

Evolved P450 Mutants as General Oxidation Catalysts for Target Synthesis *via* Early-/Late-Stage Hydroxylation

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ASLIB Abstract

Evolved P450 Mutants as General Oxidation Catalysts for Target Synthesis *via* Early-/Late-Stage Hydroxylation

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This thesis describes the adoption of $P450_{BM3}$ mutants as biocatalysts during target molecule synthesis in different stages and the efforts toward the synthesis of the azepane core type structure of natural product balanol and the enantiomer of natural product α-bulnesene.

Chapter 1 provides an introduction to the application of $P450_{BM3}$ mutants as oxidation catalysts for various structures including simple cyclic amines and sesquiterpenes. A review of the significance and various synthetic studies toward natural products balanol and α-bulnesene was provided.

Chapter 2 describes a short demonstration of the feasibility of further functionalisation of $P450_{BM3}$ hydroxylated cyclic amines by synthesising the azepane core of natural product balanol.

Finally, Chapter 3 details a short enantiospecific route to *ent*-dihydrobulnesene and an investigation into the use of $P450_{BM3}$ mutants for late-stage oxidation of sesquiterpenes, along with a comparison of $P450_{BM3}$-mediated oxidation profiles of a pair of enantiomers.

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Abbreviations

°C	Degree Celsius
2°	Secondary
1-AA	1-Aminoanthracene
Ac	Acetyl
Alk	Alkyl
APCI	Atmospheric pressure chemical ionisation
aq.	Aqueous
ATP	Adenosine triphosphate
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
br s	Broad singlet
Bz	Benzoyl
CDI	Carbonyldiimidazole
cm	Centimetre(s)
cm^{-1}	Wavenumbers
CN	Cyanide
conc.	Concentration

conv.	Conversion
COSY	Correlation spectroscopy
d	Day(s)
d	Doublet
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DCP	Dicumyl peroxide
DEA	Diethanolamine
DEAD	Diethyl azodicarboxylate
DIBAL-H	Diisobutylaluminium hydride
DIOL	High density diol
DMA	Dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMP	Dess–Martin periodinane
DMP	2,2-Dimethoxypropane
dr	Diastereomeric ratio

E. coli	Escherichia coli
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetraacetic acid
ee	Enantiomeric excess
EI	Electronic ionisation
eq.	Equivalent(s)
ESI	Electrospray ionisation
EtOAc	Ethyl acetate
Et_2O	Diethyl ether
Et_3N	Triethylamine
FAD	Flavin dinucleotide
FMN	Flavin mononucleotide
g	Gram
GC	Gas chromatograph
GDH	Glutamate dehydrogenase
h	Hour(s)
HFIP	Hexafluoroisopropanol
HMPA	Hexamethylphosphoramide

HRMS	High resolution mass spectrometry
HSQC	Heteronuclear multiple-bond correlation spectroscopy
Hz	Hertz
hν	Irradiation
IPA	Isopropyl alcohol
IR	Infrared spectroscopy
^{i}Pr	*iso*-Propyl
J	Coupling constant
HMDS	Bis(trimethylsilyl)amide
m	Multiplet
m	Medium
M	Molar (mol dm^{-3})
MCD	Methyl-β-cyclodextrin
m-CPBA	3-Chloroperbenzoic acid
Me	Methyl
MeCN	Acetonitrile
MeOH	Methanol
mg	Milligram(s)

min	Minutes(s)
mL	Millilitre(s)
mm	Millimetre(s)
mmol	Milimoles(s)
m.p.	Melting point
Ms	Methanesulfonyl
NADPH	Nicotinamide adenine dinucleotide phosphate
NIS	*N*-Iodosuccinimide
NMO	*N*-Methylmorpholine-*N*-oxide
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
PCC	Pyridinium chlorochromate
PCI	Positive chemical ionisation
PG	Protecting group
Ph	Phenyl
2-PIC	2-Picolylamine
ppm	Parts per million
q	Quartet

quant.	Quantitative
R	Generic group
R_f	Retention factor
RT	Room temperature
r.t.	Retention time
s	Singlet
SFC	Supercritical fluid chromatography
s.m.	Starting material
t	Triplet
TBAF	Tetrabutylammonium fluoride
TBHP	*tert*-Butyl hydroperoxide
TBP	Tributyl phosphate
TBS	*tert*-Butyldimethylsilyl
TCE	Trichloroethylene
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TES	triethylsilyl
Tf	Trifluoromethanesulfonyl
THF	Tetrahydrofuran

TLC	Thin layer chromatography
TPAP	Tetrapropylammonium perruthenate
TPP	Tetraphenylporphyrin
TPS	*tert*-Butyldiphenylsilyl
TPSH	2,4,6-Triisopropylbenzenesulfonyl hydrazide
Ts	4-Toluenesulfonyl
ν_{max}	Infrared absorption maximum
μ	Micro
μM	Micro Molar
w/v	Weight-to-volume ratio

Chapter 1: Introduction

Cytochrome P450 and P450$_{BM3}$

Background

Cytochrome P450s (CYP) comprise a superfamily of haem-dependent monooxygenases named for their distinctive 450 nm absorption peak in their carbon monoxide-bound form and have been extensively studied for their oxidative metabolism of xeno- and endobiotics.[1] Cytochrome P450s employ an Fe(III)-containing haem cofactor (specifically haem b, Figure 1) located in the active site; two additional axial ligands, a cysteine thiolate and water, are coordinated to the Fe(III) centre.[2]

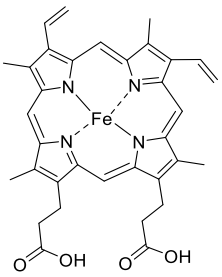
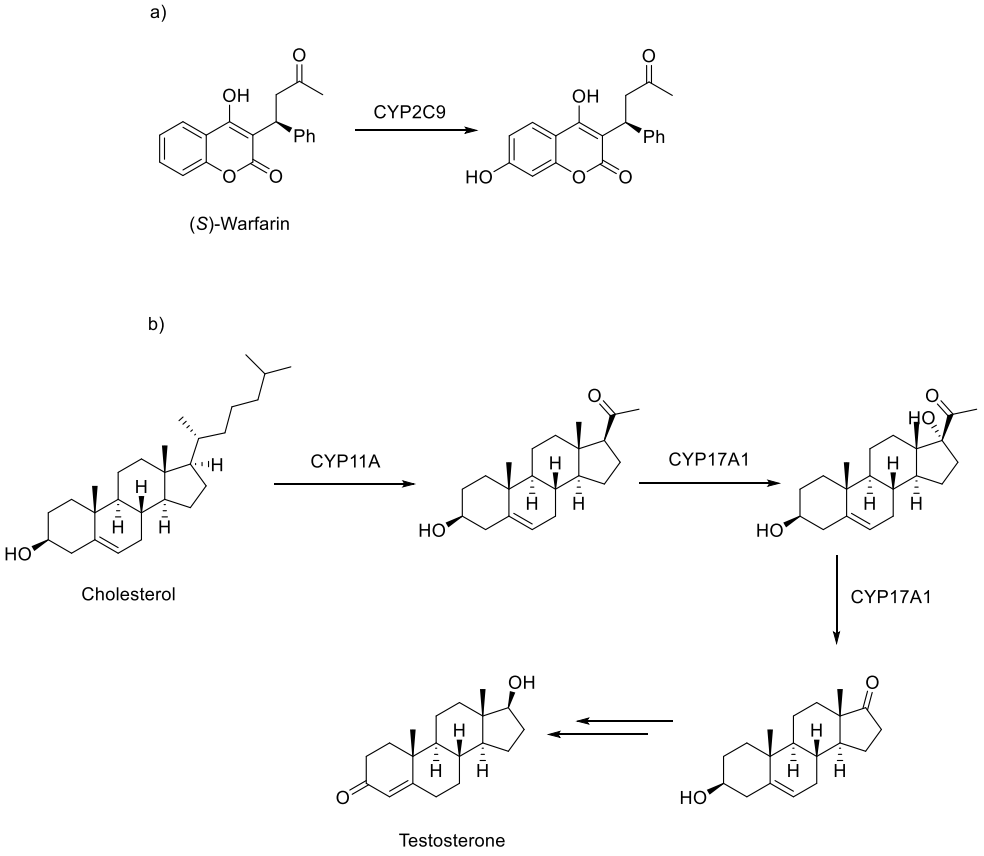


Figure 1. Structure of haem b.

Cytochrome P450s are involved in the oxidative or reductive metabolism of every class of organism; they mostly catalyse the oxidation of organic compounds in the form of C–H hydroxylation, epoxidation, or oxidative demethylation.[3] Prokaryotic P450s take part in the biosynthesis of antibiotics and catalyse reactions crucial for environmental bioremediation. In plants, cytochrome P450s are involved in the biosynthesis of phytoalexin and phytohormone, as well as various signalling molecules.[4, 5] Fungal cytochrome P450s are

crucial to the synthesis of secondary metabolites, and they facilitate pathogenesis by detoxifying the chemical defences of target host plants.[6] In mammalian tissues, cytochrome P450s are recognised as essential catalysts for the oxidative metabolism of xenobiotics (phase I drug metabolism, Scheme 1a);[7] they are also involved in oxidative steps in the biosynthesis of various natural products, such as steroid hormones and terpene structures (Scheme 1b).[8–10]



Scheme 1. a) Hydroxylation during the metabolism of (*S*)-warfarin; b) steroid hormone biosynthesis.[7, 8]

Catalytic cycle

P450 enzymes catalyse the oxidation reaction of a wide range of organic compounds by harnessing atmospheric oxygen, in which the most common reaction is the oxidation of C–H bonds to the corresponding alcohol. In turn, the activation of oxygen requires the supply of two electrons, usually through the consumption of NADH or NADPH.[11]

$$\mathbf{R–H + O_2 + NADPH + H^+ \rightarrow R–OH + H_2O + NADP^+}$$

The generally accepted form of the catalytic cycle of the enzyme is believed to be as shown in (Figure 2).[11, 12] The cycle begins with the P450 iron centre in the ferric state (**I**), where the porphyrin system supplies four equatorial nitrogen ligands with the two axial ligands being water and the thiolate ligand. The arrival of a viable substrate triggers the loss of the axial water ligand through direct displacement, which leads to an increase in the reduction potential of the species as the ferric centre changes from low-spin (S= 1/2) to high-spin (S=5/2) state (**II**). As a result, the iron centre can then receive an electron from a molecule of NADH or NADPH, thereby being reduced to the ferrous state (**III**), whereupon molecular oxygen binding gives the stable ferric-oxy species (**IV**). A ferric peroxo complex (**V**) is formed when a second electron is received; protonation of this species results in "Compound **0**", a hydroperoxy adduct (**VI**). A second protonation triggers water loss *via* heterolytic O–O bond cleavage to give "Compound **I**" (**VII**), which is a Fe(IV)-oxo species in which a radical cation is delocalised over the porphyrin and thiolate ligands, and is regarded as the active entity in most P450 oxidation reactions.[13] Abstraction of a hydrogen atom from the substrate by Compound **I** gives an alkyl radical and Fe(IV) hydroxide species (**VIII**) (also known as

Compound **II**). Radical rebound of the substrate radical onto the Fe(IV)-bound OH group generates the alcohol and leaves the enzyme in the pentacoordinated ferric state (**X**). Binding of axial water returns the enzyme back to state (**I**), thus closing the catalytic cycle.

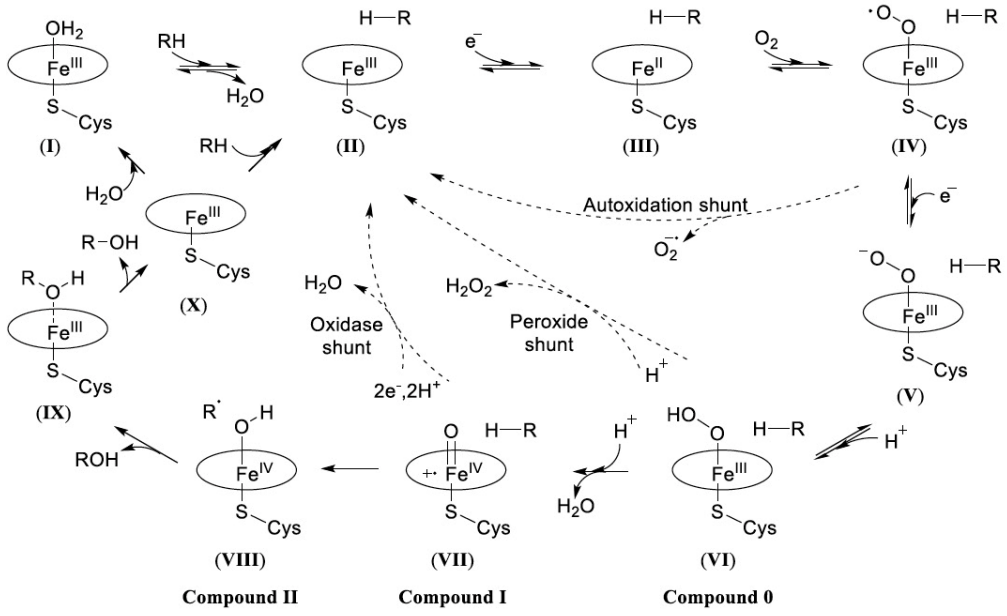


Figure 2. Cytochrome P450 catalytic cycle.[11]

Cytochrome P450$_{BM3}$ (CYP102A1)

The systematic classification of cytochrome P450s was developed by Nelson and co-workers according to the similarity between gene sequences.[14, 15] They are first assigned to a family number (e.g. CYP1, CYP2) then a subfamily letter (e.g. CYP1A, CYP2D) and finally differentiated by a number for each individual enzyme (e.g. CYP1A1, CYP2D6).[16] Amongst this superfamily, the fatty acid hydrolase P450$_{BM3}$ (CYP102A1) from *Bacillus megaterium* is commonly employed as a biocatalyst in organic synthesis as it, and its variants, possess some of the highest monooxygenase activities amongst all P450s reported to date.[17] A key feature

that distinguishes $P450_{BM3}$ from other P450 enzymes is its connected diflavin (FAD-FMN) electron-transfer reductase domain, that makes it catalytically self-sufficient to supply electrons to the active site *via* a cofactor (usually NADPH);[18] most other P450 enzymes require an external reductase cofactor to supply electrons to the active site. Additionally, $P450_{BM3}$ is stable and expressed to a high level in *E. coli*, and shows high activity for favoured substrates.[19, 20] These properties make $P450_{BM3}$ an excellent candidate for biotechnological applications. This enzyme is able to hydroxylate fatty acid bearing alkyl chain lengths ranging from 12 to 20 carbon units[17] and the X-ray crystal structure of $P450_{BM3}$ with bound palmitoleic acid reveals that the fatty acid is oriented by two main interactions: (1) Ionic interactions of the carboxylate with Arg47 and Tyr51; (2) hydrophobic interactions between the alkyl chain and amino acids at the substrate binding site (Figure 3).[21, 22]

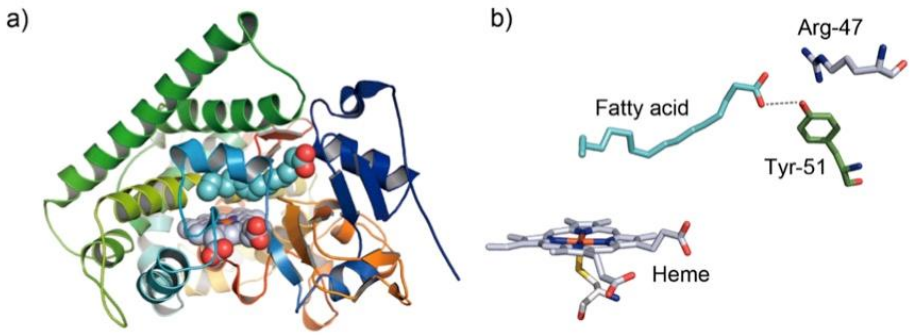


Figure 3. a) Overall structure of $P450_{BM3}$; b) palmitoleic acid at the active site.[22]

Structure

The X-ray crystal structure of the haem (BMP) dimer of $P450_{BM3}$ (CYP102A1) was first obtained in the 1990s, and reveals an α and a β domain, separated by the haem group and by an extended loop of 28 residues.[23] The haem centre is bound to cysteine-400, embedded

between the I and L helices of the α domain, with an axial water ligand hydrogen bonded to the carbonyl oxygen of Ala264. The active site haem is accessible through a hydrophobic channel constructed mostly with nonaromatic hydrophobic residues. Some of the residues are especially crucial to the binding and hydroxylation of substrates; for example phenylalanine-87 (Phe87) is responsible for the displacement of the axial water ligand upon substrate binding in the active site.[24] An acid-alcohol amino acid pair, located at residues Glu267 and Thr268 on the I helix, is used to relay protons onto iron-oxo species to progress the catalytic cycle.[25] It is suggested that the acid partner Glu267 is involved in the mechanism of activation of dioxygen and is responsible for proton transfer, while the alcohol residue Thr268 has a more complicated role involving proton transfer, substrate recognition, dioxygen activation and electron transfer.[26] Arginine-47 (Arg47) and tyrosine-51 (Tyr51) are the key active site residues for activity towards the native substrates (medium- to long-chain fatty acids). The *N*-palmitoylglycine-bound crystal structure displayed an interaction between the carboxylate group of the lipid and the phenol group of Tyr51 (Figure 4).[21, 22]

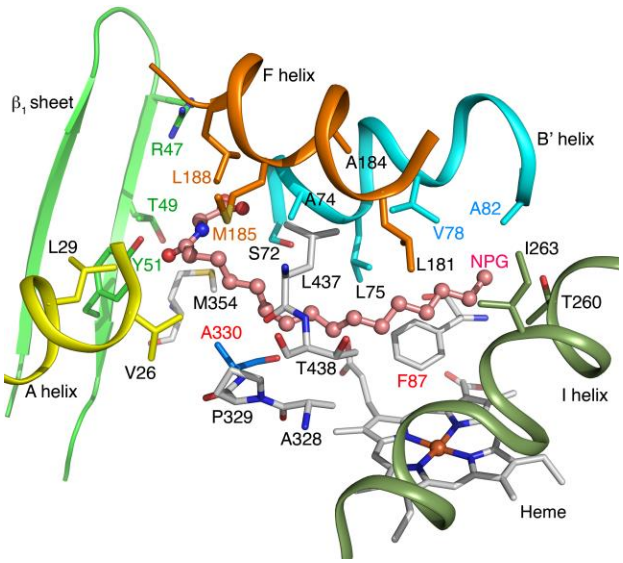


Figure 4. Crystal structure of *N*-palmitoylglycine-bound wild-type CYP102A1.[21]

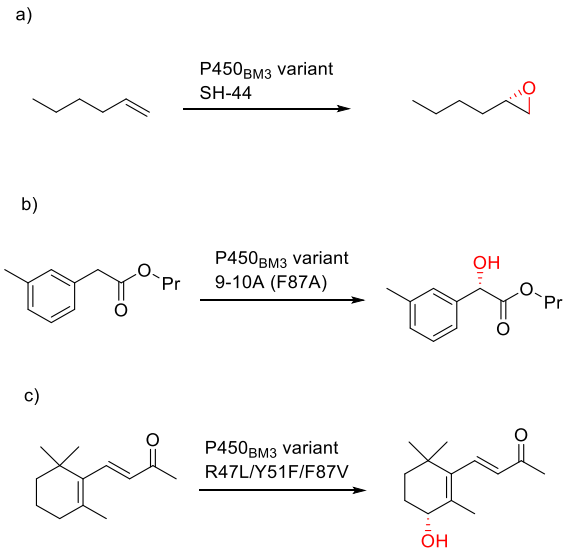
Protein engineering

With high substrate oxidation activity compared to other P450 enzymes,[27] $P450_{BM3}$ has been extensively engineered through directed evolution and rational design studies for the more efficient hydroxylation of both natural and non-natural substrates to produce fine chemicals and drug metabolites.[28] Engineering the enzyme can overcome the wild type's narrow substrate specificity (most substrates other than long-chain fatty acids do not fit the active site of the enzyme) as well as its somewhat low turnover of substrate.[29]

Mutagenesis is a useful tool to improve the activity and selectivity of $P450_{BM3}$ enzyme towards both natural and non-natural substrates.[30] Mutant variants, produced through error-prone PCR (directed evolution) and site-directed mutagenesis (rational design), can display wider substrate scope and improved reactivity compared to the wild-type.[31, 32] Error-prone PCR generates mutations at random positions and, although effective, this method does not allow for sophisticated design of mutants. Site-selective mutagenesis provides greater control by targeting desired residues. Based on structural or mechanistic analysis, specific residues are targeted due to their potential to affect activity or selectivity. Combining these two methods results in a strategy called rational evolution, which is extensively employed in the mutagenesis of P450 enzymes. A particular residue is taken as the focus for random mutagenesis; the resulting library is then further screened to identify mutants with the desired activity and selectivity, and then the process can be iterated.[33]

The engineering of $P450_{BM3}$ to increase its substrate acceptability has been well reviewed.[33–36] $P450_{BM3}$ features a long hydrophobic substrate channel connecting the protein surface to

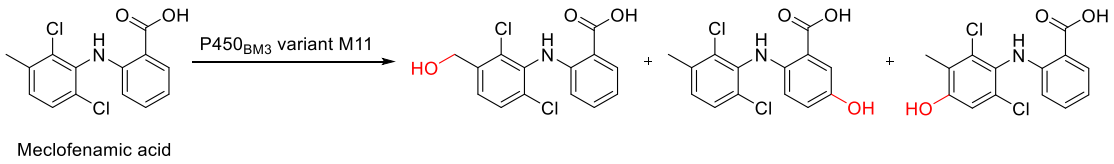
the haem pocket[23] and, with numerous amino acids extending their side chains into it, this channel provides multiple target sites for mutagenesis.[37] Amongst these target sites, F87, which is most proximal to the haem, was extensively investigated in early work.[21, 38, 39] Single mutations at F87 were found to greatly enhance hydroxylation activities towards alkenes,[40] 2-arylacetic acid esters,[41] β-ionone,[42] and cycloalkanes (Scheme 2).[43]



Scheme 2. Oxidation of various structures with engineered $P450_{BM3}$ variants.[40–43]

In wild type P450 enzymes, substrate oxidation is highly coupled to NADPH cofactor oxidation (90%–98% in $P450_{BM3}$); however, the coupling is almost invariably disrupted when non-native substrates are introduced to the active site.[37] Uncoupling leads to the formation of reactive oxygen species like O_2^- or H_2O_2, resulting in the inactivation of the enzyme and correspondingly low turnover numbers; therefore, the engineering of P450 enzymes to achieve high coupling efficiency and turnover numbers is of great importance for the hydroxylation of non-native substrates.

Commandeur reported the improved enzymatic catalysis of the conversion of meclofenamic acid to its hydroxylated analogues with $P450_{BM3}$ mutant, M11 (R47L/E64G/F81I/F87V/E143G/L188Q/E267V/G415S), while wild type $P450_{BM3}$ enzyme showed very low substrate conversion (< 0.5 %) (Scheme 3).[44]

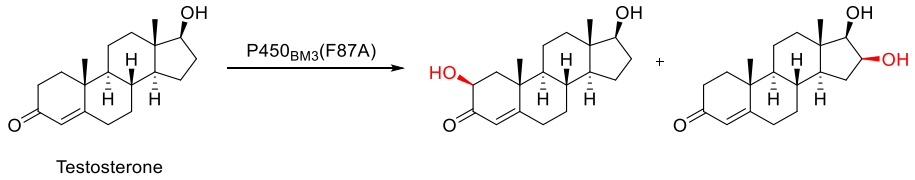


Scheme 3. Hydroxylation of meclofenamic acid with $P450_{BM3}$ variant.[44]

The control of regio- and stereoselectivity of $P450_{BM3}$ enzyme catalysts during synthesis is another challenge and has been recently studied.[34, 37] When bound to substrate, the enzyme often undergoes conformational changes due to its structural flexibility and active site plasticity.[21, 45] As a result, it is difficult to rationally design active site mutations toward a specific site in the target molecule even when the high-resolution structure of the enzyme is available.[46, 47] Another reason leading to the poor regio- or stereoselectivity of mutated P450 enzyme variants is that mutations beneficial to expanding substrate profile are usually achieved by expanding the active site, allowing substrates to bind through multiple orientations, which leads to difficulties in controlling regio- and stereochemistry.[37]

An example of engineering $P450_{BM3}$ into highly regio- and stereoselective mutants is directed evolution of $P450_{BM3}$ variants for regioselective steroid hydroxylation.[48] Starting with base mutant $P450_{BM3}$(F78A), two variants were identified, KSA-2 (R47I/T49I/Y51I/F87A) and

KSA-5 (V78L/A82F/F87A), which achieved 94% and 91% selectivity for the 2β- and 15β-positions, respectively (Scheme 4).



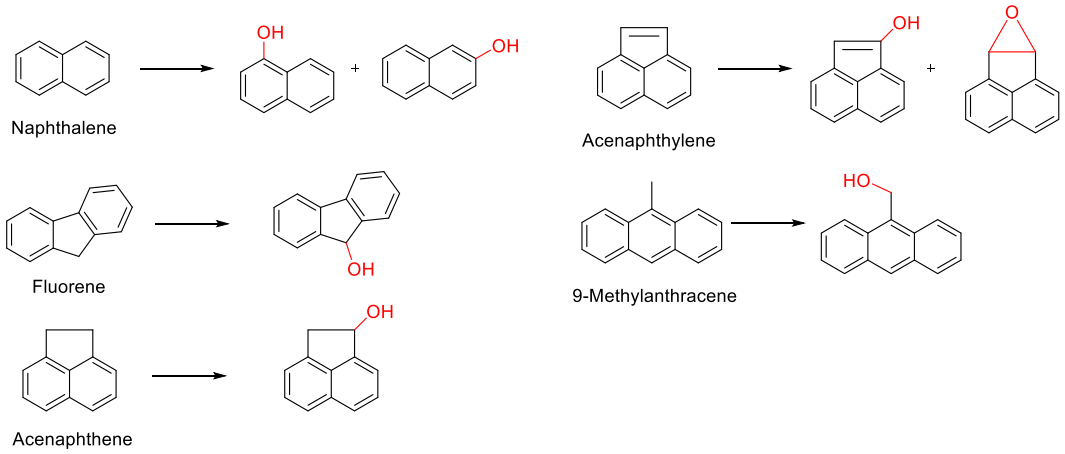
Scheme 4. Regioselective hydroxylation of testosterone with $P450_{BM3}$(F87A) variants.[48]

As an alternative to directed evolution, target substrates can be screened against small libraries of preselected P450 enzyme variants showing diverse oxidation profiles.[49] The underlying idea in this method is to identify P450 variants that show promising conversion and selectivity for each substrate. This approach was, and continues to be, favoured in the Wong group to discover $P450_{BM3}$ variants for selective functionalisation of building blocks for specific drug targets, as discussed in detail below.

Applications

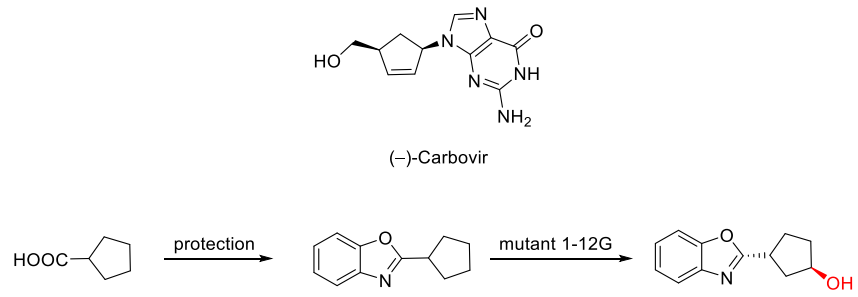
Through the engineering methods introduced above, libraries optimised for various activity or selectivity purposes have been designed and their applications have been well reviewed.[50–52] Mutant $P450_{BM3}$ enzymes have been considered to have potential novel application in the field of environmental bioremediation and the synthesis of functionalised building block molecules for natural or non-natural products.

Polycyclic aromatic hydrocarbons (PAH) are ubiquitously present in our environment and are hazardous towards humans.[53] Hydroxylated PAHs, however, are water-soluble and can be decomposed *via* further oxidation.[54] The triple $P450_{BM3}$ mutant A74G/F87V/L188Q showed promising hydroxylation results towards PAHs such as naphthalene, fluorene, acenaphthene, acenaphthylene, and 9-methylanthracene (Scheme 5). The mutant was most active towards naphthalene to generate a mixture comprising 96% 1-naphthol and 4% 2-naphthol.



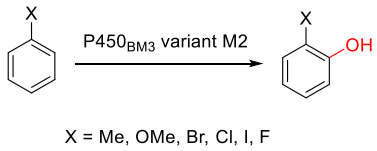
Scheme 5. Oxidation of PAHs catalysed by $P450_{BM3}$ mutant A74G/F87V/L188Q.[54]

(–)-Carbovir is a carboxylic nucleoside potentially active against HIV[55] and it was suggested that the direct hydroxylation of cyclopentane carboxylic acid would result in a useful precursor to this compound. The $P450_{BM3}$ mutant 1-12G (with the key active site mutation being A78V), developed by Arnold group, was shown to hydroxylate a derivative of this carboxylic acid with high stereoselectivity (98% *ee*) (Scheme 6).[56]



Scheme 6. Stereoselective biosynthesis of precursor to (–)-Carbovir.[56]

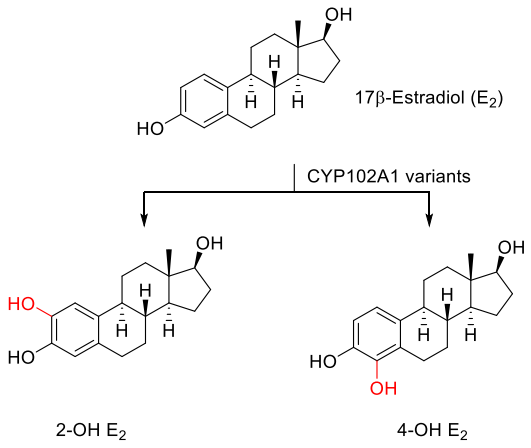
Halogenated phenols are useful building blocks for drug and vitamin synthesis.[57] Regio- and stereoselective biocatalysis of the direct hydroxylation of the benzene cores with $P450_{BM3}$ mutants provides an attractive alternative route to conventional chemosynthetic pathways. One $P450_{BM3}$ mutant, M2 (R47S/Y51W/I410M), was found to have high activity and excellent regioselectivity for the conversion of monosubstituted benzenes into *o*-substituted phenols (Scheme 7).[58]



Scheme 7. Aromatic substrates for the $P450_{BM3}$ variant M2.[58]

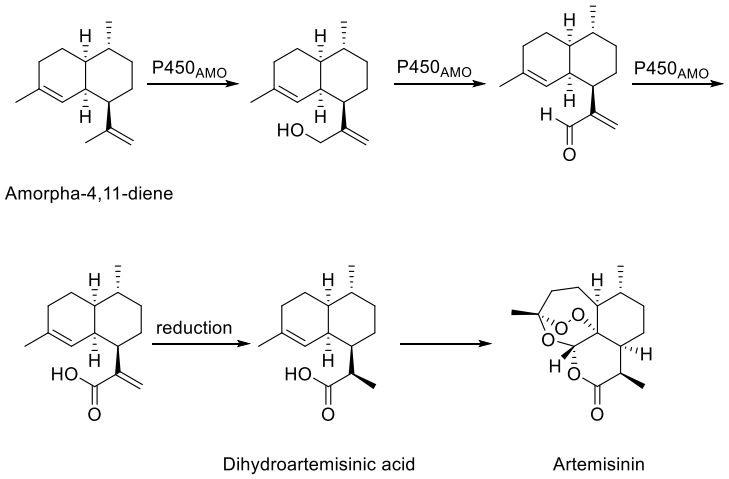
Steroids play an important role as hormones in humans, and they are widely used clinically as anticancer and anti-inflammatory agents.[59] Hydroxylated metabolites of these steroids could be carcinogenic[60] but the high cost of accessing these hydroxylated metabolites makes it difficult to evaluate their effects *in vivo* or *in vitro*.[61] The A-ring hydroxylated metabolites

of 17β-estradiol (E_2), 2-OH E_2 are difficult to access through traditional chemical methods but are readily prepared using $P450_{BM3}$ mutants (Scheme 8).



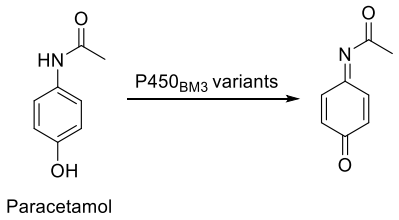
Scheme 8. 17β-estradiol can be oxidised to 2-OH E_2 and 4-OH E_2 with $P450_{BM3}$ mutants.[61]

$P450_{BM3}$ mutants are also involved in the synthesis of drugs. One example is the sequenced oxidation of amorpha-4,11-diene, a key step in the semi-biosynthetic route towards dihydroartemisinic acid, which is the precursor to the anti-malaria drug artemisinin (Scheme 9).[62]



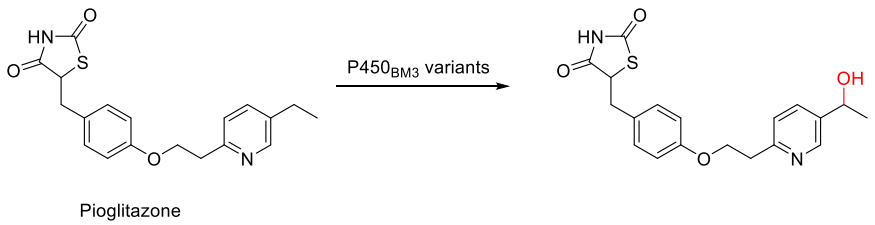
Scheme 9. Semi-biosynthetic strategy for the production of dihydroartemisinic acid.[62]

Cytochrome P450s are involved in the metabolism of over 80% of the drugs on the market, resulting in more water-soluble metabolites to facilitate urinary and biliary excretion.[63] However, the same metabolism could lead to highly reactive electrophilic metabolites that would bind to proteins and in turn influence drug efficacy and safety.[64, 65] Consequently, it is of great importance to evaluate the potential of new drugs to form electrophilic metabolites during early stage drug discovery,[66] and the preparative synthesis of drug metabolites to allow the analysis of metabolite activity and toxicity is therefore a vital aspect of drug development. Often, the chemical synthesis of drug metabolites is challenging and provides insufficient material.[67] As a consequence, both liver microsomes and heterologously expressed human P450s have been explored for their ability to synthesise drug metabolites, however their low catalytic activity and poor stability make this approach less feasible.[68–72] Bacterial P450s such as $P450_{BM3}$ are promising alternatives for the synthesis of drug metabolites due to their human-like activities yet better stability and elevated catalytic activities.[73] One example is the metabolism of a drug-like molecule paracetamol which can be effectively mimicked with triple mutant R47L/F87V/l188Q to generate the reactive *N*-acetyl-*p*-benzoquinoneimine intermediate (Scheme 10).[74]



Scheme 10. Metabolism of paracetamol with mutant R47L/F87V/l188Q.[74]

Another example is pioglitazone, a drug used to treat type II diabetes, that can be oxidised with high conversion in the ethyl side chain using $P450_{BM3}$ variants R47L/F87V/L188Q and R47L/F87V/L188Q/E267V/G415S (Scheme 11); the 2°-alcohol product is the main biologically-active metabolite of pioglitazone in humans, so-called metabolite PIO M1.[75]



Scheme 11. Metabolite formed from pioglitazone by $P450_{BM3}$ variants.[75]

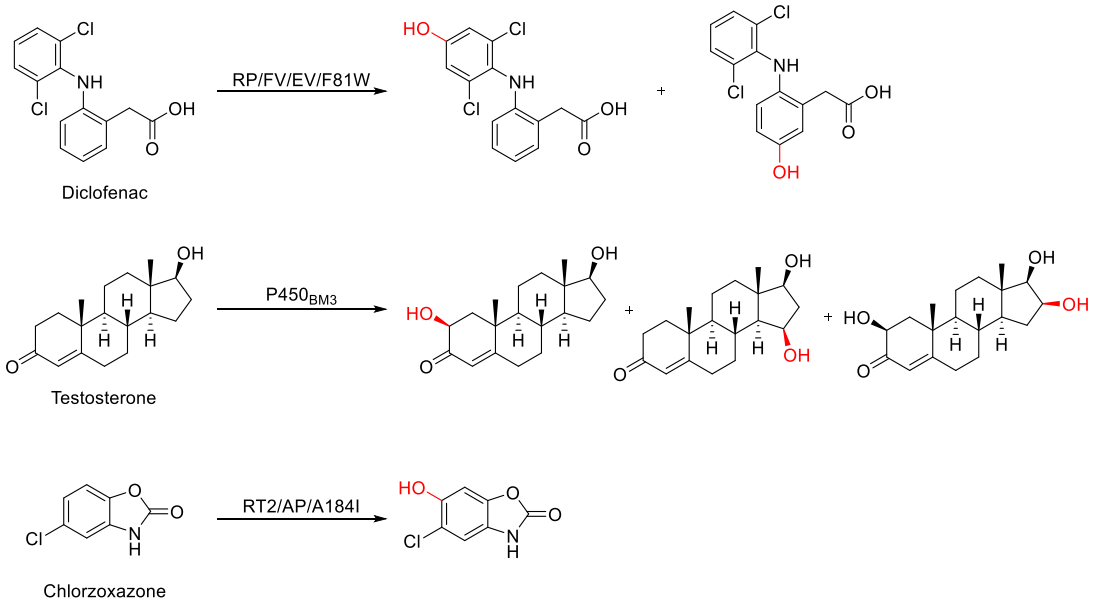
Research in the Wong Group

Focused on $P450_{BM3}$ mutant development for the purpose of improved activity and altered product selectivity, the Wong group has assembled a library of over 500 $P450_{BM3}$ mutants optimised for various substrate structures; the group's variant libraries were applied to the production or oxidation of specific structures.

The enzyme library is derived from four base mutants A330P (AP), I401P (IP), A191T/N239H/I259V/A276T/L353I (KT2) and F87A/H171L/Q307H/N319Y (KSK19) since the wild-type $P450_{BM3}$ enzyme is not usefully active towards most substrates other than fatty acids.[76] Combining these base mutants with other variants through site-specific mutagenesis or directed evolution improved the enzymes' activity towards substrates containing aromatics, alkanes and even pharmaceuticals. Moreover, the mutants were

originally, and continue to be, designed to target specific compounds through selectivity control.

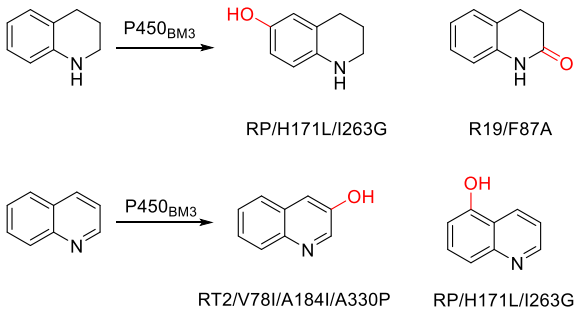
The Wong group investigated various drug metabolite syntheses and new reaction types were discovered along the way.[20] Six common drugs were selected as test substrates: naproxen, diclofenac, testosterone, chlorzoxazone, amitriptyline, and lidocaine. A library of 96 mutants identified from directed evolution studies was screened against all six drugs to find suitable mutants for preparative scale reactions. Some drugs gave more than one metabolite, and by selecting the appropriate mutant, any of the metabolites could be accessed as the major product (Scheme 12).



Scheme 12. Major metabolites from the oxidation of selected drugs with $P450_{BM3}$ mutants.[20]

By screening small libraries of preselected P450 enzyme variants against a variety of target substrates, P450s are identified with useful selectivity from which further generations of libraries can be obtained by combining mutations in the search for improved selectivity and

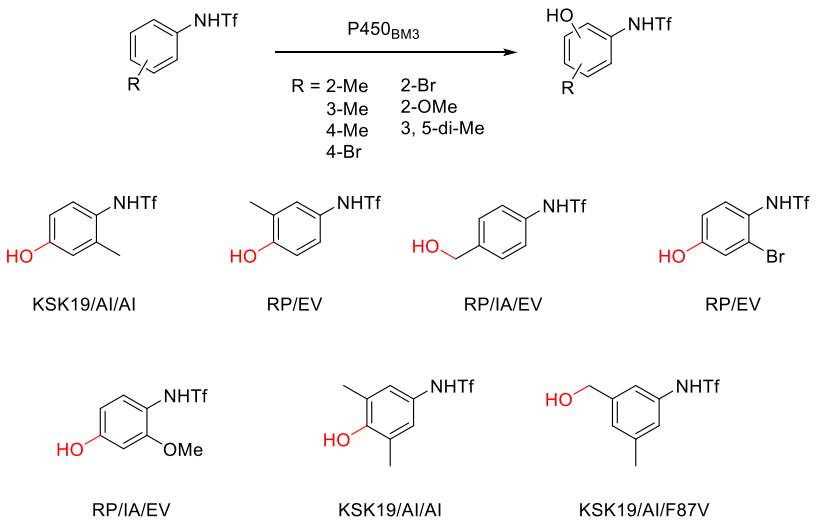
conversion. The group's more recent works have focused on substrate functionalisation *via* oxidation with their developed libraries and target compound synthesis; namely, accessing building blocks for the synthesis of drugs and other molecules with useful function. One recent example is the oxidation of quinolines and tetrahydroquinolines, which are common core motifs in drug molecules.[77] Substrates were screened against a 48-member library of $P450_{BM3}$ variants, then mutagenesis of the initial variants enabled regioselective hydroxylation with improved yield (Scheme 13).



Scheme 13. Products from the $P450_{BM3}$ mutants-catalysed oxidation.[77]

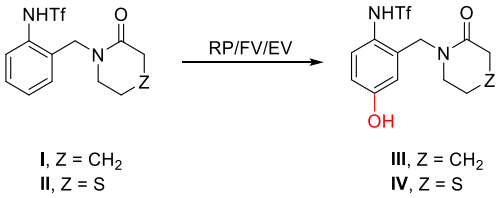
During the ongoing collaboration between the Robertson and Wong groups, our interest lies less in the expansion of the enzyme library and more in examining the utility of the library for application as general oxidation catalysts at both early and late stages in synthetic routes.

For example, direct monohydroxylation of anilides was achieved on preparative scale with $P450_{BM3}$ mutants.[78] Screening of anilides bearing simple ring substituents against a library of 48 mutants developed by the Wong group was carried out, with selective conversions to hydroxylation products being possible in all cases (Scheme 14).



Scheme 14. Preparative-scale hydroxylation of substituted anilides with $P450_{BM3}$ variants.[78]

The *para*-hydroxylation of herbicides **I** and **II** was achieved, allowing the direct formation of phenols **III** and **IV**, which were postulated to be the active form of the herbicides (Scheme 15). This work illustrated the feasibility of streamlining target synthesis by late-stage P450-hydroxylation.



Scheme 15. *para*-Hydroxylation of herbicides **I** and **II** with $P450_{BM3}$ variant RP/FV/EV.[78]

Early-stage hydroxylation: P450$_{BM3}$-mediated oxidation of simple cyclic amines

Functionalisation of simple cyclic amine structures

Nitrogen-containing heterocycles are extremely common structural components of drug fragments, present in 59% of U.S. FDA-approved small-molecule drugs.[79] Amongst these structures, saturated cyclic amines account for a great proportion of building blocks and synthetic intermediates to construct medicinal or biological molecules and are considered as prominent bioactive motifs in natural products; the most common saturated cyclic amine drug cores are pyrrolidine and piperidine, often with multiple functionalisation within the ring system (Figure 5).[79–81]

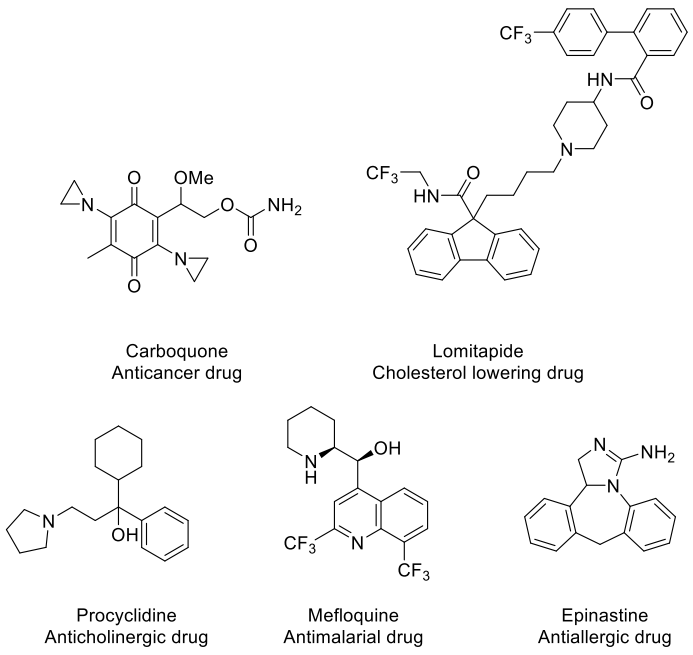
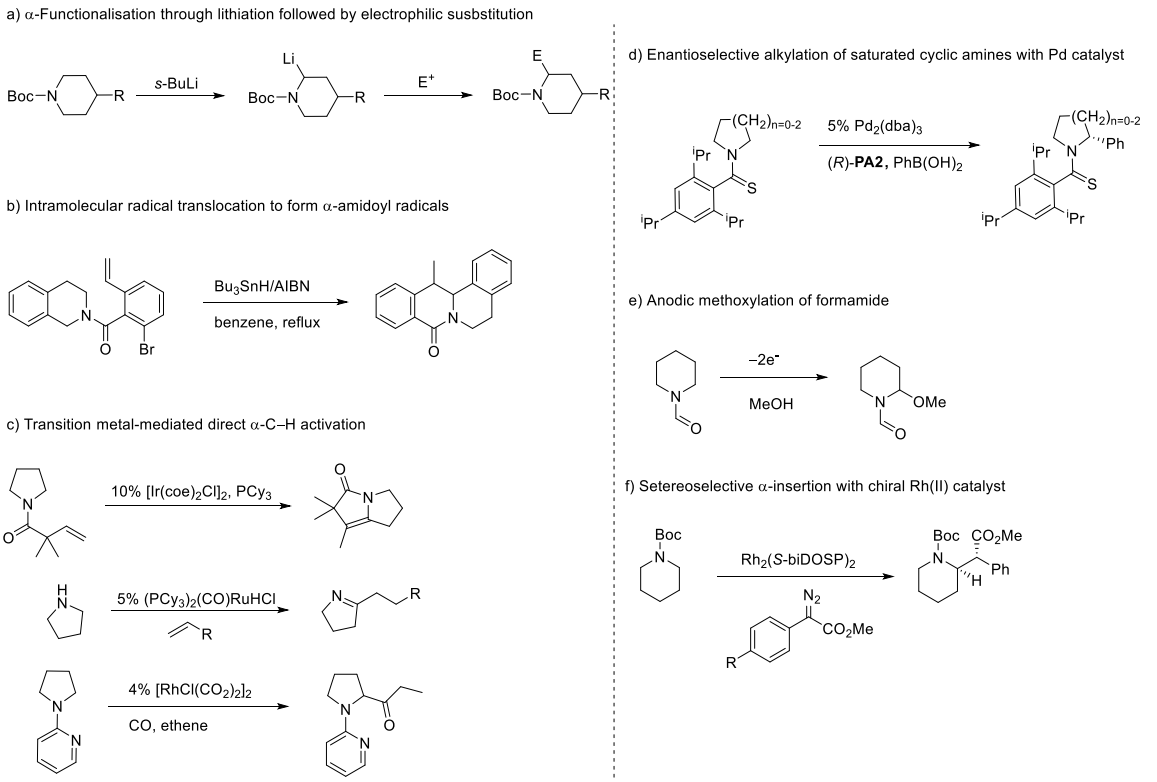


Figure 5. Examples of clinical drugs containing saturated cyclic amines.

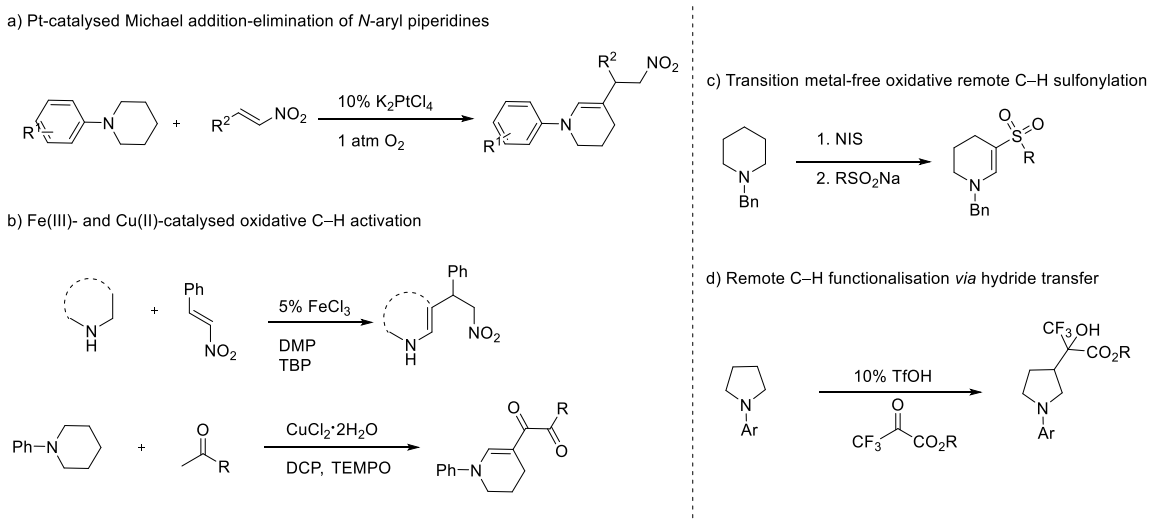
A great number of pharmaceutically active saturated cyclic amines are in clinical use as antibiotics, antidepressants, anti-HIV agents, etc;[79, 82, 83] therefore, the discovery and development of efficient and wide-scope methodologies for the C–H functionalisation of saturated cyclic amines is of great importance. The functionalisation of the α-position has been most studied and well-reviewed;[81, 84–86] functionalisation of the less accessible β- or even γ-positions within saturated cyclic amines has been reviewed by Fan's group.[87]

The most conventional reported method for direct functionalisation of sp^3 C–H bonds adjacent to nitrogen (α-position) is through α-lithiation with alkyllithium/diamine combinations to generate a dipole-stabilised carbanion for subsequent alkylation (Scheme 16a).[88] Radical based α-functionalisation of cyclic amines generates an α-amino radical for subsequent coupling with radicophiles (Scheme 16b).[89] Several reports provided examples of the use of transition metal catalysts including iridium, ruthenium and rhodium metals for direct C–H insertion adjacent to nitrogen;[90–92] moreover, Jain and co-workers reported a general method for the enantioselective alkylation of saturated cyclic amines with a Pd(II) catalyst (Scheme 16c,d).[93] In a complementary approach, C–H oxidative couplings are reported in which the *N*-heterocycle acts as an electrophile, the transformation proceeding *via* the formation and trapping by solvent of *N*-acyliminium intermediates (Scheme 16e).[94] Finally, Davies and co-workers reported a highly regio- and enantioselective incorporation of a range of aryldiazoacetates into the α-position of piperidine through chiral dirhodium-catalysed carbenoid C–H insertion (Scheme 16f).[95]



Scheme 16. Selected methodologies for the α-functionalisation of saturated cyclic amines.[88–95]

Functionalisation of the β-position in saturated cyclic amines is also reported. Liang's group reported a Pt-catalysed oxidative β-C–H activation of amines *via* dehydrogenation followed by Michael addition-elimination with nitro-olefins (Scheme 17a).[96] Related β-C–H functionalisations of various ring-sized cyclic amines were achieved with Fe(III) and Cu(II) catalysts (Scheme 17b).[97, 98] Griffiths and co-workers reported a transition metal-free oxidative β-C–H sulfonylation of *N*-functionalised piperidine (Scheme 17c).[99] Electrophile-promoted hydride-transfer, catalysed by Lewis acid to promote β-activation of pyrrolidine, was reported by Xiao and co-workers (Scheme 17d).[100]

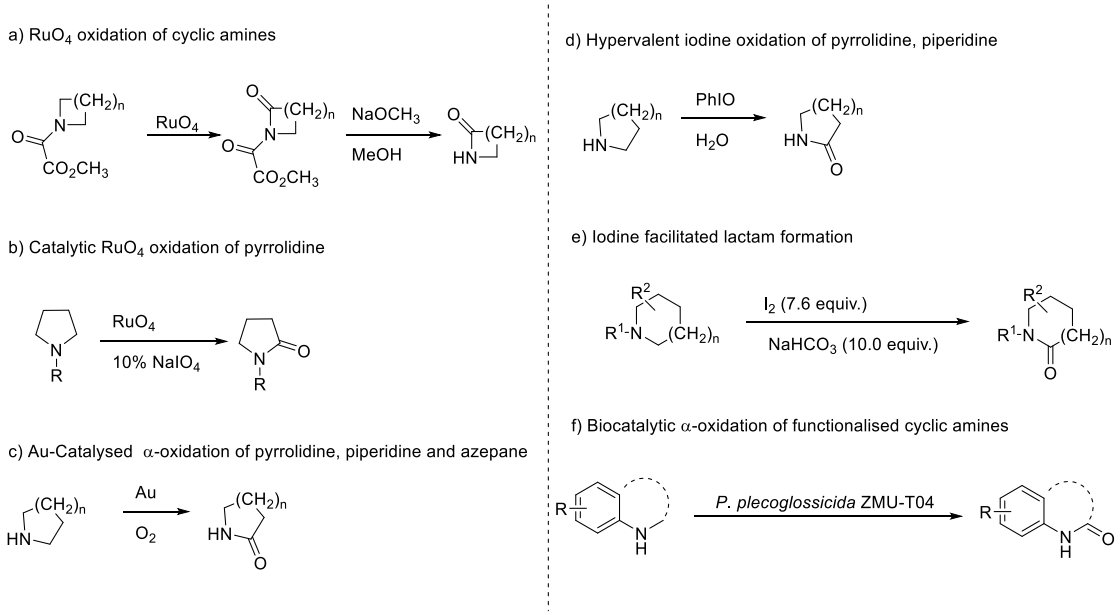


Scheme 17. Available methodologies to remote functionalisation of saturated cyclic amines.[96–100]

Oxidation of simple cyclic amines

This literature survey suggests that while the α-functionalisation of saturated cyclic amines is well-studied, methods for remote functionalisation in this type of structure remain limited, especially for the activation of the γ-position and beyond that cannot be simply accessed *via* enamine intermediates. This situation holds particularly true for the C–H oxidation of unfunctionalised cyclic amines. Tulis and Sheehan first reported the direct α-oxidation of saturated simple cyclic amines of various ring sizes with a stoichiometric amount of RuO_4 to afford the corresponding lactam; the protecting methyloxalyl group can be cleaved with sodium methoxide (Scheme 18a).[101] Kaname and co-workers reported lactam formation from protected pyrrolidines using a catalytic amount of RuO_4 with 10% $NaIO_4$ (Scheme 18b).[102] Other metal catalysts, such as gold, have been applied for a similar purpose; for example, direct lactam formation from saturated cyclic amines of various ring sizes (5 – 7) was reported

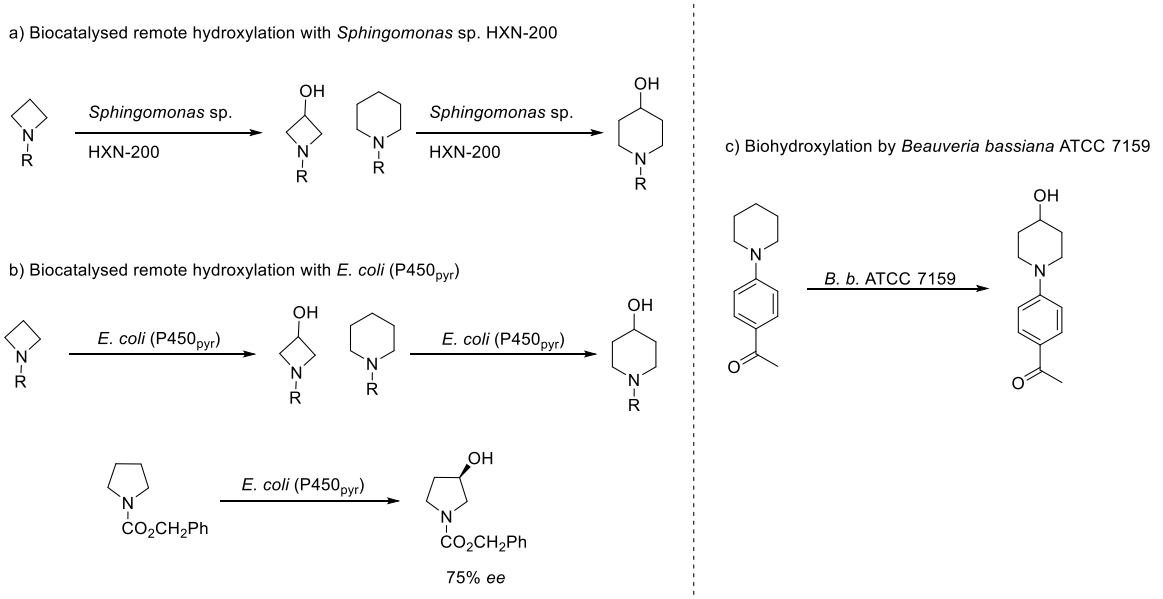
in which the substrate is treated with Au powder or Au/Al_2O_3 and O_2 in water (Scheme 18c).[103, 104] Moriarty and co-workers reported the oxidation of free pyrrolidine and piperidine by iodosobenzene in water, resulting in the corresponding pyrrolidinone and piperidinone (Scheme 18d).[105] Molecular I_2 itself was used to form lactams as reported by Griffiths and co-workers (Scheme 18e).[106] In addition to conventional chemistry methods, biocatalytic α-oxidation of cyclic amines can be achieved with *P. plecoglossicida* ZMU-T04 with *p*-xylene as inducer (Scheme 18f).[107]



Scheme 18. Previous reports on the α-oxidation of cyclic amines.[101–107]

The remote oxidation of saturated cyclic amines is more readily accomplished by biocatalysis with enzymes in whole-cell systems as shown in the following examples. Li and co-workers discovered that the bacterial strain *Sphingomonas* sp. HXN-200 (frozen/thawed cells) could regio- and stereoselectively catalyse the hydroxylation of *N*-substituted azetidines and piperidines (Scheme 19a);[108] the same authors later achieved a similar result by engineering

a recombinant *E. coli* expressing cytochrome $P450_{pyr}$ monooxygenase (Scheme 19b).[109–111] Biohydroxylation by the fungus *Beauveria bassiana* ATCC 7159 at the γ-position of an *N*-substituted piperidine was achieved by Holland and co-workers (Scheme 19c).[112]

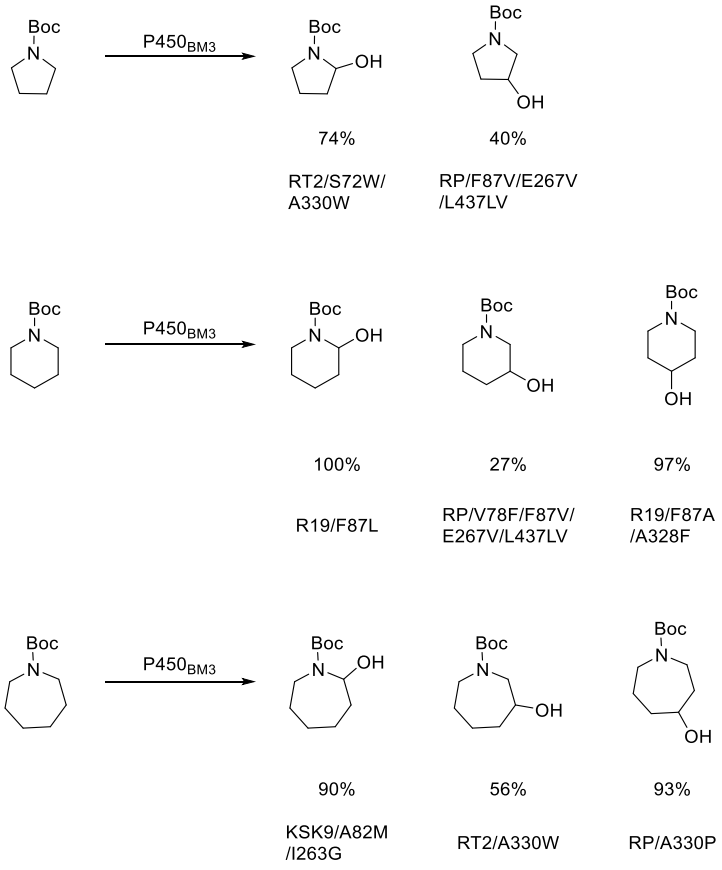


Scheme 19. Biocatalysed remote hydroxylation of saturated cyclic amines.[108–112]

Compared to conventional chemistry methods, biocatalysed oxidation has the advantage of circumventing the usage of metals, is environmentally friendly and safe, and most importantly, has the potential to hydroxylate chemically unactivated positions regio- and stereoselectively. Although microbial oxidation has been reported in previous reviews, the oxidation of cyclic amines with enzymatically engineered P450 mutants has not been thoroughly investigated. The commonly reported oxidations with P450 enzymes are *N*-oxidation and *N*-dealkylation, which are routine drug metabolism by human CYPs,[113–115] but there have been few reports on systematic investigations towards controlled remote functionalisation of cyclic amines with P450 enzymes focusing on regio- and stereoselectivity.

Remote oxidation of cyclic amines with P450$_{BM3}$ in the Wong group

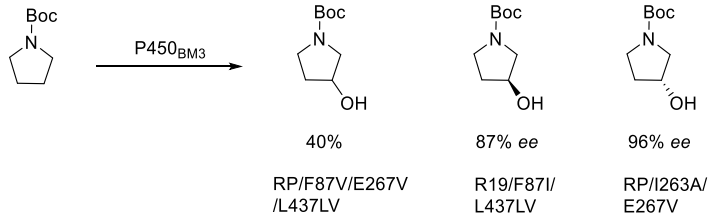
One of the main focuses of the Wong group in recent years has been the investigation of P450$_{BM3}$-mediated remote C–H bond functionalisation within cyclic amines looking for diversity and selectivity, thus expanding the usage of enzymatic oxidation in natural product and fragment molecule synthesis (Scheme 20).[116] The diversity and selectivity are achieved through the following methods: a) enzyme engineering through rational design/evolution guided by mutation-activity correlations obtained from screening results of the general panel; b) substrate engineering through functionalisation and varying *N*-protecting groups.



Scheme 20. Oxidation of *N*-Boc-cyclic amines with selected P450$_{BM3}$ variants developed by the Wong group.[117-119]

The group's initial work found that free amines such as pyrrolidine and piperidine inhibited enzyme oxidation, while Boc-protected pyrrolidine and piperidine exhibited high activity, regioselectivity and stereoselectivity during enzymatic screening, allowing access to remote non-activated C–H bonds.[117]

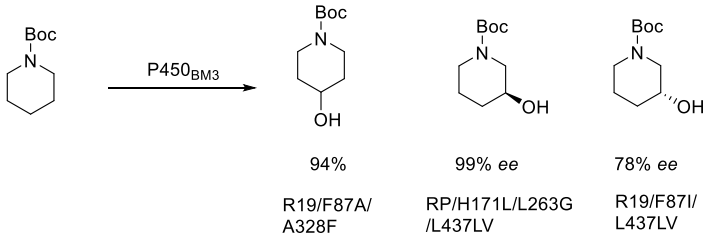
Oxidation of *N*-Boc-pyrrolidine favours α-hydroxylation but is able to produce the β-activation product with certain mutants, with the highest selectivity (40%) being found with the insertion mutant RP/F87V/E267V/L437LV; mutant R19/F87I/L437LV favours the (*S*)-enantiomer (87% *ee*) while RP/I263A/E267V gave the (*R*)-enantiomer (96% *ee*) (Scheme 21).[118, 119]



Scheme 21. Enantioselective β-hydroxylation with $P450_{BM3}$ variants.[118, 119]

In the case of *N*-Boc-piperidine, activation at all positions was observed, along with further oxidation products (Scheme 22). [118, 119] γ-Oxidation was achieved, and favoured by mutations at F87V and F87I, with R19/F87A/A328F providing the highest regioselectivity (97%). A notable mutant, RP/H171L/L263G/L437LV, gave 97% substrate conversion with 94% regioselectivity towards β-hydroxylation; even more impressively, it achieved a 99% *ee* for the (3*S*)-enantiomer. The R19/F87I/L437LV mutant achieved the highest selectivity for the (3*R*)-enantiomer (78% *ee*), although the regioselectivity was low (19%). This work allowed

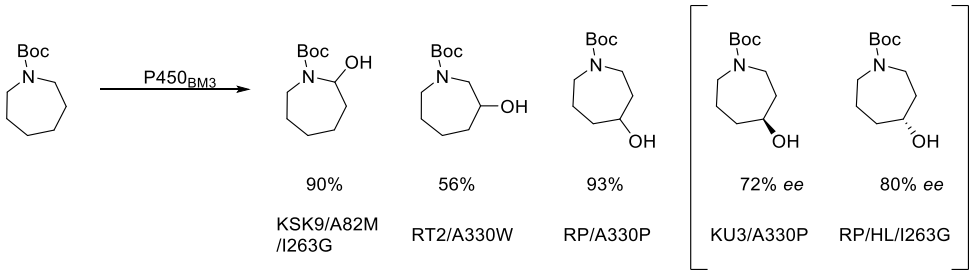
access to all remote positions on pyrrolidine and piperidine through enzymatic hydroxylation with control of regio- and enantioselectivity.



Scheme 22. Regio- and enantioselective remote hydroxylation with $P450_{BM3}$ variants. [118, 119]

$P450_{BM3}$-mediated hydroxylation of *N*-Boc-azepane and its application in drug synthesis

The Wong group has developed mutants that are effective for the selective hydroxylation of *N*-Boc-azepane, allowing access to the α-, β- and γ-positions (Scheme 23) with most variants giving conversions over 50%.[118, 119]



Scheme 23. *N*-Boc-azepane hydroxylation with $P450_{BM3}$ variants.[118, 119]

With initial focus on β-functionalisation, the most promising outcome was achieved by mutant RT2/A330W which showed 56% selectivity for this position. Among the results, a highlight was the highly regioselective formation of γ-hydroxyazepane, with up to 93% selectivity being achieved with RT2/A330P. More importantly, an *ee* of 72% for the (4*S*)-

enantiomer was found with mutant KU3/A330P, while an 80% *ee* for the (4*R*)-enantiomer was found with mutant RP/HL/I263G. This is of great interest to us since it is envisaged that these hydroxylated simple cyclic amines could serve as intermediates for the synthesis of more heavily functionalised natural products and drug molecules (Figure 6) by chemical elaboration using subsequent oxygen-tethered intramolecular C–H activation reactions.

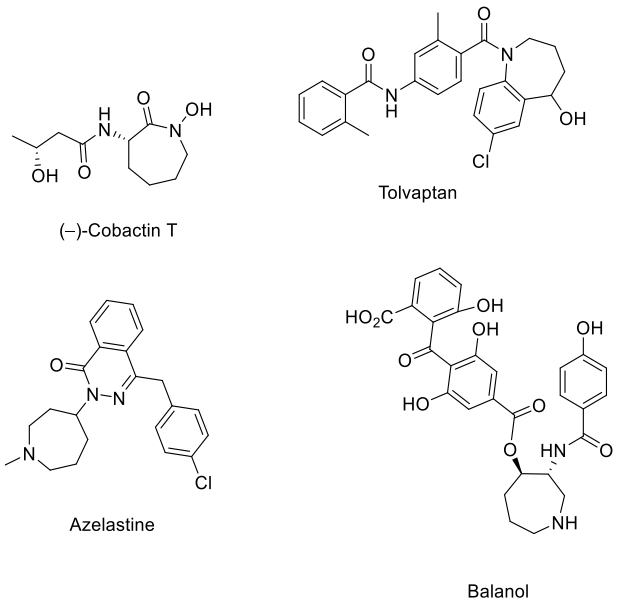


Figure 6. Examples of drug molecules containing functionalised azepane cores.

Balanol and its synthetic analogues

Balanol, a metabolic product of the fungus *Verticillium balanoides*, was first isolated and characterised by Kulanthaivel and co-workers in search of novel and potent protein kinase C (PKC) inhibitors; IC_{50} values of 4–9 nM were observed in assays against human PKC enzymes.[120] The PKCs form a family of serine/threonine-specific kinase enzymes that are involved in controlling the function of other proteins by catalysing the transfer of the γ-phosphate of ATP to substrate proteins;[121] namely, in receptor desensitisation, regulating

transcription, mediating immune responses, and regulating cell growth.[122] PKC-mediated signal transduction is known to lead to a variety of cellular responses and unregulated activation of this enzyme has been implicated in diseases such as cancer, cardiovascular dysfunctions, asthma, inflammation, and HIV infection.[123] Since PKC activity is pivotal to signal transduction pathways in many inflammatory cell types, inhibitors of PKC could potentially have therapeutic value for various diseases.[124] Mechanistically, balanol inhibits PKC through occupying the ATP binding pocket of the kinases in a similar fashion to ATP, but with a 3000 times greater affinity.[125] Balanol and its synthetic analogues (Figure 7) are reported by Nicolaou and co-workers to be potent inhibitors of purified rat brain PKC, with an IC_{50} of 4 nM being reported for balanol itself.[126] Additionally, the authors showed that balanol and its analogues also inhibit protein kinase A (PKA); its synthetic analogues showed significant selectivity for the inhibition of PKA over PKC.

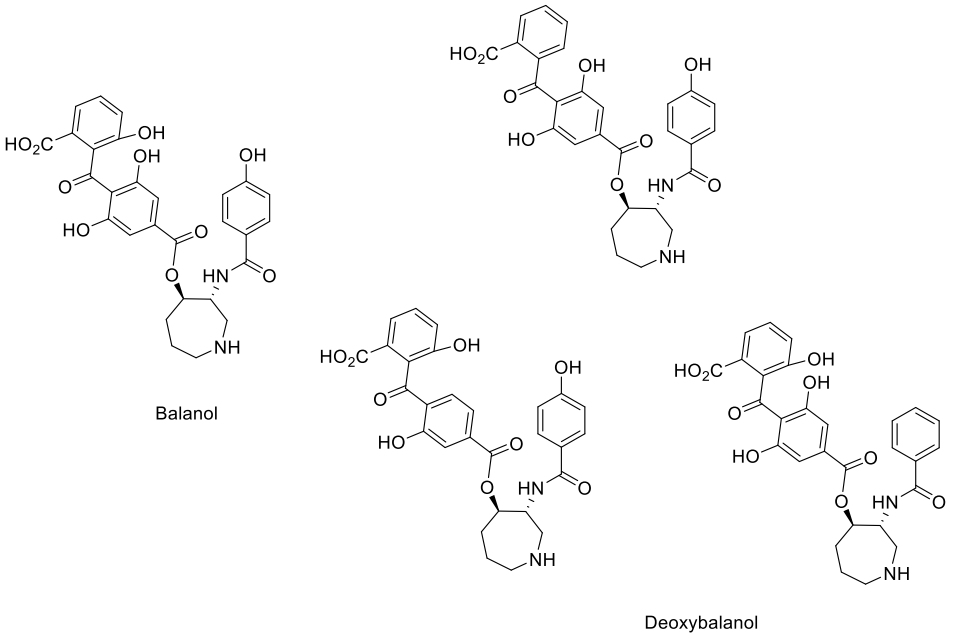
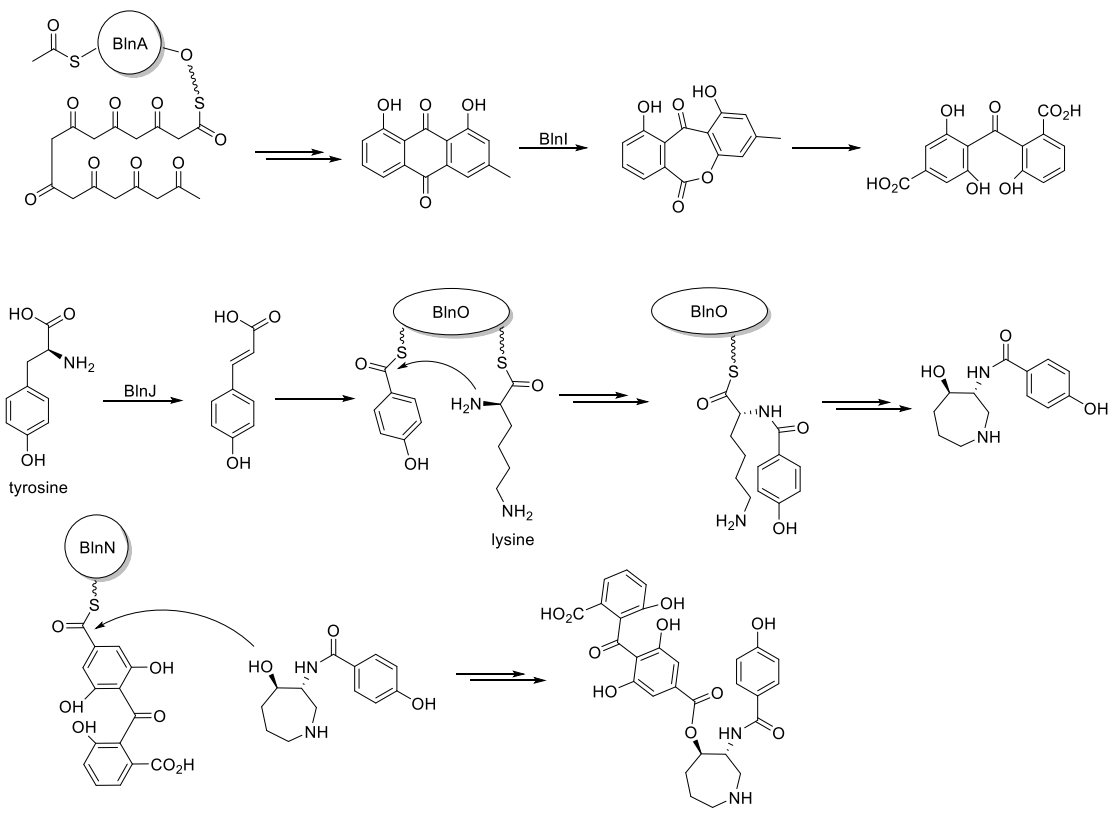


Figure 7. Balanol and three deoxy-analogues.[126]

Biosynthetic pathway of balanol

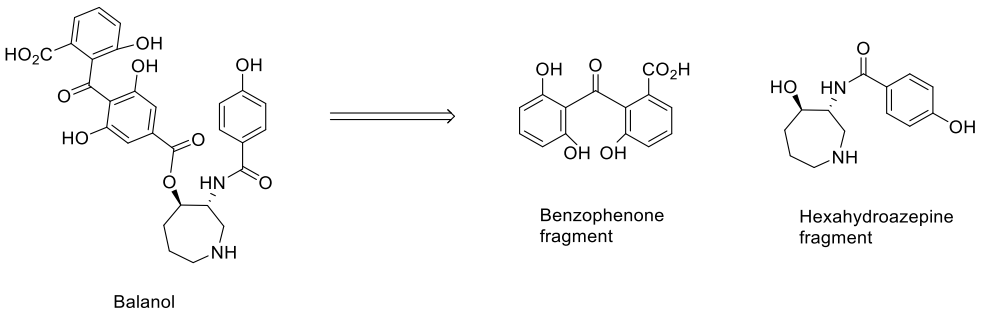
Li and co-workers first identified the balanol biosynthetic gene cluster *bln*.[127] They revealed that the biosynthesis involved two convergent synthetic pathways, in which a polyketide synthase (PKS) is responsible for generating the benzophenone fragment while the non-ribosomal peptide synthetase (NRPS) Bln *O* and other polyketides produce a dipeptide fragment. Finally, NRPS Bln *N* catalyses the coupling of the two fragments to produce balanol (Scheme 24).



Scheme 24. Biosynthetic pathway of balanol.[127]

Total synthesis of balanol

Due to its promising potential as a PKC inhibitor as well as its unique functionalised azepane structure, balanol attracted significant attention from synthetic chemists shortly after the report of its isolation. Total syntheses have been achieved by several groups. All these groups disconnected balanol at its ester and amide linkage, dividing it into two structural domains: the derivatised azepane ring fragment and a functionalised benzophenone fragment (Scheme 25).



Scheme 25. Disconnection of balanol into two synthetic fragments.

Nicolaou's group

The synthetic challenge posed at the outset was the asymmetric synthesis of the central azepane ring and the functionalised benzophenone fragment (Figure 8).[128]

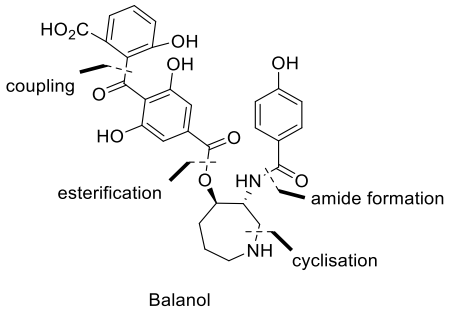
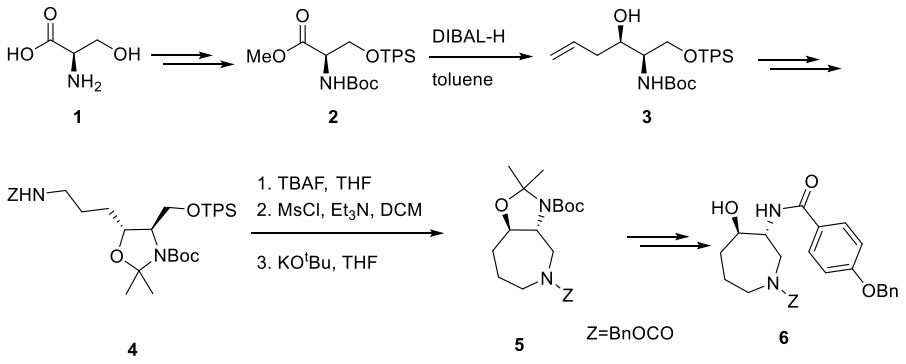


Figure 8. Nicolaou's retrosynthetic analysis of balanol.[128]

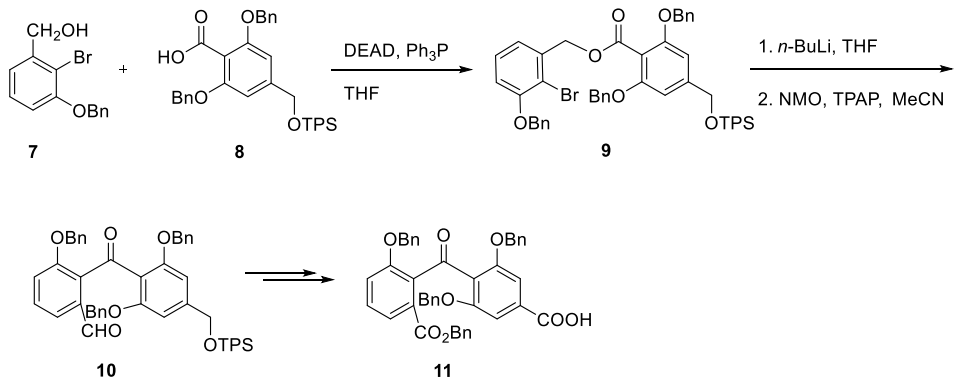
In practice, the synthesis of the azepane fragment was achieved in sixteen steps starting from amino acid D-serine **1** (Scheme 26). The correct stereochemical disposition was achieved by the reduction of ester **2** then treatment of the resulting aldehyde with Brown's diisopinocampheylborane reagent, giving amino alcohol **3** as a 12:1 mixture of *syn*/*anti* diastereoisomers, respectively. Subsequent 7-*exo*-*tet* cyclisation of an activated 1 ° alcohol

furnished the azepane ring in intermediate **5**, and treatment with *p*-(benzyloxy)benzoyl chloride afforded fragment **6** in 36% overall yield.

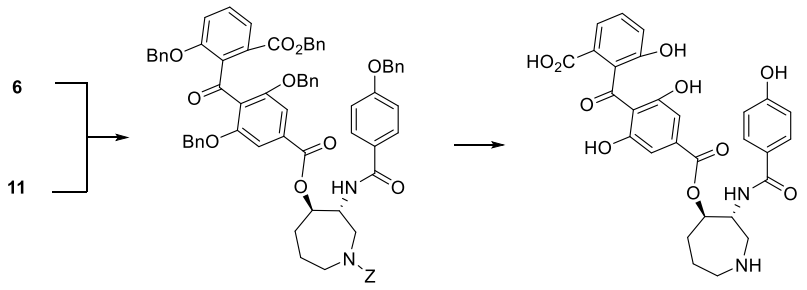


Scheme 26. Formation of the functionalised azepane fragment reported by Nicolaou.[128]

The benzophenone fragment was constructed in fifteen steps from *m*-hydroxybenzaldehyde (Scheme 27). Mitsunobu coupling between alcohol **7** and carboxylic acid **8** furnished ester **9**, which underwent acyl transfer following metal/halogen exchange with butyllithium. The so-formed benzylic alcohol was oxidised to aldehyde **10** and, following further functional group manipulations, the carboxylic acid **11** was prepared in 30% overall yield. Esterification between fragments **6** and **11** then global deprotection completed the route (Scheme 28).



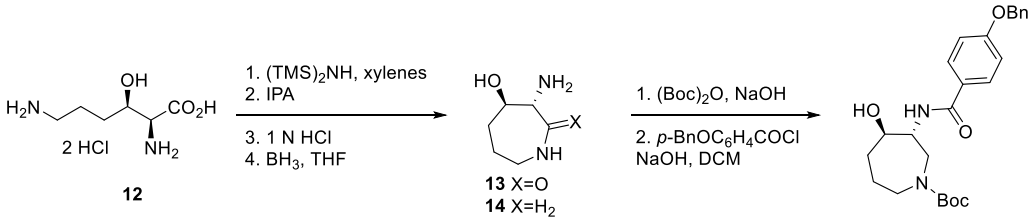
Scheme 27. Formation of the benzophenone fragment reported by Nicolaou.[128]



Scheme 28. Union of the two fragments through esterification and completion of the synthesis.[128]

Lampe's group

Lampe and co-workers started off by building the functionalised azepane core with hydroxylysine **12**, which was produced following Roemmele's procedure (Scheme 29).[129, 130] The azepane ring, as lactam **13**, was formed by heating diaminoacid **12** with hexamethyldisilazane as a dehydrating agent.



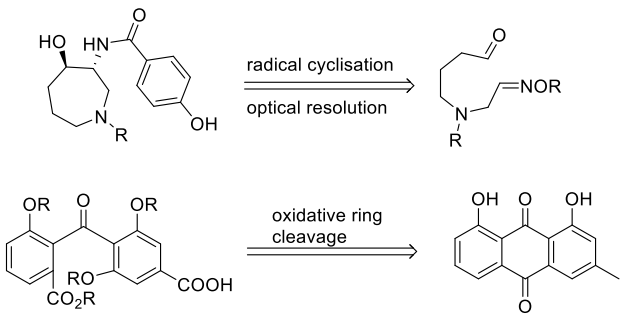
Scheme 29. Formation of the functionalised azepane fragment reported by Lampe.[129]

As for the benzophenone fragment, Lampe's group adopted the same strategy as Nicolaou's, i.e. forming the benzophenone carbon-carbon bond intramolecularly *via* rearrangement. The convergent synthesis was accomplished in eighteen total operations, proceeding in 22% yield

for the six linear steps from **12** and 4.3% yield for fourteen linear steps for the benzophenone fragment.

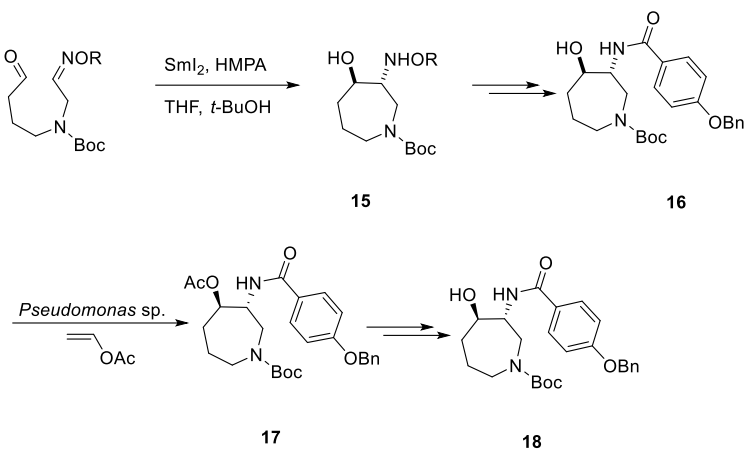
Naito's group

Naito and co-workers envisaged the preparation of an enantiomerically pure azepane fragment through a radical cyclisation followed by lipase-catalysed kinetic resolution of the racemic alcohol products.[131] In parallel, oxidative ring cleavage of chrysophanic acid, as proposed in the biosynthesis, would result in the benzophenone fragment (Scheme 30).



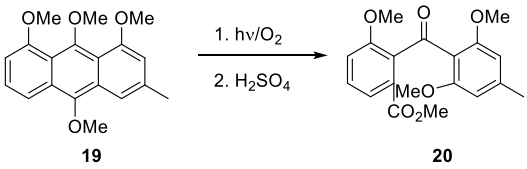
Scheme 30. Naito's retrosynthesis of the two fragments.[131]

In practice, the use of SmI_2 in the presence of HMPA afforded the reductive cyclisation product **15** with *dr* = 6.6:1 in favour of the *trans*-diastereomer (Scheme 31). Resolution was achieved later in the synthesis using enzymatic esterification of alcohol **16** with *Pseudomonas* sp., giving acetate **17** in 49% yield and 96% *ee*.



Scheme 31. Naito's synthesis of the azepane fragment.[131]

For the benzophenone fragment, anthracene **19** underwent formal [4+2] cycloaddition of singlet oxygen and the so-formed endoperoxide adduct rearranged upon treatment under acidic conditions to give the benzophenone derivative **20** (Scheme 32).

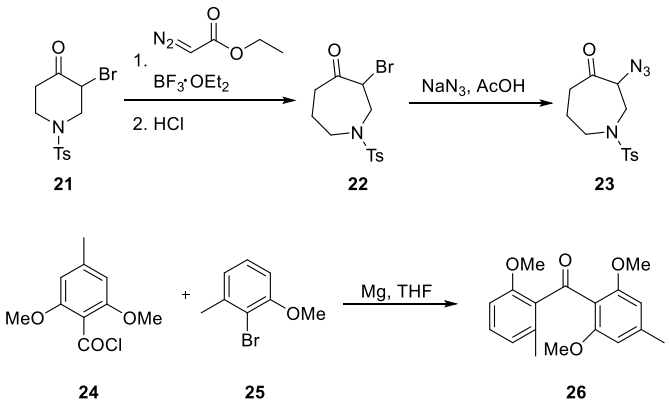


Scheme 32. Key step in Naito's synthesis of the benzophenone fragment.[131]

Vicker's group

Vicker and co-workers synthesised the azepane fragment from piperidin-4-one in ten steps, featuring a regioselective ring-expansion to give ketone **22**, which then afforded azide **23** by direct substitution (Scheme 33).[132] Subsequent $NaBH_4$ reduction gave the *trans*-isomer of hydroxyazide **23** in 26% yield, the undesired *cis*-isomer being converted into the *trans*-isomer

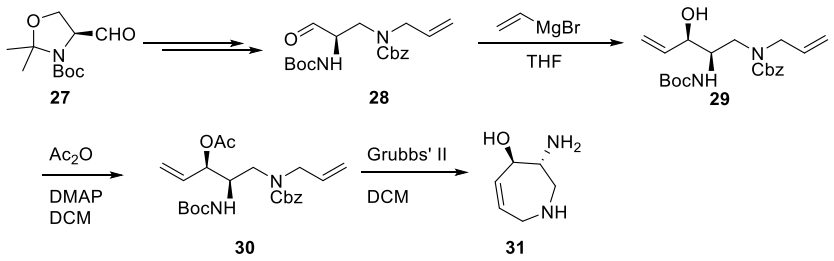
by a Mitsunobu inversion. The benzophenone fragment was achieved by oxidation of ketone **26**, which was formed, in turn, by coupling of benzoyl chloride **24** and the Grignard reagent derived from aryl bromide **25**. Coupling between the two fragments employed the procedure established in Nicolaou's synthesis.



Scheme 33. Key steps in Vicker's synthesis of the two fragments.[132]

Chattopadhyay's group

Chattopadhyay and co-workers constructed the azepane fragment in eleven steps, *via* ring-closing metathesis, with an overall 20% yield (Scheme 34).[133] The synthesis started with reductive amination of Garner's aldehyde **27**. Nucleophilic addition of vinylmagnesium bromide to aldehyde **28** gave a mixture of diastereomers, where the undesired *anti*-isomer was transformed into the *syn*-isomer **29** by a Mitsunobu-type inversion. Following acetylation of the hydroxyl group, ring-closing metathesis gave cycloalkene **31**. The benzophenone fragment was achieved in a similar fashion to Vicker's work, in eight steps with an overall 22% yield, and the coupling between the two fragments again employed Nicolaou's protocol.



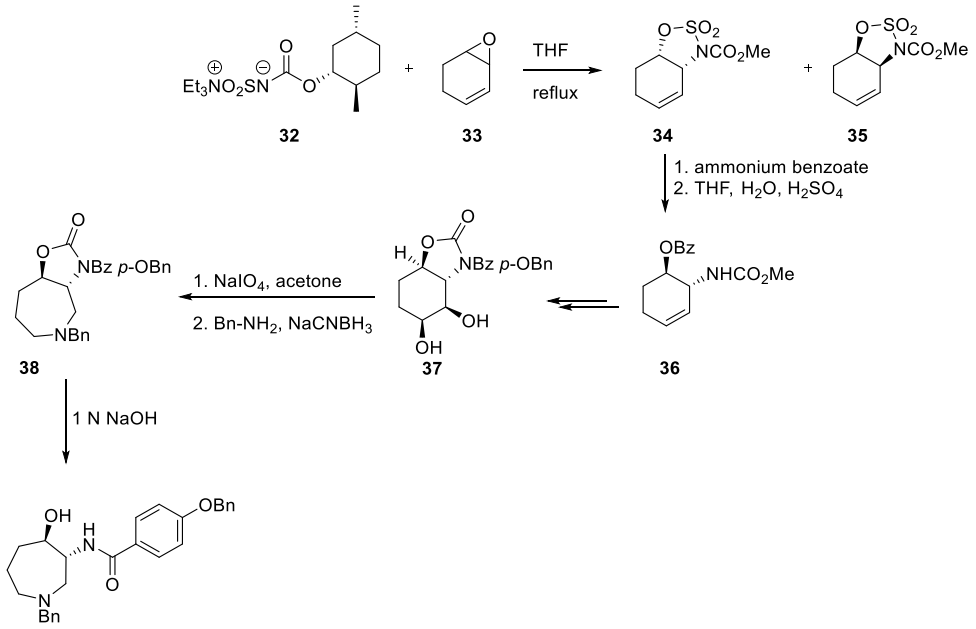
Scheme 34. Key steps in Chattopadhyay's synthesis of the azepane fragment.[133]

Panda and co-workers reported a total synthesis of balanol and all its stereoisomers in the same fashion from Garner's aldehyde.[134]

Formal synthesis of balanol

Hudlicky's group

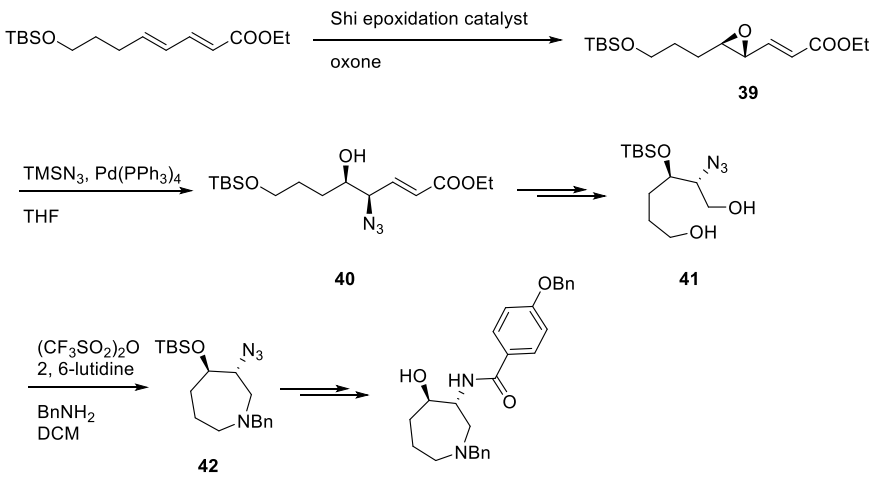
Hudlicky and co-workers reported an eight-step synthesis of the azepane fragment (Scheme 35).[135] Reaction of cyclohexadiene epoxide **33** with a chiral version of the Burgess reagent **32** proceeded by regioselective epoxide opening at the allylic site to yield two diastereomeric sulfamidates **34** and **35**; these were separated by conversion into the corresponding benzoates, with benzoate **36** possessing the desired stereochemistry. Subsequent diol cleavage and immediate treatment with benzylamine under reductive amination conditions furnished azepane carbamate **38**.



Scheme 35. Key steps in Hudlicky's synthesis of the azepane fragment.[135]

Muthyala's group

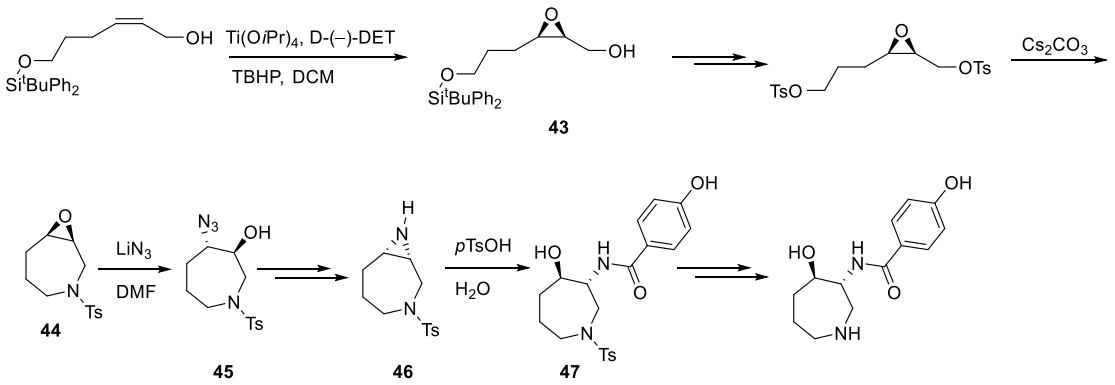
A synthesis of the azepane fragment by Muthyala and Yaragorla started with 1,5-pentanediol (Scheme 36).[136] Shi epoxidation provided epoxide **39** with 96% *ee*, from which *syn*-azido alcohol **40** was gained through Pd(0)-catalysed stereospecific azide substitution with double inversion of configuration. Ozonolysis and $NaBH_4$ reduction yielded diol **41**, which was activated as the ditriflate and treated with benzylamine to furnish azepane **42**. Overall, the azepane fragment was reached in 12 steps in 5% yield.



Scheme 36. Key steps in Muthyala's synthesis of the azepane fragment.[136]

Tanner's group

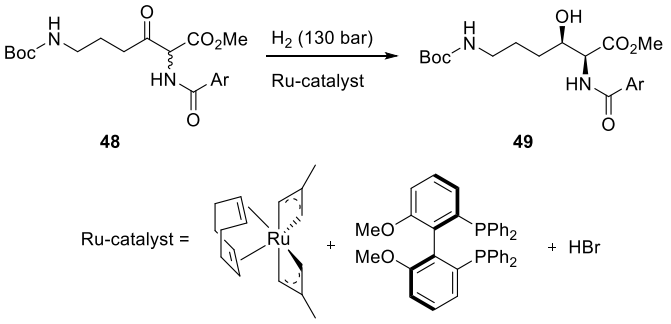
Tanner and co-workers presented a fourteen-step formal synthesis of the azepane portion of balanol from the chiral epoxide **43**,[137] which was obtained from Sharpless asymmetric epoxidation in 90% *ee* (Scheme 37).[138] Cyclisation with cesium carbonate furnished epoxide **44**, then regioselective ring-opening with LiN_3 gave azido alcohol **45** with the wrong relative disposition of nitrogen and oxygen functionality. The regiochemistry was corrected by transformation into aziridine **46**, and regioselective ring-opening to give **47** (98:2 ratio of regioisomers).



Scheme 37. Key steps in Tanner's synthesis of the azepane fragment.[137]

Genet's group

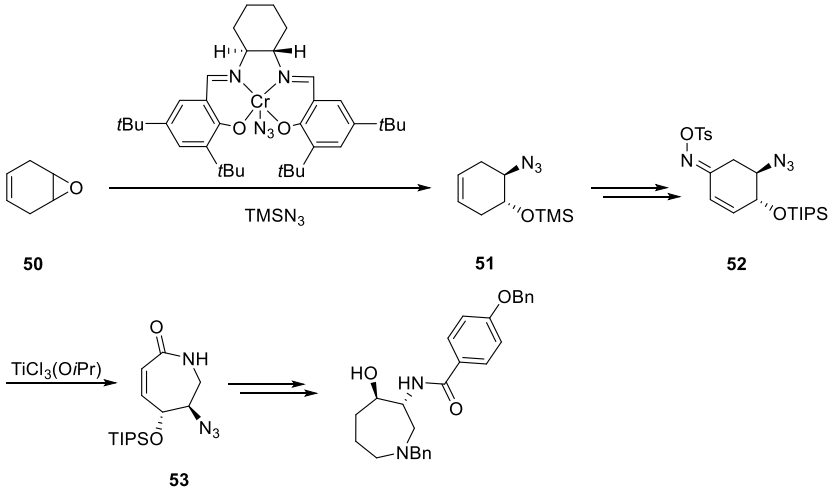
Genet and co-workers used a Ru-mediated hydrogenation of ketoester **48** to generate *syn*-**49** diastereoselectively and, with a chiral Ru(II) catalyst, dynamic kinetic resolution was achieved to give the product with 94% *ee* (Scheme 38).[139] Based on this key step, the overall route to the azepane precursor **18** was ten steps and 17.3% yield.



Scheme 38. Key steps in Genet's synthesis of the azepane fragment.[139]

Jacobsen's group

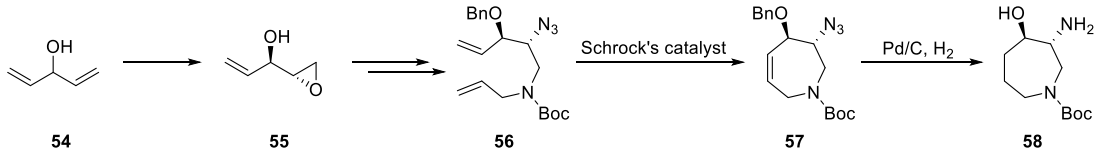
Jacobsen's group started their synthesis with enantioselective ring-opening of epoxide **50** to give silyl ether **51** with 92% *ee* (Scheme 39).[140] Subsequent ring expansion afforded lactam **53** by Beckmann rearrangement using $TiCl_3(OiPr)$ as Lewis acid. In this route, the protected azepane core was achieved in 12 steps and 31% overall yield.



Scheme 39. Key steps in Jacobsen's synthesis of the azepane fragment.[140]

Fürstner's group

Finally, Fürstner's group developed an eight-step sequence to the azepane core, starting from Sharpless epoxidation of diol **54** to generate monoepoxide **55** in essentially 100% *ee* (Scheme 40).[141] Ring-closing metathesis with Schrock's molybdenum alkylidene complex transformed diene **56** into cycloalkene **57** efficiently provided that mild reaction conditions were employed. Hydrogenation of **57** saturated the heterocyclic ring, liberated the amino group, and deprotected the benzyl ether group simultaneously.

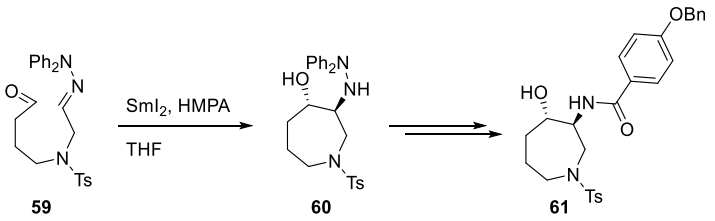


Scheme 40. Key steps in Fürstner's synthesis of the azepane fragment.[141]

Other synthetic studies

Pinacol-type cyclisation to the hexahydrazepine core by Skrydstrup' group

Skrydstrup and co-workers developed a diastereoselective and enantioselective pinacol coupling promoted by SmI_2, which was applied in the synthesis of the azepane core of balanol, generating hexahydroazepine **60** with a *trans*/*cis* ratio of 10:1, respectively (Scheme 41).[142] Hydrogenolysis in the presence of Pearlman's catalyst, then acylation with *p*-benzyloxybenzoyl chloride afforded crystalline amide **61**, which allowed the assignment of stereochemistry through single crystal X-ray crystallography.

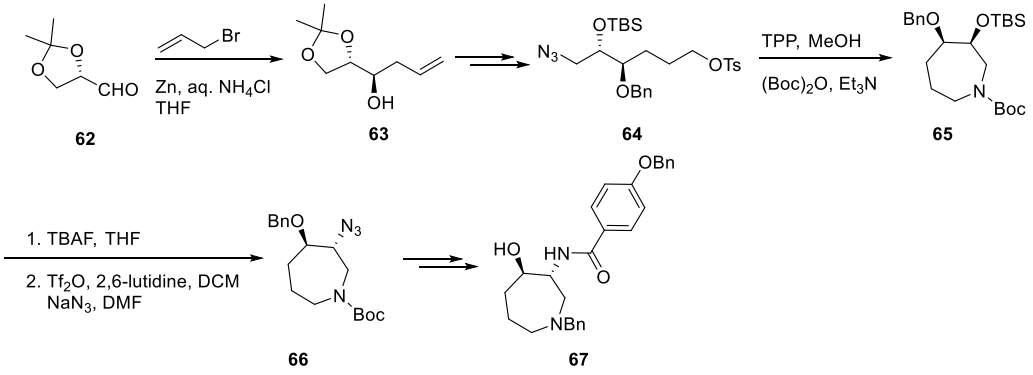


Scheme 41. Key steps in Skrydstrup's synthesis of the azepane fragment.[142]

Yadav's group

Yadav's group reported a seven-step route to the functionalised azepane **67** from aldehyde **62**,[143] which was obtained from L-ascorbic acid by a reported procedure (Scheme 42).[144]

Treatment of aldehyde **62** with allyl bromide in the presence of Zn and aq. NH_4Cl afforded the corresponding homoallyl alcohol **63** as a mixture of diastereoisomers (96:4, *anti*/*syn*). Ring formation was achieved by treating azide **64** with TPP, then Boc-protection gave the cyclised product **65**. The 2 °alcohol β- to the ring nitrogen in **65** was inverted by converting into the azide **66** *via* deprotection, triflation, and treatment with NaN_3.



Scheme 42. Key steps in Yadav's synthesis of the azepane fragment.[143]

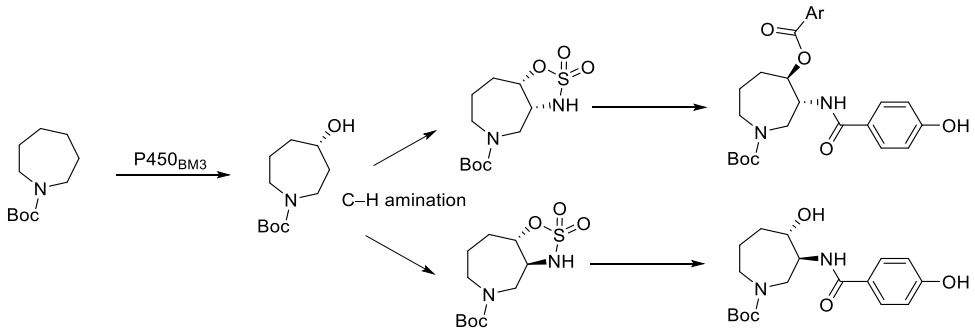
Project outline

The above review highlights the importance of saturated cyclic amines as building blocks and key intermediates in many natural products and drugs. Thus, the functionalisation of these core structures is of great importance and potential in synthesis but remote oxidation of *N*-functionalised cyclic amines remains poorly explored. As an alternative to traditional chemical reagents, biocatalytic methods hold great potential in this area. Enzymes, such as cytochrome P450 monooxygenases, can perform selective oxidation of inactivated positions in a wide range of organic molecules, especially remote C–H activation. It would be expected therefore that such enzymes could be modified to be used in a preparative context; while this

has been demonstrated for relatively simple substrates, the application of biocatalytic oxidations in the early stage of the synthesis of complex natural product molecules is under-explored. An ongoing collaboration between the Robertson and Wong groups aims to effect early-stage oxidation of saturated cyclic amines with a designed $P450_{BM3}$ mutant library, developed by the Wong group, for natural product synthesis. The group's studies on the $P450_{BM3}$-mediated Boc-azepane hydroxylation provided a potential strategy for the further functionalisation of cyclic amines by coupling enzymatic oxidation with tethered C–H activation.

To demonstrate the concept in a specific example, our project is inspired by natural compound balanol. The traditional synthetic strategies for the azepane core of balanol focus on assembling the stereodefined functionality of, and then forming, the core seven-membered ring, which can lead to lengthy synthetic routes. A novel and straightforward synthetic route could start from direct functionalisation of the bare seven-membered ring, potentially resulting in a much more concise synthesis.

The work described in the first part of this thesis aimed to develop such a route by initial stereoselective hydroxylation of *N*-protected azepane, followed by tethered C–H amination to direct the formation of the 1,2-aminoalcohol functionality present in balanol (Scheme 43). Moreover, apart from synthetic route development, this study would further our understanding of the $P450_{BM3}$ substrate/reactivity matrix by exploring the hydroxylation profiles (regioselectivity and stereoselectivity) of varied *N*-protected azepanes, starting with Boc-azepane.



Scheme 43. Envisaged hexahydroazepine fragment formation from *N*-Boc-azepane.

Late-stage hydroxylation: P450$_{BM3}$-mediated oxidation of guaiene sesquiterpenes

Guaienes: biological activities and its fragrance

The guaienes (Figure 9) are sesquiterpenes (molecular formula $C_{15}H_{24}$) with a 5,7-fused ring structure that have been isolated from a variety of plant sources, usually plant essential oils from *Cedrelopsis grevei, Xanthium strumarium L.*, *Premna microphylla*, etc.[145–148] Aromatic and medicinal plants have played an important role as therapeutic agents in history and formed the fundations of modern drugs and traditional remedies;[149–151] these plants often display a wide range of activities including antibacterial, anti-inflammatory, antioxidant and antimalarial activities, which are often a result of their sesquiterpene components.[145–148]

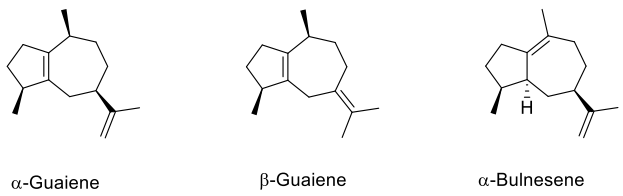


Figure 9. Structures of key members of the guaiene family.

The *Pulicaria* Gaertn genus belongs to the Inuleae tribe of the Asteraceae family; individual *Pulicaria* species are used in traditional medicine in several countries and are beneficial in the treatment of a variety of illnesses such as flu, intestinal disorders, swelling and painful boils, inflammation and heart diseases.[152, 153] The essential oil compositions of *P. vulgaris* are mainly composed of sesquiterpenes, shown by Jannet and co-workers to be rich in β-guaiene (around 60%), accounting for the essence oil's antibacterial and antioxidant activity.[145] Cocklebur (*Xanthium strumarium* L.) is an annual plant species also belonging to the Asteraceae family that, in many countries, is used in traditional remedies; extracts from this plant were found to possess antifungal, anti-inflammatory, and anticancer activities.[154, 155] Sharifi-Rad and co-workers isolated the chemical components of essential oil extracted from the leaves of *X. strumarium*, which contains around 35% β-guaiene; they also demonstrated the sesquiterpene's potential as an antimicrobial agent as well as its antifungal activity.[147] The genus *Premna* (Verbenaceae) consists of a group of over two hundred species widely distributed in tropical and subtropical areas.[156] Preparations derived from plants of this genus have been found to display a wide range of pharmacological effects involving anticancer, antibacterial, antifungal, and anti-leishmania activities.[157] For example, the leaves of *P. microphylla*, a deciduous shrub, are widely used to treat dysentery, appendicitis, and infections in China.[158] Zhang and co-workers isolated the volatile components of *P. microphylla* essential oil which contains α-guaiene (around 4%) and were then able to attribute the biological activities (antimicrobial, cytotoxicity) of the plants to these volatile components.[148]

Apart from their reputed therapeutic value, sesquiterpenes, including guaienes and their oxygenated derivatives, sesquiterpenoids, are key components of perfumes and fragrances (Figure 10). They are found widely distributed in various aromatic essential oils in the high boiling fraction. Cosmetic studies revealed that many commercial perfumery products contain a number of sesquiterpenes that contribute to the overall aroma; the characterisation of aromatic sesquiterpenes in the perfumery industry is well reviewed.[159]

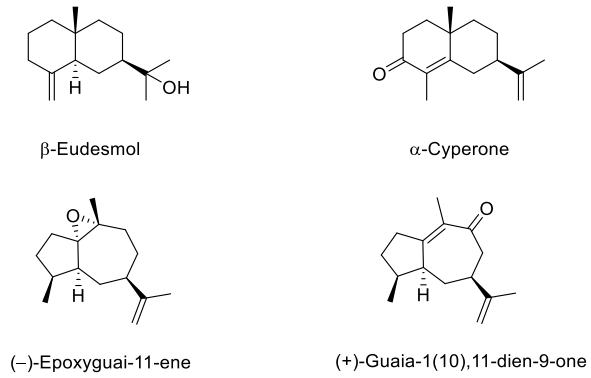
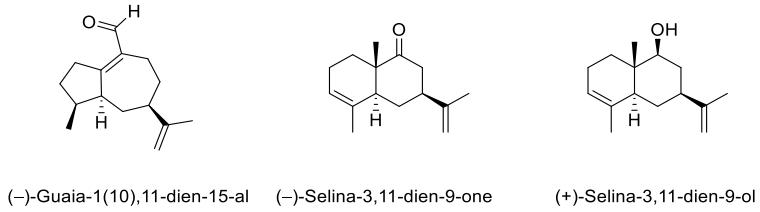


Figure 10. Examples of isolated sesquiterpenoids with fragrance profiles.[159]

Agarwood, whose production is confined to certain areas of South and Southeast Asia, is amongst the costliest woods in the world owing to its widespread use for medicinal, aromatic, and religious purposes.[160] Agarwood is the resinous heartwood of the *Aquilaria* tree; amongst the commonly traded agarwoods, *Aquilaria agallocha* (Kanankoh) is considered to have the highest quality.[161] The isolation and characterisation of the components, including descriptions of their aromas, accounting for the unique fragance of agarwood, have been reported and well reviewed.[161–164] Ishihara and co-workers first isolated and identified three sesquiterpenoids that contribute to the "gorgeous and elegant aroma" of Kanankoh (Figure 11): (–)-guaia-l(l0),l1-dien-15-al, (–)-selina-3,11-dien-9-one, and (+)-selina-3,11-dien-9-

o1.[162] The same group also isolated α-guaiene and α-bulnesene from the extract.[162] (–)-Guaia-l(l0),l1-dien-15-al has a "pleasant β-damascenone-like woody note with a touch of camphor", which is considered the most important fragrance of Kanankoh.



(–)-Guaia-1(10),11-dien-15-al (–)-Selina-3,11-dien-9-one (+)-Selina-3,11-dien-9-ol

Figure 11. Isolated aromatic sesquiterpenoids from Kanankoh.[162]

Later, Ishihara and co-workers identified more sesquiterpenoids from *A. ugallocha* Roxb. (Ryokuyu),[161] all the newly-isolated fractions being guaiene-structured sesquiterpenoids (Figure 12), including the first reported C15-functionalised examples, which remain rarely encountered in nature. These compounds were reported to have fresh and woody aromas.[163] Another notable component, (–)-rotundone, was later identified to account for the "strong spicy, peppercorn aroma" in black pepper and Australian red wine.[165] From a biosynthetic point of view, all these compounds are generated from α-guaiene and α-bulnesene through enzymatic oxidation pathways.[160]

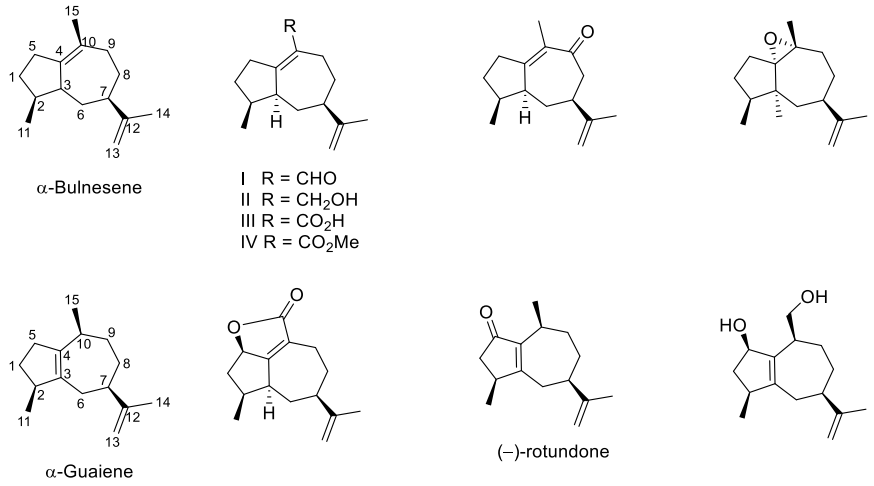


Figure 12. Isolated aromatic sesquiterpenoids from Ryokuyu.[161]

Yang and co-workers isolated and compared the chemical constituents of another high-quality agarwood, "Qi-Nan", amongst which more guaiene-structured sesquiterpenoids and further functionalised compounds were isolated and characterised; in addition to the same components previously found, some new more highly oxidised products were characterised (Figure 13).[164] Most of them possess a fresh, woody aroma of various strengths.

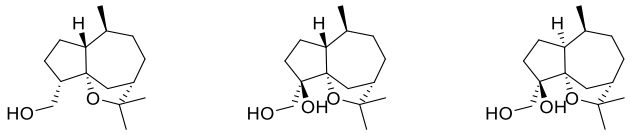


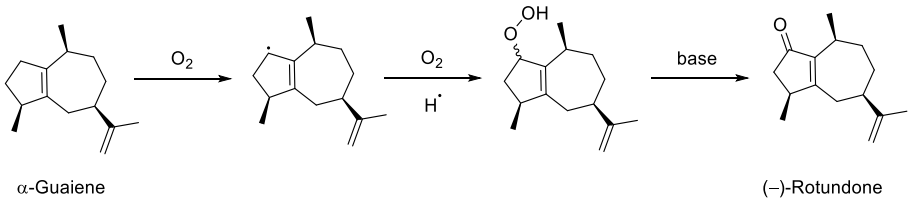
Figure 13. Isolated aromatic sesquiterpenoids from "Qi-Nan".[164]

Fragrant sesquiterpenoids from guaiene oxidation

To summarise, sesquiterpenoids, i.e. oxygenated sesquiterpene derivatives, have been of longstanding research interest due to their vast structural diversity, intriguing biological

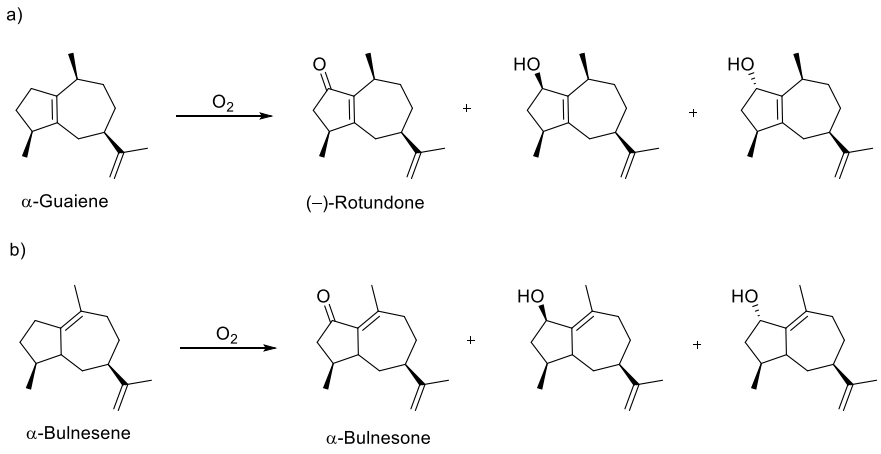
properties, and fragance profiles. The synthesis of these natural products usually proceeds by chemical oxidation of guaiene, which is readily-available from natural sources by acetate pyrolysis of guaiol.[166]

For example, one sesquiterpenoid with a distinct aroma, (–)-rotundone, is the only known impact odourant with a peppery aroma. The chemical synthesis of (–)-rotundone is of great importance due to its commercial value and low natural abundance. Huang and co-workers reported the preparation of (–)-rotundone by aerial oxidation of α-guaiene.[167] In this reaction (Scheme 44), O_2 was bubbled through a solution of pure α-guaiene in DCM; monitoring the reaction over a period of weeks revealed that (–)-rotundone was present at its maximum concentration (17.3%) at day 17 and remained constant afterwards, along with other oxidation products.



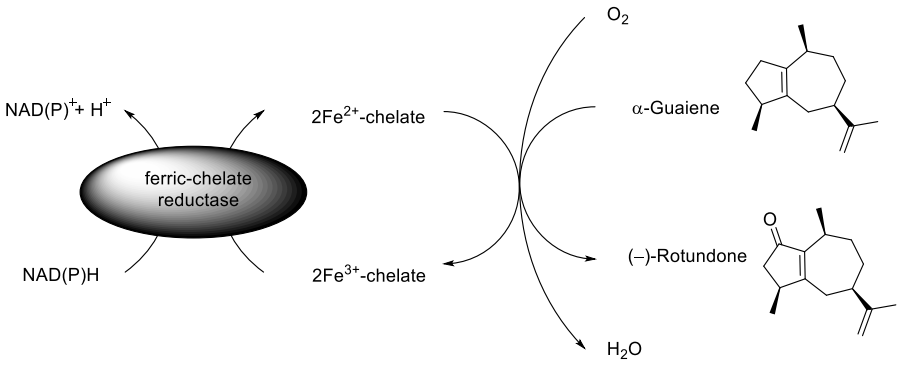
Scheme 44. Outline mechanism for the aerial oxidation of α-guaiene to (–)-rotundone.[167]

Later, Huang and co-workers became interested in the synthesis of (–)-rotundone and its derivatives from α-guaiene and α-bulnesene.[168] By coating starting materials on cellulose filter paper, aerial oxidation of α-guaiene was complete within 48 h, giving (–)-rotundone in 6.6% yield, accompanied by formation of a pair of epimers of rotundol (Scheme 45). The reaction with α-bulnesene resulted in the formation of α-bulnesone and a pair of epimers of bulnesol.



Scheme 45. Aerial oxidation of (a) α-guaiene and (b) α-bulnesene.[168]

Umezama and co-workers developed a preparative method for (–)-rotundone using a biomimetic catalyst (Scheme 46).[169] This study revealed that non-haem ferrous-chelate and ferric-chelate reductase oxidise α-guaiene to (–)-rotundone with $FeCl_3$ or Fe(III)-EDTA and YqjH (ferric-chelate reductase). The authors further improved the yield to 24% with the addition of cyclodextrin HP-β-CD to improve substrate solubility.



Scheme 46. Biomimetic catalytic synthesis of (–)-rotundone.[169]

P450$_{BM3}$-mediated oxidation of guaienes in the Wong group

As mentioned, P450$_{BM3}$ enzymes provide powerful catalysts for the oxidation and further functionalisation of natural products. The above reviewed methods for guaiene oxidation generally suffer from low solubility of substrate, low yield of product, and lack of selectivity. The Wong group is interested in the biosynthesis and quantitative production of fragrant sesquiterpenoids, and is working on the oxidation and selective functionalisation of sesquiterpenes with engineered P450$_{BM3}$ enzymes through mutagenesis.[170] With the initial aim being to produce the aroma compounds (–)-rotundone and (–)-guaia-l(l0),l1-dien-15-al enzymatically, the group is interested in assessing all oxidation products for their potential fragrance profiles.

The group's work started by screening α-guaiene against a general panel of 48 mutants, with most variants giving over 70% conversion, indicating that α-guaiene is an excellent substrate for P450$_{BM3}$ enzymes. Six metabolites were isolated and characterised, including the desired (–)-rotundone with 19% conversion by KSK19/A82M/I263G/A328G and rotundol with 55% conversion by R19/A82M/I263A (Figure 14). A second generation library was built through further mutagenesis from combining base mutants RP, K19, and RT2 with additional mutations at residues favouring C2 oxidation, which improved C2 selectively and increased the conversion for rotundol to 68% using RP/H171L/I263G/A82M.

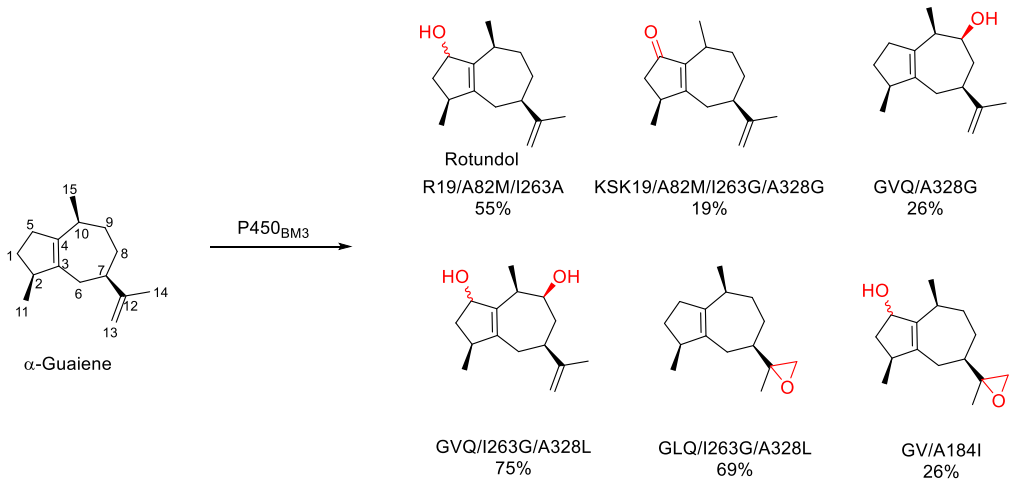


Figure 14. Metabolites from initial screening of α-guaiene against $P450_{BM3}$ mutants.[170]

The group's work with α-bulnesene showed more promising results. The work started by screening α-bulnesene against a general panel of 48 mutants, with most variants giving over 36% conversion, indicating α-bulnesene to be a suitable substrate for $P450_{BM3}$ enzymes. Seven metabolites were isolated and characterised, including the desired C15 oxidation products with highest conversion to the corresponding alcohol and aldehyde being 66% and 51%, respectively (Figure 15).

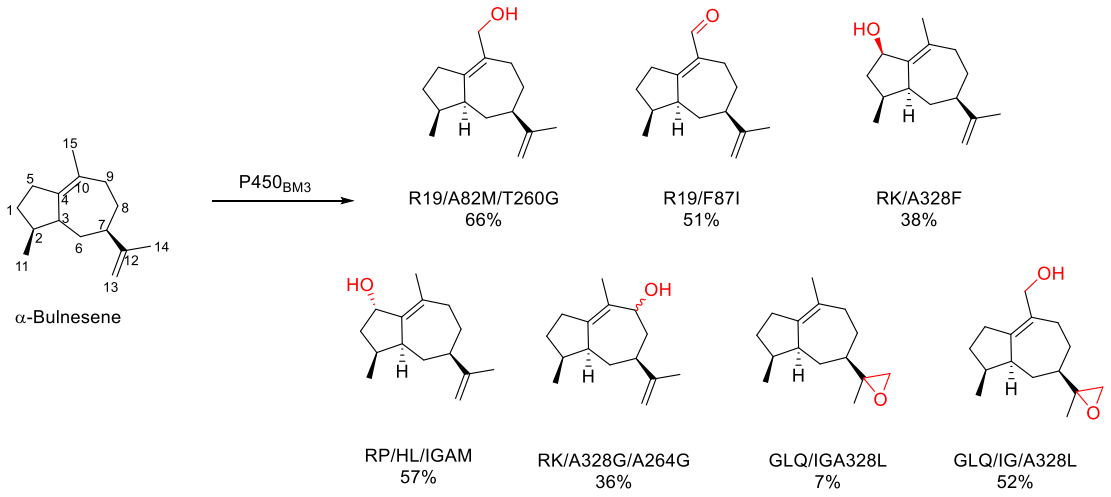


Figure 15. Metabolites from the initial screening of α-bulnesene against $P450_{BM3}$ mutants.[170]

With the desired C15 oxidation achieved, further site-directed mutagenesis was carried out based on the selectivity trend from the initial screening, where R19/F87I showed the highest C15 oxidation. A new library of mutants was generated by adding more mutants to R19/F87I, allowing oxidation selectively at the C15 position, and improving the conversion to the corresponding alcohol and aldehyde. More importantly, this library allowed the generation of the corresponding carboxylic acid, which could be employed as the precursor to produce (–)-guaia-1(10),11-diene-15,2-olide (Table 1).

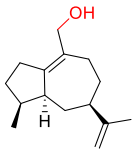
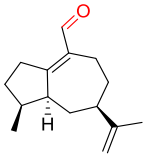
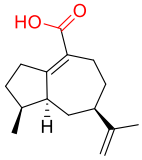
Variants	C15 conv. (%)	 %	 %	 %
R19/F87I/A82M	77	\	7	70
R19/F87I/A184I	75	\	3	72
R19/F87I/T260G	72	72	\	\
R19/F87I/A82M/A184I	82	33	19	30

Table 1. Key second generation variants and their metabolites.[170]

With these $P450_{BM3}$ mutants, the oxidations were only observed with chemically activated positions, namely epoxidation of alkene or allylic oxidations. This outcome might be due to the fact that the non-polar hydrocarbon skeletons offer little opportunity to be strongly oriented in the active site so that oxidation only occurs when weak C–H bonds approach the ferryl oxygen. Regardless, the enzymatic work conducted by the Wong group provided an efficient and environmentally benign method for the highly selective generation of these precious aroma compounds, along with further oxidation products with potential fragrance value. More importantly, this work developed a series of mutant libraries for the oxidation of guaiene structures, allowing the study and development of fragrant sesquiterpenes and their analogues.

During the ongoing collaboration between the Robertson and Wong groups, aiming to apply $P450_{BM3}$ to late-stage functionalisation in complex compound synthesis, it was discovered that $P450_{BM3}$ enzymes recognise enantiomers as different substrates which leads to different oxidation profiles with the same mutant. This phenomenon could have potentially important applications to kinetic resolution by C–H oxidation,[171] as well as the potential to access different products from each enantiomer. The Wong group's work with α-bulnesene derived from a natural source led to our interest in the enantiomer which, as a non-naturally occurring compound, required the development of a total synthesis. At the outset of this project, it was envisaged that a synthesis of *ent*-α-bulnesene and its $P450_{BM3}$ metabolites would stand as a valuable comparison with natural α-bulnesene.

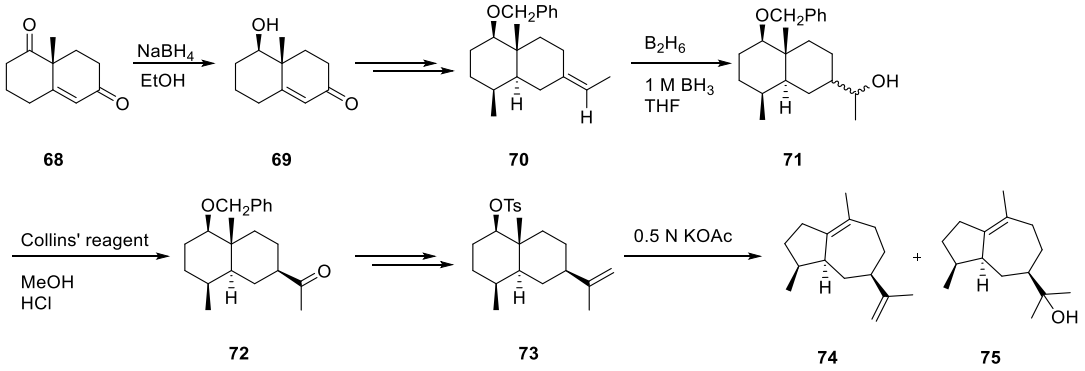
Synthetic approaches to guaiene structures

To date, no synthesis of the non-natural enantiomer of α-bulnesene, *ent*-α-bulnesene, has been reported. The syntheses of α-bulnesene and similar guaiene and sesquiterpenoid structures are thus briefly reviewed below.

Heathcock's group

Heathcock and co-workers reported the stereoselective synthesis of α-bulnesene **74**, along with its hydroxylated analogue bulnesol **75**[172] starting from unsaturated keto alcohol **69**, prepared from the Wieland–Miescher diketone **68** by the method of Boyce and Whitehurst (Scheme 47).[173] The installation of the terminal alkene was achieved with hydroboration of

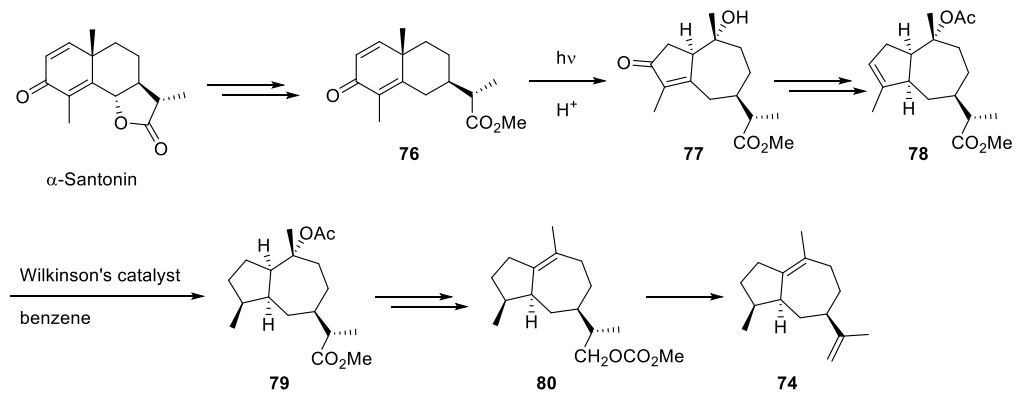
70 to give a mixture of diastereomeric alcohols **71**, which were oxidised by Collins' reagent to a mixture of methyl ketones, which, upon treatment with methanolic HCl, equilibrated, affording the pure stereoisomer **72**. Solvolysis of tosylate **73** in buffered acetic acid gave α-bulnesene **74** (16% overall yield from keto alcohol **69**) and bulnesol **75** (19% overall yield from keto alcohol **69**) in seventeen steps.



Scheme 47. Key steps in Heathcock's synthesis of α-bulnesene **74** and bulnesol **75**.[172]

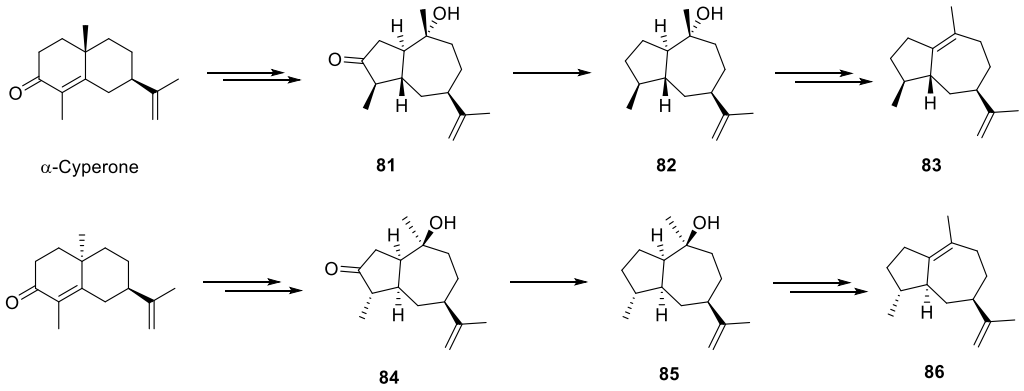
Piers' group

Piers and Cheng reported the stereoselective synthesis of α-bulnesene **74** through photochemical rearrangement (Scheme 48).[174] From keto ester **76**, which was converted from α-santonin within two steps,[175] with known absolute stereochemistry, photolysis resulted in the ring rearrangement product **77**. *Exo*-face selective hydrogenation of cyclopentene derivative **78** in the presence of Wilkinson's catalyst in benzene afforded the product **79** that, following further manipulations, was converted into α-bulnesene **74** by pyrolysis of carbonate derivative **80**.



Scheme 48. Key steps in Piers' synthesis of α-bulnesene **74**.[174]

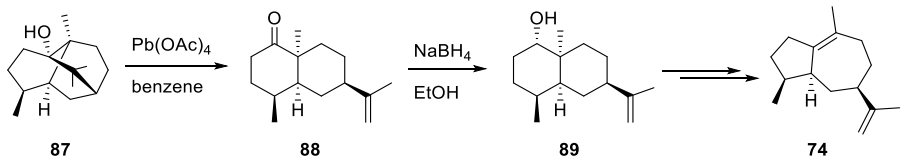
The group later reported the synthesis of 5-*epi*-α-bulnesene **83** and 4-*epi*-α-bulnesene **86** starting from naturally occurring α-cyperone and its epimer using the same strategy (Scheme 49).[176] Huang-Minlon reduction of **81** and **84** afforded the two epimers **82** and **85**, respectively.



Scheme 49. Key steps in Piers' synthesis of 5-*epi*-α-bulnesene **83** and 4-*epi*-α-bulnesene **86**.[176]

Mehta's group

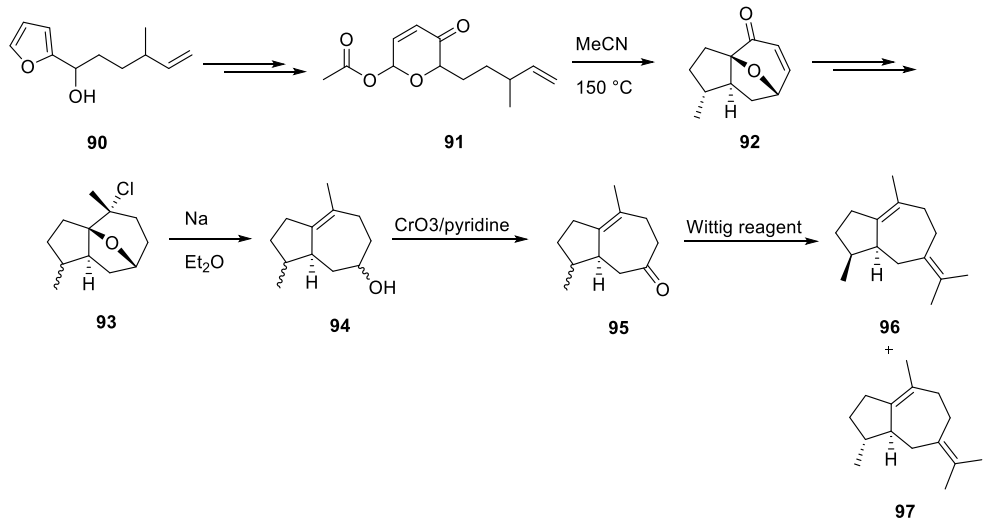
Mehta's group reported a short semi-synthetic route to α-bulnesene **74** from the naturally occurring terpenoid patchoulol **87** (Scheme 50).[177] The key oxidative cleavage step was achieved by heating **87** with $Pb(OAc)_4$ in benzene, leading to bicyclic ketone **88** which, upon stereoselective reduction from the less hindered face, generated alcohol **89**. Solvolytic rearrangement of this compound afforded the desired α-bulnesene **74** in four steps.



Scheme 50. Key steps in Mehta's synthesis of α-bulnesene **74**.[177]

Sammes' group

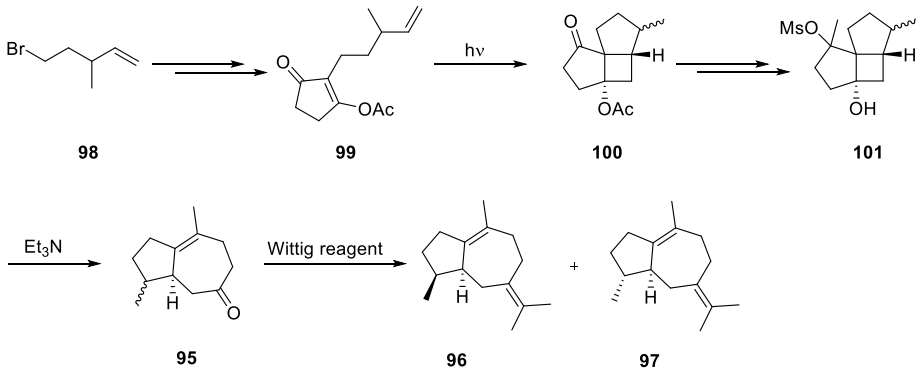
Sammes and Street reported a synthesis of β-bulnesene **96** featuring a reductive cleavage of the epoxy-bridge in an oxidopyrylium cycloadduct (Scheme 51).[178] The starting material, substituted furfuryl alcohol **90**, was prepared as a mixture of diastereomers from 3-methylpent-4-enyl bromide and 2-furaldehyde. Heating the acetate **91** gave the cycloadduct **92** as a 5:1 mixture of the two epimers. Treatment of chloride **93** with Na in ether cleaved the epoxy bridge and produced an alcohol **94** that was treated with CrO_3/pyridine to give ketone **95**. Wittig reaction completed the route, the product being obtained as a 17:83 mixture of β-bulnesene **96** and its 4-epimer **97**, respectively.



Scheme 51. Key steps in Sammes' synthesis of β-bulnesene **96** and its 4-epimer **97**.[178]

Wylie's group

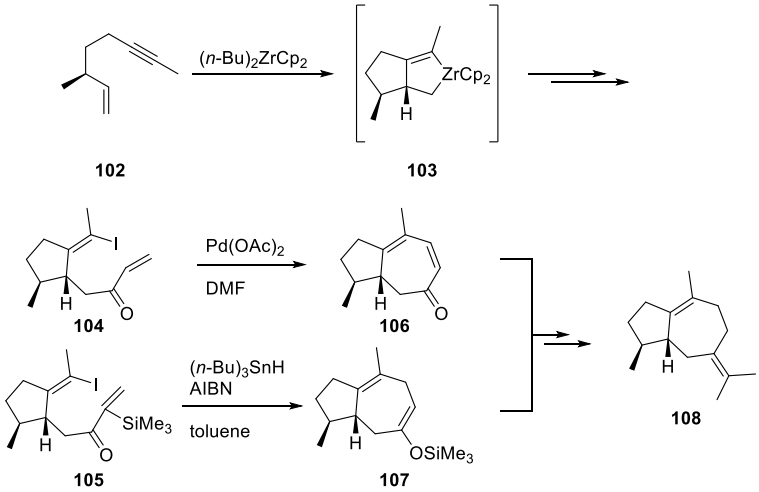
Wylie and co-workers also reported a synthesis of β-bulnesene **96** and its epimer **97**, from bromide **98** (Scheme 52).[179] The key step was an intramolecular photocycloaddition in enol acetate **99** which led to the photoadduct **100** as a mixture of diastereomers in 3.3:1 ratio. The 5,7-fused ring system **95** was achieved from fragmentation of mesylate **101**. The final products **96** and **97** were achieved overall in ten steps with a 3.3:1 ratio, respectively.



Scheme 52. Key steps in Wylie's synthesis of β-bulnesene **96** and its 4-epimer **97**.[179]

Negishi's group

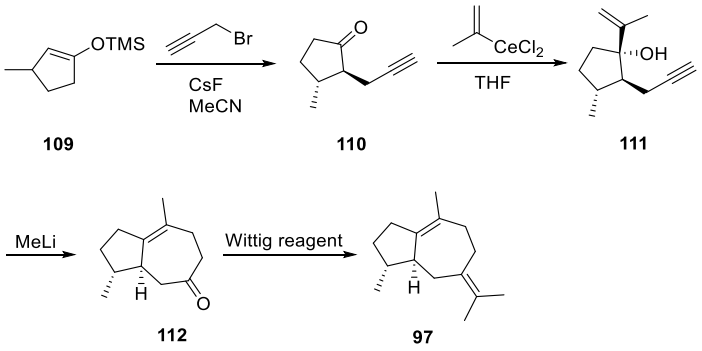
Negishi and co-workers reported a synthesis of 5-*epi*-β-bulnesene **108** *via* Zr-promoted bicyclisation of enyne **102** (Scheme 53).[180] The seven-membered ring was formed through either Heck reaction or radical cyclisation leading to 5-*epi*-β-bulnesene **108** in six steps with 16%–24% overall yield.



Scheme 53. Key steps in Negishi's synthesis of 5-*epi*-β-bulnesene **108**.[180]

Ovaska's group

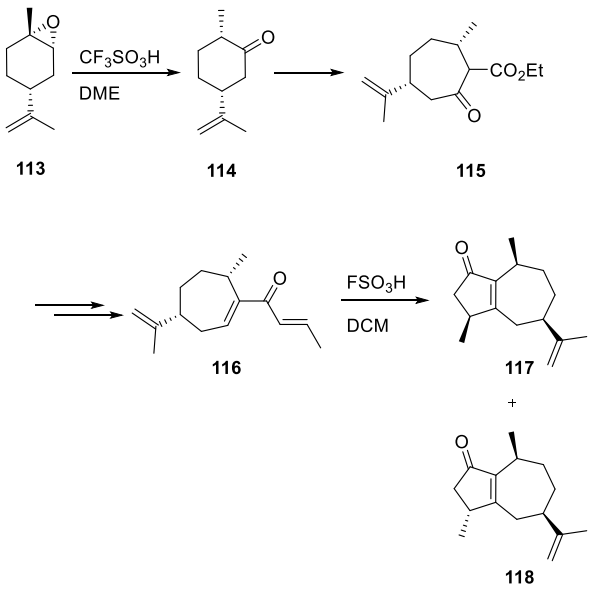
Ovaska and co-workers' stereoselective route to 4-*epi*-β-bulnesene **97** was achieved in five steps from cyclopentenone (Scheme 54).[181] Ketone **110** was obtained with 94% diastereoselectivity in favour of the *trans*-isomer by propargylation of silyl enol ether **109**. Oxycyclisation coupled with Claisen rearrangement of tertiary alcohol **111** afforded bicyclic ketone **112,** which upon Wittig reaction afforded 4-*epi*-β-bulnesene **97**.



Scheme 54. Key steps in Ovaska's synthesis of 4-*epi*-β-bulnesene **97**.[181]

Schröder's group

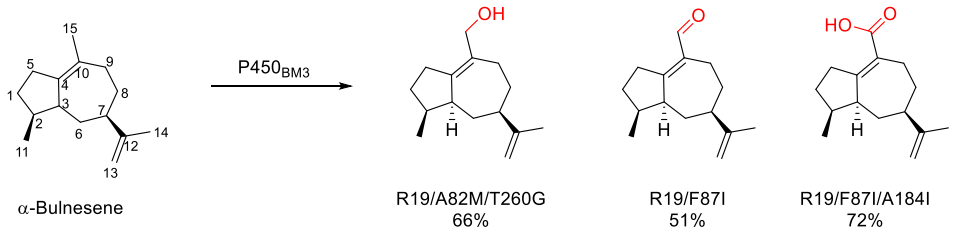
Most recently, Schröder and Rüthi reported the first synthesis of (–)-rotundone, featuring a Nazarov cyclisation (Scheme 55).[182] Treatment of commercially available (+)-(*R*)-limonene oxide **113** with CF_3SO_3H furnished *cis*-dihydrocarvone **114** as a pair of diastereomers (*dr* = 84:16). The Nazarov precursor **116** was formed *via* a Weinreb amide and treatment with 1-propenylmagnesium chloride; this sequence generated the precursor as the *cis*-configured seven membered-ring with an *E*/*Z* ratio of 87:13 in the propenyl side-chain. The final step, the Nazarov cyclisation, achieved with catalytic amounts of fluorosulfuric acid, afforded a mixture of (–)-rotundone **117** (27%) and its epimer **118** (14%).



Scheme 55. Key steps in a synthesis of (–)-rotundone **117** and *epi*-rotundone **118**.[182]

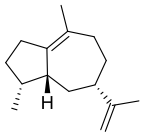
Summary and project outline

The Wong group continues to study the hydroxylation profile of naturally-occurring sesquiterpenes for the purpose of exploring biocatalytic and synthetic biologic pathways to access fragrance compounds. For this purpose, the group has developed a library for the C15 regioselective oxidation of the natural product α-bulnesene (Scheme 56).



Scheme 56. Selected oxidation products from α-bulnesene with $P450_{BM3}$ mutants.

Within the Robertson–Wong collaboration, it was discovered that $P450_{BM3}$ enzymes recognise enantiomers as different substrates, offering a potential means to achieve oxidative kinetic resolution of natural products and their enantiomers. In this sub-project, the aim was to gain additional insight into the reactivity and selectivity profiles of $P450_{BM3}$ mutants when confronted with separate enantiomers; therefore, this work set out to accomplish the following goals: (1) establish the first synthetic route to the non-natural enantiomer of α-bulnesene, *ent*-α-bulnesene (Figure 16), and study its fragrance profile; (2) apply $P450_{BM3}$-mediated oxidation as a late-stage functionalisation step to determine whether the developed enzymatic library can discriminate between the enantiomers to provide different hydroxylation products; (3) discover potentially new fragrance molecules for commercial use.



ent-α-Bulnesene

Figure 16. Structure of *ent*-α-bulnensene.

Chapter 2: Synthesis of the azepane core structure of balanol

Aims

As described in the Introduction, the aim of this project was to adopt $P450_{BM3}$-mediated hydroxylation as a functionalisation step in natural product synthesis. More specifically, the plan was to develop a synthetic route to the seven-membered ring core **119** of natural product balanol **120** (Figure 17) starting with the regio- and stereo-selective hydroxylation of protected azepane using $P450_{BM3}$ mutants.

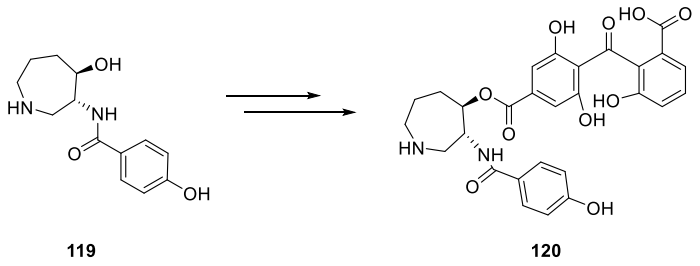
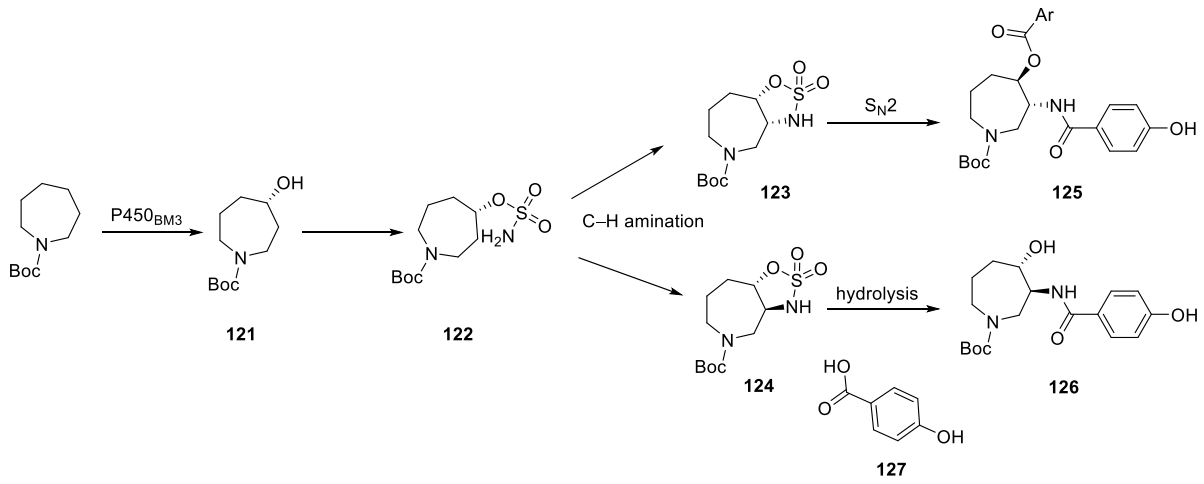


Figure 17. Structure of balanol **120** and its azepane core **119**.

Traditional synthetic strategies usually focus on assembling the functionality in, and then forming, the core seven-membered ring as described in the previous chapter, which leads to lengthy synthetic routes. It was envisaged that a plausible route would start with the regio- and stereo-selective hydroxylation of azepane, and then use the hydroxyl group as a tether for intramolecular C–H amination as outlined in Scheme 57. The hydroxyl group in **121** would come from $P450_{BM3}$-mediated oxidation, while the β-positioned amine could come from an intramolecular C–H amination from sulfamate **122**, giving either the *cis*- or *trans*-product, **123** or **124**. S_N2 reaction of the *cis*-isomer **123** followed by coupling with carboxylic acid **127**

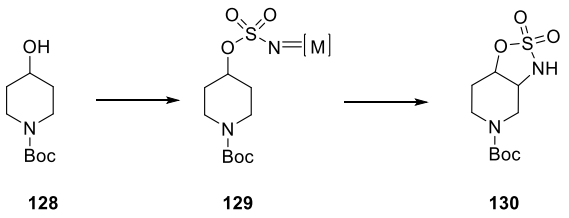
would generate the azepance core product **125**, while hydrolysis of the *trans*-isomer **124** would give the enantiomer **126**.



Scheme 57. Proposed synthetic route towards the azepane core of balanol **120**.

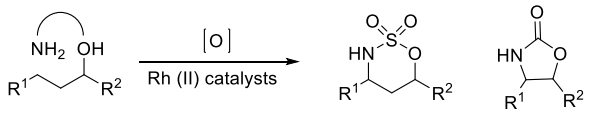
Investigation of the feasibility of Rh(II)-catalysed C–H amination

Alongside work carried out in the Wong group, where their group members had conducted preliminary screening of mutants for the selective hydroxylation of *N*-Boc-azepane, testing of the feasibility of intramolecular C–H amination *via* nitrenoid insertion was undertaken with Boc-protected 4-hydroxypiperidine **128**. This model substrate was chosen due to this model's resemblance to hydroxyazepane **121**, its commercial availability and its symmetrical structure (Scheme 58).



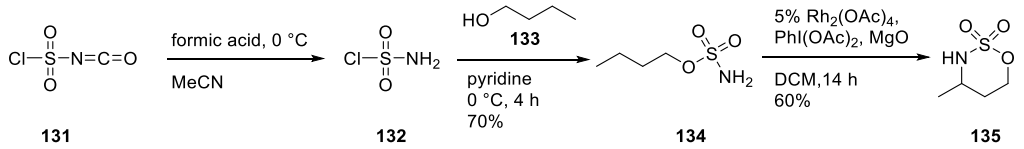
Scheme 58. Envisaged C–H amination with the 4-hydroxypiperidine model.

The Du Bois laboratory has developed amination protocols for both intermolecular and intramolecular C–H activation reactions using Rh(II) catalysts; the reaction is a concerted asynchronous two-electron insertion through a Rh-nitrenoid intermediate.[183–185] Inspired by this protocol for tethered intramolecular C–H amination with sulfamate and carbamate substrates (Scheme 59),[186] C–H activation at the β-position of 4-hydroxypiperidine **128** was attempted.



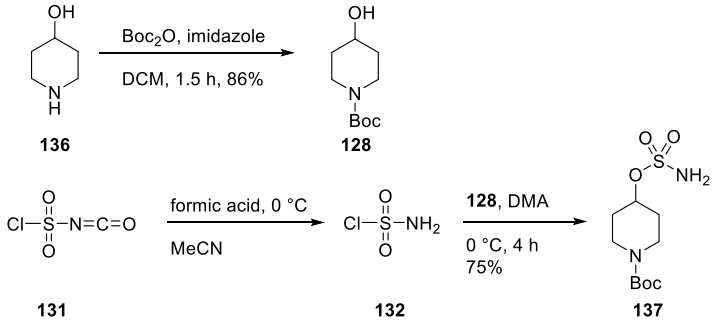
Scheme 59. Du Bois' protocol for C–H amination with Rh(II) cataysts.[186]

It was decided that a reported structure should be first examined to test out the reaction's viability. Sulfamate **134** was prepared from *n*-butanol **133** and was subjected to the standard conditions reported with $Rh_2(OAc)_4$ as catalyst;[186] comparing the ^{1}H NMR spectrum with the literature data, β-amination product **135** was observed as the major product (Scheme 60). Column chromatography gave a satisfactory yield of the desired product without notable mass loss.



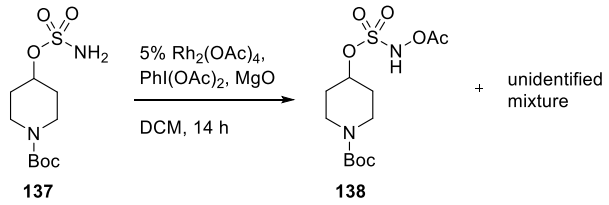
Scheme 60. Test reaction of Rh(II) catalysed C–H amination with *n*-butanol **133**.

Next, the trial began with the preparation of the corresponding sulfamate **137** with optimised conditions.[187] After Boc-protection of 4-hydroxypiperidine **136**, dropwise addition of formic acid into chlorosulfonyl isocyanate **131** at 0 ℃ generated sulfamoyl chloride **132**. Addition of a solution of alcohol **128** led to the formation of the desired sulfamate **137** in 75% isolated yield. Further investigations suggested that an inert atmosphere and vigorous stirring were crucial for efficient sulfamoyl chloride formation, and the progress could be monitored by strong gas evolution (Scheme 61).



Scheme 61. Formation of sulfamate **137** from 4-hydroxypiperidine **136**.

The intramolecular C–H amination with Rh(II) catalyst was then examined. Following the conditions reported by Du Bois,[186] sulfamate **137** was treated with 5% $Rh_2(OAc)_4$ (Scheme 62). From the NMR spectrum of the crude product, mostly starting material was observed; tiny newly formed doublet peaks around 6.7 ppm might be the NH doublets for the two rotamers; however, column chromatography afforded insufficient material to characterise. The only isolated compound possessed a new singlet resonance at 2.09 ppm compared to the starting material, which suggested that the oxidation product **138** was formed.



Scheme 62. Initial attempt to effect C–H amination with sulfamate **137** using Rh(II) catalysis.

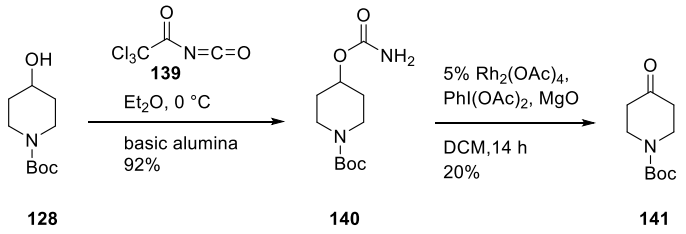
When scaling up the reaction to 0.3 mmol, along with mostly recovered starting material, some desulfamoylated alcohol **128** and a small amount of compound **138** were observed, along with an inseparable complex mixture.

These results suggested that the C–H bond was not activated in most cases. Another observation was that most of the mass from the crude was lost during column chromatography, making characterisation of the reaction difficult. It might be some form of coordination between the Rh(II) catalyst and the compounds resulted in the majority of the mass sticking to the column. TLC observations suggested that this complexation only occurred upon removal of the solvent. Washing with neutral EDTA solution proved profitable, with a stable recovery of 30% of material, which was considered reasonable compared to former recoveries.

In case the loss of material was caused by complex formation between our compounds and the catalyst, decreasing the catalyst loading and changing to bulkier or more strongly ligating rhodium catalysts could help to reduce complex formation. Moreover, the reluctance of sulfamate **137** to perform C–H activation, and the unpredictable results from these reactions, might be due to an equatorial -$OXNH_2$ group, in which the axial position is favoured for C–H insertion. Under these assumptions, extensive screening reactions were performed to try to

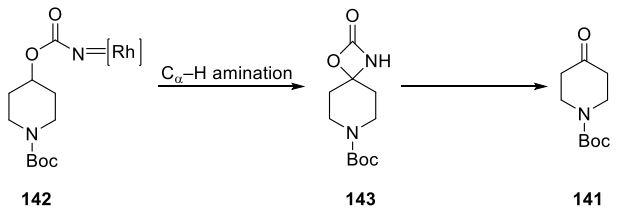
identify suitable conditions for promoting C–H activation;[188–190] however, these trials led predominantly to recovery of starting material (Appendix 1, Tables 1 and 2).

Faced with this generally unclear pattern of results from attempted C–H amination from sulfamate **137**, a further literature review suggested that, for cyclic substrates, carbamates are superior to sulfamates although the underlying reasons for this trend remain unexplained.[184, 191–193] Therefore, carbamate **140** was prepared from Boc-protected piperidine **128** and trichloroacetyl isocyanate **139** (Scheme 63).[193]



Scheme 63. Formation of carbamate **140** and attempted Rh(II)-catalysed C–H insertion.

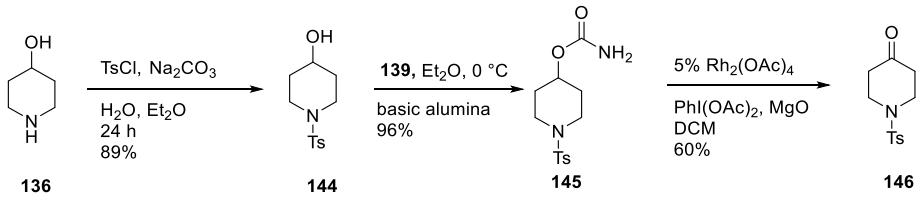
Subjecting carbamate **140** to the standard conditions with 5% $Rh_2(OAc)_4$ led to the formation of ketone **141** in 20% isolated yield (Scheme 63), which was postulated to result from the C–H insertion into the α-position to give strained spirocycle **143**, then cycloreversion to generate ketone **141** (Scheme 64).



Scheme 64. A plausible pathway for the formation of ketone **141**.

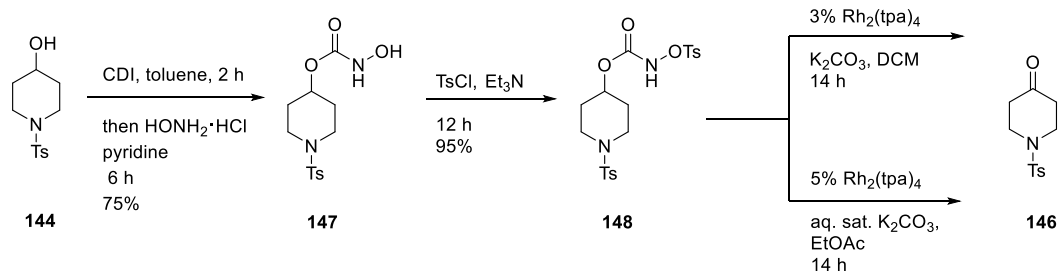
Literature discussions of similar processes generally favour this mechanism and show that ketone formation was often observed in preference to β-insertion.[194–198] An initial investigation with carbamate **140** gave generally cleaner reaction outcomes and proved the feasibility of generating a viable Rh(II)-nitrenoid intermediate although this apparently promoted the activation of only the α-C–H bond.

The Boc protecting group displays rotameric NMR spectra and may coordinate to the metal centre during the reaction and product isolation may underlie the uncertainty of the reaction outcome with substrates **137** and **140**. Accordingly, Ts-protected carbamate **145** was prepared and subjected to the same conditions (Scheme 65); however, no improvement was observed and ketone **146** was returned as the major product.



Scheme 65. Attempted C–H amination with Ts-protected carbamate **145**.

Faced with this similar result, extensive screening of carbamate **145** with a variety of reported conditions was conducted in the hope of activating the β-C–H site (Appendix 1, Table 3),[183, 184, 194, 198] but no improvement was observed. Preoxidised *N*-tosyloxycarbamate **148** was prepared and subjected to the Rh(II) catalysed conditions reported by Lebel;[199–201] however this also led only to the formation of ketone **146** (Scheme 66).[200] Other metal catalysts were also tested with carbamate **145** by applying conditions reported for cyclic structures (Appendix 1, Table 4);[191, 197, 202] again, no promising results were obtained.



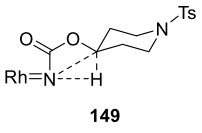
Scheme 66. Synthesis of tosyloxycarbamate **148** and attempted C–H amination.

Analysis of the above results and a further literature review revealed the main reason behind the sole formation of α-insertion products **141** and **146**:

1. Carbamate esters derived from 2 ° alcohols generally tend to afford the corresponding ketone rather than the oxazolidinone.[196, 203, 204, 205]

2. The C–H bond is more reactive towards nitrenoid insertion in an axial position.[195, 196, 205, 206] In our case the chair-like carbamate system is predominantly equatorial, which resorts to dominant ketone formation (Figure 18).

3. Insertion into the accessible equatorial hydrogen bond at the β-position would lead to a potentially strained *trans*-fused bicyclic, and conformational bias is crucial to reactivity prediction in these cyclic systems.[201]

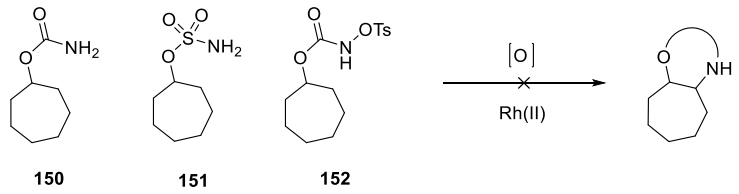


149

Figure 18. Carbamate **149** prefers α-insertion with the α-H being axial.

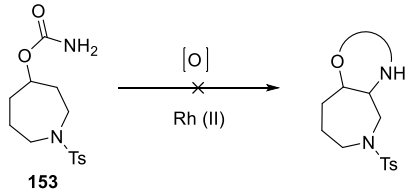
The results of metal catalysed C–H amination with hydroxypiperidine derived carbamate **145** showed the feasibility of C–H activation with Rh(II) catalysts, yet it was suspected that this structure might not be the most suitable model for the more flexible azepane system as it appears to be conformationally biased and prefers α-insertion.

Therefore, the more flexible cycloheptanol analogues were envisaged as a better model for the Du Bois chemistry. The corresponding sulfamate **150**, carbamate **151** and *N*-tosyloxycarbamate **152** derivatives were prepared and subjected to the Rh(II) catalytic conditions (Scheme 67). However, this system did not give any better results compared to the 4-hydroxypiperidine system and further optimisation with these analogues resulted in no improvement.



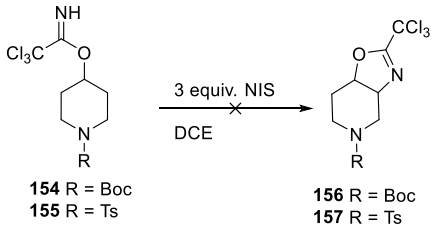
Scheme 67. Failed attempts to achieve Rh(II)-catalysed β-amination in cycloheptanol substrates.

A final attempt of the Rh(II) catalysis with the racemic azepane analogue **153** (preparation see later discussion) was also attempted but led to no improved result (Scheme 68).



Scheme 68. Attempts to achieve Rh(II)-catalysed β-amination with azepane carbamate **153**.

As a consequence of the undesired regioselectivity encountered with carbamate-derived Rh(II)-nitrenoids, alternative protocols were investigated for this C–H activation step. Analogues **154** and **155** were prepared (Scheme 69), ready for a reported radical-mediated C–H amination reaction. Following the protocol developed by He,[191] only decomposition of starting material was observed.

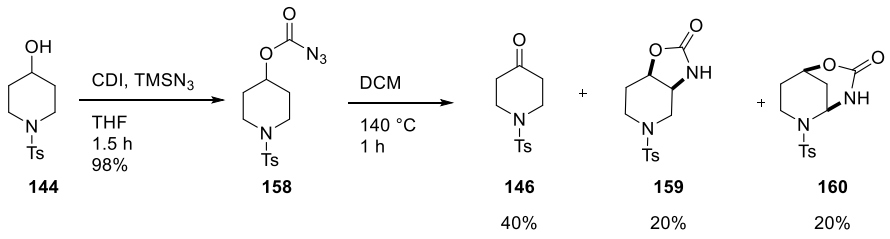


Scheme 69. Attempted radical-mediated C–H activation.

C–H amination with thermolysis

Faced with the unsuccessful β-insertion reactions discussed above, it was envisaged that a more reactive free nitrene or equivalent should give C–H amination products but it was appreciated that the gain in reactivity would be offset by a lack of selectivity. Accordingly, azidoformate **158** was prepared from alcohol **144**[207] and subjected to thermolysis by heating in DCM at 140 ℃ in a pressure tube (Scheme 70).[208] Formation of the desired amination product **159** along with ketone **146** and the bridged product **160** was observed with a ratio of 1:2:1, respectively. Analysis of the ^{1}H NMR spectra showed three pairs of CH_2 protons and two CH protons from HSQC for each of the newly formed products, indicating the success of non-α-insertion. The differentiation between the β-insertion product **159** and the γ-insertion product **160** was achieved by observing a higher resonance for the N–CH proton from the

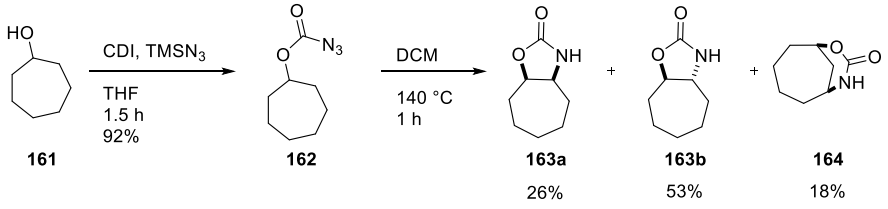
bridged-ring product **160** (5.42 ppm) compared to the fused-ring product **159** (3.93 ppm). Furthermore, coupling between the O–CH and N–CH protons could be seen in the ^{1}H-^{1}H COSY spectrum for the fused-ring product **159** but not for product **160**. The β-insertion product **159** was formed as a single diastereoisomer. Carbamate **159** was suspected to be formed in the *cis*-configuration, with a vicinal coupling constant of 7.0 Hz between the O–CH and N–CH protons; the bridged-ring product **160** can only be formed with the *cis*-configuration. This result, finally leading to activation of both the β-position and γ-position, validated the premise of applying the enzymatically-installed hydroxyl group as a tether for further functionalisation.



Scheme 70. Preparation of azide **158** and its thermolysis.

Thermolysis with cycloheptyl azidoformate **162** also showed encouraging results, activating mainly the desired β-position with a minor amount of γ-insertion (Scheme 71). Ogura and co-workers reported[209] the NMR data for the *cis*-compound in poor quality (60 MHz); reported resonances at 4.5 – 5.0 ppm for the O–CH proton and 3.7 – 4.3 ppm for the N–CH proton are consistent with the ^{1}H NMR spectrum of **163a** which showed resonances at 4.74 ppm (ddd) and 3.93 ppm (ddd), respectively. The *trans*-compound **163b** has a shifted resonance at 4.30 ppm for the O–CH proton and at 3.69 ppm for the N–CH proton, with a vicinal coupling

constant of 10.5 Hz. The bridged-ring product **164** has a slightly higher resonance at 4.77 ppm for the O–CH proton and at 3.84 ppm for the N–CH proton compared to the β-insertion products, and has a pair of CH_2 resonance at 2.28 ppm and 2.05 ppm corresponding to the central CH_2 protons. With this cycloheptyl system, none of the α-insertion products (such as the ketone) were observed. Moreover, compared to the 4-hydroxypiperidine model, the diastereoisomeric β-insertion products *trans*-**163b** and *cis*-**163a** were formed preferentially over carbamate **164** with a 3:1.5:1 ratio, respectively.



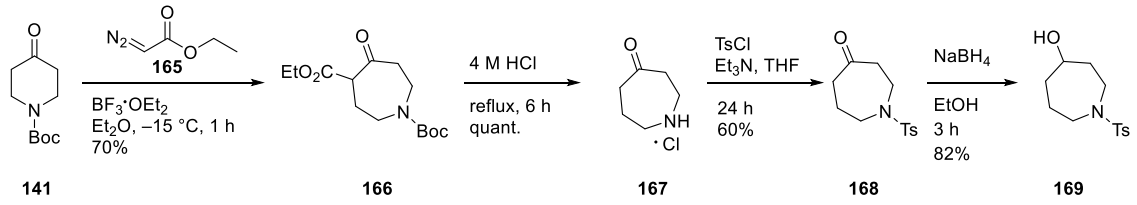
Scheme 71. Preparation of azide **162** and its thermolysis.

With this encouraging result, it was decided that the C–H activation should be tested on the azepane system relevant to balanol.

C–H amination with hydroxylazepane *via* thermolysis

Due to the relative scarcity of the enzymatically produced azepan-4-ol, it was decided that the racemic azepan-4-ol **169** should first be produced on large scale to test the azidoformate chemistry. Azepanone **167** was prepared from commercially available 4-piperidinone **141** *via* a ring-expansion reaction with ethyl diazoacetate **165** followed by hydrolysis and

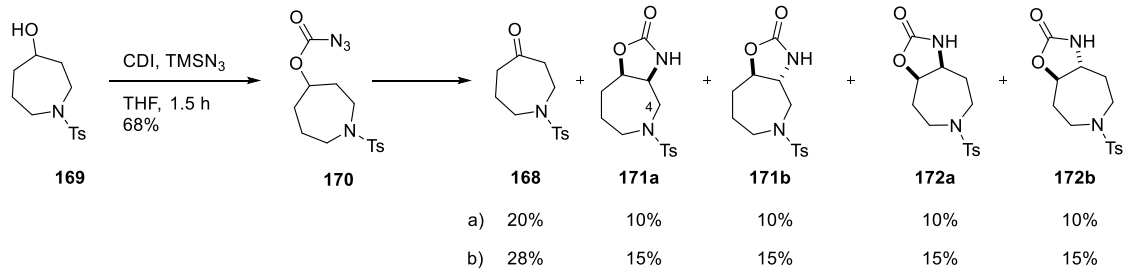
decarboxylation of ketoester **166**.[210] Protection of the resultant amine as its tosyl derivative **168**, then reduction gave the racemic alcohol **169** in 82% isolated yield (Scheme 72).



Scheme 72. Synthesis of racemic azepan-4-ol **169**.

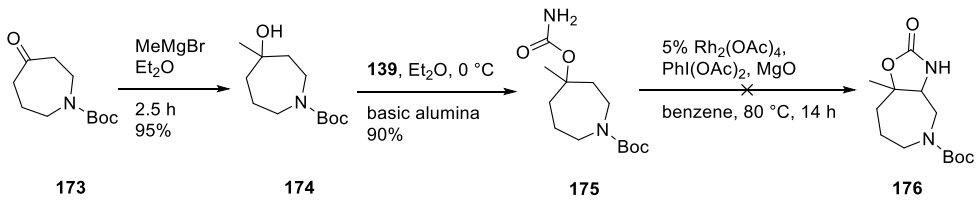
With the racemic azepan-4-ol **169** in hand, azidoformate **170** was prepared and subjected to the conditions previously established. After 1 h reaction time, the reaction was incomplete but the formation of the desired amination products **171a** and **171b** was observed, along with ketone **168** and the δ-insertion products **172a** and **172b** (Scheme 73a). Heating the substrate **170** in TCE for 2 h achieved complete reaction but avoided the dangers associated with heating DCM in pressure tubes (Scheme 73b). The β- and δ-activation gave a pair of diastereomers each, with a 1:1:1:1 ratio, respectively. The β-insertion products and the δ-insertion products were differentiated by the ^{1}H NMR of one of the H-4 resonances which, for the β-insertion products showed as a dd (3.12 ppm for *cis*-compound **171a**) while one of the H-4 resonances in the δ-insertion products showed as a ddd (3.17 ppm for *trans*-compound **172b**). Between the pair of diastereomers **171a** and **171b**, the *cis*-isomer **171a** showed J = 8.5 Hz for O–CH/N–CH, while the *trans*-isomer **171b** showed J = 11.0 Hz. Diastereomers **172a** and **172b** were differentiated by the same approach with *cis*-isomer **172a** showing J = 7.5 Hz and *trans*-isomer **172b** showing J = 10.5 Hz. No protons were observed to couple with both

the O–CH proton and the N–CH proton in these compounds, thus ruling out the possibility of bridged-ring products from these reactions.



Scheme 73. Formation of azide **170** and its thermolysis: a) DCM, 140 ℃, 1 h; b) TCE, 142 ℃, 2 h. Yield obtained from NMR analysis.

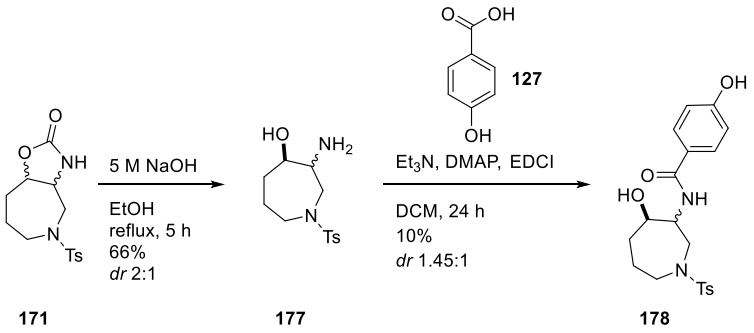
The successful formation of β-amination products under thermolysis conditions indicated that, as predicted, the energy barrier for activation of the β-position requires more forceful conditions compared to the α-position. To further support this suggestion, 4-methyl-carbamate **175** was prepared from azepan-4-one **173** in which the α-position is rendered inaccessible (Scheme 74). Subjecting carbamate **175** to optimised Du Bois conditions led only to the return of starting material, which provided further evidence for the hypothesis that the β-position is inert towards this method.



Scheme 74. Attempted β-amination with carbamate **175**.

With access to the correct regioisomers **171a** and **171b**, the final coupling step was examined.

Alkaline hydrolysis of the mixture **171** gave the expected aminoazepanol **177** as a mixture of diastereomers in 66% isolated yield, from which a single run of an EDCI/DMAP coupling produced amide **178**, as a mixture of diastereomers, in 10% total yield (Scheme 75).[211]



Scheme 75. Synthetic route to the azepane core type structure **178**.

At this point, the study had demonstrated the applicability only of azidoformate thermolysis, rather than Rh(II)-catalysis, to achieve C–H amination in this 2 °-alcohol system. Nevertheless, this sequence had validated the concept and the potential of employing the hydroxyl group as a tether for installing further functional groups within the ring system relevant to the natural product balanol. The study of C–H activation for amine formation revealed that Rh(II)-catalysed insertions into C–H bonds are less general than the literature suggests because the reported substrates are biased to avoid α-insertion by 1. installing bond-weakening groups such as benzylic or allylic functionality within the substrate scope; 2. focusing on the activation of tertiary C–H bonds; 3. deliberately blocking the α-position with cyclic secondary C–H bonds activation reactions.[183, 187, 192, 199, 212, 213]

Further investigation of this synthetic route was set aside until suitable $P450_{BM3}$ mutants were identified and optimised for the regio- and stereo-selective generation of (*S*)-azepan-4-ol **126**.

$P450_{BM3}$ hydroxylation studies of Ms-protected azepane

Investigations in the Wong group at the time suggested that the enzyme panels only tolerate and convert Boc-protected amines well but our synthetic work revealed that this Boc protecting group interferes with the chemical synthesis by partial deprotection during the C–H amination reaction and also complicates NMR analysis by the presence of interconverting rotamers. Thus, the possibility of oxidising differently-protected azepane substrate range for $P450_{BM3}$ oxidation was investigated.

The trial started with screening Ms-protected azepane **179**, which had not been previously studied against the 48-mutant panel developed for favouring γ-selective hydroxylation (full list in Experimental Section). The aim was to identify potential possibilities and differences in responses of the enzymes towards different protecting groups. Numerous mutants gave three metabolites in total without degrading the enzymes (see Experimental Section for full GC data), with mutant RP/HL/IG resulting in all three metabolites along with reasonable conversion (Figure 19). To identify and characterise the metabolites, a scale-up reaction with mutant RP/HL/IG was carried out on a 0.2 mmol scale following the same conditions. In contrast to the screening result, only two metabolites were produced after 24 h; these were found to be the desired γ-hydroxylation product **180** (4% isolated yield) and alcohol **181** (16% isolated yield), the latter arising by α-hydroxylation (**182**) then reduction (Scheme 76) of the

resulting aldehyde **183**. Based on this, the third metabolite, not observed upon scale-up, is most likely the α-hydroxylated azepane **182**. The reason for the difference in result between screening and scale-up reactions is unclear but may be due to the difference in screen-scale and large-scale expressions of the enzyme.

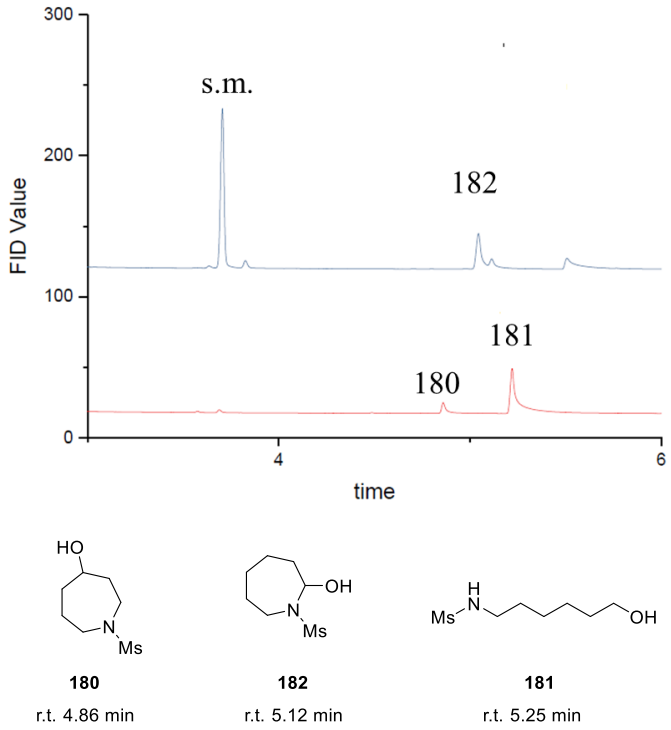
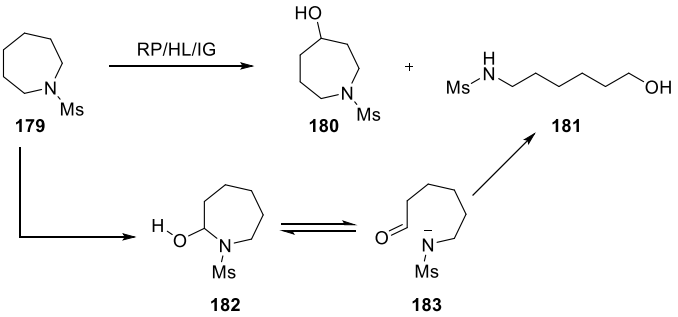


Figure 19. GC traces of scale-up reaction with mutant RP/HL/IG.



Scheme 76. Preparative scale hydroxylation of azepane **179** with mutant RP/HL/IG and alcohol **181** formation through α-oxidation then reduction of the ring-opened product **183**.

This preliminary trial demonstrated the enzymes' tolerance of Ms-protected cyclic amines as substrates, suggesting the feasibility of biocatalytic hydroxylations of azepanes and other cyclic amines with protecting groups other than Boc-protecting group to provide more stable hydroxyazepanes for further synthetic purposes. In addition, this result set the stage for the investigations of tuning the regio- and stereo-chemistry of cyclic amine hydroxylation by changing the *N*-protecting group, a concept that has become known within the group as substrate engineering.

Concusions and future studies

The studies described in this chapter provided a short demonstrative synthetic route to illustrate the feasibility of further functionalisation of hydroxylated cyclic amines by employing the newly-installed hydroxyl group as a tether. The initially planned tethered C–H amination step with Rh(II) catalysis was proved to be more problematic than expected because reported precedents were generally biased towards successful outcomes. The less selective thermolysis of azidoformates achieved C–H amination at the unactivated β-position to the ring nitrogen, this position being relatively inert in comparison with the α-position. The successful but unoptimised preparation of amide **178** by direct EDCI coupling between compound **177** and 4-hydroxybenzoic acid **127** was also demonstrated. Overall, this study provided a potentially short route to the azepane core type structure of natural product balanol, in either enantiomeric series from enzymatically produced (*S*)- or (*R*)-azepan-4-ol. Ongoing

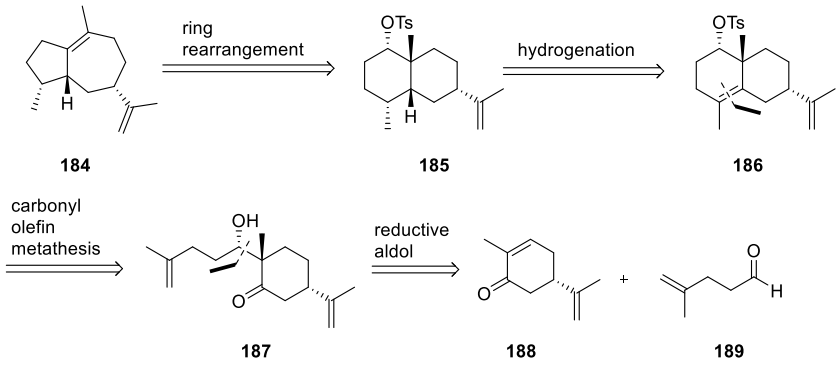
studies in the group are focusing C–H activation systems, such as that reported by He,[191] that could be used for balanol.

Finally, the preliminary enzymatic studies in this project demonstrated the tolerance of $P450_{BM3}$ mutants towards protecting groups other than Boc group, providing alternative and more stable hydroxylated cyclic amine products. This work has been continued by others within the group and interesting substrate/oxidation profiles discovered.[214]

Chapter 3: Synthesis and P450$_{BM3}$-mediated oxidation of *ent*-α-bulnesene

Aims

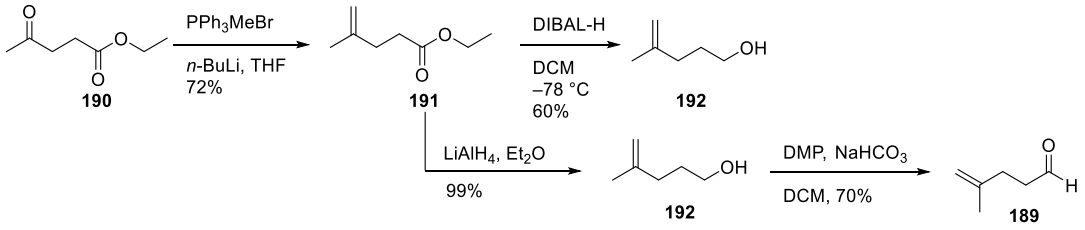
As described in the Introduction, the aim of this project was to develop a synthetic route to the enantiomer of naturally-occurring α-bulnesene **74**, *ent*-α-bulnesene **184**, which would then be subjected to P450$_{BM3}$-mediated hydroxylation and its oxidation profile compared with that of α-bulnesene **74** (Scheme 77). It was envisaged that the sesquiterpene **184** would be prepared through a ring rearrangement from the *cis*-decalin **185**, which could be obtained from diene **186** by hydrogenation. The initially-proposed carbonyl-olefin metathesis from alcohol **187** to diene **186** is unprecedented, with no reports being found on similar structures; this, therefore, was considered as a challenging step at the outset. Finally, the side chain of diene **187** would be installed through a reductive aldol reaction between commercially available (*S*)-(+)-carvone **188** and aldehyde **189**; Baran's total synthesis of hapalindole Q reported the formation of similar structures.[215]



Scheme 77. Retrosynthetic analysis of *ent*-α-bulnesene **184**.

Preparation of the carbonyl-olefin metathesis precursor

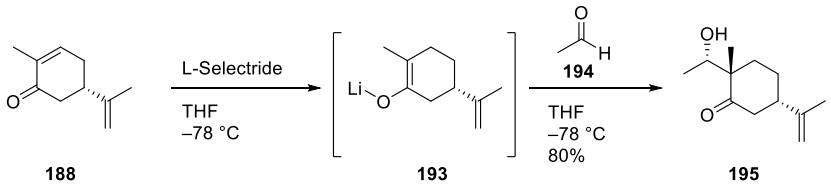
The preparation of aldehyde **189** was initially planned to follow the literature procedure reported by Zhang.[216] Commercially available ethyl levulinate **190** was subjected to Wittig reaction conditions, giving ethyl 4-methyl-4-pentenoate **191** in 72% isolated yield. The preparation of aldehyde **189** was reportedly achieved by treating ester **191** with DIBAL-H in DCM at –78 °C;[216] attempts to replicate this transformation, however, resulted in the over-reduction to alcohol **192** (Scheme 78). Therefore, to obtain aldehyde **189**, ester **191** was first reduced to the corresponding alcohol **192** with $LiAlH_4$, which was used directly in the next oxidation step, furnishing the desired aldehyde **189** in 70% isolated yield overall.[217]



Scheme 78. Attempted synthesis of aldehyde **189**; aldehyde **189** from a reduction/oxidation sequence.

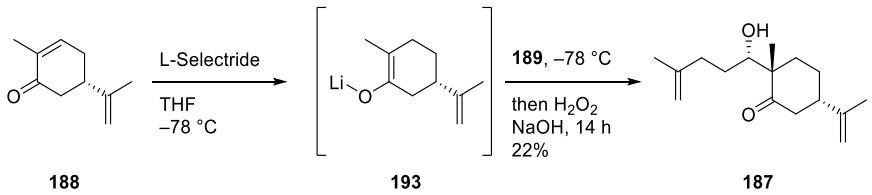
From aldehyde **189**, the conjugate reductive aldol reaction with (*S*)-(+)-carvone **188** was examined. A reaction was first carried out with acetaldehyde **194** to validate and optimise the reaction conditions reported by Baran.[215] Treatment of (*S*)-(+)-carvone **188** with L-Selectride achieved conjugate reduction to generated enolate intermediate **193**, which was then trapped by aldehyde **194**, resulting in the aldol product **195** in 80% isolated yield as a single diastereoisomer (Scheme 79). The stereochemistry of compound **195** was not confirmed at

this stage but was proposed based on axial approach to the enolate and a Zimmerman–Traxler transition state model for the aldol reaction.



Scheme 79. Test reaction with acetaldehyde **194** for the reductive aldol reaction.

Application of these conditions on a 0.5 mmol scale with aldehyde **189**, led successfully to aldol product **187**, but isolated in only 22% yield (Scheme 80). At this stage priority was given to reaching the COM precursor and testing the feasibility of the second ring formation; therefore, the stereochemistry of **187** was assumed but not yet defined (see later for discussion).

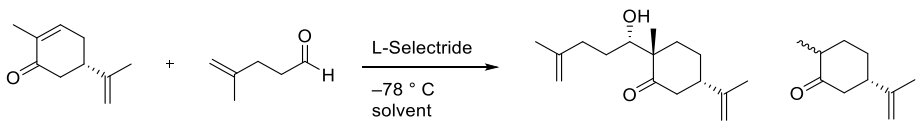


Scheme 80. Formation of the COM precursor **187**.

When the reaction was scaled up under the same conditions to a 5.0 mmol scale, only starting material was returned. At first, it was suspected that the formation of enolate **193** was sensitive to temperature; perhaps exothermicity associated with the conjugate reduction step might, on larger scale, result in localised heating during the addiction of L-Selectride. In a second

attempt at this scale, L-Selectride was added dropwise down the inside surface of the flask over 1.5 h so as not to cause a drastic change in reaction temperature; the reaction proceeded but in only 7% yield.

Repeating the reaction under the same conditions with a different bottle of L-Selectride again led to the returning of starting materials and it was suspected that the different batches of the reducing agent could influence the reaction outcome. Moreover, further literature review suggested that the concentration of the reaction could influence the outcome.[218] Therefore, the reaction was attempted with a new bottle of L-Selectride, varying the solvent and reaction concentration (Table 2). These trials gave either starting material (Entry 1) or returned non-alkylated dihydrocarvone **196** (Entry 2–5).



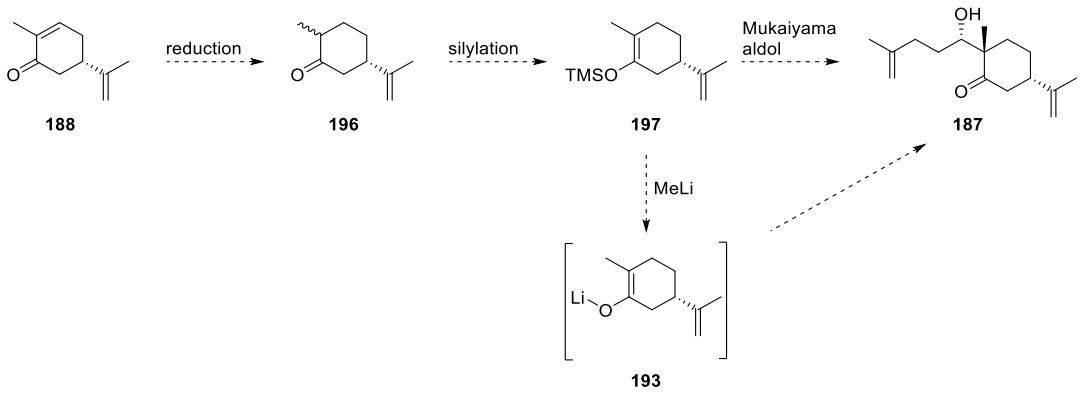
188 189 187 196

Entry	Solvent	Conc. (M)	Result
1	Et_2O	0.025	s.m.
2	Et_2O	0.50	Dihydrocarvone
3	Et_2O	1.0	Dihydrocarvone
4	THF	0.50	s.m.
5	THF	1.0	s.m.

Table 2. Variation of solvents towards **187** *via* a conjugate reduction/aldol sequence.

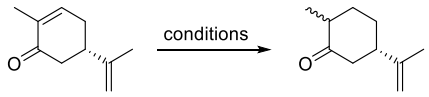
During this screening process it was discovered that the success of the conjugate reduction step highly depends on the quality of the reducing agent. When the L-Selectride was in good condition, the starting material would be reduced but the so-formed enolate was reluctant to react further; with lower-quality reagent, even the conjugate reduction was not observed.

With some insight into the source of the problem, a stepwise formation of **187**, as outlined in Scheme 81, was considered to circumvent this setback of the conjugate reduction aldol reaction. For this new route, (*S*)-(+)-carvone **188** would be reduced to dihydrocarvone **196** first, which would then be transformed into the trimethylsilyl enol ether **197**, which could regenerate the desired enolate **193** using methyllithium and undergo aldol reaction, or direct Mukaiyama aldol reaction (Scheme 81).



Scheme 81. Proposed construction of alcohol **187**.

With the conjugate reduction of (*S*)-(+)-carvone **188** with L-Selectride showing poor reproductivity, other methods for reduction were examined briefly (Table 3).[219, 220] Of these, heating a mixture of (*S*)-(+)-carvone **188** with Zn dust in aq. KOH at reflux gave the desired product **196** as a mixture of diastereomers (*dr* = 5:1) with 60% yield (Entry 4).[220]

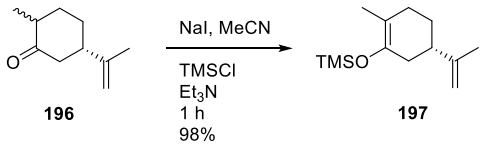


188 196

Entry	Conditions	Yield
1	Raney nickel, THF, 0 ℃, 14 h	No reaction
2	Raney nickel, THF, RT, 14 h	No reaction
3	Zn, KOH, H_2O, EtOH, 60 ℃, 14 h	No reaction
4	Zn, KOH, H_2O, EtOH, reflux, 5 h	60%

Table 3. Conditions attempted for the synthesis of dihydrocarvone **196** *via* conjugate reduction.

Dihydrocarvone **196** was transformed to the corresponding silyl enol ether **197** with trimethylsilyl chloride in 98% yield (Scheme 82).[221]

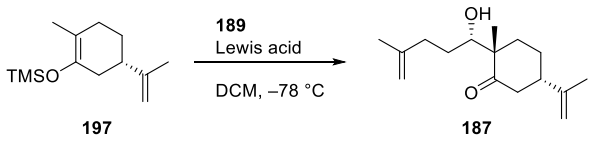


196 197

Scheme 82. Formation of silyl enol ether **197**.

From here, different Lewis acids for Mukaiyama aldol reaction were examined (Table 4). None of these was successful however (Entry 1–4), and only dihydrocarvone **196** was recovered. An attempt at fluoride catalysis of the aldol reaction with 6% TBAF at –78 ℃ was

also unsuccessful (Entry 5);[222] as was trial reaction with the enolate regenerated from the silyl enol ether with methyllithium (Entry 6).



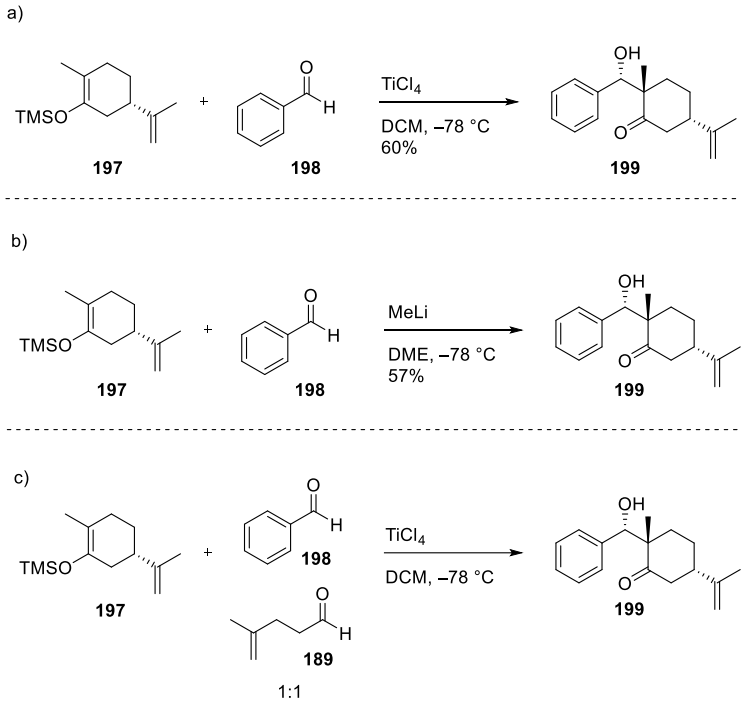
197 187

Entry	Lewis acid/Additive	Result
1	$TiCl_4$	**196**
2	BF_3 OEt_2	**196**
3	Al_2O_3	**196**
4	$SnCl_4$	**196**
5	TBAF (1.0 M in THF)	No reaction
6	MeLi	**196**

Table 4. Lewis acids screened for Mukaiyama aldol reaction.

The return of dihydrocarvone **196** from these reactions led to the suspicion that the less electrophilic aldehyde **189** was not sufficiently activated by Lewis acids towards this reaction, and instead only cleavage of the trimethylsilyl group led to the formation of dihydrocarvone **196**. Test reactions were conducted with a better precedented aldehyde, benzaldehyde **198**. With $TiCl_4$ as the Lewis acid (Scheme 83a), or with methyllithium (Scheme 83b), analysis of the ^{1}H NMR spectra showed reasonable conversion to the aldol product **199** from both

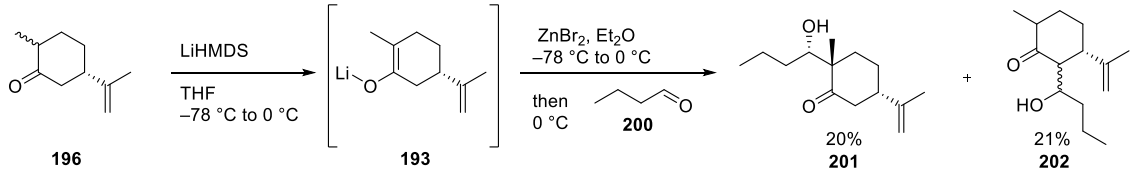
reactions. A reaction using methyllithium with a 1:1 mixture of aldehyde **189** and benzaldehyde **198** gave only keto-alcohol **199**, supporting the idea that aldehyde **189** reduced the reactivity in these Lewis acid-catalysed reactions (Scheme 83c).



Scheme 83. a). Test Mukaiyama aldol reaction with benzaldehyde **198**; b). test reaction of the lithium enolate reaction; c). test Mukaiyama aldol reaction with a mixture of benzaldehyde **198** and aldehyde **189**.

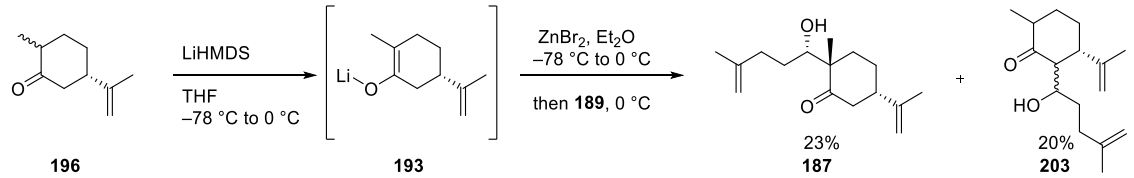
A further review of the literature revealed precedents with carvomenthone-like structures as starting materials that used Zn(II) additives in similar aldol reactions.[223, 224] A test reaction, using these modified conditions, was carried out with butyraldehyde **200** as a model for aldehyde **189**. Enolate formation with LiHMDS at –78 ℃ and warming to 0 ℃ over 1 h,

followed by the addition of $ZnBr_2$ and aldehyde **200** afforded two regioisomeric aldol products, alcohol **201** and **202**, in a 1:1 ratio, respectively (Scheme 84).



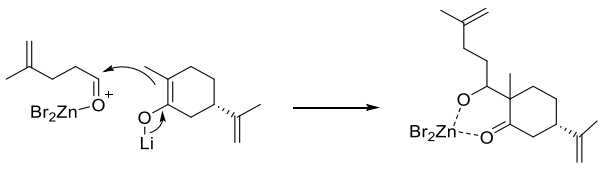
Scheme 84. Test aldol reaction with butyraldehyde **200** using $ZnBr_2$ as additive.

Following the same procedure, a similar result was produced with aldehyde **189** (Scheme 85).



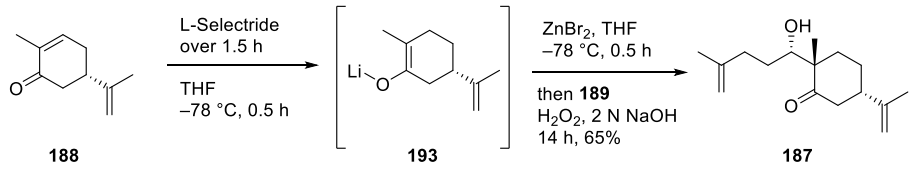
Scheme 85. Aldol reaction with aldehyde **189** in the presence of $ZnBr_2$.

The success of this procedure indicated the importance of the addition of $ZnBr_2$ for which a plausible explanation is that the additive serves both as a Lewis acid to activates the aldehyde and coordinates to both aldolate oxygens to stabilise the product against retro-aldol reaction (Scheme 86).



Scheme 86. $ZnBr_2$ as Lewis acid and prevents retro-aldol reaction.

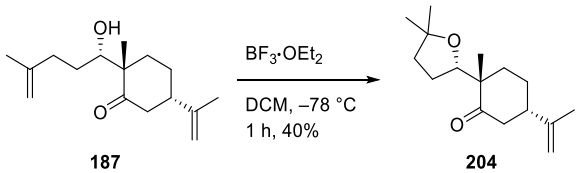
With this success, and in order to avoid the formation of aldol regioisomer **203**, the original conjugate reduction procedure was followed with an active bottle of L-Selectride, with $ZnBr_2$ added before adding the aldehyde **189** at –78 ℃. This afforded the desired alcohol **187** as a single regio- and diastereoisomer in 65% isolated yield (Scheme 87), the stereochemistry, as shown, being originally proposed by analogy and confirmed later (see discussion below).



Scheme 87. Revised conjugate reductive aldol reaction with $ZnBr_2$ additive.

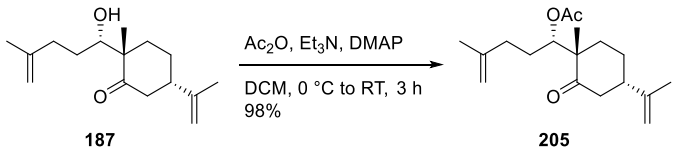
Attempted carbonyl-olefin metathesis (COM)

With the alcohol **187** precursor in hand reproducibly, the COM reaction was attempted under the most frequently employed Lewis acidic conditions using BF_3 OEt_2 at –78 ℃. However, the free hydroxyl group cyclised onto the alkene to give ketone **204** (Scheme 88).



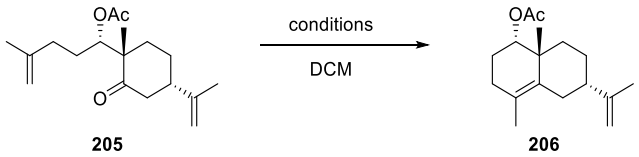
Scheme 88. Cyclisation of the hydroxyl group.

To prevent this, acetate **205** was prepared in 98% isolated yield (Scheme 89).



Scheme 89. Protecting the hydroxyl group with acetyl group.

From precursor **205**, the reaction with $BF_3 \cdot OEt_2$ was repeated but only starting material was recovered (Table 5, Entry 1). The use of $FeCl_3$ at RT led to the same result (Entry 2), and attempts to engage Grubbs II catalyst in a COM reaction led to decomposition (Entry 3 and 4).

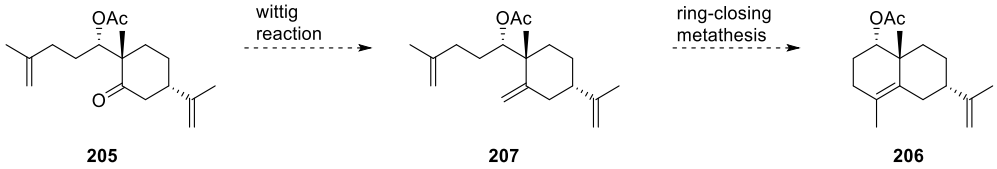


Entry	Conditions	Outcome
1	$BF_3 \cdot OEt_2$, –78 ℃, DCM	s.m.
2	$FeCl_3$, RT, DCM	s.m.
3	Grubbs II (10%), reflux, DCM	decomposed
4	Grubbs II (1 equiv.), reflux, DCM	decomposed

Table 5. Conditions attempted for the carbonyl-olefin metathesis of ketone **206**.

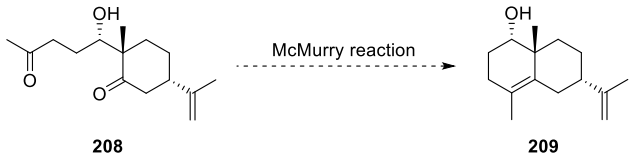
Studies towards other potential methods for the cyclisation reaction

As described earlier, since this type of COM reaction had no close precedent, other methods were investigated in parallel. For example, it was envisaged that ring-closing metathesis would be able to form the fused ring system **206** from diene **207** which, in turn, would be generated by Wittig olefination from precursor **205** (Scheme 90).



Scheme 90. Envisaged **206** formation from ring-closing metathesis.

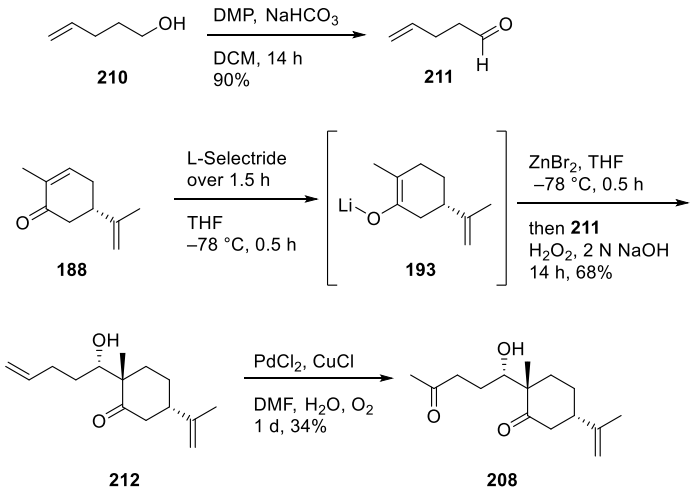
Unfortunately, Wittig reaction of ketone **205** using MTPB and either butyllithium or *t*-BuOK gave no reaction and, suspecting that the ketone was too hindered, the approach was abandoned. Next, the McMurry reaction, a low-valent titanium-induced dicarbonyl-coupling reaction,[225] was considered to form the fused-ring system (Scheme 91).



Scheme 91. Envisaged McMurry reaction.

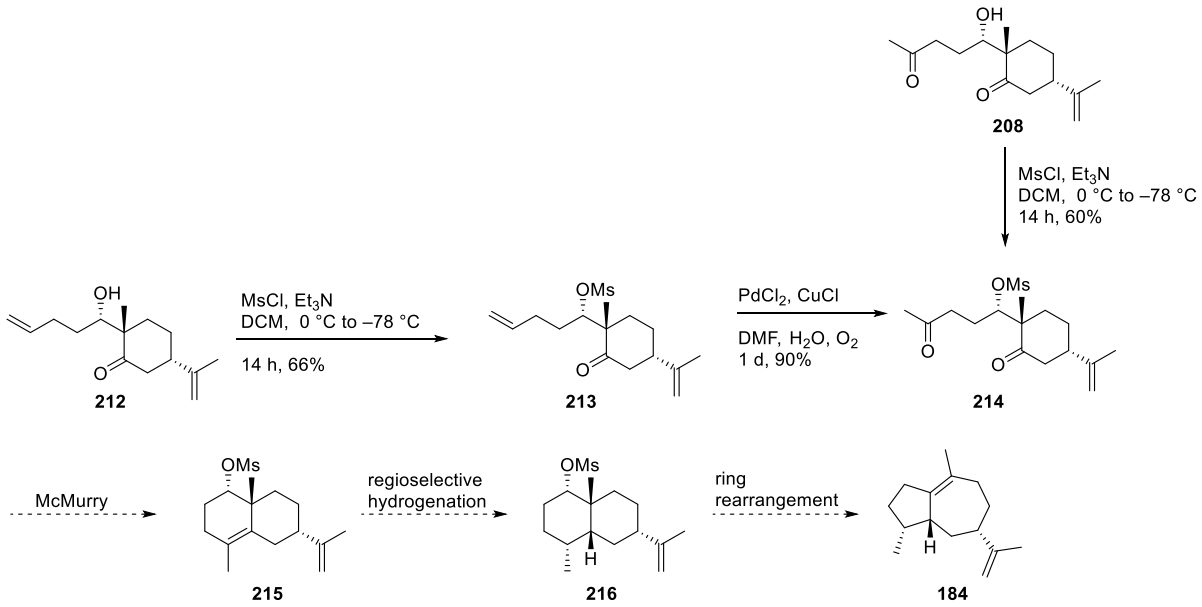
To set the stage for the investigation of the feasibility of McMurry reaction, the required diketone **208** was to be prepared by Wacker oxidation of a simple terminal alkene analogue (**212**) of aldol adduct **187**. To achieve this, the starting aldehyde **211** was prepared by

oxidation of pent-4-en-1-ol **210** with DMP in quantitative yield, and the product was used directly for the next step without further purification (Scheme 92). The $ZnBr_2$-modified reductive aldol reaction was then performed and adduct **212** was successfully generated as a single stereoisomer in 68% isolated yield. Application of the standard conditions for Wacker oxidation using $PdCl_2$ and CuCl gave an unoptimised 34% yield of the desired diketone **208**.[226]



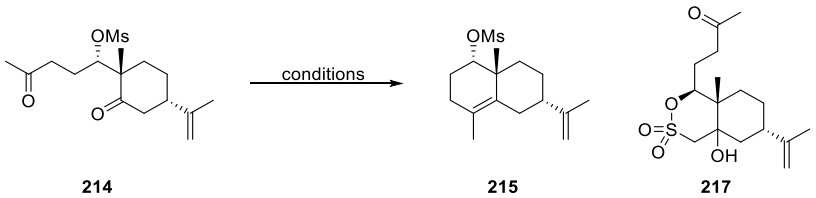
Scheme 92. Formation of the diketone McMurry precursor **208**.

Diketone **208** was protected as its mesylate (**214**) from either **208** or **213** due to the decomposition of starting material caused by free hydroxyl group, also in anticipation of the ring rearrangement step following successful McMurry reaction (Scheme 93).



Scheme 93. Ms-protection and envisaged ring rearrangement with mesylate as leaving group.

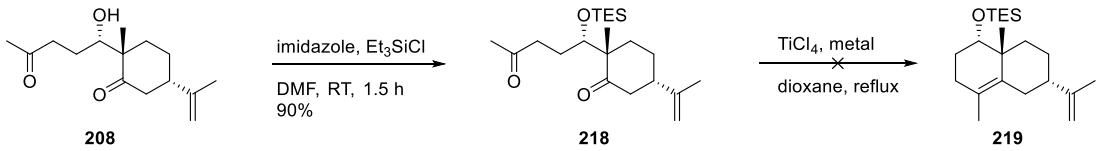
From mesylate **214**, various conditions for forming the tetrasubstituted alkene **215** by McMurry reaction were then tested; the trials are summarised in Table 6.[227, 228] In some cases, no reaction resulted but $TiCl_4$ and Zn in either THF or 1,4-dioxane at reflux led to the formation of sultone **217** (Entries 2, 3)[228] *via* deprotonation and cyclisation of the mesyl methyl group. The formation of this compound was indicated by two doublets for the diastereotopic CH_2S protons, at 3.36 and 3.07 ppm in the ^1H NMR spectrum. Using Sm dust as reducing agent led to recovery of starting material (Entry 4).



Entry	Conditions	Outcome
1	Mg, $HgCl_2$, $TiCl_4$, THF, sonication then reflux	No reaction
2	$TiCl_4$, Zn, THF, reflux	**217**
3	$TiCl_4$, Zn, dioxane, reflux	**217**
4	$TiCl_4$, Sm, dioxane, reflux	No reaction

Table 6. Conditions attempted for McMurry reaction on diketone **214**.

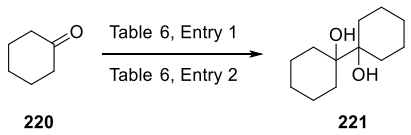
Swapping the Ms group for TES group (**218**) and applying the same conditions from Table 6, Entries 2 and 3 were performed again with diketone **218** (Scheme 94), which gave only return of starting materials.



Scheme 94. Hydroxyl protection with TES and attempted McMurry reaction.

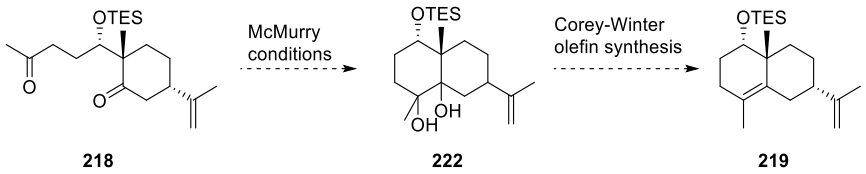
In order to ascertain the reason behind the failure of this type of reaction regarding our substrate, a test reaction was carried out with cyclohexanone **220** using the same conditions as Table 6, Entries 1 and 2 (Scheme 95). In both cases the pinacol coupled product **221** was

observed but not alkene formation, suggesting that the conditions were capable of inducing the reductive coupling of, at least, cyclohexanone **220**, but were insufficiently vigorous for the deoxygenation of 1,2-diol **221**.



Scheme 95. Pinacol coupling of cyclohexanone **220** with screened conditions.

In principle, such a coupling could potentially be followed up by a Corey–Winter olefin synthesis (Scheme 96) but the formation of even the 1,2-diol **222** was never observed in the reactions of diketone **218**.

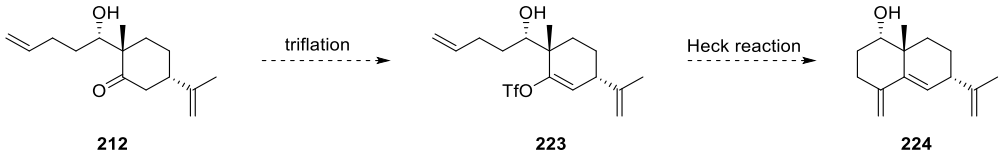


Scheme 96. Potential olefin synthesis with diol product **222**.

Investigation into the intramolecular Heck reaction

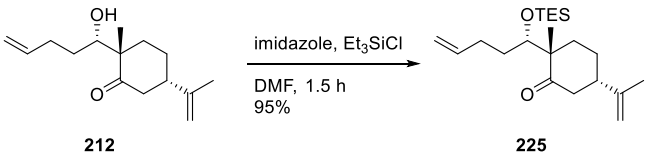
Of the various alternative methods for effecting cyclisation that presented themselves at this stage, the intramolecular Heck reaction seemed appealing. It was expected that the ketone in aldol adduct **212** could be converted into its triflate **223**, which is suitable for Heck cyclisation to give triene **224** (Scheme 97).

Compared to the previously attempted methods, this strategy had more literature precedents but would generate triene **224**, which would potentially give rise to stereochemical and chemoselectivity complications during the subsequent hydrogenation step. Despite this, this strategy was investigated.



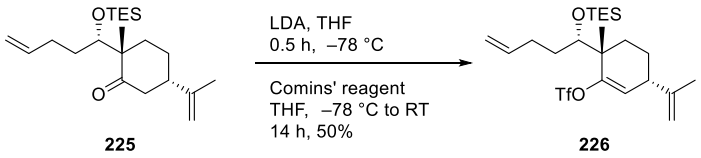
Scheme 97. Envisaged Heck reaction to achieve the cyclisation.

Earlier attempts of cyclisation described in this chapter suggested that the free hydroxyl group tended to interfere with the cyclisation process. Therefore, aldol adduct **212** was first protected as its TES derivative **225** in satisfying yield (95%) (Scheme 98).



Scheme 98. Formation of the TES derivative **225**.

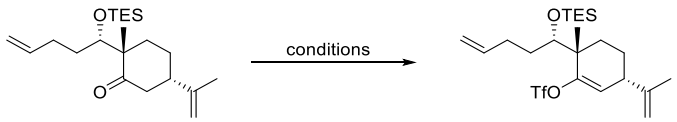
The conditions for the triflation step were inspired by Comins' original paper;[229] thus, the LDA formation, enolate generation and addition of 1.1 equiv. of commercial Comins' reagent were all conducted at –78 ℃. However, after allowing the reaction mixture to warm up to RT over 14 h, the reaction was successful but did not reach completion and a 50% isolated yield of product **226** was obtained (Scheme 99).



225 226

Scheme 99. Triflation of **225** with Comins' reagent.

Table 7 summarises attempts to optimise this reaction. The first obvious improvement was to increase the amount of base and triflating reagent (Entry 1 – 3). However, the yield did not improve further when more than 2.0 equiv. of Comins' reagent was used. Changing the solvent from THF to DME was also attempted (Entry 4), but resulted in no improvement. Using a different triflating reagent, $PhNTf_2$ (1.2 equiv.), gave a similar outcome, and changing the base to KHMDS also had little effect (Entry 5 and 6). Coincidentally, another member in the group was working on similar reactions at the same time, and it was discovered that warming up the reaction mixture to RT before adding the triflating reagent was crucial for achieving a high yield in this reaction. Thus, allowing the reaction mixture to warm up to RT after deprotonation of substrate **225** with LiHMDS at –78 ℃, then adding 1.5 equiv. of Comins' reagent to the reaction mixture at RT and stirring for 14 h generated the desired enol triflate **226** in 88% isolated yield (Entry 7).



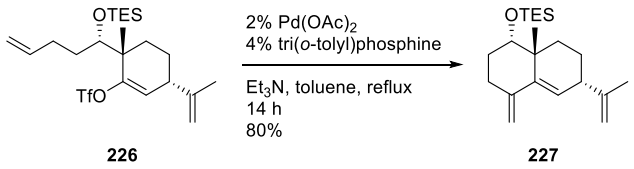
225 226

Entry	Reagent (equiv.)	Base (equiv.)	Solvent	T. (℃)	Yield
1	Comins' reagent (1.5)	LDA (1.5)	THF	–78 ℃	57%
2	Comins' reagent (2.0)	LDA (2.0)	THF	–78 ℃	60%
3	Comins' reagent (3.0)	LDA (2.0)	THF	–78 ℃	61%
4	Comins' reagent (3.0)	LDA (2.0)	DME	–78 ℃	55%
5	$PhNTf_2$ (1.2)	LDA (2.0)	THF	–78 ℃	51%
6	$PhNTf_2$ (1.2)	KHMDS (1.2)	THF	–78 ℃ → RT	53%
7	Comins' reagent (1.5)	LiHMDS (1.2)	THF	–78 ℃ → RT	88%

Table 7. Optimisation of the triflation.

With a satisfying yield and sufficient material **226** in hand, the intramolecular Heck reaction was examined on a small scale (0.5 mmol). After heating the triflate **226** with $Pd(OAc)_2$ (2%) and tri(*o*-tolyl)phosphine (4%) in toluene at reflux for 14 h, TLC indicated full consumption of starting material and a single new spot; however, removing the solvent under vacuum and directly purifying the crude product by column chromatography gave only a 20% yield of the cyclisation product **227**.

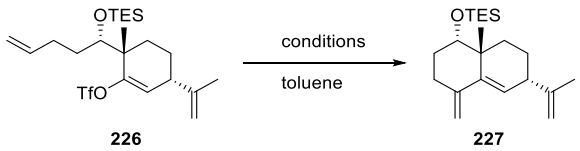
This suggested that the product might interact with the catalyst and remaining TfOH upon concentration and therefore remain on the column; with this idea, including a wash of the crude with sat. $NaHCO_3$ prior to removing the solvent significantly improved the situation and resulted in 80% isolated yield (Scheme 100).



226 **227**

Scheme 100. Small-scale Heck reaction.

Unfortunately, scaling up the reaction to a 4.0 mmol scale under the same conditions, led to the reaction stalling at a certain point, and adding more catalyst did not restart the process. The low yield and high recovery of starting material indicated that the reaction was quenched before reaching completion. Further trials with the same conditions and the same scale led to the same result or no reaction at all. It proved necessary to troubleshoot this reaction in order to progress the synthesis (Table 8). Repeating the original conditions gave 20% yield at best, and leaving the reaction for longer did not improve the outcome (Entry 1, 2). Increasing the catalyst loading quenched the reaction completely (Entry 3). However, to our surprise, reducing the catalyst loading to only 1% increased the yield to 61% (Entry 4). Finally, decreasing the concentration of the reaction mixture to 0.1 M, in addition to lowering catalyst loading to 1%, resulted in the most satisfying outcome of 80% yield (Entry 5).

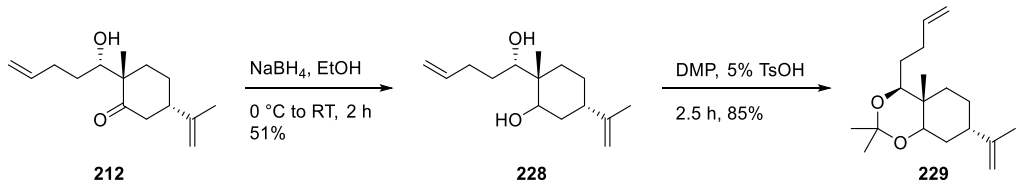


226 227

Entry	Catalyst loading	Ligand loading	Concentration (M)	Time	Yield
1	2%	4%	0.2	14 h	20%
2	2%	4%	0.2	1 day	20%
3	4%	8%	0.2	14 h	No reaction
4	1%	2%	0.2	14 h	61%
5	1%	2%	0.1	14 h	80%

Table 8. Conditions optimised for the cyclisation of **226** by intramolecular Heck reaction.

In order to confirm the stereochemical assignment of aldol adduct **212** before moving on with the synthesis, the acetonide **229** was synthesised so that a meaningful NOE analysis could be conducted.[230] Aldol product **212** was reduced to diol **228**, then subjected to the optimised ketalisation conditions shown in Scheme 101 to furnish acetonide **229** in 85% isolated yield (Scheme 101).



212 228 229

Scheme 101. Generation of the diol precursor **228** and acetonide **229** formation.

The observed NOEs showed the proximity of H_A and the bridgehead methyl group (C-16 in Figure 20); additionally, H_B is correlated to the protons on only one of the acetonide methyl groups (C-15 in the Figure 20), both being consistent with a *cis*-fused conformer. Moreover, the proximity of H-16 and H-17 was also observed. The observed proton proximities revealed the relative configuration to be as shown in Figure 20, which is the desired configuration for the formation of *ent*-α-bulnesene **184**.

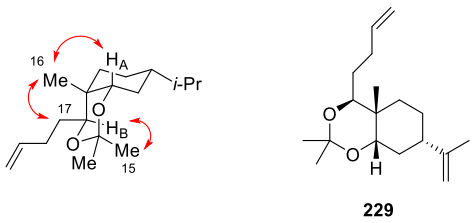
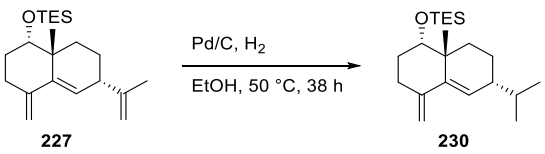


Figure 20. Characteristic NOE correlations in acetonide **229**.

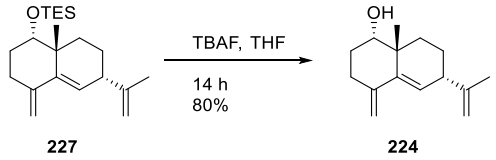
Studies towards the regio- and stereo-selective hydrogenation of triene 227

From cyclisation product **227**, attention turned to the selective reduction of the diene. General hydrogenation conditions were first tested to examine the feasibility of the diene reduction. Hydrogenation with a Pd/C catalyst resulted only in the reduction of the terminal alkene, giving **230** (Scheme 102), even after a prolonged reaction time (38 h).



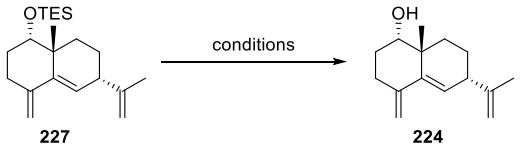
Scheme 102. Attempted hydrogenation of diene in compound **227.**

It was considered that the TES protecting group might hinder coordination of the diene to the catalyst surface; therefore, **227** was first deprotected with TBAF (Scheme 103).



Scheme 103. Removal of TES protecting group.

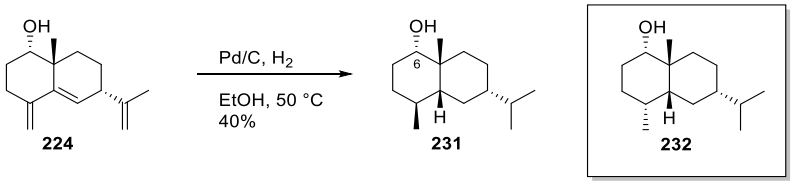
In later repeats of this apparently trivial reaction, it was discovered that the efficiency of the reaction heavily depended on the quality of the TBAF, as the decomposition of starting material was often observed (Table 9, Entry1). Thus, potentially more reliable deprotection conditions were sought. DeShong's protocol, using fluorosilicic acid (H_2SiF_6) in *t*-BuOH,[231] led only to the decomposition of starting material (Entry 2) but returning to the TBAF method, it was found that increasing the amount of TBAF and diluting the reaction concentration gave a higher yield of the product (Entry 3, 4).



Entry	Reagent (equiv.)	Concentration	Yield
1	TBAF (1.1)	0.2	Decomposition
2	H_2F_6Si (1.0)	0.1	Decomposition
3	TBAF (3.0)	0.2	50%
4	TBAF (4.0)	0.01	80%

Table 9. Conditions assessed for the TES deprotonation.

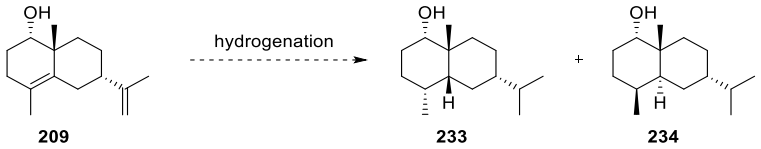
After solving the issues encountered with deprotection, hydrogenation of triene **224** with Pd/C for 4 days generated only one product in which all the alkenes had been reduced (Scheme 104). From ^{1}H NMR spectrum, the doublet of doublets at 3.24 ppm with J = 11.5, 4.5 Hz suggested an axial CHOH (H-6), indicating an equatorial hydroxyl group and a *cis*-fused decalin. Thus, we deduced the stereochemistry of the product **231** to be as shown in Scheme 104 as compound **232** would suffer from clashing between the axial methyl group and the axial isopropyl group.



Scheme 104. Hydrogenation of deprotected bicyclic compound **224**.

With the product assigned to have a *cis*-disposition of the bridgehead methyl group and hydrogen, it is proposed that the hydrogenation reaction occurred sequentially on opposite faces of the diene, resulting in a diastereomer incapable of forming *ent*-α-bulnesene **184**. Furthermore, performing the hydrogenation with PtO_2 for two days resulted in two pairs of inseparable isomers, both of which suggested the presence of alkene in the system from NMR analysis, indicating the reduction was not complete.

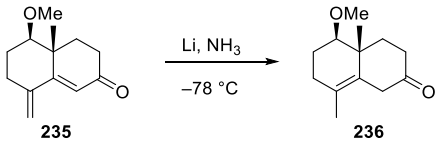
These slow reactions generating product mixtures led to an alternative strategy to be considered in which, instead of reduction of the diene, the substrate undergoing hydrogenation would have just one double bond placed between the methyl group and the bridgehead position (**209**), such that hydrogenation would result in just two stereoisomers, the *cis*- or *trans*-decalins (Scheme 105).



Scheme 105. Envisaged hydrogenation with **209** to the *cis*- and *trans*-decalins, **233** and **234**.

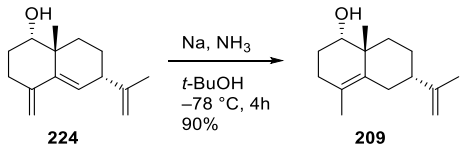
Conformational energy calculations suggested that the lowest energy conformers of alkene **209** have an axial isopropyl group which would block the lower face more effectively than the bridgehead methyl would block the upper face; therefore, it appeared likely that the hydrogenation would give the *cis*-decalin isomer **233**, which has the correct relative configuration for *ent*-α-bulnesene **184**. Watt using a Birch-type reduction condition, lithium

in liquid ammonia at –78 ℃, successfully converted conjugated enone **235** into the deconjugated enone **236** (Scheme 106).[232]



Scheme 106. Deconjugation of enone **235** with Birch reduction performed by Watt.[232]

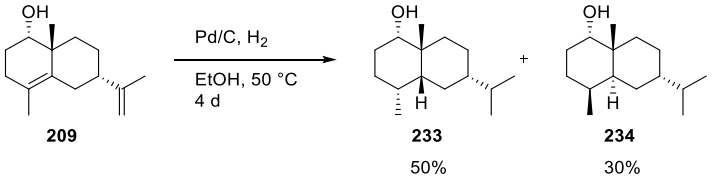
Inspired by this precedent, an analogous reduction of triene **224** to deconjugated diene **209** was attempted. Addition of pieces of sodium into liquid ammonia at –78 ℃, followed by reduction of the substrate in the presence of *t*-BuOH for 1 h, resulted in the desired diene **209** with some starting material left over. For the next trial the reaction was left to react for 4 h, which led to the full consumption of starting material, with 90% isolated yield (Scheme 107).



Scheme 107. Birch reduction of triene **224**.

With ample amount of diene **209** in hand, the catalytic hydrogenation was again attempted, with the expectation that the addition of hydrogen would proceed stereoselectively from one favoured side to give either the *cis*-decalin **233** or *trans*-decalin **234**. Under the hydrogenation conditions used previously (Pd/C, EtOH), the reaction proceeded over a number of days to

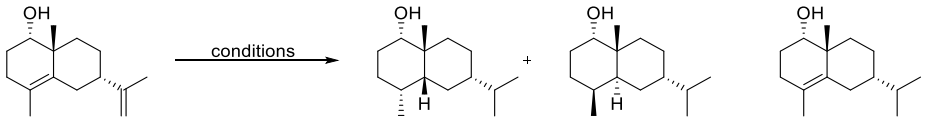
form a separable mixture of *cis*-isomer **233** and *trans*-isomer **234** in a 1.6:1 ratio, respectively, along with a mixture of inseparable isomers (Scheme 108).



Scheme 108. Hydrogenation of alkene **209**.

Though the reaction with Pd/C gave both products, the inseparable isomers were probably caused by the slow reaction, so efforts were made to improve the rate and selectivity of the reaction (Table 10). Changing the solvent to ethyl acetate and reducing the reaction temperature to RT resulted in improved selectivity with a ratio of *cis*: *trans* (2.3: 1), along with the mixture of inseparable isomers (Entry 1). Changing the catalyst to PtO_2 with acetic acid as solvent led to the sole formation of the *cis*-isomer **233** (Entry 2). Raney nickel led only to the reduction of the terminal alkene, giving alkene **237** (Entry 3). A reduction in which diimide was generated in situ from TPSH was also attempted (Entry 4) where reaction for 14 h in acetonitrile led to the formation of alkene **237**. It was suspected that the long reaction time when using Pd/C has led to the isomerisation of the alkenes present, leading to the formation of the various isomers and low yield of desired products. Speeding up the transition-metal mediated hydrogenation could potentially minimise isomerisation. Pd black, a sponge-like form of palladium, could offer a larger catalytic surface area, was thus tried out for the hydrogenation reaction. Freshly-prepared Pd black in ethanol resulted in a 2:1 ratio of *cis*-isomer **233** and *trans*-isomer **234** with a cleaner product profile as judged by ^{1}H NMR

analysis (Entry 5). Changing the catalyst to Pt/C from Pd/C resulted in the sole formation of the *cis*-isomer **233** (Entry 6). It was also hypothesised that increasing the reaction pressure would accelerate the reaction. Subjecting diene **209** to hydrogenation with Pd/C at 6 atm pressure led to a significantly faster and cleaner formation of the *cis*-isomer **233** (Entry 7).

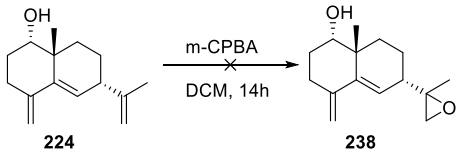


209 **233** **234** **237**

Entry	Conditions	Outcome
1	Pd/C, EtOAc, H_2, RT, 4 days	*cis*: *trans* (2.3: 1)
2	PtO_2, AcOH, H_2, RT, 2 days	**233** and **237**
3	Raney nickel, MeOH, H_2, RT, 2days	**237**
4	TPSH, Na_2CO_3, MeCN, RT, 2 days	**237**
5	Pd black, EtOH, H_2, RT, 14 h	*cis*: *trans* (2: 1)
6	Pt/C, EtOH, H_2, RT, 14 h	Only *cis*
7	10 % Pd/C, EtOH, H_2, 6 atm, RT, 2 h	Only *cis*

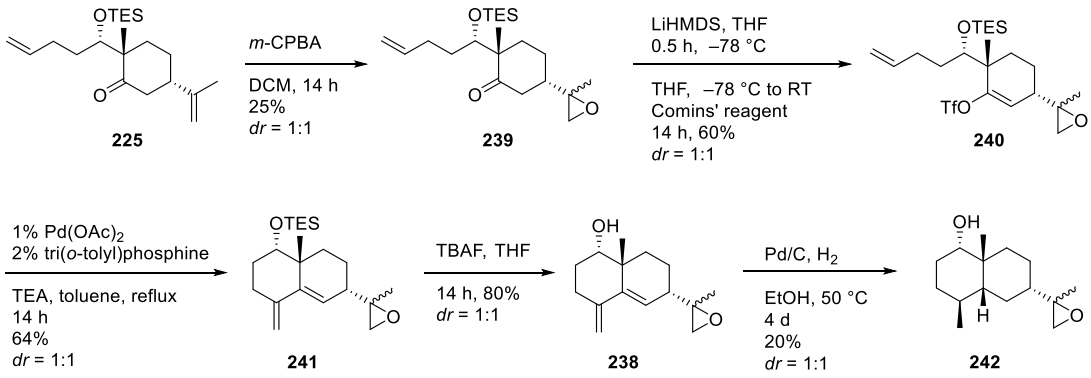
Table 10. Conditions assessed for the stereoselective hydrogenation of substrate **209**.

In the meantime, efforts were made to protect the terminal alkene through epoxidation to avoid reducing it simultaneously. Direct epoxidation with alcohol **224** proved unsuccessful, leading only to return of starting material (Scheme 109).



Scheme 109. Attempted epoxidation of alcohol **224**.

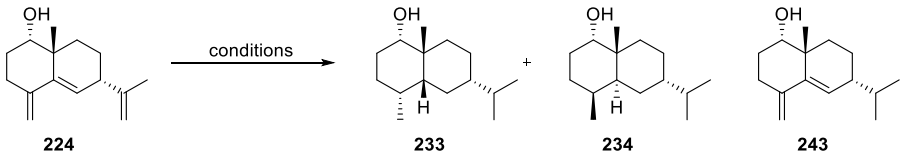
The epoxidation of diene **225** (→ **239**) was successful but the yield was rather low (25%). Moreover, the epoxide analogues reacted less efficiently in subsequent steps toward the hydrogenation precursor **238**; therefore, this route was abandoned due to excessive material loss (Scheme 110). Since re-optimising the conditions was expected to be time-consuming, it was decided that the synthesis towards constructing the 5,7-fused ring system should be progressed without epoxide protection.



Scheme 110. Synthesis of the epoxide analogue **242**.

The experience gained from the above hydrogenation study led to a reconsideration of the direct hydrogenation of triene **224**. Accelerating the reaction speed by aggravating the reaction conditions could potentially control the stereochemistry of the products (Table 11). The investigation started off with increasing the reaction pressure. In a pressure reactor, Pd/C

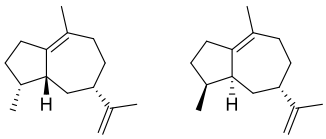
was added to a solution of triene **224** in ethanol and the system was stirred for 3 h under H_2 at 6 atm pressure (Entry 1). Surprisingly, the reaction resulted in a mixture of **243** and *trans*-product **234**. This showed again that the outcome of hydrogenation of **224** is dependent on the speed of the reaction, and therefore that controlling the reaction time would be key to controlling the stereochemical outcome. Hydrogenation with the more active Pt/C in ethanol at RT for 14 h also resulted in the formation of the *cis*-isomer **233** (Entry 2). During the study, another method was discovered that used both Pd/C and $Pd(OH)_2/C$ in a mixed solvent system for the deprotection of benzyl groups.[233] Inspired by this, hydrogenation on a 0.2 mmol scale with 2% Pd/C and 3% $Pd(OH)_2/C$ suspended in a mixture of DCM, methanol and water and stirring at RT for 3 h led to the completion of the reaction, giving a 1:1mixture of the *cis*-isomer **233** and *trans*-isomer **234** (Entry 3). It was observed that upon increasing the reaction scale, the *cis/trans* ratio would increase; on a 0.4 mmol scale, the ratio of *cis*-isomer **233** and *trans*-isomer **234** changed to 2:1 (Entry 4); when raising the reaction scale to 1.0 mmol, *cis/trans* ratio increased to 9:1 (Entry 5). It was suspected that this apparent scale-dependence in stereochemical outcome might be due to catalyst aging. A test reaction was carried out on a 0.2 mmol scale (Entry 6). Pd/C and $Pd(OH)_2/C$ was stirred under H_2 for 1 h before adding in diene **224**; this resulted in a 2:1 *cis/trans* ratio, demonstrating some influence of catalyst aging on the product stereochemistry but not explaining the extent of the difference in, for example, Entry 5.



Entry	Conditions	Outcome
1	10 % Pd/C, EtOH, H_2, 6 atm, RT, 3 h	**234** and **243**
2	10% Pt/C, EtOH, H_2, 1 atm, RT, 14 h	*cis*
3	2% Pd/C and 3% $Pd(OH)_2$/C, DCM, MeOH, H_2O, H_2, 1 atm, RT, 3 h (0.2 mmol)	*cis*:*trans* (1:1)
4	Entry 3 (0.4 mmol)	*cis*:*trans* (2:1)
5	Entry 3 (1.0 mmol)	*cis*:*trans* (9:1)
6	Entry 3 (0.2 mmol), catalyst pre-activated	*cis*:*trans* (2:1)

Table 11. Conditions assessed for accelerating the hydrogenation reaction of triene **224**.

The stable yield and reasonable control of the stereochemical outcome of the reaction using the Pd/C-$Pd(OH)_2$/C catalyst system were considered optimal for the production of both **233** and **234**; moreover, the reaction conditions are mild and allow the circumvention of Birch reduction step. It was decided that both isomers were worth investigating because, although the *cis*-decalin **233** has the desired stereochemistry for *ent*-α-bulnesene **184**, it might not undergo ring rearrangement smoothly; on the other hand, the *trans*-decalin **234** can only result in the epimer **244** (Figure 21) but looked ideal for investigating the ring rearrangement upon inverting the hydroxyl stereochemistry.

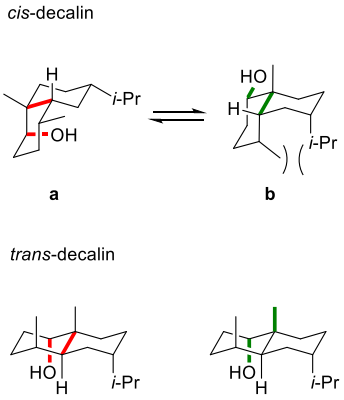


184 244

Figure 21. *ent*-α-Bulnesene **184** and its epimer **244**.

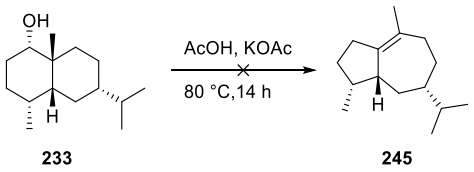
Studies on the formation of the 5,7-fused ring system through ring rearrangement

Considering the stereoelectronic requirements of the ring rearrangement step, conformational analysis suggested that the *trans*-isomer **234** would not be capable of forming the 5,7-fused ring system at this stage but is more likely to undergo [1,2]-methyl shift instead. The *cis*-isomer **233**, in conformation **b**, has the potential to rearrange to the desired product but is disfavoured due to the clashing methyl and isopropyl groups (Scheme 111).



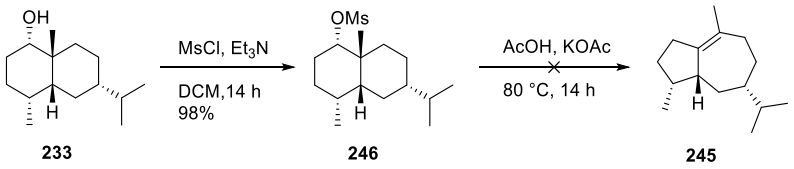
Scheme 111. Conformational analysis of *cis*-isomer **233** and *trans*-isomer **234**.

Beginning with the more abundant *cis*-isomer **233**, a trial reaction in which the alcohol was directly subjected to solvolysis conditions led to no reaction (Scheme 112) and it was deemed necessary to activate the OH group as a leaving group by sulfonylation.



Scheme 112. Attempted ring rearrangement with free alcohol as leaving group.

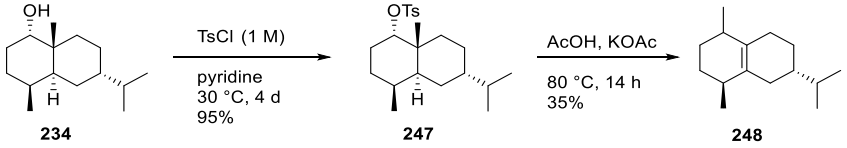
Standard mesylation conditions afforded mesylate **246** in 98% isolated yield but this substrate also proved inactive under the solvolysis conditions reported for similar reactions (Scheme 113).



Scheme 113. Generation of mesylate **246** leaving group and its attempted ring rearrangement.

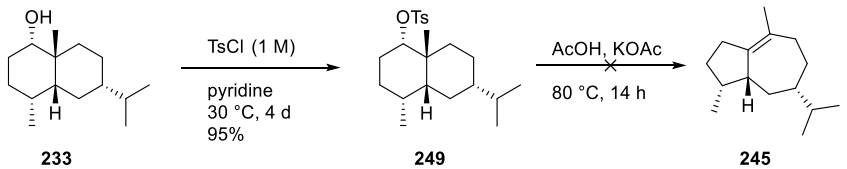
Suspecting that the mesylate substituent was constrained in its ability to leave by virtue of its relatively small size, it was considered that the bulkier tosylate, that would benefit from relief of steric crowding during ionisation, should be investigated. The tosylation reaction, first achieved with the *trans*-decalin **234** alcohol to form substrate **247**, had to be run at a relatively high concentration (≥ 1.0 M) in order to proceed at an acceptable rate and, after four days at 30 ℃, the reaction reached completion, with 95% isolated yield (Scheme 114). The ring rearrangement conditions with this *trans*-substrate **247** led to the formation of only one product with 30% isolated yield. ^{13}C NMR of this compound displayed two alkene resonance

at 128.8 ppm and 122.4 ppm, while no alkene proton resonance was observed from ^{1}H NMR. Therefore, the compound is deduced to be the [1,2]-methyl shift product **248**, which is consistent with former hypothesis.



Scheme 114. Formation of the tosylate **247** and solvolytic [1,2]-methyl shift from *trans*-isomer **234**.

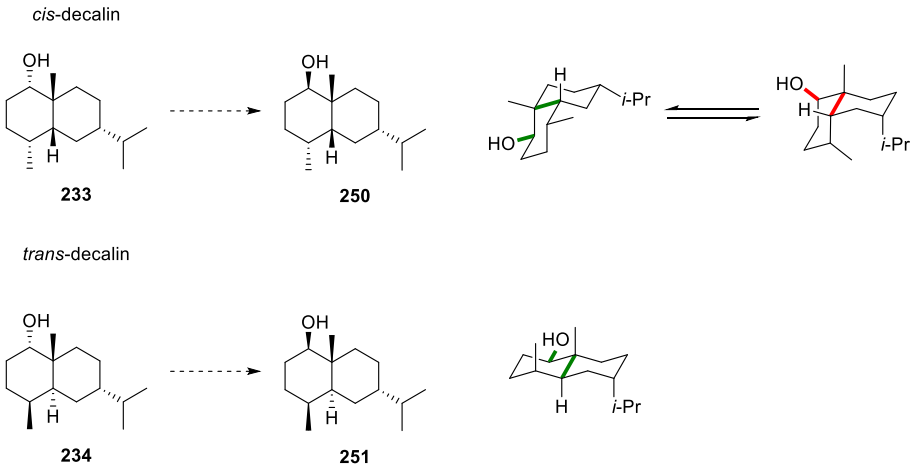
From the corresponding *cis*-isomer **233**, complete return of starting material was observed (Scheme 115), which supported the suggestion from molecular modelling that the clashing methyl and isopropyl groups would limit access to a reactive conformation.



Scheme 115. Formation of the tosylate and attempted ring rearrangement with *cis*-isomer **233**.

It was proposed that both problems would be solved by inverting the stereochemistry of the hydroxyl group. The inverted structures **250** and **251** are more likely to undergo rearrangement because the *cis*-isomer **250** can achieve this alignment without bringing the

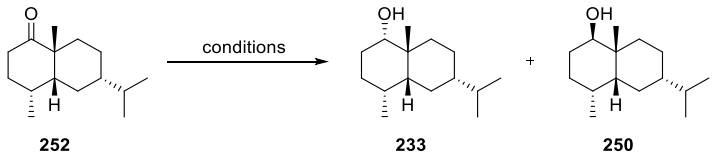
alkyl substituents into close proximity and the *trans*-isomer **251** has the natural alignment of the central C–C bond and leaving group (Scheme 116).



Scheme 116. Envisaged ring rearrangement after hydroxyl group inversion.

The inversion was planned to be realised through an oxidation/reduction sequence. Dess–Martin oxidation of the alcohol to the corresponding ketone was efficient for both *cis*-isomer **233** and *trans*-isomer **234** (Scheme 117).

The stereoselective reduction of the *cis*-isomer **252** was first attempted with simple reducing reagents (Table 12). Heating with $NaBH_4$ in ethanol resulted in a separable mixture of **250** and **233** in 2:1 ratio, respectively (Entry 1). Decreasing the reaction temperature to 0 ℃ resulted in a drop in selectivity (Entry 2). Reduction with $LiAlH_4$ in THF at 50 ℃ for 14 h led to mostly the desired isomer **250**, along with traces of **233** (Entry 3); changing the solvent to diethyl ether and decreasing the temperature to RT led to the complete formation of the desired isomer **250** (Entry 4).

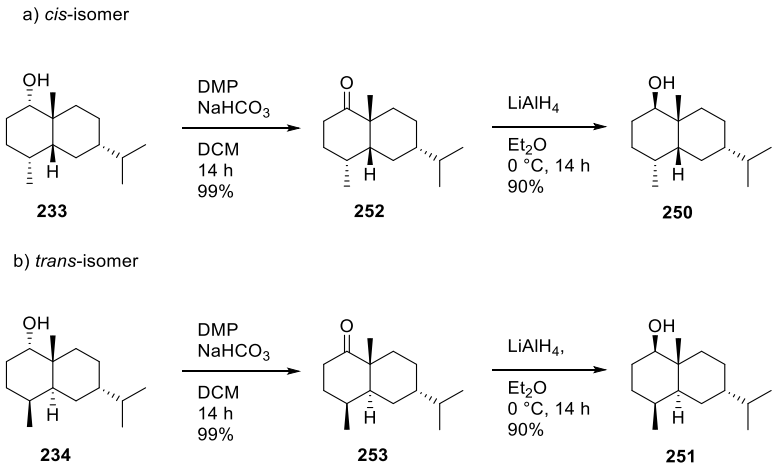


252 233 250

Entry	Reducing agent	Solvent	Temperature	Outcome
1	$NaBH_4$	EtOH	Reflux	**250**:**233** (2:1)
2	$NaBH_4$	EtOH	0 ℃	**250**:**233** (1:1)
3	$LiAlH_4$	THF	50 ℃	Mostly **250**
4	$LiAlH_4$	Et_2O	0 ℃	**250**

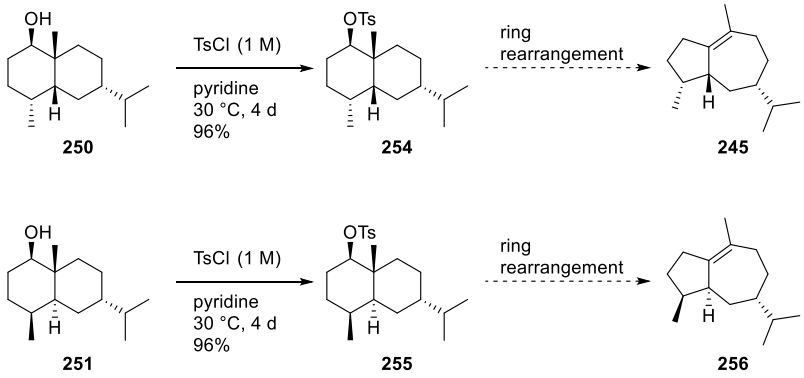
Table 12. Conditions screened for the stereoselective reduction of ketone **252**.

The inversion of stereochemistry of the hydroxyl group to *cis*-isomer **250** was successfully achieved in 90% isolated yield (Scheme 117a), and these optimised conditions were applied with *trans*-isomer **234** to give analogous result (Scheme 117b).



Scheme 117. Inversion of the stereochemistry of the hydroxyl group of a) *cis*-isomer **233**; b) *trans*-isomer **234**.

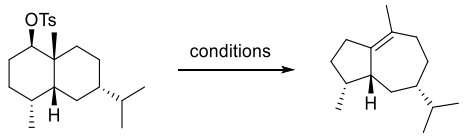
With the two inverted alcohols **250** and **251** in hand, the ring rearrangement step was attempted again, starting with tosylation. Following the conditions established for the hydroxyl epimers, 96% isolated yield was achieved for both isomers (Scheme 118).



Scheme 118. Activation of the hydroxyl group in **250** and **251** ready for ring rearrangement.

With the desired leaving group installed, conditions for the ring rearrangement were assessed with the *cis*-substrate **254**. Ratcliffe reported the stereoselective synthesis of α-bulnesene **74**

by rearrangement of a similar structure using solvolysis.[172] Inspired by this procedure, tosylate **254** was solvolysed in buffered acetic acid at 80 ℃ for 7 h, giving an inseparable mixture of hydrocarbon compound **245** and traces of unidentified isomers (Table 13, Entry 1). Changing the solvent to 80% aqueous ethanol resulted in the same products but with a higher percentage of product **245** (Entry 2). Using HFIP as solvent and heating to 70 ℃ led to decomposition of starting material, as did reducing the reaction time to 5 h (Entry 3, 4) and reducing the reaction temperature to RT led to no improvement (Entry 5).



254 **245**

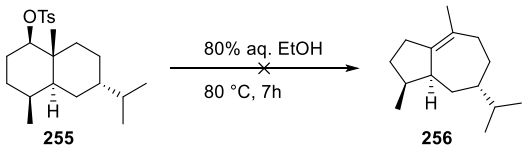
Entry	Solvent	Reaction time	Temperature	Outcome
1	5 % KOAc in AcOH	7 h	80 ℃	20% **245**
2	80% aq. EtOH	7 h	80 ℃	80% **245**
3	HFIP	14 h	70 ℃	Decomposition
4	HFIP	5 h	70 ℃	Decomposition
5	HFIP	5 h	RT	Decomposition

Table 13. Solvolysis conditions assessed with *cis*-substrate **254**.

The isomers formed from these reactions were very non-polar and difficult to purify. $AgNO_3$-impregnated silica was ineffective for purification by column chromatography, but repeated

preparative TLC separation gave 3.0 mg of relatively clean *ent*-dihydrobulnesene **245** (GC spectrum showed little impurity of the other isomer) from 20.0 mg of mixture, which was enough for enzyme screening.

With the conditions screened for *cis*-isomer **254**, the same solvolysis conditions with 80% aq. ethanol was attempted with *trans*-isomer **255** (Scheme 119). However, this reaction resulted in a mixture of non-polar hydrocarbon products that could not be separated. From analysis of the ^{1}H NMR spectra of crude products, traces of ring rearrangement product could be observed, however it was not possible to confirm if the alkene was correctly located. With time being short, and the priority of this project being to compare the contrasting P450$_{BM3}$ hydroxylation profiles of a pair of enantiomers, the solvolysis with *trans*-isomer **255** was not investigated further.



Scheme 119. Ring rearrangement with *trans*-isomer **255**.

Preparation of dihydrobulnesene and its oxidation with P450$_{BM3}$ mutants

The synthetic route developed so far naturally generated the dihydro derivative **245** of *ent*-α-bulnesene, and the route which protected the terminal alkene as an epoxide proved to significantly reduce product yield. Therefore, further optimisation on this route was considered too time consuming, and priority was given to comparing the hydroxylation of compound **245** with its enantiomer, derived from natural α-bulnesene. Additionally, it was

unlikely that the terminal alkene would have a fundamental impact on the enzymatic oxidation profile. Interestingly, a further literature review revealed that dihydrobulnesene is also a naturally occurring compound, temporarily named apitonene-1 **257**, with no stereochemistry specified, and is extracted from the resin of Apitong timber (presumed to be *Dipterocarpus gracilis*) (Figure 22).[234]

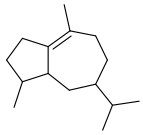
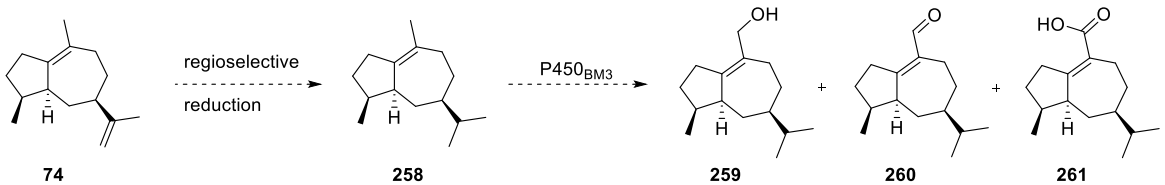


Figure 22. Apitonene-1 **257**.

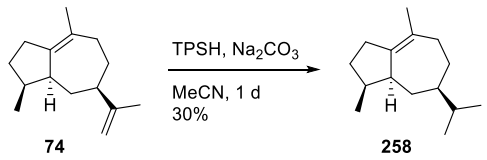
Therefore, screening and comparing the results from the two enantiomers of apitonene-1 **257** would be worth investigating. With synthetic *ent*-dihydrobulnesene **245** in hand, access to dihydrobulnesene **258** itself remained the only step before enzymatic screening could be undertaken. With the experience of the synthetic chemistry developed so far, selective reduction of the terminal alkene of α-bulnesene **74** was expected to be straightforward (Scheme 120).



Scheme 120. Envisaged dihydrobulnesene **258** formation and its oxidation with P450 mutants.

Obtaining pure α-bulnesene **74** is somewhat troublesome as it must be separated from commercially available patchouli oil, and the various terpenes present in this essence oil are very non-polar and structurally similar, so their separation by conventional column chromatography or distillation is difficult. Even with $AgNO_3$-impregnated silica, it proved very difficult to acquire pure α-bulnesene **74** this way, with some impurities remaining in the samples; nevertheless, these samples were deemed to be sufficient quality for enzymatic scale-up reactions.

Dihydrobulnesene **258** was then obtained by reducing α-bulnesene **74** with TPSH in acetonitrile, giving a 30% isolated yield (Scheme 121). Compound **258** has a pale woody smell, while α-bulnesene's woody scent is stronger.



Scheme 121. Regioselective reduction of α-bulnesene **74**.

In order to make comparisons between the enantiomers, both dihydrobulnesene **258** and its enantiomer **245** were screened against a small library containing 23 mutants (list provided in Experimental Section), plate AWG and plate AWG*, respectively, where the mutants were initially designed and selected for the regioselective oxidation at C15 position.[235]

From dihydrobulnesene **258**, the screening results with plate AWG showed three products for most mutants after 14 h reaction at RT with representative GC spectra shown below (Figure 23). Only R19/A184I and R19/A82M produced a distinct metabolite. The identity of each

metabolite was then determined by scaling up the enzymatic reactions with mutants R19/A82M, R19/FI/T88L and R19/FI/A184I/V26L following the same reaction conditions as used in the screening. Each of the GC traces corresponded to C15 hydroxylation product **259**, its further oxidation product aldehyde **260** and the derived carboxylic acid **261**; the metabolite generated by R19/A82M was decomposed during column chromatography and remains unidentified (Figure 23). Within this panel, a number of mutants were able to solely generate carboxylic acid **261**, while most of the mutants gave a mixture of alcohol **259** and aldehyde **260**, indicating that selection amongst the three metabolites could potentially be achieved by controlling the reaction time. All three metabolites possess similar pale woody odours that were difficult to differentiate upon smelling. The oxidation profile of dihydrobulnesene **258** with plate AWG is very similar to that of α-bulnesene's, supporting the hypothesis that the isopropenyl double bond has little influence on the oxidation process with P450 mutants.

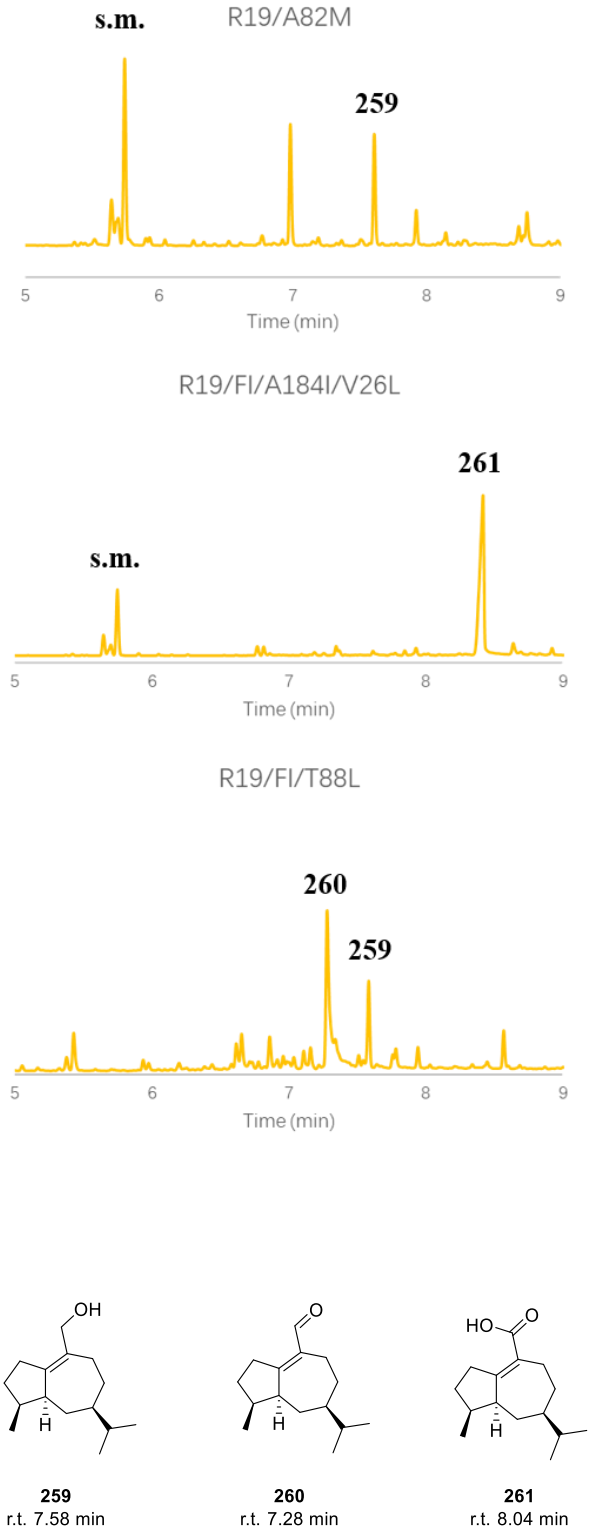
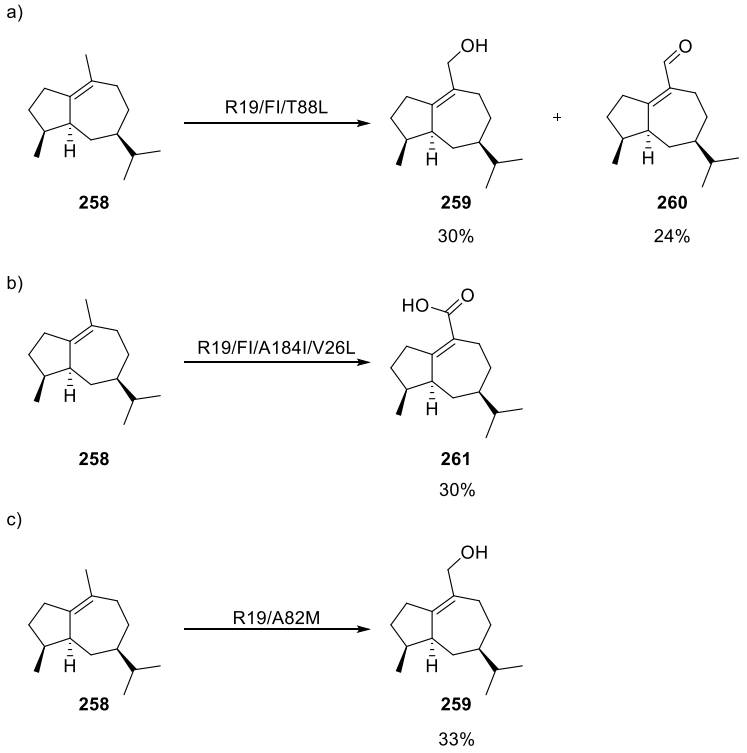


Figure 23. GC traces of selected runs showing selectivity achieved between different metabolites.

For the isolation and characterisation of these metabolites, scale-up reactions were conducted on a 0.16 mmol scale (Scheme 122). Oxidation using R19/FI/T88L furnished alcohol **259** in 30% isolated yield and aldehyde **260** in 24% isolated yield. Reaction with R19/FI/A184I/V26L, resulted in the formation of solely carboxylic acid **261** in 30% isolated yield. Lastly, reaction with mutant R19/A82M gave alcohol **259** in 33% isolated yield and 40% of the unknown metabolite (yield obtained from GC spectrum).



Scheme 122. Preparative scale oxidations of dihydrobulnesene **258**.

Compared to the screening reactions, the preparative scale reactions were generally slower and reached a lower final conversion of substrate. This could be caused by a decrease in pH in the larger-scale reaction leading to enzyme deactivation or less efficient oxygenation, difficulties that can in principle be easily overcome; however, the oxidation profiles of the scale-up reactions remained consistent with those seen during screening.

The study of the enzymatic oxidation of synthetic *ent*-dihydrobulnesene **245** was then examined. As described above, the purification of the ring rearrangement crude was rather troublesome, but 3 mg of fairly clean *ent*-dihydrobulnenesene **245** was obtained and screened against plate AWG*. The GC spectra displayed more metabolites, with six metabolites in total (Figure 24). To identify each metabolite, scaled-up enzymatic reactions were carried out with mutants R19/A82M, R19/FI/V26L and R19/FI/A184I/L437M under the same reaction conditions from screening. Upon column chromatography coupled with GC-MS spectra analysis, it was discovered that each TLC spot corresponded to a pair of peaks on GC spectra that could not be further separated by normal columns (Figure 25) and it was hypothesised that each spot represents a pair of isomers of the corresponding alcohol, aldehyde and carboxylic acid.

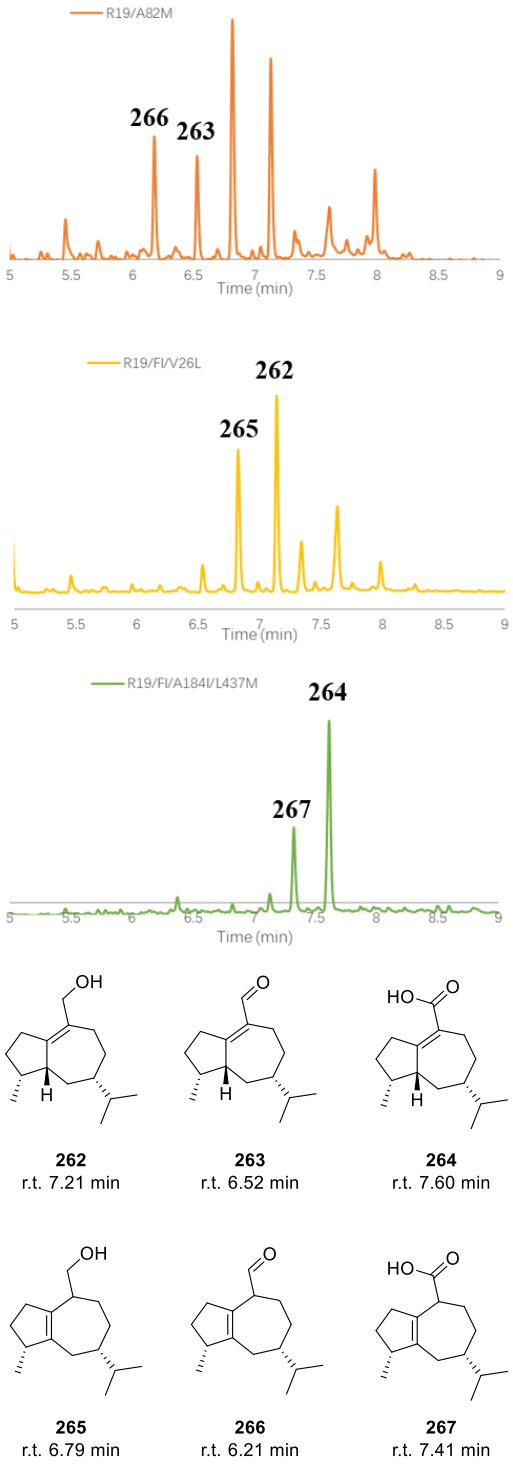


Figure 24. GC traces of selected runs showing selectivity achieved between different metabolites.

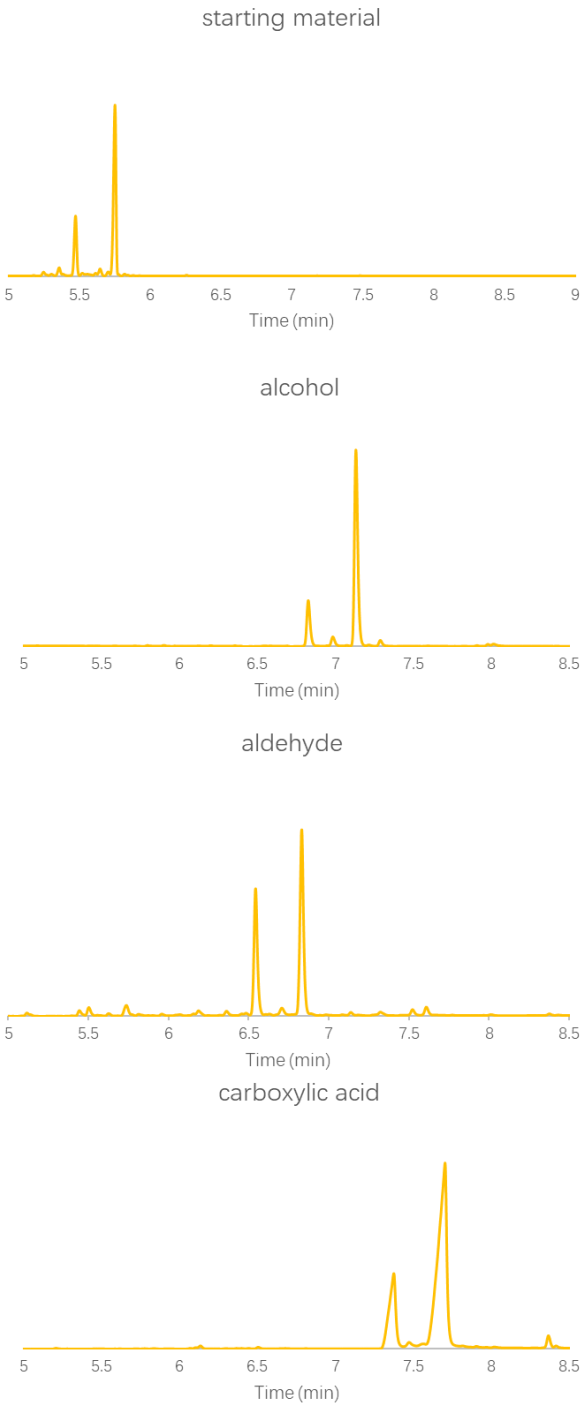


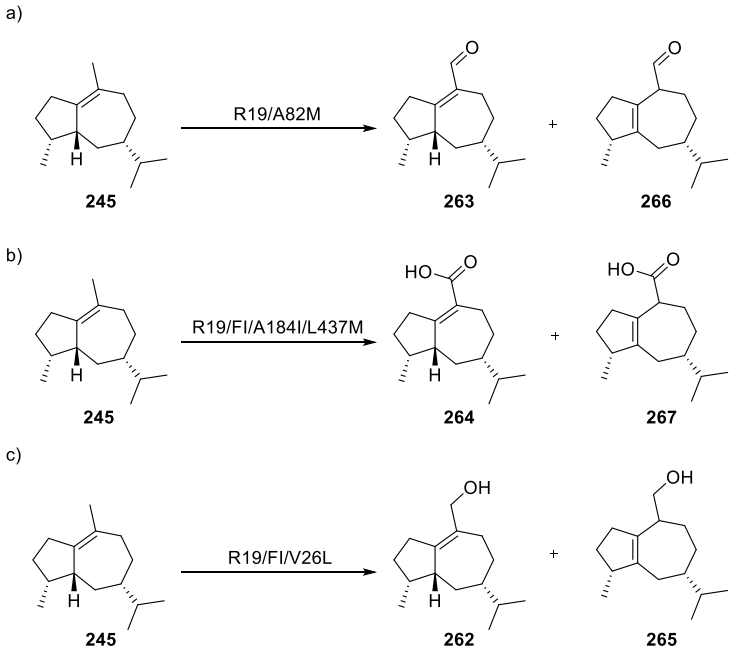
Figure 25. GC spectra of three groups of metabolites.

More *ent*-dihydrobulnesene **245** was prepared and used as a mixture for scale-up reactions due to the difficulties in separation. With these non-polar terpenenoid compounds,

supercritical fluid chromatography (SFC) was considered suitable for separation of the metabolites. Using an analytical SFC column, 8 mg of each mixture from enzymatic reactions was separated. Separation of the two components from mutant R19/FI/A184I/L437M using a DIOL column and 5% MeOH/liquid CO_2 as the mobile phase was successful and allowed the identification of carboxylic acid **264** (Figure 24). The minor isomer was collected in very low quantity due to material loss caused by the fraction collector. From ^{1}H NMR analysis, no alkene proton was observed. Moreover, a dd resonance at 3.04 ppm was observed along with a second dd resonance at 2.95 ppm, from which the structure of **267** was deduced to be as shown in Figure 24. The two aldehyde peaks generated from mutant R19/A82M were separated with a CN column with 10% MeOH/liquid CO_2 as the mobile phase, and the major peak identified as aldehyde **263**. Lastly, separation of the R19/FI/V26L hydroxylation products was achieved with a C18 column with 3% MeOH/liquid CO_2 as the mobile phase, and the major product identified as alcohol **262**. Unfortunately, although all the metabolites were separated, the minor alcohol and aldehyde products were lost during SFC separation due to material leakage from the fraction collector. Based on the identification of carboxylic acid **267**, it was hypothesised that the alcohol and aldehyde are most likely the corresponding alcohol **265** and aldehyde **266** (Figure 24).

For the isolation and characterisation of these metabolites, scale-up reactions were conducted on a 0.3 mmol scale (Scheme 123) by shaking the reaction mixtures at RT for 6 h. Compared to the screening reactions, the preparative scale reactions were surprisingly rapid; the formation of aldehyde and carboxylic acid metabolites occurred before starting material was consumed so the reaction time was reduced to maintain a spread of metabolites, however this

meant that some starting material remained for the R19/FI/V26L and R19/A82M reactions. The difference in outcomes from screening and preparative scale reactions is likely due to larger air contact.



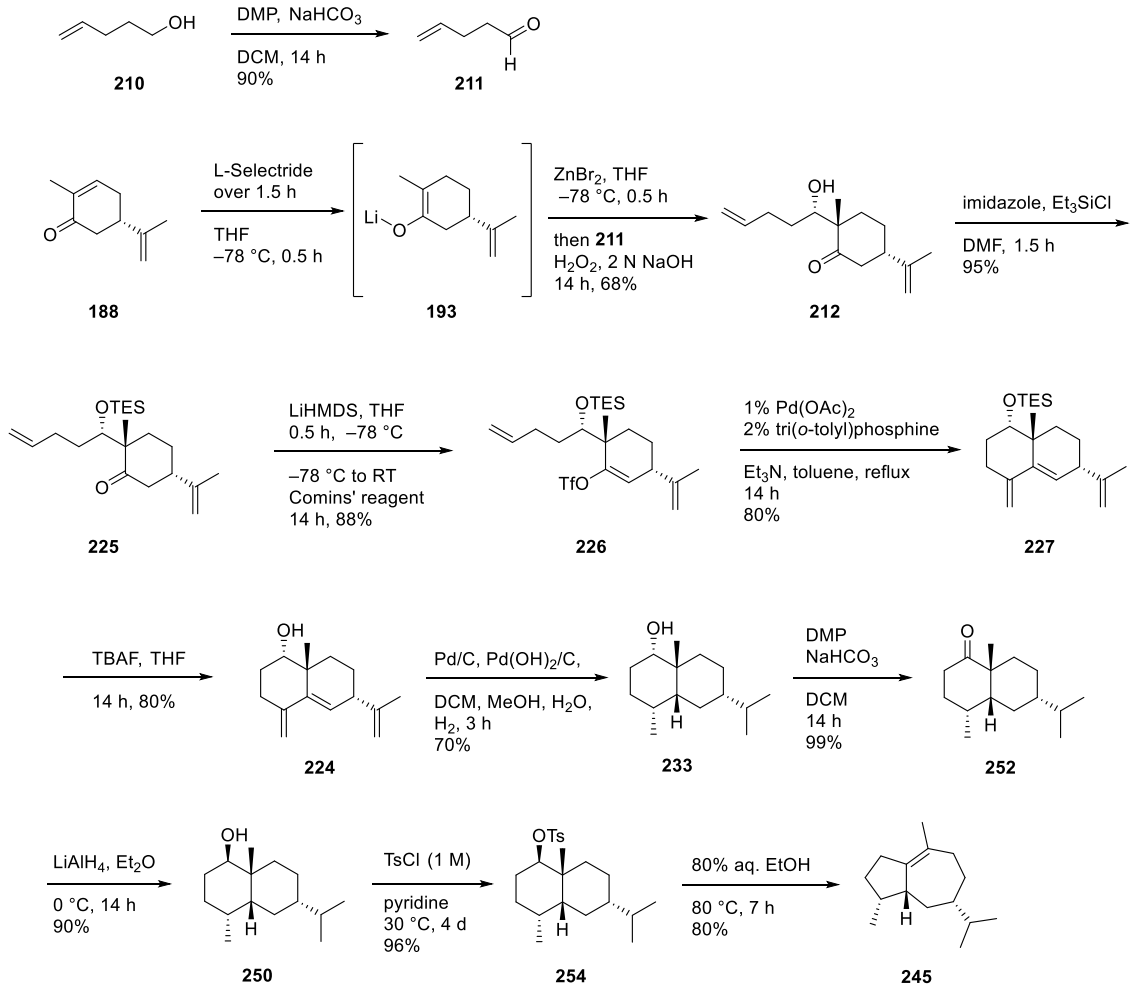
Scheme 123. Preparative scale oxidations of *ent*-dihydrobulnesene **245**.

Comparing the screening reactions of the two enantiomers, **245** and **258**, against plate AWG, for this particular structure of apitonene-1 **257**, the vinyl methyl group is the dominant factor as the hydroxylation of C15 and its further oxidation products are the key components in both cases. These hydrocarbon substrates lack polar functionality and are likely not strongly oriented within the enzymes' active site and therefore the chemical activation and steric accessibility of this methyl group leads to reaction solely at that position. The oxidation products of both enantiomers possess notable woody smells that are potentially worth

investigating for fragrance value, but the small quantities of metabolites produced during this project meant that it was not possible to properly distinguish and describe their scents.

Conclusion and future work

A short enantiospecific route to *ent*-dihydrobulnesene **245** has been developed starting from commercially available (*S*)-(+)-carvone **188** which would be equally applicable to dihydrobulnesene itself from (*R*)-(−)-carvone (Scheme 124).



Scheme 124. Total synthesis of *ent*-dihydrobulnesene **245**.

The separation of these very non-polar sesquiterpenes with both column chromatography and SFC has been extensively studied. The results obtained from both enantiomers, dihydrobulnesene **258** and *ent*-dihydrobulnesene **245**, through $P450_{BM3}$-mediated oxidation, suggest that the dominant activity of these types of structure is hydroxylation of the C15 methyl group and subsequent oxidation to the aldehyde and carboxylic acid. The metabolites all possess pale woody smells worthy of investigation. Future studies should investigate the enantiomers' hydroxylation with more general panels of P450 mutants in search of other interesting metabolites, and obtain enough materials for a complete study of their fragrance profiles.

Experimental Section

General Experimental

Unless specified otherwise, all reactions were carried out under nitrogen atmosphere in oven-dried glassware. Solvents were either used as commercially supplied or as purified by standard techniques. Tetrahydrofuran was freshly distilled over sodium and benzophenone prior to use. Toluene, DCM, DME, 1,4-dioxane and DMF were obtained from the communal stills of the Chemistry Research Laboratory, University of Oxford unless otherwise stated. All other reagents were used as obtained from the supplier.

Thin-layer chromatography (TLC) was performed on Merck DC-Alufolien 60 F254 0.2 mm pre-coated plates, and visualised with ultraviolet light (λmax = 254 nm), basic $KMnO_4$, vanillin stain, or phosphomolybdic acid (PMA) stain. Column chromatography was performed on Merck Geduran® 60 SiO_2 (40 – 60 μm) unless otherwise stated. Retention factors (R_f) of each product are quoted with the solvent system used for TLC.

Proton (^1H) and carbon (^{13}C) NMR spectra were recorded using Bruker AVG-400 (400/100 MHz), Bruker AVH-400 (400/100 MHz), Bruker AVB400 (400/100 MHz) or Bruker AVB500 (500/125 MHz) using chloroform-*d* ($CDCl_3$) as solvent. Proton chemical shifts (δH) are quoted in ppm to the nearest 0.01 ppm and carbon chemical shift (δC) are quoted in ppm to the nearest 0.1 ppm relative to tetramethylsilane (δ = 0) and referenced to the solvent residual, with signal splitting recorded as singlet (s), doublet (d), triplet (t), quartet (q), septet (sept.), multiplet (m) and broad singlet (br s). Coupling constants (*J*) are measured in Hz to the nearest 0.1 Hz. Assignments were recorded as follows: ppm (multiplicity, coupling

constants, number of protons, assignment). COSY, HSQC and NOESY experiments were carried out if necessary to aid assignment. For known compounds, ^{1}H and ^{13}C spectroscopic data were found to be consistent with the literature. Atom numbering reflects NMR peak assignments and does not necessarily correspond to IUPAC nomenclature.

Low resolution mass spectrometry was recorded on a Micromass LCT Premier spectrometer (ES). High resolution mass spectra were recorded on a Bruker μTOF mass spectrometer. High resolution values are calculated to 4 decimal places from the chemical formula with all found values being within a tolerance of 5 ppm. Gas chromatographic analysis was performed on a Thermo Finnigan TRACE gas chromatograph equipped with a flame ionisation detector (FID) and an AS3000 autosampler on a DB-1 capillary GC column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). The injector temperature was 280 ℃ and the FID was at 250 ℃. Helium was used as the carrier gas at a flow rate of 1.5 mL min^{-1}.

Infrared spectra were recorded on a Bruker Tensor 27 Fourier transform spectrometer as a thin film on a diamond ATR module. Absorption maxima (ν_{max}) are quoted in wavenumbers (cm^{-1}) and identified as broad (br), strong (s), medium (m), or weak (w) as a measure of peak intensity. Melting points were measured using Griffin melting point apparatus, and recorded in degrees Celsius (℃).

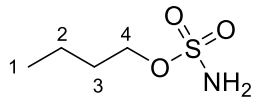
Supercritical fluid chromatographic analysis and separation were performed on a Shimazu Nexera UC chromatograph system coupled with MS-2020 using APCI source (SFE-SFC-MS system) equipped with Silica, 2-PIC, 1-AA, DEA, CN, C-18 or DIOL columns (4.6 mm, 150 mm, 6 μ) from the organic lab in Oxford Suzhou Centre for Advanced Research (OSCAR).

Gas chromatography mass spectrometric analysis was performed on a Shimazu GCMS-QP2020 NXCI system equipped with EI source and PCI source from the organic lab in Oxford Suzhou Centre for Advanced Research (OSCAR), helium was used as the carrier gas at a flow rate of 1.5 mL min^{-1}.

Compound names are as generated by ChemBioDraw Ultra 18.2.

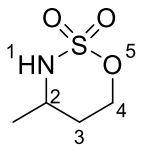
Data for Chapter 2

Butyl sulfamate (134)[236]



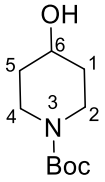
Formic acid (0.23 mL, 6.7 mmol) was added dropwise to newly purchased chlorosulfonyl isocyanate (0.59 mL, 6.7 mmol) at 0 ℃ with rapid stirring. Vigorous gas evolution was observed during the addition process and the mixture was solidified instantly. To the resulting white mass was added 6.0 mL of MeCN, and the mixture was allowed to warm up to RT over 16 h. The mixture was cooled to 0 ℃ and a solution of *n*-butanol (0.20 g, 2.7 mmol) in 4.0 mL of pyridine was added. The reaction mixture was warmed to RT and stirred for 4 h. The reaction was quenched by the addition H_2O (100 mL). The organic layer was collected; the aqueous layer was extracted with 6 × 20 mL of Et_2O. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo.* Flush column chromatography (45% Et_2O/pentane) afforded the product as a yellow solid (0.11 g, 70%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.74 (br s, 2H, N*H*$_2$), 4.23 (t, *J* = 6.5 Hz, 2H, H4), 1.74 (ddt, *J* = 8.9, 7.9, 6.5 Hz, 2H, H3), 1.49 – 1.36 (m, 2H, H2), 0.96 (t, *J* = 7.4 Hz, 3H, H1); **R$_f$** 0.7 (50% Et_2O/pentane).

4-Methyl-1,2,3-oxathiazinane 2,2-dioxide (135)[237]



To a solution of butyl sulfamate **134** (38.3 mg, 0.25 mmol) in 1.6 mL of DCM were added sequentially MgO (23.0 mg, 0.57 mmol), $PhI(OAc)_2$ (89.0 mg, 0.28 mmol), and $Rh_2(OAc)_4$ (3.8 mg, 5%). The suspension was stirred vigorously at 40 ℃ for 14 h. The reaction mixture was filtered through a small pad of $MgSO_4$ and concentrated *in vacuo.* The compounds were separated by Flush column chromatography (30% Et_2O/pentane → 50% Et_2O/pentane) to afford the product as a white solid (21.6 mg, 60%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.79 – 4.63 (m, 1H, H4), 4.59 – 4.45 (m, 1H, H4), 4.02 (br s, 1H, N*H*), 3.93 – 3.75 (m, 1H, H2), 1.81 – 1.67 (m, 2H, H3), 1.28 (d, *J* = 6.5 Hz, 3H, CH_3); **R_f** 0.4 (60% Et_2O/pentane).

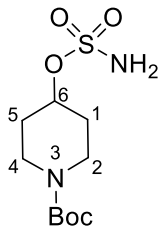
***tert*-Butyl 4-hydroxypiperidine-1-carboxylate (128)**[238]



To a solution of di-*tert*-butyl dicarbonate (1.76 g, 8.0 mmol) in DCM (36.0 mL) was added dropwise a solution of piperidin-4-ol (1.00 g, 9.0 mmol) in DCM (2.0 mL) over 15 min. CO_2 was evolved. Imidazole (760 mg, 11.1 mmol) was then added and the reaction mixture was stirred for 1 h at RT. The reaction mixture was diluted with 10 mL DCM and washed with 10 mL 1% HCl solution. The aqueous layer was extracted with DCM (2 × 10 mL). The combined

organic layers were washed with cold 1% HCl solution (100 mL), and the aqueous layer was extracted with DCM (2 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude product as a white solid (1.39 g, 86%), which was used without further purification. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 3.87 – 3.81 (m, 3H, H2, H4, H6), 3.05- 2.99 (m, 2H, H2, H4), 1.90 – 1.75 (m, 2H, H1, H5), 1.45 (m, 11H, H1, H5, C*H*$_3$); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 154.9, 79.6, 67.5, 41.3, 34.2, 28.5; **R_f** 0.5 (100% EtOAc).

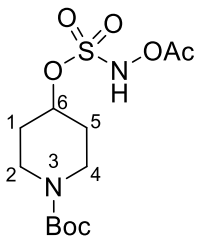
***tert*-Butyl 4-(sulfamoyloxy)piperidine-1-carboxylate (137)**



Formic acid (0.28 mL, 7.5 mmol) was added dropwise to newly purchased chlorosulfonyl isocyanate (0.65 mL, 7.5 mmol) at 0 ℃ with rapid stirring. Vigorous gas evolution was observed during the addition process and the mixture solidified instantly. To the resulting white mass was added 6.0 mL of MeCN, and the reaction mixture was allowed to warm up to RT over 16 h. The reaction mixture was cooled to 0 ℃ and a solution of alcohol **128** (0.60 g, 3.0 mmol) in 5.0 mL of DMA was added. The reaction mixture was warmed to RT and stirred for 4 h. The reaction was quenched by the addition of 100 mL of H_2O. The organic layer was collected; the aqueous layer was extracted with 6 × 20 mL of Et_2O. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. Flush column chromatography (50%

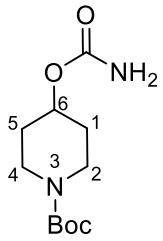
Et_2O/pentane → 80% Et_2O/pentane) afforded the product as a yellow solid (0.63 g, 75%). **IR** (film) ν_{max}/cm^{-1}: 3271br, 2974w, 2928w, 1668s, 1431m, 1171s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ:4.83 – 4.71 (m, 3H, H6, NH_2), 3.71 – 3.63 (m, 2H, H2, H4) 3.37 – 3.27 (m, 2H, H2, H4), 2.01 – 1.92 (m, 2H, H1, H5), 1.85 – 1.79 (m, 2H, H1, H5), 1.46 (s, 9H, CH_3); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 154.6, 80.0, 79.0, 44.2, 31.2, 28.4; **HRMS (ESI)** exact mass calculated for $[M+H]^+$($C_{10}H_{19}O_5N_2S$) requires *m/z* 279.1020, found *m/z* 279.1019; **M.p.**: 130 ℃**; R$_f$** 0.5 (50% Et_2O/pentane).

***tert*-Butyl 4-((*N*-acetoxysulfamoyl)oxy)piperidine-1-carboxylate (138)**



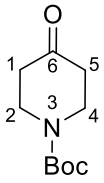
To a solution of sulfamate **137** (18) (59.6 mg, 0.20 mmol) in 1.6 mL of DCM were added sequentially MgO (9.9 mg, 0.24 mmol), $PhI(OAc)_2$ (146 mg, 0.46 mmol), and $Rh_2(OAc)_4$ (7.6 mg, 5%). The suspension was stirred vigorously at 40 ℃ for 14 h. The reaction mixture was filtered through a small pad of $MgSO_4$ and concentrated *in vacuo.* The compounds were separated by Flush column chromatography (30% Et_2O/pentane → 50% Et_2O/pentane) to afford the product as a white solid (10.0 mg, 20%). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ: 4.66 – 4.50 (m, 1H, H6), 3.68 – 3.55 (m, 2H, H2, H4), 3.56 – 3.49 (m, 2H, H2, H4), 2.50 – 2.45 (m, 2H, H1, H5), 2.09 (s, 3H, CH_3), 2.02 – 1.98 (m, 2H, H1, H5), 1.47 (s, 9H, CH_3); **R$_f$** 0.6 (30% EtOAc/pentane).

tert-Butyl 4-(carbamoyloxy)piperidine-1-carboxylate (140)



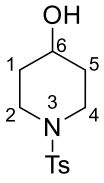
To a solution of alcohol **128** (1.28 g, 6.37 mmol) in dry Et_2O (20.0 mL) at 0 ℃ was added dropwise trichloroacetyl isocyanate (820 μL, 6.90 mmol). The mixture was allowed to warm to RT over 16 h. The reaction mixture was then pipetted onto a basic alumina pad and allowed to stand for 30 min. The alumina pad was flushed with Et_2O (100 mL) and EtOAc (100 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Flush column chromatography (50% Et_2O/pentane → 80% Et_2O/pentane) afforded the product as a white solid (1.42 g, 92%). **IR** (film) ν_{max}/cm^{-1}: 3390br, 2974w, 1716s, 1678m, 1431s, 1121s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.80-4.75 (m, 1H, H6), 4.58 (br s, 2H, NH_2), 3.73-3.68 (m, 2H, H2, H4), 3.18-3.06 (m, 2H, H2, H4), 1.93 – 1.83 (m, 2H, H1, H5), 1.61-1.56 (m, 2H, H1, H5), 1.46 (s, 9H, CH_3); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 156.0, 79.7, 70.7, 44.1, 30.9, 28.4; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{11}H_{21}O_4N_2$) requires *m/z* 245.1501, found *m/z* 245.1502; **M.p.**: 145 ℃ , **R_f** 0.8 (70% EtOAc/pentane).

tert-Butyl 4-oxopiperidine-1-carboxylate (141)[239]



To a solution of carbamate **140** (59.6 mg, 0.20 mmol) in 1.6 mL of DCM were added sequentially MgO (9.9 mg, 0.25 mmol), $PhI(OAc)_2$ (146 mg, 0.46 mmol), and $Rh_2(OAc)_4$ (7.5 mg, 5%). The suspension was stirred vigorously at 40 ℃ for 14 h. The reaction mixture was filtered through a small pad of $MgSO_4$ and concentrated *in vacuo.* The compounds were separated by Flush column chromatography (30% Et_2O/pentane → 50% Et_2O/pentane) to afford the product as a colourless liquid (8 mg, 20%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 3.71 (t, *J* = 6.2 Hz, 4H, H2, H4), 2.43 (t, *J* = 6.2 Hz, 4H, H1, H5), 1.48 (s, 9H, C*H*$_3$); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 207.1, 156.7, 79.8, 43.6, 40.7, 28.4; **R_f** 0.5 (50% Et_2O/pentane).

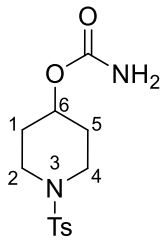
1-Tosylpiperidin-4-ol (144)[240]



To a solution of 4-hydroxypiperidine **136** (202 mg, 2.0 mmol) in H_2O (5.0 mL) was added *p*-toluenesulfonyl chloride (608 mg, 3.2 mmol) and Na_2CO_3 (275 mg, 2.6 mmol), followed by the addition of 15.0 mL of Et_2O. The reaction mixture was stirred at RT for 14 h. The reaction mixture was separated and the organic layer was concentrated *in vacuo.* Flush column

chromatography (25% EtOAc/pentane → 100% EtOAc) afforded the product as a colourless liquid (270 mg, 89%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.66 – 7.49 (m, 2H, H_{Ar}), 7.31 – 7.21 (m, 2H, H_{Ar}), 3.69 (q, *J* = 3.8 Hz, 1H, H6), 3.24 (ddd, *J* = 11.4, 7.2, 3.8 Hz, 2H, H2, H4), 2.78 (ddd, *J* = 11.8, 8.2, 3.6 Hz, 2H, H2, H4), 2.36 (s, 3H, C*H*$_3$), 1.92 – 1.74 (m, 2H, H1, H5), 1.63 – 1.55 (m, 2H, H1, H5); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 143.5, 133.2, 129.6, 127.6, 65.9, 43.1, 33.2, 21.5; **R_f** 0.3 (60% Et_2O/pentane).

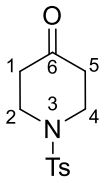
1-Tosylpiperidin-4-yl carbamate (145)



To a solution of alcohol **144** (270 mg, 1.2 mmol) in dry Et_2O (4.0 mL) at 0 ℃ was added dropwise trichloroacetyl isocyanate (150 μL, 1.3 mmol). The mixture was allowed to warm up to RT over 16 h. The reaction mixture was then pipetted onto a basic alumina pad and allowed to stand for 30 min. The alumina pad was flushed with Et_2O (30 mL) and EtOAc (30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Flush column chromatography (50% Et_2O/pentane) afforded the product as a white solid (349 mg, 96%). **IR** (film) ν_{max}/cm^{-1}: 3350br, 2930w, 2856w, 1716s, 1246s, 1163s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.65 (d, *J* = 8.3 Hz, 2H, Ar-*H*), 7.38 – 7.30 (m, 2H, Ar-*H*), 4.65 (tt, *J* = 7.8, 3.8 Hz, 1H, H6), 4.53 (br s, 2H, N*H*$_2$), 3.29 (ddd, *J* = 11.5, 7.2, 4.0 Hz, 2H, H2, H4), 2.88 (ddd, *J* = 11.9, 8.3, 3.6 Hz, 2H, H2, H4), 2.02 (dtd, *J* = 12.3, 8.1, 3.8, 2H, H1 H5), 1.77

(dtd, *J* = 12.3, 8.1, 3.8 Hz, 2H, H1, H5); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 156.4, 143.6, 133.2, 129.7, 127.6, 69.1, 43.4, 30.2, 21.5; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{13}H_{19}O_4N_2S$) requires *m/z* 299.1060, found *m/z* 299.1059; **M.p.**: 147 ℃; **R_f** 0.3 (50% EtOAc/pentane).

1-Tosylpiperidin-4-one (146)[241]



Method A

To a solution of carbamate **145** (59.6 mg, 0.20 mmol) in 1.6 mL of toluene were added sequentially MgO (9.9 mg, 0.24 mmol), $PhI(OAc)_2$ (146 mg, 0.46 mmol), and $Rh_2(OAc)_4$ (7.6 mg, 5%). The suspension was stirred vigorously at 40 ℃ for 14 h. The reaction mixture was filtered through a small pad of $MgSO_4$ and concentrated *in vacuo.* The compounds were separated by Flush column chromatography (30% Et_2O/pentane → 50% Et_2O/pentane) to afford the product as a colourless liquid (36.4 mg, 60%).

Method B

To a solution of (tosyloxy)carbamate **148** (47 mg, 0.10 mmol) in EtOAc (2.0 mL) was added $Rh_2(tpa)_4$ (15 mg, 1%) and sat. K_2CO_3 solution (20 μL, 0.15 mmol). The reaction mixture was heated at 40 ℃ for 14 h. The reaction mixture was filtered through a small pad of $MgSO_4$ and

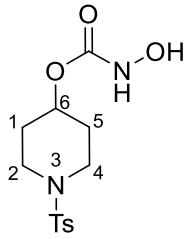
concentrated *in vacuo.* The compounds were separated by Flush column chromatography (50% EtOAc/DCM) to afford the product as a colourless liquid (13 mg, 55%).

Method C

Into a pressure tube was added azidoformate **158** (32.4 mg, 0.1 mmol), and 1.7 mL DCM. The solution was heated at 140 ℃ for 1 h, protected with a blast shield. The reaction mixture was concentrated *in vacuo* and the residue was purified by preparative thin layer chromatography (5% MeOH/DCM) to give the product as a colourless liquid (6.0 mg, 20%).

Data: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.68 (d, *J* = 8.3 Hz, 2H, Ar-*H*), 7.39 – 7.29 (d, 2H, Ar-*H*), 3.38 (t, *J* = 6.2 Hz, 4H, H2, H4), 2.53 (t, *J* = 6.2 Hz, 4H, H1, H5), 2.44 (s, 3H, C*H*$_3$); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 207.1, 143.3, 137.6, 129.3 3, 128.3 3, 70.7, 39.9, 21.4; **R$_f$** 0.6 (50% EtOAc/pentane).

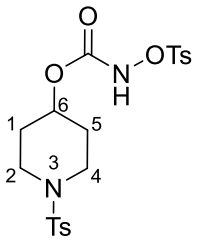
1-Tosylpiperidin-4-yl hydroxycarbamate (147)



A solution of carbonyl diimidazole (1.01 g, 6.25 mmol) in toluene (50.0 mL) was heated to 60 ℃. 1-Tosylpiperidin-4-ol **144** (1.27 g, 5.00 mmol) was added to the solution followed by addition of 5.0 mL of THF. The solution was stirred at this temperature for 14 h. after cooling down to RT, hydroxylamine solution (600 μL, 10.00 mmol) was added to the reaction mixture and stirred for 6 h. The reaction mixture was concentrated *in vacuo.* Flush column

chromatography (30% EtOAc/DCM) afforded the product as a white solid (1.00 g, 75%). **IR** (film) ν_{max}/cm^{-1}: 3323br, 2926w, 2856w, 1720m, 1493w, 1330m, 1162s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.62 – 7.47 (m, 2H, H_{Ar}), 7.39 – 7.23 (m, 2H, H_{Ar}), 6.06 (br s, 1H, N*H*), 4.69 (tt, *J* = 7.6, 3.7 Hz, 1H, H6), 3.18 (ddd, *J* = 11.5, 7.3, 3.8 Hz, 2H, H2, H4), 2.84 (ddd, *J* = 11.9, 8.1, 3.7 Hz, 2H, H2. H4), 2.38 (s, 3H, CH_3), 1.97 – 1.85 (m, 2H, H1, H5), 1.73 (dtd, *J* = 13.4, 7.9, 3.8 Hz, 2H, H1, H5); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 154.1, 143.4, 134.8, 129.8, 127.4, 68.9, 41.0, 29.8, 21.5; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{13}H_{19}O_5N_2S$) requires *m/z* 315.1009, found *m/z* 315.1010; **M.p.**: 144 ℃; **R_f** 0.3 (30% EtOAc/DCM).

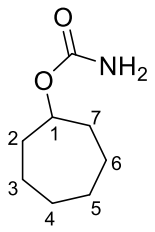
1-Tosylpiperidin-4-yl (tosyloxy)carbamate (148)



To a solution of *N*-hydroxycarbamate **147** (314 mg, 1.0 mmol) in Et_2O (10.0 mL) at 0 ℃, was added *p*-toluenesulfonyl chloride (210 mg, 1.1 mmol). Et_3N (140 μL, 1.0 mmol) was then added slowly and the resulting white suspension was stirred for 12 h at RT. The reaction mixture was washed with H2O (10 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo.* Flush column chromatography (30% EtOAc/DCM) afforded the product as a white oil (470 mg, 95%). **IR** (film) ν_{max}/cm^{-1}: 3300br, 2928w, 2856w, 1715s, 1330m, 1161s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.80 (d, *J* = 8.4 Hz, 2H, Ar-*H*), 7.63

(d, *J* = 8.3 Hz, 2H, Ar-*H*), 7.37 – 7.33 (m, 4H, Ar-*H*), 4.72 – 4.59 (m, 1H, H6), 3.12 (ddd, *J* = 11.8, 7.9, 3.7 Hz, 2H, H2, H4), 2.92 (ddd, *J* = 11.8, 7.5, 3.8 Hz, 2H, H2, H4), 2.46 (s, 3H, C*H*$_3$), 2.44 (s, 3H, C*H*$_3$), 1.87 (dtd, *J* = 13.5, 7.0, 3.7 Hz, 2H, H1, H5), 1.63 (dtd, *J* = 13.5, 7.0, 3.7 Hz, 2H, H1, H5); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 156.0, 146.8, 143.2, 138.2, 137.6, 130.3, 129.3, 128.3, 126.7, 70.5, 45.2, 28.8, 21.5, 21.4; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{13}H_{19}O_4N_2$ ^{35}Cl) requires *m/z* 469.1103, found *m/z* 469.1101; **R$_f$** 0.24 (30% EtOAc/DCM).

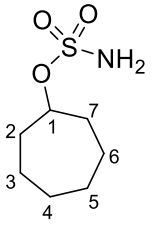
Cycloheptyl carbamate (150)



To a solution of cycloheptanol (1.37 g, 12.0 mmol) in dry Et$_2$O (40.0 mL) at 0 ℃ was added dropwise trichloroacetyl isocyanate (1.55 mL, 13.0 mmol). The mixture was allowed to warm up to RT over 16 h. The reaction mixture was then pipetted onto a basic alumina pad and allowed to stand for 30 min. The alumina pad was flushed with Et$_2$O (200 mL) and EtOAc (200 mL). The combined organic layers were dried over MgSO$_4$ and concentrated *in vacuo*. Flush column chromatography (50% Et$_2$O/pentane) afforded the product as a white solid (1.00 g, 50%). **IR** (film) ν_{max}/cm^{-1}: 3416w, 3326br, 2926m, 2857w, 176009w, 1678s, 1443m, 1040s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.80 (tt, *J* = 8.2, 4.5 Hz, 1H, H1), 4.54 (br s, 2H, N*H*$_2$), 1.98 – 1.88 (m, 2H, H2, H7), 1.80 – 1.29 (m, 10H, alkyl-*H*); **^{13}C NMR** (101 MHz,

Chloroform-*d*) δ: 219.5, 76.0, 34.0, 33.6, 28.2, 22.7, 22.5, 21.1; **HRMS (ESI)** exact mass calculated for $[M+ Na]^+$ ($C_8H_{15}O_2NNa$) requires *m/z* 180.0995, found *m/z* 180.0997; **M.p.**: 88 ℃; $\mathbf{R_f}$ 0.5 (30% EtOAc/pentane).

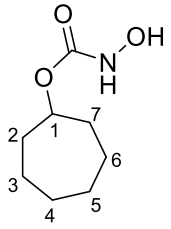
Cycloheptyl sulfamate (151)



Formic acid (940 μL g, 25.0 mmol) was added dropwise to newly purchased chlorosulfonyl isocyanate (2.15 mL, 25.0 mmol) at 0 ℃ with rapid stirring. Vigorous gas evolution was observed during the addition process and the mixture solidified instantly. To the resulting white mass was added 10.0 mL of MeCN, and the mixture was allowed to warm up to RT over 16 h. The mixture was cooled to 0 ℃ and a solution of cycloheptanol (1.14 g, 10.0 mmol) in 35.0 mL of DMA was added. The reaction mixture was warmed to RT and stirred for 6 h. The reaction was quenched by the addition of 100 mL of H_2O. The organic layer was collected; the aqueous layer was extracted with 5 × 20 mL of Et_2O. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. Flush column chromatography (30% EtOAc/pentane) afforded the product as a yellow oil (1.35 g, 70%). **IR** (film) ν_{max}/cm^{-1}: 3146br, 2926w, 2858w, 1537m, 1305s, 1266s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.86 – 4.68 (m, 3H, N*H*$_2$, H1), 2.13 – 2.00 (m, 2H, H2, H7), 1.89 – 1.80 (m, 2H, H2, H7), 1.79 –

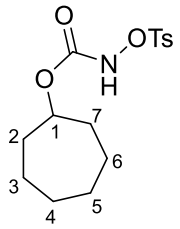
1.64 (m, 2H, H3, H6), 1.57 – 1.35 (m, 6H, alkyl-*H*); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 84.1, 35.6, 32.1, 29.1, 27.4, 23.5, 22.3; **R_f** 0.8 (50% EtOAc/pentane).

Cycloheptyl hydroxycarbamate (S1)



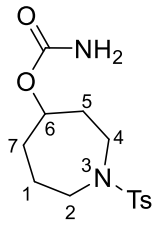
A solution of carbonyl diimidazole (20.3 g, 12.5 mmol) in toluene (100.0 mL) was heated to 60 ℃. Cycloheptanol (1.14 g, 10.0 mmol) was added to the solution followed by addition of 10.0 mL of THF. The solution was stirred at this temperature for 14 h. after cooling down to RT, hydroxylamine solution (1.27 mL, 20.0 mmol) was added to the reaction mixture and stirred for 6 h. The reaction mixture was concentrated *in vacuo.* Flush column chromatography (30% EtOAc/DCM) afforded the product as a white solid (1.19 g, 69%). **IR** (film) ν_{max}/cm^{-1}: 3328br, 2928m, 2857m, 1672s, 1508s, 1318s, 1132s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.08 (br s, 1H, N*H*), 6.43 (s, 1H, O*H*), 4.92 (tt, *J* = 8.2, 4.5 Hz, 1H, H1), 2.02 – 1.76 (m, 2H, H2, H7), 1.76 – 1.27 (m, 10H, alkyl-*H*); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 154.1, 76.3, 35.6, 33.1, 29.1, 27.4, 25.5, 22.3; **HRMS (ESI)** exact mass calculated for $[M+ Na]^+$ ($C_8H_{15}O_3NNa$) requires *m/z* 196.0944, found *m/z* 196.0946; **M.p.**: 92 ℃; **R_f** 0.4 (30% EtOAc/DCM).

Cycloheptyl(tosyloxy)carbamate (152)



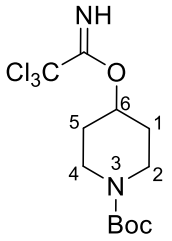
To a solution of hydroxycarbamate **S1** (98.1 mg, 0.30 mmol) in Et_2O (2.0 mL) at 0 ℃, was added *p*-toluenesulfonyl chloride (62.7 mg, 0.33 mmol). Et_3N (41 μL, 0.30 mmol) was then added slowly and the resulting white suspension was stirred for 12 h at RT. The reaction mixture was washed with H2O (5 mL) and brine (5 mL), dried over $MgSO_4$ and concentrated *in vacuo.* Flush column chromatography (30% EtOAc/DCM) afforded the product as a colourless oil (86.0 mg, 90%). **IR** (film) ν_{max}/cm^{-1}: 3275br, 2929m, 2860w, 1761m, 1734m, 1597m, 1382s, 1213s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.90 – 7.85 (m, 2H, H_{Ar}), 7.66 (s, 1H, N*H*), 7.38 – 7.32 (m, 2H, H_{Ar}), 4.76 (tt, *J* = 8.0, 4.5 Hz, 1H, H1), 2.46 (s, 3H, CH_3), 1.80 – 1.69 (m, 2H, H2, H7), 1.71 – 1.29 (m, 10H, alkyl-*H*); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 157.6, 145.2, 131.6, 129.9, 128.2, 76.0, 35.6, 33.1, 29.1, 27.4, 24.5, 22.3, 21.5; **HRMS (ESI)** exact mass calculated for $[M+ Na]^+$ ($C_{15}H_{21}O_5NNa$) requires *m/z* 350.1032, found *m/z* 350.1032; **R_f** 0.8 (30% EtOAc/DCM).

1-Tosylazepan-4-yl carbamate (153)



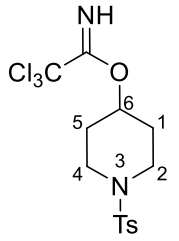
To a solution of alcohol **169** (520 mg, 1.94 mmol) in dry Et_2O (7.0 mL) at 0 ℃ was added dropwise trichloroacetyl isocyanate (395 mg, 2.09 mmol). The mixture was allowed to warm up to RT over 16 h. The reaction mixture was then pipetted onto a basic alumina pad and allowed to stand for 30 min. The alumina pad was flushed with Et_2O (15 mL) and EtOAc (10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Flush column chromatography (50% Et_2O/pentane) afforded the product as a white solid (460 mg, 76%). **^{1}H NMR** (400 MHz, Chloroform-*d*): δ 7.68 – 7.62 (m, 2H, H_{Ar}), 7.34 – 7.28 (m, 2H, H_{Ar}), 4.93 (br s, 2H, NH_2), 4.70 –4 .64 (m, 1H, H6), 3.34 – 3.27 (m, 1H, H4), 3.23 – 3.16 (m, 2H, H2, H4), 3.18 – 3.13 (m, 1H, H2), 2.40 (d, J = 1.0 Hz, 3H, CH_3), 2.26 – 2.20 (m, 1H, H5), 2.02 – 1.96 (m, 1H, H7), 1.84 –1.48 (m, 4H, H1, H5, H7); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 157.4, 143.4, 135.1, 129.8, 127.4, 76.4, 45.4, 42.9, 33.4, 29.6, 25.9, 21.5; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{14}H_{20}N_2O_4S$) requires *m/z* 335.1026, found *m/z* 335.1037; **M.p.**: 134 ℃; **R_f** 0.6 (50% EtOAc/pentane).

tert-Butyl 4-(2,2,2-trichloro-1-iminoethoxy)piperidine-1-carboxylate (154)



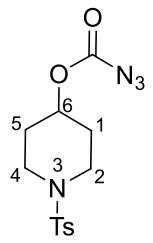
To a stirred solution of alcohol **128** (200 mg, 1.00 mmol) in DCM (2.0 mL) was added trichloroacetonitrile (150 μL, 1.50 mmol) and DBU (15 μL, 0.01 mmol). The resulting reaction mixture was stirred at RT for 16 h. The reaction mixture was diluted with H2O (10 mL), and the aqueous layer was separated and extracted with DCM (3 ×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (3% Et_3N in pentane) afforded the product as a white solid (340 mg, 98%). **IR** (film) ν_{max}/cm^{-1}: 3343br, 2975m, 2870m, 1666s, 1425m, 1170s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 8.32 (s, 1H, N*H*), 5.12-5.06 (m, 1H, H6), 3.68 – 3.30 (m, 4H, H2, H4), 1.99 – 1.75 (m, 4H, H1, H5), 1.46 (s, 9H, CH_3); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 177.6, 154.8, 79.7, 74.0, 67.8, 34.2, 29.6, 28.4; **HRMS (ESI)** exact mass calculated for $[M+ Na]^+$ ($C_{12}H_{19}O_3N_2Cl_3Na$) requires *m/z* 367.0353, found *m/z* 367.0352; **M.p.**: 55 ℃; **R_f** 0.7 (25% EtOAc/pentane).

1-Tosylpiperidin-4-yl 2,2,2-trichloroacetimidate (155)



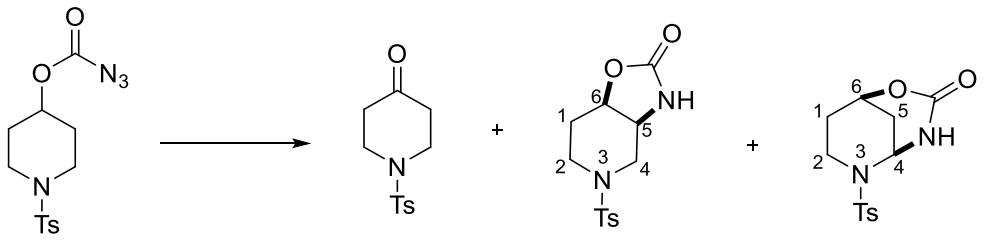
To a stirred solution of 1-tosylpiperidin-4-ol **144** (225 mg, 1.00 mmol) in DCM (2.0 mL) was added trichloroacetonitrile (150 μL, 1.50 mmol) and DBU (15 μL, 0.01 mmol). The resulting reaction mixture was stirred at RT for 16 h, the reaction mixture was diluted with H2O (10 mL), and the aqueous layer was separated and extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (3% Et_3N in pentane) afforded the product as a white solid (300 mg, 75%). **IR** (film) ν_{max}/cm^{-1}: 3331br, 2933m, 2862m, 1665s, 1348m, 1166s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 8.29 (s, 1H, N*H*), 7.65 (d, *J* = 8.3 Hz, 2H, Ar-*H*), 7.36 (d, *J* = 8.3 Hz, 2H, Ar-*H*), 4.96 (p, *J* = 4.8 Hz, 1H, H6), 3.20 (dt, *J* = 10.8, 5.1 Hz, 2H, H2, H4), 3.07 (dt, *J* = 12.2, 6.3 Hz, 2H, H2, H4), 2.44 (s, 3H, C*H*$_3$), 2.01 – 1.99 (m, 4H, H1, H5); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 161.4, 143.6, 132.9, 129.5, 127.7, 72.3, 68.2, 42.6, 28.9, 21.5; **HRMS (ESI)** exact mass calculated for $[M+ Na]^+$ ($C_{14}H_{17}O_3N_2Cl_3NaS$) requires *m/z* 420.9917, found *m/z* 420.9917; **M.p.**: 134 ℃; **R$_f$** 0.7 (25% EtOAc/pentane).

1-Tosylpiperidin-4-yl carbonazidate (158)



To a solution of 1-tosylpiperidin-4-ol **144** (127 mg, 0.5 mmol) in THF (1.5 mL) was added 1,1-carbonyldiimidazole (162 mg, 1.0 mmol) at RT, and the mixture was stirred for 30 min. To this solution were added trimethylsilyl azide (660 μL, 5.0 mmol), EtOAc (1.5 mL) and deionised H_2O (1.5 mL). After being stirred for 1.5 h, the reaction mixture was diluted with H_2O (10 mL), and the aqueous layer was separated and extracted with EtOAc (3 × 10 mL). The organic layers were combined and immediately filtered through a silica gel pad to remove remaining imidazole. Then the filtrate was concentrated *in vacuo*. Flush column chromatography (33% EtOAc/pentane) afforded the product as a white solid (160 mg, 98%). **IR** (film) ν_{max}/cm^{-1}: 2990w, 2191w, 2138w, 1725m, 1349w, 1231s, 1163s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.64 (d, *J* = 8.3 Hz, 2H, Ar-*H*), 7.39 (d, *J* = 8.3 Hz, 2H, Ar-*H*), 4.79 (tt, *J* = 7.2, 3.7 Hz, 1H, H6), 3.19 (ddd, *J* = 11.8, 7.9, 3.7 Hz, 2H, H2, H4), 3.01 (ddd, *J* = 11.7, 7.4, 3.9 Hz, 2H, H2, H4), 2.44 (s, 3H, C*H*$_3$), 2.01 (ddd, *J* = 16.6, 7.6, 3.7 Hz, 2H, H1, H5), 1.86 (dtd, *J* = 14.1, 7.3, 3.7 Hz, 2H, H1, H5); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 156.9, 143.8, 133.2, 129.8, 127.6, 72.9, 42.9, 29.8, 21.5; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{13}H_{17}O_4N_4S$) requires *m/z* 325.0965, found *m/z* 325.0964; **M.p.**: 124 ℃; **R$_f$** 0.8 (100% EtOAc).

Procedure for the thermolysis of 1-tosylpiperidin-4-yl carbonazidate 158



158 **146** **159** **160**

Into a pressure tube was added azidoformate **158** (32.4 mg, 0.1 mmol), and 1.7 mL DCM. The solution was heated at 140 ℃ for 1h, protected with a blast shield. The reaction mixture was concentrated *in vacuo* and the residue was purified by preparative thin layer chromatography (5% MeOH/DCM) to give ketone **146** (6.0 mg, 20%), compound **159** (3.0 mg, 10%) and compound **160** (3.1 mg, 10%).

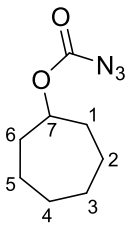
Data for **5-tosylhexahydrooxazolo[4,5-c]pyridin-2(3H)-one (159)**

IR (film) ν_{max}/cm^{-1}: 3440br, 2193m, 1720s, 1598m, 1231s, 1161s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.67 – 7.59 (m, 2H, H_{Ar}), 7.42 – 7.33 (m, 2H, H_{Ar}), 4.96 (br s, 1H, N*H*), 4.70 (dt, *J* = 6.8, 3.6 Hz, 1H, H6), 3.96 – 3.90 (m, 1H, H5), 3.68 (dd, *J* = 12.5, 5.5 Hz, 1H, H4), 3.59 –3.49 (m, 1H, H2), 2.80 – 2.72 (m, 1H, H2), 2.66 – 2.57 (m, 1H, H4), 2.45 (s, 3H, CH_3), 2.26 – 2.17 (m, 1H, H1), 2.13 – 2.04 (m, 1H, H1); **^{13}C NMR** (101 MHz, Chloroform-*d*): δ 157.3, 146.1, 142.2, 128.9, 126.4, 72.0, 49.2, 39.6, 28.6, 25.0, 20.5; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{13}H_{17}N_2O_4S$) requires *m/z* 297.0909, found *m/z* 297.0907; **M.p.**: 146 ℃; **R_f** 0.2 (50% EtOAc/pentane).

Data for **6-tosyl-2-oxa-4,6-diazabicyclo[3.3.1]nonan-3-one (160)**

IR (film) ν_{max}/cm^{-1}:3345br, 2139w, 1626s, 1349m, 1211s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.70 – 7.65 (m, 2H, H_{Ar}), 7.35 – 7.30 (m, 2H, H_{Ar}), 5.42 (br s, 1H, N*H*), 5.44 – 5.40 (m, 1H, H4), 4.78 – 4.73 (m, 1H, H6), 3.49 (ddd, *J* = 10.1, 3.5, 1.6 Hz, 1H, H5), 3.02 (ddd, *J* = 12.1, 3.5, 1.6 Hz, H5), 2.11 – 1.86 (m, 4H, H1, H2); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 155.4, 144.2, 133.1, 127.4, 127.0, 70.7, 50.3, 40.6, 30.0, 25.9, 21.6; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{13}H_{17}N_2O_4S$) requires *m/z* 297.0909, found *m/z* 297.0907; **M.p.**: 143 ℃; **R_f** 0.2 (50% EtOAc/pentane).

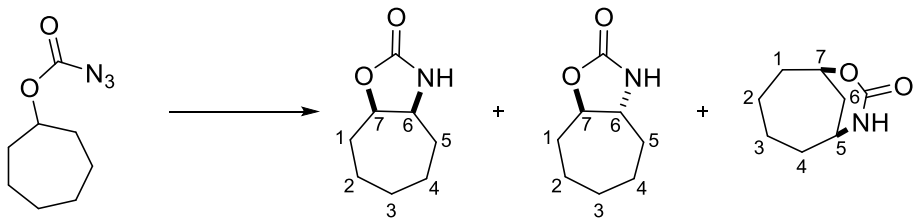
Cycloheptyl carbonazidate (162)



To a solution of cycloheptanol (228 mg, 2.0 mmol) in THF (6.0 mL) was added 1,1-carbonyldiimidazole (648 mg, 4.0 mmol) at RT, and the mixture was stirred for 30 min. To this solution were added trimethylsilyl azide (2.62 mL, 20.0 mmol), EtOAc (6.0 mL) and deionised H2O (6.0 mL). After being stirred for 1.5 h, the reaction mixture was diluted with H2O (10 mL), and the aqueous layer was separated and extracted with EtOAc (3 × 30 mL). The organic layers were combined and immediately filtered through silica gel pad to remove remaining imidazole. Then the filtrate was concentrated *in vacuo*. Flush column chromatography (33% EtOAc /pentane) afforded the product as a white solid (360 mg, 92%).

IR (film) ν_{max}/cm^{-1}: 2138m, 1768s, 1331m, 1231s, 1165s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.92-4.86 (m, 1H, H7), 2.02 – 1.89 (m, 2H, H1, H6), 1.73 – 1.65 (m, 4H, H1, H6, H2, H5), 1.56 – 1.51 (m, 4H, H2, H5, H3, H4), 1.51 – 1.36 (m, 2H, H3, H4); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 156.9, 80.5, 33.5, 28.2, 22.6; **HRMS (ESI)** exact mass calculated for $[M-H]^+$ ($C_8H_{14}N_3O_2$) requires *m/z* 184.1086, found *m/z* 184.1084; **M.p.**: 92 ℃; **R_f** 0.8 (100% EtOAc).

Procedure for the thermolysis of cycloheptyl carbonazidate 162



162 **163a** **163b** **164**

Into a pressure tube was added the cycloheptyl carbonazidate **162** (36.6 mg, 0.1 mmol) and 1.7 mL DCM. The solution was heated at 140 ℃ for 1 h, protected with a blast shield. Then the solution was concentrated *in vacuo* and the residue was purified by preparative thin layer chromatography (5% MeOH/DCM) to give the product **133a** (14.4 mg, 53%, white solid), compound **163b** (7.3 mg, 26%, white solid) and compound **164** (5.5 mg, 18%, white solid).

Data for ***cis*-octahydro-2H-cyclohepta[d]oxazol-2-one (163a)**

IR (film) ν_{max}/cm^{-1}: 3853br, 2932m, 2345w, 1700s, 1258m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 6.34 (s br, 1H, N*H*), 4.74 (ddd, *J* = 9.9, 8.9, 4.1 Hz, 1H, H7), 3.93 (ddd, *J* = 9.9, 8.6, 4.6 Hz, 1H, H6), 2.14 – 1.19 (m, 10H, alkyl-*H*); **^{13}C NMR** (126 MHz, Chloroform-

d) δ: 159.2, 80.2, 56.5, 30.9, 29.7, 25.2, 24.3, 23.5; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_8H_{14}NO_2$) requires *m/z* 156.1024, found *m/z* 156.1022; **M.p.**: 88 ℃; $\mathbf{R_f}$ 0.7 (5% MeOH/DCM).

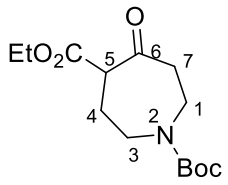
Data for ***trans*-octahydro-2H-cyclohepta[d]oxazol-2-one (163b)**

IR (film) ν_{max}/cm^{-1}: 3853br, 2932m, 1749s, 1258m; **^{1}H NMR** (500 MHz, Chloroform-*d*) δ: 5.22 (s br, 1H, N*H*), 4.30 (td, *J* = 10.3, 5.3 Hz, 1H, H7), 3.69 (td, *J* = 10.4, 4.2 Hz, 1H, H6), 2.35 – 2.23 (m, 1H, H1), 2.10 – 2.01 (m, 1H, H5), 1.80 – 1.37 (m, 8H, alkyl-*H*); **^{13}C NMR** (126 MHz, Chloroform-*d*) δ: 159.5, 83.3, 58.8, 31.0, 29.9, 26.2, 24.5, 24.3; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_8H_{14}NO_2$) requires *m/z* 156.1024, found *m/z* 156.1022; **M.p.**: 88 ℃; $\mathbf{R_f}$ 0.7 (5% MeOH/DCM).

Data for **7-oxa-9-azabicyclo[4.3.1]decan-8-one (164)**

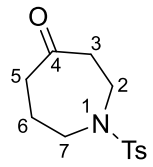
IR (film) ν_{max}/cm^{-1}: 3201br, 2974w, 2928w, 1725s, 1431s, 1321m; **^{1}H NMR** (500 MHz, Chloroform-*d*) δ: 5.41 (s br, 1H, N*H*), 4.81 – 4.75 (m, 1H, H7), 3.84 (tdd, *J* = 10.5, 4.1, 1.2 Hz, 1H, H5), 2.32 – 2.21 (m, 1H, H6), 2.12 – 2.00 (m, 1H, H6), 1.69 – 1.57 (m, 8H, alkyl-*H*); **^{13}C NMR** (126 MHz, Chloroform-*d*) δ: 207.0, 76.7, 48.4, 36.1, 30.9, 26.7, 24.5, 23.2; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_8H_{14}NO_2$) requires *m/z* 156.1024, found *m/z* 156.1022; **M.p.**: 82 ℃; $\mathbf{R_f}$ 0.4 (5% MeOH/DCM).

1-(*tert*-Butyl) 4-ethyl 5-oxoazepane-1,4-dicarboxylate (166)[242]



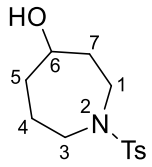
A solution of ketone **141** (156 mg, 0.78 mmol) in dry Et_2O (1.0 mL) was cooled with a IPA-dry ice bath. To this solution was added a solution of ethyl diazoacetate (116 mg, 1.02 mmol) in Et_2O (0.1 mL) and boron trifluoride diethyl ether (116 mg, 0.82 mmol) in Et_2O (0.1 mL), respectively. The reaction mixture was stirred at –25 ℃ for 4 h. The reaction was quenched with sat. K_2CO_3 with dropwise addition until gas formation ceased. The mixture was then diluted with Et_2O (5 mL) and H_2O, the aqueous layer was extracted with Et_2O (3 × 5 mL). The organic extracts were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was used directly in the next step without further purification (151 mg, 70%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.20 – 4.10 (m, 2H, H1), 3.79 – 3.70 (m, 1H, H5), 3.74 – 3.67 (m, 1H, H3), 3.48 – 3.37 (m, 1H, H3), 3.32 – 3.20 (m, 2H, H7), 2.88 – 2.75 (m, 1H, H4), 2.68 – 2.63 (m, 1H, H4), 1.44 – 1.40 (m, 12H, CH_3), 1.34 – 1.19 (m, 2H, CH_2); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 206.1, 169.6, 154.3, 80.2, 68.1, 67.1, 61.3, 60.8, 57.8, 28.3, 14.9, 14.0; **R_f** 0.2 (30% EtOAc/pentane).

1-Tosylazepan-4-one (168)[243]



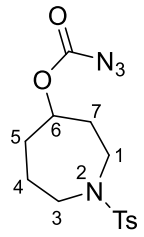
To a solution of dicarboxylate **166** (151 mg, 0.57 mmol) in aqueous hydrochloric acid solution (4.0 M, 1.50 mL) was heated at reflux for 6 h, then was concentrated *in vacuo* to afford 4-oxoazepanium chloride **167** as a white solid (100%). In a separated flask, a solution of 4-oxoazepanium chloride in THF (5.0 mL) and H_2O (2.0 mL) was added in with Et_3N (114 μL, 1.14 mmol) and 4-toluenesulfonyl chloride (129 mg, 0.68 mmol). The reaction mixture was stirred at RT for 24 h. The reaction mixture was diluted with H_2O, the aqueous layer was extracted with EtOAc (3 × 5 mL), the organic extracts were combined, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (50% EtOAc/pentane) afforded the product as a white solid (91.0 mg, 60%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.69 – 7.60 (m, 2H, H_{Ar}), 7.35 – 7.29 (m, 2H, H_{Ar}), 3.48 – 3.30 (m, 4H, H2, H7), 2.75 – 2.67 (m, 2H, H3, H5), 2.66 – 2.53 (m, 2H, H3, H5), 2.43 (s, 3H, CH_3), 1.96 – 1.80 (m, 2H, H6); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 210.5, 143.6, 135.8, 129.8, 127.0, 50.8, 44.7, 44.5, 42.5, 24.8, 21.5; **R_f** 0.3 (30% EtOAc/pentane).

1-Tosylazepan-4-ol (169)



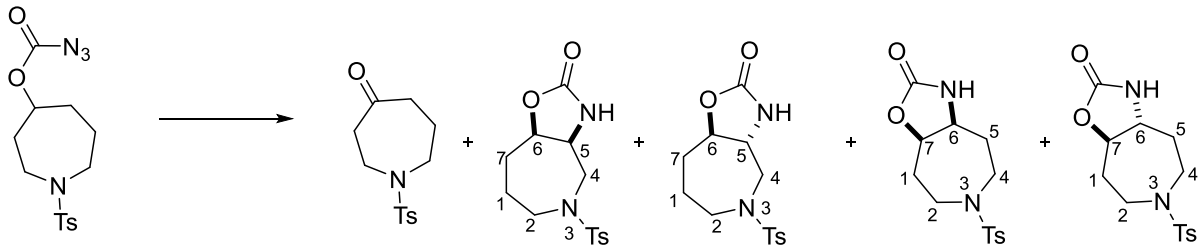
To a solution of tosylazepanone **168** (1.23 g, 4.6 mmol) in EtOH (20.0 mL) was added $NaBH_4$ (259 mg, 6.8 mmol) at 0 ℃. The reaction was mixture was allowed to warm up to RT over 3 h. The reaction was then quenched with sat. NH_4Cl, the aqueous layer was extracted with EtOAc (2 × 10 mL), the organic extracts were combined, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (50% EtOAc/pentane) afforded the product as a white solid (1.02 g, 82%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.72 – 7.59 (m, 2H, H_{Ar}), 7.32 – 7.27 (m, 2H, H_{Ar}), 4.00 – 3.88 (m, 1H, H6), 3.41 – 3.13 (m, 4H, H1, H3), 2.42 (s, 3H, CH_3), 2.01 – 1.90 (m, 1H, H7), 1.97 – 1.83 (m, 2H, H4, H5), 1.83 – 1.68 (m, 2H, H4, H5), 1.68 – 1.53 (m, 1H, H7); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 143.1, 136.1, 129.6, 127.0, 70.0, 47.7, 42.6, 37.8, 34.8, 22.4, 21.5; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{13}H_{20}NO_3S$) requires *m/z* 270.1161, found *m/z* 270.1163; **R_f** 0.3 (50% EtOAc/pentane).

1-Tosylazepan-4-yl carbonazidate (170)



To a solution of 1-tosylazepan-4-ol **169** (536 mg, 2.0 mmol) in THF (7.0 mL) was added 1,1-carbonyldiimidazole (648 mg, 4.0 mmol) at RT, and the reaction mixture was stirred for 30 min. To this solution were added trimethylsilyl azide (2.62 mL, 20.0 mmol), EtOAc (7.0 mL) and deionised H_2O (7.0 mL). After being stirred for 1.5 h, the reaction mixture was diluted with H_2O (10 mL), and the aqueous layer was separated and extracted with EtOAc (3 × 10 mL). The organic layers were combined and immediately filtered through silica gel pad to remove remaining imidazole. Then the filtrate was dried over $MgSO_4$ and concentrated *in vacuo*. Flush column chromatography (33% EtOAc/pentane) afforded the product as white solid (463 mg, 68%). **IR** (film) ν_{max}/cm^{-1}: 2974w, 2189w, 2135w, 1725m, 1696s, 1236s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.70 – 7.62 (m, 2H, H_{Ar}), 7.36 – 7.28 (m, 2H, H_{Ar}), 4.96 (ddt, *J* = 9.3, 6.8, 2.7 Hz, 1H, H6), 3.49 – 3.39 (m, 1H, H1), 3.39 – 3.30 (m, 1H, H3), 3.30 – 3.21 (m, 1H, H3), 3.21 – 3.11 (m, 1H, H1), 2.43 (s, 3H, CH_3), 2.10 (ddt, *J* = 15.5, 7.9, 3.8 Hz, 1H, H7), 2.03 – 1.78 (m, 4H, H4, H5), 1.78 – 1.64 (m, 1H, H7); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 210.2, 149.1, 143.3, 129.7, 126.9, 47.5, 42.4, 34.9, 30.7, 29.7, 22.0, 21.4; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{14}H_{19}N_4O_4S$) requires *m/z* 339.1121, found *m/z* 339.1122; **M.p.**: 124 ℃; **R_f** 0.8 (50% EtOAc/pentane).

Procedure for the thermolysis of 1-tosylazepan-4-yl carbonazidate 170



170 **168** **171a** **171b** **172a** **172b**

Method A

Into a pressure tube was added the carbonazidate **170** (135.2 mg, 0.4 mmol) and 6.0 mL DCM. The solution was heated at 140 ℃ for 1 h, protected with a blast shield. Then the solution was concentrated *in vacuo* and the residue was purified by preparative thin layer chromatography (5% MeOH/DCM) to give the product **168** (3.0 mg, 2%), compound **171a** (2.0 mg, 1%), compound **171b** (1.0 mg, 1%), compound **172a** (1.0 mg, 1%) and compound **172b** (1.0 mg, 1%).

Method B

A solution of carbonazidate **170** (101.0 mg, 0.3 mmol) in TCE (15.0 mL) was heated to reflux for 2 h. The reaction mixture was cooled down to RT, then concentrated *in vacuo*. The residue was purified by preparative thin layer chromatography (5% MeOH/DCM) to give the product **168** (26.0 mg, 28%), compound **171a** (5.0 mg, 5%), compound **171b** (5.0 mg, 5%), compound **172a** (5.0 mg, 5%) and compound **172b** (5.0 mg, 5%).

Data for ***cis*-5-tosyloctahydro-2H-oxazolo[4,5-c]azepin-2-one (171a)**

IR (film) ν_{max}/cm^{-1}: 3340br, 2190m, 1734s, 1623m, 1221s, 1161s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.64 – 7.50 (m, 2H, H_{Ar}), 7.29 – 7.20 (m, 2H, H_{Ar}), 5.33 (br s, 1H, N*H*), 4.81 (td, *J* = 8.6, 3.5 Hz, 1H, H6), 4.16 – 3.98 (m, 1H, H5), 3.36 – 3.21 (m, 2H, H2, H4), 3.12 (dd, *J* = 14.8, 8.1 Hz, 1H, H4), 3.03 – 2.95 (m, 1H, H2), 2.36 (s, 3H, CH_3), 1.99 – 1.92 (m, 2H, H7), 1.92 – 1.84 (m, 1H, H1), 1.61 – 1.49 (m, 1H, H1); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 157.1, 142.8, 134.4, 128.9, 126.0, 75.6, 54.4, 49.7, 48.1, 28.6, 27.9, 22.8; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{14}H_{19}N_2O_4S$) requires *m/z* 311.1061, found *m/z* 311.1062; **R_f** 0.2 (50% EtOAc/pentane).

Data for ***trans*-5-tosyloctahydro-2H-oxazolo[4,5-c]azepin-2-one (171b)**

IR (film) ν_{max}/cm^{-1}: 3340br, 2190m, 1734s, 1623m, 1221s, 1161s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.71 – 7.55 (m, 2H, H_{Ar}), 7.33 (d, *J* = 8.1 Hz, 2H, H_{Ar}), 5.33 (br s, 1H, N*H*), 4.45 (ddd, *J* = 11.4, 10.1, 3.6 Hz, 1H, H6), 3.77 (ddd, *J* = 10.2, 5.6, 1.4 Hz, 1H, H5), 3.60 – 3.45 (m, 2H, H2, H4), 3.35 – 3.15 (m, 1H, H4), 2.96 (ddd, *J* = 15.2, 9.8, 2.1 Hz, 1H, H2), 2.44 (s, 3H, CH_3), 2.34 – 2.30 (m, 1H, H7), 1.99 – 1.80 (m, 2H, H1), 1.77 – 1.65 (m, 1H, H7); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 169.3, 143.8, 136.1, 129.9, 127.0, 81.1, 58.4, 48.7, 45.8, 29.8, 25.8, 20.5; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{14}H_{19}N_2O_4S$) requires *m/z* 311.1061, found *m/z* 311.1062; **R_f** 0.2 (50% EtOAc/pentane).

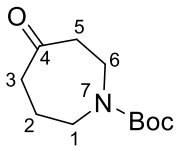
Data for ***cis*-6-tosyloctahydro-2H-oxazolo[4,5-d]azepin-2-one (172a)**

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.64 – 7.59 (m, 2H, H_{Ar}), 7.31 (dd, *J* = 8.3, 2.1 Hz, 3H, H_{Ar}), 5.63 (br s, 1H, N*H*), 4.91 (ddd, *J* = 9.1, 7.3, 3.0 Hz, 1H, H6), 4.19 – 4.05 (m, 1H, H7), 3.55 – 3.42 (m, 2H, H2, H4), 3.06 – 3.00 (m, 2H, H2, H4), 2.43 (s, 3H, CH_3), 2.29 – 2.18 (m, 1H, H5), 2.17 – 2.09 (m, 1H, H5), 2.01 – 1.96 (m, 1H, H1), 1.34 – 1.16 (m, 1H, H1); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 158.2, 143.7, 135.1, 129.9, 126.7, 78.0, 54.2, 43.8, 43.3, 32.7, 31.3, 21.5; **R_f** 0.3 (50% EtOAc/pentane).

Data for ***trans*-6-tosyloctahydro-2H-oxazolo[4,5-d]azepin-2-one (172b)**

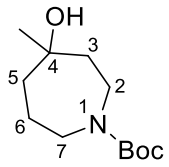
^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.59 (dd, *J* = 8.5, 2.2 Hz, 2H, H_{Ar}), 7.25 (dd, *J* = 8.0, 5.0 Hz, 2H, H_{Ar}), 5.37 (br s, 1H, N*H*), 4.36 (td, *J* = 10.6, 5.2 Hz, 1H, H6), 3.80 (ddd, *J* = 10.6, 4.4, 1.3 Hz, 1H, H7), 3.41 (dt, *J* = 14.9, 5.9 Hz, 1H, H2), 3.30 – 3.20 (m, 2H, H2, H4), 3.17 (ddd, *J* = 14.4, 7.9, 5.8 Hz, 1H, H4), 2.49 – 2.38 (m, 1H, H5), 2.36 (s, 3H, CH_3), 2.17 – 1.14 (m, 1H, H1), 1.86 (ddd, *J* = 13.0, 10.7, 6.4 Hz, 1H, H5), 1.75 – 1.61 (m, 1H, H1). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 159.1, 143.7, 136.3, 129.9, 126.7, 81.5, 57.8, 47.8, 46.5, 33.5, 31.6, 21.5; **R_f** 0.3 (50% EtOAc/pentane).

tert-Butyl 4-oxoazepane-1-carboxylate (173)[244]



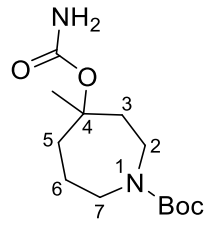
To a solution of 4-oxoazepanium chloride **167** (3.02 g, 20.0 mmol) in H_2O (45.0 mL) and THF (45.0 mL) was added $NaHCO_3$ (3.30 g, 20.0 mmol), then a solution of di-*tert*-butyl dicarbonate (3.52 g, 20.0 mmol) in THF (10.0 mL) was dropwise added to the reaction mixture. The reaction mixture was stirred at RT for 14 h. The reaction mixture washed with 10.0 mL 1% HCl solution. The aqueous layer was extracted with THF (2 × 10 mL). The combined organic layers were washed with cold 1% HCl solution (100 mL), and the aqueous layer was extracted with THF (2 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. to give the crude product as a white solid (3.00 g, 95%), which was used without further purification**.** **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 3.58 –3.45 (m, 4H, H1, H6), 2.70 – 2.55 (m, 4H, H3, H5), 1.77 – 1.60 (m, 2H, H2), 1.44 (s, 9H, CH_3); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 221.7, 154.4, 80.0, 48.8, 43.8, 43.1, 42.9, 28.3, 25.8, 25.5; **R_f** 0.3 (30% EtOAc/pentane).

4-Methyl-1-tosylazepan-4-ol (174)[245]



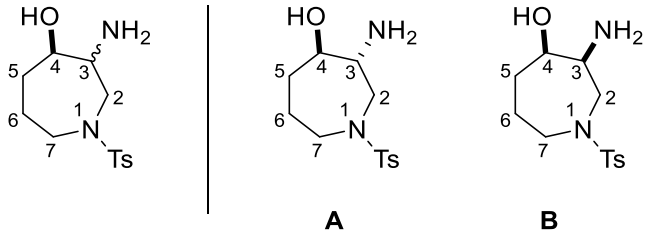
To an oven dried 25 mL round bottom flask was added azepanone **173** (213 mg, 1.0 mmol) and Et_2O (2.0 mL), the solution was cooled to 0 ℃, followed by dropwise addition of 3.0 M methyl magnesium bromide (330 μL, 1.0 mmol). The reaction mixture was allowed to warm to RT over 3 h. The reaction was quenched with sat. NH_4Cl, the aqueous layer was extracted with EtOAc (3 × 1 mL). The organic extracts were combined, dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (30% EtOAc/pentane) afforded the product as a white solid (1.02 g, 82%).**^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 3.66 – 3.40 (m, 2H, H2, H7), 3.35 – 3.11 (m, 2H, H2, H7), 2.50 – 2.31 (m, 1H, H3), 2.31 – 2.11 (m, 1H, H5), 1.80 – 1.57 (m, 4H, H3, H5, H6), 1.52 (s, 3H, CH_3), 1.45 (s, 9H, CH_3); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 155.9, 82.5, 79.2, 46.3, 41.2, 40.6, 37.0, 28.5, 26.8, 14.2; **M.p.**: 74 ℃; **R_f** 0.3 (50% EtOAc/pentane).

tert-Butyl 4-(carbamoyloxy)-4-methylazepane-1-carboxylate (175)



To a solution of alcohol **174** (500 mg, 2.18 mmol) in dry Et_2O (7.0 mL) at 0 ℃ was added dropwise trichloroacetyl isocyanate (280 μL, 2.36 mmol). The mixture was allowed to warm up to RT over 16 h. The reaction mixture was then pipetted onto a basic alumina pad and allowed to stand for 30 min. The alumina pad was flushed with Et_2O (20 mL) and EtOAc (20 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo.* Flush column chromatography (50% EtOAc/pentane) afforded the product as a white solid (613 mg, 90%). **IR** (film) ν_{max}/cm^{-1}: 3350w, 2930w, 2856w, 1716s, 1246s, 1163s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.55 (br s, 2H, NH_2), 3.66 – 3.40 (m, 2H, H2, H7), 3.35 – 3.11 (m, 2H, H2, H7), 2.50 – 2.31 (m, 1H, H3), 2.31 – 2.11 (m, 1H, H5), 1.80 – 1.57 (m, 4H, H3, H5, H6), 1.52 (s, 3H, CH_3), 1.45 (s, 9H, CH_3); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 171.1, 155.9, 82.5, 79.2, 46.3, 41.2, 40.6, 37.0, 28.5, 26.8, 14.2; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{13}H_{24}N_2O_4Na$) requires *m/z* 295.1628, found *m/z* 295.1628; **M.p.**: 75 ℃; **R_f** 0.5 (50% EtOAc/pentane).

3-Amino-1-tosylazepan-4-ol (177)



To a solution of azepinone **171** (100 mg, 0.33 mmol) in EtOH (0.4 mL) was added 0.82 mL of 5.0 M NaOH solution. The reaction mixture was heated to reflux for 5 h. The reaction mixture was then cooled down to RT, then added with 1.0 M HCl until pH is acidic. The reaction mixture was concentrated *in vacuo*. Flash column chromatography (30% EtOAc/pentane) afforded the product as a mixture of unseparated diasteriomers (*dr* = 2:1, white solid, 60 mg, 66%), isomer ratio determined by integration of the H-3 and H-4 signal in the ^{1}H NMR spectrum.

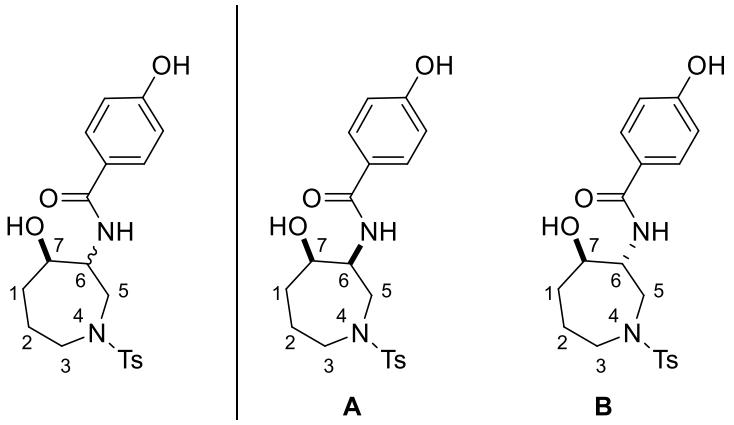
IR (film) ν_{max}/cm^{-1}: 3490br, 2926m, 2361m, 2342m, 1697m, 1606s, 1332m, 1159s; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{13}H_{20}N_2O_3SNa$) requires *m/z* 307.1082, found *m/z* 307.1087; $\mathbf{R_f}$ 0.2 (10% MeOH/DCM).

Major isomer: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.64 (d, *J* = 8.6, 2H, H_{Ar}), 7.35 – 7.28 (m, 2H, H_{Ar}), 3.54 – 3.42 (m, 1H, H2), 3.39 – 3.19 (m, 5H, H4, H7, NH_2), 3.11 – 3.00 (m, 1H, H2), 2.61 – 2.51 (m, 1H, H3), 2.41 (s, 3H, CH_3), 2.14 – 1.84 (m, 2H, H5, H6), 1.84 – 1.44 (m, 2H, H5, H6). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 143.4, 135.7, 129.7, 126.9, 71.4, 56.0, 48.1, 43.3, 28.9, 23.1, 20.9.

Minor isomer: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.64 (d, *J* = 8.6, 2H, H_{Ar}), 7.35 – 7.28 (m, 2H, H_{Ar}), 3.61 (dt, *J* = 8.8, 3.5 Hz, 1H, H4), 3.54 – 3.42 (m, 1H, H2), 3.39 – 3.19 (m, 3H,

H3, N*H*$_2$), 3.22 – 3.09 (m, 2H, H7), 3.11 – 3.00 (m, 1H, H2), 2.61 – 2.51 (m, 1H, H2), 2.41 (s, 3H, C*H*$_3$), 2.14 – 1.84 (m, 2H, H5, H6), 1.84 – 1.44 (m, 2H, H5, H6); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 143.3, 135.3, 129.7, 126.9, 74.6, 56.8, 48.3, 43.7, 33.3, 28.9, 20.9.

4-Hydroxy-*N*-4-hydroxy-1-tosylazepan-3-yl)benzamide (178)



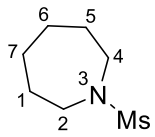
To a solution of 4-hydroxylbenzoic acid (69.0 mg, 0.50 mmol) in DCM (2.5 mL) was added Et$_3$N (170 μL, 1.25 mmol), 4-dimethylaminopyridine (28.0 mg, 0.25 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (114 mg, 0.60 mmol) at 0 ℃. The mixture was allowed to warm up to RT, followed by the addition of azepanol **177** (160 mg, 0.50 mmol). The reaction mixture was stirred at RT for 24 h. The solution was washed with sat. NaHCO$_3$ (2 × 2 mL), 10% citric acid (2 × 2 mL), brine and dried over MgSO$_4$ and concentrated *in vacuo*. Flash column chromatography (100% EtOAc) afforded the product product as a mixture of unseparated diasteriomers (*dr* = 1.45:1white solid 10 mg, 5%). Isomer ratio determined by integration of the N*H* signal in the ^{1}H NMR spectrum. **IR** (film) ν_{max}/cm^{-1}: 3001br, 2961w, 2360w, 1699m, 1606m, 1498m, 1333m, 1159s; **HRMS (ESI)**

exact mass calculated for $[M+Na]^+$ ($C_{20}H_{24}N_2O_5SNa$) requires *m/z* 427.1298, found *m/z* 427.1299; **R_f** 0.5 (10% MeOH/DCM).

Major isomer: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 8.10 – 7.96 (m, 2H, H_{Ar}), 7.87 – 7.75 (m, 2H, H_{Ar}), 7.74 – 7.62 (m, 2H, H_{Ar}), 7.02 – 6.86 (m, 2H, H_{Ar}), 5.16 (br s, 1H, N*H*), 4.48 – 4.40 (m, 1H, H7), 4.10 – 3.97 (m, 1H, H5), 3.92 – 3.82 (m, 1H, H6), 3.81 – 3.77 (m, 1H, H3), 3.75 – 3.62 (m, 2H, H3, H5), 3.23 – 2.81 (m, 1H, O*H*), 3.42 (s, 3H, CH_3), 2.12 – 1.47 (m, 4H, H1, H2); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 144.1, 132.7, 130.0, 130.0, 129.7, 129.1, 127.1, 122.2, 115.6, 77.2, 55.7, 48.5, 28.4, 22.3, 21.5.

Diagnostic signals for minor isomer: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.92 (br s, 1H, N*H*), 4.84 – 4.71 (m, 1H, H6), 3.24 – 3.07 (m, 1H, H1); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 144.1, 131.92, 130.1, 130.0, 129.4, 129.2, 127.0, 122.2, 115.5, 77.3, 55.8, 48.5, 28.4, 22.4, 21.5.

1-(Methylsulfonyl)azepane (179)[246]



To a stirred solution of azepane (1.14 mL, 10.0 mmol) in H_2O (100.0 mL) was added methanesulfonyl chloride (930 μL, 12.0 mmol) at RT and stirred for 16 h. The reaction mixture was extracted with EtOAc (4 × 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (50% Et_2O/pentane → 60% Et_2O/pentane) afforded the product as a white solid (1.06 g, 60%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 3.39 – 3.27

(m, 4H, H2, H4), 2.81 (s, 3H, CH_3), 1.82 – 1.70 (m, 4H, H1, H5), 1.65 – 1.73 (m, 4H, H6, H7); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 48.0, 36.9, 29.3, 26.9; **R_f** 0.50 (50% Et_2O/pentane).

Enzymes and Molecular Biology

The $P450_{BM3}$ enzymes were produced in *Escherichia coli* BL21 (DE3) and purified by addition of $(NH_4)_2SO_4$ to a concentration of 40% w/v and centrifugation at 37,500 g for 10 min at 4 ℃; the residue was discarded and $(NH_4)_2SO_4$ was added to a concentration of 60% w/v. The solution was submitted to centrifugation at 37,500 g for 10 min at 4 ℃, the supernatant was discarded, and the residue was suspended in phosphate buffer. The enzymes were stored as stock solutions at –20 ℃ and thawed at RT before use.

General procedure for screening of compounds

Compounds were screened using an NADPH regeneration system in 200 mM phosphate buffer (pH 7.5), at a total volume of 0.5 mL, contained in 24 well plates. The substrate was added as a 100 mM stock solution in ethanol (final concentration 2 mM), and the mutant $P450_{BM3}$ enzyme was added as a 10 μM stock solution in phosphate buffer (pH 7.5, final concentration 2 μM). The NADPH regeneration system consisted of glucose dehydrogenase (GDH), added as a 2 U/μL stock solution in 200 mM phosphate buffer (pH 7.5, final concentration 40 U/mL), glucose, added as a 1.0 M stock solution in 200 mM phosphate buffer (pH 7.5, final concentration 100 mM), and NADP+, added as a 4.0 mM stock solution in 200 mM phosphate buffer (pH 7.5, final concentration 8 μM). The reactions were shaken at RT for 14 h, extracted with 300 μL of ethyl acetate and, following centrifugation at 14,300 g for 2 minutes, the organic phase was analysed by GC.

P450$_{BM3}$ variants and the mutations in each

WY 3		Mutations
1	R19 A330W	R47L/Y51F/H171L/Q307H/N319Y/A330W
2	KSK19IG	F87A/H171L/I263G/Q307H/N319Y
3	GVQ	A74G/F87V/L188Q
4	R19FI	R47L/Y51F/F87I/H171L/Q307H/N319Y
5	K19 F87V	F87V/H171L/Q307H/N319Y
6	K19 F87V E267V	F87V/H171L/E267V/Q307H/N319Y
7	KSK19 A82M I263G	A82M/F87A/H171L/I263G/Q307H/N319Y
8	K19 F87V A328V	F87V/H171L/Q307H/N319Y/A328V
9	KSK19 F81W	F81W/F87A/H171L/Q307H/N319Y
10	KSK19 I263A	F87A/H171L/I263A/Q307H/N319Y
11	K19 F87V Q403P	F87V/H171L/Q307H/N319Y/Q403P
12	KU3 A330P A328I	N239H/I259V/A276T/A328I/A330P
13	R19 A328L A184I	R47L/Y51F/H171L/A184I/Q307H/N319Y/A328I
14	GVQ IG AL	A74G/F87V/L188Q/I263G/A328L
15	RLYF K19	R47L/Y51F/H171L/Q307H/N319Y

16	RLYF KSK19 A184I	R47L/Y51F/F87A/H171L/A184I/Q307H/N319Y
17	RLYF KSK19 A328I	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328I
18	RLYF KSK19	R47L/Y51F/F87A/H171L/Q307H/N319Y
19	GV A184I	A74G/F87V/A184I
20	RT2	R47L/Y51F/A191T/N239H/I259V/A276T/L353I
21	RT2 S72G A330W	R47L/Y51F/S72G/A191T/N239H/I259V/A276T/A330W/L353I
22	RT2 A330P V78I A184I	R47L/Y51F/V78I/A184I/A191T/N239H/I259V/A276T/A330P/L353I
23	KU3 A330P	N239H/I259V/A276T/A330P
24	RP H171L I263G	R47L/Y51F/H171L/I263G/I401P

WY4		Mutations
1	RP IA EV	R47L/Y51F/I263A/E267V/I401P
2	GVQ A328I I263A	A74G/F87V/L188Q/I263A/A328I
3	WT	WT
4	GVQ IG	A74G/F87V/L188Q/I263G

5	GVQ A264G	A74G/F87V/L188Q/A264G
6	K19 F87V A264G	F87V/H171L/A264G/Q307H/N319Y
7	K19 F87V E267V V78I	V78I/F87V/H171L/E267V/Q307H/N319Y
8	KSK19 A82M E267F	A82M/F87A/H171L/E267F/Q307H/N319Y
9	KSK19 AM	A82M/F87A/H171L/Q307H/N319Y
10	RK A328L	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328L
11	RKA S72W	R47L/Y51F/S72W/F87A/H171L/Q307H/N319Y/A328I
12	RKA V78I	R47L/Y51F/V78I/F87A/H171L/Q307H/N319Y/A328I
13	RL YF IR	R47L/Y51F/I263R
14	RLYF KSK19 A328F	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328F
15	RLYF KSK19 I263G	R47L/Y51F/F87A/H171L/I263G/Q307H/N319Y
16	RP A82W I263A	R47L/Y51F/A82W/I263A/I401P
17	RP A82M I263A	R47L/Y51F/A82M/I263A/I401P
18	RP F87V	R47L/Y51F/F87V/I401P
19	RP F87V V78I	R47L/Y51F/V78I/F87V/I401P
20	RP H171L I263G A184I	R47L/Y51F/H171L/A184I/I263G/I401P

21	R19 A328L I263G	R47L/Y51F/H171L/I263G/Q307H/N319Y/A328I
22	RP H171L I263G F87V V78I	R47L/Y51F/V78I/F87V/H171L/I263G/I401P
23	RP V78I E267V	R47L/Y51F/V78I/E267V/I401P
24	RT2 S72W A330W	R47L/Y51F/S72W/A191T/N239H/I259V/A276T/A330W/L353I

Screening data for 179

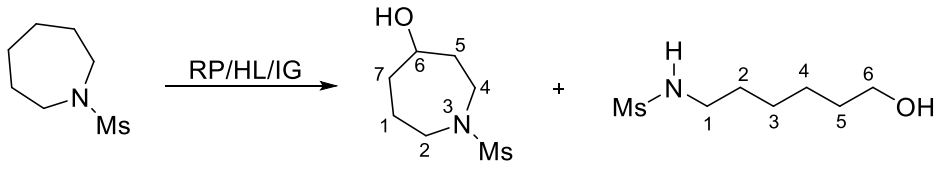
Conversion was determined by GC analysis.

Entry	Enzyme	Conversion	**180**	**182**	**181**
1	R19 A330W	1%	0%	1%	0%
2	KSK19IG	0%			
3	GVQ	0%			
4	R19FI	0%			
5	K19 F87V	10%	0%	5%	5%
6	K19 F87V E267V	3%	0%	1%	2%
7	KSK19 A82M I263G	0%			
8	K19 F87V A328V	5%	1%	0%	4%
9	KSK19 F81W	13%	3%	3%	7%
10	KSK19 I263A	0%			
11	K19 F87V Q403P	23%	52%	18%	23%
12	KU3 A330P A328I	5%	0%	32%	0%
13	R19 A328L A184I	3%	0%	50%	0%
14	GVQ IG AL	0%			
15	RLYF K19	22%	11%	4%	8%

16	RLYF KSK19 A184I	61%	40%	10%	12%
17	RLYF KSK19 A328I	17%	9%	4%	4%
18	RLYF KSK19	81%	0%	81%	0%
19	GV A184I	73%	45%	11%	18%
20	RT2	36%	18%	4%	14%
21	RT2 S72G A330W	0%			
22	RT2 A330P V78I A184I	75%	44%	8%	23%
23	KU3 A330P	47%	28%	4%	15%
24	RP H171L I263G	96%	38%	26%	31%
25	RP IA EV	1%	1%	0%	0%
26	GVQ A328I I263A	1%	1%	0%	0%
27	WT	0%			
28	GVQ IG	0%			
29	GVQ A264G	8%	1%	1%	7%
30	K19 F87V A264G	4%	0%	1%	3%
31	K19 F87V E267V V78I	2%	0%	2%	0%
32	KSK19 A82M E267F	3%	0%	3%	0%
33	KSK19 AM	38%	23%	4%	12%

34	RK A328L	7%	0%	7%	0%
35	RKA S72W	6%	0%	1%	5%
36	RKA V78I	1%	0%	0%	0%
37	RL YF IR	0%			
38	RLYF KSK19 A328F	2%	0%	2%	0%
39	RLYF KSK19 I263G	3%	0%	3%	0%
40	RP A82W I263A	2%	0%	1%	1%
41	RP A82M I263A	3%	0%	3%	0%
42	RP F87V	30%	11%	1%	15%
43	RP F87V V78I	7%	0%	3%	4%
44	RP H171L I263G A184I	24%	7%	6%	11%
45	R19 A328L I263G	2%	0%	2%	0%
46	RP H171L I263G F87V V78I	14%	4%	2%	7%
47	RP V78I E267V	23%	10%	4%	9%
48	RT2 S72W A330W	59%	29%	6%	24%

Scale-up reaction of azepane 179 with RP/HL/IG



179 **180** **181**

To a solution of 1-mesylazepane **179** (50 mg, 0.19 mmol, 100 mM solution in methanol), glucose (6.0 mL of a 1.0 M solution in H2O), GDH (0.6 mL of a 4.0 mM solution in phosphate buffer, pH 7.5), $NADP^+$ monosodium salt (0.6 mL of a 4.0 mM solution in H2O) in 200 mM phosphate buffer (pH 7.5, 42. 6mL) was added $P450_{BM3}$ mutant RP/HL/IG (2 mg, as a 20 μM solution in phosphate buffer, pH 7.5). The solution was shaken open to air for 24 h. It was then centrifuged at 9,500 g, and extracted with EtOAc (6 × 10 mL). The combined organic layers was washed with brine, dired over $MgSO_4$, and concentrated *in vacuo.* Flash column chromatography afforded the product **180** as a white solid (2 mg, 4%) and product **181** as a white solid (8 mg, 16%).

Data for **1-(methylsulfonyl)azepan-4-ol (180):**

IR (film) ν_{max}/cm^{-1}: 3270br, 2974s, 2928s, 1668s, 1431m, 1161m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.02 (dt, *J* = 8.0, 4.3 Hz, 1H, H6), 3.48 – 3.36 (m, 2H, H2, H4), 3.36 – 3.23 (m, 2H, H2, H4), 2.81 (s, 3H, CH_3), 2.09 – 0.71 (m, 6H, alkyl-*H*); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 69.9, 47.5, 42.4, 38.0, 34.7, 26.5; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_7H_{15}NO_3S$) requires *m/z* 270.1164, found *m/z* 279.1169; **M.p.**: 130 ℃; **R_f** 0.3 (100% EtOAc).

Data for ***N*-(6-hydroxyhexyl)methanesulfonamide (181)**[247]:

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 4.55 (s, 1H, N*H*), 3.64 (t, *J* = 6.5 Hz, 2H, H1), 3.12 (td, *J* = 7.1, 6.2 Hz, 2H, H6), 2.81 (s, 3H, CH_3), 1.79 – 1.29 (m, 8H, alkyl-*H*); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 69.5, 43.1, 40.2, 32.4, 30.0, 26.2, 25.2; **R_f** 0.4 (100% EtOAc).

Conformational analysis of the pair of diastereomers 163a and 163b (performed by Prof. Robertson)

(1) A conformational search (so-called Monte Carlo method) was conducted to find the major accessible conformations of both diastereomers; this was done using a simple molecular mechanics force field (MMFF).

(2) For each conformation, a higher-level equilibrium geometry calculation was conducted using a DFT model (B3LYP/6-31G*) which resulted in a reasonable conformer distribution based on the calculated energies.

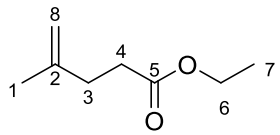
(3) The *trans*-compound gave just three conformers, two of which comprised the majority of the conformational space; the dihedral angle in each case was then calculated and assumed that the molecule spends the amount of time in each conformation based on the calculated (Boltzmann) distribution. The averaged dihedral angle is 157 °.

(4) Same procedure was conducted for the *cis*-compound; nine conformers were generated, three of which accounted for the vast majority of conformational space. The averaged dihedral angle from this was 12 °.

(5) A few of variations on the Karlplus equation, that relates dihedral angle to coupling constant were tried but they tend to yield calculated *J* values that were very similar for the two angles 157 ° and 12 °, which was not therefore helpful.

Data for Chapter 3

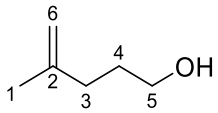
Ethyl 4-methylpent-4-enoate (191)[248]



2.5 M *n*-BuLi (20.6 mL, 51.5 mmol) was added dropwise to a solution of methyltriphenylphosphonium bromide (12.7 g, 35.7 mmol) in THF (125.0 mL) at 0 ℃ under argon. After stirring at 0 ℃ for 30 min, the mixture was cooled to –78 ℃. A solution of ethyl levulinate (5.60 g, 39.0 mmol) in THF (35.0 mL) was added dropwise *via* syringe to the above mixture at –78 ℃. The reaction mixture was warmed to RT over 19 h. The reaction mixture was quenched with 20 mL sat. NH_4Cl and extracted with pentane (4 × 25 mL). The combined organic layers were washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. Flush column chromatography (100% pentane) afforded the product as a colorless oil (3.60 g, 66%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 4.74 (d, *J* = 2.1, 1H, H8), 4.68 (d, *J* = 1.7 Hz, 1H, H8), 4.13 (q, *J* = 7.2 Hz, 2H, H6), 2.48 – 2.41 (m, 2H, H4), 2.36 – 2.28 (m, 2H, H3), 1.74 (dd, *J* = 1.5, 0.8 Hz, 3H, H1), 1.25 (t, *J* = 7.1 Hz, 3H, H7); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 173.3, 144.1, 110.3, 60.4, 32.7, 32.7, 22.5, 14.2; **R_f** 0.1 (33% EtOAc/pentane).

4-Methylpent-4-en-1-ol (192)[249]



Method A

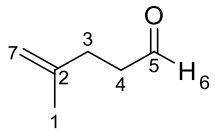
To a slurry of $LiAlH_4$ (800 mg, 21.10 mmol) in dry Et_2O (18.0 mL) was added a solution of alkene **191** (1.00 g, 7.03 mmol) in dry Et_2O (2.0 mL) at 0 ℃ under argon. The reaction mixture was stirred for 30 min at 0 ℃ before H_2O (0.8 mL) was added very cautiously. 2.0 M NaOH (0.8 mL) solution was then added slowly followed by more addition of H_2O (2.4 mL). The mixture was then stirred for 15 min, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (20% Et_2O/pentane → 40% Et_2O/pentane → 100% Et_2O) afforded the product as a colourless liquid (700 mg, 99%).

Method B

A solution of **191** (142 mg, 1.00 mmol) in DCM (5.0 mL) was cooled to –78 ℃ and dropwise added in with DIBAL-H (1.15 mL, 1.27 mmol). The reaction mixture was stirred for 3 h and added in with 0.10 mL MeOH and 0.25 mL sat. Na_2SO_4 at –78 ℃. The mixture was allowed to warm up to RT over 14 h. The layers were separated and the organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product as colourless liquid.

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 4.72 (m, 2H, H6), 3.66 (t, *J* = 6.5 Hz, 2H, H5), 2.10 (dd, *J* = 8.4, 7.0 Hz, 2H, H3), 1.79 – 1.65 (m, 2H, H4), 1.74 (s, 3H, H1); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 145.5, 110.2, 62.8, 34.1, 30.5, 22.4; **R_f** 0.3 (40% Et_2O/pentane).

4-Methylpent-4-enal (189)[248]



Method A

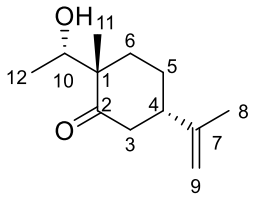
To a solution of oxalylchloride (2.54 g, 20.0 mmol) in DCM (10.0 mL) was added a solution of DMSO (3.13 g, 40.0 mmol) in DCM (10.0 mL) at –78 ℃ under argon. The solution was stirred for 45 min at –78 ℃. A solution of **192** (1.00 g, 10.0 mmol) in DCM (10.0 mL) was added slowly over 5 min to the stirred solution. The reaction mixture was stirred for a further 1 h before Et_3N (12.0 ml, 100.0 mmol) was added over 5 min and the solution was allowed to warm to RT. The solution was washed with 2.0 M HCl (25 mL), sat. $NaHCO_3$ solution (25 mL), H_2O and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flush column chromatography (10% Et_2O/pentane → 20% Et_2O/pentane) afforded the product as a colourless oil (300 mg, 50%).

Method B

To a solution of **192** (1.65 g, 16.5 mmol) in DCM (165.0 mL) was added $NaHCO_3$ (2.90 g, 34.7 mmol) and cooled to 0 ℃. DMP (10.5 g, 24.2 mmol) was added, and the mixture was allowed to warm to RT over 2 h. The mixture was washed with 1.0 M NaOH (2 × 200 mL). The aqueous layer was extracted with DCM (2 × 200 mL). The organic layers were combined, washed with H_2O and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Flush column chromatography (10% Et_2O/pentane → 20% Et_2O/pentane) afforded the product as colourless oil (1.15 g, 70%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 9.78 (s, 1H, H6), 4.77 (s, 1H, H7),

4.69 (s, 1H, H7), 2.58 (t, *J* = 7.3 Hz 2H, H4), 2.35 (t, *J* = 7.5 Hz, 2H, H3), 1.75 (s, 3H, H1); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 202.2, 143.7, 110.7, 41.8, 29.9, 22.6; **R_f** 0.8 (40% Et_2O/pentane).

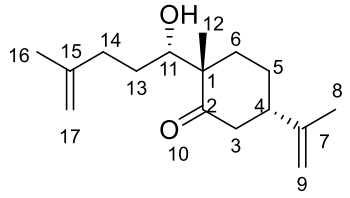
(2*S*,5*S*)-2-((*S*)-1-Hydroxyethyl)-2-methyl-5-(prop-1-en-2-yl)cyclohexan-1-one (195)



(*S*)-(+)-Carvone (56.5 mg, 0.377 mmol) was dissolved in THF (1.0 mL) and cooled to –78 ℃. A solution of L-Selectride (396 μL, 0.396 mmol) was added and stirring was continued for an additional hour. Acetaldehyde (140 μL, 2.26 mmol) was then added and the reaction mixture was stirred for 15 minutes at –78 ℃. A solution of H_2O_2 (1.3 mL, 35 wt.%) and 2.0 N NaOH (1.8 mL) was added dropwise to the reaction mixture, which was then allowed to warm to RT and stirred vigorously for 16 h. The reaction mixture was extracted with EtOAc (3 × 3 mL). The combined organic extracts were washed with H_2O (2 × 4 mL), 5 % $NaHSO_4$ (4 mL), brine, dried over $MgSO_4$ and concentrated *in vacuo.* Flush column chromatography (100% pentane → 80% pentane/EtOAc → 50% pentane/EtOAc) afforded the product as a colorless oil (30.0 mg, 60%). **IR** (film) ν_{max}/cm^{-1}: 3447br, 2979s, 2937m, 1703m, 1645m, 1377s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.82 (d, *J* = 1.0 Hz, 1H, H9), 4.67 (d, *J* = 1.0 Hz, 1H, H9), 4.12 (q, *J* = 6.4 Hz, 1H, H10), 2.75 (s, 1H, H14), 2.56 (m, 3H, H3, H4), 1.90 – 1.76 (m, 2H, H5, H6), 1.75 – 1.66 (m, 4H, H8, H6), 1.45 – 1.42 (m, 1H, H5), 1.10 (d, *J* = 6.4 Hz, 3H, H12), 1.08 (s, 3H, H11); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 217.4, 146.8, 111.2,

70.5, 51.9, 44.2, 43.1, 33.4, 24.6, 21.3, 16.7, 15.9; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{12}H_{21}O_2$) requires *m/z* 197.1536, found *m/z* 197.1537; $\mathbf{R_f}$ 0.2 (20% EtOAc/pentane).

(2*S*,5*S*)-2-((*S*)-1-Hydroxy-4-methylpent-4-en-1-yl)-2-methyl-5-(prop-1-en-2-yl)cyclohexan-1-one (187)



Method A

To a solution of LiHMDS (1.0 M, 900 μL, 0.9 mmol) in THF (1.0 mL) was added ketone **196** (76.0 mg, 0.5 mmol) at –78 ℃. The mixture was then warmed up to 0 ℃ and stirred for another 1 h. The reaction mixture was then cooled to –78 ℃, and was added in with a solution of $ZnBr_2$ (202 mg, 0.9 mmol) in THF (0.2 mL). The mixture was warmed up to 0 ℃ and stirred for 30 min. Then aldehyde **189** (98.0 mg, 1.0 mmol) was added to the mixture at 0 ℃ and stirred for 1 h, then Rochelle's salt was added to the reaction mixture. The aqueous layer was extracted with Et_2O (3 × 2 mL) and the organic extracts were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (1% Et_2O/pentane) afforded the product as a colourless oil (28.8 mg, 23%).

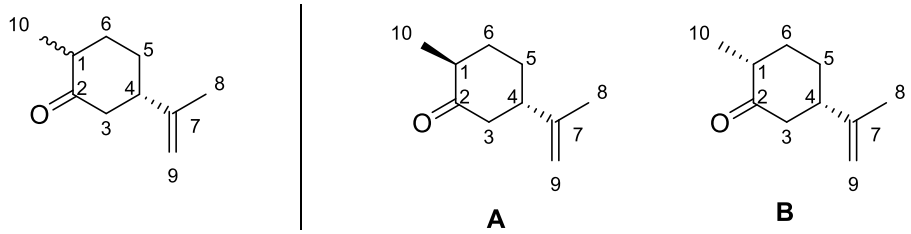
Method B

To a solution of (*S*)-(+)-carvone (195 mg, 1.30 mmol) in THF (4.0 mL) at –78 ℃ was added dropwise L-Selectride (1.0 M, 1.40 mL, 1.40 mmol), and stirred for 45 min at this temperature.

A solution of $ZnBr_2$ (527 mg, 2.30 mmol) in THF (2.0 mL) was added to the reaction mixture, and the mixture was stirred for 30 min. Then a solution of aldehyde **189** (181 mg, 1.85 mmol) was added and the mixture was stirred for 1 h at this temperature. H_2O_2 (780 μL) and 2.0 N NaOH solution (1.30 mL) were added and the mixture was allowed to warm up to RT over 14 h. The mixture was diluted with H_2O; the aqueous layer was extracted with EtOAc (3 × 4 mL). The combined organic extracts was washed with H_2O (2 × 4 mL), 5 % $NaHSO_4$ (4 mL), brine, dried over $MgSO_4$ and concentrated *in vacuo.* Flush column chromatography (33% Et_2O/pentane) afforded the product as a colourless oil (195 mg, 65%).

IR (film) ν_{max}/cm^{-1}: 3656br, 2980s, 1701m, 1459m, 1381m, 1252m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.81 (m, 1H, H9), 4.77 – 4.71 (m, 2H, H17), 4.69 (m, 1H, H9), 3.96 – 3.85 (m, 1H, H11), 2.62 – 2.51 (m, 2H, H3), 2.49 – 2.43 (m, 2H, H4, O*H*), 2.29 (ddd, *J* = 14.7, 9.4, 5.4 Hz, 1H, H14), 2.12 – 2.05 (m, 1H, H14), 2.03 (s, 3H, H8), 1.87 – 1.75 (m, 3H, H5, H6), 1.73 (m, 3H, H16), 1.60 (dddd, *J* = 13.7, 9.4, 6.7, 1.9 Hz, 1H, H13), 1.53 – 1.43 (m, 1H, H6), 1.43 – 1.33 (m, 1H, H13), 1.07 (s, 3H, H12); **^{13}C NMR** (101 MHz Chloroform-*d*) δ: 146.9, 145.7, 110.9, 110.3, 74.1, 52.2, 44.6, 43.2, 34.6, 33.7, 28.5, 24.9, 22.4, 21.0, 21.0, 14.1; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{16}H_{26}O_2Na$) requires *m/z* 273.1825, found *m/z* 273.1825; **R_f** 0.3 (33% Et_2O/pentane).

(5*S*)-2-Methyl-5-(prop-1-en-2-yl)cyclohexan-1-one (196)

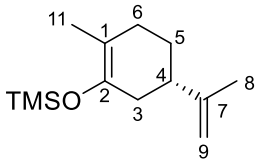


Zn dust (12.5g, 192.0 mmol) was suspended in H_2O (25.0 mL). To this suspension was added a solution of KOH (6.25 g, 56.1 mmol) in H_2O (13.0 mL), followed by a solution of (*S*)-(+)-carvone (5.00 mL, 32.0 mmol) in EtOH (62.5 mL). The reaction mixture was stirred vigorously and heated to reflux for 5 h. The white precipitate was filtered off and the EtOH was removed under vacuum. The aqueous filtrate was extracted with Et_2O (2 × 10 mL). The organic layers were washed with H_2O (10 mL) and brine, dried over $MgSO_4$ and concentrated *in vacuo.* The crude residue was purified by flush column chromatography (1% Et_2O/pentane) to give separable diastereoisomers as colourless oil (2.68 g, 50%) and colourless oil (0.53 g, 10%). **IR** (film) ν_{max}/cm^{-1}: 2931s, 1711s, 1645m, 1450m, 1376m, 1217m; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{10}H_{17}O$) requires *m/z* 153.1274, found *m/z* 153.1274; $\mathbf{R_f}$ 0.8 (33% Et_2O/pentane).

Major isomer: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.75 (m, 1H, H10), 4.74 – 4.71 (m, 1H, H9), 2.48 – 2.41 (m, 1H, H3), 2.41 – 2.21 (m, 3H, H1, H3, H4), 2.12 (tdd, *J* = 13.3, 5.8, 3.3 Hz, 1H, H5), 1.93 (tdd, *J* = 13.3, 3.3, 2.3 Hz, 1H, H6), 1.76 – 1.71 (s, 3H, H8), 1.64 (tdd, *J* = 13.3, 11.5, 3.6 Hz, 1H, H6), 1.37 ((tdd, *J* = 13.3, 5.8, 3.3 Hz, 1H, H5), 1.03 (d, *J* = 6.5 Hz, 3H, H10); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 212.6, 147.6, 109.6, 47.0, 46.8, 44.7, 34.9, 30.7, 20.4, 14.3.

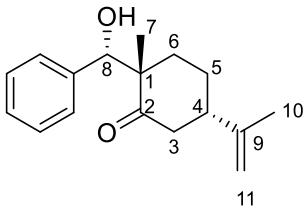
Minor isomer: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.82 (s, 1H, H10), 4.68 (s, 1H, H10), 2.59–2.52 (m, 1H, H3), 2.41–2.37 (m, 2H, H3, H4), 1.86–1.82 (m, 3H, H5, H6), 1.72 (s, 3H, H9), 1.62–1.58 (m, 1H, H5), 1.08 (d, *J* = 4.8 Hz, 3H, H11); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 212.6, 147.6, 109.6, 47.0, 46.9, 44.8, 34.9, 30.8, 20.5, 14.4.

(*S*)-Trimethyl((2-methyl-5-(prop-1-en-2-yl)cyclohex-1-en-1-yl)oxy)silane (197)[250]



A solution of NaI (3.60 g, 24.0 mmol) in 75.0 mL of dry MeCN was added dropwise to a mixture of ketone **196** (1.83 g, 12.0 mmol), trimethylsilyl chloride (3.00 mL, 24.0 mmol) and dry Et_3N (3.40 mL, 24.0 mmol), and this solution was stirred at RT for 1 h under nitrogen. The mixture was poured into 30 mL of ice and extracted with hexane (3 × 15 mL). The combined organic layers was washed with sat. NH_4Cl until neutral and dried over Na_2SO_4. Removal of the solvent *in vacuo* afforded the desired product as a colorless oil (5.20 g, 98%). The product is used directly without further purification. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.76 – 4.69 (m, 2H, H9), 2.23 (dddd, *J* = 12.8, 8.6, 4.3,2.2 Hz, 1H, H4), 2.14 – 1.90 (m, 3H, H3, H6), 1.74 – 1.71 (m, 4H, H5, H8), 1.61 – 1.52 (s, 3H, H11), 1.47 – 1.35 (m, 1H, H5), 1.35 – 1.17 (m, 1H, H6), 0.19 (s, 9H, CH_3); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 148.6, 141.6, 109.2, 107.9, 90.8, 41.8, 34.9, 29.4, 27.3, 21.6, 15.3; **R_f** 0.5 (33% Et_2O/pentane).

(2*S*,5*S*)-2-((*S*)-Hydroxy(phenyl)methyl)-2-methyl-5-(prop-1-en-2-yl)cyclohexan-1-one (199)[251]



Method A

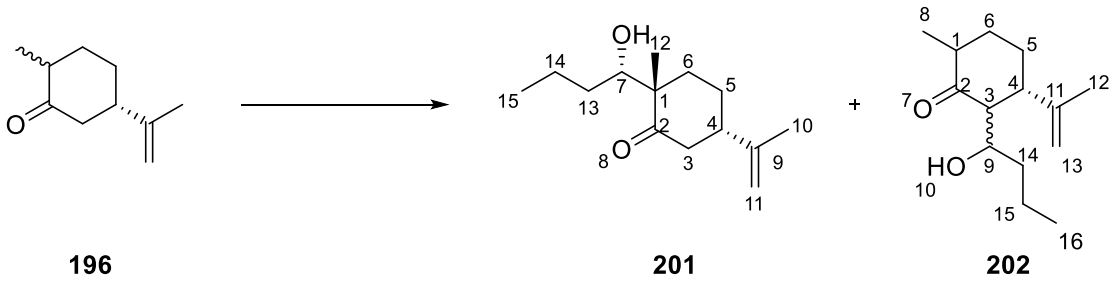
TMS-enol ether **197** (44.2 mg, 0.24 mmol) and benzaldehyde (21.2 mg, 0.20 mmol) were dissolved in DCM (0.8 mL) and cooled to –78 ℃. The reaction mixture was added in with $TiCl_4$ (37.9 mg, 0.20 mmol) and stirred for 4 h. The reaction mixture was quenched with sat. $NaHCO_3$ at –78 ℃. The two layers were separated, the aqueous layer was extracted with Et_2O (3 × 2 mL), and the organic extracts were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (10% Et_2O/pentane) afforded the product as a colourless oil (76.0 mg, 60%).

Method B

MeLi (1.6 M in hexane, 370 μL, 0.55 mmol) was added dropwise to a solution of TMS-enol ether **197** (112 mg, 0.50 mmol) in DME (2.8 mL) at –78 ℃. The mixture was warmed up to 0 ℃ and then stirred for 2 h. After cooling to –78 ℃ again, benzaldehyde (58.0 mL, 0.55 mmol) was added and the reaction mixture was stirred for 3 h at –78 ℃. The reaction mixture was quenched with NH_4Cl (30.0 mg) and then sat. NH_4Cl (2.0 mL) at –78 ℃, and warmed to RT. The two layers were separated, the aqueous layer was extracted with Et_2O (3 × 2 mL), and the organic extracts were combined, washed with brine, dried over $MgSO_4$, filtered and

concentrated *in vacuo.* Flush column chromatography (10% Et_2O/pentane) afforded the product as a colourless oil (74.0 mg, 57%).**^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.30 – 7.18 (m, 5H, H_{Ar}), 4.86 (d, *J* = 2.3 Hz, 1H, H8), 4.78 – 4.72 (m, 1H, H11), 4.71 – 4.64 (m, 1H, H11), 4.18 (d, *J* = 2.4 Hz, 1H, H9), 2.66 – 2.46 (m, 1H, H3), 2.42 – 2.22 (m, 2H, H3, H4), 1.69 (m, 4H, H5, H10), 1.61 – 1.51 (m, 2H, H5, H6), 1.34 – 1.11 (m, 4H, H6, H7); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 218.2, 146.1, 137.3, 127.1, 126.6, 126.4, 108.9, 77.5, 50.8, 45.7, 42.7, 34.8, 24.9, 21.3, 19.4; **R_f** 0.4 (33% Et_2O/pentane).

Procedure for the aldol reaction with butyraldehyde 200



196 **201** **202**

To a solution of LiHMDS (1.0 M, 900 μL, 0.9 mmol) in THF (1.0 mL) was added ketone **196** (76.0 mg, 0.5 mmol) at –78 ℃. The reaction mixture was then warmed up to 0 ℃, and stirred for another 1 h. The reaction mixture was cooled –78 ℃, and was added a solution of $ZnBr_2$ (202 mg, 0.9 mmol) in Et_2O (0.2 mL). The reaction mixture was warmed up to 0 ℃ and stirred for 30 min. Then butyraledehyde (72.0 mg, 1.0 mmol) was added to the mixture at 0 °C and stirred for 30 min, then Rochelle's salt was added to the reaction mixture. The aqueous layer was extracted with Et_2O (3 × 2 mL) and the organic extracts were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Flush column chromatography (1% Et_2O/pentane → 2% Et_2O/pentane → 4% Et_2O/pentane → 7% Et_2O/pentane) afforded the 202

product **201** as a colourless oil (20.0 mg, 20%) along with alcohol **202** (colorless oil, 21 mg, 21%).

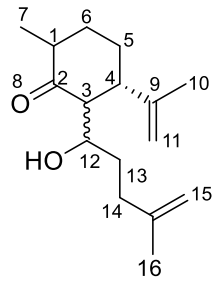
Data for **(2*S*,5*S*)-2-((*S*)-1-hydroxybutyl)-2-methyl-5-(prop-1-en-2-yl)cyclohexan-1-one (201)**

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 4.75 (d, J = 0.7 Hz, 1H, H11), 4.63 (d, J = 1.3,0.7 Hz, 1H, H11), 3.92 – 3.79 (m, 1H, H7), 2.58 – 2.38 (m, 3H, H3, H4), 2.37 – 2.30 (m, 1H, H16), 2.28 – 2.21 (m, 1H, H13), 1.81 – 1.70 (m, 3H, H5, H13, H14), 1.64 (s, 3H, H10), 1.48 – 1.16 (m, 3H, H5, H6, H14), 0.99 (s, 3H, H12), 0.91 – 0.85 (m, 3H, H15); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 216.6, 147.0, 110.8, 74.2, 65.8, 52.2, 44.7, 43.2, 33.8, 32.8, 25.0, 16.6, 15.2, 14.0; **R_f** 0.3 (33% Et_2O/pentane).

Data for **(3*S*)-2-(1-hydroxybutyl)-6-methyl-3-(prop-1-en-2-yl)cyclohexan-1-one (202)**

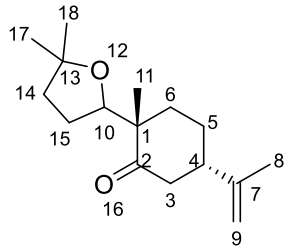
^{1}H NMR (400 MHz, Chloroform-d) δ 4.85 – 4.76 (m, 2H, H13), 3.39 (ddd, J = 11.5, 8.7, 5.1 Hz, 1H, H9), 3.00 (d, J = 11.6 Hz, 1H, H10), 2.73 – 2.62 (m, 1H, H4), 2.48 – 2.34 (m, 1H, H1), 2.29 (d, J = 12.2 Hz, 1H, H3), 2.02 (ddd, J = 13.3, 5.6, 3.4 Hz, 1H, H6), 1.81 – 1.66 (m, 3H, H5, H14, H15), 1.64 (s, 3H, H12), 1.47 – 1.26 (m, 2H. H5, H6), 1.28 – 1.14 (m, 2H, H14, H15), 0.93 (d, J = 6.4 Hz, 3H, H8), 0.83 (t, J = 7.1 Hz, 3H, H16).; **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 216.7, 145.6, 113.1, 70.4, 56.4, 51.3, 46.6, 38.7, 36.0, 31.5, 19.9, 18.6, 14.1, 14.0; **R_f** 0.8 (33% Et_2O/pentane).

(3*S*)-2-(1-Hydroxy-4-methylpent-4-en-1-yl)-6-methyl-3-(prop-1-en-2-yl)cyclohexan-1-one (203)



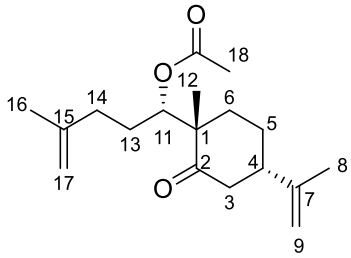
To a solution of LiHMDS (1.0 M, 900 μL, 0.9 mmol) in THF (1.0 mL) was added ketone **196** (76.0 mg, 0.5 mmol) at –78 ℃. The mixture was then warmed up to 0 ℃ and stirred for another 1 h. The reaction mixture was then cooled to –78 ℃, and was added in with a solution of $ZnBr_2$ (202 mg, 0.9 mmol) in THF (0.2 mL). The mixture was warmed up to 0 ℃ and stirred for 30 min. Then aldehyde **189** (98.0 mg, 1.0 mmol) was added to the mixture at 0 ℃ and stirred for 1 h, then Rochelle's salt was added to the reaction mixture. The aqueous layer was extracted with Et_2O (3 × 2 mL) and the organic extracts were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (1% Et_2O/pentane) afforded the product as a colourless oil (27.0 mg, 20%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.85 – 4.74 (m, 2H, H11), 4.66 – 4.56 (m, 2H, H15), 3.38 (ddd, J = 11.5, 8.6, 4.8 Hz, 1H, H12), 3.04 (d, J = 11.5 Hz, 1H, O*H*), 2.75 – 2.63 (m, 1H, H1), 2.41 (ddd, J = 13.0, 11.9, 6.4 Hz, 1H, H4), 2.31 (d, J = 12.2, 1H, H3), 2.20 –1.82 (m, 4H, H5, H6, H13, H14), 1.87 – 1.70 (m, 2H, H5, H6), 1.64 (s, 5H, H5, H16), 1.56 – 1.40 (m, 1H, H13), 1.42 – 1.26 (m, 1H, H14), 0.93 (d, J = 6.4 Hz, 3H, H7); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 216.6, 145.6, 145.4, 113.1, 109.7, 70.4, 56.6, 51.4, 46.5, 35.9, 34.7, 34.4, 31.4, 22.5, 18.6, 14.0; **R_f** 0.2 (33% Et_2O/pentane).

(5*S*)-2-(5,5-Dimethyltetrahydrofuran-2-yl)-2-methyl-5-(prop-1-en-2-yl)cyclohexan-1-one (204)



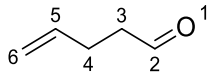
To a solution of ketone **187** (25 mg, 0.10 mmol) in DCM (2.0 mL) was added dropwise $BF_3 \cdot OEt_2$ (21 mg, 0.15 mmol) at –78 ℃, and the reaction mixture was stirred for 2 h at –78 ℃. Sat. $NaHCO_3$ solution (2 mL) was added to the reaction mixture, the aqueous layer was extracted with DCM (3 × 2 mL) and the organic extracts were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Flush column chromatography (2% Et_2O/pentane) afforded the product as a colourless oil (10 mg, 40%). **IR** (film) ν_{max}/cm^{-1}: 2980s, 2359s, 1724s, 1685m, 1442m, 1240m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.79 – 4.74 (m, 1H, H9), 4.72 (d, *J* = 1.7 Hz, 1H, H9), 4.31 – 4.12 (m, 1H, H10), 2.78 – 2.61 (m, 1H, H15), 2.46 – 2.29 (m, 2H, H4, H15), 2.09 – 1.83 (m, 3H, H5, H6, H14), 1.73 (s, 3H, H8), 1.73 – 1.50 (m, 5H, H3, H5, H6, H14), 1.19 (s, 6H, H17, H18), 0.98 (s, 3H, H11); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 214.1, 147.9, 109.7, 82.9, 80.9, 50.8, 46.2, 45.0, 37.9, 37.4, 28.5, 27.6, 27.3, 26.6, 20.4, 19.2; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{16}H_{26}O_2Na$) requires *m/z* 273.1825, found *m/z* 273.1825; **R_f** 0.5 (33% Et_2O/pentane).

(*S*)-4-Methyl-1-((1*S*,4*S*)-1-methyl-2-oxo-4-(prop-1-en-2-yl)cyclohexyl)pent-4-en-1-yl acetate (205)



To a solution of ketone **187** (250 mg, 1.00 mmol) in DCM (5.0 mL) was added 4-dimethylaminopyridine (12.1 mg, 0.10 mmol), trimethylamine (111 mg, 1.10 mmol) and acetic anhydride (103 mg, 1.01 mmol) at 0 ℃. The reaction mixture was warmed up to RT over 3 h and quenched with sat. $NaHCO_3$. The aqueous layer was extracted with DCM (3 × 3 mL) and the organic extracts were combined, washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Flush column chromatography (30% Et_2O/pentane) afforded the product as a colourless oil (219 mg, 75%). **IR** (film) ν_{max}/cm^{-1}: 2938m, 1736s, 1709m, 1453m, 1372m, 1237s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.51 (dd, *J* = 10.6, 2.2 Hz, 1H, H11), 4.78 (d, *J* = 1.5 Hz, 1H, H17), 4.77 (d, *J* = 1.6, Hz, 1H, H9), 4.74 (d, *J* = 1.6, Hz, 1H, H9), 4.72 (d, *J* = 1.5 Hz, 1H, H17), 2.88 – 2.73 (m, 1H, H3), 2.35–2.32 (m, 2H, H3, H4), 2.09 – 1.83 (m, 4H, H5, H6), 2.07 – 1.97 (s, 3H, H18), 1.82 (s, 3H, H8), 1.80 – 1.70 (m, 2H, H13, H14), 1.70 (s, 3H, H16), 1.66 – 1.58 (m, 1H, H13),1.49 (ddd, *J* = 14.5, 9.0, 7.2 Hz, 1H, H14), 1.06 (s, 3H, H12); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 213.1, 170.7, 147.2, 144.7, 110.6, 110.2, 74.8, 52.0, 46.3, 43.7, 35.9, 34.1, 26.9, 26.0, 22.6, 20.9, 20.5, 17.7; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{18}H_{28}O_3Na$) requires *m/z* 315.1936, found *m/z* 315.1936; **R_f** 0.6 (33% Et_2O /pentane).

Pent-4-enal (211)[252]



Method A

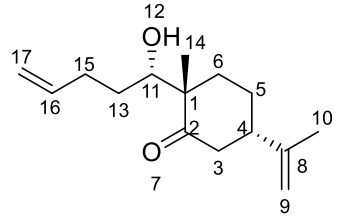
To a solution of PCC (6.47 g, 30.0 mmol) in DCM (100.0 mL) was added pent-4-ene-1-ol (2.00 mL, 20.0 mmol), and the reaction mixture was stirred at RT for 6 h. The reaction mixture was filtered through a silica/celite (3:1) pad, and the filtrate was concentrated *in vacuo.* The colourless liquid was used directly for the next step.

Method B

To a solution of pent-4-ene-1-ol (2.00 mL, 20.0 mmol) in DCM (150.0 mL) was added $NaHCO_3$ (2.34 g, 40.0 mmol) and cooled to 0 ℃. DMP (13.1 g, 30.0 mmol) was added, and the mixture was allowed to warm to RT over 2 h. The mixture was washed with 1.0 M NaOH (2 × 200 mL). The aqueous layer was extracted with DCM (2 × 100 mL). The organic layers were combined, washed with H_2O (50 mL) and brine, dried over $MgSO_4$ and concentrated *in vacuo* to give a colourless oil (1.8 g, 90%). The crude was used directly in the next step without any further purification. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 9.78 (s, 1H, H2), 6.03 – 5.68 (m, 1H, H5), 5.16 – 4.89 (m, 2H, H6), 2.66 – 2.50 (m, 2H, H3), 2.50 – 2.28 (m, 2H, H4); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 201.9, 136.4, 115.7, 42.8, 26.1; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ (C_5H_9O) requires *m/z* 84.0648, found *m/z* 84.0593; **R_f** 0.9 (33% Et_2O/pentane).

Experimental

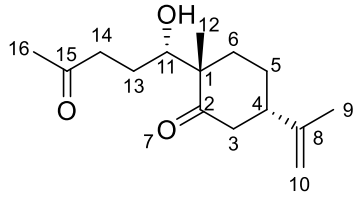
(2*S*,5*S*)-2-((*S*)-1-Hydroxypent-4-en-1-yl)-2-methyl-5-(prop-1-en-2-yl)cyclohexan-1-one (212)



To a solution of (*S*)-(+)-carvone (79.0 mg, 0.50 mmol) in THF (1.5 mL) was added L-Selectride (1.0 M, 550 μL, 0.55 mmol) dropwise at –78 ℃, and the mixture was stirred for 30 min at this temperature. A solution of $ZnBr_2$ (202 mg, 0.90 mmol) in THF (1.5 mL) was added to the reaction mixture, and the mixture was stirred for 30 min. Then a solution of aldehyde **211** (63.0 mg, 0.75 mmol) was added and the mixture was stirred for 1 h at –78 ℃. H_2O_2 (0.3 mL) and 2.0 N NaOH solution (0.5 mL) were added and the mixture was allowed to warm up to RT over 14 h. The reaction mixture was diluted with H_2O, the aqueous layer was extracted with EtOAc (3 × 4 mL) and the organic extracts were combined, washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (25% Et_2O/pentane) afforded the product as a colourless oil (159 mg, 68%). **IR** (film) ν_{max}/cm^{-1}: 3466br, 2972m, 1702s, 1644w, 1453m, 1158m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.83 (dddd, J = 17.4, 10.2, 7.3, 6.1 Hz, 1H, H16), 5.07 (ddd, J = 10.2, 2.2, 1.2 Hz, 1H, H17), 5.00 (ddd, J = 10.2, 2.2, 1.2 Hz, 1H, H17), 4.82 (d, J = 1.1 Hz, 1H, H9), 4.70 (d, J = 1.1 Hz, 1H, H9), 3.92 (ddd, J = 10.5, 5.0, 2.0 Hz, 1H, H11), 2.67 – 2.44 (m, 4H, H3, H4, H13), 2.41 – 2.34 (m, 1H, H15), 2.13 (m, 1H, H15), 1.89 – 1.75 (m, 2H, H5, H6), 1.73 (s, 3H, H10), 1.55 – 1.44 (m, 2H, H5, H6), 1.43 – 1.30 (m, 1H, H13), 1.08 (s, 3H, H14); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 216.7, 146.9, 138.4, 115.2, 111.0, 73.9, 52.1, 44.5,

43.2, 33.5, 30.6, 29.8, 24.9, 21.2, 16.6; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{16}H_{26}O_2Na$) requires *m/z* 259.1679, found *m/z* 259.1669; $\mathbf{R_f}$ 0.3 (33% Et_2O/pentane).

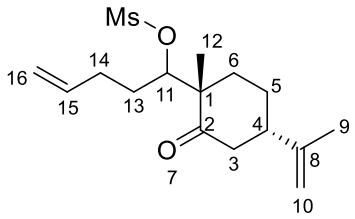
(2*S*,5*S*)-2-((*S*)-1-Hydroxy-4-oxopentyl)-2-methyl-5-(prop-1-en-2-yl)cyclohexan-1-one (208)



A suspension of $PdCl_2$ (44.0 mg, 0.25 mmol) and CuCl (248 mg, 2.50 mmol) in DMF (1.25 mL) and H_2O (250 μL, 14.1 mmol) was stirred in an oxygen atmosphere, created *via* evacuative displacement of air using an oxygen balloon, for 1 h at RT. A solution of ketone **212** (590 mg, 2.50 mmol) in 0.5 mL of DMF was then added, and the reaction mixture was stirred for 24 h at RT in the oxygen atmosphere. Aqueous HCl solution (3.0 N, 2.5 mL) was added to the reaction mixture, the aqueous layer was extracted with Et_2O (3 × 4 mL) and the organic extracts were combined, washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Flush column chromatography (15% Et_2O/pentane) afforded the product as a colourless oil (200 mg, 34%). **IR** (film) ν_{max}/cm^{-1}: 3447br, 2967s, 2935s, 2355w, 1709s, 1645s, 1458s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.81 (d, J = 1.0 Hz, 1H, H10), 4.67 (d, J = 1.0 Hz, 1H, H10), 3.82 (ddd, J = 10.8, 5.0, 2.1 Hz, 1H, H11), 3.01 (d, J = 5.0 Hz, 1H, O*H*), 2.75 – 2.49 (m, 5H, H3, H4, H14), 2.16 (s, 3H, H16), 2.04 – 1.89 (m, 2H, H6, H13), 1.88 – 1.74 (m, 3H, H9), 1.74 – 1.71 (m, 2H, H5, H6), 1.57 – 1.42 (m, 2H, H5, H13), 1.08 (s, 3H, H12); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 217.1,

209.5, 146.8, 111.1, 74.0, 52.1, 44.2, 43.1, 40.4, 33.2, 30.1, 24.6, 24.2, 21.2, 16.5; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{15}H_{24}O_3Na$) requires *m/z* 275.1618, found *m/z* 275.1620; $\mathbf{R_f}$ 0.4 (33% Et_2O/pentane).

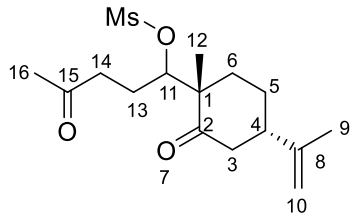
1-((1*S*,4*S*)-1-Methyl-2-oxo-4-(prop-1-en-2-yl)cyclohexyl)pent-4-en-1-yl methanesulfonate (213)



To a solution of alcohol **212** (472 mg, 2.0mmol) in DCM (6.0 mL) was added Et_3N (1.01 g, 10.0 mmol) and methanesulfonyl chloride (456 mg, 8.0 mmol) at 0 ℃. The reaction mixture was allowed to warm up to RT over 4 h. The reaction was then quenched with 1.0 M HCl, the aqueous layer was extracted with DCM (3 × 5 mL) and the organic extracts were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (4% EtOAc /pentane) afforded the product as a colourless oil (331 mg, 66%). **IR** (film) ν_{max}/cm^{-1}: 2990s, 2480w, 1700m, 1430s, 1250s; ^{1}**H NMR** (400 MHz, Chloroform-*d*) δ: 5.83 (dddd, *J* = 17.4, 10.2, 7.3, 6.0 Hz, 1H, H15), 5.36 – 5.23 (m, 1H, H11), 5.23 – 4.98 (m, 2H, H16), 4.86 – 4.70 (m, 2H, H10), 2.93 (s, 3H, C*H*$_3$), 2.88 (dd, *J* = 14.1, 12.0 Hz, 1H, H3), 2.50 – 2.43 (m, 1H, H3), 2.49 – 2.39 (m, 2H, H4, H14), 2.24 – 2.21 (m, 1H, H14), 2.02–2.00 (m, 1H, H15), 1.80 – 1.64 (m, 6H, H6, H9, H13), 1.57 – 1.46 (m, 3H, H5, H6), 1.10 (s, 3H, H12); ^{13}**C NMR** (101 MHz, Chloroform-*d*) δ: 212.7, 146.7, 136.8, 116.3, 110.5, 84.4,

52.5, 46.1, 43.5, 39.1, 35.5, 29.95, 28.7, 25.5, 20.4, 17.3; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{16}H_{26}O_4NaS$) requires *m/z* 337.1444, found *m/z* 337.1445; $\mathbf{R_f}$ 0.5 (33% Et_2O/pentane).

1-((1*S*,4*S*)-1-Methyl-2-oxo-4-(prop-1-en-2-yl)cyclohexyl)-4-oxopentyl methanesulfonate (214)



Method A

To a solution of diene **213** (314 mg, 1.0 mmol) in DMF (0.5 mL) and H_2O (0.1 mL) was charged with O_2. $PdCl_2$ (21.0 mg, 0.1 mmol) and CuCl (57.0 mg, 5.0 mmol) was then added into the solution, and the reaction mixture was stirred at RT for 14 h. The reaction was quenched with 3.0 M HCl, the aqueous layer was extracted with Et_2O (3 × 1 mL) and the organic extracts were combined, washed with sat. $NaHCO_3$, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Flush column chromatography (4% EtOAc/pentane) afforded the product as a colourless oil (282 mg, 90%).

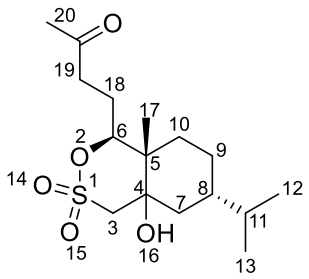
Method B

To a solution of diketone **208** (500 mg, 2.0 mmol) in DCM (6.0 mL) was added Et_3N (1.01 g, 10.0 mmol) and methanesulfonyl chloride (456 mg, 8.0 mmol) at 0 ℃. The reaction mixture was allowed to warm up to RT over 4 h. The reaction was then quenched with 1.0 M HCl, the

aqueous layer was extracted with DCM (3 × 5 mL) and the organic extracts were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (4% EtOAc/pentane) afforded the product as a colourless oil (300 mg, 60%).

IR (film) ν_{max}/cm^{-1}: 2980s, 2888w, 1707m, 1381m, 1169s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.32 (dd, *J* = 10.8, 1.8 Hz, 1H, H11), 4.86 – 4.73 (m, 3H, H16), 2.90 (s, 3H, H_{Ms}), 2.89 – 2.77 (m, 2H, H3, H14), 2.68 (dd, *J* = 6.4, 4.9 Hz, 1H, H3), 2.50 – 2.30 (m, 2H, H4, H14), 2.20 (s, 3H, H16), 2.17 – 2.07 (m, 2H, H6, H13), 1.96 – 1.79 (m, 2H, H5, H13), 1.81 – 1.75 (m, 3H, H9), 1.69 (ddd, *J* = 10.8, 6.0, 4.9 Hz, 1H, H6), 1.61 – 1.50 (m, 1H, H5), 1.08 (s, 3H, H12); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 214.2, 212.7, 146.8, 110.4, 84.1, 52.6, 46.5, 43.6, 39.0, 38.8, 36.2, 30.1, 25.6, 22.7, 20.3, 16.7; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{16}H_{26}O_5NaS$) requires *m/z* 353.1393, found *m/z* 337.1392; **R_f** 0.2 (33% Et_2O/pentane).

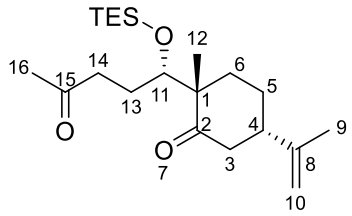
4-((1*S*,6*S*,8a*S*)-4a-Hydroxy-6-isopropyl-8a-methyl-3,3-dioxidooctahydrobenzo[*d*][1,2]oxathiin-1-yl)butan-2-one (217)



To a solution of diketone **214** (33 mg, 0.10 mmol) in 1,4-dioxane (0.5 mL) was added $TiCl_4$ (28 mg, 0.15 mmol) at –10 ℃ and stirred for 5 min. Zinc powder (19 mg, 0.30 mmol) was then added into the solution, and the reaction mixture was allowed to warm up to RT, then

heated to reflux for 14 h. The reaction was quenched with sat. K_2CO_3. The precipitate was filtered off, washed with EtOAc (3 × 1 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Flush column chromatography (30% EtOAc/pentane) afforded the product as a colourless oil (2.0 mg, 6%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.85 – 5.74 (m, 1H, H6), 5.19 – 5.05 (m, 3H, H13), 3.36 (d, *J* = 13.7 Hz, 1H, H3), 3.07 (d, *J* = 13.7 Hz, 1H, H3), 2.67 (dd, *J* = 14.8 Hz, 1H, H7), 2.46 – 2.33 (m, 1H, H8), 2.17 (m, 4H, H18, H19), 1.88 – 1.66 (m, 6H, H12, H20), 1.66 – 1.56 (m, 3H, H7, H9, H10), 1.30 – 1.12 (m, 2H, H9, H10), 1.07 (s, 3H, H17); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 147.8, 136.6, 116.6, 109.8, 73.9, 56.6, 40.9, 39.8, 38.4, 31.1, 30.9, 29.6, 27.2, 25.6, 20.6, 14.7; **R_f** 0.2 (33% Et_2O/pentane).

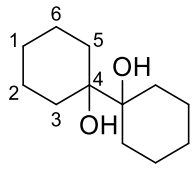
(2*S*,5*S*)-2-Methyl-2-((*S*)-4-oxo-1-((triethylsilyl)oxy)pentyl)-5-(prop-1-en-2-yl)cyclohexan-1-one (218)



To a solution of diketone **208** (350 mg, 1.0 mmol) in DMF (4.0 mL) was added imidazole (648 mg, 6.0 mmol) and trimethylsilyl chloride (425 mg, 3.0 mmol) at RT. The reaction mixture was stirred for 40 min before it was cooled to 0 ℃. H_2O was added to the reaction mixture, the aqueous layer was extracted with Et_2O (3 × 5 mL) and the organic extracts were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Flush

column chromatography (2% Et_2O/pentane) afforded the product as a colourless oil (315 mg, 90%). **IR** (film) ν_{max}/cm^{-1}: 2958w, 1705m, 1250m, 1143w, 1096m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.75 (d, *J* = 29.2 Hz, 2H, H10), 4.22 (dd, *J* = 8.3, 2.8 Hz, 1H, H11), 2.68 – 2.45 (m, 3H, H3, H4), 2.45 – 2.33 (m, 2H, H14), 2.16 (s, 3H, H16), 2.05 – 1.97 (m, 1H, H13), 1.89 (ddd, *J* = 9.3, 6.9, 2.8 Hz, 1H, H6), 1.84 – 1.77 (m, 1H, H5), 1.56 (s, 3H, H9), 1.53 – 1.41 (m, 3H, H5, H6, H13), 1.02 (s, 3H, H12), 0.98 – 0.89 (m, 9H, CH_3), 0.57 (p, *J* = 7.8 Hz, 6H, CH_2); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 214.5, 208.3, 147.4, 110.1, 84.4, 52.6, 46.1, 44.1, 40.4, 35.1, 26.3, 25.87, 20.6, 18.0, 7.0, 5.6; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{21}H_{39}O_3Si$) requires *m/z* 367.2663, found *m/z* 367.2662; **R_f** 0.7 (33% Et_2O/pentane).

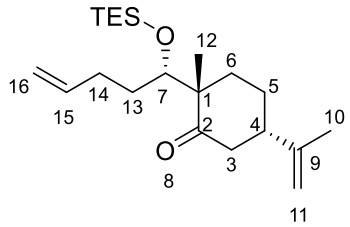
[1,1'-Bi(yclohexane)]-1,1'-diol (221)[253]



Magnesium powder (96.0 mg, 4.00 mmol) and $HgCl_2$ (238 mg, 0.88 mmol) was suspended in THF (2.0 mL) and sonicated for 30 min at RT. The suspension was then cooled to –10 ℃ and added in with $TiCl_4$ (283 mg, 1.50 mmol) dropwise under vigorous stirring. A solution of cyclohexanone (98.0 mg, 1.00 mmol) in THF (0.2 mL) was then added to the reaction mixture, which was then sonicated at 0 ℃ for 2 h. The reaction mixture was then heated to reflux for 14 h, and cooled to 0 ℃ again, followed by addition of sat. K_2CO_3 solution. After stirring for 15 min, the reaction mixture was diluted with H_2O. The resulted solid was filtered off, washed with EtOAc (5 × 1 mL). The organic layers were combined, washed with brine, dried over

$MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (50% Et_2O/pentane) afforded the product as a colourless oil (40.0 mg, 20%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 1.77 (s, 2H, O*H*), 1.73 – 1.63 (m, 6H, H4, H3, H5), 1.63 – 1.52 (m, 12H, H2, H3, H5, H6), 1.42 – 1.32 (m, 4H, H1); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 75.6, 30.7, 25.8, 21.7; **R_f** 0.4 (50% Et_2O/pentane).

(2*S*,5*S*)-2-Methyl-5-(prop-1-en-2-yl)-2-((*S*)-1-((triethylsilyl)oxy)pent-4-en-1-yl)cyclohexan-1-one (225)

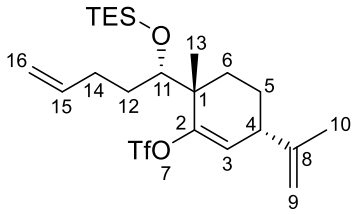


To a solution of alcohol **212** (589 mg, 2.5 mmol) in DMF (6.3 mL) was added imidazole (1.62 g, 15.0 mmol) and trimethylsilyl chloride (1.13 g, 7.5 mmol) at RT. The reaction mixture was stirred for 40 min before it was cooled to 0 ℃. H_2O was added to the reaction mixture, the aqueous layer was extracted with Et_2O (3 × 5 mL) and the organic extracts were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (2% Et_2O/pentane) afforded the product as a colourless oil (831 mg, 95%).

IR (film) ν_{max}/cm^{-1}: 2953s, 2912m, 1714s, 11643m, 1455m, 11238m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.81 (dddd, *J* = 17.2, 10.2, 6.9, 6.2 Hz, 1H, H15), 5.05 (dd, *J* = 17.2, 1.7 Hz, 1H, H16), 5.00 (dd, *J* = 10.2, 2.0 Hz, 1H, H16), 4.82 – 4.75 (m, 1H, H11), 4.71 – 4.67 (m, 1H, H11), 4.20 – 4.16 (m, 1H, H7), 2.61 (dd, *J* = 14.1, 12.7 Hz, 1H, H3), 2.40 – 2.35 (m, 1H,

H4), 2.32 (dd, *J* = 14.1, 12.7 Hz, 1H, H3), 2.33 – 2.19 (m, 1H, H14), 2.12 – 1.99 (m, 1H, H14), 1.97 (t, *J* = 4.2 Hz, 1H, H6), 1.93 (t, *J* = 4.2 Hz, 1H, H6), 1.75 – 1.70 (m, 1H, H5), 1.74 (s, 3H, H10), 1.63 (dddd, *J* = 14.1, 7.7, 6.6, 3.3 Hz, 1H, H13), 1.48 – 1.37 (m, 2H, H5, H13), 0.97 – 0.92 (m, 9H, CH_3), 0.59 (ddd, *J* = 8.2, 7.5, 1.0 Hz, 6H, CH_2); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 210.3, 146.1, 139.3, 114.9, 109.1, 73.8, 52.3, 46.1, 43.41, 35.3, 32.3, 31.3, 26.0, 21.2, 17.5, 7.0, 5.7; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{21}H_{38}O_2NaSi$) requires *m/z* 373.2533, found *m/z* 373.2533; **R_f** 0.6 (33% Et_2O/pentane).

(3*S*)-6-Methyl-3-(prop-1-en-2-yl)-6-(1-((triethylsilyl)oxy)pent-4-en-1-yl)cyclohex-1-en-1-yl trifluoromethanesulfonate (226)



Method A

To a solution of diisopropylamine (960 mg, 9.4 mmol) in THF (10.0 mL) at –78 ℃ was added dropwise *n*-BuLi (2.5 M, 3.76 mL, 9.4 mmol), and the mixture was stirred for 30 min. A solution of ketone **225** (1.50 g, 4.3 mmol) in THF (17.0 mL) was added to the reaction mixture, which was stirred for 1 h at –78 °C. A solution of Comins' reagent (3.68 g, 9.4 mmol) in THF (26.0 mL) was added, and the reaction mixture was allowed to warm up to RT overnight. Sat. $NaHCO_3$ solution was added to the mixture, the aqueous layer was extracted with EtOAc (3 × 10 mL) and the organic extracts were combined, washed with brine, dried over $MgSO_4$,

filtered and concentrated *in vacuo*. Flush column chromatography (5% EtOAc/pentane) afforded the product as a colourless oil (750 mg, 50%).

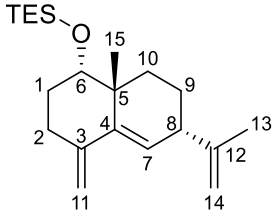
Method B

To a solution of ketone **225** (1.70 g, 4.85 mmol) in THF (24.0 mL) was added LiHMDS (1.0 M, 5.82 mL, 5.82 mmol) dropwise at –78 ℃ and the mixture wad stirred for 1 h at this temperature. The reaction mixture was then warmed up to RT and stirred for another 1 h. A solution of Comins' reagent (2.66 g, 6.79 mmol) in THF (12.0 mL) was added to the reaction mixture at RT and stirred for 14 h. The reaction mixture was quenched with sat. NH_4Cl, washed with brine. The aqueous layer was extracted with Et_2O (3 ×10 mL); the organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (5% EtOAc/pentane) afforded the product as a colourless oil (2.06 g, 88%).

IR (film) ν_{max}/cm^{-1}: 2980s, 2359s, 1643m, 1458m, 1379m, 1247m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.79 (ddt, J = 16.9, 10.3, 6.5 Hz, 1H, H15), 5.69 (d, J = 4.7 Hz, 1H, H3), 5.07 – 4.92 (m, 2H, H16), 4.88 (d, J = 0.9 Hz, 1H, H9), 4.69 (d, J = 0.9 Hz, 1H, H9), 3.74 (dd, J = 8.7, 2.2 Hz, 1H, H11), 2.92 – 2.87 (m, 1H, H4), 2.32 – 2.15 (dddd, J = 14.0, 9.1, 6.5, 2.1 Hz, 1H, H14), 2.01 (dddd, J = 14.0, 9.1, 6.5, 2.0 Hz, 1H, H14), 1.92 (ddd, J = 14.1, 10.9, 3.4 Hz, 1H, H6), 1.76 (s, 3H, H10), 1.69 – 1.59 (m, 3H, H5, H6, H12), , 1.54 – 1.44 (m, 1H, H5), 1.44 – 1.35 (m, 1H, H12), 1.22 (s, 3H, H13), 0.97 (t, J = 7.9 Hz, 9H, CH_3), 0.71 – 0.59 (q, J = 7.9 Hz, 6H, CH_2); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 154.9, 150.6, 146.0, 138.3, 114.6, 112.7, 78.7, 56.2, 44.0, 42.1, 33.3, 31.3, 26.8, 23.6, 22.5, 21.7, 7.0, 5.7; **HRMS (ESI)** exact

mass calculated for $[M+H]^+$ ($C_{22}H_{38}F_3O_4SSi$) requires *m/z* 483.2212, found *m/z* 483.2593; $\mathbf{R_f}$ 0.75 (33% Et_2O/pentane).

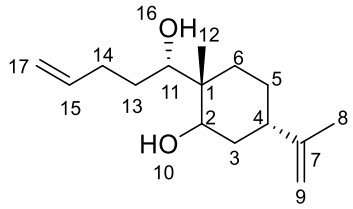
Triethyl(((1*S*,6*S*,8a*R*)-8a-methyl-4-methylene-6-(prop-1-en-2-yl)-1,2,3,4,6,7,8,8a-octahydronaphthalen-1-yl)oxy)silane (227)



Palladium(II) acetate (13.0 mg, 1%) and tri-o-tolylphosphine (35.0 mg, 2%) were suspended in toluene (40.0 mL), then to this mixture was added a solution of triflate **226** (2.75 g, 5.7 mmol) in toluene (5.0 mL). Et_3N (3.96 mL, 28.5 mmol) was added to the reaction mixture, which was then heated to reflux for 14 h. The reaction mixture was diluted with EtOAc and washed sat. $NaHCO_3$. the aqueous layer was extracted with EtOAc (3 × 10 mL), the organic layers were combined and filtered through celite and concentrated *in vacuo.* Flush column chromatography (100% pentane) afforded the product as a colourless oil (1.66 g, 80%). **IR** (film) ν_{max}/cm^{-1}: 2979s, 2360s, 1455m, 1378m, 1240s, 1120m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.56 (d, *J* = 4.7 Hz, 1H, H7), 4.82 (d, *J* = 1.7 Hz, 2H, H14), 4.79 (d, *J* = 2.5 Hz, 1H, H11), 4.61 (d, *J* = 2.6 Hz, 1H, H11), 3.53 – 3.50 (m, 1H, H6), 2.69 – 2.53 (m, 2H, H2, H8), 2.19 – 2.06 (m, 2H, H2, H9), 2.00 – 1.87 (m, 2H, H1, H9), 1.81 – 1.69 (m, 4H, H10, H13), 1.69 – 1.57 (m, 2H, H1, H10), 1.00 – 0.91 (m, 3H, H15), 0.96 – 0.92 (m, 9H, C*H*$_3$), 0.65 – 0.53 (m, 6H, C*H*$_2$); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 146.2, 138.5, 124.4, 122.0,

112.4, 108.0, 75.6, 41.9, 40.8, 29.5, 29.0, 27.9, 24.3, 22.5, 22.3, 7.1, 7.0, 5.0; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{21}H_{37}OSi$) requires *m/z* 333.2608, found *m/z* 333.2609; **R_f** 0.8 (33% Et_2O/pentane).

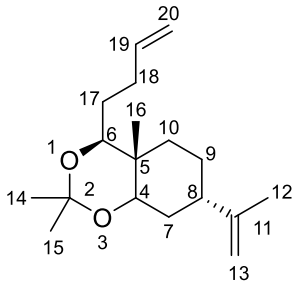
(2*S*,5*S*)-2-((*S*)-1-Hydroxypent-4-en-1-yl)-2-methyl-5-(prop-1-en-2-yl)cyclohexan-1-ol (228)



A solution of ketone **212** (47 mg, 0.5 mmol) in EtOH (2.5 mL) was cooled to 0 ℃, then $NaBH_4$ (37 mg, 1.0 mmol) was added to this solution, which was then warmed up to RT and stirred for 2 h at this temperature. The reaction was then quenched with sat. NH_4Cl, the aqueous layer was extracted with EtOAc (3 ×2 mL); the organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (10% Et_2O/pentane) afforded the product as a colourless oil (24 mg, 51%). **IR** (film) ν_{max}/cm^{-1}: 3321br, 2935s, 2867m, 1643m, 1456m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.83 (dddd, J = 17.4, 10.2, 7.3, 6.1 Hz, 1H, H15), 5.12 – 4.95 (m, 2H, H17), 4.80 – 4.62 (m, 2H, H9), 4.29 – 4.14 (m, 1H, H11), 3.63 (dd, J = 11.8, 4.5 Hz, 1H, H2), 2.49 – 2.30 (m, 1H, H4), 2.21 – 2.06 (m, 1H, H14), 2.01 (m, 2H, H3, H14), 1.83 – 1.73 (m, 2H, H6, H13), 1.72 (s, 3H, H8), 1.52 – 1.37 (m, 4H, H3, H5, H6, H13), 1.30 – 1.19 (m, 1H, H5), 0.97 (s, 3H, H12); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 145.0, 138.4, 115.0, 109.1, 80.0, 71.5, 43.9, 36.8, 35.8, 33.9, 30.3,

29.6, 26.0, 20.9, 20.4; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{27}O_2$) requires *m/z* 239.2006, found *m/z* 239.2008; $\mathbf{R_f}$ 0.5 (33% Et_2O/pentane).

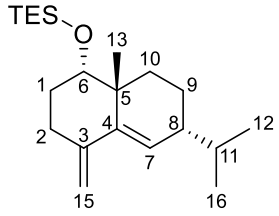
(4*S*,4a*S*,7*S*)-4-(But-3-en-1-yl)-2,2,4a-trimethyl-7-(prop-1-en-2-yl)hexahydro-4H-benzo[*d*][1,3]dioxine (229)



To a solution of diol **228** (23.8 mg, 0.1 mmol) in DMP (1.0 mL) was added tosylic acid (1.0 mg, 5%) at RT and the reaction mixture was stirred for 2.5 h. The reaction was then quenched with TEA, removed the solvent *in vacuo*. Flush column chromatography (5% Et_2O/pentane) afforded the product as a colourless oil (24.0 mg, 85%). **IR** (film) ν_{max}/cm^{-1}: 2987m, 2930s, 2874m, 1643m, 1452m, 1377m, 1223s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.81 (dddd, J = 17.5, 10.2, 7.6, 5.9 Hz, 1H, H19), 5.07 – 4.94 (m, 2H, H20), 4.75 – 4.65 (m, 2H, H13), 3.71 (dd, J = 6.9, 5.5 Hz, 1H, H6), 3.55 (dd, J = 10.4, 5.6 Hz, 1H, H4), 2.37 – 2.21 (m, 1H, H18), 2.12 – 1.95 (m, 2H, H8, H18), 1.86 (m, 1H, H7), 1.81 – 1.73 (m, 1H, H7), 1.72 (s, 3H, H12), 1.67 – 1.59 (m, 1H, H10), 1.58 (m, 1H, H9), 1.45 (m, 2H, H17), 1.40 – 1.35 (m, 6H, H14, H15), 1.36 – 1.23 (m, 1H, H9), 1.13 (ddd, J = 12.5, 8.2, 4.3 Hz, 1H, H10), 1.01 (s, 3H, H16). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 149.7, 138.7, 114.8, 108.6, 99.5, 76.7, 71.1, 40.3, 37.2, 33.1, 30.6, 30.5, 27.5, 27.5, 25.9, 25.0, 20.9, 20.7; **HRMS (ESI)** exact mass calculated

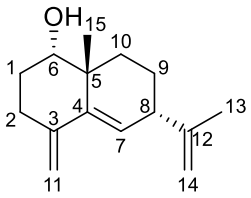
for $[M+H]^+$ ($C_{18}H_{31}O_2$) requires *m/z* 279.2319, found *m/z* 279.2320; **R_f** 0.8 (33% Et_2O/pentane).

Triethyl(((1*S*,6*R*,8a*R*)-6-isopropyl-8a-methyl-4-methylene-1,2,3,4,6,7,8,8a-octahydronaphthalen-1-yl)oxy)silane (230)



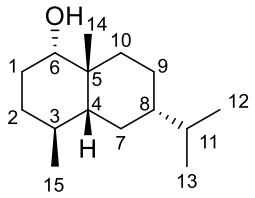
A 10 mL round bottom flask was flushed with argon. A solution of triene **227** (28 mg, 0.08 mmol) in EtOH (1.6 mL) was added to the flask, Pd/C (8.0 mg, 100mg/mmol) was then added to the solution and the flask was charged with hydrogen, and the reaction mixture was stirred at 50 ℃ for 4 days. The flask was flushed with argon before opening. The reaction mixture was filtered through celite, and then concentrated *in vacuo.* Flush column chromatography (100% pentane) afforded the product as a colourless oil (25 mg, 90%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 6.26 – 6.13 (m, 2H, H15), 3.78 (dd, *J* = 9.2, 6.1 Hz, 1H, H6), 3.52 – 3.49 (m, 2H, H2), 3.42 (d, *J* = 15.4 Hz, 1H, H7), 2.63 – 2.40 (m, 3H, H1, H8, H9), 2.38 –1.71 (m, 5H, H1, H9, H10, H11), 1.55 (s, 6H, H12, H16), 1.18 – 1.09 (m, 9H, CH_3), 1.07 – 1.00 (m, 6H, CH_2); **R_f** 0.2 (100% pentane).

(1*S*,6*S*,8a*R*)-8a-Methyl-4-methylene-6-(prop-1-en-2-yl)-1,2,3,4,6,7,8,8a-octahydronaphthalen-1-ol (224)



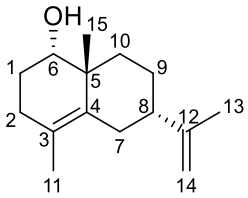
To a solution of triene **227** (100 mg, 0.3 mmol) in THF (30.0 mL) was added dropwise TBAF (1.0 M, 1.20 mL, 1.2 mmol) at 0 ℃. The reaction mixture was allowed to warm up to RT over 14 h. H_2O was added to the reaction mixture, which was then dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (100% pentane) afforded the product as a colourless oil (59.0 mg, 90%). **IR** (film) ν_{max}/cm^{-1}: 2955br, 2878m, 1641s, 1409s, 1245m, 1141m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.73 (d, *J* = 4.8 Hz, 1H, H7), 4.87 (d, *J* = 1.8 Hz, 1H, H14), 4.84 (d, *J* = 2.5 Hz, 1H, H11), 4.74 (d, *J* = 2.1 Hz, 1H, H14), 4.67 (d, *J* = 2.5 Hz, 1H, H11), 3.52 – 3.50 (m, 1H, H6), 2.65 – 2.60 (m, 1H, H8), 2.51 (ddd, *J* = 14.1, 5.3, 2.7 Hz, 1H, H2), 2.22 (ddd, *J* = 14.2, 5.1, 2.0 Hz, 1H, H2), 2.03 – 1.90 (m, 2H, H1, H10), 1.84 – 1.71 (m, 5H, H1, H9, H13), 1.70 – 1.61 (m, 1H, H9), 1.52 (s, 1H, H16), 1.15 – 1.05 (m, 1H, H10), 0.98 (s, 3H, H15); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 148.1, 147.9, 143.0, 126.3, 112.1, 109.2, 74.9, 41.73, 40.7, 28.6, 28.4, 27.6, 24.4, 22.2, 22.1; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{23}O$) requires *m/z* 219.1743, found *m/z* 219.1746; **R_f** 0.6 (33% Et_2O/pentane).

(1*S*,4*S*,4a*R*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyldecahydronaphthalen-1-ol (231)



A 10 mL round bottom flask was flushed with argon. A solution of triene **224** (30 mg, 0.14 mmol) in EtOH (2.8 mL) was added to the flask, Pd/C (15 mg, 100mg/mmol) was then added to the solution and the flask was charged with hydrogen, and the reaction mixture was stirred at 50 ℃ for 4 days. The flask was flushed with argon before opening. The reaction mixture was filtered through celite, and then concentrated *in vacuo.* Flush column chromatography (100% pentane) afforded the product as a colourless oil (28 mg, 90%). **IR** (film) ν_{max}/cm^{-1}: 3413br, 2980s, 1550s, 1459s, 1256m, 1140m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 3.30 (dd, J = 11.5, 4.5 Hz, 1H, H6), 1.75 – 1.64 (m, 3H, H2, H4, H10), 1.64 – 1.56 (m, 3H, H1, H3, H8), 1.41 – 1.27 (m, 3H, H1, H7, H9), 1.27 – 1.14 (m, 5H, H2, H7, H9, H10, H11), 1.10 (s, 3H, H15), 0.86 (d, J = 6.7 Hz, 6H, H12, H13), 0.82 (s, 3H, H14); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 79.4, 48.3, 37.7, 33.8, 33.0, 30.3, 29.2, 25.9, 25.0, 24.3, 23.5, 20.1, 19.9, 19.8; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{29}O$) requires *m/z* 225.1098, found *m/z* 225.1098; **R_f** 0.3 (33% Et_2O/pentane).

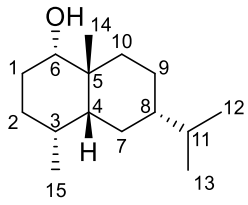
(1*S*,8a*R*)-4,8a-Dimethyl-6-(prop-1-en-2-yl)-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-ol (209)



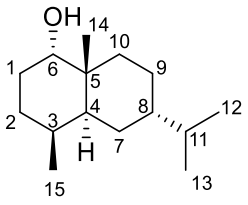
A solution of triene **224** (43 mg, 0.2 mmol) in THF (2.0 mL) was added to a cold solution of sodium (20 mg, 0.8 mmol) in liquid ammonia (3.0 mL) at –78 ℃ dropwise. The reaction mixture was stirred for 4 h at –30 ℃ before *t*-BuOH (0.4 mL) was added and stirred until the disappearance of the blue colour. A solution of sat. NH_4Cl was added to the mixture, the aqueous layer was extracted with Et_2O (3 × 1 mL) and the organic extracts were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Flush column chromatography (1% Et_2O /pentane) afforded the product as a colourless oil (24 mg, 54%). **IR** (film) ν_{max}/cm^{-1}: 3437br, 2922s, 1643m, 1452m, 1372m, 1102m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.85 – 4.78 (m, 1H, H14), 4.74 – 4.67 (m, 1H, H14), 3.39 (dd, *J* = 9.0, 4.6 Hz, 1H, H6), 2.76 (dd, *J* = 14.9, 2.4 Hz, 1H, H7), 2.36 – 2.30 (m, 1H, H8), 2.24 – 2.08 (m, 2H, H2, H7), 2.02 – 1.96 (m, 2H, H1, H10), 1.94 – 1.90 (m, 2H, H1, H7), 1.89 (ddd, *J* = 12.3, 5.2, 2.5 Hz, 1H, H10), 1.82 (ddd, *J* = 13.4, 5.0, 3.6 Hz, 1H, H9), 1.75 (m, 2H, H1, H9), 1.70 (s, 3H, H13), 1.65 (s, , 3H, H11), 1.46 (d, *J* = 8.9 Hz, 1H, O*H*), 1.08 (s, 3H, H15), 1.01 (ddd, *J* = 13.1, 3.6, 0.9 Hz, 1H, H2); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 148.1, 130.5, 123.9, 110.6, 74.8, 40.3, 39.3, 28.7, 28.4, 27.3, 25.6, 25.0, 23.6, 22.7, 19.1; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{25}O$) requires *m/z* 221.1900, found *m/z* 221.1900; **R_f** 0.5 (33% Et_2O/pentane).

(1*S*,4*R*,4a*R*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyldecahydronaphthalen-1-ol (233)

(1*S*,4*S*,4a*S*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyldecahydronaphthalen-1-ol (234)



233



234

Method A

A 10 mL round bottom flask was flushed with argon. A solution of diene **209** (30 mg, 0.14 mmol) in EtOH (2.8 mL) was added to the flask, Pd/C was then added to the solution and the flask was charged with hydrogen, and the reaction mixture was stirred at 50 ℃ for 4 days. The flask was flushed with argon before opening. The reaction mixture was filtered through celite, and then concentrated *in vacuo*. Flush column chromatography (100% pentane) to give a pair of separable diastereomers **233** as colourless oil (15 mg, 50%) and **234** as colourless oil (9 mg, 30%).

Method B

A 25 mL round bottom flask was flushed with argon. A solution of triene **224** (220 mg, 1.0 mmol) in a mixture of DCM (12.0 mL), MeOH (6.0 mL), H_2O (1.4 mL) was added to the flask, Pd/C (20.0 mg, 0.2 mmol) and $Pd(OH)_2/C$ (39 mg, 0.3 mmol) were then added to the solution and the flask was charged with hydrogen, and the reaction mixture was stirred at RT for 3 h. The flask was flushed with argon before opening. The reaction mixture was filtered through celite, and then concentrated *in vacuo*. Flush column chromatography (100% pentane)

gave a pair of separable diastereomers **233** as colourless oil (195 mg, 89%) and **234** as colourless oil (20 mg, 9%).

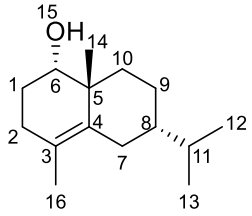
Data for **(1*S*,4*R*,4a*R*,6*S*,8a*R*)-6-isopropyl-4,8a-dimethyldecahydronaphthalen-1-ol (233)**

IR (film) ν_{max}/cm^{-1}: 3413br, 2930s, 1553s, 1459s, 1256m; **^{1}H NMR** (500 MHz, Chloroform-*d*) δ: 3.41 – 3.39 (m, 1H, H6), 2.07 – 1.97 (m, 1H, H1), 1.98 – 1.89 (m, 1H, H9), 1.78 – 1.59 (m, 4H, H2, H3, H7, H11), 1.55 (s, 3H, H14), 1.48 – 1.39 (m, 1H, H10), 1.35 – 1.27 (m, 2H, H9, H10), 1.28 – 1.20 (m, 2H, H2, H7), 1.08 (m, 2H, H4 H8), 0.93 – 0.86 (m, 9H, H12, H13, H15); **^{13}C NMR** (126 MHz, Chloroform-*d*) δ: 74.7, 45.0, 43.7, 39.4, 38.3, 37.4, 33.4, 30.4, 28.9, 26.5, 25.1, 24.4, 20.1, 19.9, 14.8; **R_f** 0.8 (33% Et_2O/pentane); **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{29}O$) requires *m/z* 225.1098, found *m/z* 225.1098.

Data for **(1*S*,4*S*,4a*S*,6*S*,8a*R*)-6-isopropyl-4,8a-dimethyldecahydronaphthalen-1-ol (234)**

IR (film) ν_{max}/cm^{-1}: 3413br, 2930s, 1553s, 1459s, 1256m; **^{1}H NMR** (500 MHz, Chloroform-*d*) δ: 3.58 –3.54 (m, 1H, H6), 1.98 – 2.05 (m, 1H, H3), 1.89 – 1.86 (m, 1H, H2), 1.84 – 1.77 (m, 1H, H9), 1.55 (s, 3H, H14), 1.54–1.65 (m, 2H, H2, H10), 1.51 – 1.40 (m, 3H, H1, H7, H11), 1.36 – 1.18 (m, 6H, H1, H7, H8, H9, H10), 1.08 – 0.99 (m, 1H, H4), 0.93 – 0.79 (m, 9H, H12, H13, H15); **^{13}C NMR** (126 MHz, Chloroform-*d*) δ: 77.5, 46.7, 43.9, 40.7, 37.0, 33.2, 31.7, 30.2, 29.2, 28.1, 25.1, 23.7, 20.1, 19.9, 19.7; **R_f** 0.7 (33% Et_2O/pentane); **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{29}O$) requires *m/z* 225.1098, found *m/z* 225.1098.

(1*S*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-ol (237)



Method A

To a solution of diene **209** (88 mg, 0.4 mmol) in acetic acid (4.0 mL) in an argon atmosphere was added PtO_2 (2.7 mg, 3%), then the reaction flask was flushed with hydrogen. The reaction mixture was stirred under hydrogen at RT for 14 h. The reaction was quenched with sat. $NaHCO_3$, the aqueous layer was extracted with EtOAc (3 × 1 mL), the organic extracts were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (6% Et_2O/pentane) afforded the product as a colourless oil (60 mg, 68%).

Method B

To a solution of diene **209** (22 mg, 0.10 mmol) in THF (2.5 mL) was added 2,4,6-triisopropylbenzenesulfonyl hydrazide (45 mg, 0.15 mmol) followed by Et_3N (0.42 mL, 0.30 mmol) at RT. The reaction mixture was heated to reflux for 1 day. The reaction mixture was filtered; the solvent was removed in *in vacuo*. Flush column chromatography with $AgNO_3$-silica (100% pentane) afforded the product as a colourless oil (6.0 mg, 30%).

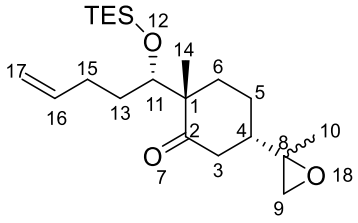
Method C

To a solution of diene **209** (22 mg, 0.1 mmol) in MeOH (2.5 mL) was added a spoon of Raney nickel in an argon atmosphere. Then the reaction flask was flushed with hydrogen. The reaction mixture was stirred under hydrogen at RT for 14 h. The reaction was filtered; the

solvent was removed in *in vacuo*. Flush column chromatography with $AgNO_3$-silica (100% pentane) afforded the product as a colourless oil (10 mg, 50%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 3.43 (dd, *J* = 5.3, 2.0 Hz, 1H, H6), 2.72 – 2.45 (m, 1H, H2), 2.25 – 2.11 (m, 1H, H2), 2.08 – 1.85 (m, 2H, H7, H9), 1.81 – 1.72 (m, 3H, H1, H7, H10), 1.63 (s, 3H, H16), 1.60 – 1.43 (m, 3H, H10, H11), 1.35 – 1.29 (m, 3H, H1, H8, H9), 1.09 (s, 3H, H14), 0.88 (d, *J* = 6.6 Hz, 3H, H12), 0.85 (d, *J* = 6.6 Hz, 3H, H13); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 130.9, 124.4, 75.2, 42.0, 39.4, 28.3, 28.3, 27.6, 26.3, 25.4, 25.4, 24.1, 21.9, 20.6, 19.3; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{27}O$) requires *m/z* 223.2056, found *m/z* 223.2058; **R_f** 0.5 (33% Et_2O/pentane).

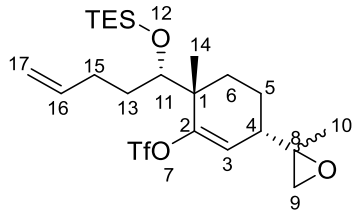
(2*S*)-2-Methyl-5-(2-methyloxiran-2-yl)-2-((*S*)-1-((triethylsilyl)oxy)pent-4-en-1-yl)cyclohexan-1-one (239)



To a solution of diene **225** (700 mg, 2.0 mmol) in DCM (100.0 mL) at 0 ℃ was added *m*-CPBA (414 mg, 2.4 mmol). The reaction mixture was allowed to warm up to RT over 14 h. Sat. K_2CO_3 solution (50 mL) was added to the solution, the aqueous layer was extracted with DCM (3 × 20 mL) and the organic extracts were combined, washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (8% Et_2O/pentane) afforded the product as a colourless oil (175 mg, 25%). The product was a

mixture of unseparated diastereoisomers with a ratio of 1:1. **IR** (film) ν_{max}/cm^{-1}: 2954s, 2363s, 1714s, 1456m, 1378m, 1237m; one isomer: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.93 – 5.71 (m, 1H, H15), 5.14 – 4.93 (m, 2H, H17), 4.16 (m, 1H, H11), 2.65 (m, 1H, H3), 2.60 – 2.45 (m, 2H, H3, H4), 2.35 (m, 1H, H15), 2.26 (m, 1H, H15), 2.12 – 2.00 (m, 1H, H6), 2.01 – 1.93 (m, 1H, H6), 1.75 – 1.56 (m, 5H, H5, H10), 1.45 – 1.40 (m, 2H, H3, H13), 1.32 – 1.28 (s, 3H, H14), 1.28 – 1.22 (m, 1H, H13), 1.03 – 0.81 (m, 9H, CH_3), 0.57 – 0.48 (m, 6H, CH_2); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 212.9, 138.3, 115.0, 75.2, 65.8, 58.4, 54.1, 53.2, 45.3, 41.5, 35.4, 32.1, 23.0, 22.3, 18.0, 15.2,7.0; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{21}H_{39}O_3Si$) requires *m/z* 367.2663, found *m/z* 367.2662; **R_f** 0.5 (33% Et_2O/pentane).

(6*S*)-6-Methyl-3-(2-methyloxiran-2-yl)-6-((*S*)-1-((triethylsilyl)oxy)pent-4-en-1-yl)cyclohex-1-en-1-yl trifluoromethanesulfonate (240)



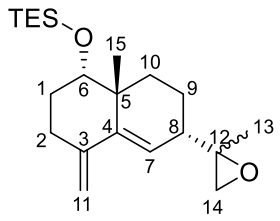
To a solution diisopropylamine (255 mg, 0.51 mmol) in THF (1.5 mL) at –78 ℃ was added dropwise *n*-BuLi (2.5 M, 470 μL, 1.17 mmol), and the reaction mixture was stirred for 30 min. A solution of epoxide **239** (333 mg, 1.06 mmol) in THF (1.0 mL) was added to the reaction mixture, which was then stirred for 1 h at –78 °C. A solution of Comins' reagent (459 mg, 1.17 mmol) in THF (3.0 mL) was added, and the reaction mixture was allowed to warm up to RT over 14 h. Sat. $NaHCO_3$ solution was added to the mixture, the aqueous layer was

extracted with EtOAc (3 ×5 mL) and the organic extracts were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (5% EtOAc/pentane) afforded the product as separated diastereoisomers **A** (102 mg, 30%, colourless oil) and **B** (100 mg, 30%, colourless oil). **IR** (film) ν_{max}/cm^{-1}: 2955s, 2878s, 1641s, 1409m, 1245m, 1141m; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{22}H_{37}F_3O_5SSiNa$) requires *m/z* 521.1975, found *m/z* 521.1974; $\mathbf{R_f}$ 0.8 (33% Et_2O/pentane).

A: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.87 – 5.72 (m, 1H, H16), 5.62 (d, *J* = 3.3 Hz, 1H, H3), 5.07 – 4.93 (m, 2H, H17), 3.71 (dd, *J* = 9.0, 2.0 Hz, 1H, H11), 2.66 – 2.61 (m, 1H, H9), 2.56 (d, *J* = 4.6 Hz, 1H, H9), 2.47 (td, *J* = 6.7, 3.3 Hz, 1H, H4), 2.06 – 1.92 (m, 1H, H15), 2.30 – 2.14 (m, 1H, H13), 1.79 (dddd, *J* = 13.7, 7.5, 6.0, 4.1 Hz, 1H, H5), 1.69 – 1.56 (m, 2H, H5, H13), 1.55 – 1.46 (m, 1H, H6), 1.40 – 1.34 (m, 1H, H6), 1.33 (s, 3H, H10), 1.18 (s, 3H, H14), 0.97 (t, *J* = 7.9 Hz, 9H, 28, CH_3), 0.64 (q, *J* = 8.1 Hz, 6H, CH_2); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 157.2, 138.1, 117.4, 115.2, 76.6, 58.3, 51.9, 43.4, 40.9, 34.2, 31.5, 28.8, 24.7, 22.0, 19.6, 7.5, 5.6.

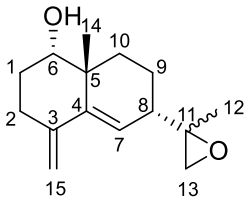
B: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.79 (dd, *J* = 17.0, 10.4 Hz, 1H, H16), 5.77 (m, 1H, 3), 5.01 (dt, *J* = 10.2, 1.7 Hz, 1H, H17), 4.96 (dt, *J* = 10.2, 1.6 Hz, 1H, H17), 3.72 (dd, *J* = 8.9, 2.0 Hz, 1H, H11), 2.63 (dd, *J* = 4.7, 0.9 Hz, 1H, H9), 2.58 (dd, *J* = 4.6 Hz, 0.9 Hz, 1H, H9), 2.23 (m, 2H, H5, H15), 2.06 – 1.92 (m, 1, 15H), 1.67 (m, 3H, H5, H6, H13), 1.60 – 1.45 (m, 1H, H13), 1.45 – 1.39 (m, 1H, H4), 1.39 – 1.23 (s, 3H, H14), 1.39- 1.23 (m,1H, H6), 1.18 (s, 3H, H10), 0.97 (t, *J* = 7.9 Hz, 9H, H30, H31, 32), 0.64 (q, *J* = 8.1 Hz, 6H, H27, H28, 29); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 155.4, 138.2, 117.8, 114.6, 76.6, 58.4, 52.7, 43.4, 42.0, 33.9, 31.4, 28.6, 24.5, 21.6, 18.8, 7.0, 5.6.

Triethyl(((1*S*,8a*R*)-8a-methyl-4-methylene-6-(2-methyloxiran-2-yl)-1,2,3,4,6,7,8,8a-octahydronaphthalen-1-yl)oxy)silane (241)



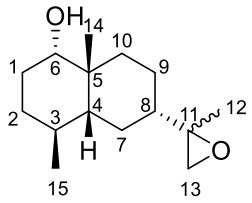
Palladium(II) acetate (2.5 mg, 0.01 mmol) and tri-o-tolylphosphine (6.0 mg, 0.02 mmol) were suspended in toluene, then to this mixture was added a solution of triflate **240** (255 mg, 0.51mmol) toluene (2.5 mL). Et_3N was added to the reaction mixture, which was then refluxed overnight. The reaction mixture was diluted with EtOAc and filtered through celite and concentrated *in vacuo.* Flush column chromatography (100 % pentane) afforded the product as a colourless oil in unseparated diastereoisomers (130 mg, 64%). **IR** (film) ν_{max}/cm^{-1}: 2980s, 2359m, 1457s, 1380s, 1239s, 1081s; one isomer: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.79 (d, *J* = 3.7 Hz, 1H, H7), 4.83 (s, 1H, H11), 4.64 (s, 1H, H11), 3.57 (t, *J* = 2.8 Hz, 1H, H6), 2.70 – 2.48 (m, 2H, H9, H14), 2.20 – 2.06 (m, 2H, H1, H2), 2.01 – 1.83 (m, 2H, H2, H8), 1.83 – 1.60 (m, 4H, H1, H9, H10, H14), 1.39 – 1.15 (m, 3H, H13), 1.16 – 1.04 (m, 1H, H10), 1.03 – 0.83 (m, 15H, CH_3), 0.65 – 0.56 (m, 6H, CH_2); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 134.5, 130.0, 122.2, 108.4, 78.5, 60.0, 53.4, 41.4, 31.0, 29.8, 25.1, 22.6, 21.7, 21.0, 14.0, 7.0, 5.0; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{21}H_{36}O_2SiNa$) requires *m/z* 371.2378, found *m/z* 371.2377; **R_f** 0.6 (33% Et_2O/pentane).

(1*S*,6*S*,8a*R*)-8a-Methyl-4-methylene-6-((R)-2-methyloxiran-2-yl)-1,2,3,4,6,7,8,8a-octahydronaphthalen-1-ol (238)



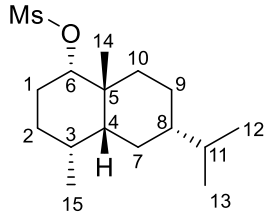
To a solution of diene **241** (131 mg, 0.37 mmol) in THF (15.0 mL) was added dropwise TBAF (1 M, 450 μL, 0.45 mmol) at 0 ℃. The reaction mixture was allowed to warm up to RT over 14 h. H_2O was added to the reaction mixture, and it was dried over $MgSO_4$, filtered and concentrated *in vacuo.* Flush column chromatography (10% Et_2O/pentane) afforded the product as a colourless oil in unseparated diastereoisomers (70 mg, 80%). **IR** (film) ν_{max}/cm^{-1}: 3457br, 2931s, 2869s, 2361w, 1628m, 1554m, 1454m; one isomer: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 5.73 – 5.64 (m, 1H, H7), 4.81 – 4.78 (m, 1H, H15), 4.66 – 4.60 (m, 1H, H15), 3.57 – 3.50 (m, 1H, H6), 2.76 – 2.66 (m, 1H, H13), 2.58 – 2.42 (m, 4H, H2, H8, H13), 2.33 – 2.07 (m, 1H, H10), 1.95 – 1.67 (m, 3H, H9, H10), 1.40 (s, 3H, H12), 1.34 – 1.13 (m, 2H, H1), 1.02 (s, 3H, H14); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 123.1, 122.0, 109.4, 108.8, 75.2, 52.6, 39.8, 38.1, 29.6, 28.9, 28.4, 25.0, 21.8, 21.2, 20.4; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{15}H_{22}O_2Na$) requires *m/z* 257.1512, found *m/z* 257.1512; **R_f** 0.5 (33% Et_2O/pentane).

(1*S*,4*S*,4a*R*,6*S*,8a*R*)-4,8a-Dimethyl-6-((*R*)-2-methyloxiran-2-yl)decahydronaphthalen-1-ol (242)



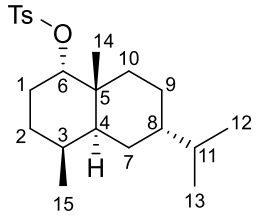
A 10 mL round bottom flask was flushed with argon. A solution of diene **238** (30 mg, 0.14 mmol) in EtOH (2.8 mL) was added to the flask, Pd/C was then added to the solution and the flask was charged with hydrogen, and the reaction mixture was stirred at 50 ℃ for 4 days under hydrogen. The flask was flushed with argon before opening. The reaction mixture was filtered through celite, and then concentrated *in vacuo.* Flush column chromatography (100% pentane) afforded the product as unseparated diasoereomers as colourless oil (6.0 mg, 20%). **IR** (film) ν_{max}/cm^{-1}: 3658br, 2980s, 2888m, 1461mw, 1382m, 1251m, 1152m; one isomer: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 3.17 – 3.02 (m, 1H, H6), 1.67 – 1.03 (m, 14H, H_{ring}), 0.97 – 0.71 (m, 12H, CH_3); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 81.6, 44.2, 37.3, 34.0, 33.8, 32.8, 30.4, 29.3, 25.0, 24.9, 22.8, 20.0, 19.8, 16.9, 14.1; **R_f** 0.8 (33% Et_2O/pentane).

(1*S*,4*R*,4a*R*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyldecahydronaphthalen-1-yl methanesulfonate (246)



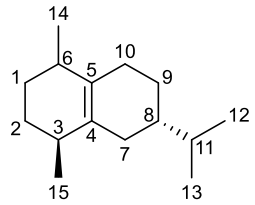
To a solution of alcohol **233** (2 mg, 0.01 mmol) in DCM (0.5 mL) was added in with Et_3N (5 mg, 0.05 mmol), and the solution was cooled to 0 ℃. To this cooled solution was added methanesulfonyl chloride (6 mg, 0.04 mmol), and the reaction mixture was allowed to warm up to RT over 14 h. The reaction mixture was washed with 1.0 M HCl, H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (5% Et_2O/pentane) afforded the product as a colourless oil (3 mg, 98%). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ: 4.46 – 4.39 (m, 1H, H6), 2.07 – 1.97 (m, 1H, H1), 1.98 – 1.89 (m, 1H, H9), 1.78 – 1.59 (m, 4H, H1, H2, H3, H7, H11), 1.55 (s, 3H, H14), 1.48 – 1.39 (m, 1H, H10), 1.55 (s, 3H, H_{Ms}), 1.35 – 1.27 (m, 2H, H9, H10), 1.28 – 1.20 (m, 2H, H2, H7), 1.08 (m, 2H, H4, H8), 0.93 – 0.86 (m, 9H, H12, H13, H15); **R_f** 0.8 (33% Et_2O/pentane).

(1*S*,4*S*,4a*S*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyldecahydronaphthalen-1-yl 4-methylbenzenesulfonate (247)



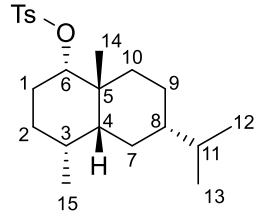
To a solution of alcohol **234** (154 mg, 0.7 mmol) in pyridine (1.0 mL) was added *p*-toluenesulfonyl chloride (119 mg, 1.0 mmol) at RT. The reaction mixture was heated at 30 ℃ and stirred for 4 days at this temperature. The reaction mixture was poured onto ice, the aqueous layer was extracted with Et_2O (3 × 5 mL). The organic extracts were combined, washed with 2.0 M HCl, H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (5% Et_2O/pentane) afforded the product as a colourless oil (285 mg, 95%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.85 – 7.69 (m, 2H, H_{Ar}), 7.39 – 7.27 (m, 2H, H_{Ar}), 4.82 – 4.72 (m, 1H, H6), 2.43 (s, 3H, CH_3), 2.11 – 1.84 (m, 3H, H1, H3), 1.85 – 1.52 (m, 11H, alkyl-*H*, H14), 1.52 – 1.08 (m, 16H, alkyl-*H*), 1.03 – 0.60 (m, 9H, H12, H13, H15); **R_f** 0.6 (33% Et_2O/pentane).

(4*S*,6*S*)-6-Isopropyl-1,4-dimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene (248)



Tosylate **247** (30 mg, 0.08 mmol) was dissolved in a buffered acetic acid (0.30 mL, 0.5 N potassium acetate in acetic acid). The reaction mixture was heated to reflux for 14 h. The reaction mixture was diluted with H_2O, the aqueous layer was extracted with DCM (3 × 1 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a colourless oil (10 mg, 35%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 2.81 – 2.59 (m, 1H, H1), 2.52 – 1.68 (m, 4H, alkyl-*H*), 1.68 – 1.14 (m, 10H, alkyl-*H*), 1.12 – 0.54 (m, 12H, C*H*$_3$). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 128.8, 122.4, 48.4, 46.1, 38.9, 33.0, 30.3, 29.7, 22.3, 19.5, 14.0; **R$_f$** 0.8 (100% pentane).

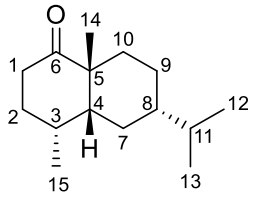
(1*S*,4*R*,4a*R*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyldecahydronaphthalen-1-yl 4-methylbenzenesulfonate (249)



To a solution of alcohol **233** (170 mg, 0.77 mmol) in pyridine (1.0 mL) was added *p*-toluenesulfonyl chloride (119 mg, 1.00 mmol) at RT. The reaction mixture was heated at 30 ℃ and stirred for 4 days at this temperature. The reaction mixture was poured onto ice, the

aqueous layer was extracted with Et_2O (3 × 5 mL). The organic extracts were combined, washed with 2.0 M HCl, H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (5% Et_2O/pentane) afforded the product as a colourless oil (268 mg, 95%). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ: 7.93 (d, *J* = 8.5 Hz, 1H, H_{Ar}), 7.41 (d, *J* = 8.5 Hz, 1H, H_{Ar}), 3.41 – 3.39 (m, 1H, H6), 2.07 – 1.97 (m, 1H, H1), 1.98 – 1.89 (m, 1H, H9), 1.78 – 1.59 (m, 4H, H2, H3, H7, H11), 1.55 (s, 3H, H14), 1.48 – 1.39 (m, 1H, H10), 1.35 – 1.27 (m, 2H, H9, H10), 1.28 – 1.20 (m, 2H, H2, H7), 1.08 (m, 2H, H4, H8), 0.93 – 0.86 (m, 9H, H12, H13, H15); **R_f** 0.6 (10% Et_2O/pentane).

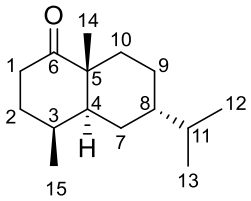
(4*R*,4a*R*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyloctahydronaphthalen-1(2H)-one (252)



To a solution of alcohol **233** (220 mg, 1.0 mmol) in DCM (10.0 mL) was added $NaHCO_3$ (176 mg, 2.1 mmol) and cooled to 0 ℃. DMP (636 mg, 1.5 mmol) was added, and the reaction mixture was allowed to warm to RT over 14 h. The mixture was washed with 1.0 M NaOH (2 × 5 mL). The aqueous layer was extracted with DCM (2 × 5 mL). The organic layers were combined, washed with H_2O (5 mL) and brine, dried over $MgSO_4$ and concentrated *in vacuo* to give a colourless oil (220mg, 99%). The crude was used directly in the next step without any further purification. **IR** (film) ν_{max}/cm^{-1}: 2955s, 2928m, 2867m, 2363w, 1707s, 1465m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 2.77 (ddd, *J* = 14.4, 4.7, 2.7 Hz, 1H, H1), 2.11 (ddd, *J* = 14.4, 4.7, 2.7 Hz, 1H, H1), 1.92 – 1.89 (m, 1H, H2), 1.83–1.78 (m, 1H, H3), 1.77 – 1.69

(m, 2H, H4, H8), 1.70 – 1.63 (m, 3H, H2, H7, H9), 1.54 – 1.50 (m, 1H, H9), 1.42 – 1.24 (m, 4H, H7, H10, H11), 1.07 (s, 3H, H14), 1.03 (d, *J* = 7.4 Hz, 3H, H15), 0.81 (d, *J* = 6.9 Hz, 3H, H12), 0.80 (d, *J* = 6.9 Hz, 3H, H13); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 217.3, 47.2, 44.6, 37.1, 35.3, 34.1, 32.8, 32.7, 32.3, 29.4, 24.8, 19.9, 19.8, 19.1, 14.8; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{27}O$) requires *m/z* 223.2056, found *m/z* 223.2058; **R_f** 0.9 (33% Et_2O/pentane).

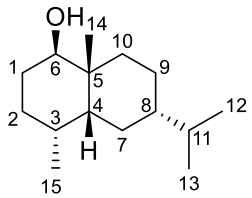
(4*S*,4a*S*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyloctahydronaphthalen-1(2H)-one (253)



To a solution of **234** (220 mg, 1.0 mmol) in DCM (10 mL) was added $NaHCO_3$ (176 mg, 2.1 mmol) and cooled to 0 ℃. DMP (636 mg, 1.5 mmol) was added, and the reaction mixture was allowed to warm to RT over 14 h. The mixture was washed with 1.0 M NaOH (2 × 5 mL). The aqueous layer was extracted with DCM (2 × 5 mL). The organic layers were combined, washed with H_2O (5 mL) and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give a colourless oil (220mg, 99%). The crude was used directly in the next step without any further purification. **IR** (film) ν_{max}/cm^{-1}: 2955s, 2928m, 2867m, 2363w, 1707s, 1465m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 2.59 (ddd, *J* = 14.9, 4.4, 2.7 Hz, 1H, H1), 2.21 (ddd, *J* = 14.9, 4.4, 2.7 Hz, 1H, H1), 2.08 – 1.95 (m, 1H, H3), 1.92 – 1.74 (m, 2H, H2, H8), 1.67 – 1.62 (m, 1H, H7), 1.59 – 1.46 (m, 1H, H9), 1.42 – 1.27 (m, 2H, H2, H11), 1.27 – 1.16 (m, 5H, H4, H7, H9, H10), 1.10 (s, 3H, H14), 0.87 (d, *J* = 6.5 Hz, 3H, H15), 0.81 (d, *J* = 6.9

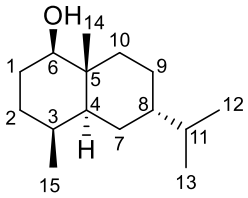
Hz, 3H, H12), 0.80 (d, *J* = 6.9 Hz, 3H, H13); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 216.6, 49.3, 48.7, 38.0, 37.2, 34.7, 32.8, 31.7, 29.5, 28.5, 26.4, 24.9, 19.8, 19.7, 19.6; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{27}O$) requires *m/z* 223.2056, found *m/z* 223.2058; **R_f** 0.9 (33% Et_2O/pentane).

(1*R*,4*R*,4a*R*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyldecahydronaphthalen-1-ol (250)



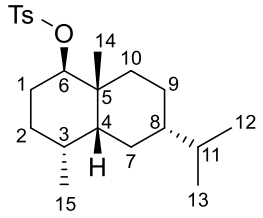
To a solution of ketone **252** (220 mg, 1.0 mmol) in Et_2O (10.0 mL) at 0 ℃ was added $LiAlH_4$ (111 mg, 3.0 mmol). The reaction mixture was allowed to warm up to RT over 14 h. The reaction mixture was cooled to 0 ℃ and added 0.1 mL H_2O, 0.3 mL of 15% aq. NaOH followed by 0.3 mL of H_2O. the mixture was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (20% Et_2O/pentane) afforded the product as a colourless oil (195 mg, 90%). **IR** (film) ν_{max}/cm^{-1}: 3402br, 2930s, 2866s, 1459m, 1384m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 3.22 (dd, *J* = 11.5, 4.4 Hz, 1H, H6), 1.81 – 1.75 (m, 1H, H10), 1.73 – 1.37 (m, 8H, H1, H2, H3, H4, H8, H9, H10, H11), 1.25 – 1.15 (m, 4H, H1, H2, H7, H9), 0.93 – 0.81 (m, 12H, H12, H13, H14, H15); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 70.3, 45.0, 40.4, 39.3, 34.2, 33.4, 33.0, 31.5, 31.3, 29.9, 26.3, 25.1, 20.0, 19.9, 14.9; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{29}O$) requires *m/z* 225.1098, found *m/z* 225.1098; **R_f** 0.6 (33% Et_2O/pentane).

(1*R*,4*S*,4a*S*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyldecahydronaphthalen-1-ol (251)



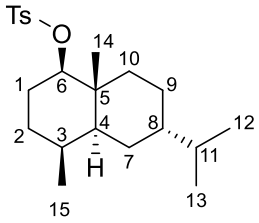
To a solution of ketone **253** (220 mg, 1.0 mmol) in Et_2O (10.0 mL) at 0 ℃ was added $LiAlH_4$ (111 mg, 3.0 mmol). The reaction mixture was allowed to warm up to RT over 14 h. The reaction mixture was cooled to 0 ℃ and added 0.1 mL H_2O, 0.3 mL of 15% aq. NaOH followed by 0.3 mL of H_2O. the mixture was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (20% Et_2O/pentane) afforded the product as a colourless oil (195 mg, 90%). **IR** (film) ν_{max}/cm^{-1}: 3402br, 2930s, 2866s, 1459m, 1384m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 3.48 (dd, *J* = 11.5, 4.4 Hz, 1H, H6), 1.93 – 1.80 (m, 1H, H10), 1.80 – 1.14 (m, 16H, H1, H2, H3, H4, H7, H8, H9, H10, H11), 0.93 – 0.77 (m, 12H, H12, H13, H14); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 75.7, 46.4, 40.1, 37.3, 34.2, 33.4, 33.0, 31.5, 31.3, 29.9, 26.3, 24.8.1, 21.6, 19.8, 14.6; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{29}O$) requires *m/z* 225.1098, found *m/z* 225.1098; **R_f** 0.6 (33% Et_2O/pentane).

(1*R*,4*R*,4a*R*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyldecahydronaphthalen-1-yl 4-methylbenzenesulfonate (254)



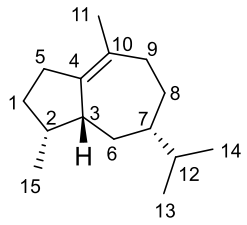
To a solution of alcohol **250** (170 mg, 0.77 mmol) in pyridine (1.0 mL) was added *p*-toluenesulfonyl chloride (119 mg, 1.00 mmol) at RT. The reaction mixture was heated at 30 ℃ and stirred for 4 days at this temperature. The reaction mixture was poured onto ice, the aqueous layer was extracted with Et_2O (3 × 5 mL). The organic extracts were combined, washed with 2.0 M HCl, H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography (5% Et_2O/pentane) afforded the product as a colourless oil (279 mg, 96%). **IR** (film) ν_{max}/cm^{-1}: 2956m, 2928s, 2869m, 1716w, 1363m, 1175s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.78 (d, *J* = 8.4 Hz, 2H, H_{Ar}), 7.37 (d, *J* = 8.4 Hz, 2H, H_{Ar}), 4.22 (dd, *J* = 11.9, 4.4 Hz, 1H, H6), 2.44 (s, 3H, C*H*$_3$), 1.88 – 1.80 (m, 1H, H1), 1.79 – 1.71 (m, 1H, H1), 1.70 – 1.60 (m, 3H, H3, H4, H10), 1.54 (m, 7H, H2, H9, H10, H14), 1.47 – 1.30 (m, 3H, H7, H9, H11), 1.35 – 1.18 (m, 1H, H7), 1.12 – 0.93 (m, 1H, H8), 0.90 (d, *J* = 0.7 Hz, 3H, H15), 0.86 (d, *J* = 6.9 Hz, 3H, H12), 0.85 (d, *J* = 6.9 Hz, 3H, H13); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 144.7, 134.0, 129.7, 128.1, 87.7, 46.4, 41.6, 36.9, 34.0, 33.0, 32.8, 30.6, 27.9, 27.0, 25.9, 22.3, 21.5, 19.8, 19.7; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{22}H_{34}O_3NaS$) requires *m/z* 401.2121, found *m/z* 401.2122; **R$_f$** 0.5 (10% Et_2O/pentane).

(1*R*,4*S*,4a*S*,6*S*,8a*R*)-6-Isopropyl-4,8a-dimethyldecahydronaphthalen-1-yl 4-methylbenzenesulfonate (255)



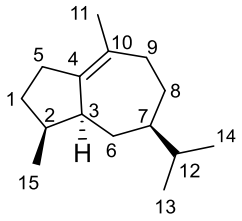
To a solution of alcohol **251** (154 mg, 0.70 mmol) in pyridine (1.0 mL) was added *p*-toluenesulfonyl chloride (119 mg, 1.00 mmol) at RT. The reaction mixture was heated at 30 ℃ and stirred for 3 days at this temperature. The reaction mixture was poured onto ice, the aqueous layer was extracted with Et_2O (3 × 5 mL). The organic extracts were combined, washed with 2.0 M HCl, H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo.* Flush column chromatography (5% Et_2O/pentane) afforded the product as a colourless oil (270 mg, 95%). **IR** (film) ν_{max}/cm^{-1}: 2956m, 2928s, 2869m, 1716w, 1363m, 1175s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 7.93 (d, J = 8.5 Hz, 2H, H_{Ar}), 7.48 (d, J = 8.5 Hz, 2H, H_{Ar}), 3.48 (dd, *J* = 11.9, 4.4 Hz, 1H, H6), 2.49 (s, 3H, C*H*$_3$), 1.95 – 1.79 (m, 4H, H1, H3, H10), 1.78 – 1.32 (m, 9H, H2, H4, H9, H10, H14), 1.31 – 0.93 (m, 10H, H7, H8, H11), 0.92 – 0.72 (m, 9H, H12, H13, H15); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 130.2, 129.6, 127.6, 127.0, 92.2, 46.4, 40.1, 37.4, 32.8, 32.6, 31.3, 29.7, 24.8, 24.1, 21.6, 19.9, 19.7, 14.7, 14.6; **HRMS (ESI)** exact mass calculated for $[M+Na]^+$ ($C_{22}H_{34}O_3NaS$) requires *m/z* 401.2121, found *m/z* 401.2122; **R$_f$** 0.5 (10% Et_2O/pentane).

(3*R*,3a*R*,5*S*)-5-Isopropyl-3,8-dimethyl-1,2,3,3a,4,5,6,7-octahydroazulene (245)



Tosylate **254** (30 mg, 0.08 mmol) was dissolved in 80% aqueous EtOH (0.6 mL) and heated at 80 ℃ for 6 h. The reaction mixture was diluted with H_2O and extracted with DCM (3 × 2 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flush column chromatography with $AgNO_3$-silica (100% pentane) afforded the product as a colourless oil (10 mg, 80%). **IR** (film) ν_{max}/cm^{-1}: 2957m, 2920s, 2851m, 2362w, 1736m, 1676m, 1260m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 2.42 – 2.38 (m, 1H, H2), 2.37 – 2.26 (m, 1H, H9), 2.25 – 2.09 (m, 3H, H3, H5, H9), 2.09 – 1.92 (m, 1H, H5), 1.75 – 1.48 (m, 5H, H1, H6, H8, H12), 1.65, (s, 3H, H11), 1.39 – 1.24 (m, 2H, H7, H8), 1.10 (dddd, *J* = 13.1, 11.7, 10.1, 1.8 Hz, 1H, H1), 0.89 (d, *J* = 7.1 Hz, 3H, H15), 0.84 (d, *J* = 6.9 Hz, 3H, H13), 0.83 (d, *J* = 6.9 Hz, 3H, H14); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 141.8, 128.8, 48.4, 46.1, 38.9, 34.6, 34.3, 33.0, 30.8, 30.3, 29.2, 22.2, 19.6, 19.5, 15.3; **HRMS (ESI):** stable ion was not found in ESI; **R_f** 0.9 (100% pentane).

(3*S*,3a*S*,5*S*)-5-Isopropyl-3,8-dimethyl-1,2,3,3a,4,5,6,7-octahydroazulene (258)



To a solution of bulnesene (57.0 mg, 0.28 mmol) in THF (7.0 mL) was added 2,4,6-triisopropylbenzenesulfonyl hydrazide (125 mg, 0.42 mmol) followed by Et_3N (660 μL, 0.84 mmol) at RT. The reaction mixture was heated to reflux for 1 day. The reaction mixture was filtered; the solvent was removed in *in vacuo.* Flush column chromatography with $AgNO_3$-silica (100% pentane) afforded the product as a colourless oil (17.0 mg, 30%). **IR** (film) ν_{max}/cm^{-1}: 2957m, 2851m, 2362w, 1736m, 1676m, 1260m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 2.42 – 2.38 (m, 1H, H2), 2.37 – 2.26 (m, 1H, H9), 2.25 – 2.09 (m, 3H, H3, H5, H9), 2.09 – 1.92 (m, 1H, H5), 1.75 – 1.48 (m, 5H, H1, H6, H8, H12), 1.65, (s, 3H, H11), 1.39 – 1.24 (m, 2H, H7, H8), 1.10 (dddd, *J* = 13.1, 11.7, 10.1, 1.8 Hz, 1H, H1), 0.89 (d, *J* = 7.1 Hz, 3H, H15), 0.84 (d, *J* = 6.9 Hz, 3H, H13), 0.83 (d, *J* = 6.9 Hz, 3H, H14); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 141.8, 128.8, 48.4, 46.1, 38.9, 34.6, 34.3, 33.0, 30.8, 30.3, 29.2, 22.2, 19.6, 19.5, 15.3; **HRMS (ESI):** stable ion was not found in ESI; **R_f** 0.9 (100% pentane).

Enzymes and Molecular Biology

The $P450_{BM3}$ enzymes were produced in *Escherichia coli* BL21 (DE3) and purified by addition of $(NH_4)_2SO_4$ to a concentration of 40% w/v and centrifugation at 37,500g for 10 min at 4 ℃; the residue was discarded and $(NH_4)_2SO_4$ was added to a concentration of 60% w/v. The solution was submitted to centrifugation at 37,500 g for 10 min at 4 ℃, the supernatant was discarded, and the residue was suspended in phosphate buffer. The enzymes were stored as stock solutions at –20 ℃ and thawed at RT before use.

General procedure for screening of compounds

Compounds were screened using an NADPH regeneration system in 200 mM phosphate buffer (pH 7.5), at a total volume of 0.5 mL, contained in 24 well plates. The substrate was added as a 100 mM stock solution in ethanol (final concentration 2 mM), and the mutant $P450_{BM3}$ enzyme was added as a 10 μM stock solution in phosphate buffer (pH 7.5, final concentration 2 μM). The NADPH regeneration system consisted of glucose dehydrogenase (GDH), added as a 2 U/μL stock solution in 200 mM phosphate buffer (pH 7.5, final concentration 40 U/mL), glucose, added as a 1.0 M stock solution in 200 mM phosphate buffer (pH 7.5, final concentration 100 mM), and NADP+, added as a 4.0 mM stock solution in 200 mM phosphate buffer (pH 7.5, final concentration 8 μM). MCD was added as a 3.0 mM stock solution in phosphate buffer to increase substrate solubility. The reactions were shaken at RT for 14 h, extracted with 300 μL of ethyl acetate and, following centrifugation at 14,300 g for 2 minutes, the organic phase was analysed by GC.

General procedure for scaled-up reaction

To a 250 mL conical flask the following materials were added in sequence: phosphate buffer (pH = 8.3), 0.5 mL GDH (20 U/mL in phosphate buffer, pH 7.5), 0.5 mL NADP+ (40 μM in H_2O), 2.5 mL MCD (3 mM in phosphate buffer, pH 7.5), 0.45 g glucose, substrate stock solution (0.2 M in methanol) and enzyme (concentration is independent for each batch), the final concentration ratio of substrate to enzyme is 2000:1 (4 mM:2 μM), the total volume of the reaction mixture is made up 50.0 mL. The flask was then placed on a shaker under 20 ℃. The reaction was monitored by pH meter and GC. After completion, the reaction mixture was extracted and centrifuged with EtOAc for 3 times. The organic layers were combined, washed with sat. $NaHCO_3$, H_2O, brine, dried over $MgSO_4$ and concentrated *in vacuo*.

Data for enzymatic work with dihydrobulnesene 258

P450$_{BM3}$ variants and the mutations in each for plate AWG

		Mutations
1	R19 FI	R47L/Y51F/H171L/Q307H/N319Y/F87I/
2	R19 FI V26L	R47L/Y51F/H171L/Q307H/N319Y/F87I/V26L
3	R19 FI L181F	R47L/Y51F/H171L/Q307H/N319Y/F87I/ L181F
4	R19 A184I	R47L/Y51F/H171L/Q307H/N319Y/A184I
5	R19 FI A82M A184I A330V	R47L/Y51F/H171L/Q307H/N319Y/F87I/A82M/A184I/A330V
6	R19 FI T88L	R47L/Y51F/H171L/Q307H/N319Y/F87I/T88L
7	R19 A82M	R47L/Y51F/H171L/Q307H/N319Y/A82M
8	R19 FI T88F	R47L/Y51F/H171L/Q307H/N319Y/F87I/T88F
9	R19 FI V178W	R47L/Y51F/H171L/Q307H/N319Y/F87I/V178W
10	R19 FI K440W	R47L/Y51F/H171L/Q307H/N319Y/F87I/K440W
11	R19 FI T88M	R47L/Y51F/H171L/Q307H/N319Y/F87I/T88M
12	R19 FI V26L L71M	R47L/Y51F/H171L/Q307H/N319Y/F87I/V26L L71M
13	R19 FI V26M	R47L/Y51F/H171L/Q307H/N319Y/F87I/

14	R19 A82M A184I T260G A330I	R47L/Y51F/H171L/Q307H/N319Y/ A82M/A184I/T260G/A330I
15	R19 FI V26M L71M	R47L/Y51F/H171L/Q307H/N319Y/F87I/V26M/L71M
16	R19 A184I T260G A330I	R47L/Y51F/H171L/Q307H/N319Y/A184I/T260G/A330I
17	R19 FI A184I LLV	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/L437LV
18	R19 FI A184I S72L	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/S72L
19	R19 FI A184I V26M	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/V26M
20	R19 FI A184I L437F	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/L437F
21	R19 FI A184I V26L	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/V26L
22	R19 FI A184I L437M	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/L437M
23	R19 FI A184I E267F	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/E267F

Screening data for dihydrobulnesene 258

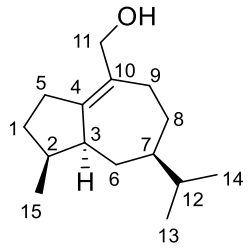
Conversion was determined by GC analysis.

Mutant	Conversion	Unknown	**260**	**259**	**261**
R19 FI	92%	0%	18%	54%	19%
R19 FI V26L	81%	0%	33%	41%	7%
R19 FI L181F	84%	0%	26%	40%	18%
R19 A184I	75%	45%	31%	0%	0%
R19 FI A82M A184I A330V	81%	0%	0%	0%	81%
R19 FI T88L	83%	0%	65%	18%	0%
R19 A82M	74%	37%	29%	8%	0%
R19 FI T88F	81%	0%	66%	15%	0%
R19 FI V178W	73%	0%	73%	0%	0%
R19 FI K440W	78%	0%	60%	17%	0%
R19 FI T88M	82%	0%	29%	36%	9%
R19 FI V26L L71M	81%	0%	35%	39%	7%
R19 FI V26M	80%	0%	28%	42%	10%
R19 A82M A184I T260G A330X(I)	75%	0%	45%	30%	0%
R19 FI V26M L71M	85%	0%	38%	40%	8%

R19 A184I T260G A330I	53%	0%	53%	0%	0%
R19 FI A184I LLV	67%	0%	38%	18%	0%
R19 FI A184I S72L	85%	0%	28%	46%	11%
R19 FI A184I V26M	76%	0%	0%	0%	76%
R19 FI A184I L437F	72%	0%	0%	17%	33%
R19 FI A184I V26L	78%	0%	0%	0%	78%
R19 FI A184I L437M	78%	0%	0%	12%	67%
R19 FI A184I E267F	71%	0%	49%	21%	0%

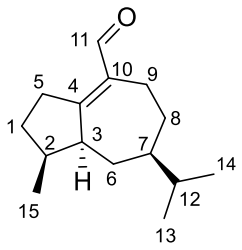
Data for scale-up reactions with dihydrobulnesene 258:

((1*S*,7*R*,8a*S*)-7-Isopropyl-1-methyl-1,2,3,5,6,7,8,8a-octahydroazulen-4-yl)methanol (259)



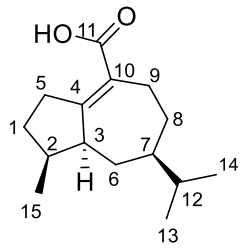
Following the general procedure, dihydrobulnesene **258** (23 mg, 0.28 mL stock solution, 0.11 mmol) with mutant R19/A82M (0.72 mL stock solution, 135 μM) afforded the product as a colourless oil (10 mg, 33%) with flush column chromatography (20% EtOAc/pentane). **IR** (film) ν_{max}/cm^{-1}: 3402br, 2930s, 2866s, 1653m, 1459m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.15 – 4.01 (m, 2H, H11), 2.54 – 2.41 (m, 1H, O*H*), 2.33 – 2.28 (m, 2H, H3, H9), 2.21 – 2.07 (m, 2H, H2, H9), 1.76 – 1.63 (m, 2H, H1, H5), 1.63 – 1.51 (m, 3H, H1, H5, H12), 1.51 – 1.20 (m, 5H, H6, H7, H8), 0.91 (d, *J* = 7.1 Hz, 3H, H15), 0.85 (d, *J* = 6.9 Hz, 3H, H13), 0.83 (d, *J* = 6.9 Hz, 3H, H14); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 147.4, 132.4, 65.8, 48.8, 46.4, 38.3, 34.4, 33.0, 30.5, 30.2, 29.9, 29.4, 19.5, 19.3, 15.2; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{2527}O$) requires *m/z* 223.2056, found *m/z* 223.2058; **R_f** 0.5 (30% Et_2O/pentane).

(1*S*,7*R*,8a*S*)-7-Isopropyl-1-methyl-1,2,3,5,6,7,8,8a-octahydroazulene-4-carbaldehyde (260)



Following the general procedure, dihydrobulnesene **258** (23 mg, 0.28 mL stock solution, 0.11 mmol) with mutant R19/FI/T88L (0.72 mL stock solution, 135 μM) afforded the product as a colourless oil (7 mg, 24%) with flush column chromatography (5% EtOAc/pentane **IR** (film) ν_{max}/cm^{-1}: 2957s, 2362m, 1736s, 1368m, 1260s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 9.92 (s, 1H, H11), 3.07 – 2.90 (m, 1H, H9), 2.79 – 2.70 (m, 2H, H3, H9), 2.27 – 2.12 (m, 1H, H2), 1.91 – 1.76 (m, 2H, H1, H8), 1.71 – 1.84 (m, 3H, H5, H6, H7), 1.62 – 1.49 (m, 4H, H5, H6, H8, H12), 1.49 – 1.36 (m, 1H, H1), 0.98 (d, *J* = 7.1 Hz, 3H, H15), 0.85 (d, *J* = 6.9 Hz, 3H, H13), 0.84 (d, *J* = 6.9 Hz, 3H, H14); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 191.4, 136.06, 106.9, 49.2, 48.7, 37.0, 34.3, 32.9, 29.2, 28.8, 23.4, 19.4, 19.2, 14.9, 1.0; **HRMS (ESI):** stable ion was not found in ESI; **R_f** 0.7 (30% Et_2O/pentane).

(1*S*,7*R*,8a*S*)-7-Isopropyl-1-methyl-1,2,3,5,6,7,8,8a-octahydroazulene-4-carboxylic acid (261)



Following the general procedure, dihydrobulnesene **258** (33 mg, 0.30 mL stock solution, 0.11 mmol) with mutant R19/FI/A184I/V26L (0.70 mL stock solution, 138 μM) afforded the product as a colourless oil (9 mg, 30%) with flush column chromatography (30% EtOAc/pentane). **IR** (film) ν_{max}/cm^{-1}: 2931br, 2850m, 1759s, 1672m, 1384m, 1256s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 2.93 – 2.72 (m, 1H, H9), 2.67 – 2.51 (m, 2H, H9, H3), 2.16 – 2.00 (m, 2H, H2, H5), 1.76 – 1.60 (m, 3H, H1, H5, H8), 1.56 – 1.52 (m, 1H, H7), 1.52 – 1.44 (m, 1H, H8), 1.42 – 1.28 (m, 2H, H6, H12), 1.27 – 1.21 (m, 1H, H6), 1.00 – 0.95 (m, 1H, H1), 0.91 (d, *J* = 7.0 Hz, 3H, H15), 0.77 (d, *J* = 6.9 Hz, 3H, H13), 0.75 (d, *J* = 6.9 Hz, 3H, H14); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 173.4, 169.9, 125.7, 58.4, 49.2, 49.1, 37.8, 34.2, 34.1, 33.1, 28.9, 28.0, 19.4, 19.2, 15.0; **HRMS (ESI)** exact mass calculated for $[M+H]^+$ ($C_{15}H_{25}O_2$) requires *m/z* 237.1849, found *m/z* 237.1849; **R_f** 0.2 (30% Et_2O/pentane).

P450$_{BM3}$ variants and the mutations in each for plate AWG-HEART

			Mutations
	1	R19 FI	R47L/Y51F/H171L/Q307H/N319Y/F87I/
	2	R19 FI V26L	R47L/Y51F/H171L/Q307H/N319Y/F87I/V26L
	3	R19 FI L181F	R47L/Y51F/H171L/Q307H/N319Y/F87I/ L181F
	4	R19 A184I	R47L/Y51F/H171L/Q307H/N319Y/A184I
	5	R19 FI A82M A72L	R47L/Y51F/H171L/Q307H/N319Y/A82M/A72L
	6	R19 FI T88L	R47L/Y51F/H171L/Q307H/N319Y/F87I/T88L
	7	R19 A82M	R47L/Y51F/H171L/Q307H/N319Y/A82M
	8	R19 FI T88F	R47L/Y51F/H171L/Q307H/N319Y/F87I/T88F
	9	R19 FI V178W	R47L/Y51F/H171L/Q307H/N319Y/F87I/V178W
	10	R19 FI A330I A184I	R47L/Y51F/H171L/Q307H/N319Y/F87I/A330I/A184I
	11	R19 FI T88M	R47L/Y51F/H171L/Q307H/N319Y/F87I/T88M
	12	R19 FI V26L L71M	R47L/Y51F/H171L/Q307H/N319Y/F87I/V26L L71M
	13	R19 FI V26M	R47L/Y51F/H171L/Q307H/N319Y/F87I/
	14	R19 A82M A184I T260G A330I	R47L/Y51F/H171L/Q307H/N319Y/ A82M/A184I/T260G/A330I
	15	R19 FI V26M L71M	R47L/Y51F/H171L/Q307H/N319Y/F87I/V26M/L71M

	16	R19 A184I T260G A330I	R47L/Y51F/H171L/Q307H/N319Y/A184I/T260G/A330I
	17	R19 FI A184I LLV	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/L437LV
	18	R19 FI A184I S72L	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/S72L
	19	R19 FI A184I V26M	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/V26M
	20	R19 FI A184I L437F	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/L437F
	21	R19 FI A184I V26L	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/V26L
	22	R19 FI A184I L437M	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/L437M
	23	R19 FI A184I E267F	R47L/Y51F/H171L/Q307H/N319Y/F87I/A184I/E267F

Screening data for *ent*-dihydrobulnesene 245

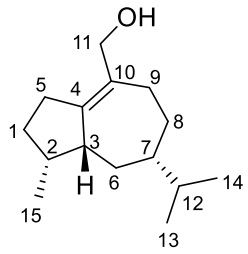
Conversion was determined by GC analysis.

Mutant	Conversion	**265**	**262**	**266**	**263**	**267**	**264**
R19 FI	92%	2%	5%	2%	19%	43%	21%
R19 FI V26L	81%	2%	3%	2%	12%	32%	31%
R19 FI L181F	87%	4%	10%	3%	18%	37%	16%
R19 A184I	100%	7%	19%	2%	19%	41%	12%
R19 FI A82M A72L	92%	4%	12%	4%	17%	38%	17%
R19 FI T88L	97%	9%	24%	4%	18%	34%	8%
R19 A82M	78%	4%	8%	20%	17%	25%	4%
R19 FI T88F	97%	5%	11%	1%	21%	47%	12%
R19 FI V178W	98%	17%	45%	0%	9%	24%	2%
R19 FI A330I A184I	58%	2%	5%	0%	9%	23%	19%
R19 FI T88M	67%	2%	4%	3%	9%	26%	22%
R19 FI V26L L71M	85%	1%	2%	2%	14%	37%	28%
R19 FI V26M	88%	3%	7%	3%	16%	38%	22%
R19 A82M A184I T260G A330I	76%	1%	3%	3%	20%	34%	14%
R19 FI V26M L71M	84%	1%	3%	2%	16%	38%	24%

R19 A184I T260G A330I	88%	9%	22%	3%	15%	36%	4%
R19 FI A184I LLV	96%	13%	38%	6%	14%	22%	3%
R19 FI A184I S72L	86%	2%	5%	2%	19%	41%	16%
R19 FI A184I V26M	79%	4%	9%	1%	12%	30%	23%
R19 FI A184I L437F	64%	2%	5%	12%	12%	23%	10%
R19 FI A184I V26L	72%	3%	6%	1%	10%	27%	24%
R19 FI A184I L437M	67%	2%	5%	2%	12%	26%	20%
R19 FI A184I E267F	92%	22%	58%	0%	3%	9%	0%

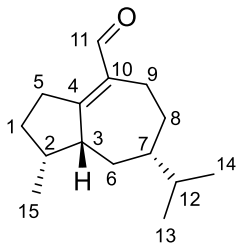
Data for scale-up reactions with *ent*-dihydrobulnesene 245:

((1*R*,7*S*,8a*R*)-7-Isopropyl-1-methyl-1,2,3,5,6,7,8,8a-octahydroazulen-4-yl)methanol (262)



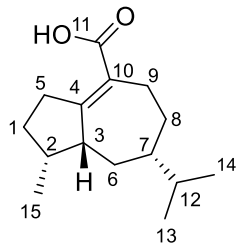
Following the general procedure, *ent*-dihydrobulnesene **245** (33 mg, 0.30 mL stock solution, 0.11 mmol) with mutant R19/FI/V26L (0.84 mL stock solution, 119 μM) afforded the product as a colourless oil (<1 mg). Purification of the product was achieved with supercritical fluid chromatography **(**C18 column, 3% MeOH/liquid CO_2, 1.5 mL/min flow). **IR** (film) ν_{max}/cm^{-1}: 3402br, 2930s, 2866s, 1653m, 1459m; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 4.15 – 4.01 (m, 2H, H11), 2.54 – 2.41 (m, 1H, O*H*), 2.33 – 2.28 (m, 2H, H3, H9), 2.21 – 2.07 (m, 2H, H2, H9), 1.76 – 1.63 (m, 2H, H1, H5), 1.63 – 1.51 (m, 3H, H1, H5, H12), 1.51 – 1.20 (m, 5H, H6, H7, H8), 0.91 (d, *J* = 7.1 Hz, 3H, H15), 0.85 (d, *J* = 6.9 Hz, 3H, H13), 0.83 (d, *J* = 6.9 Hz, 3H, H14); **^{13}C NMR** (101 MHz, , Chloroform-*d*) δ: 147.4, 132.4, 65.8, 48.8, 46.4, 38.3, 34.4, 33.0, 30.5, 30.2, 29.9, 29.4, 19.5, 19.3, 15.2; **R_f** 0.5 (30% Et_2O/pentane); indicative **GC-MS** trace: 222 (exact mass calculated for $C_{15}H_{24}O_2$ requires *m/z* 222.1984).

(1*R*,7*S*,8a*R*)-7-Isopropyl-1-methyl-1,2,3,5,6,7,8,8a-octahydroazulene-4-carbaldehyde (263)



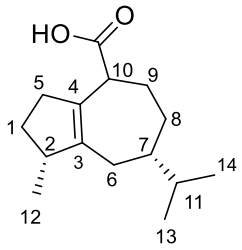
Following the general procedure, *ent*-dihydrobulnesene **245** (33 mg, 0.30 mL stock solution, 0.11 mmol) with mutant R19/A82M (0.84 mL stock solution, 120 μM) afforded the product as a colourless oil (<1 mg). Purification of the product was achieved with supercritical fluid chromatography **(**CN column, 10% MeOH/liquid CO_2, 1.5 mL/min flow). **IR** (film) ν_{max}/cm^{-1}: 2957s, 2362m, 1736s, 1368m, 1260s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 9.92 (s, 1H, H11), 3.07 – 2.90 (m, 1H, H9), 2.79 – 2.70 (m, 2H, H3, H9), 2.27 – 2.12 (m, 1H, H2), 1.91 – 1.76 (m, 2H, H1, H8), 1.71 – 1.84 (m, 3H, H5, H6, H7), 1.62 – 1.49 (m, 4H, H5, H6, H8, H12), 1.49 – 1.36 (m, 1H, H1), 0.98 (d, *J* = 7.1 Hz, 3H, H15), 0.85 (d, *J* = 6.9 Hz, 3H, H13), 0.84 (d, *J* = 6.9 Hz, 3H, H14); **^{13}C NMR** (101 MHz, , Chloroform-*d*) δ: 191.4, 136.0, 106.9, 49.2, 48.7, 37.0, 34.3, 32.9, 29.2, 28.8, 23.4, 19.4, 19.2, 14.9, 1.0; **R_f** 0.7 (30% Et_2O/pentane); indicative **GC-MS** trace: 220 (exact mass calculated for $C_{15}H_{24}O_2$ requires *m/z* 220.1827).

(1*R*,7*S*,8a*R*)-7-Isopropyl-1-methyl-1,2,3,5,6,7,8,8a-octahydroazulene-4-carboxylic acid (264)



Following the general procedure, *ent*-dihydrobulnesene **245** (33 mg, 0.30 mL stock solution, 0.11 mmol) with mutant R19/FI/A184I/L437M (0.60 mL stock solution, 170 μM) afforded the product as a colourless oil (<1 mg). Purification of the product was achieved with supercritical fluid chromatography **(**DIOL column, 5% MeOH/liquid CO_2, 1.5 mL/min flow). **IR** (film) ν_{max}/cm^{-1}: 2931br, 2850m, 1759s, 1672m, 1384m, 1256s; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 2.93 – 2.72 (m, 1H, H9), 2.67 – 2.51 (m, 2H, H9, H3), 2.16 – 2.00 (m, 2H, H2, H5), 1.76 – 1.60 (m, 3H, H1, H5, H8), 1.56 – 1.52 (m, 1H, H7), 1.52 – 1.44 (m, 1H, H8), 1.42 – 1.28 (m, 2H, H6, H12), 1.27 – 1.21 (m, 1H, H6), 1.00 – 0.95 (m, 1H, H1), 0.91 (d, *J* = 7.0 Hz, 3H, H15), 0.77 (d, *J* = 6.9 Hz, 3H, H13), 0.75 (d, *J* = 6.9 Hz, 3H, H14); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ: 173.4, 169.9, 125.7, 58.4, 49.2, 49.1, 37.8, 34.2, 34.1, 33.1, 28.9, 28.0, 19.4, 19.2, 15.0; **R$_f$** 0.2 (30% Et_2O/pentane); indicative **GC-MS** trace: 236 (exact mass calculated for $C_{15}H_{24}O_2$ requires *m/z* 236.1776).

(1*R*,7*S*)-7-Isopropyl-1-methyl-1,2,3,4,5,6,7,8-octahydroazulene-4-carboxylic acid (267)



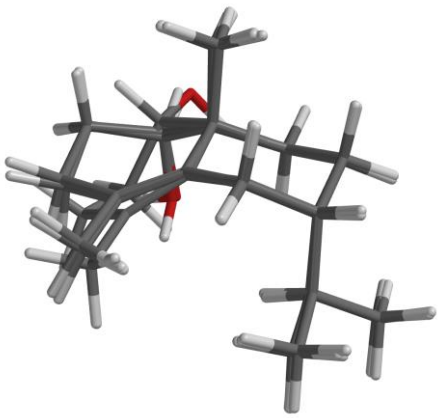
Following the general procedure, *ent*-dihydrobulnesene **245** (33 mg, 0.30 mL stock solution, 0.11 mmol) with mutant R19/FI/A184I/L437M (0.60 mL stock solution, 170 μM) afforded the product as a colourless oil (< 1 mg). Purification of the product was achieved with supercritical fluid chromatography **(**DIOL column, 5% MeOH/liquid CO_2, 1.5 mL/min flow). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ: 3.04 (dd, J = 18.2, 6.9 Hz, 1H, H10), 2.95 (dd, J = 14.8, 7.5 Hz, 1H, H2), 2.58 –1.46 (m, 12H, alkyl-*H*), 1.08 – 0.68 (m, 9H, H12, H13, H14); **R_f** 0.2 (30% Et_2O/pentane).

Conformational analysis of Birch reduction product XX (performed by Prof. Roberson)

(1) Monte Carlo conformational search was run using the molecular mechanics force field MMFF (in the software Spartan for Mac ’18); this generated 38 conformers that was ranked by lowest to highest in energy.

(2) DFT calculations were ran on the bottom 25 of these in energy terms to get a more accurate energy (force field = B3LYP/6-31G*).

(3) The bottom three of these accounted for 96% of the conformational space deduced by Boltzmann-weighting each conformer which was then overlaid to give the graphic shown below.



References

1. Klingenberg, M., *Arch. Biochem. Biophys.*,**1958**, *75*, 376.

2. Omura, T., Sato, R. *J. Biol. Chem*., **1964**, *239*, 2370.

3. Sono, M., Roach, M. P., Coulter E. D. and Dawson, J. H., *Chem. Rev.*, **1996**, *96*, 284.

4. Jeandet, P., Clément, C., Courot, E. and Cordelier, S., *Int. J. Mol. Sci.*, **2013**, *14*, 14136.

5. Bolwell, G.P., Bozak, K. and Zimmerlin, A., *Phytochemistry*, **1994**, *37*, 1491.

6. Shin, J., Kim, J., Lee, Y. and Son, H., *Toxins*, **2018**, *10*, 112.

7. *Cytochrome P450: Structure, Mechanism, and Biochemistry, ed P. R. Ortiz de Montellano, Plenum Publishers, New York, 3rd edn*., **2005**.

8. Waterman, M. R. and Keeney, D. S., *Horm. Res*., **1992**, *38*, 217.

9. Kaspera, R. and Croteau, R., *Phytochem. Rev.* **2006**, *5*, 433.

10. Zhao, Y., Cheng, Q., Su, P., Chen, X., Wang, X., Gao, W. and Huang, L., *Appl. Microbiol. Biotechnol.* **2014**, *98*, 2371.

11. Rowlatt, B., Yorke, J. A., Strong, A. J., Whitehouse, C. J., Bell, S. G. and Wong, L. L., *Protein & cell*, **2011**, *2*, 656.

12. Whitehouse, C. J. C., Bell, S. G., Wong, L.-L. *Chem. Soc. Rev.*, **2012**, *41*, 1218.

13. Rittle, J. and Green, M. T., *Science*, **2010**, *330*, 933.

14. Nebert, D. W., Nelson, D. R., Coon, M. J., Estabrook, R. W., Feyereisen, R., Fujii-kuriyama, Y., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Loper, J. C., Sato, R., Waterman, M. R. and Waxman, D. J., *DNA Cell Biol.*, **1991**, *10*, 1.

15. Nelson, D. R., Koymans, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D. J., Waterman, M. R., Gotoh, O., Coon, M. J., Estabrook, R. W., Gunsalus, I. C. and Nebert, D. W., *Pharmacogenetics*, **1996**, *6*, 1.

16. Moktali, V., Park, J., Fedorona-Abrams, N. D., Park, B., Choi, J., Lee, Y and Kang, S., *BMC Genomic*, **2012**, *13*, 525.

17. Girvan, H. M., Marshall, K. R., Lawson, R. J., Leys, D., Joyce, M. G., Clarkson, J., Smith, W. E., Cheesman, M. R. and Munro, A. W., *J. Biol. Chem.*, **2004**, *279*, 23274.

18. Narhi, L. O., Kim, B. H., Stevenson, P. M. and Fulco, A. J., *Biochem. biophys. Res. Commun.*, **1983**, *116*, 851.

19. Pflug, S., Richter, S. M. and Urlacher, V. B., *J. Biotechnol.*, **2007**, *129*, 481.

20. Ren, X., Yorke, J. A., Taylor E., Zhang T., Zhou, W. and Wong L. L., *Chemistry*, **2015**, *21*, 15039.

21. Li, H. Y. and Poulos, T. L., *Nat. Struct. Biol*., **1997**, *4*, 140.

22. Shoji, O., Aiba, Y. and Watanabe, Y., *Acc. Chem. Res*., **2019**, *52*, 925.

23. Ravichandran, K. G., Boddupalli, S. S., Hasemann, C. A., Peterson, J. A. and Deisenhofer, J., *Science*, **1993**, *261*, 731.

24. Haines, D. C., Tomchick, D. R., Machius, M. and perterson J. A., *Biochemistry*, **2001**, *40*, 13456.

25. Monro, A. W., McLean, K., J., Grant, J., L. and Makris T. M., *Biochem. Soc. Trans.*, **2018**, *46*, 183.

26. Clark, J. P., Miles, C. S., Mowat, C. G., Walkinshaw, M. D., Reid, G. A., Daff, S. N. and Chapman S. K., *J. Inorg. Biochem.*, **2006**, *100*, 1075.

27. Li, Q., Ogawa, J., Schmid, R. D. and Shimizu, S., *Biosci. Biotechnol. Biochem.*, **2005**, *69*, 293.

28. Bell, S.G., Hoskins, N., Whitehouse, C.J.C., and Wong, L. L., *Metal Ions Life Sci*, **2007**, *3*, 437.

29. Celik, A., Sperandio, D., Speight, R. E. and Turner, N. J., *Org. Biomol. Chem.*, **2005**, *3*, 2688.

30. Bloom, J. D., Meyer, M. M., Meinhold, P., Otey, C. R., MacMillan, D. and Arnold, F. H., *Curr. Opin. Biotechnol.*, **2005**, *15*, 447.

31. S. Lutz, *Curr. Opin. Biotechnol*., **2011**, *21*, 734.

32. Butler, C. F., Peet, C., Mason, A. E., Voice, M. W., Leys, D. and Munro, A. W., *J. Biol. Chem.*, **2013**, *288*, 25387.

33. Gillam, E. M., Aguinaldo, A. M., Notley, L. M., Kim, D., Mundkowski, R. G., Volkov, A. A., Arnold, F. H., Soucek, P., DeVoss J. J. and Guengerich, F. P., *Biochem. Biophys. Res. Commun.*, **1999**, *265*, 469.

34. Le-Huu, P., Heidt, T., Claasen, B., Laschat, S. and Urlacher, V. B., *ACS Catal.*, **2015**, *5*, 1772.

35. Roiban, G. D. and Reetz, M. T., *Chem Commun*, **2015**, *51*, 2208.

36. Li, Z., Jiang, Y., Guengerich, F. P., Ma, L., Li, and Zhang, W., *JBC Reviews*, **2020**, *295*, 833.

37. Fasan, R., *ACS Catal.*, **2012**, *2*, 647.

38. Roberts, A., Katayama, J., Kaspera, R., Ledwitch, K. V., Le Trong, I., Stenkamp, R. E., Thompson, J. A. and Totah, R. A., *Biochim. Biophys. Acta.*, **2016**, *1860*, 669.

39. Oliver, C. F., Modi, S., Sutcliffe, M. J., Primrose, W. U., Lian, L. Y., Roberts, G. C., *Biochemistry*, **1997**, *36*, 1567.

40. Kubo, T., Peters, M. W., Meinhold, P. and Arnold, F. H., *Chemistry*, **2006**, *12*, 1216.

41. Landwehr, M., Hochrein, L., Otey, C. R., Kasrayan, A., Backvall, J. E.and Arnold, F. H., *J. Am. Chem. Soc*., **2006**, *128*, 6058.

42. Urlacher, V. B., Makhsumkhanov, A. and Schmid, R. D., *Appl. Microbiol. Biotechnol.*, **2006**, *70*, 53.

43. Weber, E., Seifert, A., Antonovici, M., Geinitz, C., Pleiss, J. and Urlacher, V. B., *Chem. Commun. (Camb.)*, **2011**, *47*, 944.

44. Venkataraman, H., Verkade-Vreeker, M. C. A., Capoferri, L., Geerke, D. P., Vermeulen, N. P. E. and Commandeur, J. N, M., *Bioorg. Med. Chem.*, **2014**, *22*, 5613.

45. Pochapsky, T. C., Kazanis, S. and Dang, M., *Antioxid. Redox. Signaling*, **2010**, *13*, 1273.

46. Bell, S. G., Chen, X. H., Sowden, R. J., Xu, F., Williams, J. N., Wong, L. L. and Rao, Z. H. J., *Am. Chem. Soc.*, **2003**, *125*, 705.

47. Kumar, S., Scott, E. E., Liu, H. and Halpert, J. R. J., *Biol. Chem.*, **2003**, *278*, 17178.

48. Kille, S., Zilly, F. E., Acevedo, J. P. and Reetz, M. T., *Nat. Chem.* **2011**, *3*, 738.

49. Rentmeister, A., Arnold, F. H. and Fasan, R., *Nat. Chem. Biol.*, **2009**, *5*, 26.

50. Reilly, E. O., Kohler, V., Flitsch, S. L. and Turner, N. J., *Chem. Commun.*, **2011**, *47*, 2490.

51. Jung, S. T., Lauchli, R. and Arnold, F. H., *Curr. Opin. Biotechnol*., **2012**, *22*, 809.

52. Urlacher, V. B. and Girhard, M., *Trends Biotechnol.*, **2012**, *30*, 26.

53. Carmichael, A. B. and Wong, L. L., *Eur. J. Biochem.*, **2001**, *268*, 3117.

54. Li, Q., Ogawa, J., Schmid, R. D. and Shimizu, S., *Appl. Environ. Microb.*, **2001**, *67*, 5735.

55. Parker, W. B., White, E. L., Shaddix, S. C., Ross, L. J., Buckheit, R. W., Germany, J. M., Secrist, J. A., Vince, R. and Shannon, W. M., *J. Biol. Chem.*, **1991**, *266*, 1754.

56. Munzer, D. F., Meinhold, P., Perters, M. W., Feichtenhofer, S., Griengl, H., Arnold, F. H., Glieder, A. and Raadt A., *Chem. Commun.*, **2005**, 2597.

57. Bhunia, S., Saha, D. and Koner S., *Langmuir*, **2011**, *27*, 15322.

58. Dennig, A., Lulsdorf, N., Liu, H. and Schwaneberg, U., *Angew. Chem. Int. Ed*., **2013**, *52*, 8459.

59. Gregoraszczuk, E. L., Ptak, A and Wrobel, A., *Endocrine Regulations*, **2011**, *45*, 125.

60. Turan, V. K., Sanchez, R. I., Li, J. J., Li, S. A., Reuhl, K. R., Thomas, P. E., Conney, A. H., Gallo, M. A., Kauffman, F. C. and Mesia-Vela, S., *J. of Endocrinology*, **2004**, *183*, 91.

61. Cha, G. S., Ryu, S. H., Ahn, T. and Yun C., *Biotechnol. Lett.*, **2014**, *36*, 2501.

62. Dietrich, J. A., Yoshikuni, Y., Fisher, K.J., Woolard, F. X., Ockey, D., McPhee, D. J., Renninger, N. S., Chang, M. C. Y., Baker, D. and Keasling, J. D., *ACS Chem. Biol.*, **2009**, *4*, 261.

63. Evans, W. E. and Relling M. V., *Science*, **1999**, *286*, 487.

64. Damsten, M. C., van Vugt-Lussenburg, B. M. A., Zeldenthuis, T., de Vlieger, J. S. B., Commandeur, J. N. M. and Vermeulen, N. P. E., *Chemico-Biological Interactions*, **2008**, *171*, 96.

65. Beyer, N., Kulig, J. K., Fraaije, M. W., Hayes, M. A. and Janssen, D. B., *ChemBioChem*, **2018**, *19*, 326.

66. Baillie, T. A., *Chem. Res. Toxicol*., **2006**, *19*, 889.

67. Bernhardt, R. and Urlacher, V. B., *Applied Microbiology and Biotechnology*, **2014**, *98*, 6185.

68. Parikh, A., Gillam, E. M. and Guengerich, F. P., *Nat. Biotechnol*., **1997**, *15*, 784.

69. Vail, R. B., Homann, M. J., Hanna, I. and Zaks, A., *J. Ind. Microbiol. Biotechnol.*, **2005**, *32*, 67.

70. Rushmore, T. H., Reider, P. J., Slaughter, D., Assang, C. and Shou, M., *Metab. Eng*., **2000**, *2*, 115.

71. Guengerich, F. P., *Nat. Rev. Drug Discovery*, **2002**, *1*, 359.

72. Julsing, M. K., Cornelissen, S., Behler, B. and Schmid, A., *Curr. Opin. Chem. Biol.*, **2008**, *12*, 177.

73. Bernhardt, R., *J. Biotechnol.*, **2006**, *124*, 128.

74. Van Vugt-Lussenburg, B. M., Damsten, M. C., Maasdijk, D. M., Vermeulen, N. P. E. and Commandeur, J. N. M., *Biochem. Biophys. Res. Commun.*, **2006**, *346*, 810.

75. Van Vugt-Lussenburg, B. M., Stjernschantz, E., Lastdrager, J., Oostenbrink, C., Vermeulen, N. P. E. and Commandeur, J. N. M., *J. Med. Chem.*, **2007**, *50*, 455.

76. Whitehouse, C. J. C., Bell, S. G., Tufton, H., G., Kenny, R. J. P., Ogilvie, L. C. I. and Wong, L. L., *Chem. Commun*., **2008**, *28*, 966.

77. Li, Y. and Wong, L. L., *Angew. Chem. Int. Ed.*, **2019**, *58*, 9551.

78. O' Hanlon, J. A., Ren, X., Morris, M., Wong, L. L. and Robertson, *J., Org. Biomol. Chem.*, **2017**, *15*, 8780.

79. Vitaku, E., Smith, D. T. and Njardarson, J. D., *J. Med. Chem.*, **2014**, *57*, 10257.

80. Aldeghi, M., Malhotra, S., Selwood, D. L. and Chan, A. W. E., *Chem. Biol. Drug Des*., **2014**, *83*, 450.

81. Mitchell, E. A., Peschiulli, A., Lefevre, N., Meerpoel, L. and Maes, B. U. W., *Chem. Eur. J.*, **2012**, *18*, 10092.

82. Taylor, R.D., MacCoss, M., Lawson, A.D.G., *J. Med. Chem.* **2014**, *57*, 5845.

83. Ismail, F.M., Levitsky, D.O., Dembitsky, V.M., *Eur. J. Med. Chem.* **2009**, *44*, 3373.

84. Campos, K. R., *Chem. Soc. Rev.*, **2007**, *36*, 10692017.

85. Cheng, M. X. and Yang, S. D., *Synlett*, **2017**, *28*, 159.

86. Beatty, J. W. and Stephenson, C. R. J., *Acc. Chem. Res*., **2015**, *48*, 1474.

87. He, Y., Zheng, Z., Yang, J., Zhang, X. and Fan, X., *Org. Chem. Front*, **2021**, *8*, 4582.

88. Beak, P. and Lee, W. K., *Tetrahedron. Lett*, **1989**, *30*, 1197.

89. Snieckus, V., Cuevas, J. C., Sloan, C. P., Liu H., and Curran, D. P., *J. Am. Chem. Soc*., **1990**, *112*, 898.

90. DeBoef, B., Pastine, S. J. and Sames, D., *J. Am. Chem. Soc.*, **2004**, *126*, 6556.

91. Yi, C. S., Yun, S. Y. and Guzei, I. A., *Organometallics*, **2004**, *23*, 5392.

92. Chatani, N., Asaumi, T., Ikeda, T., Yorimitsu, S., Ishii, Y., Kakiuchi F. and Murai, S., *J. Am. Chem. Soc.*, **2000**, *122*, 12882.

93. Jain, P., Verma, P., Xia, G. and Yu, J., *Nat. Chem.*, **2017**, *9*, 140.

94. Speckamp, W. N. and Hiemstra, H., *Tetrahedron*, **1985**, *41*, 4367.

95. Davies, H. M. L., Venkatarmani, C., Hansen, T. and Hopper, D. W., *J. Am. Chem. Soc.*, **2003**, *125*, 6462.

96. Xia, X., Shu, X., Ji, K., Yang, Y., Shaukat, A., Liu, X. and Liang, Y., *J. Org. Chem.* **2010**, *75*, 2893.

97. Takasu, N., Oisaki, K. and Kanai, M., *Org, Lett*., **2013**, *15*, 1918.

98. Wang, F., Zhang, X., He, Y. and Fan, X., *J. Org., Chem*., **2020**, *85*, 2220.

99. Griffiths, R.J., Kong, W. C., Richards, S. A., Burley, G. A., Willis, M. C. and Talbot, E. P. A., *Chem. Sci*., **2018**, *9*, 2295.

100. Zhou, L., Shen, Y., An, X., Li, X., Li, S., Liu, Q. and Xiao, J., *Org, Lett*, **2019**, *21*, 8543.

101. Sheehan, J. C. and Tulis, R. W., *J. Org. Chem*., **1974**, *39*, 2264.

102. Kaname, M., Yoshifuji, S. and Sashida, H., *Tetrahedron Lett.*, **2008**, *49*, 2786.

103. Klobukowsiki, E. R., Mueller, M. L., Angelici, R. J. and Woo L. K., *ACS Catal*., **2011**, 1, 703.

104. Jin, X., Kataoka, K., Yatabe, T., Yamaguchi, K. and Mizuno, N., *Angew. Chem*., **2016**, *128*, 7328.

105. Moriarty, R. M., Vaid, R. K. and Ducan M. P., *Tetrahedron Lett.*, **1988**, *29*, 6913.

106. Griffiths, R. J., Burley, G. A. and Talbot, E. P. A., *Org. Lett*. **2017**, *19*, 870.

107. Zheng, D., Zhou, X., Cui, B., Han, W., Wan, N. and Chen, Y., *Chem. Cat. Chem*., **2017** *9*, 937.

108. Chang, D., Feiten, H., Engesser, K., van Beilen, J. B., Witholt, B. and Li, Z., *Org. Lett.*, **2002**, *4*, 1859.

109. Zhang, W., Tang, W., Wang, Z. and Li, Z., *Adv. Synth. Catal*., **2010**, *352*, 3380.

110. Pham, S. Q., Pompidor G., Liu, J. Li, X. and Li, Z., *Chem. Commun*., **2012**, *38*, 4618.

111. Yang, Y. and Li, Z., *CHIMIA*, **2015**, *69*, 136.

112. Holland, H. L., Morris, T. A., Nava P. J. and Zabic, M., *Tetrahedron*, **1999**, *55*, 7441.

113. Karki, S. B. and Dinnocenzo, J. P., *Xenobiotica*, **1995**, *25*, 711.

114. Kim, D. and Guengerich, F. P., *Annu. Rev. Pharmacol. Toxicol*., **2005**, *45*, 27.

115. Peterlin Masic, L., *Curr. Drug Metab.*, **2011**, *12*, 35.

116. Li, Y. and Wong, L.L., *Angew. Chem*., **2019**, *131*, 9651.

117. Dphil thesis of Yushu Li, University of Oxford, 2020.

118. Part II thesis of Sophie Cooke, University of Oxford, 2018.

119. Unpublished work of Yuan Zhang, University of Oxford.

120. Kulanthaivel, P., Hallock, Y. F., Boros, C., Hamilton, S. M., Janzen, W. P., Ballas, L. M., Loomis, C.R., Jiang, J. B., and Katz, B. *J. Am. Chem. Soc.*, **1993**, *115*, 6452.

121. Wilson, C. H., Ali, E. S., Scrimgeour, N., Martin, A. M., Hua, J., Tallis, G. A., Rychkov, G. Y. and Barritt, G. J., *Biochem. J*., **2015**, *466*, 379.

122. Staal, J., Driege, Y., Haegman, M., Kreike, M, Iliaki, S., Vanneste, D., Lork, M., Afonina, I. S., Braun, H. and Beyaert, R., *FEBS Journal*, **2020**, *288*, 1630.

123. Jakobovits, A., Rosenthal, A. and Capon, D. J., *EMBO Journal*, **1990**, *9*, 1165.

124. Bradshaw, D., Hill, C. H., Nixson, J. S. and Wilknson, S. E., *Agents Actions*, **1993**, *38*, 135.

125. Pande, V., Ramos, M. J. and Gago, F., *Anticancer Agents Med. Chem.*, **2008**, *8*, 638.

126. Koide, K., Bunnage, M. E., Paloma, L. G., Kanter, J. K., Taylor, S. S., Brunton, L. L. and Nicolaou, K. C., *Chem. Biol.*, **1995**, *2*, 601.

127. He, X., Zhang, M., Guo, Y., Mao, X., Chen, X. and Li, Y., *Org. Lett.*, **2018**, *20*, 6323.

128. Nicolaou, K. C., Bunnage, M. E. and Koide, K., *J. Am. Chem. Soc.*, **1994**, *116*, 8402.

129. Lampe, J. W., hughes, P. F., Biggers, C. K., Smith, S. H. and Hu, H., *J. Org. Chem.*, **1994**, *59*, 5147.

130. Roemmele, R. C. and Rapoport, H., *J. Org. Chem.*, **1989**, *54*, 1866.

131. Miyabe, K., Torieda, M., Inoue, K., Tajiri, K., Kiguchi, T. and Naito, T., *J. Org. Chem.*, **1998**, *63*, 4397.

132. Adams, C. P., farway, S. M., Hardy, C. J., Hibbs, D. E., Hurthouse, M. B., Morley, A. D., Sharp, B. W., Vicker, N. and Warner, I., *J. Chem. Soc. Perkin. Trans.*, **1995**, *1*, 2355.

133. Saha, T., Maitra, R. and Chattopadhyay, S. K., *Beilstein J. Org. Chem.*, **2013**, *9*, 2910.

134. Kumar, A. and Panda, G., *Chem., Eur. J.*, **2008**, *14*, 4675.

135. Sullivan, B., Gilmet, J., Leisch, H. and Hudlicky, T., *J. Nat. Prod.*, **2008**, *71*, 346.

136. Yaragorla, S. and Muthyala, R., *Tetrahedron. Lett.*, **2010**, *51*, 467.

137. Tanner, D., Almario, A. and Hogberg, T., *Tetrahedron*, **1995**, *51*, 6061.

138. Suzuki,T., Sato, O., Hirama, M., Yamamoto, Y., Murata, M., Yasumoto, T., Harada, N. *Tetrahedron Lett.*, **1991**, *32*, 4505.

139. Phansavath, P., de Paule, S. D., Rotavelomanana-Vidal, V. and Genet, J. P., *Euro. J. Org. Chem.*, **2000**, *23*, 3903.

140. Wu, M. H. and Jacobsen, E. N., *Tetrahedron Lett.*, **1997**, *38*, 1693.

141. Furstner, A. and Thiel, O. R., *J. Org. Chem.*, **2000**, *65*, 1738.

142. Riber, D., Hazell, R. and Skrydstrup, T., *J. Org, Chem*., **2000**, *65*, 5382.

143. Srinivas, C. and Yadav, J. S., *Tetrahedron Lett*., **2002**, *43*, 3837.

144. Jung, M. E., Shaw, T. J., *J.Am.Chem.Soc*., **1980**,*102*, 6304.

145. Zardi-Bergaoui, A., Jelassi, A., Daami-Remadi, M., Harzallah-Skhiri, F., Flamini, G, Ascrizzi, R. and Jannet, H. B., *J. Essent. Oil Res.*, **2020**, *32*, 111.

146. Afoulous, S., Ferhout, H., Raoelison, E. G., Valentin, A., Moukarzel, B., Couderc, F. and Bouajila, J., *Food Chem. Toxicol.*, **2013**, *56*, 352.

147. Sharifi-Rad, J., Hoseini-Alfatemi, S. M., Sharifi-Rad, M., Sharifi-Rad, M., Iriti, M., Sharifi-Rad, M., Sharifi-Rad, R. Sara Raeisi, S., *Molecules*, **2015**, *20*, 7034.

148. Zhang, H., Gao, Y. and Lai, P., *Molecules*, **2017**, *22*, 381.

149. Rad, J.S., Alfatemi, M.H., Rad, M.S., Sen, D.J., *Am. J. Advan. Drug. Deliv*. **2013**, *1*, 1.

150. Rad, J.S., Alfatemi, S.M.H., Rad, M.S., *Int. J. Biosci*. **2014**, *4*, 217.

151. Sharifi-Rad, J., Hoseini-Alfatemi, S.M., Sharifi-Rad, M., Miri, A., Sharifi-Rad, M., *Minerva Biotecnol*. **2014**, *26*, 287.

152. Liu, L. Yang, J. and Shi, Y., *Chem. Biodivers.*, **2010**, *7*, 327.

153. Ei-Gleel, V. and Hassanien, M. F. R., *Acta Alimentaria*, **2012**, *41*, 143.

154. Lavault, M., Landreau, A., Larcher, G., Bouchara, J. P., Pagniez, F., Pape, P.L., Richomme, P., *Fitoterapia* **2005**, *76*, 363.

155. Mandala, S.C., Dharab, A.K., Kumara, C.K.A., Maitic, B.C., *Herbs Spices Med. Plants*. **2001**, *8*, 69.

156. Ayinampudi, S. R. and Domala, R., *Fitoterapia*, **2012**, *83*, 88.

157. Suresh, G. and Babu, K. S., *Tetrahedron Lett*., **2011**, *52*, 1273.

158. Zhong, C. and Wang, D., *Indian J. Heterocycl. Chem.*, **2002**, *12*, 1273.

159. Ansari, H. R. and Curtis, A. J., *J. Soc. Cosmet. Chem*., **1974**, *25*, 203.

160. Naziz, P. S., Das, R. and Sen S., *Front. Plant Sci.*, **2019**, *10*, 1.

161. Ishihara, M., Tsuneya, T. and Uneyama, K., *Phytochemistry*, **1991**, *30*, 3343.

162. Ishihara, M., Tsuneya, T., Shiga, M. and Uneyama, K., *Phytochemistry*, **1991**, *30*, 563.

163. Naef, R., *Flavour Frag. J.*, **2011**, *26*, 73.

164. Yang, L., Yang, J., Dong, W., Wang, Y., Zeng, J., Yuan, J., Wang, H., Mei, W. and Dai, H., *Molecules*, **2021**, *26*, 436.

165. Wood, C., Siebert, T., E., Parker, M., Capone, D. L., Elsey, G., M., Pollnitz, A. P., Eggers, M., Meier, M., Vossing, T., Widder, S., Krammer, G., Sefton, M. A. and Herderich, M. J., *J. Agric. Food Chem*., **2008**, *56*, 3738.

166. Bates, R. B. and Slagel R.C., *J. Am. Chem. Soc.*, **1962**, *84*, 1307.

167. Huang, A., Burrett, S., Sefton, M. A. and Taylor, D. K., *J. Agric. Food Chem*., **2014**, *63*, 1932.

168. Huang, A., Sefton, M. A. and Taylor, D. K., *J. Agric. Food Chem*., **2015**, *62*, 10809.

169. Umezama, S., Konishi, S. and Kino, K., *Biosci., Biotech. Bioch.*, **2019**, *83*, 1875.

170. Unpublished work of Alice Wong, University of Oxford.

171. Harwood, L. A., Wong, L. L. and Robertson, J., *Angew. Chem. Int. Ed.* **2021**, *60*, 4434.

172. Heathcock, C. H. and Ratcliffe, R., *J. Am. Chem. Soc.*, **1971**, *93*, 1746.

173. Boyce, C. B. C. and Whitehurst, J. S., *J. Chem. Soc.*, **1960**, *0*, 2680.

174. Pier, E. and Cheng, K. F., *Chem. Comm.*, **1969**, *447*, 562.

175. Pier, E. and Cheng, K. F., *Canadian J. Chem.*, **1968**, *46*, 377.

176. Pier, E. and Cheng, K. F., *Canadian J. Chem.*, **1970**, *48*, 2234.

177. Mehta, G. and Singh, B. P., *Tetrahedron Lett.*, **1975**, *50*, 4495.

178. Sammes, P. G. and Street, L. J., *J. Am. Soc. Chem. Commun*, **1983**, *12*, 666.

179. Oppolzer, W. and Wylie, R. D., *Helvetica Chimica Acta*, **1980**, *63*, 1198.

180. Negishi, E., Ma, S., Sugihara, T. and Noda, Y., *J. Org. Chem.*, **1997**, *62*, 1922.

181. Kumar, J. S. R., O'Sullivan, M. F., Reisman, S. E., Hulford, C. A. and Ovaska, T. V., *Tetrahedron Lett*., **2002**, *43*, 1939.

182. Ruthi, F. and Schroder, F., *Helv. Chim. Acta,* **2020**, 103, e2000129.

183. Fiori, K. W. and Du Bois, J., *J. Am. Chem. Soc*., **2007**, *129*, 56.

184. Du Bois, J., *Org Process Res Dev.*, **2011**, *15*, 758.

185. Bess, E. N., DeLuca, R. J., Tindall, D. J., Oderinde, M. S., Roizen, J. L., Du Bois, J. and Sigman, M. S., *Am. Chem. Soc.*, **2014**, *136*, 5783.

186. Fiori, K. W., Espino, C. G., Brodsky, B. H. and Du Bois, J., *Tetrahedron*, **2009**, *65*, 3042.

187. Winum, J., Vullo, D., Casini, A., Montero, J., Scozzafava, A. and Supuran, C. T., *J. Med. Chem.*, **2003**, *46*, 5471.

188. Espino, C. G., Fiori, K. W., Kim, M. and Du Bois, J, *J. Am. Chem. Soc.*, **2004**, *126*, 15378.

189. Narina, S. V., Kumar, T. S., George, S. and Sudalai, A., *Tetrahedron Lett.*, **2007**, *48*, 65.

190. Perry, R. H., Ill, T. J. C., Roizen, J. L., Du Bois, J. and Zare, R. N., *PNAS*, **2012**, *109*, 18295.

191. Cui, Y., and He, C. *Angew. Chem. Int. Ed*. **2004**, 43, 4210.

192. Espino, C. G. and Du Bois, J., *Angew. Chem. Int. Ed.*, **2001**, *40*, 598.

193. Kocovsky, P., *Tetrahedron Lett.*, **1986**, *27*, 5521.

194. Yakura, T., Sato, S., and Yoshimoto, Y. *Chem. Pharm. Bull.* **2007,** 55, 1284.

195. Toumieux, S., Compain, P., Martin, O. R., and Selkti, M. *Org. Lett.*, **2006**, *8*, 4493.

196. Gupta, R., Sogi, K. M., Bernard, S. E., Decatur, J. D., and Rojas, C. M. *Org. Lett*, **2009**, *11*, 1527.

197. Huters, A. D., Quasdorf, K. W., Styduhar, E. D. and Garg, N. K., *J. Am. Chem. Soc.*, **2011**, *133*, 15797.

198. Yamaguchi, M., Hayashi, M., Hamada, Y. and Nemoto T., *Org. Lett*., **2016**, *18*, 2347.

199. Lebel, H., Huard, K. and Lectard, S., *J. Am. Chem. Soc.*, **2005**, *127*, 14198.

200. Huard, K. and Lebel, H., *Chem. Eur. J.*, **2008**, *14*, 6222.

201. Lebel, H, Laparra, L. M., Khalifa, M., Trudel, C., Audubert, C., Szponarski, M., Leduc, C. D., Azek, E. and Ernzerhof, M., *Org. Biomol. Chem.*, **2017**, *15*, 4144.

202. Levites-Agababa, E., Menhaji, E., Person, L. N. and Rojas, C. M., *Org. Lett*., **2002**, *4*, 863.

203. Espino, C. G., Du Bois, J. In Modern Rhodium-Catalyzed Organic Reactions, Evans, P. A., *Ed., Wiley-VCH: Weinheim*, **2005**, 379.

204. Kim, M., Mulcahy, J. V., Espino, C. G. and Du Bois, J., *Org. Lett*., **2006**, *8*, 1073.

205. Hurlocker, B., Abascal, N. C., Repka, L. M., Santizo-Deleon, E., Smenton, A. L., Baranov, V., Gupta, R., Bernard, S. E., Chowdhury, S. and Rojas, C. M., *J. Org. Chem*., **2011**, *76*, 2240.

206. Toumieux, S., Compain, P. and Martin, O. R., *Tetrahedron Lett.*, **2005**, *6*, 4731.

207. 1995Yoshimitsu, T., Ino, T., Futamura, N., Kamon, T. and Tanaka, T., *Org. Lett*., **2009**, *11*, 3402.

208. Marais, P. C. and Meth-Cohn, O., *J. Chem. Soc. Perkin Trans*., **1987**, *1*, 1553.

209. Hu, N. X., Aso, Y., Otsubo, T. and Ogura, *J. Org., Chem.*, **1989**, *54*, 4398.

210. Dubinina, G. G., Yoshida, W. Y. and Chain, W. J., *Tetrahedron. Lett.*, **2010**, *51*, 5325.

211. Strukil, V., Bartolec, B., Portada, T., Dilovic, I., Halasz, I. and Margetic, D., *Chem. Commun.*, **2012**, *48*, 12100.

212. Wehn, P. M., Lee, J. and Du Bois, J., *Org. Lett.*, **2003**, *5*, 4823.

213. Espino, C. G., Wehn, P. M., Chow, J. and Du Bois, J., *J. Am. Chem. Soc.*, **2001**, *123*, 6935.

214. Unpublished works within the Robertson and Wong groups: a) Mary Wilson; b) Pol Hernandez Llado; c) Yuan Zhang, University of Oxford.

215. Richter, J. M., Ishihara, Y., Masuda, T., Whitefield, B. W., Llamas, T., Pohjakallio, A. and Baran, P. S., *J. Am. Chem. Soc*., **2008**, *130*, 17938.

216. Qin, X., Chen, B., Fu, J., Shang, L., Lei, X. and Zhang, W., *Euro. J. Med. Chem.*, **2015**, *102*, 256.

217. Clarke, P. A., Grist, M., Ebden, M., Wilson, C. and cBlake, A. J., *Tetrahedron*, **2005**, *61*, 353.

218. Fortunato, J. M. and Ganem, B., *J. Org. Chem.*, **1976**, *41*, 2194.

219. Barrero, A. F., Alvarez-Manzaneda, E. J., Chahboun, R. and Meneses, R,*Synlett*, **1999**, *10*, 1663.

220. Mou, L., Zhu, L., Lin, Z. and Liang, X., *JANPR*, **2001**, *3*, 103.

221. Solladie-Cavallo, A., Jierry, L., Bouerat, L. and Taillasson, P., *Tetrahedron Asymmetry*, **2001**, *12*, 883.

222. Nakamura, E., Shimizu, M., Kuwajima, I., Sakata, J., Yokoyama, K. and Noyori, R., *J. Org. Chem.*, **1983**, *48*, 932.

223. Tanino, K., Onuki, K., Asano, K., Miyashita, M., Nakamura, T., Takahashi, Y. and Kuwajima, I., *J. Am. Chem. Soc.*, **2003**, *125*, 1498.

224. Liu, Y., Cheng, L., Yue, H., Che, W., Xie, J. and Zhou Q., *Chem. Sci*., **2016**, *7*, 4725.

225. McMurry, J. E., *Acc. Chem. Res.*, **1983**, *16*, 405.

226. Jiang, X and Takacs, J. M., *Curr. Org. Chem.*, **2003**, *7*, 369.

227. McMurry, J. E., Lectka, T. and Cornell, J. G., *J. Org. Chem.*, **1989**, *54*, 3748.

228. Mukaiyama, T., Sato, T. and Hanna, J., *Chem. Lett.*, **1973**, 1041.

229. Comins, D. L. and Dehghani, A., *Tetrahedron Lett.*, **1992**, *33*, 6299.

230. Stoyanova, M.P., Shivachev, B. L., Nikolova, R. P. and Dmitrov, V., *Tetrahedron Asymmetry*, **2013**, *24*, 1426.

231. Pilcher, A. S. and Deshong, P., *J. Org. Chem.*, **1993**, *58*, 5130.

232. Kim, M., Kawada, K. and Watt, D. S., *Syn. Comm.*, **1989**, *11*, 2017.

233. Mishra, V. K., personal communication to Prof. Jeremy Robertson, 2020.

234. Kitao, K. and Ikeda T., *Zairyo/J. Soc. Materials Science*, **1967**, *16*, 848.

235. Plate Alice Wong, developed by Alice Wong, University of Oxford.

236. Bess, E. N., DeLuca, R. J., Tindall, D. J., Oderinde, M. S., Roizen, J. L., Du Bois, J. and Sigman M. S., *J. Am. Chem. Soc*. **201**4, *136*, 5783.

237. Fruit, C. and Mueller, P., *Helvetica Chimica Acta*, **2004**, *7*, 1607.

238. Albrecht, M., Begall, J., Craen, D., Großkurth, J., Himmel, L., Isaak, E., Linnenberg, O., *Beilstein J. Org. Chem.*, **2020**, *16*, 2338.

239. Watson, D. W., Gill, M., Kemmitt, P., Lamont, S. G., Popescu, M. V., Simpson, I., *Tetrahedron Lett.*, **2018**, 59, 4479.

240. Sarkar, D., Ghosh, M., Rout, N., *Tetrahedron Lett.*, **2018**, *59*, 2360.

241. Nikitas, N. F., Voutyritsa, E., Gkizis, P. L., Kokotos, C., *Euro. J. Org. Chem.*, **2021**, *2021*, 96.

242. Dvorak, C. A., Rudolph, D. A., Nepomuceno, D., Dvorak, L., Lord, B., Fraser, I., Bonaventure, P., Lovenberg, T., Carruthers, N., *Bioorganic and Medicinal Chem. Lett.*, **2021**, *31*, 127669.

243. Clement, H. A., Hall, D., *Tetrahedron Lett.*, **2018**, *59*, 4334.

244. Schultz, D. M., Lévesque, F., DiRocco, D. A., Reibarkh, M., Ji, Y., Joyce, L. A., Dropinski, J. F., Sheng, H., Sherry, B. D., Davies, I. W. *Angew. Chem.*, **2017**, *129*, 15476.

245. Crop, S. B., Casillas, L. N., Jamieson, C., Marquis, R., WO2006029154(A2), 2006.03.16.

246. Lin, Z., Huang, L. and Yuan, G., *Chem. Comm.*, **2021**, *57*, 3579.

247. Kim, I., Tsai, H., Nishi, K., Kasagami, T., Morisseau, C., Hammock, B. D., *J. Med. Chem.*, **2007**, *50*, 5217.

248. Qin, X., Chen, B., Fu, J., Shan, L., Lei, X., Zhang, W., *Eur. J. Med. Chem.*, **2015**, *102*, 256.

249. Allemann, R. K., Benton, J. C. R., Dunbabin, A., Johnson, L. A., Mart, R. J., *Angew. Chem. Int. Ed.*, **2020**, *59*, 8486.

250. Verstegen-Haaksma, A. A., Swarts, H. J., Jansen, B. J. M. and Groot, A., *Tetrahedron*, **1994**, *50*, 10073.

251. Quesnel, Y., Toupet, L., Duhamel, L., Duhamel, P. and Poirier, J., *Tetrahedron Asymmetry.*, **1999**, *10*, 1015.

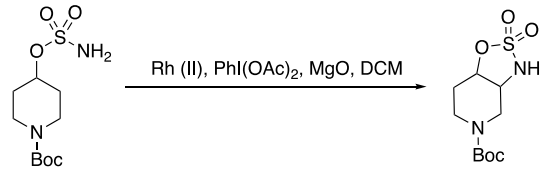
252. Griffith, G. A., Percy, J. M., Pintat, S., Smith, C. A., Spencer, N., Uneyama, E., *Org. Biomol. Chem.*, **2005**, *3*, 2701.

253. Li, J., Niu, Q., Li, S., Sun, Y., Zhou, Q., Lv, X., Wang, X., *Tetrahedron Lett.*, **2017**, *58*, 1250.

Appendices

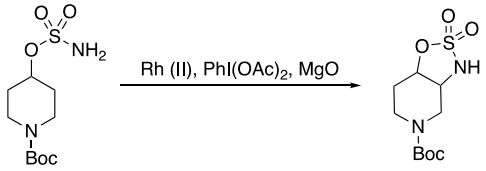
The numbering system used in the following appendices reflects that used in the Experimental Section.

Appendix 1: Tables of Reaction Screenings from Chapter 2



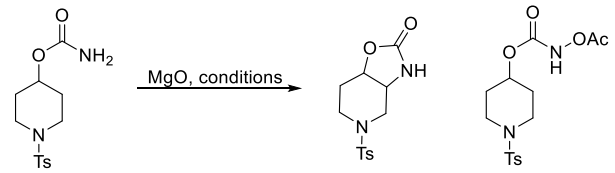
Entry	Catalyst	Catalyst loading	Outcome
1	$Rh_2(OAc)_4$	1%	s.m., **138**
2	$Rh_2(OAc)_4$	2%	s.m., **138**, unidentified mixture
3	$Rh_2(OAc)_4$	5%	s.m., **138**, unidentified mixture
4	$Rh_2(esp)_2$	2%	s.m.
5	$Rh_2(esp)_2$	5%	s.m.

Table 1. Optimisation of Catalysts for C–H activation with sulfamate **137**.



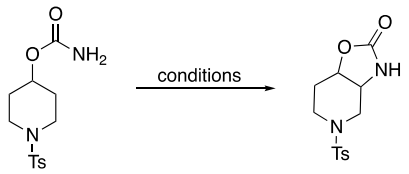
Entry	catalyst	Solvent	Temperature	Outcome
1	$Rh_2(OAc)_4$	DCM	40 ℃	s.m., **128**, **138**
2	$Rh_2(esp)_2$	DCM	40 ℃	s.m.
3	$Rh_2(OAc)_4$	MeCN	RT	s.m.
4	$Rh_2(OAc)_4$	MeCN	80 ℃	Decomposition

Table 2. Optimisations of solvents for C–H activation with sulfamate **137**.



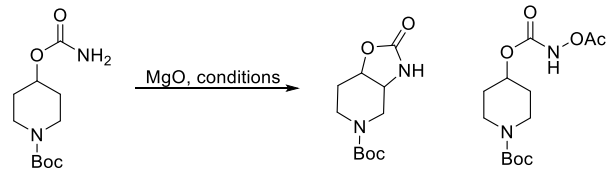
Entry	Catalyst (10%)	Additive	Solvent	Temperature	Outcome
1	$Rh_2(OAc)_4$	$PhI(OAc)_2$	DCM	40 ℃	**146**, **S2**
2	$Rh_2(OAc)_4$	$PhI(OAc)_2$	Benzene	80 ℃	**146**, **S2**
3	$Rh_2(esp)_2$	$PhI(OAc)_2$	Benzene	80 ℃	**146**, **S2**
4	$Rh_2(esp)_2$	$PhI(OAc)_2$	Toluene	80 ℃	**146**
5	$Rh_2(tpa)_4$	$PhI(OAc)_2$	Benzene	80 ℃	**146**, **S2**
6	$Rh_2(OAc)_4$	$PhI(Opiv)_2$	Benzene	80 ℃	**146**
7	$Rh_2(esp)_2$	$PhI(Opiv)_2$	Benzene	80 ℃	**146**
8	$Rh_2(esp)_2$	$PhI(OAc)_2$	MeCN	80 ℃	**146**

Table 3. Conditions screened for β-amination with Ts protected carbamate **145**.



Entry	Conditions	Outcome
1	5% $Fe(acac)_3$, $PhI(OAc)_2$, MeCN, 80 ℃, 14 h	s.m.
2	4 % $AgNO_3$, 4% *t*-Bu_3tpy, $PhI(OAc)_2$, MeCN, 80 ℃, 14 h	**146**
3	4 % $AgNO_3$, 4% *t*-Bu_3tpy, $PhI(Opiv)_2$, MeCN, 80 ℃, 14 h	**146**
4	AgOTf (0.5 equiv,), $PhI(OAc)_2$, bathophenanthroline (0.5 equiv.), MeCN, 80 ℃, 1 day	**146**
5	10% $Cu(MeCN)_4PF_6$, *t*-BuOH, PhI=O, DCM, 14 h	s.m.

Table 4. Attempted β -insertion with carbamate **145** using different metal catalysts.



Entry	Catalyst (10%)	Additive	Solvent	Temperature	Outcome
1	$Rh_2(OAc)_4$	$PhI(OAc)_2$	DCM	40 ℃	**S3**
2	$Rh_2(OAc)_4$	$PhI(OAc)_2$	Benzene	80 ℃	**141**, **S3**
3	$Rh_2(esp)_2$	$PhI(OAc)_2$	Benzene	80 ℃	**141**, **S3**
4	$Rh_2(esp)_2$	$PhI(OAc)_2$	Toluene	80 ℃	**141, S3**
5	$Rh_2(esp)_2$	$PhI(OAc)_2$	MeCN	80 ℃	**S3**

Table 5. Conditions screened for β-amination with Boc protected carbamate **140**.

Appendix 2: NOESY spectrum for compound 229

