bioactive molecules, especially pharmaceuticals, but require chemical handles around them from which to build interactions with the biological target and, hence, their actual activity.^{79–82}

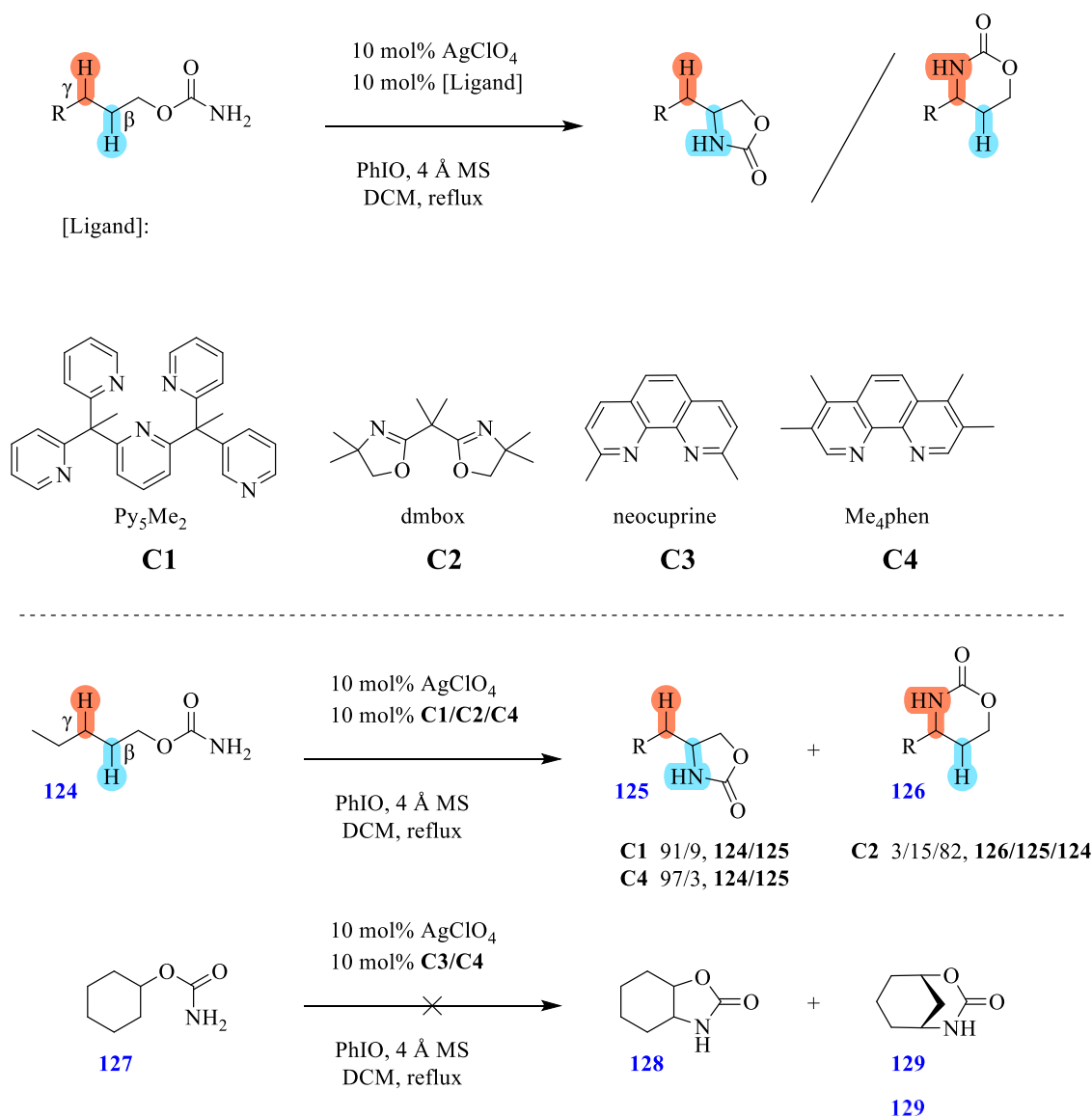
Previous strategies for the synthesis of such functionalised heterocycles start either from functionalised cyclic precursors, an expensive and limiting approach, or building the necessary functionality in a linear precursor prior to ring-closing steps.^{83,84} Both these strategies increase the number of reactions needed to be achieved within the complete synthesis and neither are particularly amenable to a diversity-oriented approach to the generation of focused molecular libraries based on the cyclic amine core. There is, therefore, a need for methods which convert bare cyclic amines into functionalised derivatives, more apt to build from. The seven-membered ring class of compounds are especially hard to close due to the negative entropic factor.⁸⁵ The work presented in this chapter is focused on finding an appropriate C–H amination method, using an hydroxo- moiety as a tether for the insertion.

Previous Work in the Robertson Group

Previous investigations in the Robertson group ruled out the azidoformate pathway⁸⁶ and the use of silver catalysis chemistry,⁸⁷ the first due to poor selectivity, the second due to no activity with cyclic substrates (**Scheme 35 to 37**).

Lee, in the context of fragment diversification, explored⁸⁸ the silver catalyst systems developed by the Schomaker group. From carbamate substrates, the insertion is reported to be tunable to either the β position or the γ position.⁸⁸ To afford the correct regioisomer for the balanol core, the insertion needed to take place at the β position. Thus, the chosen ligands were

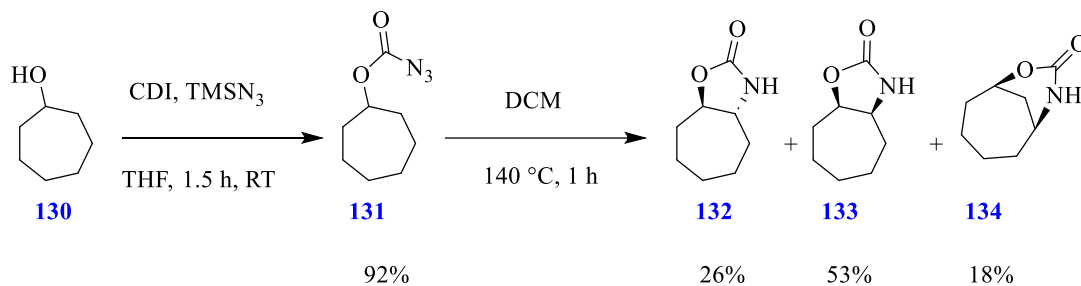
Py₅Me₂ **C1** and Me₄phen **C4**, reported to give the highest β insertion. In Lee's hands, however, the insertion could not be replicated on cyclic substrate **127**, and only linear substrates could be activated albeit with low conversions. This method, then, was considered unpromising for the current project.



Scheme 35 Lee's exploration of Schomaker's chemistry.

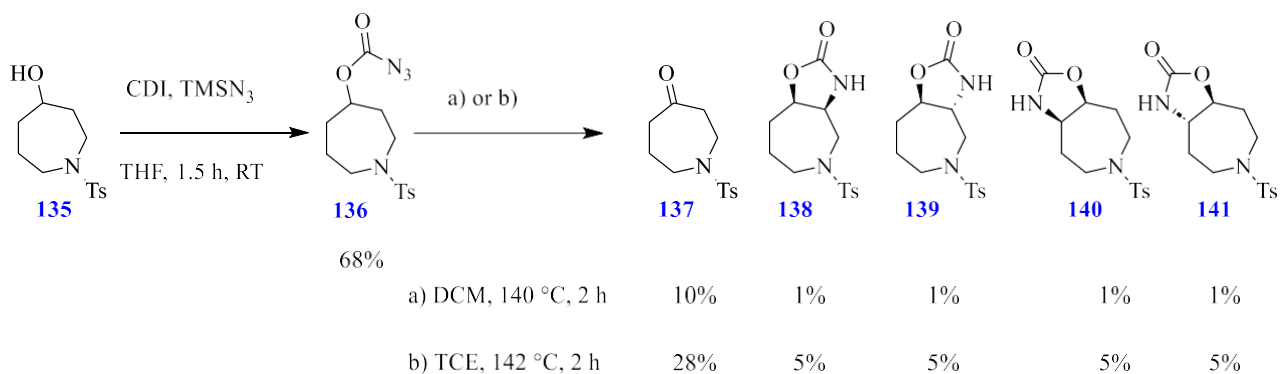
In separate work, Zhang examined the thermolysis of azidoformates which are known to undergo C–H amination in the absence of nearby alkenes.^{86,89} The high reactivity of the nitrene intermediates in these reactions is balanced by an inherent lack of selectivity.⁹⁰

Nevertheless, Zhang's studies with cycloheptanol were encouraging, and both *cis* and *trans* β -insertion products were observed in moderate yields along with γ -insertion as a minor pathway (**Scheme 36**).



Scheme 36 Formation of the model azidoformate **131** and its thermolysis.

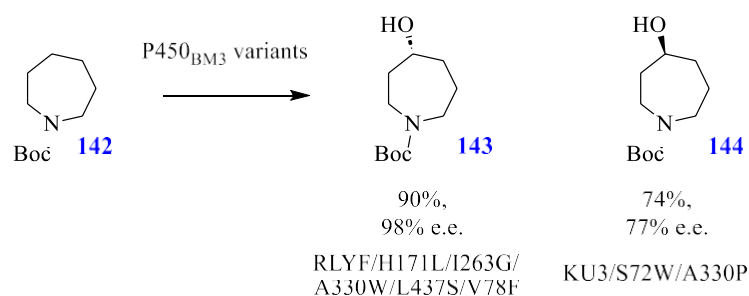
The thermolysis of the azidoformate derivative **136** of the selected *N*-protected azepanol **135** gave low yields of target products **138** and **139** (**Scheme 37**). At the time, these results were considered insufficiently positive to form the basis of a balanol synthesis and, in this project, the azidoformate approach was also not considered.



Scheme 37 Formation of the target azidoformate **136** and its thermolysis.

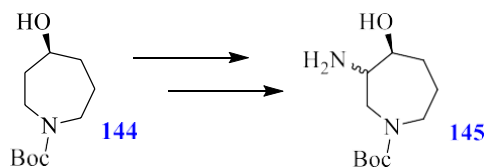
Enzymatic Synthesis of Azepanols via P450_{BM3}-Mediated Hydroxylation

A joint effort by the Robertson and Wong groups led to an enzymatic method to hydroxylate cyclic amines enantioselectively and at every position around each ring, including the unactivated C–H positions.⁹¹ This work offers new starting points for the synthesis of both existing targets and previously unknown structures. This chapter describes attempts to develop this idea, aiming to build on the enzymatic synthesis of the azepanols **143** and **144** (Scheme 38).



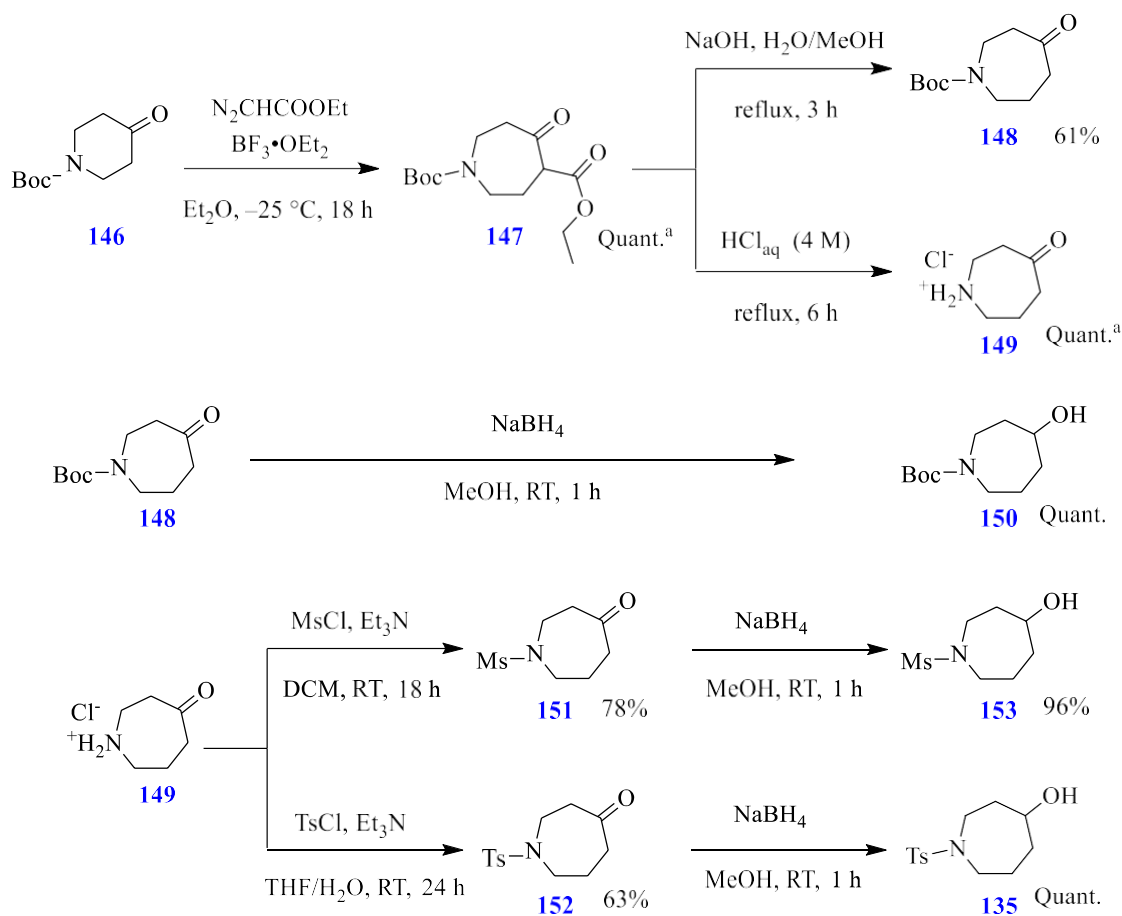
Scheme 38 Enantioselective oxidation of azepane by engineered P450_{BM3}.

The goal in this project was to use this newly-installed chemical handle to achieve proximal C–H amination. This tethered insertion would form a 1,2-amino-alcohol motif, the core of balanol **145**, overall constituting the conversion of an isolated –CH₂CH₂– unit into a –CH(OH)CH(NH₂)– unit with full control of the stereochemical configuration.



Scheme 39 Tethered C–H amination.

and **135**. These simple, high-yielding steps were reliable and provided a consistent supply of the desired alcohols.



Scheme 41 Preparation of *N*-protected (±)-4-hydroxyazepane.

^aCrude yield calculated from NMR.

C–H Amination

Following previous work from Lee and Zhang (see above, under **Previous work**), different approaches were then tested to form the desired 1,2-aminoalcohol with improved yields and selectivity. Apart from the previously-explored methods, only the groups of Nagib, He, Du Bois, and Lebel were able to build β C–H amino-alcohols, using hydrogen atom transfer (Nagib),¹⁰¹ silver chemistry (He),¹⁰² or carbamates and rhodium couples (Du Bois and Lebel).^{103,104} It was decided to first test the Nagib method,^{94,101} later extended by He,⁹³ as it was supposed to work with a wide range of cyclic secondary alcohol substrates at not obviously

activated centres,^{93,94,101} whereas the other routes were more efficient with primary alcohols or activated positions.^{97,103,104}

Radical Iodination

First, the chemistry developed by the Nagib group,^{94,95} and extended by the He group,^{92,93} was explored, based on a radical iodination process (See **Scheme S1** in Appendices).

The imidate substrates required for these reactions were prepared from the corresponding alcohols using a variety of methods as summarised in **Table 1**. With these imidates in hand, radical iodination was attempted, the results of which are summarised in **Schemes 42** and **43**.

In all cases, low yields were achieved, explained by the instability of the imidates which, being prone to hydrolysis, afforded the corresponding amide as the major side-product. Cyclisation was attempted following two main methods, the first being the use of NIS and Ag₂O.⁹² Imidates **155–162** (except for **157** and **160**) could not be cyclised and only resulted in decomposed materials. Only **157** led to the insertion product (**Scheme 42**) with the NIS/Ag₂O method, and it was found that Ag₂O was unnecessary for the cyclisation to occur (its reported role is primarily as an iodine scavenger to avoid the abstraction of chlorine by iodine, and hence the isolated product was the dichloro-compound **164**), and amide **164** was obtained in 12% yield from **157**. Indeed, the trichloroacetimidate-derived products were reported to be unstable on silica⁹² and crude product mixtures were therefore directly hydrolysed. Several conditions for the hydrolyses were explored,^{96,105–107} and only hydrated *p*-toluenesulfonic acid in pyridine opened the ring (stronger conditions, such as concentrated HCl in organic solvents, resulted in degradation). ¹H-¹H NMR coupling constants (*trans* protons), COSY and HSQC spectra, and comparisons with known structures¹⁰⁸ assured the regiochemistry and relative configuration of the product. Attempts to optimise the reaction were unsuccessful and the conversion could not be reproduced with other substrates.

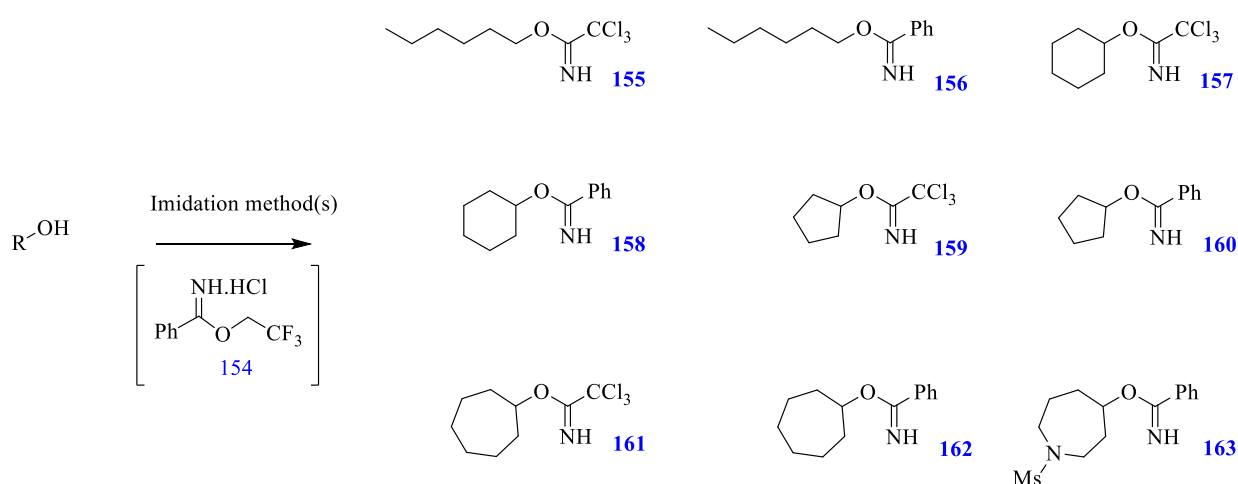
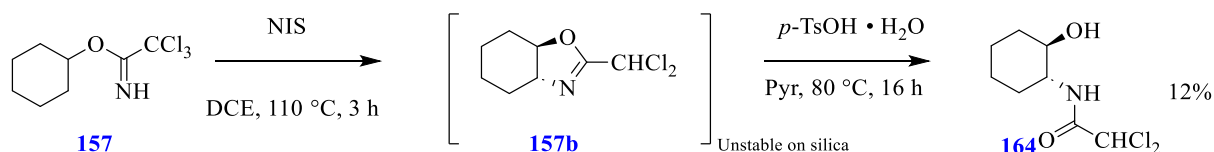


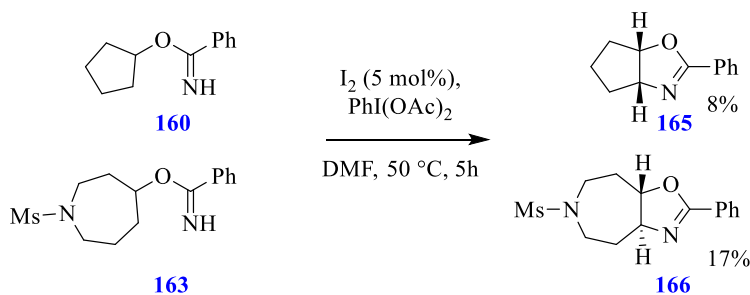
Table 1 Formation of imidates.

Entry	Alcohol	Imidation reagent	Additive	Solvent, T, t	Outcome (% Yield)
A	1-Hexanol	Cl ₃ CCN	DBU	DCM RT, 12 h	155 (64%)
B	1-Hexanol	PhCN	AcCl	None RT, 65 h	156 (Quant.)
C	Cyclohexanol	Cl ₃ CCN	DBU	DCM RT, 22 h	157 (53%)
D	Cyclohexanol	PhCN	HCl	1,4-dioxane RT, 75 h	158 (10%)
E	Cyclopentanol	Cl ₃ CCN	DBU	DCM RT, 24 h	159 (45%)
F	Cyclopentanol	PhCN	HCl	1,4-dioxane RT, 75 h	160 (11%)
G	Cyclopentanol	154	—	MeCN 50 °C, 48 h	160 (5%)
H	Cycloheptanol	Cl ₃ CCN	DBU	DCM RT, 24 h	161 (27%)
I	Cycloheptanol	PhCN	HCl	1,4-dioxane RT, 24 h	162 (9%)
J	153	154	—	MeCN 50 °C, 54 h	163 (9%)



Scheme 42 Synthesis of 1,2-amidoalcohol **165**.

Focus turned to the method developed by the Nagib group⁹⁴ which uses I_2 as catalyst, along with iodobenzene diacetate as stoichiometric oxidant, in DMF solution (the proposed catalytic mechanism⁹⁴ is presented in **Scheme S1** of the Appendices). The method was first trialled with imidate **160**, and the target product **165** was isolated, albeit in a lower yield (8%) than that reported (63%).⁹⁵ With this partial success, alcohol **153** was converted into the imidate **163** and cyclised, again with partial success, generating the cyclic imidate **166**. Coupling constants (for *trans* protons), along with COSY and HSQC, supported the β' -*trans*-insertion product shown (**Scheme 43**).



Scheme 43 Cyclisation using Nagib's method.

Although C–H amidation had been achieved, the β -insertion product required for balanol was not observed and so the Nagib method was not progressed further and a different system was evaluated.

Nitrenoid Chemistry

Next, the general approach developed by Du Bois^{103,109} and extended by Lebel⁹⁷ was explored (the accepted catalytic mechanism is presented in **Scheme S2** of the Appendices).

Variations of Lebel's published protocol were tested with cyclohexanol and cycloheptanol (**Table 2**) and, although the *N*-hydroxycarbamate formed as reported, the second step proved problematic. The main difficulty was in preventing a second mesylation of the desired product and so the yields of substrate derived from cyclohexanol and especially cycloheptanol were low. Nevertheless, treatment of substrates **167** and **168** with a rhodium catalyst gave moderate yields of β -insertion products (**170-171**, and **172-173** from **167** and **168** respectively) as roughly equimolar mixtures of the *cis* and *trans* diastereomers (**Table 2**, entries **A** and **B**, identification via comparison with known structures and 2D NMR). Their mechanism of formation is presented in the Appendices (**Scheme S2**), and is typical of metal nitrenoid reactivity (a three-member cycle as the transition state) These positive test results could not be extended to the azepanol derivatives **150** and **153** (entries **C-F**), from which no C-H insertion substrate could be isolated. Accordingly, this method was also abandoned.

C-H activation, Carbenoid Chemistry

Given the low success rate of the C-H amination reactions, especially with the azepanol-derived substrates, it was decided to explore the tandem C-H insertion/olefination method developed by Unsworth (for which the proposed mechanism is presented in **Scheme S3** of the Appendices).⁹⁸⁻¹⁰⁰ One of the literature examples was reproduced successfully (**Table 3**, entry **A**) although the yield of the insertion/olefination step (20%) was lower than that reported (73%).⁹⁸ The procedure translated reasonably well to the azepanol derivatives **150** (entry **B**) and **135** (entry **C**) but the extra conformational flexibility in the seven-membered ring resulted in a lack of regio- and stereocontrol; coupled with the low overall yield of the final step in both cases, this chemistry was not investigated further. The products were identified with 2D NMR and pattern identification from existing structures, notably with the coupling constants between the hydrogen at the base of the cyclopentyl and their CH₂ neighbours (for their *cis/trans* configuration), as well the COSY coupling between the proton β to the olefin and the CH₂ next to the cyclic amine (giving information on the insertion site, one intermediate atom meaning

184, and two meaning **185**). The absence of CHO peak in **186** proves the spiro motif.^{98–100}

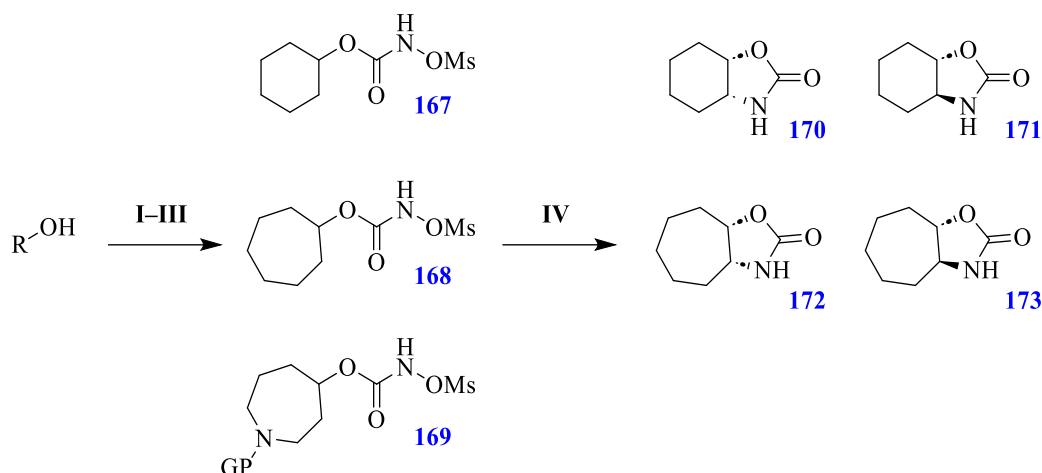


Table 2 Model studies and applications of Lebel's protocols towards C–H amination.

Reagents and conditions: **I** CDI, NH_2OH , toluene, 60 °C, 4h then $MsCl$, Et_3N , Et_2O , 0 °C, 2 h

II CDI, $NH_2OH \cdot HCl$, imidazole, MeCN, 0 °C, 6 h

III CDI, DBU (10 mol%), THF, RT, 3 h then *p*-TsOH, acetone, RT, 0.5 h
 $NH_2OH \cdot HCl$, imidazole, RT, 2 h

IV $Rh_2(tpa)_4$ (4 mol%), aq. K_2CO_3 , EtOAc, 40 °C, 16 h

Entry	Alcohol	<i>N</i> -hydroxycarbamate formation method (yield)	Outcome (yield)
A	Cyclohexanol	I (45%)	170 (11%) 171 (12%)
B	Cycloheptanol	I (2%)	172 (20%) 173 (20%)
C	150	I (n.d.)	—
D	153	I (n.d.)	—
E	153	II (n.d.)	—
F	153	III (n.d.)	—

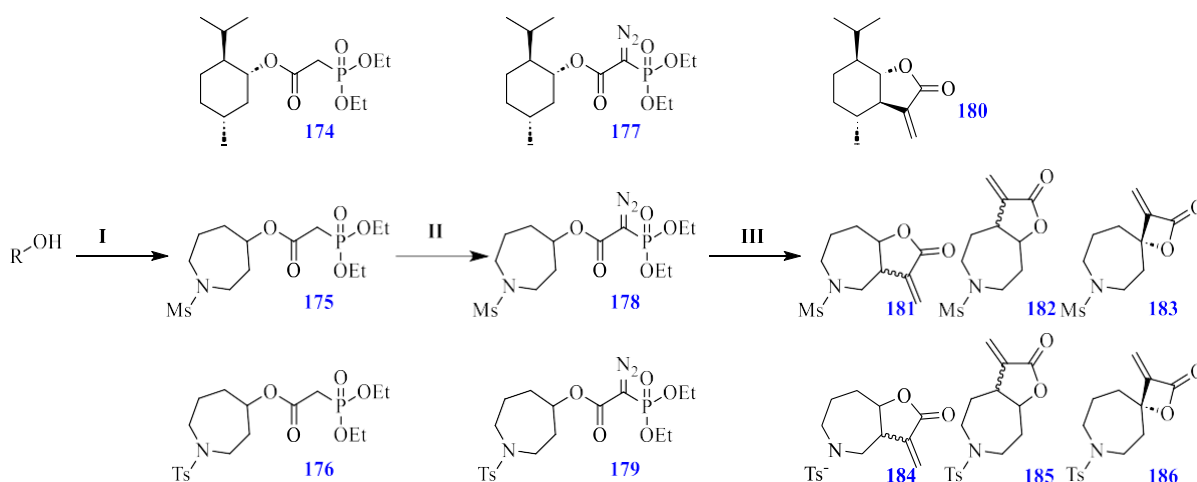


Table 3 Model studies and applications of Unsworth's protocols towards C–H insertion.

Reagents and conditions: **I** DEPAA, DIPEA, T₃P, Toluene, RT, 45 min

II LHMDs, *p*-ABSA, THF, 0 °C, 1 h

III Rh₂(Oct)₄ (2 mol%), DCM, 45 °C, 18 h ; *tert*-BuOK, (CH₂O)_n, RT, 2 h

Entry	Alcohol	Ester yield (%)	Diazo-ester yield (%)	Insertion outcomes (yield %)*
A	(–)-menthol 187	174 (Quant.)	177 (69%)	180 (20%)
B	150	175 (95%)	178 (53%)	181 (4%) 182 (11%) 183 (5%)
C	135	176 (49%)	178 (Quant.)	184 (8%) 185 (10%) 186 (8%)

Conclusion Chapter 2

The partially successful tethered C–H insertion reactions in the azepane systems were insufficiently encouraging for targeted application to the balanol core or similar azepane-containing compounds and it was decided to bring this sub-project to an end.

*Structures of **181** to **186** are proposed, though a lack of some data, namely HRMS and IR, precludes a definitive assignment.

Chapter 3 – Cephalotaxine

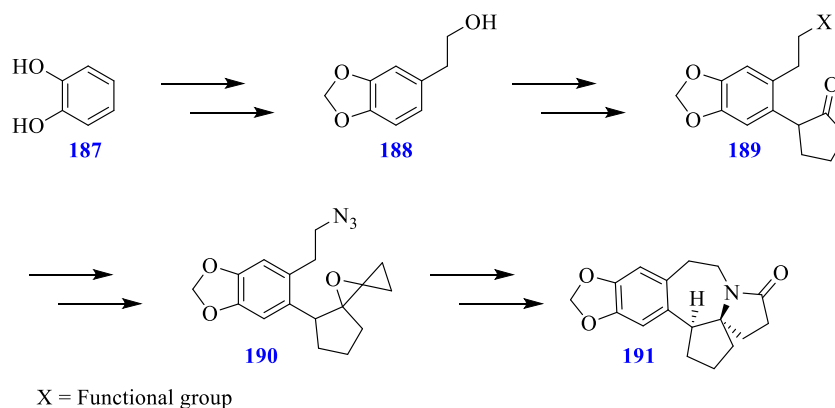
Project Aims and Strategy

This second project had three initial objectives. First, to establish a new route to the carbon skeleton of cephalotaxine, lactam **191**. Second, to explore the ability of engineered P450_{BM3} variants to hydroxylate the sp³ C–H bonds within this pentacycle, with special interest in reactions at the unactivated positions. Third, and building from the second aim, to obtain positional analogues of cephalotaxine and related potentially bioactive ester derivatives.

Setting sail apart from previous syntheses, it was decided to start from an affordable and readily-available starting material, catechol **187** (**Scheme 44**). The synthetic strategy was divided into three objectives, corresponding to three key intermediates. The first of these was alcohol **188**, for which the “upper” branch of the molecule was to be prepared by a new route, see below.¹¹⁰ From this alcohol, the second objective was to append the “lower” branch of the molecule, the cyclopentanone moiety, in ketone **189**. The possibilities to achieve this objective were numerous, and it was expected for the 1° alcohol to be converted into other groups to allow these methods to be tested. The final intermediate was identified as oxaspirocyclopentanone **190**. It was proposed this compound could undergo rearrangement and intramolecular Schmidt reaction to create the azepane ring, with an embedded spirobicyclic lactam, in target **191**.^{111–114} Between these intermediates, modifications would need to be conducted to allow the correct reactivity to take place, resulting in an estimated 10-step synthesis.

This approach offers a new point of view in the synthesis of cephalotaxine and its derivatives, by building the two appendages on the aromatic ring and only then joining them, where most of the reported procedures first built one complex chain and closed it directly on the benzenoid core.⁶⁴ Only Fuchs in 1988¹¹⁵ and Danishefsky in 1990¹¹⁶ used this approach within their synthesis of cephalotaxine, Fuchs using a [4+2]-cycloaddition and Danishefsky a

carbenoid cyclisation.

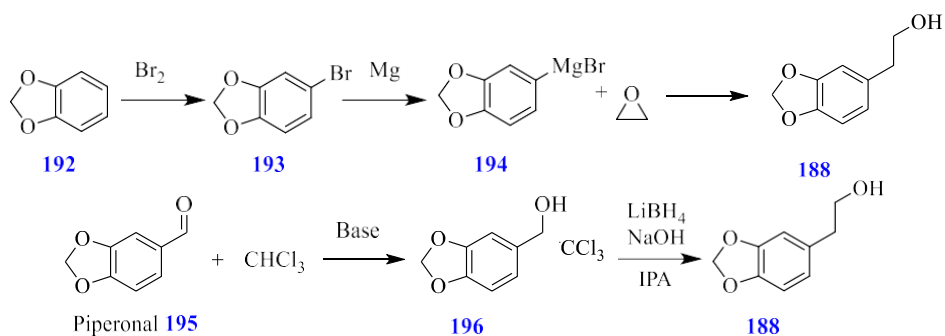


Scheme 44 Outline of the proposed route towards the cephalotaxine skeleton **191**.

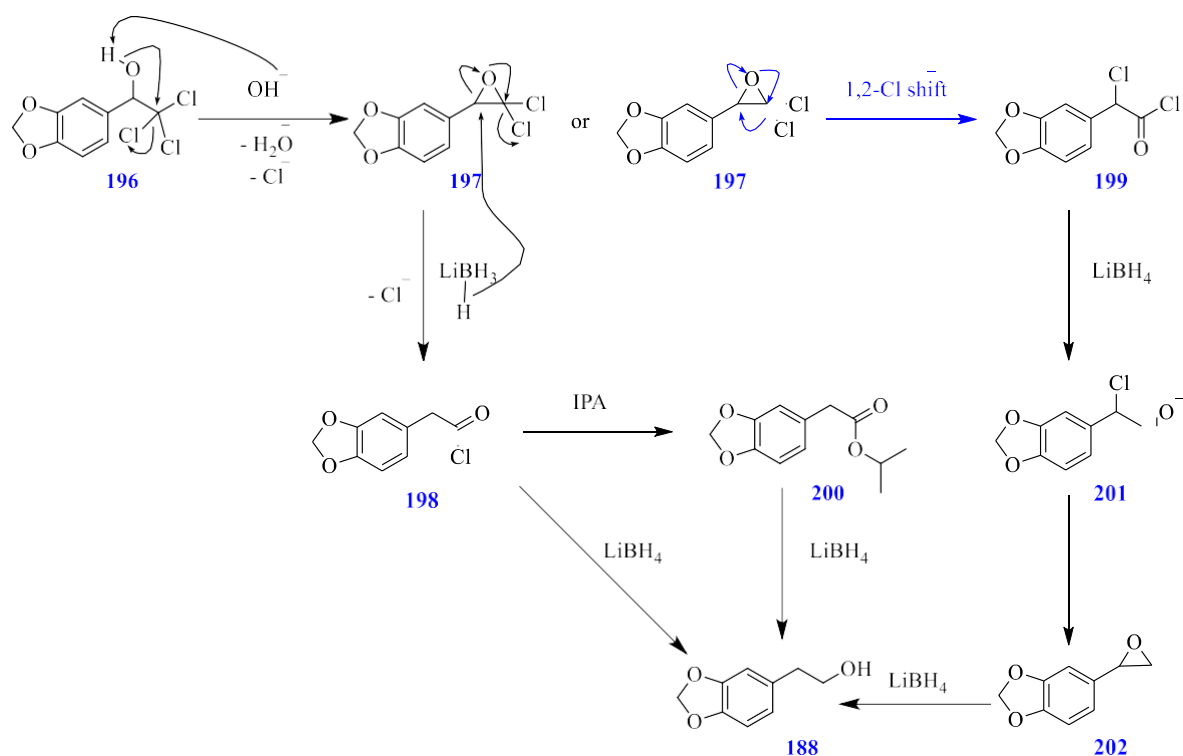
Synthesis of Alcohol **188**

The synthetic work offered the opportunity to evaluate a new strategy towards the first key intermediate **188** which would also circumvent the need to use gaseous ethylene oxide in combination with Grignard reagent **194** (**Scheme 45**), the current main method for its synthesis.^{117,118} Alcohol **188** is known and commonly used in synthetic strategies, whether academic^{119–121} or industrial.^{122–124}

The new plan would use a modification of a published method previously not applied in a total synthesis setting, a Jovic-type reaction enabling a one-carbon homologation of aldehydes.¹¹⁰ The proposed mechanism is outlined **Scheme 46**. The Jovic–Reeve reaction is known to create carboxylic acids from trichloromethylcarbinols in the presence of a base.^{125,126} Reasoning that the nucleophile could be a hydride instead of a hydroxide, the Snowden group managed to synthesise primary alcohols from these trichloromethylcarbinols via the Jovic–Reeve pathway (mechanistic example, applied to a piperonal type structure, in **Scheme 46**).¹¹⁰



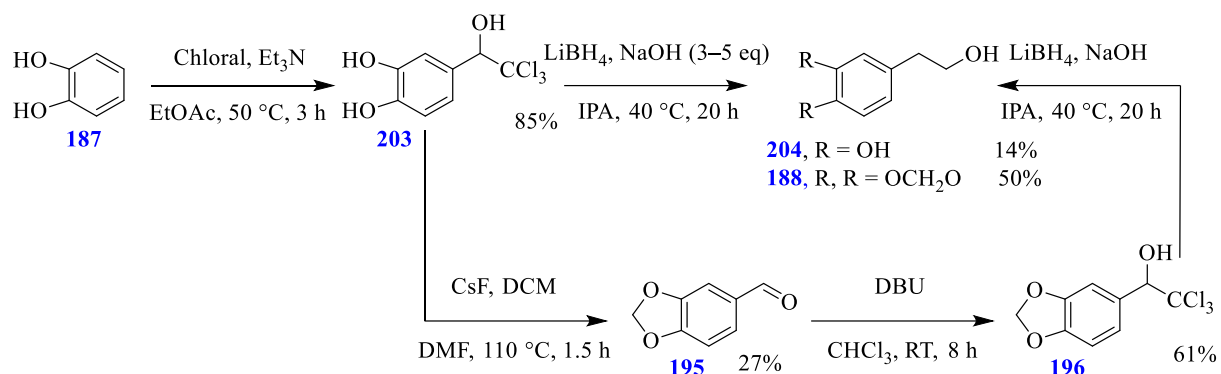
Scheme 45 Precedented versus proposed synthesis of alcohol **188**.



Scheme 46 Jovic-type proposed mechanism by Snowden.

The relevant starting aldehyde **195** (piperonal) is a precursor of the illegal psychoactive drug MDMA¹²⁷ and so its use and commercial availability are tightly controlled. For this reason, catechol **187** was chosen as the starting point and, with the aim of converting it into carbinol **196**, it was first transformed into alcohol **203** with chloral.^{128,129} Attempted homologation using Snowden's conditions (NaOH, LiBH₄, aq. IPA) was hampered by the phenols, even with additional NaOH, and a low yield of the 1° alcohol **204** was obtained. Protection of the catechol functionality in **203** as the benzodioxole was accompanied by elimination of chloroform to

deliver piperonal **195**. This was converted back into the trichloromethyl carbinol **196**, from which the overall homologated 1° alcohol **188** was obtained in a more acceptable yield. With this substrate, the Snowden homologation was more efficient when the reaction was run at a lower temperature for longer [40 °C, 20 h (50%) vs. 85 °C, 1 h (4%)]. This route is presented in **Scheme 47**.



Scheme 47 Route to alcohol **188** from catechol.

The overall yields from catechol (12% and 7%) were deemed too low at this early stage in the synthesis and although it would probably have been possible to optimise each step, a potentially more promising starting point was chosen, 1,3-benzodioxole **205**, similarly-priced to catechol. This compound was inert to chloral^{128,129} (**Table 4**, entry **E**) and, as summarised in **Table 4** was also resistant to trichloroacetylation^{20,130} and acetylation¹³¹ with the corresponding acid chlorides (entries **A,B**), and Vilsmeier–Haack reaction²⁰ (entry **D**). Friedel–Crafts acetylation was possible using acetic anhydride,¹³² leading to ketone **207** (entry **C**) in quantitative yield. This ketone was decomposed by TCCA action²⁰ (entry **F**), but a variant¹³³ of the Kosower¹³⁴ reaction yielded dichloroketone **208**.

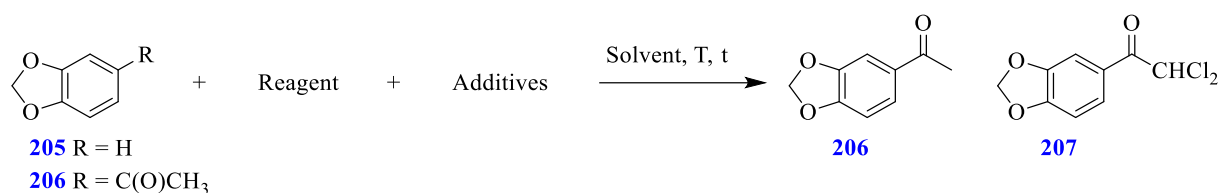


Table 4 Functionalisation of arene **205** and **206**.

Entry	Arene	Reagent	Additive	Solvent, T, t	Outcome (yield %)
A	205	Cl_3CCOCl	AlCl_3	DCM, 0 °C, 1 h	205 (–)
B	205	CH_3COCl	AlCl_3	DCM, RT, 30 min	205 (–)
C	205	Ac_2O	–	TFA, RT, 18 h	206 (Quant. by NMR)
D	205	DMF	POCl_3	MeCN, RT, 1 h	205 (–)
E	205	Cl_3CCHO	Et_3N	EtOAc, 50 °C, 3 h	205 (–)
F	206	TCCA	–	AcOH, reflux, 3 h	Decomposition
G	206	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$	LiCl	DMF, 90 °C, 4 h	207 (35%)

Indeed, Atkinson, a previous D.Phil student in the group, had shown that dichloro- (rather than trichloro-) acetophenone derivatives could be effective substrates for Snowden's reductive homologation conditions. Optimising this variation of the Kosower method (see **Table 5**), the yield of desired ketone **207** was substantially raised (entry **G**) and, inconsequentially, small amounts of the trichloroacetophenone **208** were also produced. Both were submitted to Snowden's conditions, and both were transformed into a mixture of the alcohols **188** and **209** (**Scheme 49**). The dichloroacetyl ketone **207** afforded both alcohols in a 50:50 ratio, while the reaction of the trichloroacetyl ketone **208** favoured the 1° alcohol (66:34 ratio). A lower reaction temperature and longer reaction time were again found to improve the yield of the desired alcohol (as tested with dichloroacetophenone **207**). The use of LiBH_4 in solution in THF (2.0 M) improved the overall alcohol yield compared to solid LiBH_4 (quant. vs. 68%) without modifying the selectivity.

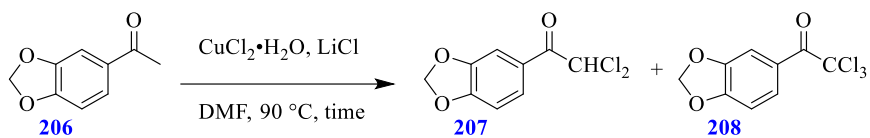
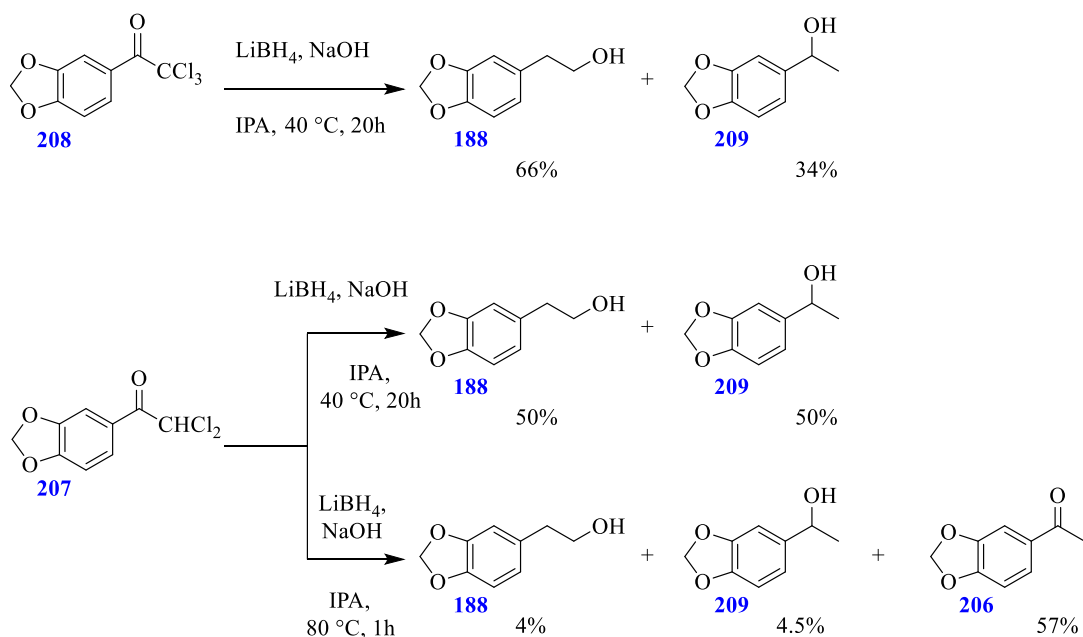
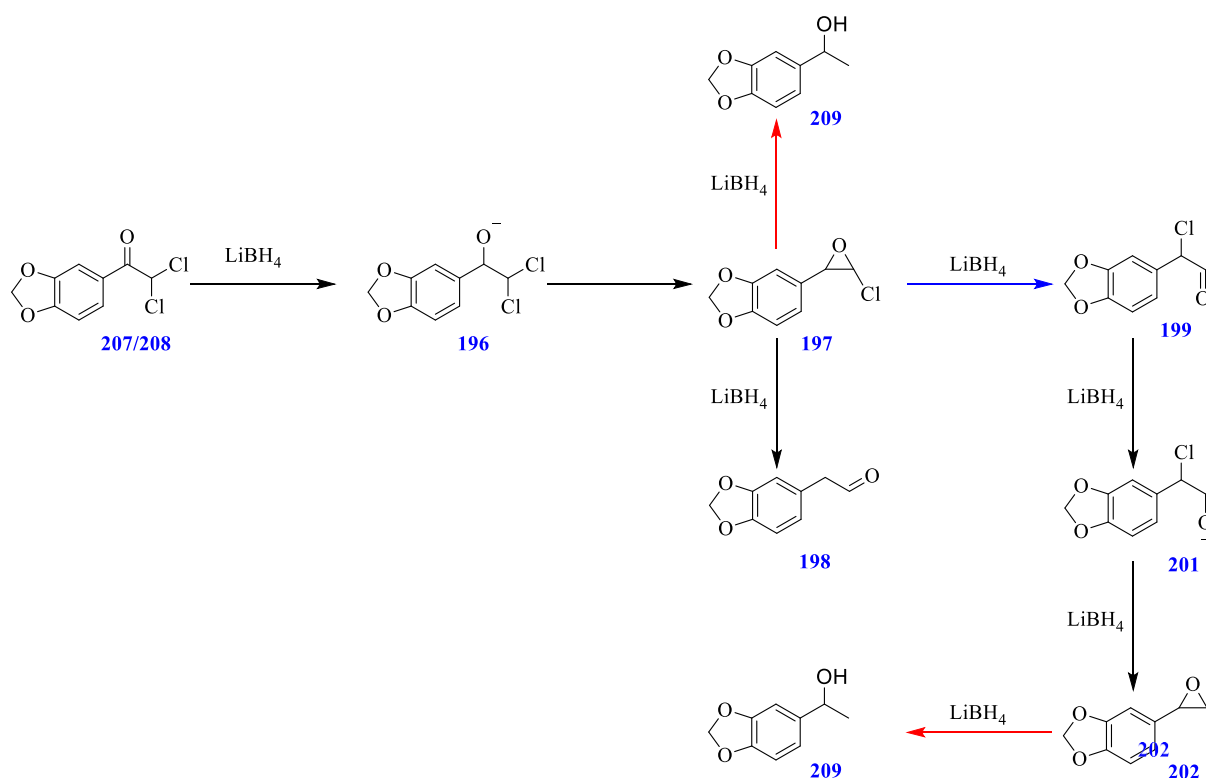


Table 5 Optimisation of the Kosower method for ketone dichlorination.

Entry	$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (equivalents)	LiCl (equivalents)	Time (h)	Yield 207	Yield 208
A	5	5	4	35%	n.d
B	6	6	24	9%	n.d
C	5	5	3	45%	n.d
D	5	5	5	48%	1.6%
E	5	5	6	26%	9%
F	6	6	4.5	69%	1%
G	6.5	6.5	6	70%	1%



Scheme 48 Homologation of ketones **207** and **208**.



Scheme 49 Amended proposed mechanism to account for **209**.

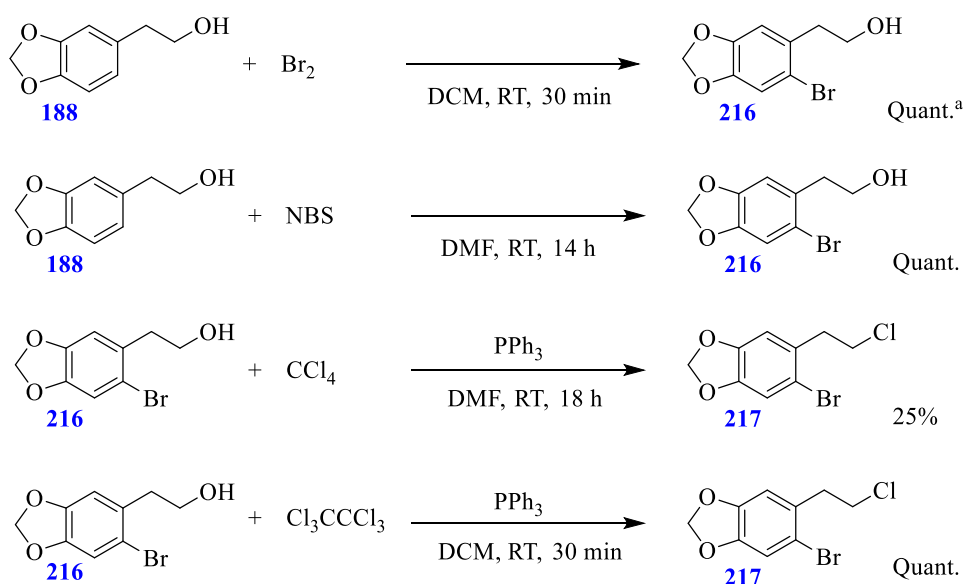
The different ratios of **188** and **209** obtained from the *dichloro*-substrates is explained by reference to **Scheme 46** and **49**. With *dichloro*-substrates the non-benzylic part of epoxide **197** is less hindered and thus more prone to be attacked by LiBH_4 , leading to the red pathway and the 2° alcohol. Even with the blue pathway, the 2° alcohol can be created with the final epoxide (**202**), as the benzylic position is more hindered and thus less prone to be attacked by LiBH_4 despite electronic activation by the adjacent aromatic π -system. These alternative pathways are more likely with *dichloro*- substrates compared to the *trichloro*- analogues, hence the disparity observed in **Scheme 48** (assuming the ketones **207** and **208** are similarly reduced by an additional equivalent of LiBH_4).

The overall yield for the desired 1° alcohol **188** in this three-step route from 1,3-benzodioxole (41%) was considered sufficient for continuing the synthesis.

Appending the Cyclopentanone Ring

With the desired alcohol in hand, installing the cyclopentane moiety was next in sight. The first method tested was an α -arylation developed by the Dong group.¹³⁵ This method was chosen first as it required the minimal number of additional steps to convert alcohol **188** into ketone **189**; moreover, it was reported to be selective for monoarylation, to support free alcohols (saving protection/deprotection steps), and worked even in sterically hindered systems.

Still anticipating potential complications with free alcohol **188**, the hydroxy group was converted into the corresponding chloride **217** via a modified Appel reaction,¹³⁶ using hexachloroethane instead of carbon tetrachloride (**Scheme 50**). This transformation was quick and efficient but the highly non-polar product co-eluted on silica with triphenylphosphine. To solve this purification problem, the work-up was modified to include an H_2O_2 wash, to oxidise the phosphine to its oxide, and an aqueous $\text{Na}_2\text{S}_2\text{O}_3$ wash to destroy excess peroxide. This work-up allowed easy separation of the so-formed phosphine oxide from the product by chromatography.



Scheme 50 Bromination of **188** and modified Appel reaction of **216**.

^aCrude yield calculated from NMR.

With the desired substrates, along with the model compounds **218**–**222** (all prepared from the corresponding arene with Br₂ in DCM, to the exception of **220**, which needed NBS and FeCl₃ in MeCN, and the commercially available **218**, **219**, and **221**), studies on the α -arylation were undertaken, with the results compiled in **Table 6**.

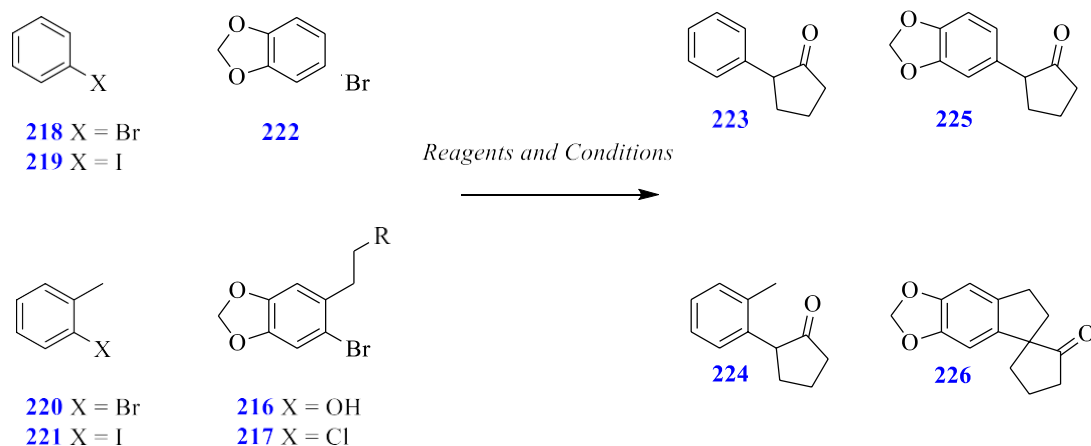


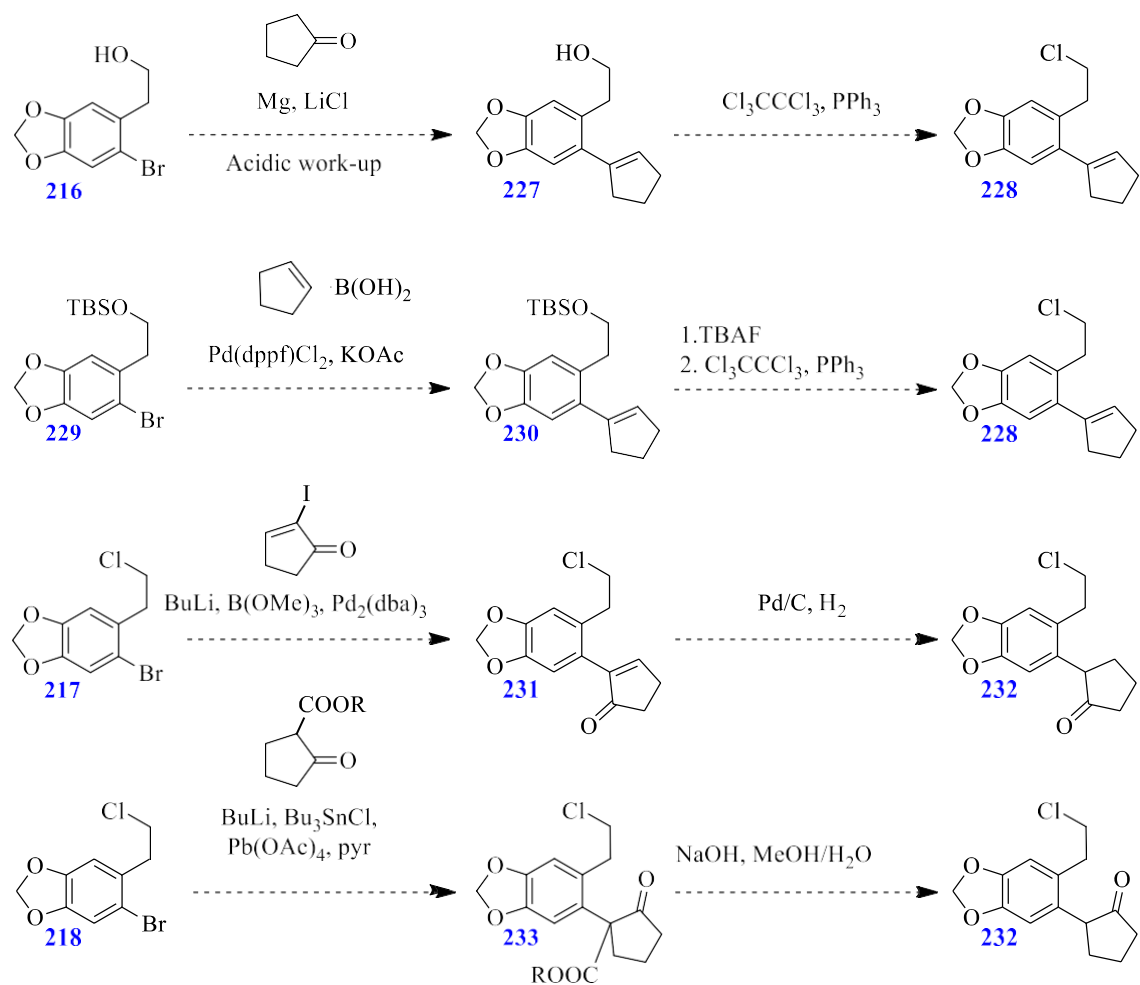
Table 6 Studies of the α -arylation of cyclopentanones.
Reagents and conditions Cyclopentanone, Pd(OAc)₂ (2.5 mol%), P(*o*-Tol)₃, pyrrolidine, NaOAc, *tert*-octylamine, 1,4-dioxane, 110 °C, 12 h.

Entry	Substrate	Outcome (yield %)
A	218	223 (29 %)
B^a	218	223 (30%)
C	219	223 (27%)
D	220	224 (11%)
E	221	224 (n.d.)
F	222	225 (28%)
G	216	n.d.
H	217	226 (10%)

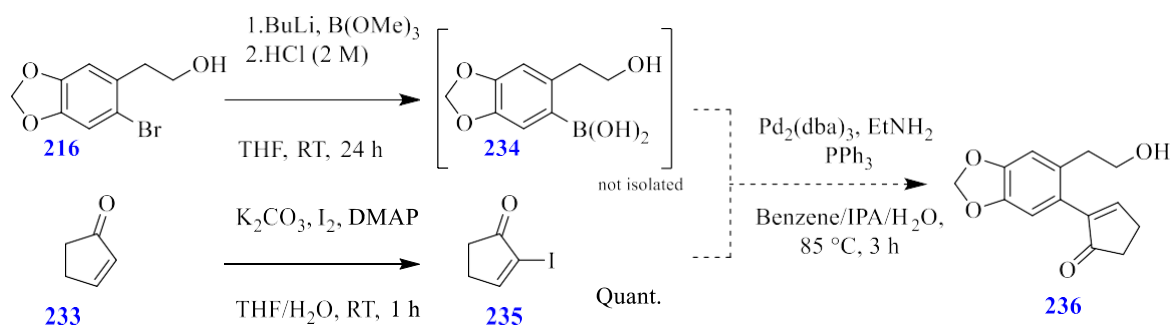
^a same reagents and conditions with the exception of *tert*-octylamine (absent here).

This study showed that the reaction proceeds in the same manner with or without *tert*-octylamine (entries **A,B**), despite its role being an unexplained key for Dong and coworkers.¹³⁷ It also demonstrated that bromine and iodine are similarly effective (entries **A,C**), that electron-donating aryl substituents are tolerated (entries **A,F**), and that substrates bearing *ortho*-substituents suffer a clear drop in efficiency as, for example, the yield with *o*-bromotoluene was about one-third of that for bromobenzene (entries **A,E**). Compound **225** (entry **F**) was considered as an interesting product as it could open a new synthetic pathway towards target lactam **191**; however, the relatively high cost of the reaction deterred its use in the very first step of the synthesis project. Application to the substrate bearing the alcohol side-chain gave no tangible outcome (entry **G**), which was attributed to the potentially complicating presence of the free hydroxyl group. The chlorinated substrate was made for this reason, however, the only product from this substrate (entry **G**) was identified as spirocyclic ketone **226** (from 2D NMR spectroscopy and HRMS), so this method of appending the cyclopentanone was discontinued.

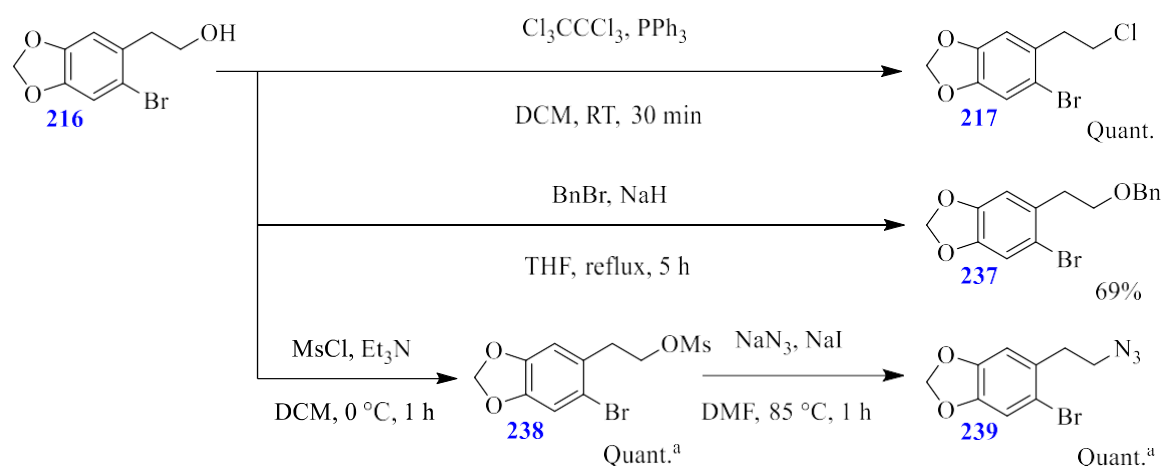
Several alternatives to the cyclopentanone α -arylation were considered (**Scheme 51**),^{138–144} among which the first Suzuki–Miyaura coupling (from **229**) appeared to be the most direct and the intermediates **216** and **217** could still be used in the new synthetic plan. The reversed Suzuki–Miyaura coupling (from **217** via the corresponding aryl boronic acid) would need an additional hydroboration/oxidation step to afford the desired ketone. Vinyl iodide **235** (**Scheme 52**) was prepared from cyclopentenone **233**,¹⁴⁵ and the aryl bromide **216** was converted into the boronic acid **234** via lithium/halogen exchange, addition of trimethylborate, and hydrolysis by hydrochloric acid.¹⁴⁶ Anticipating potential complications from the alcohol side-chain, a number of alternative substrates were prepared (**Scheme 53**) and tested (**Table 7**).



Scheme 51 Alternative routes to the α -arylation.



Scheme 52 Suzuki–Miyaura coupling between 234 and 235.



Scheme 53 Alternative substrates for the Suzuki–Miyaura coupling.

^aCrude yield calculated from NMR.

A reported procedure, in which chloride **217** was the substrate, gave a low yield when followed (entry **A**). The alcohol substrate **216** was transformed with satisfactory yield (entry **B**), and the benzyl-protected **237** version afforded an even better outcome (entry **C**). The azide functionality in **239** was not tolerated, presumably reacting with the triphenylphosphine in the Staudinger reaction,¹⁴⁷ preventing the phosphine from acting as the intended ligand (entry **D**).

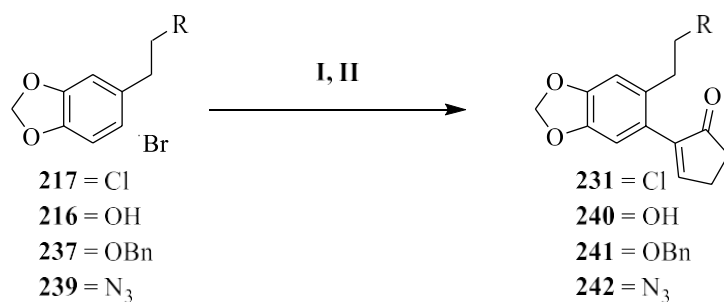


Table 7 Comparison of substrates in the Suzuki–Miyaura coupling with iodocyclopentenone **235**.

Reagents and Conditions: **I** BuLi, THF, $-78\text{ }^{\circ}\text{C}$, 30 min ; then B(OMe)_3 , RT, 12 h ;

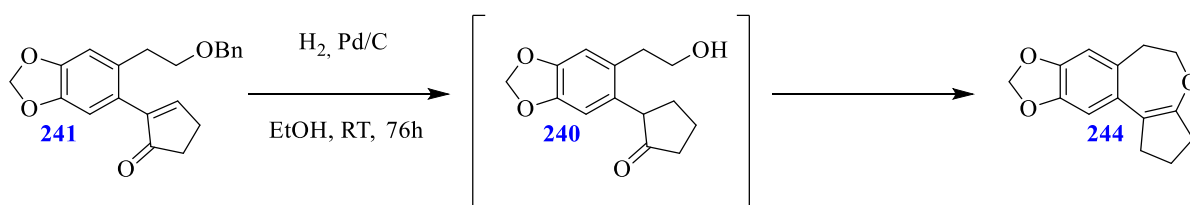
then HCl_{aq} (2 M), RT, 12 h.

II $\text{Pd}_2(\text{dba})_3$, **235**, EtNH_2 , PPh_3 , benzene, IPA, H_2O , $85\text{ }^{\circ}\text{C}$, 3 h.

Entry	Substrate	Outcome (yield %)
A	217	231 (24 %)
B	216	240 (63%)
C	237	241 (90%)
D	239	242 (n.d.)

Progress Towards Lactam 191

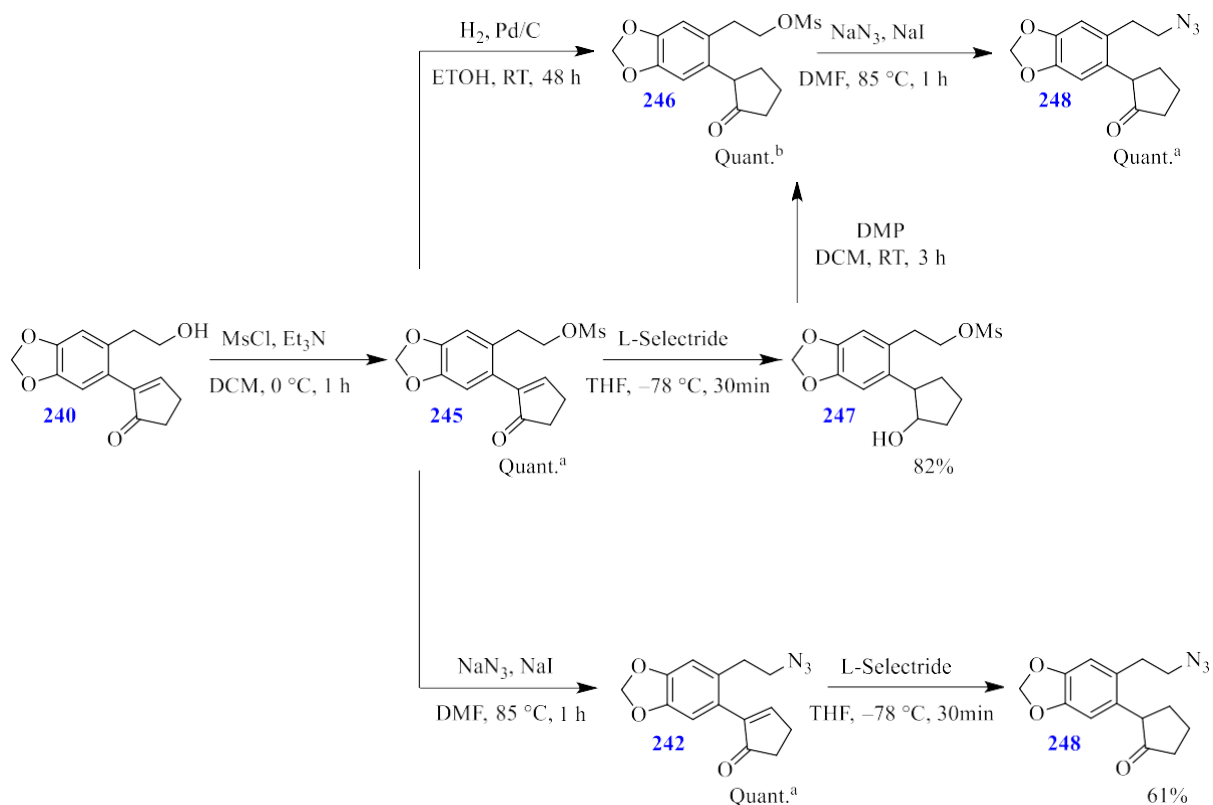
From the highest-yielding coupling product **241**, the focus turned to deprotecting the benzyl ether and reducing the enone in a single combined hydrogenolysis/hydrogenation step. This overall transformation worked well but the newly formed alcohol **243** quickly attacked the ketone (while being inert with the enone), leading to unwanted condensation to give tricyclic enol ether **244** (Scheme 54), unstable on silica. This enol ether was identified through the resonances of the lower cyclopentane (a tt at 2.74 ppm for the lowest CH₂, and a shifted multiplet for the CH₂ between the benzylic CH₂ and the enol ether). This particular result brought back the alcohol **216** as the desired substrate for the Suzuki coupling, to ensure the best compromise between yields and number of steps.



Scheme 54 Undesired cyclisation of alcohol **243**.

Knowing that the alcohol and the ketone cannot coexist, the alcohol was then mesylated both to protect it from cyclising and to activate it for subsequent azide displacement. Reduction of the enone **245** was subsequently conducted as before (with H₂ and Pd/C), and with L-Selectride[®] (Scheme 55). The H₂ route was easier to conduct (fewer columns) and yielded more ketone **248**. Surprisingly, the enone functionality in **245** was reduced fully with L-Selectride[®], to alcohol **247** but this product could be quickly oxidised back to ketone **246** with Dess–Martin periodinane. The mesylate was substituted using NaN₃²⁴⁶ to afford the desired keto-azide **248**. Enone reduction of azide **242**, in contrast, gave the same azide **248**, albeit in slightly lower yield.

The final two steps were the oxaspirocyclopentanone formation followed by rearrangement and intramolecular Schmidt reaction.¹¹²

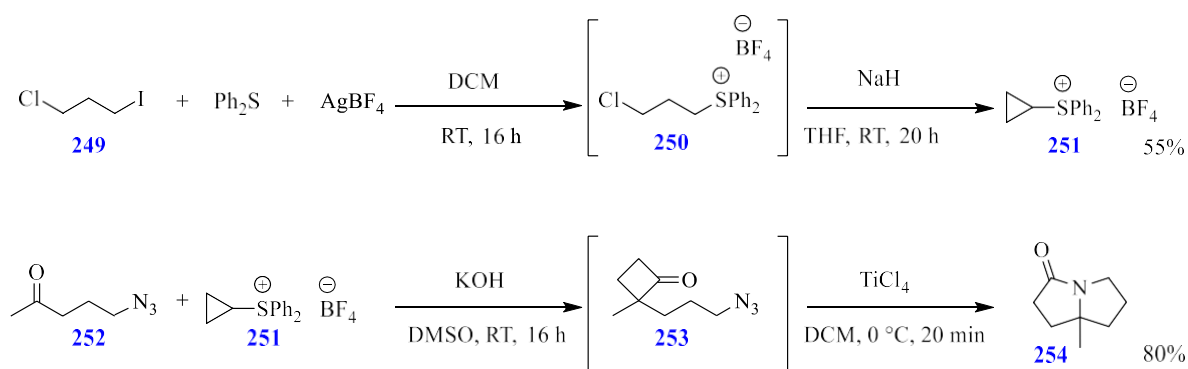


Scheme 55 Routes towards azide **248**.

^aCrude yield calculated by NMR

^bSame yield for both steps

Cyclopropyldiphenyl-sulfonium tetrafluoroborate **251**¹⁴⁸ was prepared and the two steps tested successfully using model keto- azide **252**, readily made from its chloro-analogue with NaN₃, leading to bicyclic lactam **254**, whose ¹H NMR spectrum perfectly matched what was reported in the literature. These conditions were then applied to mesyl substrate **246** but the reaction gave only the previously-observed cyclised product **244**, presumed to arise following hydrolysis of the mesylate (**Table 8**, entry **A**). The reaction was then attempted by first creating the ylid *in situ* with one equivalent of NaH then adding ketone **246** to the pre-formed ylid. A similar outcome was observed (entry **B**), suggesting that, if not hydrolysis by adventitious KOH, then deprotonation of the mesylate methyl group had occurred with release of the alcohol along with sulfene (H₂C=SO₂). The reaction was also attempted with azide **248** but this led merely to substrate decomposition, with no signs of the desired oxaspirocyclopentanone or the cyclobutanone (entries **C,D**).



Scheme 56 Schmidt rearrangement in a model system.

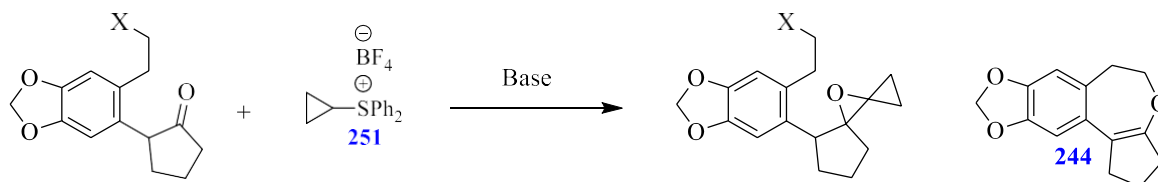


Table 8 Attempts to form the oxaspirocyclopentanone motif.

Entry	X	Base	Solvent, T, t	Outcome (% Yield)
A	–OMs	KOH	DMSO RT, 16 h	244 (Quant.) ^c
B^a	–OMs	NaH	DMSO RT, 16 h	244 (Quant.) ^c
C^b	–N ₃	KOH	DMSO RT, 16 h	Decomposition
D^a	–N ₃	BuLi	THF 0°C, 5 h	No reaction

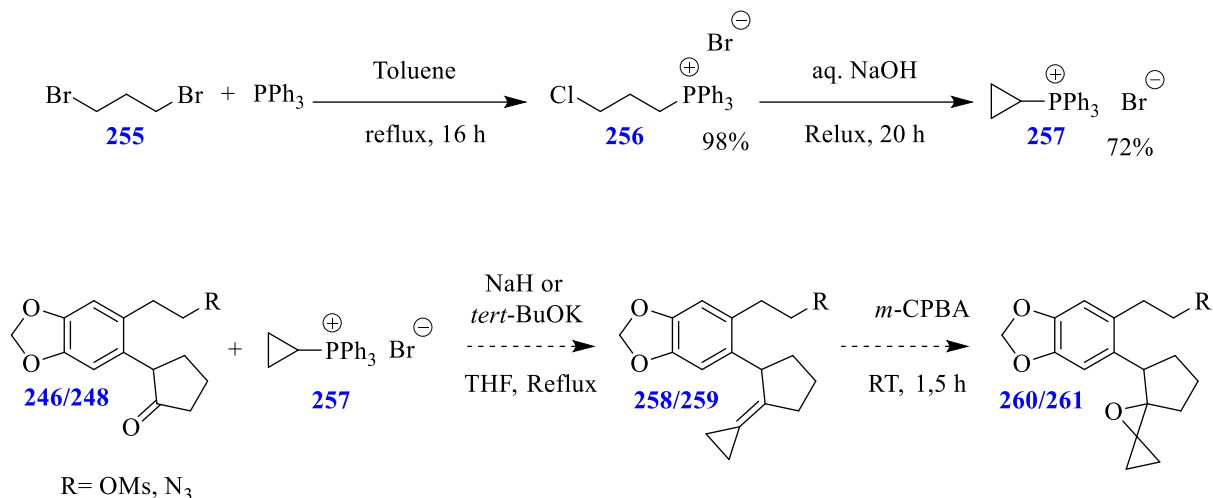
^a Ylid formed first.

^b Repeated in DMSO-*d*₆ with the same outcome.

^c Crude yields determined by NMR analysis.

With limited time remaining to progress the synthesis, an attempt was made to form the oxaspirocyclopentanone via a Wittig reaction with phosphonium salt **257**¹⁴⁹ but both methods tested failed to deliver the cyclopropylidene product (**Scheme 57**). Part of the crude product

from the first step was isolated but its ^1H NMR data fitted no desired structure, and was subsequently discarded after failing to react with *m*-CPBA.

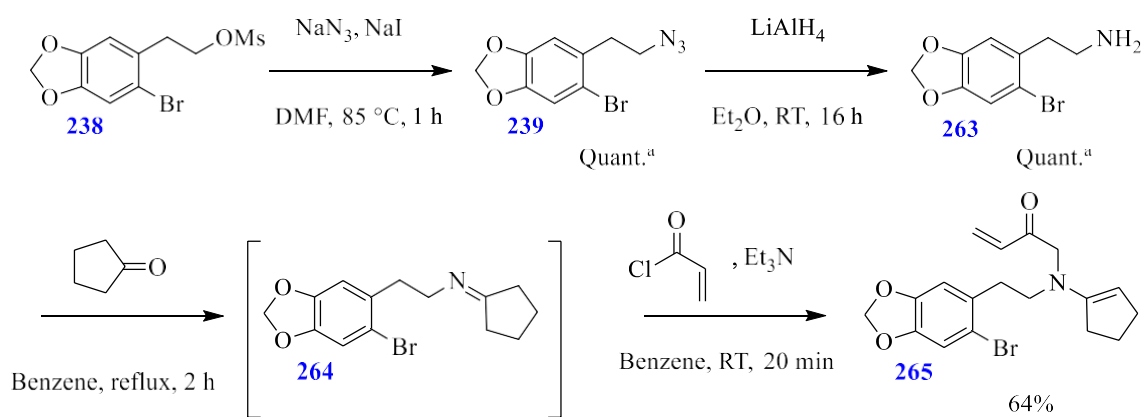


Scheme 57 Alternative pathway via Wittig chemistry.

Contingency Plan, the Radical Cascade

In parallel with exploring the final steps of the above route, and as it became clear that these might not be successful, it was decided to prepare the target lactam **191** using a reported tandem 7-*endo-trig*/5-*endo-trig* radical cyclisation from substrate **265** (**Scheme 59**; see **Scheme 60** for the proposed mechanism).^{150,151} The necessary intermediate, amine **263**, was obtained by effecting azide displacement from **238** then reducing the azide **262** with LiAlH₄.[†] The amine was used to form an imine (**264**) with cyclopentanone under a Dean–Stark apparatus, then *N*-acylation of this imine *in situ* with acryloyl chloride provided the enamide **265** as the substrate for the radical cyclisation. This short route is presented **Scheme 58**.

[†] Attempted reduction of azide **225** by catalytic hydrogenation gave a complex product mixture with no clear signs of the target.

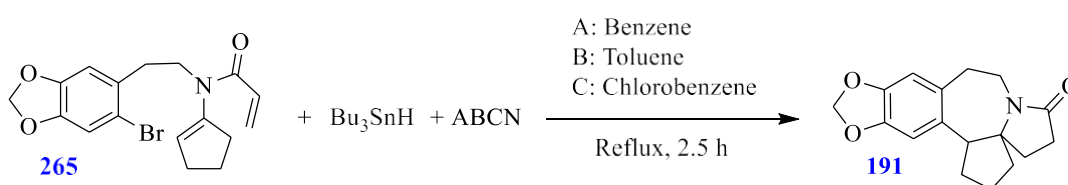


Scheme 58 Synthesis of radical precursor **265**.

^aCrude yield calculated by NMR.

Treating enamide **265** with a degassed solution of tributyltin hydride with 1,1'-azobis(cyclohexanecarbonitrile) (ABCN)[‡] in boiling toluene (**A**, **Scheme 59**), according to the published procedure, gave the desired cyclised product **191** but only in trace yield. The bulk of the reaction crude was found to correspond to reduction products (by ¹H NMR spectroscopic analysis).

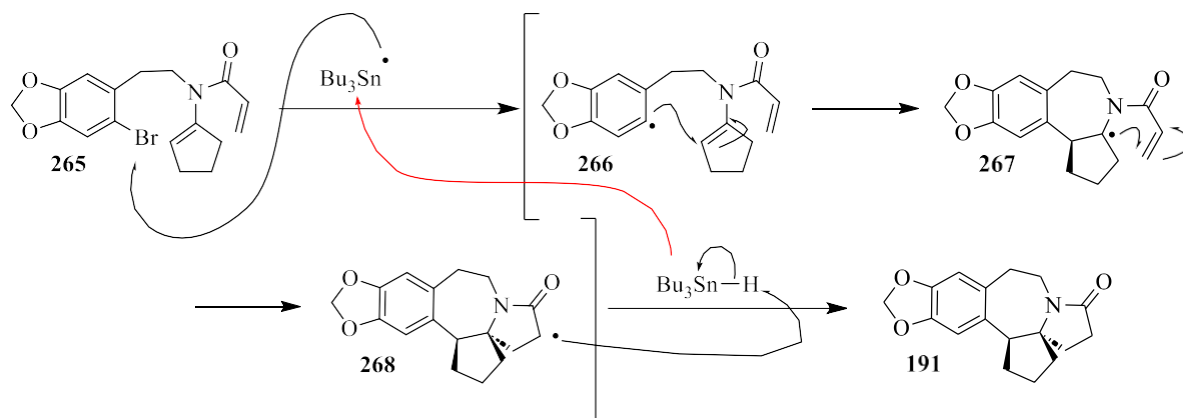
Considering that the methyl hydrogen atoms of the toluene solvent could be abstracted by reactive radical intermediates,¹⁵² the reaction was repeated in boiling benzene (**B**, **Scheme 59**) but the lower reaction temperature stopped the reaction from progressing. Finally, repeating the reaction in boiling chlorobenzene (**C**, **Scheme 59**)¹⁵³ gave more than satisfactory results, with the desired lactam **191** being the major product, and with an isolated yield 2.2 times greater than that reported (70% instead of 32%).



Scheme 59 Study of the reaction solvent and temperature for the tandem radical cyclisation leading to pentacyclic lactam **191**.

[‡] ABCN has a 10 h half-life in benzene at reflux.

The radical cascade is proposed to initiate with a 7-*endo* cyclisation, affording radical **267**, which then undergoes (Baldwin disfavoured) 5-*endo-trig* cyclisation to generate intermediate **268** *en route* to lactam **191**.

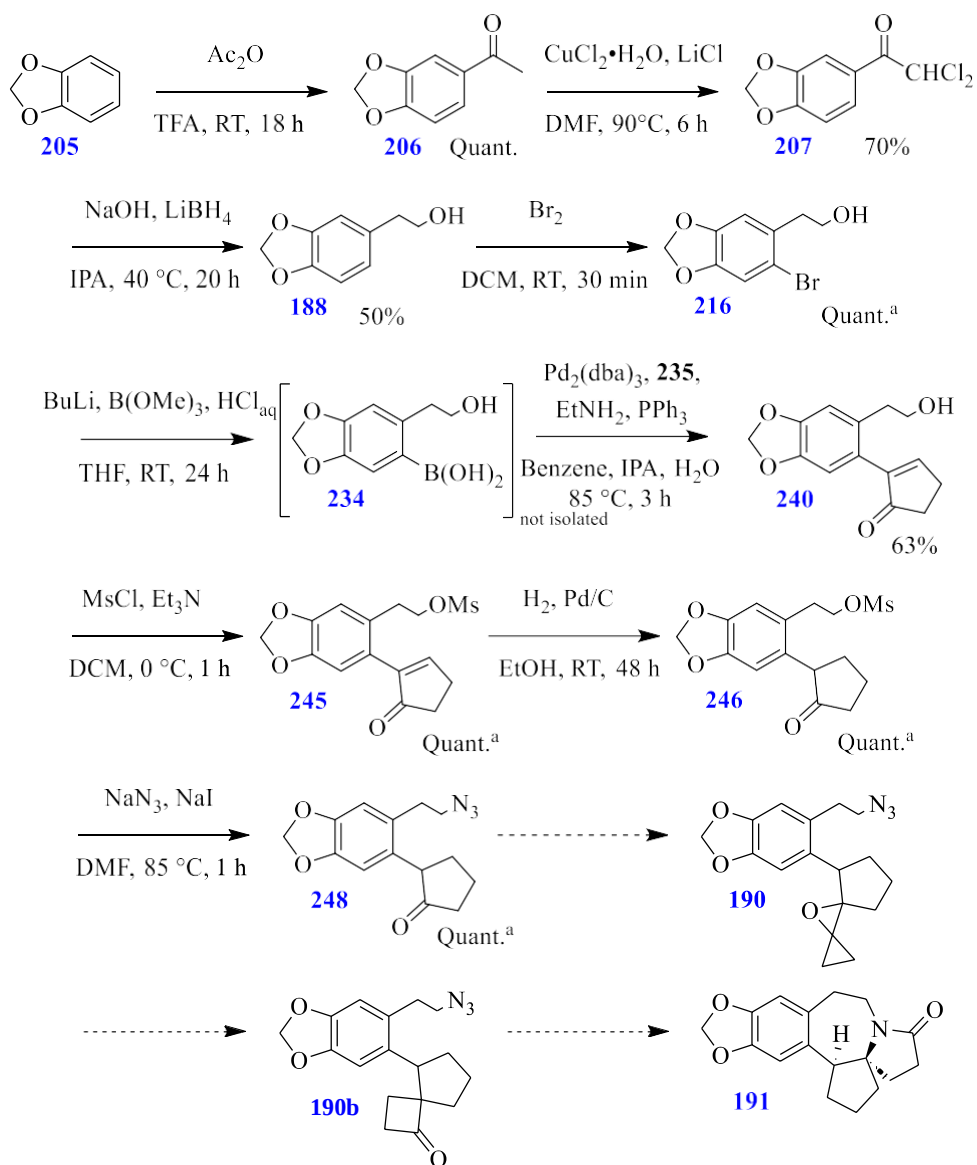


Scheme 60 Proposed radical mechanism to afford lactam **191**.

Several purifications by column chromatography were necessary in order to afford the lactam completely free from impurities that might otherwise interfere with the enzymatic screening. In particular, all tin species needed to be removed as they are highly toxic to cells and interfere with enzymes.

Conclusion Chapter 3

Complications in forming the oxaspirocyclopentanone structure and hence the cyclobutanone required to test the intramolecular Schmidt rearrangement meant that the new synthetic route remains unfinished (summary presented in **Scheme 61**). Nevertheless, the target lactam **191** was prepared by a published method, and the efficiency of the final radical reaction was greatly improved by replacing the recommended solvent (toluene) with chlorobenzene. The final goal of this project was now within reach, and the results of the primary enzymatic screening study are the focus of **Chapter 4**.



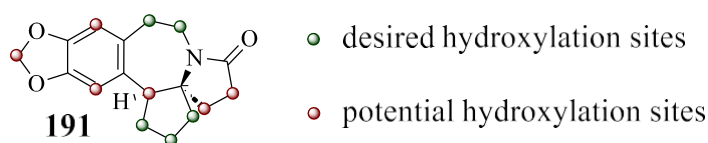
Scheme 61 Summary of the synthesis to intermediate **248** towards lactam **191**.

^aCrude yield calculated by NMR.

Chapter 4 Late-Stage Diversification of Lactam **191**

Projects Aims and Context

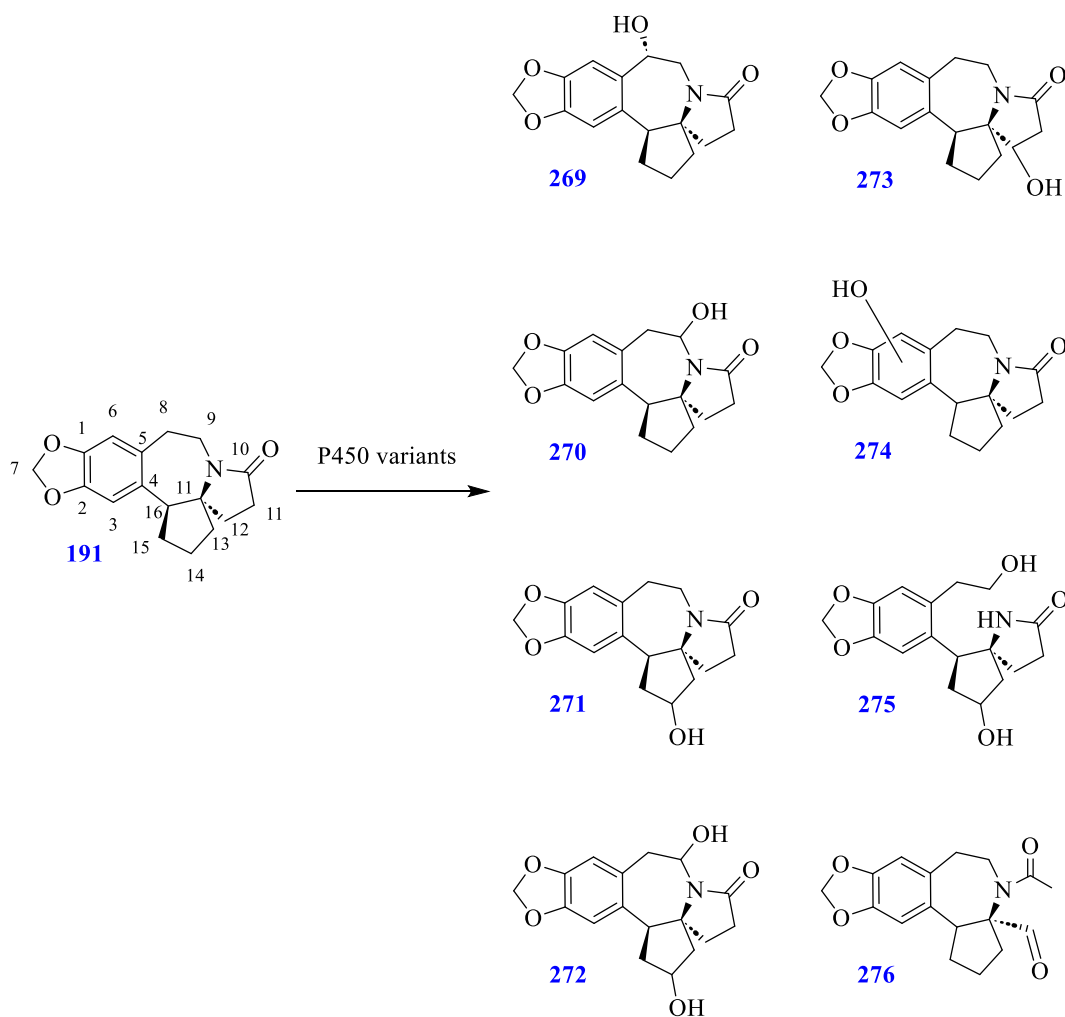
This fourth chapter describes a study which built upon the synthetic chemistry results in the previous one; that is, from the cephalotaxine core skeleton **191**. This compound possesses the carbon skeleton of the alkaloid, and thus offers a unique chance to study late-stage diversification (see **Chapter 1** for details) within the cephalotaxine alcohols family, thanks in no little part to P450_{BM3} and its variants' activity. Indeed, the diversification means adding a new reactive handle to the skeleton, but in **191** the lower cyclopentane is hardly activated (apart from the benzylic position), and many of its (sp³) C-H bonds will resist reaction with traditional synthetic methods (see **Chapter 1** for details, and **Scheme 62** for desired hydroxylation sites); however, they should be within the reach of suitable enzymes, as many have shown before.^{18,24,154} Lactam **191** was thus screened against the extensive enzymatic library that the Wong group has built over the years, giving precious insights into the reactivity of compound **191** in this new synthetic context. It was intended for the most promising enzymes to be scaled-up to enable isolation and characterisation of new products of interest, placing new branches in the *Cephalotaxus* alkaloids' family tree.



Scheme 62 Cephalotaxine core lactam **191** and its potential sites of hydroxylation.

Relevance of each characterised metabolite within *Cephalotaxus* alkaloids class

To put the following section's results into the context of the *Cephalotaxus* alkaloids, each identified metabolite is linked to one of the existing alkaloids or an intermediate within their synthesis, as the only difference between lactam **191** and cephalotaxine is the oxidation state of three of its carbons (positions 10, 14 and 15). Thus, each potential alcohol is one step closer to the base member of the *Cephalotaxus* alkaloids family. The identified metabolites' structures are presented in **Scheme 63**, and detailed below.



Scheme 63 Identified metabolites from P450 oxidation of **191**.

The first alcohol isolated during this work was compound **269** (isolated with a KSK191 A82M scale-up), with the hydroxo group at its benzylic position. Despite this position being, in general, known to be activated by means other than enzymatic (notably by photochemistry,¹⁵⁵ or via the action of transition metal oxidants¹⁵⁶), this result presents a new *Cephalotaxus* alcohol, and is tied to the important C-8 oxygenated sub-family of alkaloids. From it many alkaloids are derived, such as drupacine, drupangtonine, 8-hydroxy-cephalotaxine, torryafargesine, cephalocyclidin, and many others.^{158–160} NMR data* were key to determine its structure, as its exact mass measurement indicated an alkene, most likely due to it being easily dehydrated upon protonation in the mass spectrometer. ¹H-¹H COSY correlations between the *CHOH* resonance and those for the protons α -to the nitrogen (at C-9) sealed the structure. The relative configuration is thought to be *S* because the *CHOH* resonance appears as a triplet in ¹H NMR ($J = 8$ Hz), more consistent with the structure with two equivalent C-H dihedral angles; in contrast, the *R* configuration ($\theta \sim 180^\circ$ and 60°) would give rise to a double-doublet resonance. This metabolite was found to be fairly unstable and decomposed after a few days at room temperature and would need to be used quickly in subsequent manipulations.

The second metabolite of interest is the C-9 oxidised molecule **270**, obtained from a scale-up reaction with variant KSK19 A82M A264G A328G. This metabolite was, unsurprisingly, one of the most common found (see screening results in the next sub-chapter) since the neighbouring heteroatom imparts enhanced reactivity to the C-H bond. Again, NMR spectroscopic data* uncovered this structure, confirmed by HRMS, by comparison of the C-9 protons (still coupling to the more shifted benzylic ones, but now integrating for one proton) in the metabolite and those in substrate **191**.

* ¹³C NMR and derived 2D spectra could not be acquired due to the high-field NMR machines being out of use for several months over the summer, up to the point of writing. Measures have been taken to ensure their acquisition when the machines are back in service. At the time of writing, this project has been taken up by Dr Ziyue Xiong at OSCAR (the Oxford Suzhou Centre for Advanced Research), and ¹³C NMR spectra for alcohols **270** and **271** have been acquired, sent, and have confirmed the structures.

Though the stereochemistry of this metabolite could not be elucidated in time, equilibrium to the more stable epimer through a rapid reversible ring-opening and ring-closure is expected to take place. Its stability is higher than that of **269** as it survived several days at room temperature.

This type of structure is rather unheard of in the field of *Cephalotaxus* compounds, most of the work being focused on the positions 8, 9, 13, 14, and 15.^{60,64} This represents a great opportunity to measure its bioactivity, and create even new analogues of the known esters (such as the anti-leukemia homoharringtonine, see **Chapter 1**) and test their own activity; however due to time constraints this work will need to be done in the future.

A third and more interesting metabolite is alcohol **271**, with its 14-position oxygenated, also obtained in a scale-up reaction with variant KSK19 AM AG AG. Its structure was elucidated by comparison with close known structures,¹⁶¹ and by 2D NMR* which indicated coupling between the C-14 proton and those at C-15, which in turn coupled to the distinctive C-16 proton. This metabolite is extremely interesting as its ketone counterpart, which can be easily obtained with Dess–Martin periodinane¹⁶² preceded by amide reduction,¹⁶³ is a known intermediate of cephalotaxine,¹⁶⁴ and can also be found in Fan's investigations on the *Cephalotaxus* alkaloids.¹⁶⁵

With the insights provided by the last two results, identification of the product obtained with GV A184I was straightforward. Indeed, resonance patterns from both compounds were present in the ¹H NMR spectrum (4.6 ppm, t for the C-14-alcohol, and 5.5 ppm, dd for the C-9-alcohol) but, with only two resonances for the dioxole bridge (5.9 ppm, one doublet for each diastereotopic proton), the conclusion of a single compound containing both functionalities was justified (along with ¹³C NMR resonances for two alcohols); this was further cemented with an accurate mass consistent with a diol-type structure. This metabolite was obtained by lowering the substrate to enzyme ratio from 500:1 to 100:1, which led unexpectedly to double oxidation. This diol **272** is valuable both by virtue of its new twin moieties, and by the presence

of its di-ketone analogues in many syntheses of cephalotaxine (Hanaoka's intermediate most notably).^{76,166-168} Again, amide reduction and oxidation of the hydroxy groups would afford known intermediates, offering another formal synthesis of cephalotaxine.

The next isolated oxidation product is **273**. This insertion site, rather than the other lactam ring (C-11) position, tends to be susceptible because the active ferryl intermediate may be viewed as an "electrophilic" radical, which should prefer to attack electron-rich sites, hence at C-12 rather than at C-11. Data to support this structure are mainly from the ¹H NMR spectrum, as the resonances for the cyclopentane and the C-8 and C-9 positions were intact, yet the measured accurate mass was only compatible with a single alcohol moiety. The coupling (from the COSY spectrum) between the *CHOH* proton and the carbonyl-adjacent methylene established oxidation at C-12. Functionalisation at this position is completely new among the *Cephalotaxus* compounds, and further investigations into its derivatives' bioactivity would add precious insights into the pharmacokinetics of the wider family.

Finally, three more compounds were assigned tentatively, on the basis of the NMR data*, though their structure is loosely confirmed because of a lack of more concrete evidence and the low amounts of material (these structures are at best an educated guess and should be treated as such in future discussions). The first one, **274**, according to proton ¹H NMR analysis, also from KSK19 AM AG AG, is thought to contain a phenol moiety in addition to the benzodioxole, as only one of the aromatic peaks is visible in the ¹H NMR spectrum; the rest of the NMR spectrum also suggested no other oxidation but the isolated quantity was insufficient for convincing ¹³C NMR data, meaning that this assignment could not be confirmed. This type of reactivity is common with electron-rich aryl systems and was, for example, explored in previous work by Harwood²⁴ in the context of the aromatic moiety of the trigoxyphin core. It is, however, an infrequent mode of reaction for the Wong group's enzyme library, and worthy of further investigation, notably to identify the site of the phenol position (C-3 or C-6). The *Cephalotaxus* family has no member with an aromatic moiety differing from the benzodioxole,

so these new phenolic structures might possess interesting or unseen bioactivity, and should be explored in the future.

The second one, **275**, seems to be correlated with diol **272**, as its production is proposed to arise from reduction (made possible by the overall reductive environment created by glucose, GDH, and NADP⁺) of the ring-opened aldehyde in equilibrium with the C-9 alcohol. In the ¹H NMR spectrum, a complex 1H multiplet at 4.4 ppm and a 2H triplet at 3.6 ppm indicated the presence of the 2° and 1° alcohols, respectively. This type of structure, with a spiro moiety and a free side chain, is rarely seen within previous syntheses of this type of alkaloids (see Fuchs' and Danishefsky's approaches in Project Aims and Strategy subchapter in **Chapter 3**). Most often, the spiro motif is installed beforehand, there is no side chain and the azepane ring is formed by reacting with the benzenoid core.^{115,116,169,170} The structure of this metabolite is loosely related to that of (–)-amathaspiramide F, a marine alkaloid under scrutiny for potential bioactivity.¹⁷¹

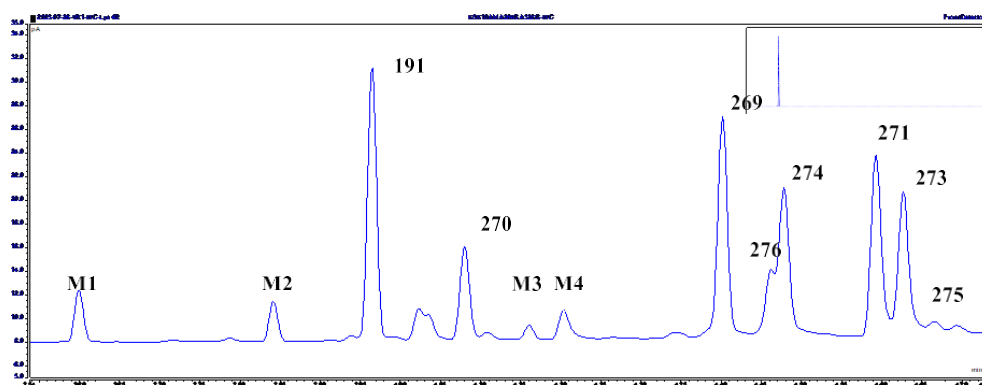
The final one to be tentatively identified is aldehyde **276**, based mainly on the singlet aldehyde peak at 9.4 ppm. Being a singlet means the aldehyde is next to a 4° centre, plus an untouched benzodioxole moiety (same s-s-dd resonance pattern) means the only possible 4° centre is C-11 with the aldehyde at C-12. It is proposed that this metabolite arises from retro-aldol reaction of metabolite **273**; however, the acetamide methyl resonance was not clearly seen due to contamination in the 2-ppm region of the ¹H NMR spectrum and this structure remains unconfirmed. Assuming a correct supposition, an intermediate with a similar structure can be found in Yang's synthesis of cephalotaxine (compound **16**).¹⁷²

Screening Results

First, lactam **191** was screened against 46 enzymes (2 24-wells plates, labelled Common Plate 5 and 6), each presenting different mutations, exhibiting a wide array of potential

selectivity, ideal to identify active mutations from which to build upon. The procedure for the screening process is presented in the Appendices. A screening takes place in a 24-well plate, already containing the desired variants, in which are added glucose, NADPH, GDH, and phosphate buffer, and then a solution of lactam **191** (200 μ M solution) dissolved in ethanol (the substrate to enzyme ratio was 250 to 1 in all screenings to the exception of Common plate 5, which was 500 to 1).

A typical gas chromatogram is presented **Scheme 64**. Some compounds, despite a relatively high intensity on the chromatogram, could not be isolated (**M1** to **M4**) and so their structures are unknown. The peak at 4.02 min is attributed to an impurity in the substrate as it is present throughout every reaction. Diol **272** was only seen once, with a retention time of 5.14 min.



Scheme 64 Gas chromatogram of KSK19 AM AG AG variant against **191**.

Vertical axis : intensity (pA, arbitrary unit).

Horizontal axis : retention time (minutes).

Following screening, the various metabolites were identified from products formed in scale-up reactions (usually with 5 to 10 mg of substrate, compared to the screening scale, which was 0.005 mg of substrate per well) with P450_{BM3} variants chosen either for their conversion or their selectivity profile. These first screening results are presented in **Tables 9** and **10**, obtained following **General Procedure A**. Other unidentified products are designated by their retention time. Potential missed aspects of selectivity arise from unidentified products whose GC retention times are close to the solvent and therefore unresolved.

Table 9 Common Plate 5 screening results.

Entry	Enzyme variant	Conversion (%)	Outcomes (% selectivity)						
			269	270	271	273	274	275	276
A	RL YF KSK19 A184I	86	7	76	0	12	0	5	0
B	KSK19 A82M	73	47	13	16	11	7	2	4
C	GV A184I	72	3	49	39	1	3	3	2
D	RK A328G	72	0	83	6	4	2	3	2
E	S72A A82M F87A A184I T260G E435M	45	24	52	24	0	0	1	0
F	KSK19 A82M I263G	35	25	7	5	27	24	0	12
G	RKAI TG A328G	32	10	69	18	0	0	3	0
H	KT2 L188G I263G	20	0	100	0	0	0	0	0
I	K19 F87V	19	5	60	35	0	0	0	0
J	K19 F87V Q403P	15	0	60	40	0	0	0	0
K	RKA	12	0	95	0	5	0	0	0
L	RT2 V78I A184I A330P	11	20	80	0	0	0	0	0
M	KSK19 I263A	10	0	100	0	0	0	0	0
N	KU3 AP S72W	10	0	100	0	0	0	0	0
O	RK I263G	10	0	100	0	0	0	0	0
P	RT2	10	0	100	0	0	0	0	0
Q	KU3 AP IA	9	0	100	0	0	0	0	0
R	R19	9	0	36	64	0	0	0	0
S	R19 FI LLV	9	0	100	0	0	0	0	0
T	GVQ	8	0	100	0	0	0	0	0
U	R19 FI	8	0	100	0	0	0	0	0
V	RP H171L I263G	7	0	100	0	0	0	0	0

Table 10 Common Plate 6 screening results.

Entry	Enzyme variant	Conversion (%)	Outcomes (% selectivity)						
			269	270	271	273	274	2.7 min	276
A	K19 FV IG	100	0	0	0	0	0	100	0
B	R19 SG A330W	77	3	84	4	4	0	4	2
C	GQ I263G A328L A330L S332F	69	38	8	14	14	7	16	2
D	R19FI L437F	29	0	0	0	0	0	100	0
E	AM FA	28	0	27	8	0	0	65	0
F	WT	26	0	100	0	0	0	0	0
G	K19 F87V E267V V78I	24	0	71	7	0	0	22	0
H	RP HL IG A184I	22	0	69	18	0	0	14	0
I	R19FI L437F	19	0	0	0	0	0	100	0
J	R19 F87I L437LV	18	0	100	0	0	0	0	0
K	GLQ IG A328G	17	0	29	9	0	0	61	0
L	GVQ A328G	15	0	0	0	0	0	100	0
M	RK F81W TG A328G	14	0	34	66	0	0	0	0
N	VQ SG AW	14	0	69	12	0	0	18	0
O	GVQ A264G	13	0	63	37	0	0	0	0
P	R19 A328L I263A	13	0	0	0	0	0	100	0
Q	RP A82M I263A	13	0	0	0	0	0	0	0
R	RK T260G	12	0	69	0	0	0	31	0
S	RK A328G P329G A330G	11	0	0	0	0	0	100	0
T	GVQ I263G A328L	10	0	0	0	0	0	100	0
U	GVQ IG	0	0	0	0	0	0	0	0
V	K19 F87V A264G	0	0	0	0	0	0	0	0
W	RKA S72W	0	0	0	0	0	0	0	0

The overall trend is of a lack of conversion of the substrate by these variants, with 65% of them exhibiting conversions below 20%. The main products are **269**, **270**, and **271** and, for these, the conversion is good. The other products tend to appear when the conversion is lower, implying a roughly inverse correlation of selectivity with substrate conversion. The most active variants from the screen were found to be:

KSK19 A82M, RL YF KSK19 A184I, GV A184I,
KSK19 FV IG, R19 SG A330W,

with a clear pattern of KSK19 and AI mutations being key. To further probe this apparent pattern, lactam **191** was screened against a plate of enzymes based on the KSK19 A82M variant previously prepared by Barros Metlova, a member of the Wong group. Results are compiled in **Table 11**.

Table 11 Screening results for a second-generation panel derived from KSK19 AM.

Entry	Enzymes	Conversion (%)	Outcomes (% selectivity)							
			269	270	271	273	274	275	276	4.57–4.75 min
A	KSK19	100	0	2	0	0	0	0	0	98
B	KSK19 AM L262G	99	42	12	20	8	18	3	3	0
C	RL YF KSK19	99	4	21	0	0	0	0	0	75
D	KSK19 A82M A264G A328G	98	10	3	45	21	35	8	6	0
E	KSK19 AM A330W	97	42	7	16	7	2	6	23	0
F	KSK19 AM A264G	96	38	2	24	14	13	1	13	0
G	KSK19 AM S270G	95	52	3	22	6	20	0	3	0
H	KSK19 AM	94	43	13	21	9	16	2	2	0
I	KSK19 AM I263G	91	53	22	7	6	6	0	6	0
J	KSK19 AM F261G	79	46	10	20	14	8	2	4	0
K	KSK19 AM T268S	78	47	27	10	8	5	0	4	0
L	KSK19 AM T260G	75	43	16	30	2	3	1	7	0
M	KSK19 AM I401M	63	53	15	14	8	5	1	5	0
N	KSK19 AM I263W	38	24	56	9	10	0	0	0	0

It is immediately clear that these KSK19 AM variants are highly active, with only one variant exhibiting conversion lower than 50% (KSK19 AM I263W, entry **N**), possibly due to the bulkier tryptophan substitution of isoleucine restricting substrate access to the heme. This screening revealed a plethora of products, and scale-up reactions with some of these variants

enabled the identification of seven metabolites (entries **A,D**). The variants chosen for scale-up were so due to a combination of factors: availability, diversity of expression, and a balance between conversion and selectivity. The column designated “4.57–4.75 min” represents a cluster of four close peaks in the GC trace whose products could not be isolated despite several scale-up reactions with KSK19. Their relatively longer retention time (4.6–4.7 min) could hint at poly-oxidation products. Characterisation of these metabolites will be a key objective for future work.

The substrate was also screened against another pre-prepared enzyme plate based on variant K19 F87V as these enzymes were found in recent results by the Wong group to be active with spirolactams. **Table 12** shows that these mutations did not afford higher conversions (30% at best in entry **C**), and mainly gave products **270** and **271**.

In this plate two further variants based on A82M (entries **A** and **B**) were added, as this was an active mutation, as seen in the preliminary screenings (with KSK19 A82M, entry **B** **Table 9**), and this gave an opportunity to test this mutation in isolation. The conversion was higher and the selectivity profile close to KSK19 A82M. This, plus the results of KSK19 alone, mean that the A82M mutation, rather than KSK19, is crucial for the activity observed in the third screening. These results offer a key insight into the interactions between the substrate and P450 variants.

All the above screening and scale-up reactions were conducted with harvested enzymes, in which the cells have been sonicated and their cytoplasm removed to afford a crude isolate of the enzymes without other compounds. To better understand how the substrate would behave in a biological environment (as the final goal of this project would be to afford new bioactive compounds), it was decided to screen the substrate against whole cells and lysate. “Whole cell reactions” are those in which the enzymes are still inside the *E. Coli*, and “lysate reactions” refer to the use of a medium containing *E. coli* cells whose walls have been disrupted by

sonication, but with no purification steps so that the cytoplasmic content is present in the reaction medium.

Table 12 Screening results for a third panel derived from K19 FV

Entry	Enzymes	Conversion (%)	Outcomes (% selectivity)					
			269	270	271	273	274	276
A	A82M F87A	96	46	11	12	4	15	4
B	A82M F87A A184I	83	39	34	31	13	14	4
C	K19 F87V Q403P	30	0	58	59	0	0	0
D	K19 F87V	29	0	78	78	0	0	0
E	K19 V78I F87V	26	0	81	81	0	0	0
F	K19 F87V I263G	17	12	73	82	0	0	0
G	K19 FV I263V	12	0	75	75	0	0	0
H	K19 F87V E267G	8	0	100	100	0	0	0
I	K19 FV I263G A264G	7	0	100	100	0	0	0
J	K19 FV A184V	5	0	100	100	0	0	0
K	K19 F87V A264G	4	0	87	100	0	0	0
L	K19 F87V A328V	2	0	53	100	0	0	0
M	K19 F87V E267V	2	0	37	37	0	0	0
N	K19 F87V A328I	0	0	0	0	0	0	0
O	K19 FV I263G	0	0	0	0	0	0	0

By comparing the reactions of isolated enzymes with whole-cell and lysate reactions, the cell permeability of the substrate can be tested and possible side-reactions with cytoplasmic contents identified. Furthermore, the concentration of enzymes in the cell is locally higher than in a harvested reaction, sometimes resulting in improved conversion. Interactions with cytoplasmic medium can also modify the enzyme's activity and can lead to different selectivity profiles, or even new compounds.

Two very active and diverse variants (KSK19 and KSK19 AM AG AG) were chosen for this test. The results presented in **Table 13** support the substrate's ability to cross the cell's

walls. Lysate appeared to be less active than whole cells and harvest, presumably from side-interactions with the rest of the cytoplasmic contents once liberated from the cellular environment. As evident in the reactions of KSK19 AM AG AG, whole cell conditions push conversion when it is not already complete; however, the selectivity profiles are the same across the three types of “enzyme delivery”. For this reason, the KSK19 AM AG AG scale-up was carried out with whole cells rather than harvested enzymes (entry **D**).

Table 13 Cell permeability tests and enzyme delivery medium comparison.

Entry	Enzymes	Conversion (%)	Outcomes (% selectivity)							
			269	270	271	273	274	275	276	4.57–4.75 min
A	KSK19-Whole Cell	100	0	4	0	0	0	0	0	96
B	KSK19-Lysate	97	0	4	0	0	0	0	0	96
C	KSK19-Harvest	100	0	4	0	0	0	0	0	96
D	KSK19 AM A264G A328G-Whole Cell	83	25	9	21	18	19	2	0	0
E	KSK19 AM A264G A328G-Lysate	63	25	9	21	18	19	2	0	0
F	KSK19 AM A264G A328G-Harvest	72	25	9	21	18	19	2	0	0

Whole cells are less freezer-stable than harvested enzymes and so they needed to be re-grown for the scale-up reactions. An appropriate colony, grown and mutated from competent cells²⁴ by Barros Metlova (a Wong group member), was picked, inoculated, and induced to produce a batch of whole cells with the correct enzyme variant. Monitoring the temperature during these steps is crucial, as a 37 °C temperature, required for the inoculation, is detrimental for the production of competent enzymes during the induction period (at 20 °C), often resulting in protein misfolding and lack of activity.

A final screening was conducted, based on further KSK19 AM variants and mutated K19, as K19 is a base residue within KSK19, with the results compiled in **Table 14**. The reactions of the KSK19 AM based variants continued the trend of high activity evident in **Table 11**, with the K19 variants exhibiting generally good conversions; however, the selectivity shifted towards the production of unidentified products eluting (on GC) just before the substrate. From past experience in the Wong group^{20,86,87} it was assumed that these metabolites are dehydrated compounds (i.e. alkenes arising from hydroxylation and elimination, overall a dehydrogenation); however, without additional data this assumption was not able to be confirmed. Further work will be necessary to elucidate their structure.

Table 14 Final screening results with a set of KSK19 AM and K19 variants.

Entry	Enzymes		Conversion (%)	Outcomes (% selectivity)							3.7 min
				269	270	271	273	274	275	276	
A	KSK19 A330W	A82M	86	0	7	0	0	0	0	0	93
B	KSK19 I401M	A82M	90	31	6	11	11	14	1	13	27
C	KSK19 A264G 2	A82M	72	38	11	7	6	6	1	18	19
D	K19 V78I F87V		61	0	39	0	0	0	0	0	61
E	K19 FV I263V		54	0	48	0	0	0	0	0	52
F	K19 F87V E267G		46	29	8	4	4	1	0	1	54
G	K19 FI A184I LLV		17	0	0	0	0	0	0	0	100

Conclusion Chapter 4

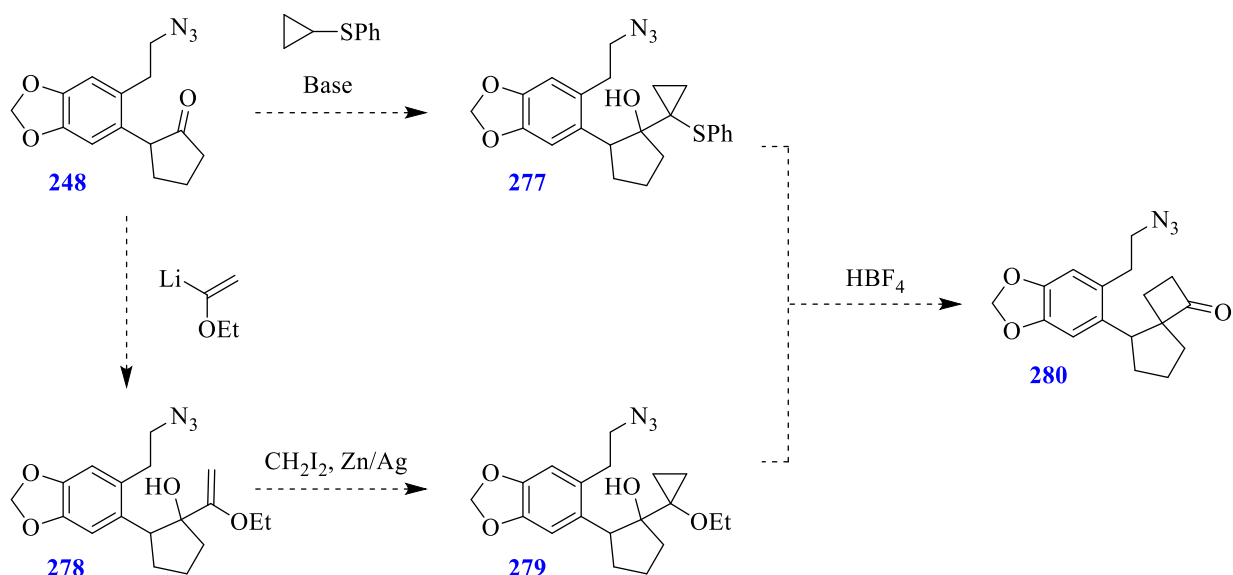
In collaboration with members of the Wong group, a wide panel of enzymes have been tested, outlining the general reactivity of lactam **191** against P450_{BM3} variants. The most active mutations were identified and built upon, offering high conversion and/or high selectivity for all characterised alcohols, and a solid basis for potential subsequent chemical transformations. Future work would include an investigation of kinetic resolution processes operating on the

racemic substrate by establishing chromatographic assays for the enantiomers of both the substrate and products. To probe this issue, future work will study the less activated dimethoxy analogue, knowing that the di-*O*-methyl ethers can be efficiently deprotected and converted into the dioxole bridge.⁶⁷ The P450_{BM3} metabolites of lactam **191** form a new sub-family of the *Cephalotaxus* alkaloids genre, and open the way towards new bioactive ester derivatives.

Chapter 5 – Summary and Future Work

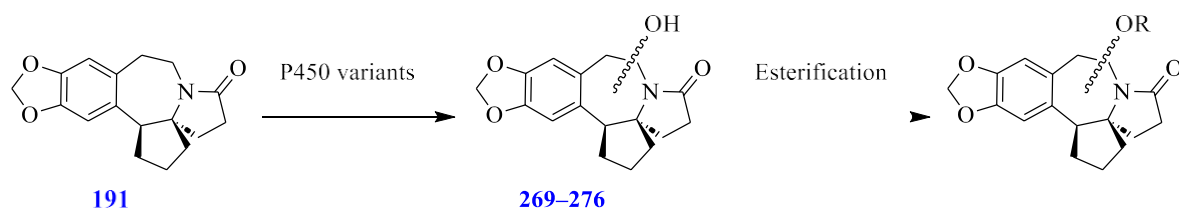
The original goal of the first project, the C–H amination of azepanol to create the core of balanol, could not be completed, yet a better idea of the substrates' behaviour within the types of reactions tested was formed. With future developments in chemical methodology, new C–H amination reagents may be discovered whose substrate scope will allow the consecutive 1,n-oxyamination of unactivated 1,n-methylene positions to be achieved.

The synthetic goal of the second project, a new synthesis of lactam **191**, fell two steps short (out of 11) of reaching completion and, due to a lack of time at the end of the project, two proposed solutions^{173,174} (**Scheme 65**) could not be tested. A previously reported method¹¹⁷ was greatly improved (from 32% to 70% yield for the key transformation, the radical cascade) which allowed the final part of this second project to be tackled.



Scheme 65 Future work towards cyclobutanone **280**.

The activity profile of lactam **191** and P450 variants has been mapped out by the results summarised in **Chapter 4**, with up to 117 different variants tested, along with a short cell permeability study. Several new compounds were isolated, enriching the family of *Cephalotaxus* alkaloid derivatives with several members, offering new routes to known intermediates in reported total syntheses, and opening the way to bioactive ester derivatives (**Scheme 66**). Future work would include further enzymatic screening, with a different library to further increase conversion and selectivity, allowing the isolation and characterisation of low-abundance metabolites and elimination products; a study of their kinetic resolution; esterifications of the newly-acquired alcohols; and an assessment of their bioactivity. Dr Ziyue Xiong, at OSCAR, is continuing this project at the time of writing.



Scheme 66 Final chemical transformation towards bioactive *Cephalotaxus* esters.

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Chapter 6 – Procedures and Characterisation

General Experimental Details

All solvents were used as commercially supplied or were dispensed from solvent drying units in the Chemistry Research Laboratory. All reagents were used as commercially supplied, unless stated otherwise.

Flash column chromatography (FCC) was performed on Merck Silicagel60, particle size 40–63 μm . Analytical thin-layer chromatography (TLC) was conducted on Merck aluminium-backed Silicalgel DC60 F254 0.2 mm pre-coated plates, with visualisation via ultra-violet light ($\lambda_{\text{max}} = 254 \text{ nm}$) or TLC staining agents (phosphomolybdic acid, basic potassium permanganate, or vanillin). Retention factors (R_f) are quoted with the solvent system used for TLC, unless stated otherwise.

Media components, kanamycin and IPTG were supplied by Melford Laboratories, UK; Lysozyme was from Sigma-Aldrich, NADP^+ was from Proxomix and glucose dehydrogenase (GDH) was from Codexis, USA. Oligonucleotides were supplied by Eurofins Genetic Service, UK. Standard phosphate buffer used as reaction media in biotransformations (2.4 g KH_2PO_4 and 31.7 g K_2HPO_4 dissolved in 1 L deionised H_2O , adjusted to pH 7.9 before use).

^1H and ^{13}C NMR spectra were recorded at 295 K, using Bruker AVF-400, AVG-400, AV600, AV700, or NEO600 machines as specified, in deuterated solvents. Chemical shifts (δ_{H} , δ_{C}) are quoted in parts per million (ppm, to the closest 0.01 ppm, and 0.1 ppm for ^1H and ^{13}C , respectively), and referenced during processing in MestReNova to non-deuterated solvent traces (7.26 ppm for ^1H NMR and 77.23 ppm for ^{13}C NMR for CHCl_3 traces in CDCl_3 , for example). Coupling constants (J), are quoted in Hertz (Hz), rounded to the nearest 0.5 Hz. Signal multiplicities are described as: singlet (s), doublet (d), triplet (t), quartet (q), broad (br), or multiplet (m), and combinations. Assignments were made with the assistance of 2D (COSY,

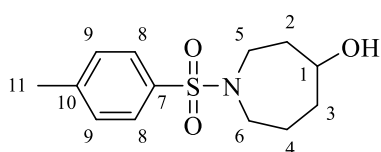
HSQC, HMBC, and NOESY) NMR spectra. Where an assignment could not be made unambiguously, proposed assignments are listed.

Infrared spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer. Absorption maxima ($\nu_{\max}$) are reported in wavenumbers (cm^{-1}). Low resolution mass spectrometry (LRMS) was conducted on a Waters LCT Premier (ESI). High resolution mass spectrometry (HRMS) was conducted on a Bruker Daltronics MicroTOF spectrometer (ESI). Mass to charge ratios (m/z) are reported in Daltons. Accurate mass was measured on an Agilent 5977B GC/MSD (EI).

Compound names were as generated by CambridgeSoft ChemBioDraw Ultra 18.2.

Procedures and Compounds, Chapter 2

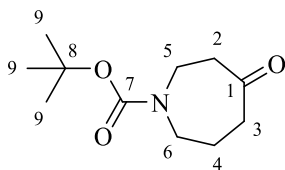
1-tosylazepan-4-ol **135**



Synthesised by analogy to a reported procedure.¹ To a solution of ketone **152** (1.13 g, 4.23 mmol) in methanol (27.5 mL), NaBH_4 (80.5 mg, 2.13 mmol) was slowly added. The solution was stirred at room temperature for 40 min, concentrated *in vacuo*, and quenched with sat. aq. NH_4Cl (25 mL). The solution was extracted with EtOAc (4×30 mL); the combined organic layers were dried (MgSO_4), filtered, and concentrated *in vacuo* to afford the title compound as a white solid (1.16 g, quant.). **R_f** 0.32 (1/50 MeOH/DCM); **¹H NMR** (400 MHz, CDCl_3) δ 7.71 – 7.63 (m, 2H, H-8), 7.33 – 7.27 (m, 2H, H-9), 3.94 (tdd, $J = 8.0, 4.0, 2.5$ Hz, 1H, H-1), 3.42 – 3.12 (m, 4H, H₂-5/H₂-6), 2.42 (s, 3H, H₃-11), 2.07 – 1.53 (m, 6H, H₂-2/H₂-3/H₂-4); **¹³C NMR** (101 MHz, CDCl_3) δ 143.3 (C7), 136.3 (C8), 129.9 (C9), 127.2 (C10), 70.3 (C1), 47.9 (C5), 42.8 (C6), 38.0 (C2), 35.0 (C3), 22.7 (C4), 21.7 (C11).

Data consistent with literature.²

tert*-Butyl 4-oxoazepane-1-carboxylate **148*

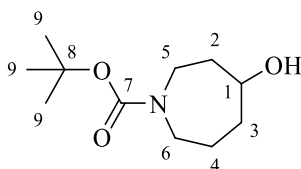


Synthesised following a reported procedure.¹ To a solution of ethyl diazoacetate (2.80 mL, 26.7 mmol), BF₃.Et₂O (3.21 mL, 25.3 mmol), and diethyl ether (100 mL) was added dropwise 1-boc-piperidone (4.00 g, 20.0 mmol) at -25 °C. The solution was stirred at -25 °C for 18 h. Sat. aq. NaHCO₃ (50 mL) was cautiously added, the solution allowed to warmed to room temperature, and stirred for 1 h. The solution was extracted with EtOAc (4 × 50 mL); the combined organic fractions were washed with brine (10 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo* to afford the crude product (5.4 g).

The crude product was diluted in MeOH (11 mL), and NaOH (553 mg, 13.8 mmol) was added. The solution was stirred at reflux for 3 h. Aq. HCl (12 mL, 1 M) was added and the solution was warmed to room temperature, extracted with diethyl ether (3 × 20 mL); the combined organic layers were washed with water (20 mL) and brine (20 mL), dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the title compound as a white powder (1.56 g, 61%). **R_f** 0.32 (1/100 MeOH/DCM) ; **¹H NMR** (400 MHz, CDCl₃) δ 3.55 (m, 4H, H₂-2/H₂-3), 2.62 (m, 4H, H₂-5/H₂-6), 1.82 – 1.73 (m, 2H, H₂-4), 1.43 (s, 9H, H₃-9) ; **¹³C NMR** (101 MHz, CDCl₃) δ 212.1 (C7), 154.6 (C1), 80.2 (C8), 49.8 (C5) 49.0 (C6), 44.1 (C2), 43.1 (C3), 28.6 (C9), 25.8 (C4).

Data consistent with literature.³

tert*-Butyl 4-hydroxyazepane-1-carboxylate **150*

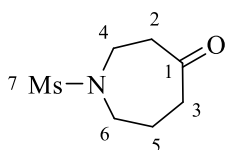


Synthesised by analogy to a reported procedure.⁴ To a solution of ketone **148** (812 mg, 3.81 mmol) in MeOH (24 mL) was added NaBH₄ (63 mg, 1.66 mmol). The solution was stirred at room temperature for 1 hour, then concentrated *in vacuo*. The resulting solid was quenched with sat. aq. NH₄Cl (20 mL),

the solution was extracted with EtOAc (4 × 40 mL); the combined organic layers were dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the pure product (820 mg, quant.). **R_f** 0.29 (1/1 pentane/EtOAc) ; **¹H NMR** (400 MHz, CDCl₃) δ 4.27 (s, 1H, H–O), 3.85 (tdd, *J* = 8.5, 4.0, 2.5 Hz, 1H, H–1), 3.43 (t, *J* = 8.0 Hz, 2H, H₂–5/H₂–6), 3.36 – 3.20 (m, 2H, H₂–5/H₂–6), 2.00 – 1.50 (m, 6H, H₂–2/H₂–3/ H₂–4), 1.44 (s, H₃–9) ; **¹³C NMR** (101 MHz, CDCl₃) δ 209.0 (C7), 155.8 (C1), 79.5 (C8), 46.4 (C5), 45.7 (C6), 37.3 (C2), 35.3 (C3), 22.3 (C4), 28.5 (C9).

Data consistent with literature.⁵

1-(Methylsulfonyl)azepan-4-one 151

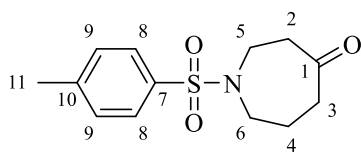


Synthesised by analogy to a reported procedure.^{1,6} To a solution of ethyl diazoacetate (2.8 mL, 26.7 mmol), BF₃.Et₂O (3.21 mL, 25.3 mmol), and diethyl ether (100 mL) was added dropwise 1-boc-piperidone (4.00 g, 20.0 mmol) at -25 °C. The solution was stirred at -25 °C for 18 h. Sat. aq. NaHCO₃ (50 mL) was cautiously added, the solution allowed to warm to room temperature, and stirred for 1 h. The solution was extracted with EtOAc (4 × 50 mL); the combined organic fractions were washed with brine (10 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo* to afford the crude product (5.4 g). Part of the crude product (4.72g) was mixed with aq. HCl (4 M, 70 mL), stirred at reflux for 6 hours, and concentrated *in vacuo* to afford the crude salt (2.60 g).

The crude product was suspended in boiling DCM (150 mL). The solution was cooled to 0 °C, and MsCl (1.33 mL, 17.3 mmol) and Et₃N (4 mL, 17.3 mmol) were added dropwise. The solution was warmed to room temperature and stirred for 20 h. The solution was quenched with water (100 mL), extracted with DCM (3 × 150 mL); the combined organic fractions were washed with brine (300 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo*. FCC (EtOAc) afforded the title compound as a colourless oil (2.58 g, 78%). **R_f** 0.39 (EtOAc) ; **IR** ν_{max}/cm⁻¹ 2931 (C–H), 1702 (C=O), 1320 (S=O), 1260 (C–N) ; **¹H NMR** (600 MHz, CDCl₃) δ 3.54 (m, 4H, H₂–4/H₂–6), 2.85 (s, 3H, H₃–7), 2.75 – 2.66 (m, 4H, H₂–2/H₂–3), 1.93 (m, 2H, H₂–5) ; **¹³C NMR** (151 MHz, CDCl₃) δ 210.3 (C1), 50.6 (C2), 45.1 (C3), 44.4 (C4), 42.5 (C6),

38.1 (C7), 25.4 (C5) ; **HRMS** (ESI+) calculated for $[M+H]^+$ ($C_7H_{14}NO_3S$) requires m/z 192.0694, found 192.0696.

1-Tosylazepan-4-one 152

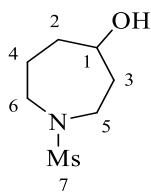


Synthesised by analogy to a reported procedure.^{1,4} To a solution of ethyl diazoacetate (2.8 mL, 26.7 mmol), $BF_3 \cdot Et_2O$ (3.21 mL, 25.3 mmol), and diethyl ether (100 mL) was added dropwise 1-boc-piperidone (4.00 g, 20 mmol) at $-25^\circ C$. The solution was stirred at $-25^\circ C$ for 18 h. Sat. aq. $NaHCO_3$ (50 mL) was cautiously added, the solution allowed to warm to room temperature, and stirred for 1 h. The solution was extracted with EtOAc (4×50 mL); the combined organic fractions were washed with brine (10 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford the crude product (5.4 g). Part of the crude product (4.72 g) was mixed with aq. HCl (4 M, 70 mL), stirred at reflux for 6 hours, and concentrated *in vacuo* to afford the crude salt (2.60 g).

Part of the crude product (1.00 g, 6.67 mmol) was suspended in THF (58 mL). Et_3N (1.88 mL, 13.3 mmol) and *para*-tosyl chloride (1.59 g, 8 mmol) were added and the solution was stirred for 24 hours at room temperature. The solution was diluted with water (20 mL), extracted with EtOAc (3×40 mL); the combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. FCC (1/1 pentane/EtOAc) afforded the title compound as an orange oil (1.13 g, 63%). **R_f** 0.32 (1/1 pentane/EtOAc) ; **¹H NMR** (400 MHz, $CDCl_3$) δ 7.72 – 7.63 (m, 2H, H-8), 7.35 – 7.29 (m, 1H, H-9), 3.46 – 3.35 (m, 4H, H₂-5/H₂-6), 2.73 – 2.66 (m, 2H, H-2/H-3), 2.62 – 2.54 (m, 2H, H-2/H-3), 2.43 (s, 3H, H₃-11), 1.94 – 1.84 (m, 2H, H₂-4) ; **¹³C NMR** (101 MHz, $CDCl_3$) δ 210.4 (C1), 143.5 (C7), 135.7 (C8), 129.8 (C9), 127.0 (C10), 50.8 (C5), 44.7 (C6), 44.4 (C2), 42.4 (C3), 24.8 (C4), 21.4 (C11).

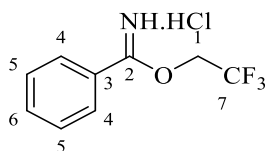
Data consistent with literature.⁷

1-(Methylsulfonyl)azepan-4-ol **153**



Synthesised by analogy to a reported procedure.⁴ To a solution of ketone **151** (1.29 g, 6.68 mmol) in dry methanol (40 mL) was added NaBH₄ (127 mg, 3.37 mmol). The solution was stirred at room temperature for 1 hour, concentrated *in vacuo*, and quenched with sat.aq. NH₄Cl (30 mL). The solution was extracted with EtOAc (4 × 40 mL); the combined organic layers were dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the title compound as a white solid (1.6 g, quant.). **R_f** 0.15 (1/50 MeOH/DCM) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 2926 (C–H), 1323 (S=O) ; **¹H NMR** (400 MHz, CDCl₃) δ 4.02 (dd, *J* = 7.5, 4 Hz, 1H, H–1), 3.52 – 3.24 (m, 4H, H₂–5/H₂–6), 2.81 (s, 3H, H₃–C), 2.11 – 1.62 (m, 6H, H₂–2/H₂–3/H₂–4) ; **¹³C NMR** (101 MHz, CDCl₃) δ 70.1 (C1), 60.6 (C7), 47.76 (C5), 42.62 (C6), 38.29 (C3), 34.96 (C2), 22.76 (C4) ; **HRMS** (ESI+) calculated for [M+H]⁺ (C₇H₁₆NO₃S) requires *m/z* 194.0850, found 194.0851.

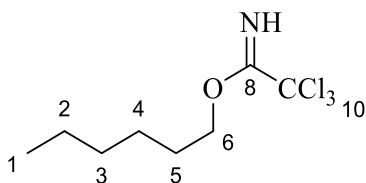
2,2,2-Trifluoroethyl benzimidate hydrochloride **154**



Synthesised following a reported procedure.⁸ To a solution of cyanobenzene (6.5 mL, 63.2 mmol) and 2,2,2-trifluoroethanol (5 mL, 69.5 mmol), HCl(g) was bubbled into at room temperature (bubbling rate: 1 bubble per second) for 8 hours in an oven-dried pressure tube. The pressure tube was then sealed with a PTFE cap, stirred at room temperature for 54 hours, and left for a week at room temperature. The solution was washed with pentane and cold acetonitrile to afford the product as a white solid (2.34 g, 15%). **¹H NMR** (400 MHz, CDCl₃) δ 8.12 – 8.05 (m, 2H, H–4), 7.67 – 7.58 (m, 2H, H–5), 7.55 – 7.42 (m, 1H, H–6), 4.70 (quart, *J* = 8.5 Hz, 2H, H₂–1).

Data consistent with literature.⁸

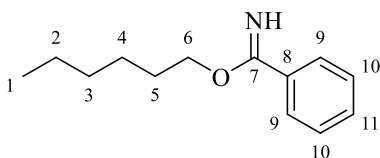
Hexyl 2,2,2-trichloroacetimidate **155**



Synthesised following a reported procedure.⁹ To a solution of 1-hexanol (1.24 mL, 10 mmol) in dry DCM (20 mL) were added DBU (0.15 mL, 0.1 mmol) and trichloroacetonitrile (1.5 mL, 15 mmol). The solution was stirred at room temperature and followed by TLC (10/1 pentane/EtOAc). After 12 h, the solution was diluted with water (15 mL) and extracted with DCM (2 × 15 mL). The combined organic fractions were washed with brine (2 × 20 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (3/20/77 Et₃N/EtOAc/ pentane) afforded the title compound as a colourless oil (1.50 g, 64%). **R_f** 0.84 (3/20/77 Et₃N/EtOAc/ pentane). **¹H NMR** (400 MHz, CDCl₃) δ 8.22 (s, 1H, H–N), 4.28 (t, *J* = 6.5 Hz, 2H, H₂–6), 1.77 (tt, *J* = 9.0, 6.5 Hz, 2H, H₂–5), 1.50 – 1.38 (m, 2H, H₂–4), 1.38 – 1.30 (m, 4H, H₂–3/H₂–2), 0.96 – 0.84 (m, 3H, H₃–1); **¹³C NMR** (101 MHz, CDCl₃) δ 163.3 (C8), 91.9 (C10), 69.7 (C6), 31.3 (C5), 28.2 (C4), 25.4 (C3), 22.5 (C2), 14.0 (C1).

Data consistent with literature.⁹

Hexyl benzimidate 156

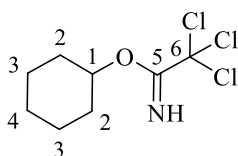


Synthesised following a reported procedure.⁹ To a solution of 1-hexanol (0.103 mL, 1 mmol) in benzonitrile (2.5 mL, 20 mmol) was added acetyl chloride (0.11 mL, 10 mmol) at 0 °C. The solution was warmed at room temperature and stirred for 65 h. The solution was extracted with DCM (3 × 15 mL); the combined organic fractions were washed sequentially with sat. aq. NaHCO₃ (2 × 20 mL), and brine (2 × 20 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (3/80/17 Et₃N/pentane/EtOAc) afforded the title compound as a colourless oil (273 mg, quant.). **R_f** 0.21 (3/80/17 Et₃N/pentane/EtOAc); **¹H NMR** (400 MHz, CDCl₃) δ 7.72 (s, 1H, N–H), 7.50 – 7.37 (m, 5H, H–Ar), 3.64 (t, *J* = 6.5 Hz, 2H, H₂–6), 1.86 – 1.75 (m, 2H, H₂–5), 1.56 (tt, *J* = 8.0, 6 Hz, 2H, H₂–4), 1.42 – 1.21 (m, 4H, H₂–3/H₂–2), 0.97 – 0.82 (m, 3H, H₃–1); **¹³C NMR** (101 MHz, CDCl₃) δ 164.1 (C7), 130.8 (C8),

128.5 (C9–C10), 126.6 (C11), 63.1 (C6), 32.8 (C5), 31.6 (C4), 25.4 (C3), 22.6 (C2), 14.0 (C1).

Data consistent with literature.¹⁰

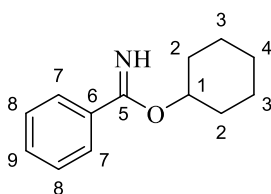
Cyclohexyl 2,2,2-trichloroacetimidate 157



Synthesised following a reported procedure.⁹ To a solution of cyclohexanol (1.04 mL, 10 mmol) in dry DCM (20 mL) were added DBU (0.15 mL, 0.10 mmol) and trichloroacetonitrile (1.5 mL, 15 mmol). The solution was stirred at room temperature and followed by TLC (1/1 pentane/EtOAc). After 22 h, the solution was diluted with water (20 mL) and extracted with DCM (2 × 15 mL). The combined organic fractions were washed with brine (2 × 15 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (3/97 Et₃N/pentane) afforded the title compound as a colourless oil (1.31 g, 53%). **R_f** 0.23 (3/97 Et₃N/pentane) ; **¹H NMR** (400 MHz, CDCl₃) δ 8.20 (s, 1H, N–H), 4.94 (tt, *J* = 8.0, 3.5 Hz, 1H, H–1), 1.99 – 1.87 (m, 2H, H₂–2), 1.84 – 1.70 (m, 2H, H₂–2), 1.64 (tt, *J* = 8.5, 3.5 Hz, 2H, H₂–3), 1.58 – 1.48 (m, 2H, H₂–3), 1.41 (dd, *J* =, 10.5, 6.0 Hz, 2H, H₂–4) ; **¹³C NMR** (101 MHz, CDCl₃) δ 162.19 (C5), 92.12 (C6), 46.29 (C1), 30.59 (C2), 25.49 (C3), 23.26 (C4).

Data consistent with literature.⁹

Cyclohexyl benzimidate 158

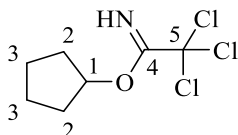


Synthesised by analogy to a reported procedure.⁹ A solution of benzonitrile (0.1 mL, 1 mmol) and cyclohexanol (0.09 mL, 1.0 mmol) diluted in HCl in 1,4-dioxane (4 M, 5 mL) was stirred at room temperature for 75 h, and concentrated *in vacuo*. The solution was diluted in DCM (10 mL), washed with sat. aq. NaHCO₃ (10 mL), and brine (10 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo*. FCC (1/40 MeOH/ DCM) afforded the title compound as a colourless oil (20 mg, 10%). **R_f** 0.24 (1/20 MeOH/ DCM) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.79 – 7.70 (m, 2H, H–Ar), 7.50 – 7.35 (m, 3H, H–Ar),

5.00 (tt, $J = 8.5, 3.5$ Hz, 1H, H-1), 2.07 – 1.96 (m, 2H, H₂-2), 1.80 (ddd, $J = 8.5, 6.5, 2.0$ Hz, 2H, H₂-2), 1.68 – 1.25 (m, 6H, H₂-3/H₂-4); ¹³C NMR (101 MHz, CDCl₃) δ 167.2 (C5), 133.8 (C6), 130.9 (C7), 128.6 (C8), 162.9 (C9), 73.4 (C1), 31.6 (C2), 29.9 (C3), 25.9 (C4).

Data consistent with literature.⁸

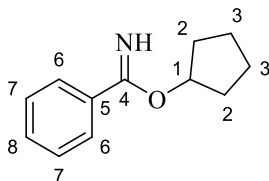
Cyclopentyl 2,2,2-trichloroacetimidate 159



Synthesised following a reported procedure.⁹ To a solution of cyclopentanol (0.91 mL, 10 mmol) in dry DCM (20 mL) were added DBU (0.15 mL, 0.1 mmol) and trichloroacetonitrile (1.5 mL, 15 mmol). The solution was stirred at room temperature and followed by TLC (1/1 pentane/EtOAc). After 24 h, the solution was diluted with water (20 mL) and extracted with DCM (2 × 15 mL). The combined organic fractions were washed with brine (2 × 15 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (3/97 Et₃N/pentane) afforded the title compound as a colourless oil (1.04 g, 45%). R_f 0.43 (3/97 Et₃N/pentane); ¹H NMR (400 MHz, CDCl₃) δ 8.19 (s, 1H, N-H), 5.29 (m, 1H, H-1), 1.88 – 1.72 (m, 6H, H₂-2/H₂-3), 1.71 – 1.55 (m, 2H, H₂-3); ¹³C NMR (101 MHz, CDCl₃) δ 162.3 (C4), 91.9 (C5), 82.1 (C1), 32.1 (C2), 23.6 (C3).

Data consistent with literature.⁹

Cyclopentyl benzimidate 160

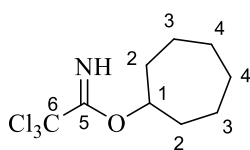


Synthesised by analogy to a reported procedure.⁸ A solution of benzonitrile (0.1 mL, 1 mmol) and cyclopentanol (0.09 mL, 1.0 mmol) diluted in HCl in 1,4-dioxane (4 M, 5 mL) was stirred at room temperature for 75 h, and concentrated *in vacuo*. The solution was diluted in DCM (10 mL), washed

with sat.aq. NaHCO_3 (10 mL), and brine (10 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo*. FCC (1/20 MeOH/ DCM) afforded the title compound as a colourless oil (20 mg, 11%). **R_f** 0.26 (1/20 MeOH/ DCM); **¹H NMR** (400 MHz, CDCl_3) δ 7.50 – 7.36 (m, 2H, H-6), 5.33 (m, 3H, H-7/H-8), 3.69 – 3.61 (m, 1H, H-1), 2.03 – 1.88 (m, 2H, H₂-2), 1.85 – 1.78 (m, 2H, H₂-2), 1.65 (m, 4H, H₂-3); **¹³C NMR** (101 MHz, CDCl_3) δ 165.2 (C4), 133.6 (C5), 130.9 (C6), 128.6 (C7), 126.9 (C8), 78.1 (C1), 32.91 (C2), 24.2 (C3).

Data consistent with literature.⁸

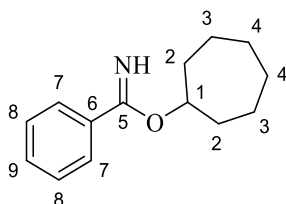
Cycloheptyl 2,2,2-trichloroacetimidate 161



Synthesised following a reported procedure.⁹ To a solution of cycloheptanol (2.7 mL, 10 mmol) in dry DCM (20 mL) were added DBU (0.15 mL, 0.10 mmol) and trichloroacetonitrile (1.5 mL, 15.0 mmol). The solution was stirred at room temperature and followed by TLC (1/1 pentane/EtOAc). After 24 h, the solution was diluted with water (20 mL) and extracted with DCM (2 × 15 mL). The combined organic fractions were washed with brine (2 × 15 mL), dried (MgSO_4), filtered, and concentrated *in vacuo*. FCC (3/97 Et₃N/pentane) afforded the title compound as a colourless oil (693 mg, 27%). **R_f** 0.41 (3/97 Et₃N/pentane); **¹H NMR** (400 MHz, CDCl_3) δ 8.18 (s, 1H, H-N), 5.08 (tt, J = 7.5, 4.5 Hz, 1H, H-1), 2.05 – 1.91 (m, 2H, H-2), 1.91 – 1.39 (m, 10H, H-2/H₂-3/H₂-4); **¹³C NMR** (101 MHz, CDCl_3) δ 162.3 (C5), 92.4 (C6), 80.2 (C1), 33.0 (C2), 28.5 (C3), 22.9 (C4).

Data consistent with literature.⁸

Cycloheptyl benzimidate 162

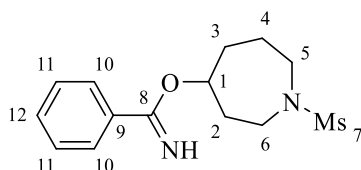


Synthesised by analogy to a reported procedure.⁸ A solution of benzonitrile (0.1 mL, 1 mmol) and cyclohexanol (0.09 mL, 1.0 mmol) diluted in HCl in 1,4-dioxane (4 M, 5 mL) was stirred at room

temperature for 75 h, and concentrated *in vacuo*. The solution was diluted in DCM (10 mL), washed with sat.aq. NaHCO₃ (10 mL), and brine (10 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo*. FCC (1/40 MeOH/DCM) afforded the title compound as a colourless oil (20 mg, 9%). **R_f** 0.17 (1/40 MeOH/DCM) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.78 – 7.70 (m, 2H, H-7), 7.52 – 7.36 (m, 3H, H-8/H-9), 5.16 (s, 1H, H-N), 3.85 (tt, *J* = 8.5, 4.5 Hz, 1H, H-1), 2.12 – 2.01 (m, 2H, H₂-2), 1.96 – 1.82 (m, 6H, H₂-2/H₂-3), 1.80 – 1.33 (m, 6H, H₂-3/H₂-4) ; **¹³C NMR** (101 MHz, CDCl₃) δ 164.5 (C5), 134.1 (C6), 131.2 (C7), 128.9 (C8), 127.3 (C9), 73.3 (C1), 34.1 (C2), 29.0 (C3), 23.5 (C4).

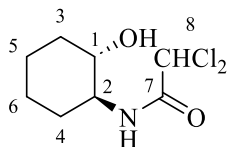
Data consistent with literature.⁸

1-(Methylsulfonyl)azepan-4-yl benzimidate **163**



Synthesised by analogy to a reported procedure.⁸ To a solution of alcohol **154** (203 mg, 1.04 mmol), in dry acetonitrile (5.2 mL), benzimidate **34** (250 mg, 1.04 mmol) was added. The solution was stirred at 60 °C. The reaction was monitored by ¹H NMR. After the equilibrium was reached, the salt was suspended in diethyl ether, and sat. aq. NaHCO₃ was added dropwise until the salt was thoroughly dissolved. The organic layer was separated and concentrated *in vacuo*. FCC (1/99 Et₃N/EtOAc) afforded the title compound as a clear oil (28.7 mg, 9%). **R_f** 0.44 (1/99 Et₃N in EtOAc) ; **IR** ν_{max}/cm⁻¹ 2935 (C–H), 1687 (C=N), 1475 (C=C), 1322 (C–N), 1146 (C–O) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.79 – 7.71 (m, 1H, H-12), 7.68 – 7.60 (m, 2H, H-10), 7.54 – 7.31 (m, 2H, H-11), 5.24 (m, 1H, H-1), 3.50 – 3.18 (m, 4H, H₂-5/H₂-6), 2.81 – 2.73 (m, 3H, H₃-7), 2.22 – 1.67 (m, 6H, H₂-2/H₂-3/H₂-4) ; **¹³C NMR**: sample decomposed ; **HRMS** (ESI+) calculated for [M+H]⁺ (C₁₄H₂₁N₂O₃S) requires *m/z* 297.1272, found 297.1272

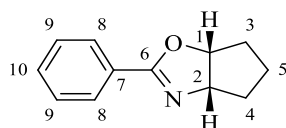
2,2,2-Trichloro-*N*-[(1*S**,2*S**)-2-hydroxycyclohexyl]acetamide **164**



Synthesised following a reported procedure.^{9,10} In an oven-dried pressure tube sealed with a PTFE cap. To a solution of imidate **157** (100 mg, 0.4 mmol) in DCE (2 mL) was added NIS (276 mg, 1.2 mmol). The solution was stirred at 110 °C for 18 h. The resulting solution was concentrated *in vacuo*. The crude product was mixed with *para*-toluenesulfonic acid (70 mg, 0.4 mmol), water (1 mL), and pyridine (4.5 mL), and stirred at 80 °C for 18 h. The solution was diluted in diethyl water (15 mL), the organic fraction was washed with water (15 mL) and brine (15 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo*. FCC (5/1 pentane/EtOAc) afforded the title compound as a white solid (10 mg, 12%). **R_f** 0.44 (5/1 pentane/EtOAc) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 3290 (N–H), 2945 (C–H), 1678 (C=O), 1450 (O–H), 1219 (C–O) ; **¹H NMR** (600 MHz, CDCl₃) δ 6.50 (s, 1H, H–8), 3.88 – 3.78 (m, 2H, H–1/H–2), 2.30 (ddt, *J* = 10.5, 3.5, 2.0 Hz, 1H, H–3), 2.21 (ddt, *J* = 11.0, 3.5, 2.0 Hz, 1H, H–4), 1.86 – 1.78 (m, 1H, H–3), 1.79 – 1.71 (m, 1H, H–4), 1.49 – 1.26 (m, 4H, H₂–5/H₂–6) ; **¹³C NMR** (151 MHz, CDCl₃) δ 163.9 (C7), 66.7 (C8), 61.9 (C1), 56.4 (C2), 36.2 (C3), 32.2 (C4), 25.5 (C5), 24.3 (C6).

Data consistent with literature.¹¹

(3*aS**,6*aR**)-2-Phenyl-3*a*,5,6,6*a*-tetrahydro-4*H*-cyclopenta[d]oxazole **165**

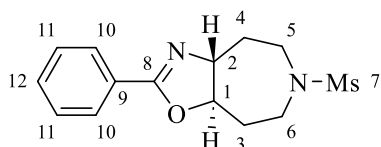


Synthesised following a reported procedure.⁸ To a solution of diiodide (5 mol%, 0.01 M in DMF, 1.18 mL, degassed before use), were added benzimidate **159** (30 mg, 0.23 mmol) and phenyliodobisacetate (91 mg, 0.28 mmol, ground and dried before use). The solution was stirred at 50 °C in a flame-dried round-bottomed flask for 4 hours. The solution was concentrated *in vacuo*. FCC (7/1 pentane/EtOAc) afforded the title compound as a colourless oil (3.4 mg, 7.7%). **R_f** 0.23 (7/1 pentane/EtOAc) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.97 – 7.88 (m, 2H, H–8), 7.57 – 7.35 (m, 3H, H–9/H–10), 5.16 – 5.08 (m, 1H, H–1), 4.73 (m, 1H, H–2), 2.18 – 1.89 (m, 2H, H₂–3), 1.75 – 1.66 (m, 2H, H₂–4), 1.58 – 1.41 (m, 2H, H₂–

5).

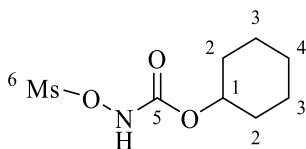
Data consistent with literature.⁸

(3aR*,8aR*)-6-(Methylsulfonyl)-2-phenyl-3a,5,6,7,8,8a-hexahydro-4H-oxazolo[4,5-d]azepine 166



Synthesised by analogy to a reported procedure.⁸ To a solution of diiodide (5 mol%, 0.01 M in DMF, 0.49 mL, degassed before use) were added benzimidate **163** (29 mg, 0.10 mmol) and phenyliodobisacetate (38 mg, 0.12 mmol, ground and dried before use). The solution was stirred at 50 °C in a flame-dried round-bottomed flask for 4 hours. The solution was concentrated *in vacuo*. FCC (20% → 100% EtOAc in pentane) afforded the title compound (5 mg, 17%) as an inseparable solution with benzimidate **163** (compound **167** being the minor constituent). **R_f** 0.22 (2/1 EtOAc/pentane) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 2968 (C–H), 1654 (C=N), 1339 (S=O), 1153 (C–O) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.92 – 7.82 (m, 1H, H–12), 7.76 – 7.60 (m, 2H, H–10), 7.41 – 7.30 (m, 2H, H–11), 4.89 (d, *J* = 4.0 Hz, 1H, H–1), 4.65 – 4.50 (m, 1H, H–2), 3.71 – 3.14 (m, 4H, H₂–5/H₂–6), 2.78 (s, 3H, H₃–7), 2.15 – 1.96 (m, 4H, H₂–3/H₂–4) ; **LRMS** (ESI⁺) calculated for [M+H]⁺ (C₁₄H₁₉N₂O₃S) requires *m/z* 295.1116, found 295.1112.

Cyclohexyl [(methylsulfonyl)oxy]carbamate 167



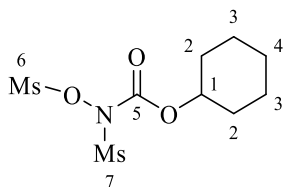
Synthesised following a reported procedure.⁶ To a solution of 1,1'-carbonyldiimidazole (404 mg, 2.50 mmol) in dry toluene (20 mL) was added cyclohexanol (1.92 mL, 2.00 mmol). The solution was stirred at 60 °C and monitored by TLC (3/2 pentane/EtOAc). After 3 h, the solution was cooled to room temperature, and hydroxylamine (1.18 mL, 4.00 mmol, 50% in water,) was added. The solution was

stirred vigorously and monitored by TLC (3/2 pentane/EtOAc). After 2 h, the reaction was quenched with aq. HCl (12 mL, 1.2 M), and extracted with EtOAc (2 × 30 mL); the combined organic layers were washed with brine (50 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo*.

Part of the crude product (265 mg, 1.67 mmol) was dissolved in dry diethyl ether (50 mL) in a flame-dried RBF and cooled to 0 °C. Freshly distilled methanesulfonyl chloride (0.13 mL, 1.67 mmol) and freshly distilled Et₃N (0.23 mL, 1.67 mmol) were cautiously added. The solution was warmed to room temperature, stirred, and monitored by TLC (3/2 pentane/EtOAc). The solution was quenched with water (30 mL), extracted with EtOAc (3 × 30 mL); the combined organic layers were washed with brine (50 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo*. FCC (1/5 → 1/3 EtOAc/pentane with a gradient of 1/20) afforded the title compound as a white solid (194 mg, 48%). **R_f** 0.67 (1/5 EtOAc/pentane); ¹H NMR (400 MHz, CDCl₃) δ 8.73 (s, 1H, H–N), 4.83 – 4.65 (m, 1H, H–1), 3.15 (s, 3H, H₃–6), 1.93 – 1.80 (m, 2H, H–2), 1.76 – 1.64 (m, 2H, H–2), 1.57 – 1.14 (m, 6H, H₂–3/H₂–4); ¹³C NMR (101 MHz, CDCl₃) δ 156.3 (C5), 77.9 (C1), 36.2 (C6), 31.5 (C2), 25.1 (C3), 23.5 (C4).

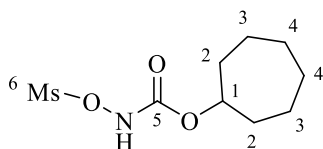
Data consistent with literature.⁶

Cyclohexyl *N*-(methanesulfonyl)-*N*-[(methanesulfonyl)oxy]carbamate **281**



Compound **281** was isolated from the same experiment as compound **167** (colourless oil, 105 mg, 20%). **R_f** 0.22 (5/1 pentane/EtOAc); **IR** ν_{max} /cm⁻¹ 2940 (C–H), 1725 (C=O), 1370 (S=O); ¹H NMR (400 MHz, CDCl₃) δ 4.97 (tt, *J* = 8.0, 3.5 Hz, 1H, H–1), 3.37 (s, 3H, H₃–6), 3.30 (s, 3H, H₃–7), 1.97 – 1.87 (m, 2H, H–2), 1.84 – 1.73 (m, 2H, H–2), 1.66 (dtd, *J* = 12.5, 9.0, 4.0 Hz, 2H, H–3), 1.41 (m, 4H, H–3/H₂–4); ¹³C NMR (101 MHz, CDCl₃) δ 151.02 (C5), 79.46 (C1), 40.54 (C6), 38.76 (C7), 30.98 (C2), 24.93 (C3), 22.91 (C4); **HRMS** (ESI⁺) calculated for [M+H]⁺ (C₉H₁₈NO₇S₂) requires *m/z* 316.0524, found 316.0528.

Cycloheptyl [(methanesulfonyl)oxy]carbamate **168**

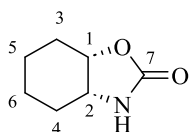


Synthesised by analogy to a reported procedure.⁶ To a solution of 1,1'-carbonyldiimidazole (1.93 g, 11.9 mmol) in dry toluene (50 mL) was added cycloheptanol (1.05 mL, 8.77 mmol). The solution was stirred at 60 °C and monitored by TLC (3/2 pentane/EtOAc). After 3 h, the solution was cooled to room temperature, and hydroxylamine (0.56 mL, 17.6 mmol, 50% in water) was added. The solution was stirred vigorously and monitored by TLC (3/2 pentane/EtOAc). After 2 h, the reaction was quenched with aq. HCl (30 mL, 1.2 M), and extracted with EtOAc (2 × 30 mL); the combined organic layers were washed with brine (50 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo*.

Part of the crude product (190 mg, 1.03 mmol) was dissolved in dry diethyl ether (30 mL) in a flame-dried RBF and cooled to 0 °C. Freshly distilled methanesulfonyl chloride (79 µL, 1.03 mmol) and freshly distilled Et₃N (0.139 mL, 1.03 mmol) were cautiously added. The solution was warmed to room temperature, stirred, and monitored by TLC (3/2 pentane/EtOAc). The solution was quenched with water (15 mL), extracted with EtOAc (3 × 20 mL); the combined organic layers were washed with brine (30 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo*. FCC (1/5 → 1/3 EtOAc/pentane with a gradient of 1/20 afforded the title compound as a white solid (21 mg, 2%). **R_f** 0.71 (3/2 pentane/EtOAc); **¹H NMR** (400 MHz, CDCl₃) δ 8.11 (s, 1H, H–N), 4.98 (tt, *J* = 8.2, 4.5 Hz, 1H, H–1), 3.18 (s, 3H, H₃–6), 2.06 – 1.86 (m, 4H, H₂–2), 1.85 – 1.33 (m, 8H, H₂–3/H₂–4); **¹³C NMR** (101 MHz, CDCl₃) δ 155.7 (C5), 79.8 (C1), 36.6 (C6), 33.9 (C2), 28.3 (C3), 22.7 (C4).

Data consistent with literature.⁶

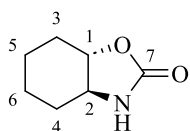
(3a*R**,7a*S**)-Hexahydrobenzodioxazol-2(3H)-one **170**



Synthesised following a reported procedure.⁶ To a solution of *N*-mesyloxycarbamate **167** (80 mg, 0.34

mmol) in EtOAc (10 mL), $\text{Rh}_2(\text{tpa})_4$ (17 mg, 4 mol%) was added and the solution was stirred until complete dissolution of $\text{Rh}_2(\text{tpa})_4$. Sat. aq. K_2CO_3 (50 μL , 1.2 mmol) was added and the solution was stirred at 40 °C for 16 h. The crude solution was filtered on Celite and concentrated *in vacuo*. FCC (1/1 pentane/EtOAc) afforded the title compound (5.3 mg, 11%). R_f 0.13 (3/2 pentane/EtOAc); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.47 (s, 1H, H–N), 4.75 (ddd, J = 9.0, 4.0, 2.5 Hz, 1H, H–1), 3.79 – 3.63 (m, 1H, H–2), 2.09 – 1.80 (m, 2H, H–3/H–4), 1.74 (m, 2H, H–3/H–4), 1.62 – 1.16 (m, 4H, H₂–5/H₂–6); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 164.2 (C7), 83.8 (C1), 75.1 (C2), 32.0 (C3), 31.0 (C4), 26.7 (C5), 21.7 (C6). Data consistent with literature.⁶

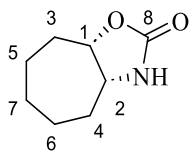
(3aS*,7aS*)-Hexahydrobenzo[d]oxazol-2(3H)-one 171



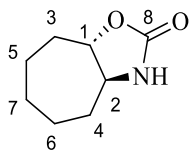
Compound 171 was isolated from the same experiment as compound **170** (6 mg, 12%). R_f 0.32 (1/1 pentane/EtOAc); $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 5.00 (s, 1H, N–H), 3.89 (td, J = 10.5, 3.0 Hz, 1H, H–1), 3.31 (td, J = 10.5, 3.5 Hz, 1H, H–2), 2.23 – 2.14 (m, 1H, H–3), 2.08 – 1.99 (m, 1H, H–4), 1.96 – 1.58 (m, 2H, H–3/H–4), 1.54 – 1.22 (m, 4H, H₂–5/H₂–6); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 164.5 (C7), 84.0 (C1), 61.1 (C2), 31.7 (C3), 29.4 (C4), 28.8 (C5), 23.8 (C6).

Data consistent with literature.⁶

(3aR*,8aS*)-Octahydro-2H-cyclohepta[d]oxazol-2-one 172



[(3aS*,8aS*)-Octahydro-2H-cyclohepta[d]oxazol-2-one 173

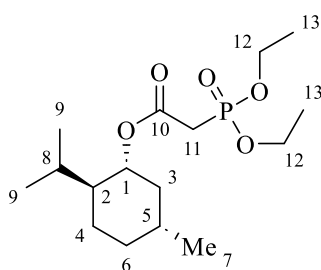


Synthesised by analogy to a reported procedure.⁶ To a solution of *N*-mesyloxycarbamate **168** (21 mg, 0.08 mmol) in EtOAc (5 mL), $\text{Rh}_2(\text{tpa})_4$ (6 mg, 4 mol%) was added and the solution was stirred until

complete dissolution of $\text{Rh}_2(\text{tpa})_4$. Sat. aq. K_2CO_3 (12.5 μL , 0.3 mmol) was added and the solution was stirred at 40 °C for 16 h. The crude solution was filtered on Celite and concentrated *in vacuo*. FCC (2/1 pentane/EtOAc) afforded the title compound **172** and **173** (5 mg, 40%) as an inseparable mixture. R_f 0.07 (2/1 pentane/EtOAc) ; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 4.87 (s, 2H, H–N), 4.79 (td, J = 9.5, 4.0 Hz, 1H, H–1_**172**), 4.30 (td, J = 10.5, 5.0 Hz, 1H, H–1_**173**), 4.01 – 3.91 (m, 1H, H–2_**172**), 3.74 – 3.64 (m, 1H, H–2_**173**), 2.11 – 1.64 (m, 16H, H₂–4/H₂–5/H₂–6/H₂–7 **172** and **173**).

Data consistent with literature.^{12–14}

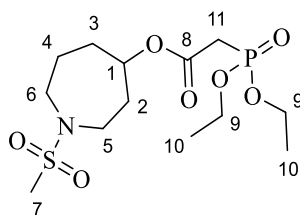
(1*R,2*S**,5*R**)-2-Isopropyl-5-methylcyclohexyl 2-[ethoxy(methoxy)phosphoryl]acetate **174****



Synthesised following a reported procedure.¹⁵ To a solution of (–)-menthol (100 mg, 0.64 mmol) in dry toluene (5 mL) were added DEPAA (0.11 mL, 0.67 mmol), DIPEA (0.29 mL, 1.66 mmol), and T3P (0.48 mL, 0.83 mmol, 50% in EtOAc). The solution was stirred at room temperature for 45 min. The solution was diluted in water (10 mL), extracted with EtOAc (3 × 10 mL) ; the combined organic layers were washed with aq. HCl (10%, 5 mL) and brine (10 mL), dried (MgSO_4), filtered, and concentrated *in vacuo* to afford the title compound as a clear oil (221 mg, quant.). R_f 0.23 (1/1 pentane/EtOAc) ; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 4.72 (td, J = 11, 4.5 Hz, 1H, H–1), 4.16 (quart, J = 7.0 Hz, 4H, H₂–12), 2.94 (d, J = 22 Hz, 2H, H₂–11), 2.06 – 1.90 (m, 2H, H₂–3), 1.74 – 1.63 (m, 1H, H–2), 1.56 – 1.16 (m, 8H, H–5/H–4/H₂–8), 1.16 – 0.67 (m, 14H, H₂–6/H₃–9/H₃–7/H₃–13) ; $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 165.4 (C10), 75.7 (C1), 62.6 (t, J = 6.0 Hz, C11), 46.9 (C12), 40.7 (C3), 34.8 (C2), 31.4 (C4), 25.8 (C5), 23.2 (C8), 22.0 (C6), 20.8 (C7), 16.3 (C13), 16.1 (C9).

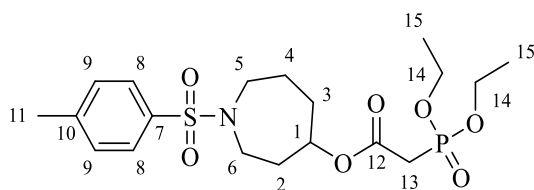
Data consistent with literature.¹⁵

1-(Methylsulfonyl)azepan-4-yl 2-(diethoxyphosphoryl)acetate 175



Synthesised by analogy to a reported procedure.¹⁵ To a solution of alcohol **150** (250 mg, 1.29 mmol) in dry toluene (10 mL) were added DEPAA (0.22 mL, 1.34 mmol), DIPEA (0.58 mL, 3.33 mmol) and T3P (1.18 mL, 50% in EtOAc, 1.66 mmol). The solution was stirred at RT for 30 min, then diluted with water (15 mL) and the aqueous portion was extracted with EtOAc (3 × 15 mL); the combined organic fractions were washed sequentially with aq. HCl (8 mL, 10%), sat. aq. NaHCO₃ (8 mL), and brine (15 mL), then dried (MgSO₄), filtered, and concentrated *in vacuo*. The crude product was purified by FCC (EtOAc) to afford the title compound as a colourless oil (450 mg, 95%). **R_f** 0.11 (EtOAc); **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 2929 (C–H), 1734 (C=O); **¹H NMR** (400 MHz, CDCl₃) δ 5.08 (tt, *J* = 7.0, 3.0 Hz, 1H, H–1), 4.25 – 4.09 (m, 4H, H–9), 3.51 – 3.27 (m, 4H, H–5/H–6), 2.95 (d, *J* = 22 Hz, 2H, H₂–11), 2.81 (s, 3H, H–7), 2.11 – 1.86 (m, 4H, H–2/H–3), 1.34 (m, 8H, H–4/H–10); **¹³C NMR** (101 MHz, CDCl₃) δ 165.2 (C8), 73.8 (C1), 62.9 (C9), 47.6 (C5), 42.9 (C6), 37.1 (C7), 35.5 (d, *J* = 7.5 Hz, C11), 34.2 (C2), 30.9 (C3), 22.7 (C4), 16.6 (C10); **HRMS** (ESI+) calculated for [M+H]⁺ (C₁₃H₂₇NO₇PS) requires *m/z* 372.1240, found 372.1245.

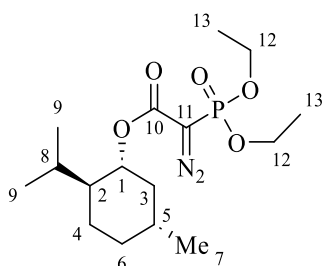
1-Tosylazepan-4-yl 2-(diethoxyphosphoryl)acetate 176



Synthesised by analogy to a reported procedure.¹⁵ To a solution of alcohol **135** (300 mg, 1.11 mmol) in dry toluene (15 mL), DEPAA (0.19 mL, 1.16 mmol), DIPEA (0.51 mL, 0.03 mmol), and T3P (1.42 mL, 1.42 mmol, 1 M in EtOAc) were added. The solution was stirred at room temperature for 1 hour. The solution was diluted with water (10 mL), extracted with EtOAc (3 × 15 mL); the combined organic layers were washed with sat. aq. HCl (10%, 5 mL), sat. aq. NaHCO₃ (5 mL), and brine (10 mL); dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the title compound as a yellow oil (404 mg, 49%). **R_f** 0.34

(EtOAc) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 2919 (C–H), 1733 (C=O), 1335 (S=O), 1273 (C–N), 1159 (C–O) ; **^1H NMR** (400 MHz, CDCl_3) δ 7.71 – 7.62 (m, 2H, H–8), 7.33 – 7.28 (m, 2H, H–9), 5.11 – 4.97 (m, 1H, H–1), 4.25 – 4.06 (m, 4H, H₂–14), 3.47 – 3.15 (m, 4H, H₂–5/H–6), 2.92 (d, J = 22 Hz, 2H, H₂–13), 2.42 (s, 3H, H₃–11), 2.04 (m, 2H, H–2/H–3), 1.96 – 1.82 (m, 4H, H–2/H–3/H₂–4) ; **^{13}C NMR** (101 MHz, CDCl_3) δ 165.1 (C12), 129.9 (C7), 129.2 (C8), 128.4 (C9), 127.2 (C10), 73.9 (C1), 62.9 (C14), 47.8 (C6), 42.9 (C5), 35.8 (d, J = 6.3 Hz, C13), 35.2 (3), 30.9 (C2), 22.4 (C4), 21.7 (C11), 16.6 (C15); **HRMS** (ESI+) calculated for $[\text{M}+\text{H}]^+$ ($\text{C}_{13}\text{H}_{27}\text{NO}_7\text{PS}$) requires m/z 372.1245, found 372.1240.

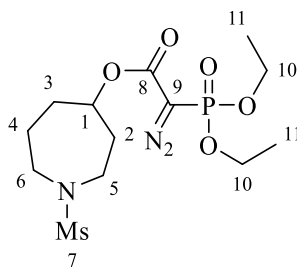
(1*R,2*S**,5*R**)-2-Isopropyl-5-methylcyclohexyl-2-diazo-2 [(diethoxyphosphoryl)]acetate 177**



Synthesised following a reported procedure.¹⁵ A solution of acetate **174** (120 mg, 0.36 mmol) in dry THF (7 mL) was cooled to -78°C . LHMDs (0.43 mL, 0.43 mmol, 1 M in THF) was added. The solution was warmed to room temperature and stirred for 10 min. *p*-ABSA (103 mg, 0.43 mmol) was added, and the solution was stirred for 1 hour, then diluted with water (10 mL) and diethyl ether (10 mL). The aqueous fraction was extracted with diethyl ether (3×10 mL); the combined organic layers were washed with sat. aq. NaHCO_3 (2×10 mL), dried (MgSO_4), filtered, and concentrated *in vacuo*. FCC (2/1 pentane/EtOAc) afforded the title compound as a colourless oil (90 mg, 69%). **R_f** 0.45 (2/1 pentane/EtOAc) ; **^1H NMR** (400 MHz, CDCl_3) δ 4.79 (td, J = 11, 4.0 Hz, 1H, H–1), 4.29 – 4.07 (m, 4H, H₂–12), 2.02 (m, 2H, H₂–3), 1.89 (m, 1H, H–2), 1.73 – 1.65 (m, H, H₂–6/H₂–4/H–5/H–8), 1.36 (dd, J = 7.0, 1 Hz, 6H, H₃–9), 1.13 – 0.95 (m, 3H, H₃–7), 0.91 (dd, J = 7, 4.5 Hz, 6H, H₃–13).

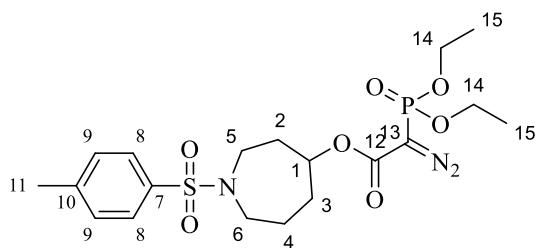
Data consistent with literature.¹⁵

1-(Methylsulfonyl)azepan-4-yl 2-diazo-2-(diethoxyphosphoryl)acetate 178



Synthesised by analogy to a reported procedure.¹⁵ To a solution of azepane **175** (200 mg, 0.54 mmol) in dry THF (10 mL) was added LHMDS (0.64 mL, 0.64 mmol, 1 M in THF). The solution was stirred at –78 °C, then warmed to room temperature and stirred for 10 min. *p*-ABSA (154 mg, 0.64 mmol) was added and the solution was stirred at room temperature for 1 hour. The solution was diluted with diethyl ether (15 mL) and water (15 mL). The solution was extracted with diethyl ether (3 × 20 mL); the combined organic layers were washed with sat. aq. NaHCO₃ (2 × 15 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (2/1 EtOAc/pentane) afforded the product as a colourless oil (113.5 mg, 53%). **R_f** 0.1 (2/1 EtOAc/pentane) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 2932 (C–H), 1704 (C=O), 2138 (N=N), 1704 (C=O), 1330 (S=O), 1285 (C–N) ; **¹H NMR** (600 MHz, CDCl₃) δ 5.20 – 5.14 (m, 1H, H-1), 4.32 – 4.12 (m, 4H, H₂-10), 3.55 – 3.23 (m, 4H, H₂-5/H₂-6), 2.82 (s, 3H, H₃-7), 2.08 (m, 2H, H-2/H-3), 2.04 – 1.94 (m, 2H, H-2/H-3), 1.78 (m, 2H, H₂-4), 1.37 (t, *J* = 7.0 Hz, 6H, H₃-11) ; **¹³C NMR** (151 MHz, CDCl₃) δ 163.0 (C8), 74.0 (C1), 63.8 (C9), 63.8 (C10), 47.6 (C5), 42.8 (C6), 37.1 (C7), 35.8 (C2), 31.1 (C3), 22.5 (C4), 16.4 (C11) ; **HRMS** not found.

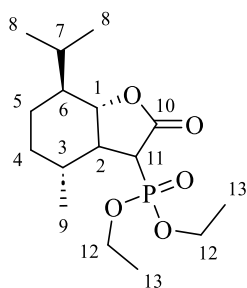
1-Tosylazepan-4-yl 2-diazo-2-(diethoxyphosphoryl)acetate **179**



Synthesised by analogy to a reported procedure.¹⁵ To a solution of azepane **176** (404 mg, 0.90 mmol) in dry THF (4.2 mL) was added LHMDS (1.09 mL, 1.09 mmol, 1 M/L in THF). The solution was stirred at –78 °C, then warmed to room temperature and stirred for 10 min. *p*-ABSA (260 mg, 1.09 mmol) was added and the solution was stirred at room temperature for 1 hour. The solution was diluted with diethyl

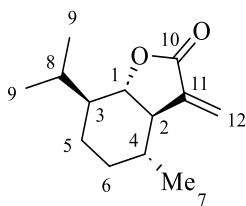
ether (10 mL) and water (10 mL). The solution was extracted with diethyl ether (3×10 mL); the combined organic layers were washed with sat. aq. NaHCO_3 (2×10 mL), dried (MgSO_4), filtered, and concentrated *in vacuo*. FCC (2/1 EtOAc/pentane) afforded the product as a colourless oil (426 mg, quant.). **R_f** 0.35 (2/1 EtOAc/pentane); **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 2932 (C–H), 2133 (N=N), 1704 (C=O), 1371 (S=O), 1281 (C–N), 1163 (C–O); **¹H NMR** (400 MHz, CDCl_3) δ 7.90 (d, $J = 9$ Hz, 1H, H–8), 7.78 (d, $J = 9$ Hz, 2H, H–9), 5.11 (tt, $J = 6.5, 3.0$ Hz, 1H, H–1), 4.26 – 3.95 (m, 4H, H₂–14), 3.44 – 3.13 (m, 2H, H₂–5/H₂–6), 2.33 (m, 6H, H₂–15), 2.04 (s, 3H, H₃–11), 1.93 – 1.82 (m, 6, H₂–2/H₂–3/H₂–4); **¹³C NMR** (101 MHz, CDCl_3) δ 163.1 (C12), 143.2 (C13), 129.5 (C7), 129.2 (C8), 128.4 (C9), 127.0 (C10), 73.8 (C1), 65.3 (C13), 62.0 (C14), 46.0 (C6), 42.3 (C5), 35.0 (C3), 30.8 (C2), 22.3 (C4), 21.7 (C11), 16.5 (C15); **HRMS** not found.

Diethyl [(4*R,7*S**,7*aS**)-7-isopropyl-4-methyl-2-oxooctahydrobenzofuran-3-yl]phosphonate 282**



Synthesised following a reported procedure.¹⁵ In an oven-dried tube sealed with a PTFE cap. To a solution of compound **177** (40 mg, 0.11 mmol) in DCM (2.2 mL) was added $\text{Rh}_2(\text{Oct})_4$ (3.4 mg, 4 mol%). The solution was stirred at 45 °C for 18 h and concentrated *in vacuo*. FCC (1/2 EtOAc/pentane) afforded the title compound as a colourless oil (8 mg, 22%). **R_f** 0.4 (1/2 EtOAc/pentane); **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 2959 (C–H), 1743 (C=O), 1250 (C–O); **¹H NMR** (400 MHz, CDCl_3) δ 4.86 (m, 1H, H–1), 4.56 – 4.38 (m, 1H, H–11), 4.30 – 4.12 (m, 4H, H₂–12), 2.18 – 2.00 (m, 1H, H–2), 2.00 – 1.80 (m, 1H, H–6), 1.70 (m, 10H, H₂–4/H₂–5/H₃–8), 1.57 – 1.41 (m, 3H, H–3/H–6/H–7), 1.35 (dd, $J = 4.5, 2.0$ Hz, 6H, H₃–13), 1.25 (m, 3H, H₃–9); **¹³C NMR** (101 MHz, CDCl_3) δ 169.5 (C10), 84.5 (C1), 69.8 (C12), 47.1 (d, $J = 2.5$ Hz, C11), 40.9 (C2), 40.5 (C2), 31.6 (C3), 26.4 (C5), 22.9 (C4), 22.1 (C7), 20.9 (C8), 16.5 (C13), 15.7 (C9); **³¹P NMR** (162 MHz, CDCl_3) δ 19.5; **HRMS** not found.

(3aR*,4R*,7S*,7aS*)-7-Isopropyl-4-methyl-3-methylenehexahydrobenzofuran-2(3H)-one 180



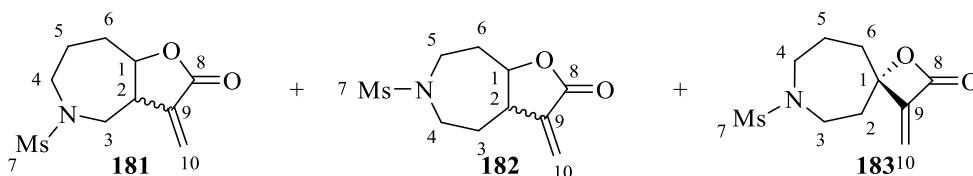
Synthesised following a reported procedure.¹⁵ A solution of compound **280** (88 mg, 0.247 mmol) in dry THF (5 mL) was stirred and cooled to 0 °C. Potassium *tert*-butoxide (34 mg, 0.30 mmol) was added and the solution stirred at 0 °C for 1 hour, then cooled to –78 °C. Paraformaldehyde (15 mg, 0.50 mmol) was added and the solution was stirred at –78 °C for 15 min, then 2 hours at room temperature and quenched with sat.aq. NH₄Cl (12 mL). The solution was extracted with EtOAc (3 × 15 mL); the combined organic layers were dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (8/1 pentane/EtOAc) afforded the product as a colourless oil (10 mg, 20%). *R*_f 0.55 (8/1 pentane/EtOAc) ; ¹H NMR (400 MHz, CDCl₃) δ 6.10 (d, *J* = 3.0 Hz, 1H, H–12), 5.63 (d, *J* = 3.0 Hz, 1H, H–12), 3.60 (t, *J* = 10.5 Hz, 1H, H–1), 2.19 (t, *J* = 10.5 Hz, 1H, H–2), 2.00 (heptd, *J* = 7.0, 3.5 Hz, 1H, H–8), 1.85 – 1.60 (m, 3H, H₃–7), 1.20 – 1.06 (m, 6H, H₃–9), 0.94 (m, 1H, H–3), 0.89 (m, 4H, H₂–5/H₂–6).

Data consistent with literature.¹⁵

(3aR*,8aS*)-3-Methylene-5-(methylsulfonyl)octahydro2H-[furo3,2-*c*]azepin-2-one 181,

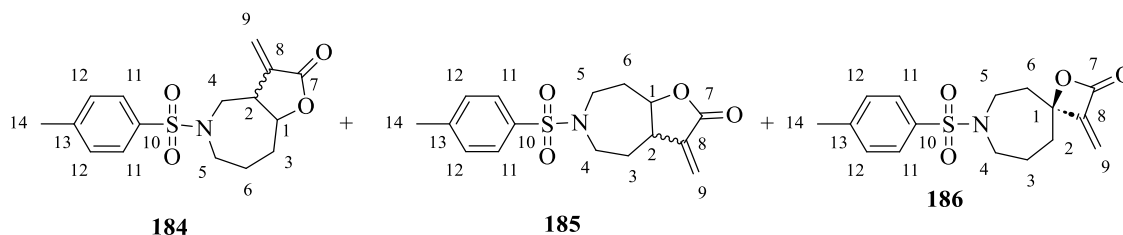
(3aS*,8aS*)-3-Methylene-6-(methylsulfonyl)octahydro-2H-[furo2,3-*d*]azepin-2-one 182,

and 3-Methylene-7-(methylsulfonyl)-1-oxa-7-azaspiro[3.6]decan-2-one 183



Synthesised by analogy to a reported procedure.¹⁵ In an oven-dried pressure tube sealed with a PTFE cap. To a solution of compound **178** (88 mg, 0.23 mmol) in dry DCM (5.2 mg), Rh₂(Oct)₄ (7.2 mg, 4 mol%) was added and the solution was stirred at 45 °C for 18h and concentrated *in vacuo*. The crude product was diluted in dry THF (5 mL) and the solution was cooled to 0 °C. Potassium *tert*-butoxide (33 mg, 0.29 mmol) was added and the solution was stirred at 0 °C for 1 hour. The solution was cooled

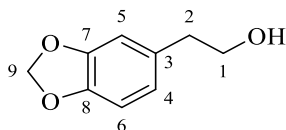
to $-78\text{ }^{\circ}\text{C}$, paraformaldehyde (14.6 mg, 0.49 mmol) was added, and the solution was stirred at $-78\text{ }^{\circ}\text{C}$ for 15 min, then at room temperature for 2 hours, and quenched with sat. aq. NH_4Cl (10 mL). The solution was extracted with EtOAc ($3 \times 15\text{ mL}$); the combined organic layers were dried (MgSO_4), filtered, and concentrated *in vacuo*. FCC (2/1 pentane/EtOAc) afforded the title compounds as inseparable mixtures (**181**, 2.3 mg, 4%; **182**, 6.5 mg, 11%; and **183**, 3 mg, 5%). Structures are supposed (**HRMS** not found and **IR** spectra not conclusive). **Data for 181:** R_f 0.21 (2/1 pentane/EtOAc) ; ^1H **NMR** (400 MHz, CDCl_3) δ 6.24 (d, $J = 3.5\text{ Hz}$, 1H, H-10), 5.52 (d, $J = 3.0\text{ Hz}$, 1H, H-10), 4.31 (m, 1H, H-1), 3.71 – 3.28 (m, 4H, $\text{H}_2\text{-3/H}_2\text{-4}$), 2.87 (s, 3H, $\text{H}_3\text{-7}$), 2.61 (m, 1H, H-2) 2.31 – 1.57 (m, 4H, $\text{H}_2\text{-5/H}_2\text{-6}$) ; ^{13}C **NMR** (101 MHz, CDCl_3) δ 170.8 (C8), 149.1 (C10), 120.6 (C9), 81.3 (C1), 48.8 (C3), 44.7 (C4), 37.8 (C7), 33.9 (C6), 38.1 (C2), 29.5 (C5) ; **LRMS** (ESI+) calculated for $[\text{M}+\text{H}]^+$ ($\text{C}_{10}\text{H}_{15}\text{NO}_4\text{S}$) requires m/z 246.1, found 246.1. **Data for 182:** R_f 0.21 (2/1 pentane/EtOAc) ; ^1H **NMR** (400 MHz, CDCl_3) δ 6.38 (dd, $J = 3.0\text{ Hz}$, 1H, H-10), 5.62 (d, $J = 3.0\text{ Hz}$, 1H, H-10), 4.30 (m, 1H, H-1), 3.86 – 3.25 (m, 4H, $\text{H}_2\text{-4/H}_2\text{-5}$), 2.85 (s, 3H, $\text{H}_3\text{-7}$), 2.62 (m, 1H, H-2) 2.21 – 1.56 (m, 4H, $\text{H}_2\text{-3/H}_2\text{-6}$) ; ^{13}C **NMR** (101 MHz, CDCl_3) δ 170.2 (C8), 148.6 (C10), 119.7 (C9), 80.9 (C1), 50.7 (C4), 46.9 (C5), 38.1 (C7), 33.9 (C6), 29.5 (C3) ; **LRMS** (ESI+) calculated for $[\text{M}+\text{H}]^+$ ($\text{C}_{10}\text{H}_{15}\text{NO}_4\text{S}$) requires m/z 246.1, found 246.1. **Data for 183:** R_f 0.57 (2/1 pentane/EtOAc) ; ^1H **NMR** (400 MHz, CDCl_3) δ 5.89 (d, $J = 2.0\text{ Hz}$, 1H, H-10), 5.64 – 5.50 (d, $J = 2.0\text{ Hz}$, 1H, H-10), 3.73 – 3.63 (m, 1H, H-3), 3.44 – 3.36 (m, 2H, $\text{H-3/H}_2\text{-4}$), 3.23 – 3.12 (m, 1H, H-4), 2.85 (s, 3H, $\text{H}_3\text{-7}$), 2.39 – 1.85 (m, 6H, $\text{H}_2\text{-2/H}_2\text{-5/H}_2\text{-6}$) ; ^{13}C **NMR** (101 MHz, CDCl_3) δ 168.9 (C8), 150.1 (C10), 114.8 (C9), 87.6 (C1), 47.1 (C3), 42.4 (C4), 39.8 (C7), 36.9 (C2), 34.5 (C6), 22.3 (C5) ; **LRMS** (ESI+) calculated for $[\text{M}+\text{H}]^+$ ($\text{C}_{10}\text{H}_{15}\text{NO}_4\text{S}$) requires m/z 246.1, found 246.1.

3-Methylene-5-tosyloctahydro-2H-furo[3,2-c]azepin-2-one 184**3-Methylene-6-tosyloctahydro-2H-furo[2,3-d]azepin-2-one 185****(S*)-3-Methylene-7-tosyl-1-oxa-7-azaspiro[3.6]decan-2-one 186**

Synthesised by analogy to a reported procedure.¹⁵ To a solution of compound **179** (450 mg, 0.74 mmol) in dry DCM (21.6 mL), Rh₂(Oct)₄ (29.6 mg, 4 mol%) was added. The solution was stirred at 45 °C overnight in an oven-dried pressure tube sealed with a PTFE cap. The solution was concentrated *in vacuo*, diluted in dry THF (15.5 mL), and cooled to 0 °C. Potassium *tert*-butoxide (102 mg, 0.91 mmol) was added. The solution was stirred at 0 °C for 1 hour, and cooled to –78 °C. Paraformaldehyde (44.4 mg, 1.48 mmol) was added, the solution was stirred for 15 min at –78 °C, then 2 hours at room temperature, and quenched with sat. aq. NH₄Cl (15 mL). The solution was extracted with EtOAc (3 × 30 mL); the combined organic layers were dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (4/1 EtOAc/pentane) afforded a solution of inseparable products (**184**, 10% ; **185**, 8% ; **186**, 8.5%). The yields are calculated through NMR analysis. The structures are supposed by comparisons with ¹H NMR data comparisons with previous and known¹² structures. **Data for 184:** R_f 0.24 (4/1 EtOAc/pentane) ; ¹H NMR (400 MHz, CDCl₃) δ 7.76 – 7.56 (m, 2H, H–11), 7.32 (m, 2H, H–12), 6.24 (d, *J* = 3.5 Hz, 1H, H–9), 5.51 (d, *J* = 3.5 Hz, 1H, H–9), 3.55 – 3.48 (m, 1H, H–1), 3.42 – 3.10 (m, 4H, H₂–4/H₂–5), 2.51 – 2.42 (m, 1H, H–2), 2.05 (s, 3H, H₃–14), 2.18 – 1.63 (m, 4H, H₂–3/H₂–6). **Data for 185:** R_f 0.24 (4/1 EtOAc/pentane) ; ¹H NMR (400 MHz, CDCl₃) δ 7.66 (d, *J* = 6.5 Hz, 2H, H–11), 7.37 – 7.26 (m, 2H, H–12), 5.98 (d, *J* = 2.0 Hz, 1H, H–9), 5.42 (d, *J* = 2.0 Hz, 1H, H–9), 3.71 (m, 1H, H–1), 3.42 – 3.10 (m, 4H, H₂–4/H₂–5), 2.98 – 2.90 (m, 1H, H–2), 2.05 (s, 3H, H₃–14), 1.78 – 1.63 (m, 4H, H₂–3/H₂–6). **Data for 186:** R_f 0.24 (4/1 EtOAc/pentane) ; ¹H NMR (400 MHz, CDCl₃) δ 7.66 (d, *J* = 6.5 Hz, 2H, H–11), 7.37 – 7.26 (m, 2H, H–12), 5.42 (d, *J* = 2.0 Hz, 1H, H–9), 5.36 (d, *J* = 2.0 Hz, 1H, H–9), 4.29 – 4.18 (m, 1H, H₂–4/H₂–5), 2.05 (s, 3H, H₃–14), 2.04 – 1.63 (m, 4H, H₂–2/H₂–3/H₂–6).

Procedures and Compounds, Chapter 3

2-(Benzo[d][1,3]dioxol-5-yl)ethan-1-ol **188**



Method 1: Synthesised by analogy to a reported procedure.^{16,17} To a solution of ketone **207** (40 mg, 0.17 mmol) in isopropanol (0.7 mL), were added LiBH₄ (0.43 mL, 2 M in THF, 0.86 mmol) and powdered NaOH (20 mg, 0.51 mmol). The solution was stirred at 40 °C for 20 h. The solution was cooled to room temperature, quenched with sat. aq. NH₄Cl (3 mL), saturated with solid NaCl, and extracted with EtOAc (5 × 15 mL). The combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (4/1 pentane/EtOAc) afforded the title compound (14 mg, 50%).

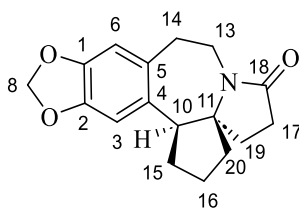
Method 2: Synthesised by analogy to a reported procedure.^{16,17} To a solution of ketone **208** (11 mg, 0.041 mmol) in isopropanol (0.6 mL), were added LiBH₄ (0.103 mL, 2 M in THF, 0.205 mmol) and powdered NaOH (5 mg, 0.123 mmol). The solution was stirred at 40 °C for 20 h. The solution was cooled to room temperature, quenched with sat. aq. NH₄Cl (3 mL), saturated with solid NaCl, and extracted with EtOAc (5 × 15 mL). The combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (4/1 pentane/EtOAc) afforded the title compound (4.5 mg, 66%).

Method 3: Synthesised by analogy to a reported procedure.¹⁶ To a solution of alcohol **196** (38 mg, 0.141 mmol) in isopropanol (1 mL) were added solid LiBH₄ (15.5 mg, 0.71 mmol) and crushed NaOH (16.8 mg, 0.42 mmol). The solution was stirred at 40 °C for 20 h. The solution was cooled to room temperature, quenched with sat. aq. NH₄Cl, saturated with solid NaCl, and extracted with EtOAc (5 × 10 mL), the combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (8/1 pentane/EtOAc) afforded the title compound as a colourless oil (12 mg, 50%).

R_f 0.04 (4/1 pentane/EtOAc) ; **¹H NMR** (400 MHz, CDCl₃) δ 6.83 – 6.64 (m, 3H, H-4/H-5/H-6), 5.93 (s, 2H, H₅-9), 3.82 (t, *J* = 6.5 Hz, 2H, H₂-1), 2.79 (t, *J* = 6.5 Hz, 2H, H₂-2) ; **¹³C NMR** (101 MHz, CDCl₃) δ 148.0 (C7), 146.4 (C8), 132.4 (C3), 122.1 (C4), 109.5 (C5), 108.6 (C6), 101.1 (C9), 64.0 (C1), 39.1 (C2).

Data consistent with literature.¹⁸

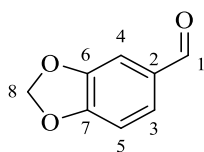
(3aR*,14bS*)-1,2,3,4,5,8,9,14b-Octahydro-6H-[1,3]dioxolo[4',5':4,5]benzo[1,2-d]cyclopenta[b]pyrrolo[1,2-a]azepin-6-one 191



Synthesised by analogy to a reported procedure.¹⁹ To a solution of amide **265** (21 mg, 0.05 mmol) in dry degassed chlorobenzene (6 mL) stirred at reflux was added a solution of tributyltin hydride (41 μ L, 0.08 mmol – correction made after titration) and ABCN (1.3 mg, 10 mol%) over 1.5 h. The solution was stirred 1h at reflux after the addition, cooled down, concentrated *in vacuo*, diluted in acetonitrile (20 mL), washed with pentane (3 $\times$ 15 mL), and concentrated *in vacuo*. FCC FCC (1/1 pentane/EtOAc) afforded the title compound as a white crystal (10 mg, 70%). *R*_f 0.38 (3/1 pentane/EtOAc) ; ¹H NMR (400 MHz, CDCl₃) δ 6.62 (s, 1H, H-6), 6.59 (s, 1H, H-3), 5.94 – 5.87 (m, 2H, H₂-8), 4.10 (dd, *J* = 8.5, 3.5 Hz, 1H, H-13), 4.06 (dd, *J* = 8.5, 3.5 Hz, 1H, H-13), 3.30 – 3.12 (m, 1H, H-14), 3.11 – 3.07 (m, 1H, H-14), 2.98 (dd, *J* = 11.5, 8.5 Hz, 1H, H-10), 2.60 (m, 1H, H-14), 2.36 – 2.13 (m, 2H, H₂-19), 1.98 – 1.87 (m, 2H, H₂-17), 1.81 – 1.71 (m, 2H, H₂-15), 1.70 – 1.48 (m, 2H, H₂-16), 1.34 (m, 2H, H₂-20).

Data consistent with literature.¹⁹

Benzo[d][1,3]dioxole-5-carbaldehyde 195

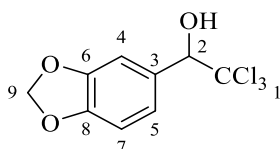


Synthesised by analogy to a reported procedure.²⁰ To a solution of alcohol **203** (453 mg, 1.76 mmol) in dry DMF (5.6 mL) was added CsF (134 mg, 8.80 mmol). The solution was shaken until it became warm to the touch, and let to cool to room temperature. DCM (124 μ L, 1.94 mmol) was added, and the solution was stirred at 115 °C for 1.5 h. The solution was cooled to room temperature, extracted with EtOAc (50 mL), the organic fractions was washed with H₂O (3 $\times$ 20 mL), sat. aq. Na₂CO₃ (20 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (1/1 pentane/EtOAc) afforded the title compound as a

colourless solid (70 mg, 27%), also known as piperonal. **R_f** 0.58 (1/1 pentane/diethyl ether) ; **¹H NMR** (400 MHz, CDCl₃) δ 9.81 (s, 1H, H-1), 7.42 (dd, *J* = 8.0, 1.5 Hz, 1H, H-5), 7.34 (d, *J* = 1.5 Hz, 1H, H-4), 6.94 (d, *J* = 8.0 Hz, 1H, H-3) ; **¹³C NMR** (101 MHz, CDCl₃) δ 190.5 (C1), 153.3 (C6), 148.9 (C7), 132.1 (C5), 128.9 (C2), 108.6 (C4), 107.1 (C3), 102.3 (C8).

Data consistent with literature.²¹

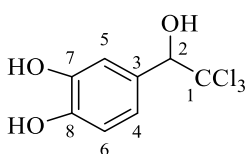
1-(Benzo[d][1,3]dioxol-5-yl)-2,2,2-trichloroethan-1-ol **196**



Synthesised by analogy to a reported procedure.¹⁶ To a solution of aldehyde **195** (35 mg, 0.23 mmol) in CHCl₃ (37 μL, 0.46 mmol) was added dropwise DBU (34 μL, 0.23 mmol). The solution was stirred at room temperature for 8 h. The solution was diluted in DCM (5 mL), and quenched with sat. aq. NH₄Cl (5 mL). The solution was extracted with DCM (3 × 5 mL); the combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (8/1 pentane/EtOAc) afforded the title compound as a colourless solid (38 mg, 61%). **R_f** 0.47 (5/1 pentane/EtOAc) ; **IR** *v*_{max}/cm⁻¹ 3531 (O-H), 2917 (C-H), 1387 (O-H), 1254 (C-O) 742 (C-Cl). **¹H NMR** (400 MHz, CDCl₃) δ 7.13 (d, *J* = 2.0 Hz, 1H, H-7), 7.06 (dd, *J* = 8.0, 2.0, Hz, 1H, H-4), 6.81 (d, *J* = 8.0 Hz, 1H, H-5), 5.99 (s, 2H, H₂-9), 5.14 (d, *J* = 3.5 Hz, 1H, H-2), 3.41 (br, 1H, O-H) ; **¹³C NMR** (101 MHz, CDCl₃) δ 148.7 (C6), 147.4 (C8), 128.7 (C7), 123.7 (C4), 121.1 (C5), 115.6 (C3), 107.8 (C9), 101.5 (C2), 84.5 (C1).

Data consistent with literature.²²

4-(2,2,2-Trichloro-1-hydroxyethyl)benzene-1,2-diol **203**

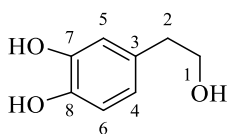


Synthesised following a reported procedure.^{23,24} To a solution of catechol (220 mg, 2.00 mmol) in dry EtOAc (0.2mL) was added chloral (311 μL, 3.20 mmol). The solution was stirred at 0 °C. Et₃N (54 μL) was slowly added, and the solution was stirred at 50 °C for 3 h, and cooled with an ice bath. Aq. HCl (1 M, 0.7 mL) and EtOAc (2.5 mL) were added and the fractions separated. The organic fraction was

washed with H₂O (3 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (4/1 pentane/EtOAc) afforded the title compound as a yellow oil (330 mg, 64%). **R_f** 0.08 (3/1 pentane/EtOAc) ; **¹H NMR** (400 MHz, Acetone) δ 8.04 (s, 1H, Ar–O–H), 7.95 (s, 1H, Ar–O–H), 7.18 (d, *J* = 2.0 Hz, 1H, H–C), 6.99 (dd, *J* = 8.0, 2.0 Hz, 1H, H–4), 6.81 (d, *J* = 8.0 Hz, 1H, H–6), 6.01 (m, 1H, O–H), 5.19 – 5.13 (m, 1H, H–2) ; **¹³C NMR** (101 MHz, Acetone) δ 146.7 (C7), 145.1 (C8), 129.5 (C3), 122.4 (C4), 117.4 (C5), 115.0 (C6), 84.8 (C2).

Data consistent with literature.²⁴

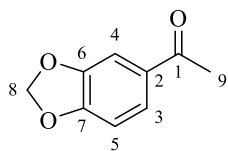
4-(2-Hydroxyethyl)benzene-1,2-diol **204**



Synthesised following a reported procedure.¹⁶ To a solution of alcohol **203** (50 mg, 0.19 mmol) in isopropanol (0.7 mL) were added LiBH₄ (0.38 mL, 0.76 mmol, 2 M in THF) and powdered NaOH (23 mg, 0.57 mmol). The solution was stirred at 40 °C for 20 h, and cooled to room temperature. The solution was quenched with sat.aq. NH₄Cl (5 mL), diluted with EtOAc (10 mL), and saturated with solid NaCl. The solution was extracted with EtOAc (5 × 15 mL); the combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (2/1 pentane/EtOAc) afforded the title compound as a white solid (4 mg, 14%). **R_f** 0.3 (2:1 EtOAc/pentane) ; **¹H NMR** (400 MHz, CDCl₃) δ 6.90 – 6.71 (m, 3H, H–6/H–5/H–4), 3.82 (t, *J* = 6.5 Hz, 2H, H₂–1), 2.76 (t, *J* = 6.5 Hz, 2H, H₂–2).

Data consistent with literature.²⁵

1-(Benzo[d][1,3]dioxol-5-yl)ethan-1-one **206**

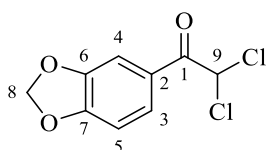


Synthesised by analogy to a reported procedure.²⁶ To a solution of 1,3-benzodioxole (520 μL, 2.00 mmol) in TFA (1.07 mL), was added acetic anhydride (189 μL, 2.00 mmol). The solution was stirred at room temperature in an oven-dried round-bottomed flask for 18 h. The solution was diluted with sat. aq. NaHCO₃ (50 mL), extracted with EtOAc (3 × 20 mL) ; the combined organic fractions were dried

(MgSO₄), filtered, and concentrated *in vacuo*. FCC (5/1 pentane/diethyl ether) afforded the title compound as a white powder (328 mg, quant.). **R_f** 0.25 (5/1 pentane/diethyl ether) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.54 (dd, *J* = 8.0, 1.5 Hz, 1H, H-3), 7.42 (d, *J* = 1.5 Hz, 1H, H-4), 6.84 (d, *J* = 8.0 Hz, 1H, H-5), 6.03 (s, 2H, H₂-8), 2.46 (s, 3H, H₃-9) ; **¹³C NMR** (101 MHz, CDCl₃) δ 196.41 (C1), 151.94 (C6), 148.34 (C7), 132.28 (C3), 124.94 (C2), 108.13 (C4), 107.99 (C5), 102.03 (C8), 26.64 (C9).

Data consistent with literature.²⁷

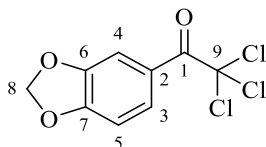
1-(Benzo[d][1,3]dioxol-5-yl)-2,2-dichloroethan-1-one **207**



Synthesised following a reported procedure.²⁸ To a solution of ketone **206** (2.45g, 14.9 mmol) in dry DMF (70 mL), were added CuCl₂•2H₂O (15.3 g, 89.9 mmol), and LiCl (3.81 g, 89.9 mmol). The solution was stirred at 90 °C for 4 h 30. The solution was cooled down to room temperature, diluted with DCM (200 mL), and washed with H₂O (160 mL). The aqueous fraction was extracted with DCM (200 mL); the combined organic fractions were washed with H₂O (160 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (8/1 pentane/diethyl ether) afforded the title compound as a white solid (2.4 g, 70%). **R_f** 0.41 (5/1 pentane/diethyl ether) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.71 (dd, *J* = 8.0, 2 Hz, 1H, H-3), 7.53 (d, *J* = 2 Hz, 1H, H-4), 6.90 (d, *J* = 8.0 Hz, 1H, H-5), 6.61 (s, 1H, H-9), 6.09 (s, 2H, H₂-8) ; **¹³C NMR** (101 MHz, CDCl₃) δ 184. (C1), 153.30 (C6), 148.6 (C7), 126.7 (C3), 125.8 (C2), 109.6 (C4), 108.5 (C5), 102.5 (C8), 68.0 (C9).

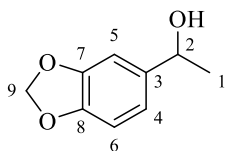
Data consistent with literature.²⁸

1-(Benzo[d][1,3] dioxol-5-yl)-2,2,2-trichloroethan-1-one **208**



Compound **208** was isolated from the same experiment as ketone **207** as a yellow oil (40 mg, 1%). **R_f** 0.63 (5/1 pentane/diethyl ether) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 2949 (C–H), 1701 (C=O), 1635 (C=C), 846 (C–Cl), 1223 (C–O) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.96 (dd, J = 8.5, 2 Hz, 1H, H–3), 7.70 (d, J = 2 Hz, 1H, H–4), 6.89 (d, J = 8.5 Hz, 1H, H–5), 6.09 (s, 2H, H₂–8) ; **¹³C NMR** (101 MHz, CDCl₃) δ 179.8 (C1), 153.0 (C6), 148.0 (C7), 129.0 (C3), 122.9 (C2), 111.4 (C4), 108.2 (C5), 102.5 (C8), 66.1 (C9) ; **HRMS** (ESI+) calculated for [M+H]⁺ (C₉H₆Cl₃O₃) requires m/z 266.9382, found 266.9383.

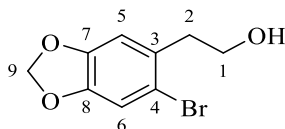
1-(Benzo[d][1,3]dioxol-5-yl)ethan-1-ol **209**



Alcohol **209** was isolated from the same experiment as alcohol **188** as a white solid. **R_f** 0.16 (4/1 pentane/EtOAc) ; **¹H NMR** (400 MHz, CDCl₃) δ 6.90 (d, J = 1.5 Hz, 1H, H–6), 6.85 – 6.74 (m, 2H, H–4/H–5), 5.95 (s, 2H, H₂–9), 4.82 (quart, J = 6.5 Hz, 1H, H–2), 1.46 (d, J = 6.5 Hz, 3H, H₃–1) ; **¹³C NMR** (101 MHz, CDCl₃) δ 148.0 (C8), 147.1 (C7), 140.1 (C6), 118.9 (C5), 108.3 (C4), 106.2 (C3), 101.2 (C9), 70.5 (C2), 29.9 (C1).

Data consistent with literature.²⁹

2-(6-Bromobenzo[d][1,3]dioxol-5-yl)ethan-1-ol **216**

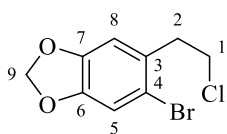


Method 1: Synthesised by analogy to a reported procedure.³⁰ To a solution of alcohol **188** (25 mg, 0.15 mmol) in dry DCM (1 mL) was added Br₂ (11 μ L) dropwise. The solution was stirred 30 minutes at room temperature. Sat. aq. Na₂SO₃ (1 mL) was added, and the solution was extracted with DCM (3 $\times$ 5 mL). The combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the title compound as a white solid (37 mg, quant.).

Method 2: Synthesised by analogy to a reported procedure.³¹ To a solution of alcohol **188** (25 mg, 0.15 mmol) in dry DMF (1 mL) under intense stirring, was added NBS (31 mg, 0.175 mmol) slowly at 0 °C. The solution was stirred at room temperature for 16 h, diluted in H₂O (5 mL), extracted with EtOAc (3 × 5 mL). The combined organic fractions were washed with brine (10 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. The crude solution was dissolved in DMF (1 mL) under intense stirring, and NBS (10 mg, 0.06 mmol) was slowly added at 0 °C. The solution was stirred at room temperature for 16 h, diluted in H₂O (5 mL), extracted with EtOAc (3 × 5 mL). The combined organic fractions were washed with brine (10 mL), dried (MgSO₄), filtered and concentrated *in vacuo*. FCC (3/1 pentane/EtOAc) afforded the title compound as a white solid (37 mg, quant.). **R_f** 0.52 (3/1 pentane/EtOAc) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.01 (s, 1H, H-6), 6.77 (s, 1H, H-5), 5.96 (s, 2H, H₂-9), 3.84 (t, *J* = 6.5 Hz, 3H, H₂-1), 2.94 (t, *J* = 6.5 Hz, 3H, H₂-2).

Data consistent with literature.³²

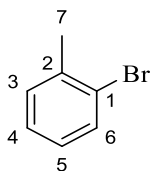
5-Bromo-6-(2-chloroethyl)benzo[d][1,3]dioxole **217**



Synthesised by analogy to a reported procedure.³³ To a solution of alcohol **216** (20 mg, 0.08 mmol) in dry DCM (1 mL) were added triphenyl phosphine (32 mg, 0.12 mmol) and hexachloroethane (29 mg, 0.12 mmol). The solution was stirred at room temperature for 30 minutes. The solution was quenched with H₂O (2 mL), extracted with diethyl ether (3 × 5 mL); the combined organic fractions were washed with 10% aq. H₂O₂ (2 × 5 mL), sat. aq Na₂SO₃ (2 × 5 mL), brine (10 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (10/1 pentane/EtOAc) afforded the title compound as a clear oil (34 mg, quant.). **R_f** 0.53 (1/10 Et₂O/pentane) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.00 (s, 1H, H-5), 6.76 (s, 1H, H-8), 5.97 (s, 2H, H₂-9), 3.68 (t, *J* = 7.5 Hz, 2H, H₂-1), 3.10 (t, *J* = 7.5 Hz, 2H, H₂-2) ; **¹³C NMR** (101 MHz, CDCl₃) δ 147.7 (C6), 147.6 (C7), 130.5 (C4), 114.8 (C5), 113.1 (C8), 111.1 (C3), 102.0 (C9), 43.6 (C2), 39.5 (C1).

Data consistent with literature.³²

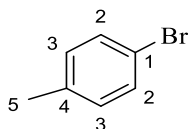
1-Bromo-2-methylbenzene **220**



Synthesised following a reported procedure.³⁴ To a solution of dry toluene (1.061 mL, 10 mmol) in dry MeCN (5 mL) were added FeCl₃ (162 mg, 1 mmol) and NBS (1780 mg, 10 mmol). The solution was stirred at room temperature for 20 min. The solution was diluted with H₂O, extracted with EtOAc (3 × 30 mL); the combined organic fractions were washed with H₂O (50 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. The crude solution was purified with a silica plug and eluted with cyclohexane, to afford the title compounds as an inseparable solution (779 mg, 45% conversion, 1/1 ratio between **283** and **220**). **R_f** 0.8 (15/1 pentane/EtOAc) ; ¹H NMR (400 MHz, CDCl₃) δ 7.44 – 7.34 (m, 2H, H-6/H-5), 7.13 – 7.03 (m, 2H, H-4/H-3), 2.43 (s, 3H, H₃-7) ; ¹³C NMR (101 MHz, CDCl₃) δ 137.0 (C1), 131.4 (C6), 131.0 (C5), 131.0 (C4), 127.4 (C3), 125.2 (C2), 27.1 (C7).

Data consistent with literature.³⁴

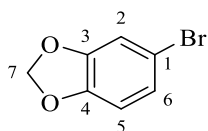
1-Bromo-4-methylbenzene **283**



Compound **283** was isolated from the same experiment as **220** (779 mg, 45% conversion, 1/1 ratio) ; ¹H NMR (400 MHz, CDCl₃) δ 7.56 (d, *J* = 8.0 Hz, 2H, H-2), 7.31 – 7.19 (m, 2H, H-3), 2.33 (s, 3H, H₃-5) ; ¹³C NMR (101 MHz, CDCl₃) δ 138.1 (C1), 132.5 (C2), 127.5 (C3), 119.3 (C4), 23.1 (C5).

Data consistent with literature.³⁵

5-Bromobenzo[d][1,3]dioxole **222**

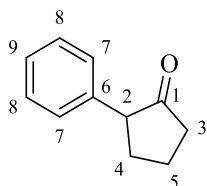


Synthesised by analogy to a reported procedure.³⁰ To a solution of 1,3-benzodioxole (115 μL, 1 mmol) in dry DCM (5 mL) was added Br₂ (72 μL, 1.4 mmol) dropwise. The solution was stirred at room temperature for 30 min. Sat. aq. Na₂SO₃ (5 mL) was added, and the solution was extracted with DCM (3 × 5 mL). The combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo*.

FCC (cyclohexane) afforded the title compound as a colourless oil (203 mg, quant.). **R_f** 0.7 (10/1 pentane/EtOAc) ; **¹H NMR** (400 MHz, CDCl₃) δ 6.96 (s, 1H, H-2), 6.94 (d, *J* = 2.0 Hz, 1H, H-5), 6.73 – 6.65 (m, 1H, H-6), 5.97 (s, 2H, H₂-7) ; **¹³C NMR** (101 MHz, CDCl₃) δ 148.8 (C3), 147.2 (C4), 124.5 (C1), 113.2 (C2), 112.5 (C6), 109.8 (C5), 101.8 (C7).

Data consistent with literature.³⁵

2-Phenylcyclopentane-1-one 223

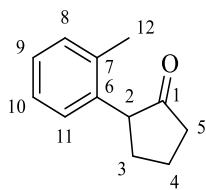


Synthesised following a reported procedure.³⁶ In an oven-dried pressure tube filled with argon and sealed with a PTFE cap, Pd(OAc)₂ (5.6 mg, 2.5 mol%), P(*o*-Tol)₃ (15 mg, 5 mol%), NaOAc (82 mg, 1 mmol), cyclopentanone (88 μL, 1.0 mmol), bromobenzene (105 μL, 1.0 mmol), pyrrolidine (25 μL, 0.3 mmol), *tert*-octylamine (48 μL, 0.3 mmol, *optional*), 1,4-dioxane (4 mL) were added. The solution was stirred at 110 °C for 16 h. The solution was cooled to room temperature, filtered through a silica plug and eluted with EtOAc. The filtrate was concentrated *in vacuo*. FCC (10/1 pentane/EtOAc) afforded the title compound as a yellow oil (46 mg, 29%).

Alternatively, the same procedure can be applied with iodobenzene instead of bromobenzene to yield the title compound (43 mg, 27%). **R_f** 0.39 (7/1 pentane/EtOAc) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.44 – 7.19 (m, 5H, H-Ar), 3.44 – 3.31 (m, 1H, H-2), 2.64 – 2.45 (m, 2H, H-3), 2.42 – 2.11 (m, 2H, H-4/H-5), 2.08 – 1.89 (m, 2H, H-4/H-5) ; **¹³C NMR** (101 MHz, CDCl₃) δ 218.2 (C1), 138.6 (C9), 128.8 (C8), 128.3 (C7), 127.1 (C6), 55.5 (C2), 38.6 (C3), 31.9 (C4), 21.0 (C5).

Data consistent with literature.³⁶

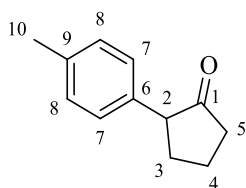
2-(*p*-Tolyl)cyclopentane-1-one **224**



Synthesised following a reported procedure.³⁶ In an oven-dried vial sealed with a screw cap, Pd(OAc)₂ (5.6 mg, 2.5 mol%), P(*o*-Tol)₃ (15 mg, 5 mol%), NaOAc (82 mg, 1.00 mmol), *tert*-octylamine (48 μ L, 0.30 mmol), cyclopentanone (84 μ L, 1 mmol), bromide **220** (171 mg, 1.00 mmol), pyrrolidine (25 μ L, 0.30 mmol), *tert*-octylamine (48 μ L, 0.30 mmol), 1,4-dioxane (4 mL) were added. The solution was stirred at 110 °C for 12 h. The solution was cooled to room temperature, filtered through a silica plug and eluted with EtOAc, and the filtrate was concentrated *in vacuo*. FCC (1/15 EtOAc/pentane) afforded the title compound **224** as a yellow oil (19 mg, 11%). *R*_f 0.34 (1/15 EtOAc/pentane) ; ¹H NMR (400 MHz, CDCl₃) δ 7.21 – 7.12 (m, 4H, H-Ar), 3.53 (dd, *J* = 8.0, 1.5 Hz, 1H, H-2), 2.57 – 2.46 (m, 2H, H₂-5), 2.32 (s, 3H, H₃-12), 2.24 – 2.12 (m, 2H, H₃-3), 2.11 – 1.96 (m, 2H, H₂-4) ; ¹³C NMR (101 MHz, CDCl₃) δ 219.1 (C1), 131.0 (C9), 129.7 (C10), 128.2 (C8), 127.8 (C11), 127.3 (C6), 126.7 (C7), 53.4 (C2), 39.1 (C5), 32.2 (C3), 21.5 (C4), 20.3 (C12).

Data consistent with literature.³⁶

2-(*o*-Tolyl)cyclopentane-1-one **284**

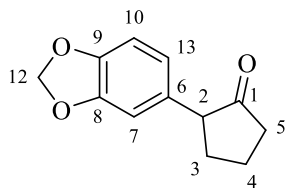


Compound **284** was isolated from the same experiment as **224** as a yellow solid (2.4 mg, 1.4%).

*R*_f 0.25 (1/15 EtOAc/pentane) ; ¹H NMR (400 MHz, CDCl₃) δ 7.24 – 7.06 (m, 4H, H-Ar), 3.64 (t, *J* = 6.5 Hz, 1H, H-2), 2.70 (dd, *J* = 7.5, 2.5 Hz, 2H, H₂-5), 2.64 – 2.56 (m, 2H, H₂-3), 2.56 – 2.48 (m, 2H, H₂-4), 2.36 (s, 3H, H₃-10).

Data consistent with literature.³⁷

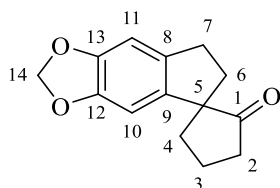
2-(Benzo[d][1,3]dioxol-5-yl)-cyclopentan-1-one 225



Synthesised by analogy to a reported procedure.³⁶ In an oven-dried pressure tube filled with argon and sealed with a PTFE cap, Pd(OAc)₂ (5.6 mg, 2.5 mol%), P(*o*-Tol)₃ (15 mg, 5 mol%), NaOAc (82 mg, 1.0 mmol), cyclopentanone (88 μ L, 2.0 mmol), halogen **222** (201 μ L, 2.0 mmol), *tert*-octylamine (48 μ L, 0.3 mmol), pyrrolidine (25 μ L, 0.3 mmol), 1,4-dioxane (4 mL) were added. The solution was stirred at 110 °C for 12 h. The solution was cooled to room temperature, filtered through a silica plug and eluted with EtOAc, and the filtrate was concentrated *in vacuo*. FCC (7/1 pentane/EtOAc) afforded the title compound as a yellow oil (62 mg, 28%). **R_f** 0.23 (10/1 pentane/EtOAc) ; **¹H NMR** (400 MHz, CDCl₃) δ 6.78 (s, 1H, H-7), 6.71 – 6.59 (m, 2H, H-10/H-13), 5.93 (s, 2H, H₂-12), 3.29 – 3.19 (m, 1H, H-2), 2.54 – 2.37 (m, 2H, H₂-5), 2.27 (m, 2H, H₂-3), 2.20 – 2.08 (m, 2H, H₂-4) ; **¹³C NMR** (101 MHz, CDCl₃) δ 218.3 (C1), 148.0 (C9), 146.7 (C8), 132.3 (C10), 121.5 (C6), 108.5 (C13), 108.3 (C7), 101.2 (C12), 55.3 (C2), 38.4 (C5), 32.1 (C3), 22.5 (C4)

Data consistent with literature.³⁸

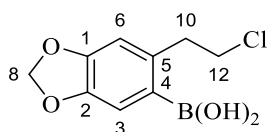
6',7'-Dihydrospiro[cyclopentane-1,5'-indeno[5,6-d][1,3]dioxol]-2-one 226



Synthesised by analogy to a reported procedure.³⁶ In an oven-dried vial fitted with a screw cap, Pd(OAc)₂ (3.1 mg, 2.5 mol%), P(*o*-Tol)₃ (8.7 mg, 5 mol%), NaOAc (47 mg, 0.57 mmol), cyclopentanone (50 μ L, 0.57 mmol), halogen **217** (150 mg, 0.57 mmol), *tert*-octylamine (8.7 μ L, 0.17 mmol), pyrrolidine (12 μ L, 0.17 mmol), 1,4-dioxane (2 mL) were added. The solution was stirred at 110 °C for 12 h. The solution was cooled to room temperature, filtered through a silica plug and eluted with EtOAc, and the filtrate was concentrated *in vacuo*. FCC (10/1 pentane/EtOAc) immediately followed by a second FCC (40/1 pentane/EtOAc) afforded the title compound as yellow crystals (11 mg, 8%). **R_f**

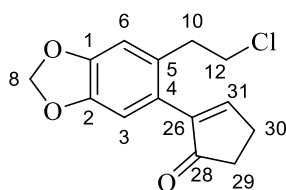
0.23 (9/1 pentane/EtOAc); **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 1737 (C=O) ; **^1H NMR** (600 MHz, CDCl_3) δ 6.70 (s, 1H, H-11), 6.47 (s, 1H, H-10), 5.92 (s, 2H, H₂-14), 3.43 (t, J = 7.5 Hz, 2H, H₂-2), 3.01 – 2.84 (m, 2H, H₂-4), 2.60 (t, J = 6.0 Hz, 2H, H₂-7), 2.57 – 2.07 (m, 2H, H₂-6), 1.85 (quart, J = 6.0 Hz, 2H, H₂-3) ; **^{13}C NMR** (151 MHz, CDCl_3) δ 221.3 (C1), 147.40 (C13), 146.8 (C12), 137.9 (C11), 137.8 (C10), 108.6 (C8), 108.0 (C9), 101.1 (C14), 47.4 (C2), 37.6 (C5), 37.4 (C7), 30.5 (C3), 28.1 (C4), 22.7 (C6) ; **HRMS** (ESI+) mass calculated for $[\text{M}+\text{Na}]^+$ ($\text{C}_{14}\text{H}_{14}\text{O}_3\text{Na}$) requires m/z 253.0841, found m/z 253.0851.

[6-(2-Chloroethyl)benzo[d][1,3]dioxol-5-yl]boronic acid **285**



Synthesised following a reported procedure.³² A solution of chlorine **217** (39 mg, 0.15 mmol) in dry THF (1 mL) was cooled to -78°C . *n*-BuLi (100 μL , 0.16 mmol, 1.6 M in hexanes) was added dropwise. The solution was stirred 30 minutes at -78°C . Trimethyl borate (33 μL , 0.30 mmol) was slowly added. The solution was warmed to room temperature, stirred for 5 h, quenched with aq. HCl (2 M, 2 mL) and stirred for 16 h. The solution was extracted with chloroform ($3 \times 10\text{ mL}$), and the combined organic fractions were dried (MgSO_4), filtered, and concentrated *in vacuo*. The crude material was used without further purification.

2-[6-(2-Chloroethyl)benzo[d][1,3]dioxol-5-yl]cyclopent-2-en-1-one **231**

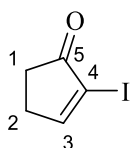


Synthesised following a reported procedure.³² In an oven-dried vial fitted with a screw cap, to a solution of boronic acid **283** (30 mg, 0.14 mmol), freshly made vinyl iodine **235** (36 mg, 0.17 mmol) in benzene (1 mL) and isopropanol (0.3 mL) was added triphenylphosphine (4 mg, 10 mol%). The solution was degassed with argon for 5 minutes. $\text{Pd}_2(\text{dba})_3$ (13 mg, 10 mol%) was added, and the solution stirred for 15 min at room temperature. Diethylamine (22 μL , 0.21 mmol) and H_2O (0.3 mL) were added, and the solution was degassed with argon for 5 min. The solution was warmed to 85°C and stirred for 2 h 30. The solution was diluted with Et_2O (10 mL), washed with aq. NaOH (0.2 M, 5 mL) and aq. HCl (0.5 M,

5 mL), the organic fraction was dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (5/1 pentane/diethyl ether) afforded the title compound as a red oil (9 mg, 24% over two steps). **R_f** 0.07 (5/1 pentane/Et₂O) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.62 (td, *J* = 8.0, 2.0 Hz, 1H, H-31), 6.70 (s, 1H, H-3), 6.68 (s, 1H, H-6), 5.86 (s, 2H, H₂-8), 3.75 (t, *J* = 6.5 Hz, 2H, H₂-12), 2.72 (t, *J* = 6.5 Hz, 2H, H₂-10), 2.65 (td, *J* = 7.0, 3.5 Hz, 2H, H₂-29), 2.41 – 2.29 (m, 2H, H₂-30).

Data consistent with literature.³²

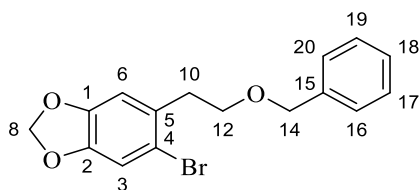
2-Iodocyclopent-2-en-1-one 235



Synthesised following a reported procedure.³⁹ To a solution of cyclopentanone (0.3 mL, 3 mmol) in dry THF (7.5 mL) and H₂O (7.5 mL) were added potassium carbonate (498 mg, 3.6 mmol), molecular iodine (1143 mg, 4.5 mL) and 4-Dimethylaminopyridine (DMAP, 72 mg, 0.6 mmol). The solution was stirred at room temperature for 1 h, diluted with EtOAc and washed with sat. aq. Na₂SO₃ (20 mL) and aq. HCl (0.1M, 20 mL). The aqueous fractions were extracted with EtOAc (3 × 20 mL). The combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (7/3 pentane/diethyl ether) afforded the title compound as clear white crystals (600 mg, 96%). The compound is unstable and must be used quickly. **R_f** 0.23 (7/3 pentane/Et₂O) ; **¹H NMR** (400 MHz, CDCl₃) δ 8.02 (t, *J* = 3.0 Hz, 1H, H3), 2.78 (dd, *J* = 7.5, 2.0 Hz, 2H, H₂-1), 2.55 – 2.48 (m, 2H, H₂-2).

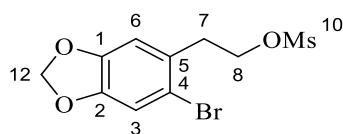
Data consistent with literature.³⁹

5-[2-(Benzyloxy)ethyl]-6-bromobenzo[d][1,3]dioxole 237



Synthesised following a reported procedure.⁴⁰ To a solution of alcohol **216** (50 mg, 0.2 mmol) in dry THF (2 mL) was added NaH (43% suspended in mineral oil, 12 mg, 0.22 mmol). The solution was stirred 5 minutes at room temperature. Benzyl bromide (26 μ L, 0.22 mmol) was added dropwise. The solution was warmed to reflux for 5 h, cooled to room temperature, diluted with H₂O (2 mL), and extracted with Et₂O (4 $\times$ 5 mL). The combined organic fractions were washed with brine (5 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo*. FCC (8/1 pentane/Et₂O) afforded the title compound as a clear oil (34 mg, 69%). **R_f** 0.44 (8/1 pentane/Et₂O) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 1250 (O-C14) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.41 – 7.29 (m, 5H, H–Benzyl), 6.99 (s, 1H, H–3), 6.78 (s, 1H, H–6), 5.94 (s, 2H, H₂–8), 4.53 (s, 2H, H₂–14), 3.65 (t, J = 7.0 Hz, 2H, H₂–12), 2.98 (t, J = 7.0 Hz, 2H, H₂–10) ; **¹³C NMR** (101 MHz, CDCl₃) δ 128.6 (C1/C2), 127.8 (C4), 127.8 (C5), 112.9 (C–Bn), 110.9 (C–Bn), 101.8 (C8), 73.1 (C14), 69.7 (C12), 36.6 (C10) ; **HRMS** (ESI+) mass calculated for [M+Na]⁺ (C₁₆H₁₅BrO₃Na) requires m/z 357.0102 found m/z 357.0105.

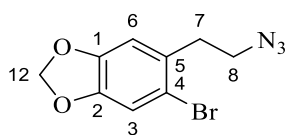
2-(6-Bromobenzo[d][1,3]dioxol-5-yl)ethyl methanesulfonate 238



Synthesised by analogy to a reported procedure.⁴¹ A solution of alcohol **216** (80 mg, 0.32 mmol), triethylamine (104 μ L, 0.75 mmol) in dry DCM (1 mL) was stirred at 0 °C, and methanesulfonyl chloride (30 μ L, 0.39 mmol) was added dropwise. The solution was stirred at 0 °C for 1 h, quenched with aq. NH₄Cl (2 M, 5 mL), extracted with Et₂O (3 $\times$ 10 mL), washed with sat. aq. NH₄Cl (2 $\times$ 5 mL), the combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the title compound as a clear oil (104 mg, quant). **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 1558 (S=O) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.01 (s, 1H, H–3), 6.77 (s, 1H, H–6), 5.98 (s, 2H, H₂–12), 4.39 (t, J = 7.0 Hz, 2H, H₂–8), 3.16 – 3.07 (m, 2H, H–7), 2.94 (s, 3H, H₃–10) ; **¹³C NMR** (101 MHz, CDCl₃) 128.4 (C1), 127.7 (C2), 127.6 (C4/C5), 113.1

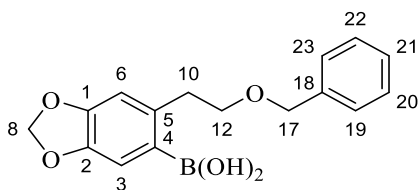
(C3), 110.9 (C6), 101.9 (C12), 68.4 (C8), 35.9 (C7) ; **HRMS** (ESI+) mass calculated for $[M+Na]^+$ ($C_{10}H_{11}BrNaO_5S$) requires m/z 344.9408, found m/z 344.9403.

5-(2-Azidoethyl)-6-bromobenzo[d][1,3]dioxole **239**



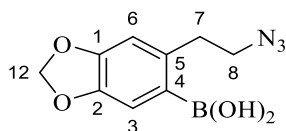
Synthesised by analogy to a reported procedure.³⁹ A solution of compound **238** (81 mg, 0.25 mmol), NaI (49 mg, 0.33 mmol), NaN_3 (36 mg, 0.55 mmol) in dry DMF (1.5 mL) was stirred 2 h at room temperature, and 1 h at 85 °C. The solution was diluted with H_2O (10 mL), extracted with Et_2O (4×10 mL), the combined organic fractions were washed with H_2O (3×10 mL) and brine (20 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford the title compound as a clear oil (67 mg, quant.). **IR** ν_{max}/cm^{-1} 2102 (N=N=N) ; **^1H NMR** (400 MHz, $CDCl_3$) δ 7.00 (s, 1H, H-3), 6.74 (s, 1H, H-6), 5.96 (s, 2H, H₂-12), 3.46 (t, $J = 7.0$ Hz, 2H, H₂-8), 2.97 – 2.91 (m, 2H, H₂-7) ; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 147.5 (C2), 147.4 (C1), 130.1 (C4), 114.5 (C3), 112.9 (C6), 110.5 (C5), 101.8 (C12), 50.9 (C8), 35.6 (C7) ; **HRMS** (ESI+) mass calculated for $[M+Na]^+$ ($C_9H_8BrN_3O_2Na$) requires m/z 291.9697, found m/z 291.9693.

(6-(2-(Benzyloxy)ethyl)benzod1,3dioxol-5-yl)boronic acid **286**



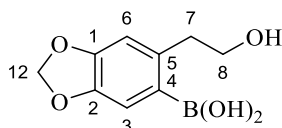
Synthesised by analogy to a reported procedure.³² A solution of benzyl **237** (34 mg, 0.10 mmol) in dry THF (1 mL) was cooled to –78 °C. *n*-BuLi (167 μ L, 0.11 mmol, 1.6 M in hexanes) was added dropwise. The solution was stirred 30 minutes at –78 °C. Trimethyl borate (22 μ L, 0.20 mmol) was slowly added. The solution was warmed to room temperature, stirred for 5 h, quenched with aq. HCl (2 M, 2 mL) and stirred for 16 h. The solution was extracted with chloroform (3×5 mL), and the combined organic fractions were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude material was used without further purification.

[6-(2-Azidoethyl)benzo[d][1,3]dioxol-5-yl]boronic acid **287**



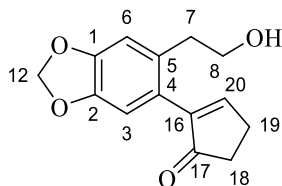
Synthesised by analogy to a reported procedure.³² A solution of azide **239** (67 mg, 0.25 mmol) in dry THF (2.7 mL) was cooled to $-78\text{ }^{\circ}\text{C}$. *n*-BuLi (169 μL , 0.27 mmol, 1.6 M in hexanes) was added dropwise. The solution was stirred 30 minutes at $-78\text{ }^{\circ}\text{C}$. Trimethyl borate (55 μL , 0.50 mmol) was slowly added. The solution was warmed to room temperature, stirred for 5 h, quenched with aq. HCl (2 M, 2 mL) and stirred for 16 h. The solution was extracted with chloroform ($3 \times 10\text{ mL}$), and the combined organic fractions were dried (MgSO_4), filtered, and concentrated *in vacuo*. The crude material was used without further purification.

[6-(2-Hydroxyethyl)benzo[d][1,3]dioxol-5-yl]boronic acid **288**



Synthesised by analogy to a reported procedure.³² A solution of alcohol **216** (60 mg, 0.08 mmol) in dry THF (1 mL) was cooled to $-78\text{ }^{\circ}\text{C}$. *n*-BuLi (104 μL , 0.17 mmol, 1.6 M in hexanes) was added dropwise. The solution was stirred 30 minutes at $-78\text{ }^{\circ}\text{C}$. Trimethyl borate (18 μL , 0.16 mmol) was slowly added. The solution was warmed to room temperature, stirred for 15 h, quenched with aq. HCl (2 M, 7 mL) and stirred for 16 h. The solution was extracted with chloroform ($3 \times 10\text{ mL}$), and the combined organic fractions were dried (MgSO_4), filtered, and concentrated *in vacuo*. The crude material was used without further purification.

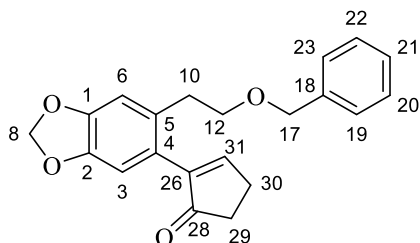
2-[6-(2-Hydroxyethyl)benzo[d][1,3]dioxol-5-yl]cyclopent-2-en-1-one **240**



Synthesised by analogy to a reported procedure.³² In an oven-dried vial fitted with a screw cap, to a solution of boronic acid **288** (30 mg, 0.15 mmol), freshly made vinyl iodine **235** (40 mg, 0.19 mmol) in

benzene (1 mL) and isopropanol (0.3 mL) was added triphenylphosphine (4 mg, 10 mol%). The solution was degassed with argon for 5 minutes. Pd₂(dba)₃ (14 mg, 10 mol%) was added, and the solution stirred for 15 min at room temperature. Diethylamine (23 µL, 0.23 mmol) and H₂O (0.3 mL) were added, and the solution was degassed with argon for 5 min. The solution was warmed to 85 °C and stirred for 3h. The solution was diluted with EtOAc (10 mL), washed with aq. NaOH (0.2 M, 5 mL) and aq. HCl (0.5 M, 5 mL), the organic fraction was dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (2/1 EtOAc/pentane) afforded the title compound as an orange oil (31 mg, 63% over two steps). **R_f** 0.09 (1/1 pentane/EtOAc) ; **IR** ν_{max} /cm⁻¹ 1734 (C=O) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.62 (t, *J* = 3.0 Hz, 1H, H-20), 6.79 (s, 1H, H-3), 6.57 (s, 1H, H-6), 5.94 (s, 2H, H₂-12), 3.77 (t, *J* = 6.5 Hz, 2H, H₂-8), 2.86 – 2.73 (m, 2H, H₂-18), 2.66 (t, *J* = 6.5 Hz, 2H, H₂-7), 2.63 – 2.51 (m, 2H, H₂-19) ; **¹³C NMR** (101 MHz, CDCl₃) δ 209.1 (C17), 162.9 (C20), 148.5 (C1), 146.7 (C2), 132.7 (C6), 131.3 (C3), 129.0 (C5), 125.8 (C4), 110.3 (C16), 101.7 (C12), 63.7 (C8), 36.4 (C7), 33.3 (C18), 29.7 (C19) ; **HRMS** (ESI+) mass calculated for [M+Na⁺] (C₁₄H₁₄NaO₄) requires *m/z* 269.0789, found *m/z* 269.0785.

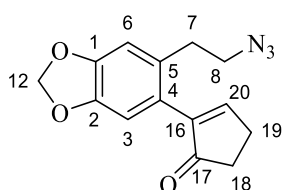
2-{6-[2-(Benzyloxy)ethyl]benzo[d][1,3]dioxol-5-yl}cyclopent-2-en-1-one **241**



Synthesised by analogy to a reported procedure.³² In an oven-dried vial fitted with a screw cap, to a solution of boronic acid **286** (28 mg, 0.09 mmol), freshly made vinyl iodine **235** (24 mg, 0.12 mmol) in benzene (0.6 mL) and isopropanol (0.2 mL) was added triphenylphosphine (3 mg, 10 mol%). The solution was degassed with argon for 5 minutes. Pd₂(dba)₃ (8.5 mg, 10 mol%) was added, and the solution stirred for 15min at room temperature. Diethylamine (14 µL, 0.14 mmol) and H₂O (0.2 mL) were added, and the solution was degassed with argon for 5 min. The solution was warmed to 85 °C and stirred for 2h30. The solution was diluted with Et₂O (10 mL), washed with aq. NaOH (0.2 M, 5 mL) and aq. HCl (0.5 M, 5 mL), the organic fraction was dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (5/1 pentane/diethyl ether) afforded the title compound as a dark red oil (30 mg, 90% over two steps).

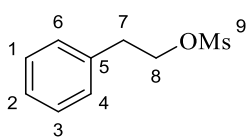
R_f 0.05 (5/1 pentane/Et₂O) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 1735 (C=O) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.45 (t, J = 3.0 Hz, 1H, H-31), 7.31 – 7.16 (m, 5H, H-Benzyl), 6.71 (s, 1H, H-3), 6.51 (s, 1H, H-6), 5.86 (s, 2H, H₂-8), 4.40 (s, 2H, H₂-17), 3.49 (t, J = 7.5 Hz, 2H, H₂-12), 2.73 – 2.59 (m, 4H, H₂-10/29), 2.49 – 2.42 (m, 2H, H₂-30) ; **¹³C NMR** (101 MHz, CDCl₃) δ 208.0 (C28), 161.5 (C31), 128.4 (C1), 127.6 (C2), 127.6 (C4/C5), 109.8 (C3), 109.6 (C6), 101.1 (C8), 73.0 (C17), 71.0 (C12), 60.4 (C26), 34.7 (C10), 33.5 (C29), 31.9 (C30) ; **HRMS** (ESI+) mass calculated for [M+Na]⁺ (C₂₁H₂₀O₄Na) requires m/z 359.1259, found m/z 359.1253.

2-[6-(2-Azidoethyl)benzo[d][1,3]dioxol-5-yl]cyclopent-2-en-1-one 242



Synthesised by analogy to a reported procedure.³⁹ A solution of compound **245** (6 mg, 0.02 mmol), NaI (4 mg, 0.03 mmol), NaN₃ (3 mg, 0.05 mmol) in dry DMF (0.5 mL) was stirred 1 h at 85 °C. The solution was diluted with H₂O (5 mL), extracted with Et₂O (4 × 10 mL), the combined organic fractions were washed with H₂O (3 × 10 mL) and brine (10 mL), dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the title compound as a clear oil (4 mg, quant.). **R_f** 0.18 (3/1 pentane/Et₂O) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 2089 (N=N=N), 1736 (C=O) ; **¹H NMR** (400 MHz, CDCl₃) δ 7.61 (t, J = 3.0 Hz, 1H, H-20), 6.74 (s, 1H, H-3), 6.58 (s, 1H, H-6), 5.95 (s, 2H, H₂-12), 3.37 (t, J = 7.5 Hz, 2H, H₂-8), 2.77 (d, J = 7.5 Hz, 2H, H₂-7), 2.73 – 2.64 (m, 2H, H₂-18), 2.62 – 2.55 (m, 2H, H₂-19) ; **¹³C NMR** (101 MHz, CDCl₃) δ 207.9 (C17), 162.4 (C20), 149.9 (C2), 148.1 (C1), 132.4 (C3), 132.3 (C6), 128.8 (C5), 128.7 (C4), 125.7 (C16), 101.4 (C12), 66.1 (C8), 52.5 (C18), 34.9 (C7), 29.6 (C19) ; **HRMS** (ESI+) mass calculated for [M+Na]⁺ (C₁₅H₁₆O₆SN₃) requires m/z 294.0854, found m/z 294.0856.

Phenethyl methanesulfonate 289

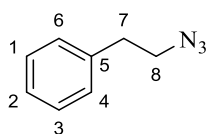


Synthesised following a reported procedure.⁴¹ A solution of 2-phenylethanol (120 μ L, 1.00 mmol), triethylamine (320 μ L, 3.00 mmol) in dry DCM (2 mL) was stirred at 0 °C, and methanesulfonyl chloride

(93 μ L, 1.20 mmol) was added dropwise. The solution was stirred at 0 °C for 1 h, quenched with aq. NH_4Cl (2 M, 10 mL), extracted with Et_2O (3×10 mL), washed with sat. aq. NH_4Cl (2×10 mL), the combined organic fractions were dried (MgSO_4), filtered, and concentrated *in vacuo* to afford the title compound as a clear oil (200 mg, quant.). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.38 – 7.22 (m, 5H, H–Ar), 4.43 (t, J = 6.9 Hz, 2H, H_2 –8), 3.06 (t, J = 6.9 Hz, 2H, H_2 –7), 2.84 (s, 3H, H_3 –9).

Data consistent with literature.³⁹

(2-Azidoethyl)benzene 290

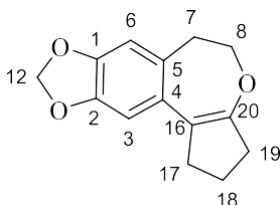


Synthesised following a reported procedure.⁴² A solution of compound **285** (200 mg, 1 mmol), NaI (195 mg, 1.3 mmol), NaN_3 (143 mg, 2.2 mmol) in dry DMF (1.3 mL) was stirred 2 h at room temperature, and 1 h at 85 °C. The solution was diluted with H_2O (10 mL), extracted with Et_2O (4×10 mL), the combined organic fractions were washed with H_2O (3×10 mL) and brine (20 mL), dried (MgSO_4), filtered, and concentrated *in vacuo* to afford the title compound as a clear oil (100 mg, 72%). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.38 – 7.15 (m, 5H, H–Ar), 3.51 (t, J = 7.5 Hz, 2H, H_2 –8), 2.90 (t, J = 7.5 Hz, 2H, H_2 –7).

Data consistent with literature.⁴³

Compounds **289** and **290** were made to test the azide transfer steps on a model system.

2,3,5,6-Tetrahydro-1H-[1,3]dioxolo[4',5':4,5]benzo[1,2-d]cyclopenta[b]oxepine **244**



Method 1 To a solution of enone **240** (10 mg, 0.04 mmol) in dry THF (1 mL), cooled to -78 °C, L-Selectride® (50 μ L, 0.05 mmol, 1 M in THF) was added dropwise. The solution was stirred at -78 °C for 30 minutes, quenched with sat. aq. NH_4Cl (5 mL), warmed to room temperature, and extracted with EtOAc (4×10 mL). The combined organic fractions were washed with brine (10 mL), dried (MgSO_4), filtered, and concentrated *in vacuo* to afford the title compound (12 mg, quant.).

Method 2 Synthesised by analogy to a reported procedure.⁴⁴ A solution of enone **240** (23 mg, 0.07 mmol), palladium on activated carbon (10 mg, 10 mol%) in absolute ethanol was placed under hydrogen atmosphere. The solution was stirred for 76 h, filtered on Celite®, and concentrated *in vacuo* to afford the title compound.

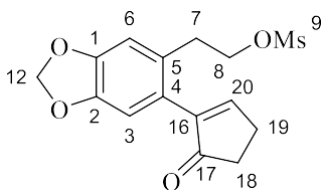
Method 3 Synthesised by analogy to a reported procedure.⁴⁵ To a solution of ketone **246** (5 mg, 0.015 mmol), cyclopropyl salt **59** (5.6 mg, 0.018 mmol) in DMSO (0.5 mL) was added dry powdered KOH (2 mg, 0.03 mmol). The solution was stirred at room temperature for 16 h, diluted in sat. aq. NaHCO_3 (5 mL), extracted with Et_2O (3×10 mL), the combined organic fractions were washed with brine (10 mL), dried (MgSO_4), filtered, and concentrated *in vacuo* to afford the crude title compound.

Method 4 Synthesised by analogy to a reported procedure.⁴⁵ To a solution of salt **246** (15 mg, 0.048 mmol) in DMSO (0.5 mL) stirred at room temperature, was added NaH (2.6 mg, 0.048 mmol, 43% in mineral oil). The solution was stirred 30 min at room temperature, and ketone

246 (10 mg, 0.04 mmol) was added. The solution was stirred 16 h, diluted in sat. aq. NaHCO₃ (5 mL), extracted with Et₂O (3 × 10 mL), the combined organic fractions were washed with brine (10 mL), dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the crude title compound.

The title compound is quickly degraded upon purification by FCC, and full analysis could not be conducted. ¹H NMR (400 MHz, CDCl₃) δ 6.66 (s, 1H, H-3), 6.53 (s, 1H, H-6), 5.90 (s, 2H, H₂-12), 4.26 – 4.18 (m, 2H, H₂-8), 3.04 – 2.96 (m, 2H, H₂-19), 2.74 (tt, *J* = 7.5, 1.5 Hz, 2H, H₂-17), 2.59 (m, 2H, H₂-7), 1.97 – 1.83 (m, 2H, H₂-17) ; ¹³C NMR (101 MHz, CDCl₃) δ 153.8 (C20), 146.2 (C2), 144.6 (C1), 134.48 – 132.1 (m, C3/C4/C5/C6), 109.2 (C16), 101.0 (C12), 70.1 (C8), 47.0 (C16), 38.6 (C7), 35.6 (C19), 35.3 (C17), 29.9 (C18).

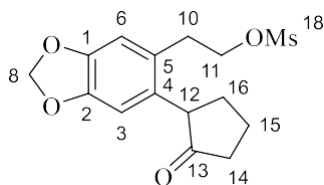
2-[6-(5-Oxocyclopent-1-en-1-yl)benzo[d][1,3]dioxol-5-yl]ethyl methanesulfonate **245**



Synthesised by analogy to a reported procedure.⁴¹ A solution of alcohol **240** (8 mg, 0.03 mmol), triethylamine (11 µL, 0.08 mmol) in dry DCM (1 mL) was stirred at 0 °C, and methanesulfonyl chloride (3 µL, 0.04 mmol) was added dropwise. The solution was stirred at 0 °C for 1 h, quenched with sat. aq. NH₄Cl (5 mL), extracted with Et₂O (3 × 10 mL), washed with brine (5 mL), the combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the title compound as a clear oil (9 mg, quant.). IR ν_{max}/cm⁻¹ 1735 (C=O), 1588 (S=O) ; ¹H NMR (400 MHz, CDCl₃) δ 7.61 (t, *J* = 3.0 Hz, 1H, H-20), 6.75 (s, 1H, H-3), 6.59 (s, 1H, H-6), 4.27 (t, *J* = 7.5 Hz, 2H, H₂-8), 2.91 (s, 3H, H₃-9), 2.88 (t, *J* = 7.5 Hz, 2H, H₂-7), 2.83 – 2.74 (m, 2H, H₂-18), 2.62 – 2.55 (m, 2H, H₂-19) ; ¹³C NMR (101 MHz, CDCl₃) δ 208.2 (C17), 162.3 (C20), 147.6 (C2), 145.9 (C1), 132.1 (C3), 132.0 (C6), 128.5 (C16), 110.0 (C4), 109.7

(C5), 101.4 (C12), 70.2 (C8), 52.3 (C9) 37.3 (C7), 34.8 (C18), 29.7 (C19) ; **HRMS** (ESI+) mass calculated for $[M+Na]^+$ ($C_{15}H_{16}O_6SNa$) requires m/z 347.0565, found m/z 347.0564.

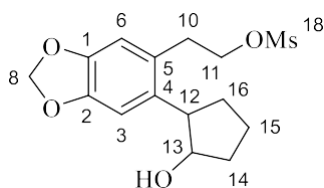
2-[6-(2-Oxocyclopentyl)benzo[d][1,3]dioxol-5-yl]ethyl methanesulfonate **246**



Method 1 Synthesised by analogy to a reported procedure.⁴⁶ To a solution of alcohol **247** (15 mg, 0.05 mmol) in DCM (0.3 mL) stirred at 0 °C, Dess-Martin periodinane (23 mg, 0.06 mmol) was added. The solution was warmed to room temperature and stirred for 3 h. The solution was diluted in DCM, washed with sat. aq. $Na_2S_2O_3$ (2×5 mL), sat. aq. $NaHCO_3$ (2×5 mL). The organic phase was filtered through Celite, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. FCC (3/1 Et_2O /pentane) afforded the title compound as a yellow oil (15 mg, quant.).

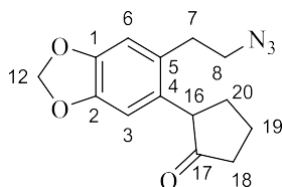
Method 2 Synthesised by analogy to a reported procedure.⁴⁵ Enone **245** (95 mg, 0.29 mmol) and Pd/C (50 mg, 10 mol%, 5% in weight) were placed under a hydrogen atmosphere. Ethanol (5 mL) was added, and the solution was stirred for 48 h. The solution was filtered on Celite, and concentrated *in vacuo* to afford the title compound as a yellow oil (96 mg, quant.). **R_f** 0.35 (3/1 Et_2O /pentane) ; **IR** ν_{max}/cm^{-1} 1735 (C=O) ; **¹H NMR** (400 MHz, $CDCl_3$) δ 6.69 (s, 1H, H-3), 6.48 (s, 1H, H-6), 5.94 (d, $J = 1.5$ Hz, 1H, H-8), 5.90 (d, $J = 1.5$ Hz, 1H, H-8), 4.40 – 4.24 (m, 2H, H₂-11), 3.48 (m, 1H, H-12), 3.06 – 2.93 (m, 2H, H₂-10), 2.90 (s, 3H, H₃-18), 2.56 – 2.42 (m, 2H, H₂-14), 2.07 – 1.89 (m, 2H, H₂-15), 1.98 (m, 2H, H₂-16) ; **¹³C NMR** (101 MHz, $CDCl_3$) δ 218.6 (C13), 147.1 (C2), 146.6 (C1), 131.1 (C3), 128.3 (C6), 110.0 (C10), 107.7 (C4), 101.1 (C8), 70.2 (C11), 65.7 (C12), 52.1 (C18), 37.1 (C14), 33.3 (C15), 32.8 (C16) ; **HRMS** (ESI+) mass calculated for $[M+Na]^+$ ($C_{15}H_{18}O_6SNa$) requires m/z 349.0722, found m/z 349.0726.

2-[6-(2-Hydroxycyclopentyl)benzo[d][1,3]dioxol-5-yl]ethyl methanesulfonate **247**



To a solution of enone **245** (22 mg, 0.07 mmol) in dry THF (4 mL), cooled to $-78\text{ }^{\circ}\text{C}$, L-Selectride® (150 μL , 0.150 mmol, 1 M in THF) was added dropwise. The solution was stirred at $-78\text{ }^{\circ}\text{C}$ for 30 minutes, quenched with sat. aq. NH_4Cl (15 mL), warmed to room temperature, and extracted with Et_2O ($3 \times 25\text{ mL}$). The combined organic fractions were washed with brine (10 mL), dried (MgSO_4), filtered, and concentrated *in vacuo*. FCC (2/1 Et_2O /pentane) afforded the title compound as a yellow oil (18 mg, 82%). **R_f** 0.26 (3/1 Et_2O /pentane) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 1584 (S=O), 1071 (C–OH) ; **¹H NMR** (400 MHz, CDCl_3) δ 6.93 (s, 1H, H–3), 6.71 (s, 1H, H–6), 5.93 (m, 2H, H₂–8), 4.41 – 4.26 (m, 2H, H₂–11), 4.20 (td, $J = 5.0, 2.0\text{ Hz}$, 1H, H–12), 3.18 – 3.07 (m, 1H, H–13), 3.07 – 2.96 (m, 2H, H₂–10), 2.92 (s, 3H, H₃–18), 2.18 – 1.91 (m, 2H, H₂–16), 1.91 – 1.72 (m, 4H, H₂–14/H₂–15) ; **¹³C NMR** (101 MHz, CDCl_3) δ 146.6 (C1), 147.2 (C2), 131.6 (C3), 127.8 (C6), 110.1 (C5), 109.1 (C4), 101.1 (C8), 74.6 (C11), 69.9 (C13), 60.4 (C12), 52.3 (C18), 47.1 (C14), 37.5 (C10), 34.6 (C15), 32.7 (C16) ; **HRMS** (ESI+) mass calculated for $[\text{M}+\text{Na}]^+$ ($\text{C}_{15}\text{H}_{18}\text{O}_6\text{SNa}$) requires m/z 351.0878, found m/z 351.0881.

2-[6-(2-Azidoethyl)benzo[d][1,3]dioxol-5-yl]cyclopentan-1-one **248**

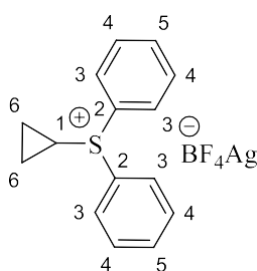


Method 1 To a solution of enone **242** (13 mg, 0.05 mmol) in dry THF (1 mL), cooled to $-78\text{ }^{\circ}\text{C}$, L-Selectride® (50 μL , 0.05 mmol, 1 M in THF) was added dropwise. The solution was stirred at $-78\text{ }^{\circ}\text{C}$ for 30 minutes, quenched with sat. aq. NH_4Cl (5 mL), warmed to room temperature, and extracted with Et_2O ($3 \times 10\text{ mL}$). The combined organic fractions were washed

with brine (10 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (4/1 pentane/Et₂O) afforded the title compound as an orange oil (8 mg, 61%).

Method 2 Synthesised by analogy to a reported procedure.⁴² A solution of compound **246** (4 mg, 0.01 mmol), NaI (3 mg, 0.02 mmol), NaN₃ (2 mg, 0.03 mmol) in dry DMF (0.5 mL) was stirred 1 h at 85 °C. The solution was diluted with H₂O (5 mL), extracted with Et₂O (4 × 10 mL), the combined organic fractions were washed with H₂O (3 × 10 mL) and brine (10 mL), dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the title compound as a clear oil (3.5 mg, quant.). **R_f** 0.26 (3/1 pentane/Et₂O) ; **IR** $\nu_{\text{max}}/\text{cm}^{-1}$ 2900 (C–H), 2100 (N=N=N), 1736 (C=O) ; **¹H NMR** (700 MHz, CDCl₃) δ 6.68 (s, 1H, H–3), 6.48 (s, 1H, H–6), 5.95 (d, *J* = 1.5 Hz, 1H, H–12), 5.89 (d, *J* = 1.5 Hz, 1H, H–12), 3.43 (m, 3H, H₂–8/H–16), 2.80 (t, *J* = 7.5 Hz, 2H, H₂–18), 2.49 (tt, *J* = 12.0, 7.0 Hz, 2H, H₂–7), 2.25 – 2.15 (m, 2H, H₂–19), 2.07 – 1.91 (m, 2H, H₂–20) ; **¹³C NMR** (176 MHz, CDCl₃) δ 218.0 (C17), 146.9 (C1), 141.8 (C2), 136.0 (C6), 130.9 (C3), 110.0 (C5), 108.0 (C4), 101.4 (C12), 52.7 (C8), 52.5 (C16), 38.7 (C18), 32.8 (C19), 32.1 (C20) ; **HRMS** (ESI+) mass calculated for [M+Na]⁺ (C₁₅H₁₈O₆SNa) requires *m/z* 296.1011, found *m/z* 296.1008.

Cyclopropyldiphenylsulfonium-tetrafluoroborate silver salt **251**

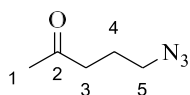


Synthesised following a reported procedure.⁴⁷ To a solution of diphenylsulfide (93 mg, 0.38 mmol), 1-chloro-3-iodo-propane (365 mg, 1.3 mmol) in DCM (5 mL) shielded from light, AgBF₄ (59 mg, 0.3 mmol) was added. The solution was stirred at room temperature for 16 h. DCM (7 mL) was added, the solution was filtered through a sintered glass funnel filled with Florisil®, and the solid was washed with DCM. The organic phase was concentrated *in vacuo*

until a solid precipitated. Et₂O (5 mL) was added to complete the precipitation. The solution was placed at –20 °C for 1 h, and the crystals were collected, washed with Et₂O and concentrated *in vacuo*. The crystals were dissolved in dry THF (5 mL), and stirred at room temperature. NaH (14.3 mg, 0.29 mmol, 43% in mineral oil) was added, and the solution was stirred at room temperature for 20 h. The solution was quenched with 48% HBF₄/NaBF₄ in water, and stirred 5 min. DCM was added, the organic phase was washed with water (5 mL). The aqueous fraction was extracted with DCM (3 × 10 mL), dried (Na₂SO₄), filtered, and concentrated *in vacuo*. Et₂O was added to precipitate the product. The crystals formed were washed with Et₂O and recrystallised from hot EtOH to afford the title compound (66 mg, 55%). ¹H NMR (400 MHz, CDCl₃) δ 8.00 – 7.61 (m, 10H, H-3/H-4/H-5), 3.81 (tt, *J* = 7.5, 4.5 Hz, 1H, H-1), 1.77 – 1.61 (m, 2H, H-6), 1.49 – 1.39 (m, 2H, H-6).

Data consistent with literature.⁴⁷

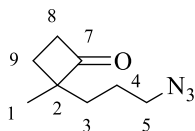
5-Azidopentan-2-one 252



Synthesised by analogy to a reported procedure.⁴⁵ A solution of 5-chloropentan-2-one (114 µL, 1.00 mmol), NaI (225 mg, 1.50 mmol), NaN₃ (163 mg, 2.50 mmol) in dry DMF (3 mL) was stirred at 85 °C for 2 h. The solution was diluted with water (10 mL), extracted with Et₂O (4 × 20 mL), the organic fractions were washed with water (water (3 × 20 mL), brine (20 mL), dried (MgSO₄), filtered, concentrated *in vacuo* to afford the title compound as a yellow oil (115 mg, 91%). ¹H NMR (400 MHz, CDCl₃) δ 3.29 (t, *J* = 7.0 Hz, 2H, H₂-5), 2.52 (t, *J* = 7.0 Hz, 2H, H₂-3), 1.83 (quint, *J* = 7 Hz, 2H, H₂-4), 1.28 – 1.17 (m, 2H, H₃-1).

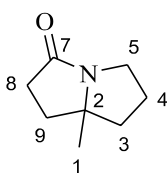
Data consistent with literature.⁴⁵

2-(3-Azidopropyl)-2-methylcyclobutan-1-one 253



Synthesised following a reported procedure.⁴⁵ To a solution of compound **252** (16 mg, 0.13 mmol), cyclopropyl salt **251** (47 mg, 0.15 mmol) in DMSO (2 mL) was added dry powdered KOH (14 mg, 0.25 mmol). The solution was stirred at room temperature for 16 h. The solution was diluted with sat. aq. NaHCO₃ (10 mL), extracted with Et₂O (3 × 15 mL), the combined organic fractions were washed with brine (10 mL), dried (MgSO₄), filtered, and concentrated *in vacuo* to afford the crude product (41 mg). The crude material was directly used in the next step.

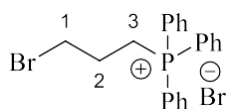
7a-methylhexahydro-3H-pyrrolizin-3-one 254



Synthesised following a reported procedure.⁴⁵ To a solution of the crude ketone **253** (21 mg, 0.13 mmol) in dry DCM (0.5 mL), stirred at 0 °C, TiCl₄ (504 µL, 1 M in toluene) was added dropwise. Gas evolution was observed. The solution was stirred 20 min at 0 °C, diluted with EtOAc (20 mL), washed with sat. aq. NaHCO₃ (5 mL), brine (5 mL), dried (Na₂SO₄), filtered, concentrated *in vacuo*. FCC (EtOAc) afforded the title compound (14 mg, 80%). ¹H NMR (400 MHz, CDCl₃) δ 3.55 (dt, *J* = 12.0, 8.0 Hz, 1H, H-8), 3.05 – 2.88 (m, 1H, H-8), 2.86 – 2.68 (m, 2H, H₂-5), 2.39 (dd, *J* = 9.0, 2.5 Hz, 2H, H-4), 2.35 (dd, *J* = 9.0, 2.5 Hz, 1H, H-4), 1.85 – 1.67 (m, 2H, H₂-9), 1.76 (dd, *J* = 7.0, 3.0 Hz, 2H, H-3), 1.72 (dd, *J* = 7.0, 3.0 Hz, 1H, H-3), 1.23 (s, 3H, H₃-1).

Data consistent with literature.⁴⁸

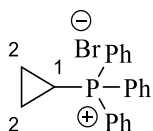
(3-Bromopropyl)triphenylphosphonium bromine salt **256**



Synthesised following a reported procedure.⁴⁹ To a solution of triphenylphosphine (4.89 g, 18.7 mmol) in dry toluene (20 mL) was added 1,3-dibromopropane (2.00 mL, 19.6 mmol). The solution was stirred at reflux for 16 h, cooled down and filtered. The crystals were washed with toluene and dried *in vacuo* to afford the title compound (8.52 g, 98%). ¹H NMR (400 MHz, DMSO) δ 7.99–7.80 (m, 15H, H–Ph), 3.75–3.61 (m, 4H, H₂–3/H₂–1), 2.08 (m, 2H, H₂–2).

Data consistent with literature.⁴⁹

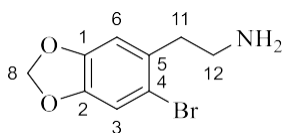
Cyclopropyltriphenylphosphonium bromine salt **257**



Synthesised following a reported procedure.⁴⁹ A solution of phosphonium salt **256** (8.5 g, 19.1 mmol) in aq. NaOH (1 M, 18 mL) was stirred at reflux for 20 h, cooled down, diluted in CHCl₃ (30 mL), extracted with CHCl₃ (3 × 30 mL). The combined organic fractions were concentrated *in vacuo* until a precipitate formed. Et₂O was added to complete the precipitation. The solid was filtered, washed with Et₂O, and dried *in vacuo* to afford the title compound (5 g, 72%). ¹H NMR (400 MHz, DMSO) δ 8.02–7.75 (m, 15H, H–Ph), 2.94–2.81 (m, 1H, H–1), 1.64–1.52 (m, 2H, H–2), 0.77–0.62 (m, 2H, H–2).

Data consistent with literature.⁴⁹

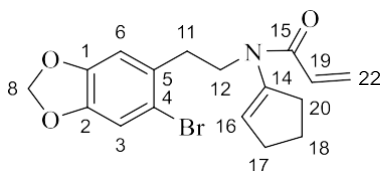
2-(6-Bromobenzo[d][1,3]dioxol-5-yl)ethan-1-amine 263



Synthesised by analogy to a reported procedure.⁵⁰ To a solution of compound **239** (112 mg, 0.41 mmol) in dry Et₂O (2.5 mL) was added a solution of LiAlH₄ (63 mg, 1.66 mmol) in dry Et₂O (2.5 mL). The solution was stirred at reflux for 3 h, then 16 h at room temperature. The solution was cooled to 0 °C, quenched with water (5 mL), extracted with DCM (4 × 20 mL), the combined organic fractions were washed with brine (20 mL) and concentrated *in vacuo* to afford the title compound as a colourless oil (100 mg, quant.). ¹H NMR (400 MHz, CDCl₃) δ 7.00 (s, 1H, H-3), 6.74 (s, 1H, H-6), 5.95 (s, 2H, H₂-8), 2.96 (ddd, *J* = 7.5, 6.5, 1.0 Hz, 2H, H₂-12), 2.89 – 2.78 (m, 2H, H₂-11), 1.31 (br.s, 2H, NH₂).

Data consistent with literature.⁵⁰

N-[2-(6-Bromobenzo[d][1,3]dioxol-5-yl)ethyl]-*N*-(cyclopent-1-en-1-yl)acrylamide 265



Synthesised following a reported procedure.¹⁹ A solution of amine **263** (216 mg, 0.86 mmol), cyclopentanone (92 µL, 1.04 mmol) in dry benzene (4.5 mL) was stirred at reflux for 2 h with an azeotropic removal of water. The solution was cooled down, and Et₃N (239 µL, 1.77 mmol) and acryloyl chloride (105 µL, 1.3 mmol) were added. The solution was stirred 20 min at room temperature, diluted with water (15 mL), and extracted with EtOAc (3 × 30 mL). The combined organic fractions were washed with brine (15 mL), dried (MgSO₄), filtered, and concentrated *in vacuo*. FCC (8/1 pentane/EtOAc) afforded the title compound as a crystalline solid (166 mg, 53%). *R*_f 0.51 (5/1 pentane/EtOAc) ; ¹H NMR (400 MHz, CDCl₃) δ 6.94 (s, 1H, H-3), 6.76 (s,

1H, H-6), 6.54 – 6.42 (m, 1H, H-22), 6.39 – 6.29 (m, 1H, H-22), 5.92 (s, 2H, H₂-8), 5.58 (dd, $J = 10.0, 2.0$ Hz, 1H, H-19), 5.41 (t, $J = 2.0$ Hz, 1H, H-16), 3.71 – 3.53 (m, 2H, H₂-12), 2.95 – 2.81 (m, 2H, H₂-11), 2.40 – 2.29 (m, 4H, H₂-17/H₂-20), 1.98 – 1.86 (m, 2H, H₂-18).

Data consistent with literature.¹⁹

Procedures and Compounds, Chapter 4

General procedure A, enzymatic screenings

Substances	Concentration	Volume added
Lactam 191 (solution in EtOH)	200 μ M	1.25 μ L
Phosphate buffer (pH 7.9)	10 M	277.5 μ L
NADP ⁺	3 mg/mL	10 μ L
GDH	8 mg/mL	10 μ L
Enzyme	2 μ M	100 μ L
Glucose	1 M	100 μ L

Table S1 Components for each screening reaction (amount given per well).

Lactam **191** was screened against common plates CP5 and CP6, and plates containing specific mutations (kindly prepared by the Wong group) in a substrate to enzyme ratio of 500:1 (Common Plate 5, volume added: 2.5 μ L) and 250:1 (Common Plate 6 and further plates). Each plate was prepared by adding the amounts of each substance as presented in **Table S1**. The volume of EtOH used was calculated to ensure that the concentration of the lactam was equal to 200 μ M.

Plates were shaken at 120 rpm for 16 h at 20 °C. The contents of each well were transferred into Eppendorf tubes, and diluted with 300 μ L of EtOAc. The Eppendorf tubes were

vortexed for 15 s and centrifuged for 1 min at 13 300 rpm. 200 μ L of the organic phase was transferred into a 200 μ L glass GC tube. Each result was analysed by GC, with the following temperature profile: Initial oven temperature = 120 °C and held for 30 s; temperature raised to 270 °C over 75 s, then to 300 °C in 75 s; temperature maintained at 300 °C for 135 s.

General procedure B, scale-up reactions

Lactam **191** was dissolved in the volume of EtOH to ensure the concentration was equal to 200 μ M. This solution was added to a conical flask containing a solution of the relevant P450 variant (suspended in phosphate buffer, to the desired ratio), glucose (9 g per 50 mL of phosphate buffer solution), NADP⁺ (3 mg per mL, phosphate buffer solution), and GDH (8 mg per mL, phosphate buffer solution). The solution was stirred at 120 rpm at 20 °C for 16 h. The resulting aqueous solution was dissolved in EtOAc (3 times the reaction volume), transferred to Falcon tubes, vortexed for 30 s, and centrifuged at 9,500 rpm for 3 min. The organic fractions were transferred to a conical flask. This extraction was repeated three times. The combined organic fractions were dried (MgSO₄), filtered, and concentrated *in vacuo*. The crude products were then purified by FCC to afford the different metabolites.

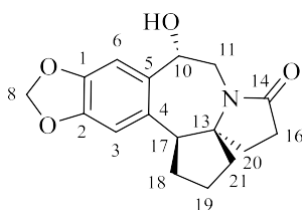
General procedure C, “whole cells” variant preparation

For some experiments, variants in whole cells were used. To prepare them, a colony of competent cells previously modified by mutagenesis containing the desired mutation(s), were picked, and dissolve in a LB solution⁵¹ (5 mL) and shaken at 180 rpm for 6 h at 37 °C. Inoculation solution was prepared,⁵¹ and both solutions (1 mL for bacteria solution, 1.5 mL for inoculation solution) were added to a conical flask containing 40 mL of pre-FB solution⁵¹ and 500 mL of double deionised H₂O, and the solution was stirred at 120 rpm for 16 h at 37 °C. The solution was then cooled to 20 °C, and induction solution⁵¹ (1 mL) and tryptone (500 mg) were added. The solution was shaken at 120 rpm at 20 °C. The induction protocol was repeated on days 3, 4, and 5 (the solution was continuously shaken at 120 rpm and 20 °C). Once ready, the

solution (now a dark red) was transferred to centrifuge tubes and centrifuged at 4,500 rpm for 10 min. The surfactant was discarded and the cells re-suspended in 25 mL of phosphate buffer. The resulting solution was used immediately afterwards, but can be frozen for a few weeks for later use, as the source of enzyme. The rest of the reaction follows **General procedure B**.

(3aR,9S*,14bS)-9-Hydroxy-1,2,3,4,5,8,9,14b-octahydro-6H-

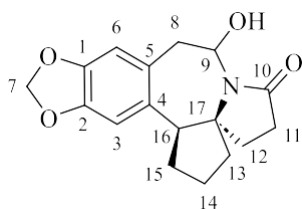
[1,3]dioxolo[4',5':4,5]benzo[1,2-d]cyclopenta[b]pyrrolo[1,2-a]azepin-6-one 269



Alcohol **269** was isolated after scale-up with KSK19 A82M in 43% yield following **General Procedure B**. R_t (with oven method described above) 4.41 min ; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 6.82 (s, 1H, H-6), 6.64 (s, 1H, H-3), 5.96 – 5.93 (m, 2H, H₂-8), 5.04 (t, J = 8.0 Hz, 1H, H-10), 4.50 (dd, J = 14.5, 8.0 Hz, 1H, H-11), 3.15 – 3.03 (m, 1H, H-11), 2.89 (dd, J = 12.5, 8.0 Hz, 1H, H-17), 2.44 – 1.74 (m, 10H) ; $^{13}\text{C NMR}$ (151 MHz, CDCl_3) δ 112.7 (C6), 112.5 (C3), 101.3 (C8), 73.1 (C13), 58.6 (C10), 42.5 (C11). (Partial data extracted from HSQC data) ; m/z (EI) for $[\text{M}+\text{H}]^+$, calculated for $(\text{C}_{17}\text{H}_{20}\text{NO}_4)$, requires 302.13, found 302.13.

(3aR*,14bS*)-8-Hydroxy-1,2,3,4,5,8,9,14b-octahydro-6H-

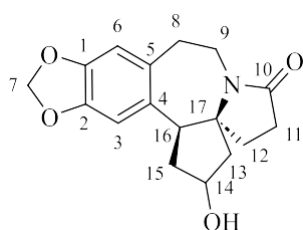
[1,3]dioxolo[4',5':4,5]benzo[1,2-d]cyclopenta[b]pyrrolo[1,2-a]azepin-6-one 270



Alcohol **270** was isolated after a scale-up with KSK19 A82M A264G A328G in 9% yield following **General Procedure C**. R_t 4.08 min ; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 6.69 (s, 1H, H-6), 6.61 (s, 1H, H-3), 5.94 – 5.89 (m, 2H, H₂-7), 5.51 (dd, J = 11.5, 5.5 Hz, 1H, H-9), 3.30 (dd,

$J = 11.5, 2.5$ Hz, 1H, H-8), 2.91 (m, 2H, H-8/H-16), 2.43 (m, 2H, H₂-11), 2.37 – 1.83 (m, 8H, H₂-12/H₂-13/H₂-14/H₂-15) ; ^{13}C NMR (101 MHz, CDCl₃) δ 178.3 (C10), 146.9 (C1), 146.8 (C2), 133.2 (C6), 127.4 (C3), 111.46 (C5), 111.4 (C4), 101.3 (C7), 76.6 (C17), 70.3 (C9), 57.5 (C8), 38.2 (C11), 37.8 (C16), 36.5 (C12), 34.7 (C15), 30.0 (C13), 24.5 (C14) ;⁵² m/z (EI) for $[\text{M}+\text{H}]^+$, calculated for (C₁₇H₂₀NO₄), requires 302.13, found 302.13.

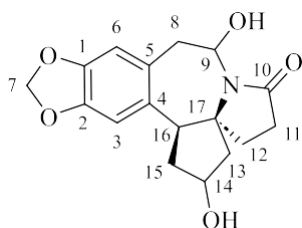
(3aS*,14bS*)-2-Hydroxy-1,2,3,4,5,8,9,14b-octahydro-6H-[1,3]dioxolo[4',5':4,5]benzo[1,2-d]cyclopenta[b]pyrrolo[1,2-a]azepin-6-one 271



Alcohol **271** was isolated after a scale-up with KSK19 A82M A264G A328G in 21% yield following **General Procedure C**. R_t 4.59 min ; ^1H NMR (400 MHz, CDCl₃) δ 6.63 (s, 1H, H-6), 6.62 (s, 1H, H-3), 5.91 – 5.89 (m, 2H, H₂-7), 4.56 (t, $J = 4.0$ Hz, 1H, H-14), 4.20 – 4.02 (m, 1H, H-9), 3.48 (dd, $J = 11.5, 8.5$ Hz, 1H, H-8), 3.12 – 3.02 (m, 2H, H-8/H-9), 3.01 – 2.91 (m, 1H, H-16), 2.61 (dd, $J = 10.5, 6.0$ Hz, 1H, H₂-15), 2.46 (dd, $J = 10.5, 4.0$ Hz, 2H, H₂-11), 2.41 – 1.93 (m, 4H, H₂-12/H₂-13) ; ^{13}C NMR (101 MHz, CDCl₃) δ 175.7 (C10), 146.9 (C1), 146.6 (C2), 132.7 (C6), 129.9 (C3), 111.32 (C5), 111.1 (C4), 101.2 (C7), 72.0 (C17), 69.6 (C14), 54.8 (C9), 43.6 (C8), 42.5 (C16), 40.5 (C15), 38.6 (C11), 30.5 (C13), 30.2 (C12) ;⁵² m/z (EI) for $[\text{M}+\text{H}]^+$, calculated for (C₁₇H₂₀NO₄), requires 302.13, found 302.13.

(3aS*,14bS*)-2,8-Dihydroxy-1,2,3,4,5,8,9,14b-octahydro-6H-

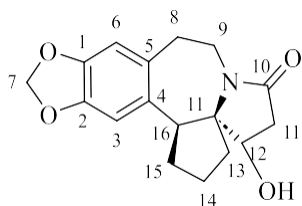
[1,3]dioxolo[4',5':4,5]benzo[1,2-d]cyclopenta[b]pyrrolo[1,2-a]azepin-6-one 272



Alcohol **272** was isolated after a scale-up with GV A184I in 43% yield following **General Procedure C**. *R_t* 5.15 min ; ¹H NMR (600 MHz, CDCl₃) δ 6.68 (s, 1H, H-6), 6.65 (s, 1H, H-3), 5.93 (d, *J* = 7.5 Hz, 1H, H-7), 5.91 (d, *J* = 7.5 Hz, 1H, H-7), 5.54 (dd, *J* = 12.5, 5.5 Hz, 1H, H-9), 4.58 – 4.52 (m, 1H, H-14), 3.47 (dd, *J* = 12.5, 8.0 Hz, 1H, H-8), 3.30 – 3.23 (m, 1H, H-5), 2.90 (dd, *J* = 10.5, 5.5 Hz, 1H, H-16), 2.76 – 2.69 (m, 2H, H₂-11), 2.35 – 2.13 (m, 8H, H₂-12/H₂-13/H₂-15) ; ¹³C NMR (151 MHz, CDCl₃) δ 178.5 (C10), 147.0 (C1), 147.0 (C2), 132.5 (C6), 127.1 (C3), 111.6 (C5), 111.5 (C4), 101.3 (C7), 76.3 (C17), 72.1 (C9), 70.3 (C8), 54.1 (C14), 45.4 (C16), 43.4 (C11), 39.5 (C15), 32.1 (C13), 30.7 (C12). *m/z* (EI) for [M+H]⁺, calculated for (C₁₇H₂₀NO₅), requires 318.13, found 318.13.

(3aS*,4R*,14bS*)-4-Hydroxy-1,2,3,4,5,8,9,14b-octahydro-6H-

[1,3]dioxolo[4',5':4,5]benzo[1,2-d]cyclopenta[b]pyrrolo[1,2-a]azepin-6-one 273



Alcohol **273** was isolated after a scale-up with KSK19 A82M A264G A328G in 18% yield following **General Procedure C**. *R_t* 4.64 min ; ¹H NMR (400 MHz, CDCl₃) δ 6.61 (s, 1H, H-6), 6.56 (s, 1H, H-3), 5.99 – 5.71 (m, 2H, H₂-7), 4.09 (td, *J* = 13.0, 6.5 Hz, 1H, H-9), 4.01 (dd, *J* = 5.5, 3.0 Hz, 1H, H-12), 3.18 (dd, *J* = 12.5, 6.5 Hz, 1H, H-8), 3.15 (dd, *J* = 12.5, 6.5 Hz, 1H, H-8), 3.12 – 3.03 (m, 1H, H-9), 3.03 – 2.90 (m, 1H, H-16), 2.53 – 2.38 (m, 2H, H₂-11),

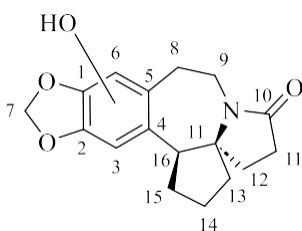
2.31 – 1.81 (m, 6H, H₂–13/H₂–14/H₂–15) ; *m/z* (EI) for [M+H]⁺, calculated for (C₁₇H₂₀NO₄), requires 302.13, found 302.13.

(3aS*,14bS*)-10-Hydroxy-1,2,3,4,5,8,9,14b-octahydro-6H-

[1,3]dioxolo[4',5':4,5]benzo[1,2-d]cyclopenta[b]pyrrolo[1,2-a]azepin-6-one or

(3aS*,14bS*)-14-hydroxy-1,2,3,4,5,8,9,14b-octahydro-6H-

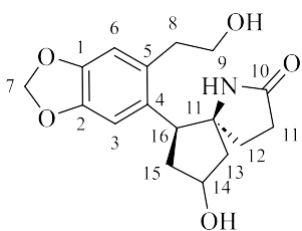
[1,3]dioxolo[4',5':4,5]benzo[1,2-d]cyclopenta[b]pyrrolo[1,2-a]azepin-6-one 274



Phenol **274** was isolated after a scale-up with KSK19 A82M A264G A328G in 19% yield following **General Procedure C**. *R*_t 4.47 min ; ¹H NMR (600 MHz, CDCl₃) δ 6.59 (s, 1H, H–3 or H–6), 6.06 – 5.99 (m, 2H, H₂–7), 4.07 – 3.95 (m, 1H, H–9), 3.21 – 3.05 (m, 3H, H–8/H–9/H–16), 2.64 (m, 1H, H₂–8), 2.35 (m, 2H, H₂–11), 2.12 – 1.8 (m, 8H, H₂–12/H₂–13/H₂–14/H₂–15) ; *m/z* (EI) for [M+H]⁺, calculated for (C₁₇H₂₀NO₄), requires 302.13, found 302.13.

(5S*,6S*)-8-Hydroxy-6-[6-(2-hydroxyethyl)benzo[d][1,3]dioxol-5-yl]-1-

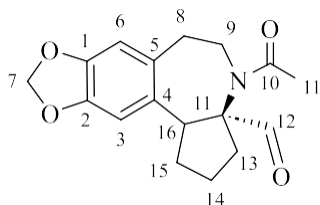
azaspiro[4.4]nonan-2-one 275



Alcohol **275** was isolated after a scale-up with KSK19 A82M A264G A328G in 2% yield following **General Procedure C**. *R*_t 4.67 min ; ¹H NMR (600 MHz, CDCl₃) δ 6.69 (s, 1H, H–6), 6.66 (s, 1H, H–3), 5.94 (m, 2H, H₂–7), 4.53 – 4.41 (m, 1H, H–14), 3.59 (t, *J* = 6.0 Hz, 2H, H₂–9), 3.20 – 3.08 (m, 2H, H₂–8), 2.79 (d, *J* = 9.0 Hz, 1H, H–16), 2.68 (d, *J* = 6.0 Hz, 1H, H–

11), 2.65 (d, $J = 6.0$ Hz, 1H, H-11), 2.20 – 1.82 (m, 6H, H₂-12/H₂-13/H₂-15) ; m/z (EI) for $[M+H]^+$, calculated for (C₁₇H₂₁NO₅), requires 320.14, found 320.14.

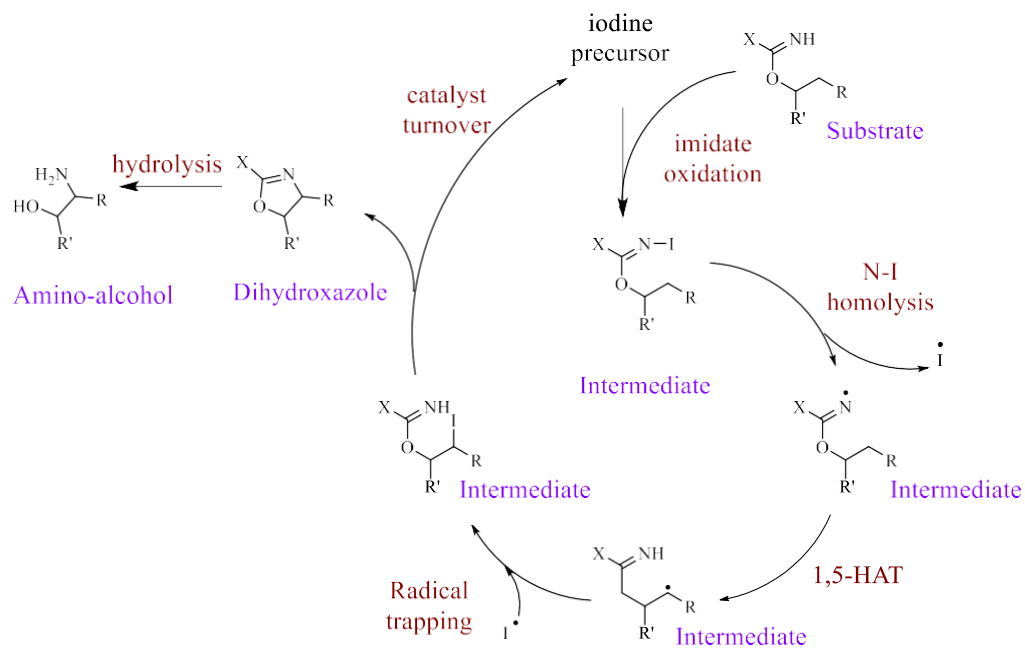
(3aS*)-4-Acetyl-2,3,4,5,6,11b-hexahydro-[1,3]dioxolo[4',5':4,5]benzo[1,2-d]cyclopenta[b]azepine-3a(1H)-carbaldehyde 276



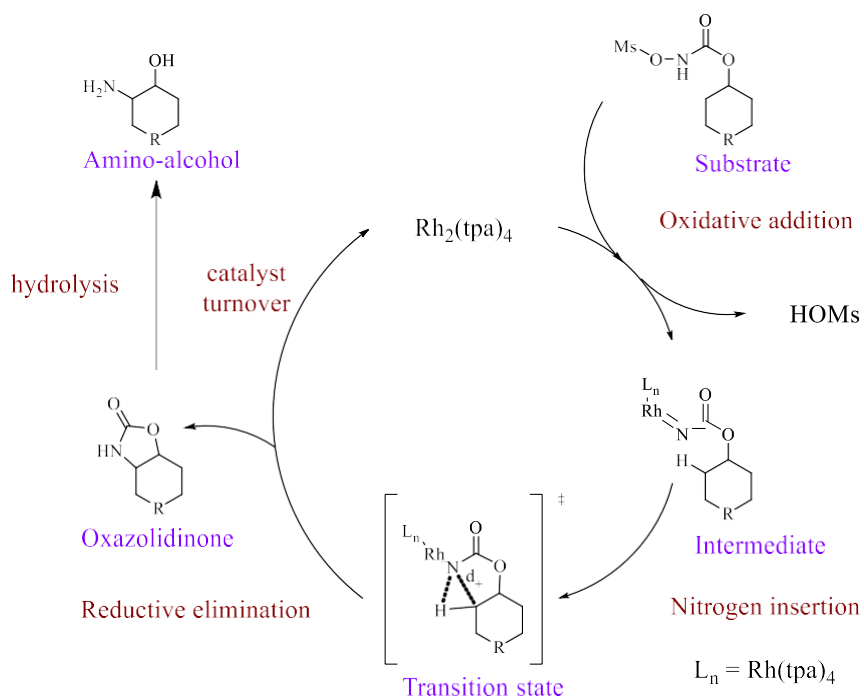
Alcohol **276** was isolated after a scale-up with KSK19 A82M A264G A328G in 1% yield following **General Procedure C**. R_t 4.46 min ; $^1\text{H NMR}$ (600 MHz, CDCl₃) δ 9.74 (s, 1H, H-12), 6.62 (s, 1H, H-6), 6.57 (s, 1H, H-3), 5.90 (dd, $J = 9.5, 1.5$ Hz, 1H, H-7), 5.89 (d, $J = 1.5$ Hz, 1H, H-7), 4.11 (dd, $J = 9.0, 7.0$ Hz, 1H, H-9), 3.18 (m, 1H, H-8), 3.10 (m, 2H, H-8/H-9), 2.95 (dd, $J = 11.0, 8.0$ Hz, 1H, H-16), 2.60 (m, 1H, H₂-8), 2.03 – 1.87 (m, 6H, H₂-13/H₂-14/H₂-15). m/z (EI) for $[M+H]^+$, calculated for (C₁₇H₂₀NO₄), requires 302.13, found 302.13.

Appendices

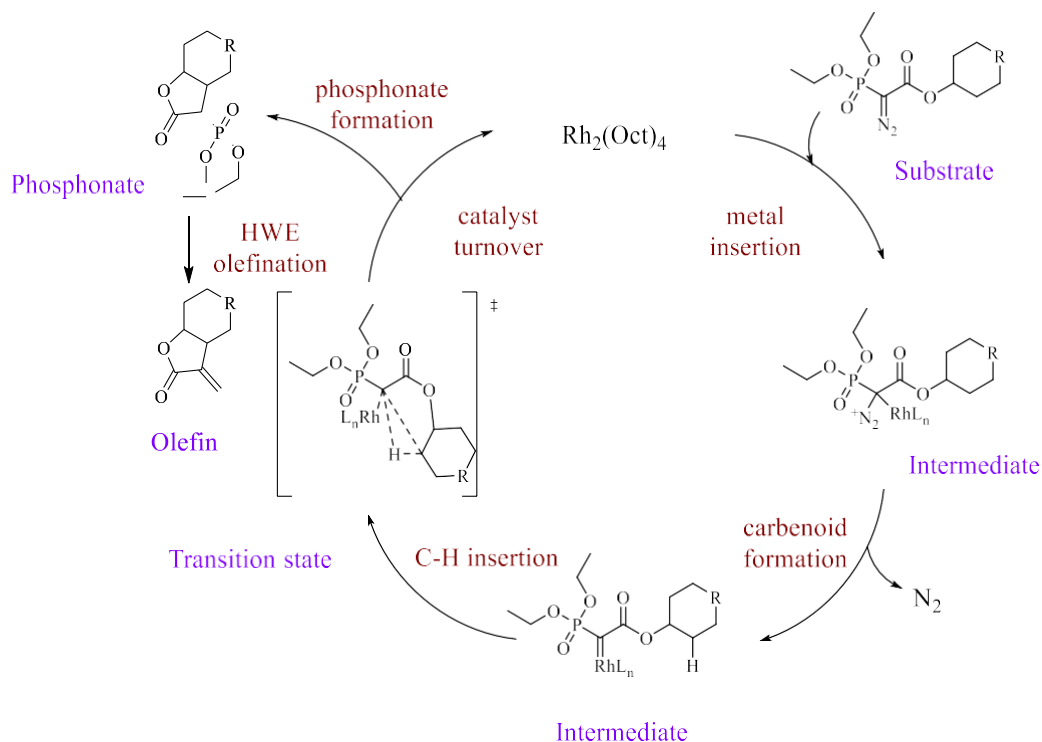
Proposed mechanisms for C–H insertions (Chapter 2)



Scheme S1 Proposed catalytic mechanism for C–H amination via a radical process.



Scheme S2 Proposed catalytic mechanism for Lebel type C-H insertion.



Scheme S3 Proposed catalytic mechanism for C-H insertion via metal carbenoid, followed by HWE olefination.

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