Evolved P450 Mutants for Streamlining Synthesis *via* Late-Stage C-H Activation

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Abstract

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This thesis describes synthetic studies towards the carbon framework of the natural product viridiol and the application of mutant P450 enzymes to effect late-stage oxidations on these substrates.

The introduction provides a short summary of existing C-H activation and biocatalytic strategies in synthesis. An overview of P450 enzymes is also given and the background of viridiol and past synthetic studies are discussed.

Chapter 1 explores the construction of the ABE tricycle of viridiol, starting from the furan unit.

Chapter 2 introduces an alternative synthetic approach, focusing on the synthesis of the steroidal core from cyclohexenyl and 4-indanyl fragments. The challenges encountered in the preparation of single regioisomers of the indanyl system are also discussed.

Chapter 3 describes the development of an efficient route to 4-substituted indanes.

Chapter 4 continues the synthesis of the steroidal core from the 4-indanyl unit and details the completion of the viridiol carbon framework.

Chapter 5 investigates the late-stage oxidation of both tri and tetracyclic substrates with mutant P450 enzymes.

Contents

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Abbreviations

Ac	acetyl
ap.	apparent
APCI	atmospheric pressure chemical ionisation
aq.	aqueous
Ar	aryl or argon
Arg	arginine
atm	atmosphere or atmospheric
ATP	adenosine triphosphate
BAIB	(diacetoxyiodo)benzene
9-BBN	9-borabicyclo[3.3.1]nonane
BHT	butylated hydroxytoluene
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
bp	boiling point
br	broad
Bu	butyl
Bz	benzoyl
calc.	calculated
conc.	concentrated
COSY	correlation spectroscopy
Cys	cysteine
d	doublet
DBB	4,4′-di-*tert*-butylbiphenyl
DCE	dichloroethane
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
dec.	decomposed or decomposition
DIPEA	*N*,*N*-diisopropylethylamine

DMA	dimethylacetamide
DMAP	4-dimethylaminopyridine
DMDO	dimethyldioxirane
DME	dimethoxyethane
DMF	dimethylformamide
DMP	Dess–Martin periodinane
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dppf	1,1'-bis(diphenylphosphino)ferrocene
DVE3	triethyleneglycol divinyl ether
E^+	electrophile
EE	ethoxyethyl
ee	enantiomeric excess
EI	electrospray ionisation
eq	equivalents
ESI	electrospray ionisation
Et	ethyl
FAD	flavin adenine dinucleotide
FCC	flash column chromatography
FdR	FAD-containing reductase
Fdx	ferredoxin
FMN	flavin mononucleotide
GC	gas chromatography
GCMS	gas chromatography–mass spectrometry
GDH	glucose dehydrogenase
Glu	glutamic acid
h	hours
hex	hextet
HFIP	hexafluoro-2-propanol
HMBC	heteronuclear multiple bond correlation

HMPA	hexamethylphosphoramide
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
hν	irradiation
i-	*iso*-
imid	imidazole
IPA	isopropanol
IPTG	isopropyl β-D-1-thiogalactopyranoside
IR	infrared
J	coupling constant
KHMDS	potassium bis(trimethylsilyl)amide
lit.	literature
LRMS	low-resolution mass spectrometry
lut	lutidine
Lys	lysine
m	multiplet
m-	*meta*-
m/z	mass/charge ratio
Me	methyl
MHAT	metal hydride H-atom transfer
min	minutes
mp	melting point
mP450	mutant cytochrome P450
Ms	methanesulphonyl chloride
n-	*neo*-
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NBS	*N*-bromosuccinimide
NFSI	*N*-fluorobenzenesulphonimide
NHC	*N*-heterocyclic carbene

NHMDS	sodium bis(trimethylsilyl)amide
NMO	*N*-methylmorpholine N-oxide
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
Nu	nucleophile
o-	*ortho*-
obs.	observed
p	pentet
p-	*para*-
PCR	polymerase chain reaction
PDP	pyridoxal-5’-diphosphate
PE	petroleum ethers
PFC	perfluorocarboxylic acid
PG	protecting group
Ph	phenyl
Phe	phenylalanine
phen	phenanthroline
Phth	phthaloyl
PI_3 kinase	phosphoinositide 3-kinase
PIFA	(bis(trifluoroacetoxy)iodo)benzene
Piv	pivaloyl
ppm	parts-per-million
PPTS	pyridinium *p*-toluenesulphonate
Py	pyridine
q	quartet
quant.	quantitative
R_f	retention factor
rt	room temperature

R_t	retention time
s	singlet
s-	*sec*-
sat.	saturated
sec	seconds
SET	single electron transfer
SFC	supercritical fluid chromatography
SM	starting material
SRS	substrate recognition site
t	triplet
t-	*tert*-
TBAB	tetrabutylammonium bromide
TBS	*tert*-butyldimethylsilyl
TCCA	trichloroisocyanuric acid
TEA	triethylamine
TES	triethylsilyl
Tf	trifluoromethanesulfonate
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
TFDO	methyl(trifluoromethyl)dioxirane
THF	tetrahydrofuran
Thr	threonine
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMEDA	tetramethylethylenediamine
TMS	trimethylsilyl
tol	toluene
TON	turnover number
TPSH	2,4,6-triisopropylbenzenesulphonyl hydrazide
Ts	4-toluenesulfonyl

Tyr	tyrosine
UV	ultraviolet
WT	wild type
Δ	heating to reflux
δ	chemical shift
λ_{max}	maximum wavelength

Introduction

C-H Activation in Synthesis

Organic synthesis requires a diverse and robust range of reactions in order to rapidly construct molecular complexity. Tremendous progress has been made in the last century in both the availability of suitably complex building blocks, and the overall elegance of total synthesis. Our ability to manipulate functional groups makes reactive handles particularly important for introducing diversity to synthesis but this has limited functionalisation to certain reactive regions of compounds. C-H bonds are ubiquitous, and their inherent lack of reactivity has been relied upon by traditional synthetic methods for over a century. However, selective modification of a single C-H bond, either in the presence of more reactive functional groups, or simply in preference over other similar bonds, is a highly desirable transformation at all stages of synthesis and over the last 20 years, significant advances in C-H functionalisation have allowed novel disconnections.

Low value feedstock or waste chemicals may be enhanced by insertion of a synthetic handle, enabling their ready incorporation into useful syntheses. One example is the C-H oxidation of remote positions in simple amines and amides, providing direct access to critical building blocks, currently inaccessible by other means (***Figure 0.01***).[1] Functionalised aliphatic amines such as **1**, **2**, and **3** are particularly high value products, owing to the prevalence of this motif in bioactive compounds.

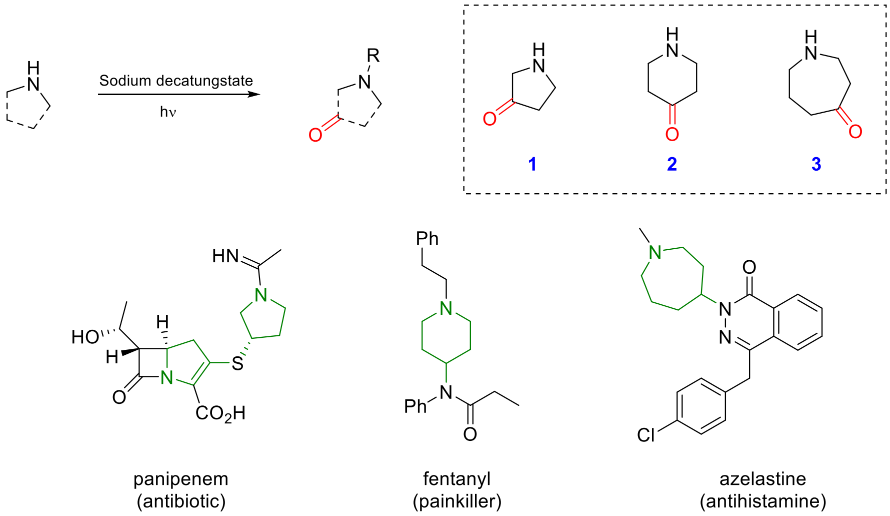
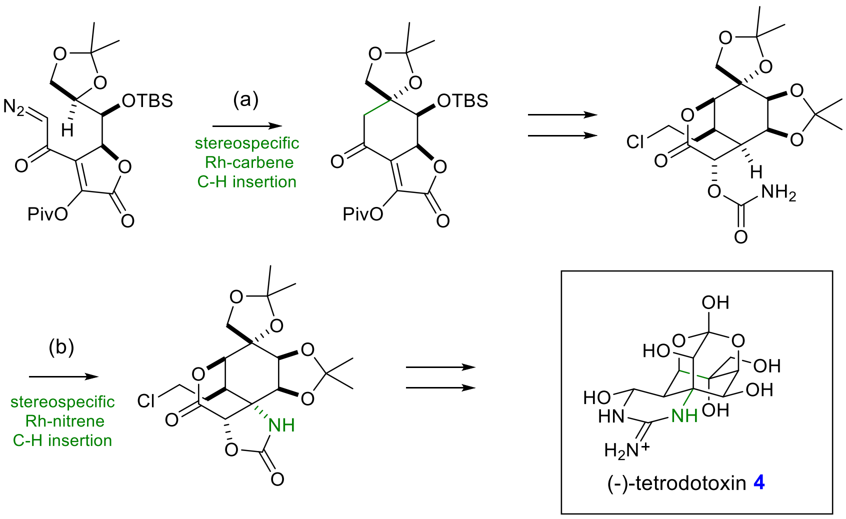


Figure 0.01: *Pharmaceutical building blocks via remote C-H oxidation of simple amines and example pharmaceuticals containing these motifs.*

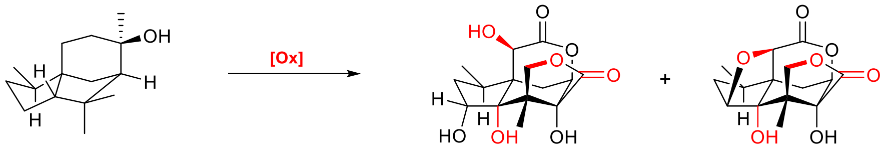
But perhaps more important is the role of C-H activation as a retrosynthetic disconnection. The universal nature of C-H bonds permits incorporation of this step at any stage in the synthesis, making it a highly versatile tool.

C-H activation as another synthetic step is very common, and there are many examples of total syntheses for which this is the key transformation.[2,3] A famous example is Du Bois' synthesis of (–)-tetrodotoxin in 2003 (***Scheme 0.01***), applying two Rh-catalysed C-H insertions: (**a**) to construct the cyclohexane core, and (**b**) to install the tetra-substituted carbinolamine centre.[4]



Scheme 0.01: *Rh-Catalysed C-H insertion disconnections in Du Bois' synthesis of (–)-tetrodotoxin* ***4****.*

A late-stage approach is an efficient method of streamlining synthesis. From a single synthetic intermediate, multiple products may be rapidly accessed by C-H functionalisation. The Maimone group envisaged multiple selective C-H oxidations of the abundant terpenoid (+)-cedrol **5**, enabling the first total synthesis of (–)-majucin **6** and several of its congeners (***Scheme 0.02***).[5] Similarly, this same strategy is often applied to natural products to determine whether additional functionality changes the properties.[6,7] This facilitates the ready creation of compound libraries, particularly important for drug development.



(+)-cedrol **5** (-)-majucin **6** (-)-jiadifenoxolane A **7**

Scheme 0.02: *Oxidative strategy towards majucin series.*

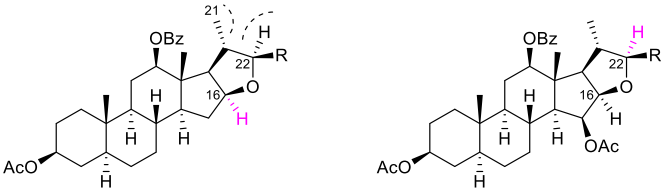
Influencing the Selectivity of C-H Activation

However, the abundance and stability of these bonds present a stern challenge and the difficulty remains of overcoming this intrinsic lack of reactivity and differentiating between multiple similar bonds. As the nominal nucleophile, electron rich C-H bonds are more reactive towards activation by an external species. But steric interactions also play a significant role, governing the ability of the activating species to become proximal to the C-H bond in question. As such, there are a number of subtle factors which influence the observed selectivity in different activations.

- *Type of bond* – a balance between increasing hyperconjugative stabilisation and steric crowding. Generally $3^y > 2^y > 1^y$ reactivity is observed except where crowding is extreme.

- *Hyperconjugative donation* – from proximal lone pairs into C-H antibonding orbitals increases reactivity towards activation.
- *Inductive effects* – proximal electron-withdrawing groups exert the greatest effect but, owing to the similarity of C-H bonds, even distal electron-withdrawing groups can influence selectivity.

The interplay of these factors is demonstrated in Fuchs' steroidal oxidations towards cephalostatin, using CrO_3/Bu_4NIO_4 to generate a monoperoxo chromate (IV) species *in situ* (***Figure 0.02***).[8] Positions 16 and 22 are both activated by hyperconjugative donation from the neighbouring oxygen atom but C-22 is sterically hindered by the C-21 methyl group. For **8**, this means oxidation is exclusively observed at C-16, whereas in **9**, C-16 is deactivated by the adjacent acetate, reversing the selectivity.



8 **9**

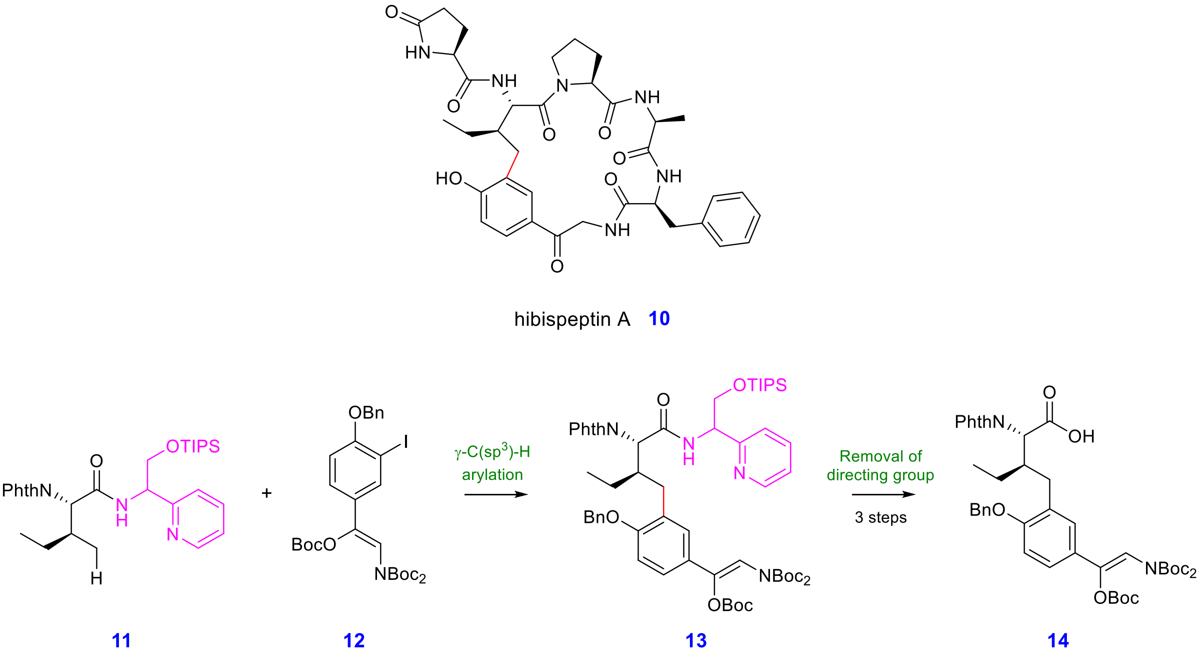
Figure 0.02: *Selectivity factors in steroidal oxidations.*

Unfortunately, the most activated or accessible bond is often not the one we want to functionalise and the difficulty arises of overcoming the inherent reactivity of a particular compound. The earliest reports of C-H activation required harsh conditions. Widely considered the first example, Fenton reported the oxidation of tartaric acid with a mixture of $FeSO_4$ and H_2O_2,[9] although Dimroth's $Hg(OAc)_2$-mediated H/D exchange is perhaps closer to the modern definition of C-H activation.[10,11] Later work by Hodges and Garnett,[12,13] and Shilov[14] respectively, applied Pt(II) and Pd(II) catalysis under milder conditions on unactivated alkane substrates and these seminal papers were the foundation for the explosion of research in the field subsequently.[15] Functional group tolerance, regioselectivity, and over-reaction still remain challenges but significant strides have been made in recent decades to improve the general applicability of this process.[16–22]

Directed C-H Activation

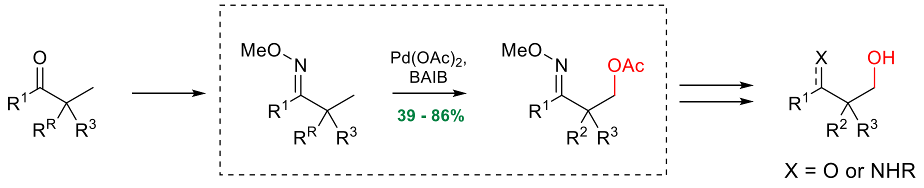
In some cases, more drastic alterations to the substrate are required to enforce the desired selectivity, usually *via* a directing group which biases proximity of the activating species to the relevant C-H bond. This generally requires the installation of specific functionality earlier in the synthesis which is able to chelate to the activating species and hence direct activation.

Chen *et al.* exemplify this strategy in the total synthesis of hibispeptin A **10**, the key step being a γ-C(sp^3)-H arylation of an isoleucine unit **11** with a sterically hindered aryl iodide **12** (***Scheme 0.03***).[23] Various directing groups delivered the expected product in poor yield, forcing the group to develop a novel sub-class of pyridylmethylamine auxiliaries for the transformation. Whilst ultimately successful, a cumbersome three-step process was required to remove this auxiliary, demonstrating the limitations of this approach.



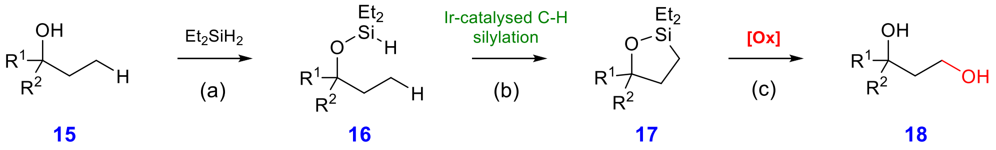
Scheme 0.03: *Chen's γ-C(sp^3)-H arylation using a pyridylmethylamine-based directing group towards the synthesis of hibispeptin A* ***10****.*

Use of more established functionality to direct activation is preferable, generally being operationally simpler, and avoiding the difficulties of installation and removal. An early example of this is Sanford's Pd(II)-catalysed acylation of unactivated oxime substrates, easily accessed from the parent ketone, using BAIB as a stoichiometric oxidant (***Scheme 0.04***).[24] This strategy is both highly regio- and chemo-selective, specifically targeting primary, β- C-H bonds and hence providing a simple route to β-hydroxy ketones and β-amino alcohols.



Scheme 0.04: *Sanford's chelate-directed oxidation of unactivated sp^3 C-H bonds.*

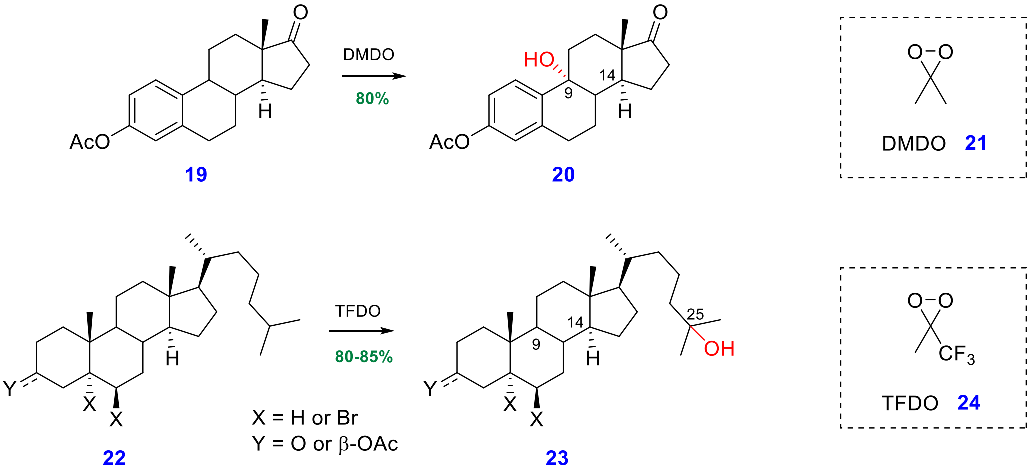
However, the most valuable transformations are those making use of an *in situ* directing group which is incorporated into the product. In 2012, Hartwig described a hydroxyl-directed 1,3-oxidation of primary C-H bonds with a wide substrate scope ranging from simple building blocks to natural products (***Scheme 0.05***).[25] The hydroxyl group forms a silyl ether **16** (a) which undergoes iridium-catalysed C-H silylation (b) to oxalosilane **17**, subsequently subjected to Tamao–Fleming conditions (c) to afford the product diol **18**, demonstrating selective reactivity towards primary C-H bonds in the presence of more reactive O-H bonds, and secondary and tertiary C-H bonds.



Scheme 0.05: *Hartwig's hydroxyl-directed oxidation of unactivated primary C-H bonds.*

Non-Directed C-H Activation

The historical approach to non-directed activation has typically relied upon high-energy electrophilic oxidants such as dioxiranes. The most common are dimethyldioxirane (DMDO) **21** and methyl(trifluoromethyl)dioxirane (TFDO) **24** which are generated *in situ* under very rigorous conditions to prevent their decomposition into carbon-centred radicals (***Scheme 0.06***). Regioselective oxidation is possible in complex systems but reaction occurs almost exclusively at the least sterically hindered of the most activated positions.[26] This has been exploited in the derivatisation of steroids; for example, estrone **19** is hydroxylated exclusively at C-9 in preference over C-14, owing to the activating effect of the proximal aromatic ring.[27] The cholestone derivatives demonstrate the steric selectivity, hydroxylating at C-25, the least crowded tertiary C-H bond.[28]

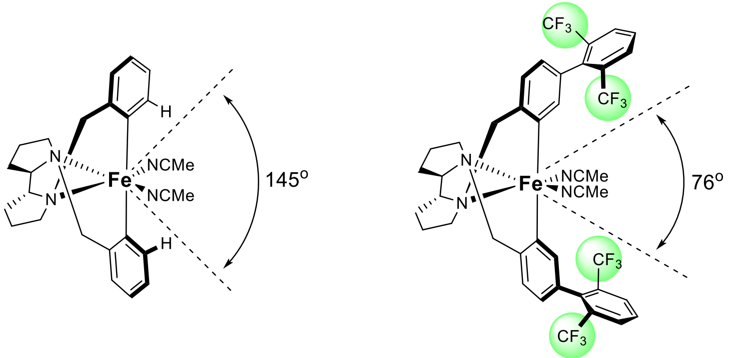


Scheme 0.06: *Dioxirane oxidations of steroids.*

However, with no means of controlling or tuning the regioselectivity without modification of the substrate, the wider application of dioxiranes in synthesis is limited.

Non-heme iron complexes are a more recent development to overcome some of these challenges. Envisaged as structural mimics of non-heme iron oxidases, their modular design facilitates steric and electronic tuning, enabling refinement of observed selectivity.[29] Early catalysts were promising but exhibited limited selectivity and TON.[30,31]

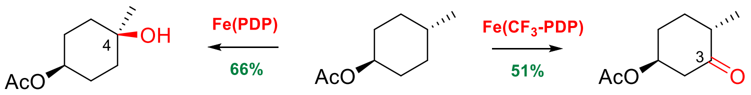
In 2007 the White group developed Fe(PDP)[32,33] and demonstrated that selectivity is a delicate interplay between the inherent reactivity of the substrate (i.e. which bonds are activated) and catalyst-substrate interactions.[34] The tunability of the Fe(PDP) system enabled White to change the relative importance of these factors, altering the reaction outcome without modification of the substrate. The Fe(PDP) **25** system comprises an Fe(II) centre surrounded by a bulky amine framework, leaving a 145° cone of approach to the oxidising centre (***Figure 0.03***). As such, both steric and electronic factors are able to influence the reaction outcome. The modified Fe(CF_3-PDP) **26** system has a greatly restricted cone of approach (76°) and consequently weights steric factors much more highly.[35]



Fe(PDP) **25** Fe(CF_3-PDP) **26**

Figure 0.03: *Structures of the Fe(PDP) and Fe(CF_3-PDP) catalyst systems.*

This is exemplified by the reaction of each system with *trans*-4-methylcyclohexyl acetate **28** (***Scheme 0.07***). Fe(PDP) is principally influenced by electronic factors for this substrate, oxidising in the more hindered but more activated tertiary C-4 position **27**. Conversely, Fe(CF_3-PDP) is directed by sterics and oxidises the electronically disfavoured but more accessible secondary C-3 position **29**.



27 **28** **29**

Scheme 0.07: *Reaction of trans-4-methylcyclohexyl acetate* ***28*** *with Fe(PDP) and Fe(CF_3-PDP).*

Other groups have likewise taken a biomimetic approach such as Costas' use of non-heme iron and manganese complexes,[36] or Groves' manganese porphyrins for selective halogenation.[37] This is inspired by the innate selectivity demonstrated by enzymes, using their complex three-dimensional structures to enforce the desired reactivity on specific substrates.

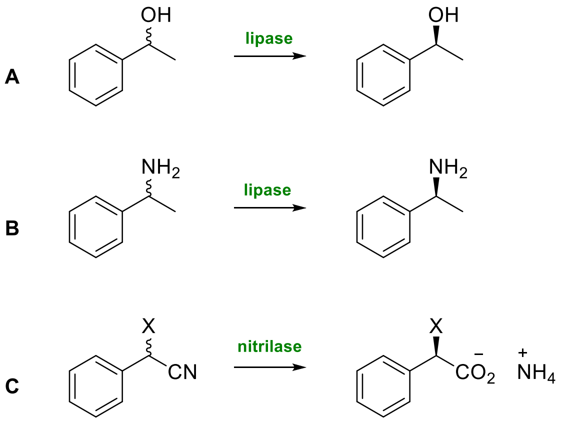
Biocatalysis

The application of enzymes or microbes in chemical synthesis has a number of advantages over traditional metallo- and organo-catalytic methods.[38] The exquisite selectivity, and the mild operating conditions of enzymatic reactions should make them a powerful tool in both industry and academia, but they have been historically underused owing to perceived limitations of practical difficulty, stability, and specificity.

The field of biocatalysis was born over a hundred years ago when it was first recognised that cells, or components thereof, were able to effect useful chemical transformations. One of the earliest reports by Rosenthaler details the emulsin-catalysed addition of hydrogen cyanide to benzaldehyde, and the hydrolysis of benzaldehyde cyanohydrin.[39] Later reports of condensation reactions between glucose and glycerol, also with emulsin,[40] highlighted the limitations of the existing categorisation of enzymes as either synthetic or hydrolytic.[41] In 1961, the International Union of Biochemistry classified all enzymes based on the reactivity they catalyse, and this system is still used today.[42,43]

- *Oxidoreductases* – oxidation/reduction by oxygenation or gain/loss of hydrogen atoms.
- *Transferases* – functional group transfer.
- *Hydrolases* – hydrolysis.
- *Lyases* – bond cleavage by elimination, or addition to double bonds.
- *Isomerases* – isomerisation
- *Ligases* – bond formation with concomitant hydrolysis of a nucleotide triphosphate.

Enzymes have been incorporated into industrial processes since these initial observations. From the late 20th century, BASF employed lipases to generate enantiopure alcohols (***Scheme 0.08,* A**) and amines (**B**) as feedstock chemicals for the pharmaceutical industry.[44] This programme was later expanded to include nitrilases which provided access to multi-ton quantities of enantiopure amino and hydroxycarboxylic acids from racemic nitriles (**C**).[45]



Scheme 0.08: *Industrial application of biocatalysis at BASF in synthesis of chiral* ***A)*** *alcohols;* ***B)*** *amines;* ***C)*** *hydroxyl- and amino-carboxylic acids.*

However, multiple challenges limited the wider use of industrial biocatalytic strategies at this time.[46] Solubility and stability problems restricted the conditions under which enzymes could be used, although immobilisation and development of suitable biphasic reaction mixtures addressed these concerns.[47] More important though, were considerations of cost and activity.

Biocatalytic reactions may either be conducted in whole-cell systems, or using the isolated enzyme. In whole-cell systems the enzyme is over-expressed in the host organism and reaction occurs directly without further processing. This method is cheap but can be unpredictable, owing to the presence of other enzymes within the cell. On the other hand, the free enzyme may be extracted following over-expression and whilst this gives more consistent results, the purification process is expensive. The development of recombinant systems, systems in which the gene encoding the enzyme is transferred to a different host for expression, improved both the quality and availability of enzymes for synthesis and advances in protein engineering enabled mutation of specific enzymes to increase both the range of possible reactions and the tolerance of non-natural substrates.[48,49]

Enzyme Design

Protein engineering and enzyme design have been thoroughly reviewed and the key methods are summarised below.[38,49,50]

Rational design:[50] this technique requires a significant amount of information about the 3-D structure of the enzyme in both its free and substrate-bound forms. Crystallographic data are used to identify key residues which may be exchanged *via* site-directed mutagenesis to improve function. However, this approach is often unsuccessful owing to the unclear relationship between structure and function for many enzymes (***Figure 0.04,*** **A**).

Directed evolution:[51] this technique is used where there are insufficient data for rational design. The wild-type enzyme is subjected to random mutagenesis; reliant on the error-prone polymerase chain reaction (PCR), the number of mutations introduced to the DNA can be controlled, but their position is completely random. The mutants produced by random mutagenesis are screened for the desired activity and the best mutant selected and re-subjected to mutation. Multiple iterations of this cycle are completed until an enzyme with the desired characteristics is obtained. Directed evolution has the advantage of highlighting desirable mutations which otherwise would not have been of obvious importance (***Figure 0.04*, B**).

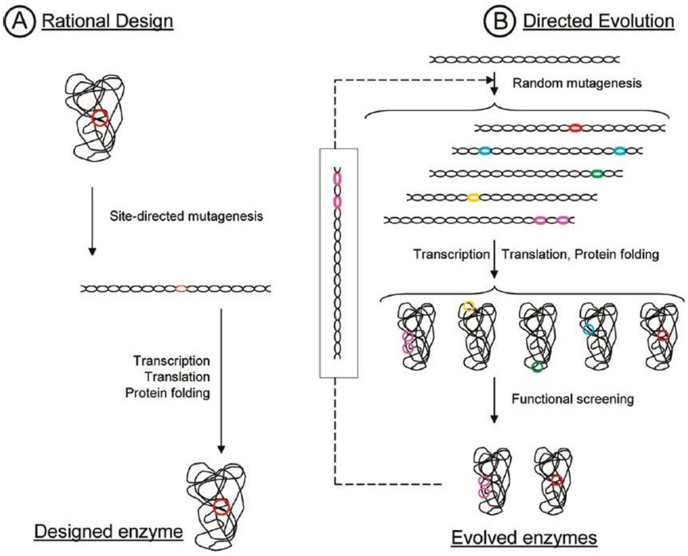


Figure 0.04: *Enzyme design by:* ***A)*** *Rational design;* ***B)*** *Directed evolution.*[49]

Site-saturation mutagenesis:[52] this usually follows rational design or directed evolution when a residue of interest has been identified. Site-directed mutagenesis enables the replacement of this residue with every other amino acid to identify the best mutant (***Figure 0.05***).

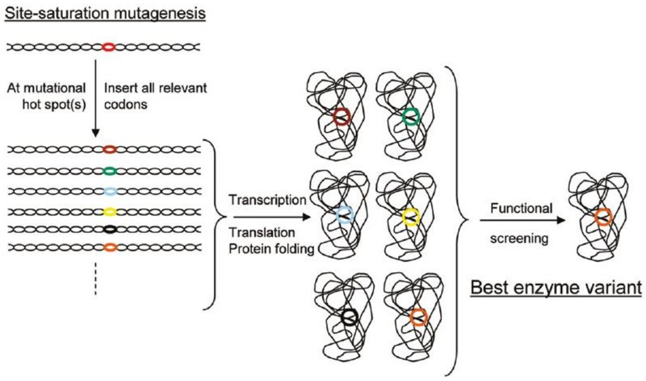
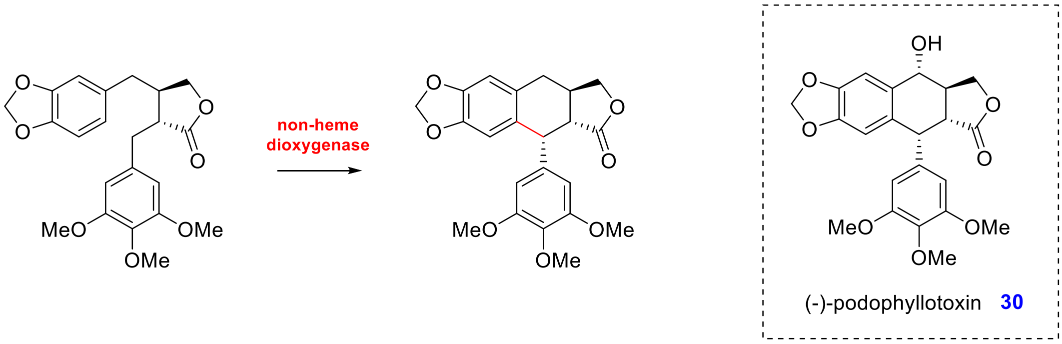


Figure 0.05: *Enzyme design by site-saturation mutagenesis.*[49]

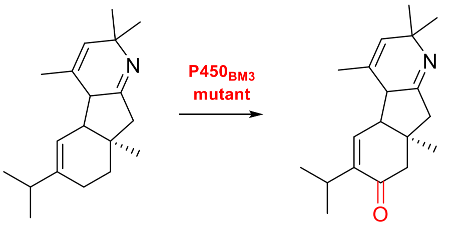
Chemoenzymatic Synthesis

Although still considered a niche area, the utility of enzymes in total synthesis has already been demonstrated and reviewed.[53–56] In 2019 Renata reported a concise chemoenzymatic synthesis of (–)-podophyllotoxin **30**, applying an enzyme-mediated oxidative C-C coupling as the key step (***Scheme 0.09***).[57] This strategy was so successful that the group applied the same methodology to synthesise five of the related aryltetralin lignans.



Scheme 0.09: *Key chemoenzymatic step from Renata's synthesis of (–)-podophyllotoxin* ***30****.*

Equally powerful is the application of enzymes as a tool for late-stage functionalisation, a strategy employed by Arnold and Stoltz in the synthesis of nigelladine A **32** (***Scheme 0.10***).[58] Using an evolved cytochrome P450$_{\mathrm{BM3}}$ mutant, selective C-7 oxidation was achieved on the highly conjugated core **31**, resulting in a 5% yield over a concise total synthesis of 12 steps.



Scheme 0.10: *Arnold and Stoltz's enzymatic late-stage functionalisation to nigelladine A* ***32***.

Cytochrome P450s have been extensively used in this capacity and the remainder of this thesis will focus on their application to total synthesis.[59,60]

Cytochromes P450

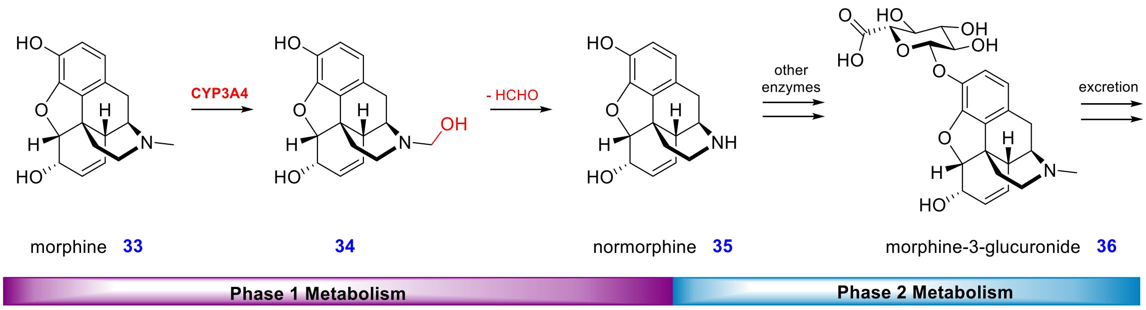
The cytochromes P450 are a superfamily of heme mono-oxygenases: iron-containing oxidative enzymes. In 1951 a novel hemochromagen was isolated from mammalian livers[61] which Garfield[62] and Klingenberg[63] subsequently reported as a CO-binding pigment with a distinctive absorption peak at 450 nm. Omura and Sato later purified this pigment and identified it as a hemeprotein which became known as cytochrome P450.[64,65]

Since their discovery, these enzymes have been found almost ubiquitously through life and their presence in extremophile archaea such as *Sulfolobus solfataricus* (thermoacidophilic archaeon found in sulphurous volcanoes),[66] and *Halobacterium salinarum* (halophilic archaeon found in salt lakes)[67] suggests ancient origins. Despite catalysing numerous anabolic and catabolic processes in nature, the omnipresence of these enzymes remains unexplained, as they appear to have no role in universal life-sustaining functions such as respiration. It has therefore, been postulated that their unusual reactivity is the result of evolution in response to increasing atmospheric oxygen.[68]

Indeed, the majority of reactions catalysed by P450s are oxidations, the most famous being hydroxylation *via* the insertion of a single atom from molecular dioxygen into a C-H bond. Other oxidative processes such as dehydrogenation, dealkylation, heteroatom oxidation, and C=C bond formation and cleavage are generally well understood and have been much reviewed,[69] but the mechanisms of the more unusual reactivity exhibited by these enzymes (reductions, isomerisations, rearrangements) are less clear.[70]

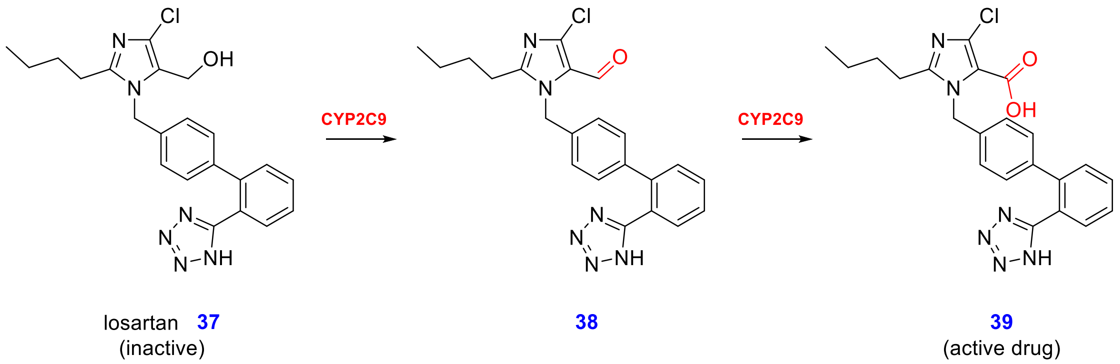
Such a diverse range of activity particularly adapts the P450 enzymes for metabolic processes and they are correspondingly found in high concentrations in human liver cells. Human xenobiotic metabolism occurs in two stages: Phase 1 (modification), usually P450-mediated, involves chemical reactions such as oxidation, reduction, or hydrolysis to change the substrate by introducing polar groups. The metabolites of this process have different biological activities from the starting substrate and an understanding of this is a key part of drug development. Inactive metabolites proceed to Phase 2 metabolism (conjugation) where conjugation with a charged co-factor forms a compound soluble enough for excretion in the urine.[71]

Morphine **33** is deactivated by Phase 1 metabolism: hydroxylation of the *N*-methyl group by CYP3A4 produces unstable hemiaminal **34** which rapidly collapses to normorphine **35** by loss of formaldehyde (***Scheme 0.11***).[72] The inactive normorphine metabolite proceeds to Phase 2 metabolism and is excreted as normorphine-3-glucuronide **36**.[73]



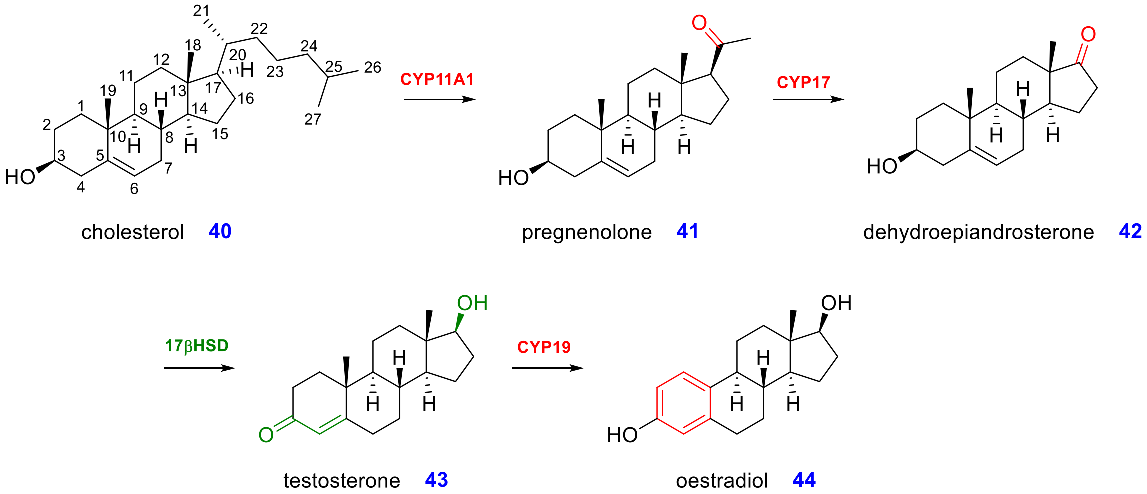
Scheme 0.11: *Metabolism of morphine in humans.*

However, for some drugs, Phase 1 metabolism is necessary to generate the bioactive compound and such substrates are known as prodrugs. Losartan is a blood pressure medication, administered orally as the inactive alcohol **37** (***Scheme 0.12***). Phase 1 metabolism by CYP2C9 produces first the aldehyde **38** through a slow oxidation, then the bioactive acid **39** through a second rapid oxidation. These differing rates of oxidation enable control of the bioavailability of the active drug.[74]



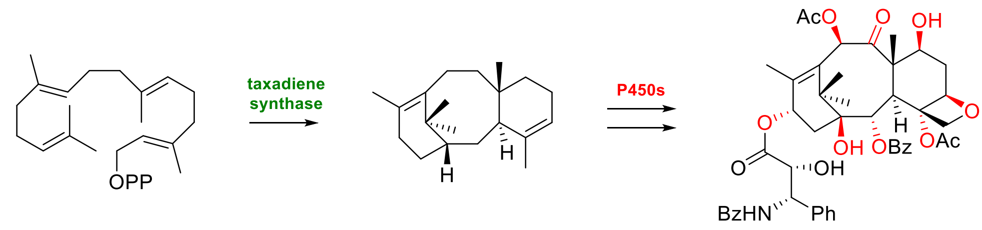
Scheme 0.12: *Activation of prodrug losartan* ***37*** *by Phase 1 metabolism.*

The cytochromes P450 also have an important biosynthetic function. In humans, they are the key enzymes involved in steroidogenesis. Steroid hormones are oxidised derivatives of cholesterol, a lipid stored as cholesterol esters within the lipid vesicles of mammalian cells. Following hydrolysis of these esters, free cholesterol **40** undergoes three successive oxidations (hydroxylation at C-22, hydroxylation at C-20, and cleavage of the side chain at C-20), all mediated by CYP11A to form pregnenolone **41**. A range of oxidative transformations then convert pregnenolone to different steroid hormones, dependent on the gender of the individual, and the gland in which steroidogenesis is occurring. ***Scheme 0.13*** outlines the biosynthesis of oestradiol **44** as a representative example. CYP17 enables hydroxylation of pregnenolone **41** at C-17, followed by C-C bond cleavage in the same position, to give dehydroepiandrosterone **42**. Two dehydrogenase-catalysed steps follow (alcohol oxidation at C-3 and reduction at C-17), forming testosterone **43** which is aromatised by CYP19 to give oestradiol **44**. [75,76]



Scheme 0.13: *Biosynthesis of oestradiol* ***44****.*

Similarly, these enzymes also mediate many of the oxidative steps in plant-terpene biosynthesis.[77] About half of the steps in the (19 step) biosynthesis of the diterpenoid anti-cancer drug taxol **47**, extracted from members of the *Taxus* genus, have been found to involve P450 enzymes.[78] Cyclisation of geranylgeranyl diphosphate **45** by taxadiene synthase gives taxadiene **46** which then undergoes eight separate P450-mediated oxidative functionalisations to decorate the taxane core (***Scheme 0.14***). Following several other functionalisations, taxol **47** is produced.



geranylgeranyl diphosphate **45** taxadiene **46** taxol **47**

Scheme 0.14: *Biosynthesis of taxol **47**.*

Structure & Catalytic Cycle

The range of specific transformations catalysed by these enzymes is testament to the sheer magnitude of this superfamily. But despite this diverse reactivity and substrate tolerance, all members share the same basic structure. The prosthetic group centres around a low spin Fe(III) species, contained within the cavity of porphyrin IX to form the heme B complex (***Figure 0.06,* A**). A cysteine residue ligates this to the protein component, a highly conserved arrangement of twelve α-helices and five β-sheets, creating the characteristic protein fold (***Figure 0.06,* B**). Within the pocket created by this is a water molecule, occupying the final octahedral coordination site when the enzyme is in its resting state.[79]

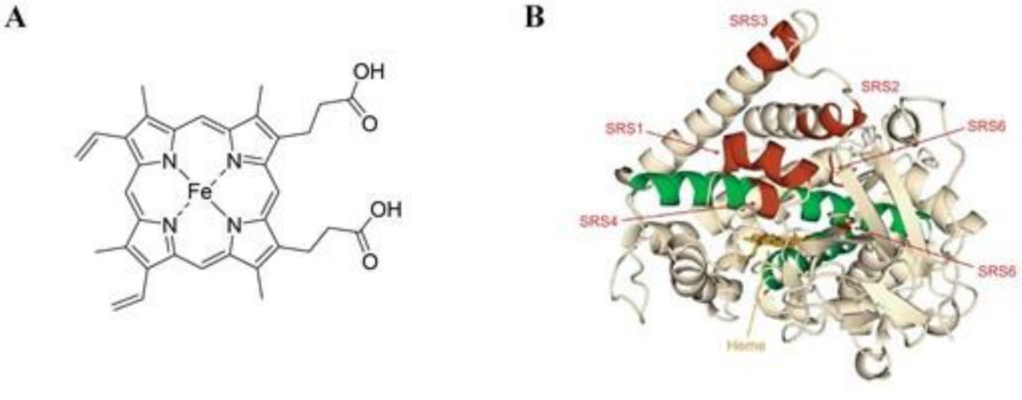
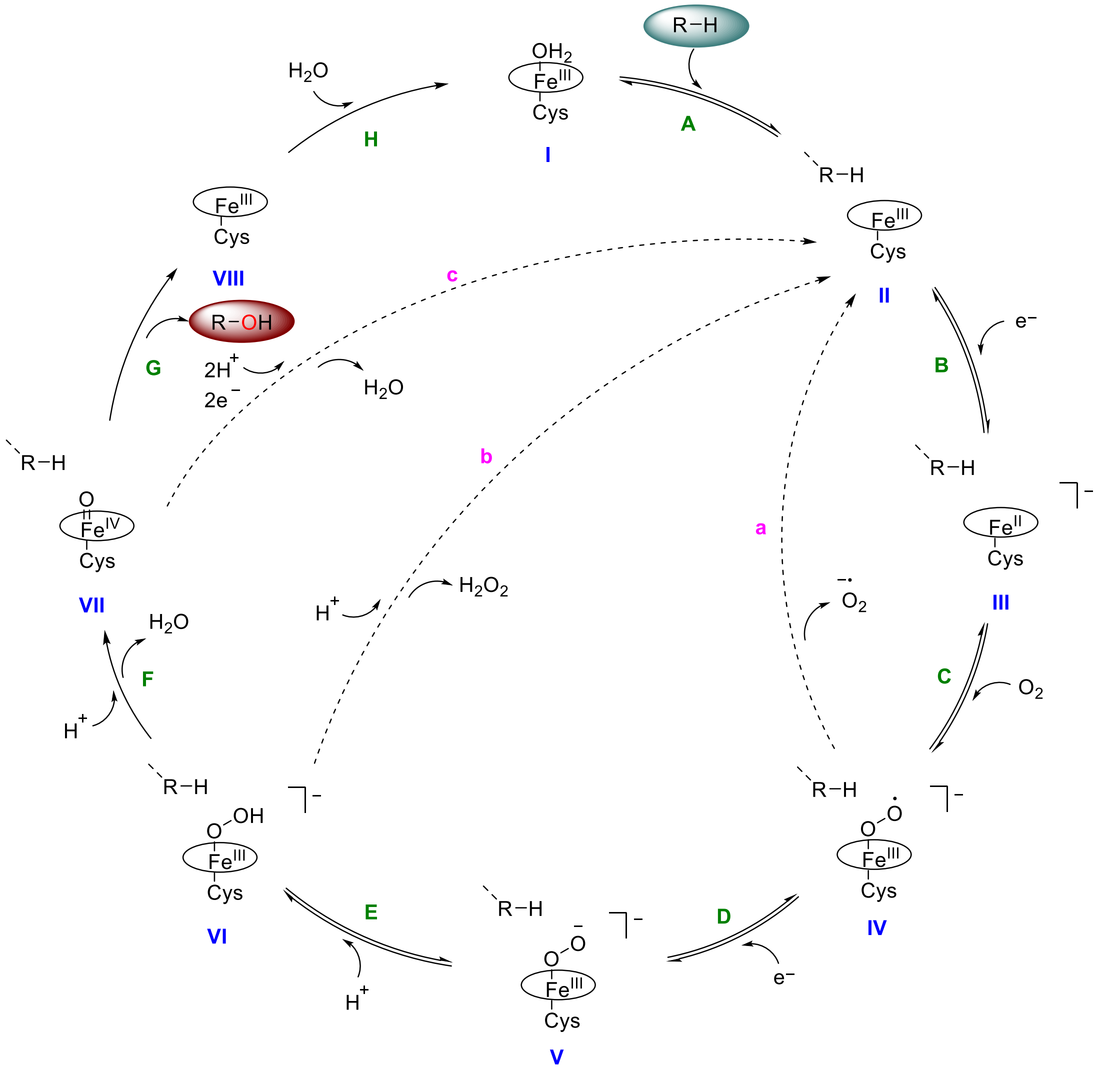


Figure 0.06:* *A) *Structure of heme-b complex;* ***B)*** *Model of the tertiary structure of a cytochrome P450. Heme-b is highlighted in orange, substrate recognition sites (SRS1 – SRS6) in red, and the heme-co-ordinating helices (I and L) in green.*[80]

The catalytic cycle has been widely studied and is outlined in ***Scheme 0.15*** below.[79,69]



Scheme 0.15: *Catalytic cycle for cytochrome P450s. Incoming substrate indicated in teal. Oxidised product indicated in burgundy.*

A – The resting enzyme **I** is first perturbed by the arrival of the substrate into the active site which necessarily involves the loss of the axial water ligand. This change forces the iron to adopt a high spin configuration which results in a corresponding increase in ionic radius and hence displacement from the cavity of the porphyrin ring.

B – Subsequent electron transfer is rapid and the ferrous complex **III** is formed, with the negative charge delocalised over the entire prosthetic group.

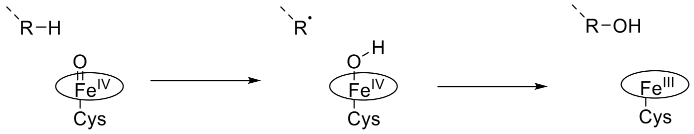
C – A stable iron-oxygen bond is formed as one electron is donated from each the Fe(II) centre and molecular dioxygen to form the oxy-complex **IV**.

D – A further reduction occurs, to produce dianionic iron-peroxo complex **V**. This step is usually rate determining in wild-type enzymes.

E – Species **V** may act as a nucleophile directly, but rapid protonation to hydroperoxo **VI** is more likely. Although this complex is less nucleophilic than **V**, it is still sufficiently competent to enable formation of the critical iron-oxo species.

F – This second protonation is likely assisted by the negative charge delocalised over the prosthetic group. Electron density is released from the iron to break the weak O–O bond and form water, and the reactive iron-oxo species **VII**.

G – The mechanism of hydroxylation is still under debate and several theories have been proposed. The most widely accepted of these is the oxygen rebound mechanism. (***Scheme 0.16***).



Scheme 0.16: *Oxygen rebound mechanism of hydroxylation.*

C-H abstraction: a proton is abstracted from the substrate by the reactive iron-oxo species **VII**.

Alkyl rotation: in some cases, the carbon radical may undergo rearrangement (e.g. inversion) to give a more favourable orientation for rebound.

Rebound: The carbon radical forms a bond with the oxygen, leaving a ferric alcohol complex which may then expel the newly hydroxylated substrate to re-enter the cycle.

H – The product diffuses out of the active site, leaving pentacoordinate ferric species **VIII**. Water is re-bound in the vacant site to return the enzyme to its resting state **I**.

However, not every iteration of this cycle leads to a productive oxidation. Several competing uncoupling pathways back to **II** are possible:

a – *Superoxide uncoupling:* where oxygen binding is hindered, the second electron transfer (**D**) may be too slow to prevent direct loss of superoxide.

b – *Peroxide uncoupling:* in some cases, a loosely bound substrate may insufficiently shield the iron-bound oxygen atom from protonation by vicinal water. Where this occurs, hydrogen peroxide is lost directly, back to **II**. However, at high peroxide concentrations, this process reverses, enabling oxidation to be driven by hydrogen peroxide, in place of oxygen and NAD(P)H. This is known as the peroxide shunt and it can result in oxidation of some amino acids within the active site and is consequently detrimental to the enzyme.

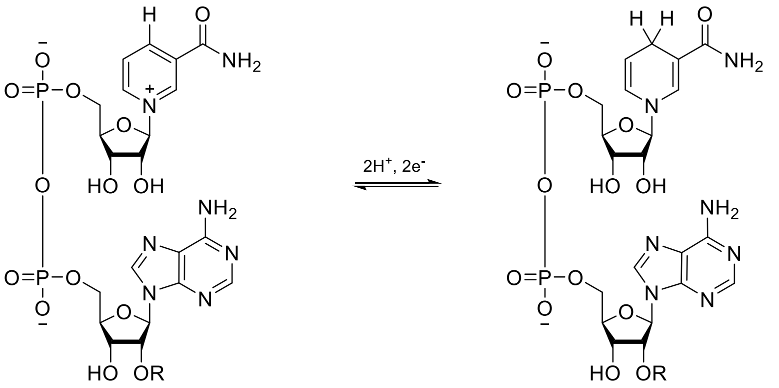
c – *Oxidase uncoupling:* where there are no vicinal hydrogen atoms for abstraction, the iron-oxo species is instead reduced to water and **II**.

Each successful cycle may be summarised by ***Equation 0.01*** and results in the insertion of one atom from molecular dioxygen into a C-H bond, the other being reduced to water.

$$R - H + O_2 + 2H^+ + \ 2e^- \ \rightarrow R - OH + H_2O$$

Equation 0.01: *Overall reaction for each successful catalytic cycle.*

The cytochromes P450 are external monooxygenases, meaning that the electrons required for the reductive steps are obtained from cofactors. NADH **50** and NADPH **51** are reduced pyridine nucleotides which act as redox partners in these reactions (***Scheme 0.17***).

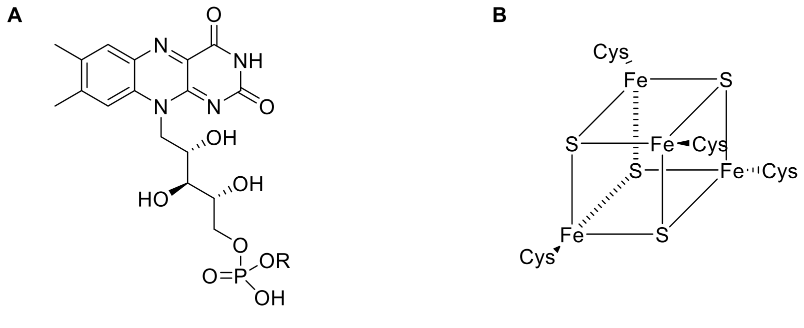


R = H NAD$^+$ **48**
R = PO_3^{2-} NADP$^+$ **49**

R = H NADH **50**
R = PO_3^{2-} NADPH **51**

Scheme 0.17: *NADH and NADPH redox cofactors.*

An electron transfer system is necessary to shuttle these electrons to the active site of the enzyme and P450 variants may be classified by the structure of this system.[81] The majority use a sequence of external reductases, cofactors, or ferredoxin clusters which may be either membrane-bound, or cytosolic (***Figure 0.07***).



R = H FMN **52**
R = adenyl FAD **53**

[4Fe-4S] ferredoxin **54**

Figure 0.07: *External reductases:* ***A)*** *FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide) cofactors and,* ***B)*** *Example iron-sulfur cluster [4Fe-4S] ferredoxin.*

For example, ***Figure 0.08*** illustrates the electron transport chain for Class I bacterial systems. An FAD-containing reductase (FdR) accepts electrons from NADH, which in turn reduces a ferredoxin (Fdx). This component then transfers the electrons to the cytochrome P450 itself. As the entire process occurs in the cytoplasm, the rate of electron transfer is limited by diffusion and availability of redox partners, and hence the rate limiting step.

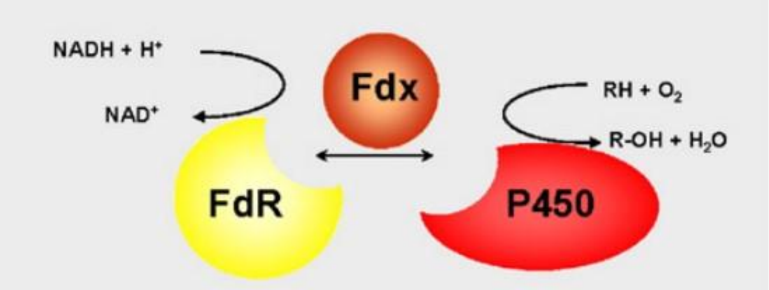


Figure 0.08: *Electron transport chain for Class I bacterial systems.*[81]

The unusual structure of Class VIII systems overcomes these problems, containing a reductase partner fused to the cytochrome P450 unit in a single polypeptide chain (***Figure 0.09***).

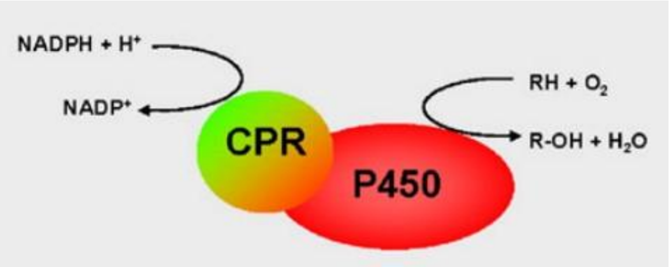


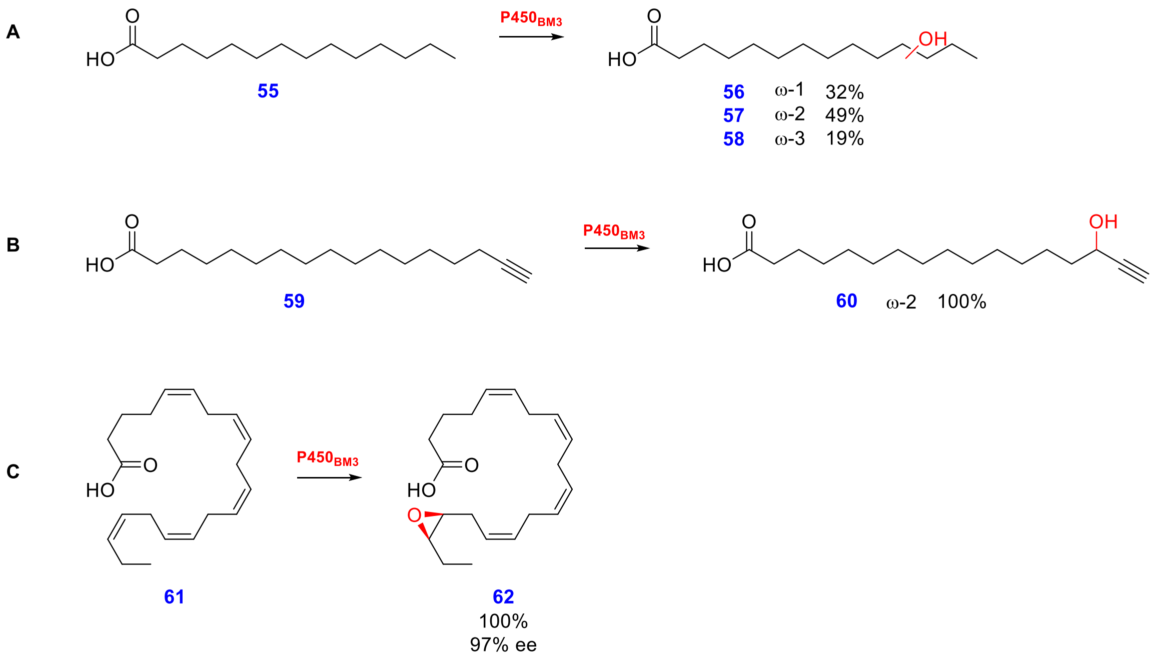
Figure 0.09: *Electron transport chain for Class VIII bacterial systems.*[81]

Crucially, this makes a catalytically self-sufficient enzyme which is therefore more active and easier to handle in a laboratory setting. CYP102A1 (also known as cytochrome P450_{BM3}) is the most extensively studied member of this group and is the specific enzyme of interest in this project.

P450$_{BM3}$ (CYP102A1)

Cytochrome P450$_{BM3}$ is so named because it is the third P450 enzyme isolated from *Bacillus megaterium,* as reported in 1975 by Fulco *et al*.[82] Initially characterised as a single 119 kDa protein,[83] subsequent proteolysis studies identified it as a combination of two smaller protein domains (55 and 66 kDa respectively),[84] linked by a short amino acid chain.[85] The smaller of these is the heme-domain (BMP), containing the active site of the enzyme, described previously, whilst the larger reductase domain (BMR) comprises an equimolar ratio of the prosthetic flavins FAD **53** and FMN **52** (***Figure 0.07,* A**). The proximity of these domains critically enables a far higher turnover in comparison to similar enzymes and their separation results in a complete loss of monooxygenase activity which is not recovered upon recombination.[86]

The precise physiological role is unclear but P450$_{BM3}$ is a fatty acid hydroxylase, typically acting on medium to long chain fatty acids.[79,82] Saturated substrates are hydroxylated in sub-terminal positions, but generally without selectivity (***Scheme 0.18,* A**).[87] Unsaturated fatty acids demonstrate greater control, being either selectively hydroxylated in the ω-2 position (***Scheme 0.18,* B**),[87] or preferentially undergoing epoxidation (***Scheme 0.18,* C**).[88]



Scheme 0.18: *Fatty acid substrates for P450*$_{BM3}$*:* ***A)*** *saturated;* ***B)*** *mono-unsaturated;* ***C)*** *poly-unsaturated.*

Analysis of the original crystal structure revealed a long, hydrophobic, non-aromatic access channel to the active site, but calculations suggested that this relatively open conformation would not be able to bind these known substrates effectively. Active site re-organisation was proposed upon substrate binding and crystal structures of substrate-bound[89] and substrate-free[90] P450$_{BM3}$ were compared to begin to identify the residues responsible. Further crystal structures and site-selective mutagenesis have since been used to clarify the roles of the most significant amino acids:

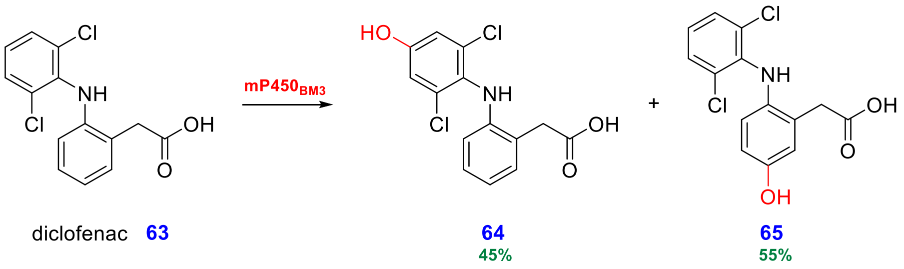
- **Arg47 and Tyr51** are two residues at the entrance of the active site, shown in the original crystal structure to be closely associated with the fatty acid substrate. Mutation of either residue reduces both hydroxylation activity and binding affinity, indicating that Arg47 and Tyr51 are responsible for controlling access, orientation, and binding of substrates within the active site.[91]
- **Phe87** extends into the active site and sits close to the heme centre. Upon substrate binding, this projection undergoes a 90° rotation, displacing active site water and inducing further active site reorganisation to bring the substrate closer to the

heme.[92] Mutations at this residue vary the volume of this ‘active cavity’ and consequently enable reaction of larger substrates.[91]

- **Glu267** is hydrogen bonded to at least one water molecule in the substrate-free enzyme. Mutation reduces oxidation activity, both through disruption of proton transfer, and substrate positioning.[79]
- **Thr268** has a more complicated role and has been implicated in various processes: proton delivery, dioxygen activation, and intermediate stabilisation. Mutation does not deactivate the enzyme but changing product profiles are observed for different substrates.[79]
- **Cys400**, the ligating cysteine residue, increases the reactivity of the iron oxo complex **VII** towards C-H bonds *via* electron donation. Substitution with less electron-donating ligands such as histidine results in a complete loss of monooxygenase activity,[93,94] whereas replacement with more nucleophilic selenocysteine augments the reactivity towards C-H bonds.[95]

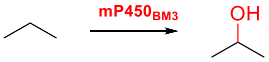
The identification of these key residues has facilitated the development of whole libraries of mutant enzymes through protein engineering.[96–98] These mutant P450s, often exhibiting significantly different activities and selectivities from the wild type, have found numerous applications in drug development and traditional synthesis.[99]

Synthesis of metabolites is an important example. An understanding of the toxicity and activity of breakdown products is a critical part of drug discovery and metabolites are required on a preparative scale for these studies.[100] Human liver enzymes are both expensive and unreliable for *in vitro* study but the development of mutant P450 libraries has proven a practical alternative. Following screening against one such library, Wong *et al*. were able to develop a mutant able to fully metabolise diclofenac **63** into the two hydroxylated metabolites **64** and **65** (***Scheme 0.19***).[101] This process has been applied by numerous other groups to give access to hundreds of drug metabolites *via* evolved P450 mutants.[102–107]



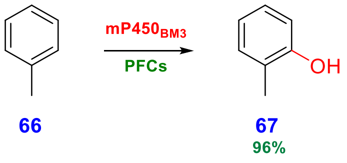
Scheme 0.19: *Metabolism of diclofenac* ***63*** *by mutant P450 enzymes (mP450$_{BM3}$).*[101]

Mutant P450s are also valuable tools in the synthesis of diverse functionalised building blocks and hydroxylation of small molecules is particularly important in this respect.[108] In 2003, Arnold reported the hydroxylation of small linear alkanes with complete regioselective control (***Scheme 0.20***).[109,110]



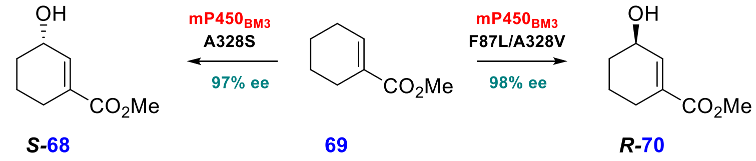
Scheme 0.20: *Arnold's selective hydroxylation of propane.*

Later work by Wong expanded this scope to include aromatics and nitrogen-containing molecules in conjunction with decoy molecules (***Scheme 0.21***).[111–113] These are chemically inert 'promoters' of oxidation and work by filling part of the active site to enable the substrate to bind closer to the heme centre, boosting the activity without influencing the selectivity.



Scheme 0.21: *Hydroxylation of toluene with mP450$_{BM3}$ and perfluorocarboxylic acid (PFC) decoy molecules.*[113]

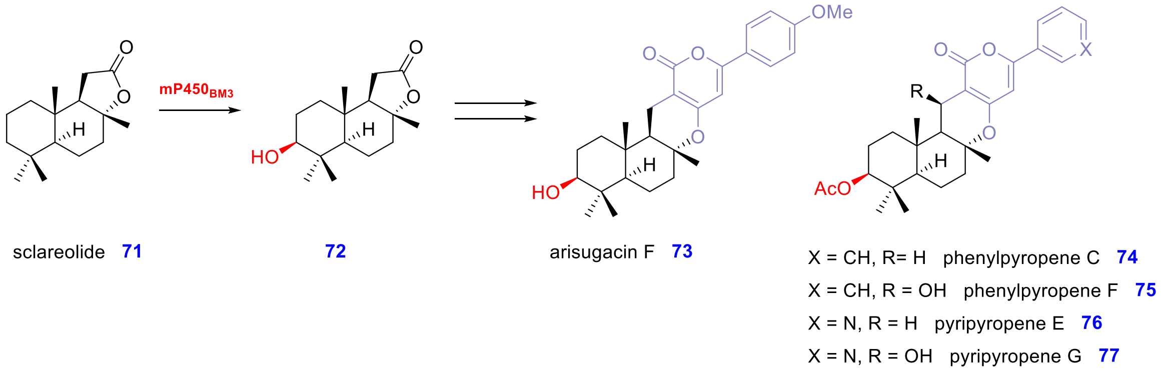
Enantioselective hydroxylation is also well precedented.[114–119] The Reetz group demonstrated exquisite enantiocontrol in the hydroxylation of cyclohexene-1-carboxylic acid methyl ester **69**, using directed evolution to develop two mutants with complementary selectivity (***Scheme 0.22***).[120]



Scheme 0.22: *Reetz's enantioselective hydroxylation of* ***69****. A328S and F87L/A328V refer to specific residue mutations.*

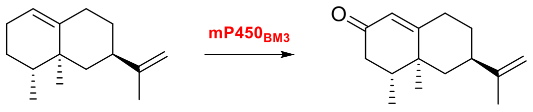
More recently, the exploitation of other oxidative reactivity such as cyclopropanation[121] and carbene transfer[122,123] has been possible, giving access to a greater diversity of small molecule building blocks.

The improved substrate tolerance of engineered mutants has enabled their application more generally in natural product synthesis. Renata *et al*. demonstrated the merit of including P450-mediated oxidation steps in a retrosynthetic plan, achieving the concise synthesis of eight oxidised meroterpenoids from two molecular scaffolds.[124] C-3 hydroxylation of sclareolide **71** gave a common intermediate which was subsequently diversified into **74** to **77** (***Scheme 0.23***).



Scheme 0.23: *Renata's divergent synthesis of oxidised meroterpenoids* ***74*** *to* ***77****.*

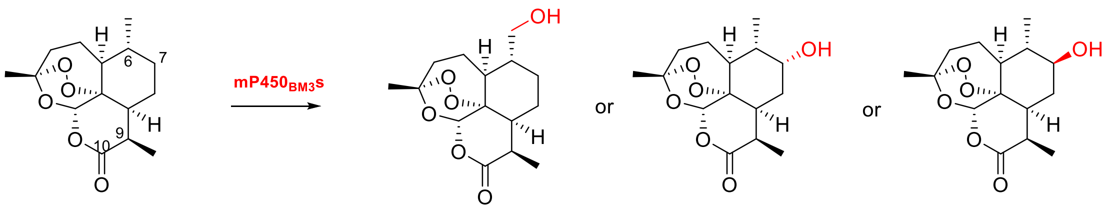
Enzymatic oxidation in natural product synthesis is also commercially significant. (+)-Nootkatone **79** is a fragrant sesquiterpenoid isolated from grapefruits but natural supplies fail to meet demand from the flavours and fragrances industry. The chemical oxidation of abundant (+)-valencene **78**, isolated from orange oil, is reported but requires either harsh conditions or metal catalysis to produce synthetic grade (+)-nootkatone.[125,126] P450-mediated oxidation of **78**, developed by Wong *et al*., now operates at industrial scale to produce natural grade (+)-nootkatone which is a higher value product (***Scheme 0.24***).[127]



(+)-valencene **78** (+)-nootkatone **79**

Scheme 0.24:*Enzymatic oxidation of (+)-valencene* ***78*** *to (+)-nootkatone* ***79****.*

Arnold's synthesis of nigelladine A **32** has already demonstrated the value of a late-stage approach in total synthesis, avoiding the use of protecting groups by installing functionality at the end (***Scheme 0.10***). This same logic has been applied to natural product diversification, and screening of natural products and natural product fragments against mutant P450 enzymes has generated a number of chemically-inaccessible compounds, providing a convenient method of expanding compound libraries for drug development.[128] A famous example is the oxidation of artemisinin **80**, an antimalarial sesquiterpene. Previous chemical attempts had successfully functionalised at C-9 and C-10 but C-H bonds in the upper part of the structure proved inaccessible. Fasan *et al.* developed a series of P450 mutants able to oxidise at the elusive C-6 methyl and C-7 positions, both implicated in the rapid metabolism of artemisinin-based antimalarials (***Scheme 0.25***).[129]



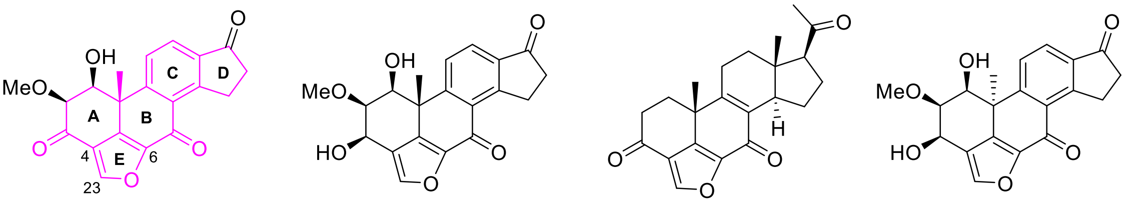
artemisinin **80** C6a **81** C7(*R*) **82** C7(*S*) **83**

Scheme 0.25: *P450-mediated oxidation of artemisinin* ***80****.*

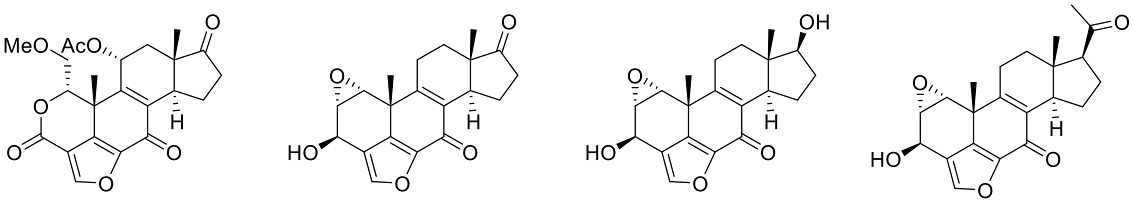
The remainder of this thesis focuses on this last aim with respect to the natural product viridiol and its synthetic precursors.

The Viridin Family of Steroidal Antibiotics

The viridin family of steroidal antibiotics is part of the wider natural product family of furanosteroids. The parent member, viridin **84**, was isolated in 1945 from *Trichoderma viride* and was immediately identified as a potent fungistatic compound.[130] Other members of this family were subsequently isolated and new compounds are still being discovered (***Figure 0.10***).[131–140]



viridin **84** viridiol **85** virone **86** 9-*epi*-viridiol **87**



wortmannin **88** wortmannolone **89** wortmannolol **90** epoxyvirone **91**

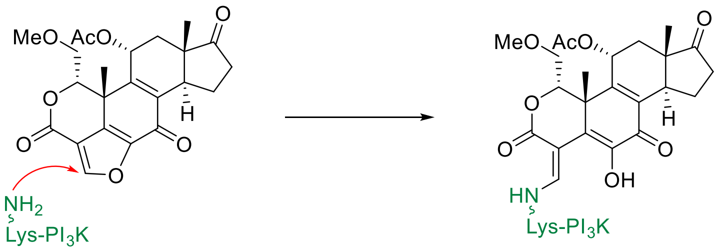
Figure 0.10: *Representative members of the viridin family with the key structural elements indicated in pink.*

All members contain the same characteristic structural feature: a furan ring fused to the steroidal framework between C-4 and C-6. The crystal structure of viridin demonstrates the strain caused by this arrangement: the A ring is significantly buckled whilst the B ring is almost flattened.[141] This strain augments the already highly electrophilic character of the furan ring, arising from the two vicinal carbonyls.

Biological Activity

This ABE tricyclic unit is responsible for much of the observed biological activity of these compounds. Originally described only as antifungals, more recently, generalised enzyme inhibition has been demonstrated by several members.

Wortmannin **88** is the most studied of the group, with several total syntheses having been completed in the last three decades.[142–145] It is best known for its potent anti-proliferative activity and is able to irreversibly bind to the ATP pocket of PI$_3$ kinases through nucleophilic attack of a lysine residue on the electrophilic furan (***Scheme 0.26***).[146]

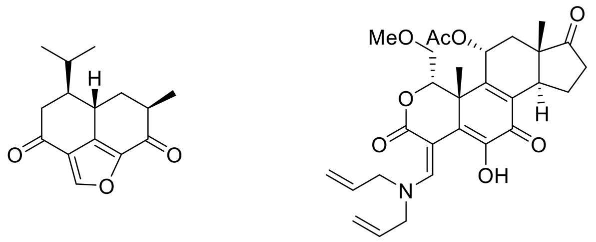


wortmannin **88**

92

Scheme 0.26: *Irreversible binding of PI$_3$K to wortmannin* ***88****.*

Surprisingly, structurally related furanosesquiterpene hibiscone C **93** (***Figure 0.11***) was found to be significantly less active, despite containing the ABE unit.[147] Non-covalent interactions arising from the C and D rings of wortmannin are therefore significant. Further studies showed that blocking of the electrophilic carbon of wortmannin did not eliminate PI$_3$ kinase activity, it merely reduced the potency to a more viable level.[148] One such of these compounds (**94**) has proceeded through to clinical trials. Whilst the diacyl furan unit is required for irreversible binding, it appears that appropriate non-covalent interactions are also sufficient for inhibitive activity.



hibiscone C **93**　　PX-866 **94**

Figure 0.11: *Structures of hibiscone C* ***93*** *and PX-866* ***94****.*

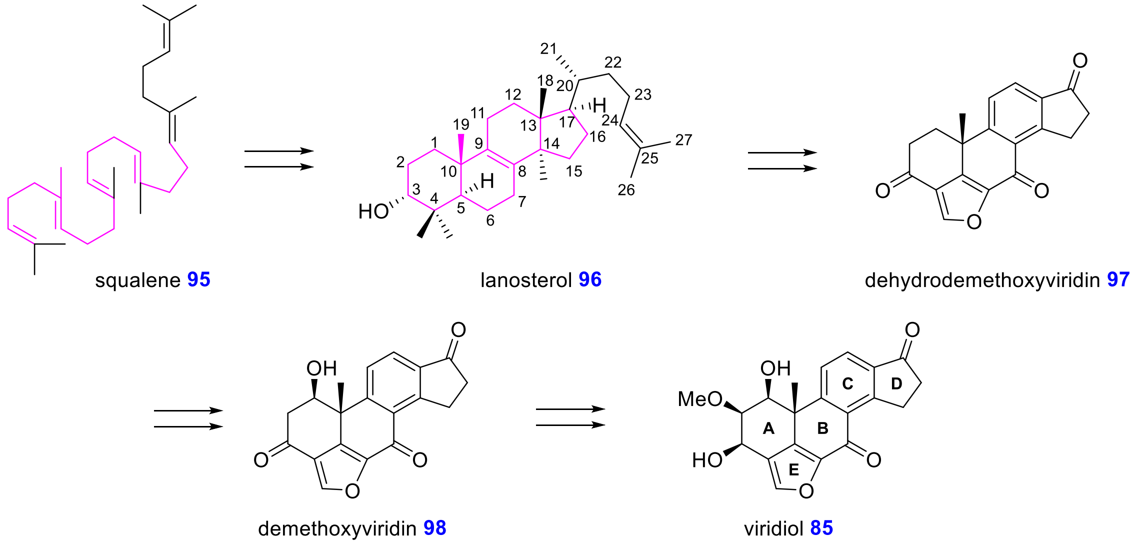
However, this potent activity resulting from the activated furan unit causes problems of general toxicity for all of the viridins, severely limiting their applications medicinally and agriculturally. Instability has limited biological testing and proved a challenge during multiple attempted syntheses; therefore, a new synthetic route, providing access to larger amounts of material, and also permitting divergence to analogues, is highly desirable. The remainder of this thesis will address this challenge, focussing specifically on viridiol **85**.

Viridiol

Viridiol **85** was first isolated as a fungal metabolite of *Gliocladium virens* in 1969, related to the known viridin as the dihydro-derivative.[133] The crystal structure shows the same unusual geometry: flattened and buckled rings with substituents bent well out of the plane.[148] Far less is known about viridiol than other group members; it is less generally toxic than its congeners, likely owing to the reduced electrophilicity of the singly activated furan, and it is unknown whether it possesses any useful medicinal properties. Although instability prevents it from being used directly as a herbicide, dried, ground preparations of *Gliocladium virens* known to be expressing viridiol have been used as effective treatments of pigweed infested soil, without damage to crop plants.[149] More recently, viridiol has been found to inhibit the synthesis of aflatoxins, highly carcinogenic toxins produced by fungi on plants in humid areas which are able to accumulate in the food chain.[150,151] As such, further research on this compound is highly desirable.

Biosynthesis

The complete biosynthesis of viridiol is not known but numerous labelling studies have led to the proposal of the general pathway outlined in ***Scheme 0.27***.[152] Early studies with (*R*)-mevalonic acid suggested a steroidal route *via* squalene **95** for the synthesis of viridin.[153] Later experiments using derivatives of lanosterol **96** confirmed the importance of this intermediate although further studies have been unable to determine the exact sequence of processes by which lanosterol becomes viridiol **85**.[154] Both dehydrodemethoxyviridin **97** and demethoxyviridin **98** have been isolated during labelling experiments and are presumed to be intermediates in this chain.



Scheme 0.27: *Outline biosynthesis of viridiol* ***85****.*

The conversion of lanosterol to viridiol involves four key processes; formation of the furan ring, aromatisation of the C ring, oxidation of the A ring, and loss of alkyl groups at carbons 13, 14, and 17. The aforementioned experiments have enabled some analogy to be drawn with known steroid syntheses. The methyl group at C-14 is lost as formic acid, a step mediated by sterol 14-demethylase, as in the biosynthesis of cholesterol, and the side chain at C-17 has been demonstrated to follow a mammalian pathway of cleavage, breaking first between C-20 and C-22, then between C-17 and C-20.[155]

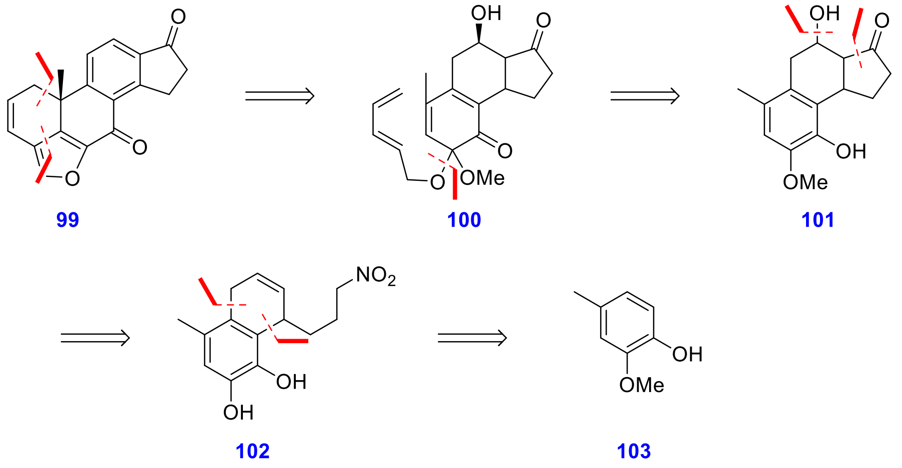
Previous Syntheses

The viridins have been targets of interest since their discovery but instability of the intermediates and the difficulty of achieving the desired selective oxidations on the A ring have hindered their synthesis. Several abortive synthetic attempts demonstrate these difficulties:

Rodrigo *et al.* (1999)

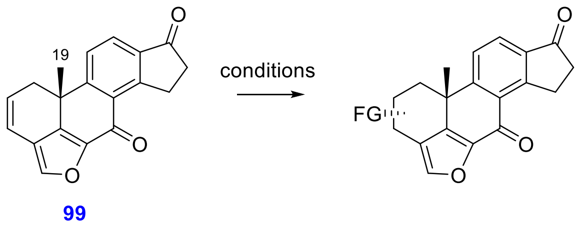
Rodrigo *et al.* reported the first synthesis of the pentacyclic core shared by all the viridins. Although a total synthesis of viridiol was not achieved, the approach was significant in informing subsequent attempts.[156] This strategy is outlined in ***Scheme 0.28***.

Diels–Alder disconnection of the A-ring of **99** revealed *o*-benzoquinone monoketal **100**, accessible *via* a precedented procedure within the group from benzindanone **101**.[157] The D-ring would be closed by nitrile oxide cycloaddition onto dihydronaphthalene **102**, obtainable from commercial reagents following a Diels–Alder cycloaddition and functional group modifications.



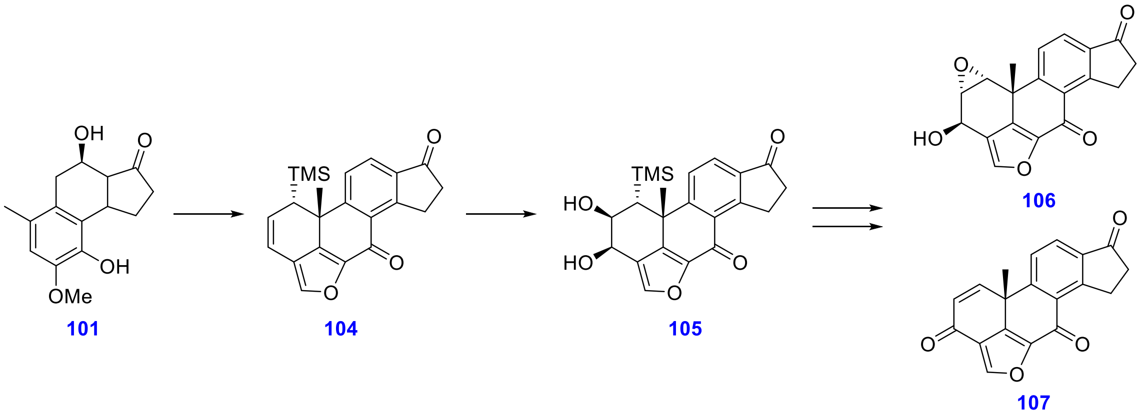
Scheme 0.28: *Rodrigo's retrosynthetic analysis.*

Overall, core **99** was successfully obtained in 12% yield over nine steps. However, attempts to install any functionality on the A-ring with regio- and stereo-control proved to be in vain, the α-face being consistently preferred (***Scheme 0.29***).



Scheme 0.29: *C-19 β-Me directs functionalisation onto α-face under all attempted conditions.*

To overcome the steric influence of the methyl group, Rodrigo and coworkers repeated the synthesis of a modified target by incorporating a bulky blocking group in the neighbouring position.[158] Minor modifications to the previous synthesis afforded BCD ring structure **101** in 60% yield over six steps (*cf.* 52% over eight steps) and with inclusion of a TMS group on the dienol, Diels–Alder and Cope rearrangement gave the desired core **104** (***Scheme 0.30***).

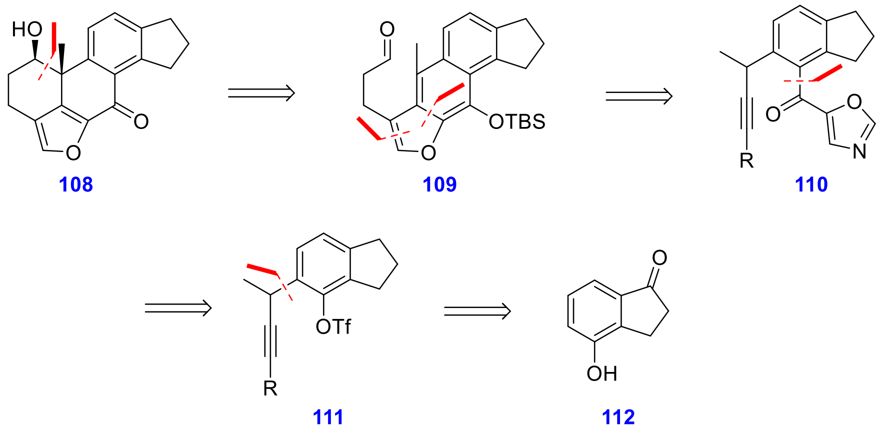


Scheme 0.30: *Synthesis towards and functionalisation of alternative core* ***104****.*

Osmylation of **104** was slow but did successfully occur on the β-face to afford diol **105**. Unfortunately, following this initial functionalisation, poor stereocontrol was observed for all subsequent reactions, with a return to preferential reaction on the α-face to give **106**, or loss of stereochemistry to give **107**. The instability of **105** and its derivatives was also a limiting factor and ultimately the group abandoned the synthesis.

Jacobi *et al.* (2006)

The Jacobi group also reported several attempts towards the synthesis of viridin and its analogues, relying on an intramolecular Diels–Alder/retro-Diels–Alder strategy.[159–161] The initial disconnection of **108** was a Mukaiyama aldol reaction from tetracycle **109**. **110** was obtained by cross-coupling of the requisite oxazole with **111**, accessible from *ortho* functionalisation of hydroxyindanone **112** (***Scheme 0.31***).



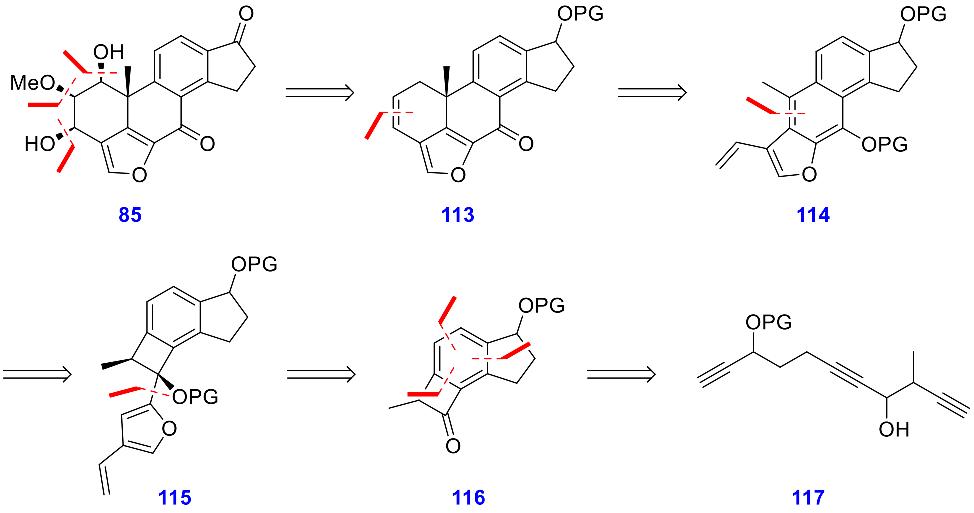
Scheme 0.31: *Jacobi's retrosynthetic analysis.*

In practice, the desired Mukaiyama aldol cyclisation proved troublesome; the electronic properties of the C-17 carbon exerted a strong effect on the reaction outcome with no cyclisation observed for the C-17 ketone (hence its removal early in the synthesis). Product instability also presented a challenge; no reaction was observed at –78 °C, but only slow decomposition occurred at 0 °C. Ultimately, stringently degassed dichloromethane was found to permit reaction with four equivalents of $TiCl_4$ at room temperature to afford pentacycle **108**. However, no successful functionalisations of this pentacycle were subsequently reported.

Despite the difficulty of synthesising this family, three successful syntheses have been completed, in chronological order, by Sorensen, Guerrero, and Gao:

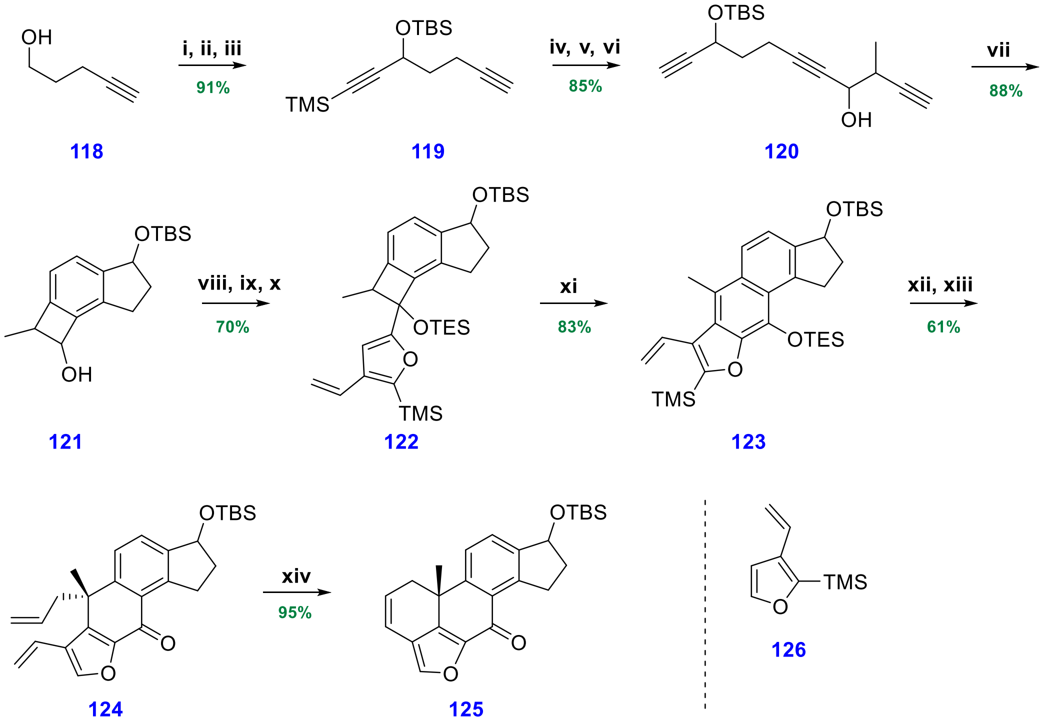
Sorensen *et al.* (2004)

Sorensen reported the first successful synthesis of racemic viridiol **85** in 2004, in 4.7% overall yield over 28 steps.[162] Oxidative functionalisation of the A-ring of **113** was the final step and their strategy addressed the reported instability of the acyl furan intermediates by forming this unit at a late stage in the synthesis *via* annulation of **114-**type intermediates (***Scheme 0.32***). These would be obtained through thermal rearrangement of benzocyclobutane **115**, accessed from **116**, itself formed from an alkyne trimerisation reaction of **117**.



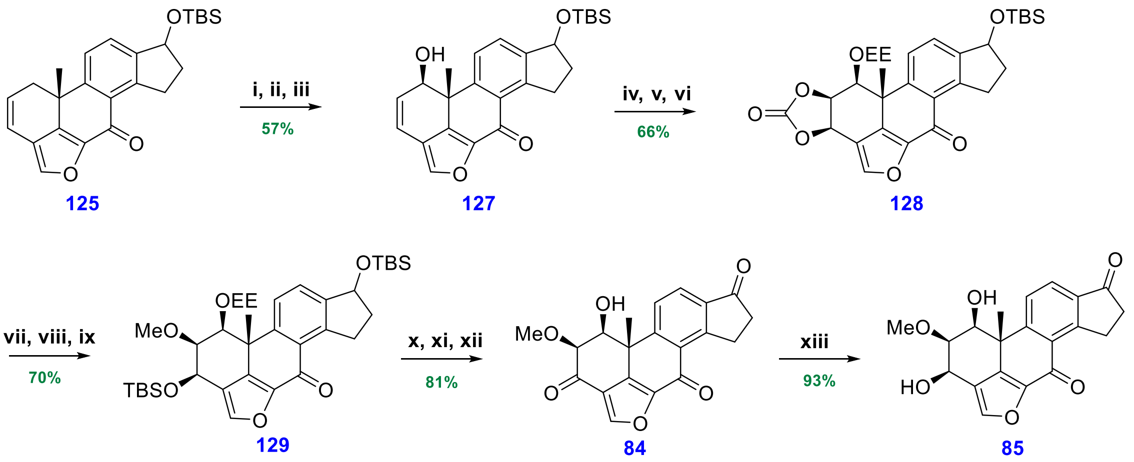
Scheme 0.32: *Sorensen's retrosynthetic analysis.*

Oxidation, alkynylation, and protection of 4-pentyn-1-ol **118** gave **119**, which was formylated, alkynylated, and deprotected to afford triyne **120** (***Scheme 0.33***). Then, treatment of **120** with $RhCl(PPh_3)_3$ in refluxing ethanol induced the expected cyclotrimerisation to **121** in excellent yield. Oxidation of the alcohol **121** under Swern conditions enabled the trapping of lithiated **126** and protection of the resultant alcohol to give **122**. Heating at reflux in xylene under basic conditions effected the desired electrocyclic ring opening of the cyclobutane and subsequent 6π electrocyclisation with the furan to give tetracycle **123**. The handle on the B-ring was next elaborated to generate a suitable substrate for annulation. In a one-pot process, the TES group was removed and the deprotected alcohol allylated. Heating in mesitylene triggered allyl migration to afford **124**, which underwent ring closing metathesis to pentacycle **125** when treated with Grubbs II catalyst.



Scheme 0.33: *Assembly of pentacyclic core* ***125***. **Reagents and conditions: i)** $(COCl)_2$, TEA, DMSO; **ii)** TMSCCH, BuLi, THF, –78 °C; **iii)** imid, DMAP, TBSCl, DCM, rt; **iv)** BuLi, DMF, THF, –40 °C; **v)** 2-bromo-3-butyne, Zn, $HgCl_2$, THF, 65 °C; **vi)** MeOH, K_2CO_3; **vii)** $RhCl(PPh_3)_3$, EtOH, Δ; **viii)** $(COCl)_2$, TEA, DMSO; **ix) 126**, BuLi, THF, –78 °C; **x)** imid, DMAP, TESCl, DMF, 0 °C; **xi)** DIPEA, xylene, Δ, then DDQ, rt; **xii)** CsF, allyl bromide, DMF, 0 °C; **xiii)** DIPEA, mesitylene, Δ; **xiv)** Grubbs II, DCM, rt.

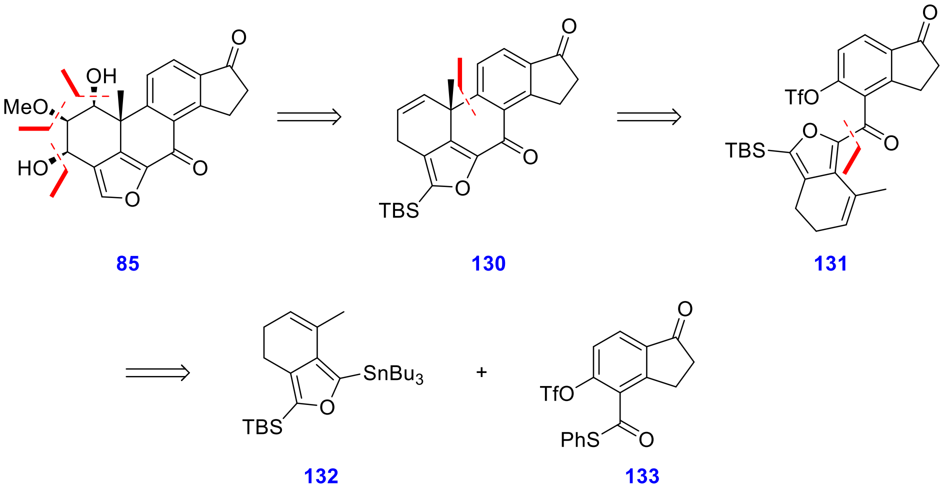
Multiple redox and protecting group sequences were required to achieve correct functionalisation of the A-ring (***Scheme 0.34***). Allylic oxidation of **125** with SeO_2 hydroxylated on the α-face and selective oxidation/reduction afforded the desired alcohol **127**. This hydroxyl group was successfully employed to direct dihydroxylation of the double bond onto the β-face to give the triol. Treatment with triphosgene formed the cyclic carbonate, and the C-1 hydroxyl group was protected as the ethoxyethyl (EE) ether **128**. Hydrolysis of the carbonate and selective silylation on the more accessible C-3 hydroxyl group enabled differentiation of the triol. Methylation of the remaining free alcohol afforded **129**. Deprotection and oxidation of the silyl alcohols, followed by deprotection of the ethoxyethyl ether gave viridin **84** in 5% overall yield. A selective reduction of the C-3 ketone, proceeding in 93% yield, afforded viridiol **85**.



Scheme 0.34: Functionalisation of A-ring. **Reagents and conditions: i)** SeO_2, dioxane, 100 °C; **ii)** DMP, DCM, 0 °C; **iii)** $NaBH_4$, EtOH, 0 °C; **iv)** TMEDA, OsO_4, DCM, –78 °C; **v)** Py, triphosgene, DCM, –78 °C; **vi)** ethyl vinyl ether, PPTS, DCM, rt; **vii)** LiOH, THF, rt; **viii)** TBSOTf, 2,6-lut, DCM, –78 °C; **ix)** NaHMDS, MeOTf, THF, –78 °C; **x)** Bu_4NF, THF, rt; **xi)** DMP, DCM, 0 °C; **xii)** PPTS, MeOH, DCM, rt; **xiii)** $NaBH_4$, EtOH, DCM, 0 °C.

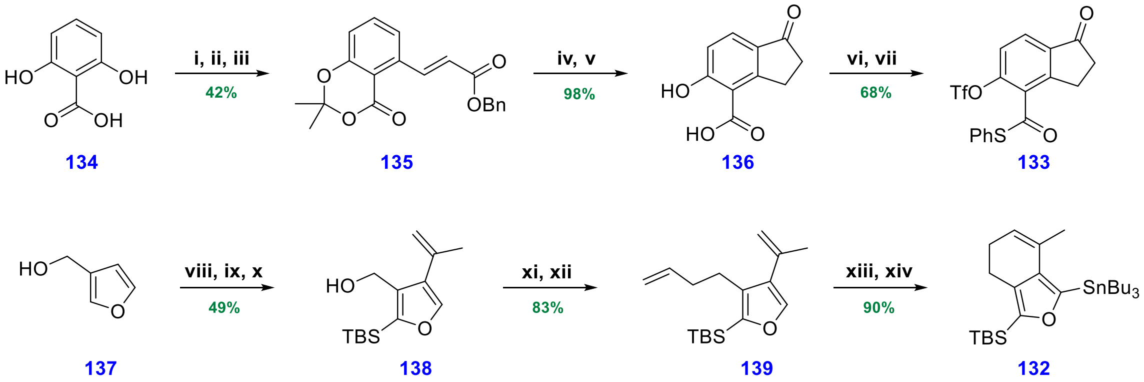
Guerrero *et al.* (2018)

In 2018 the Guerrero group reported the first enantioselective synthesis of viridiol in 1.6% yield over 23 steps, employing an intramolecular Heck reaction to set the stereochemistry as the core was formed.[163] The retrosynthesis of the successful route is outlined below in ***Scheme 0.35*** and was completed by a sequence of redox transformations to correctly oxidise the A-ring. Core **130** was constructed by the aforementioned enantioselective intramolecular Heck reaction of **131**, which is accessible from the coupling of reported fragments **132** and **133**.



Scheme 0.35: *Guerrero's retrosynthetic analysis.*

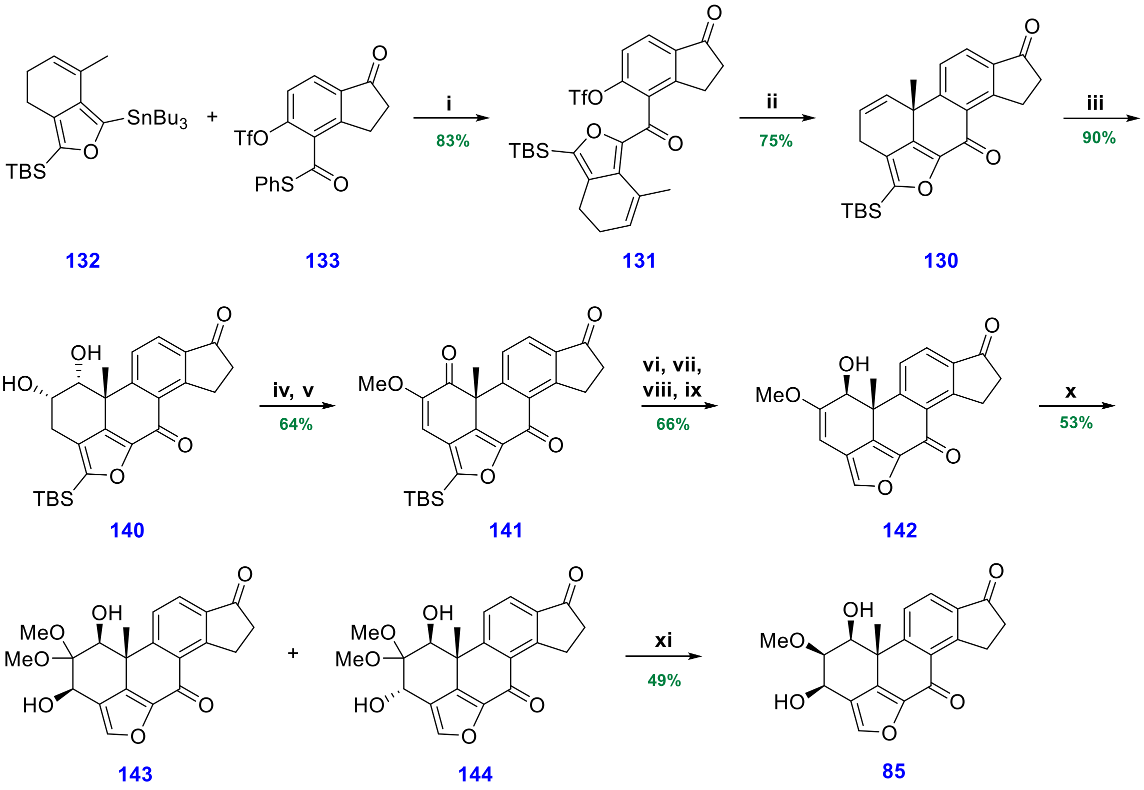
Both fragments **132** and **133** were accessed in seven steps, following literature precedent (***Scheme 0.36***). 2,6-Hydroxybenzoic acid **134** was protected as the cyclic ester, triflated, and alkenylated to give **135**. Deprotection of the benzyl group and subsequent Friedel–Crafts acylation formed indanone **136**, which underwent sequential thioesterification and triflation to give **133** in 27% overall yield. Cross-coupling of protected, silylated **137** afforded **138**, which was chlorinated on the alcohol and allylated to **139**. Ring closing metathesis furnished the bicycle and stannylation gave completed fragment **132** in 37% yield.



Scheme 0.36: *Synthesis of reported fragments* ***132*** *and* ***133****.* **Reagents and conditions: i)** acetone, $SOCl_2$, DMAP, DME, 20 °C; **ii)** Tf_2O, Py, 0 °C; **iii)** benzyl acrylate, $Pd(OAc)_2$, PPh_3, K_2CO_3, Me_4NBr, DMF, 100 °C; **iv)** H_2, Pd/C, 1:1 EtOAc/MeOH, rt; **v)** $ClSO_3H$, 0 °C; **vi)** $(PhS)_2$, Bu_3P, DCM, rt; **vii)** Tf_2O, Py, DCM, 0 °C; **viii)** TBSCl, imid, DMF, rt; **ix)** BuLi, HMPA, THF, –78 °C; **x)** BuLi, DME, –78 °C, then $B(OMe)_3$, rt, then $Pd(PPh_3)_4$, 2-bromopropene, 70 °C; **xi)** MsCl, TEA, DCM, 0 °C; **xii)** allylMgCl, THF, Δ; **xiii)** Grubbs II, DCM, Δ; **xiv)** BuLi, THF, –78 °C, then Me_3SnCl, 0 °C.

Assembly of these fragments began with a Liebeskind stannane-thioester coupling which afforded **131** in high yield (***Scheme 0.37***). The crucial enantioselective Heck reaction to construct the pentacycle likewise proceeded in high yield and **130** was obtained in >99% ee. A number of redox transformations were required to install the correct oxygenated functionality. Upjohn dihydroxylation of the A-ring alkene resulted in α-face functionalisation to give **140**, which was immediately subjected to double Swern oxidation to give the diketone. The tautomer of this 1,2-diketone was quickly trapped by methylation (**141**), providing an indirect means of oxidising the otherwise inaccessible

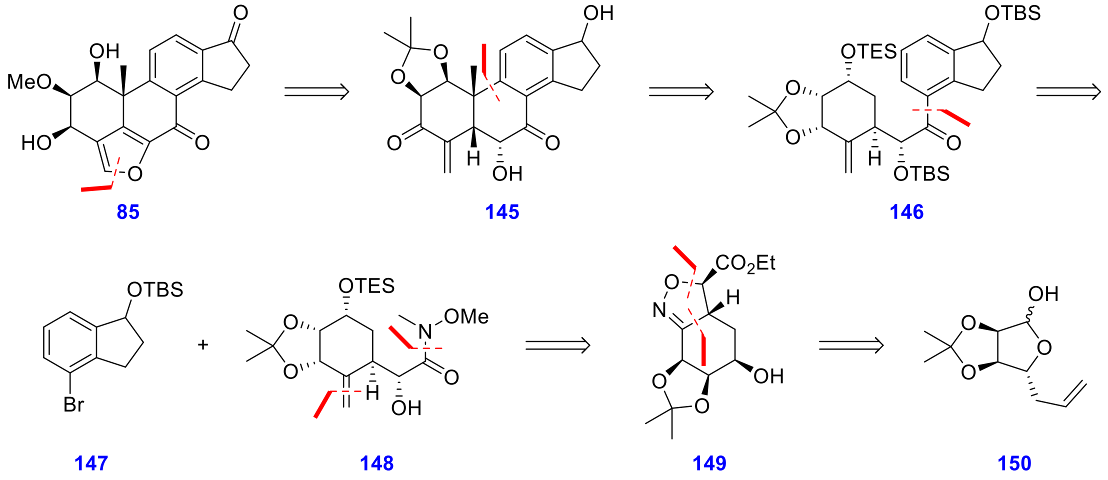
C-3 position. Removal of the furylic TBS group followed, then another three-step sequence to set the stereochemistry of the C-3 hydroxyl group: the D-ring ketone was first protected with TBSOTf, the A ring ketone selectively reduced with borane, and the D-ring ketone deprotected to give **142**. An alkene epoxidation/reduction sequence was next applied to give a 1:2 mixture of **143** and **144** which was separated and mono-demethylated to give (–)-viridiol **85** in 1.6% overall yield.



Scheme 0.37: *Completion and functionalisation of the pentacycle.* **Reagents and conditions: i)** PdCl$_2$(MeCN)$_2$, TFP, CuOP(O)Ph$_2$, DMF, rt; **ii)** Pd(OAc)$_2$, (*S*)-*t*-Bu PHOX, PMP, tol, µw, 175 °C; **iii)** OsO$_4$, NMO, acetone, rt; **iv)** DMSO, TFAA, DCM, –60 °C, then DIPEA, then AcOH, rt; **v)** KHMDS, Me$_2$SO$_4$, THF, 0 °C; **vi)** 1:1 TFA/MeCN, rt; **vii)** TBSOTf, 2,6-lut, DCM, –30 °C; **viii)** BH$_3$·THF, –30 °C; **ix)** TEA·3HF, –30 °C; **x)** AcOOH, MeOH, rt; **xi)** TMSOTf, BH$_3$·SMe$_2$, DCM, –30 °C, then AcOH, Py, TEA·3HF.

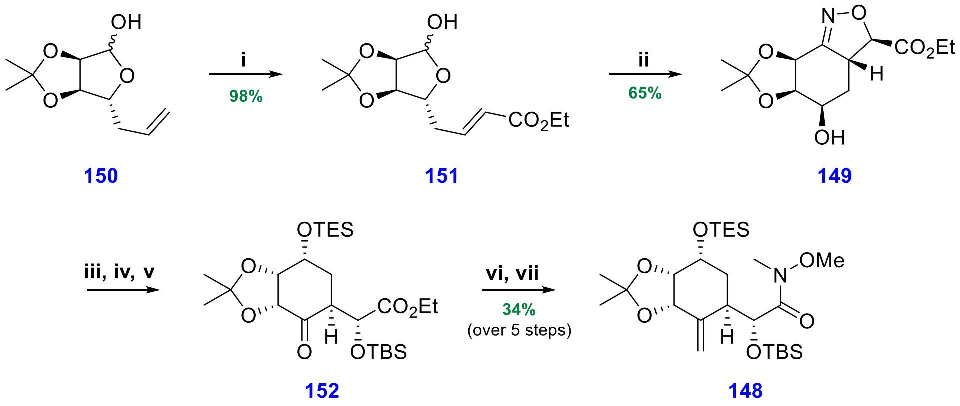
Gao *et al.* (2019)

A complementary approach was reported in 2019 by Gao *et al*., likewise achieving an enantioselective synthesis in 18 steps.[163] Starting from commercially available L-ribose, the challenging oxidised functionality was installed at the beginning of the synthesis. The key retrosynthetic disconnections are outlined in ***Scheme 0.38***, including a cobalt-catalysed metal hydride H-atom transfer to form the tetracyclic core, and a nitrile oxide-alkene cycloaddition to furnish the A-ring.



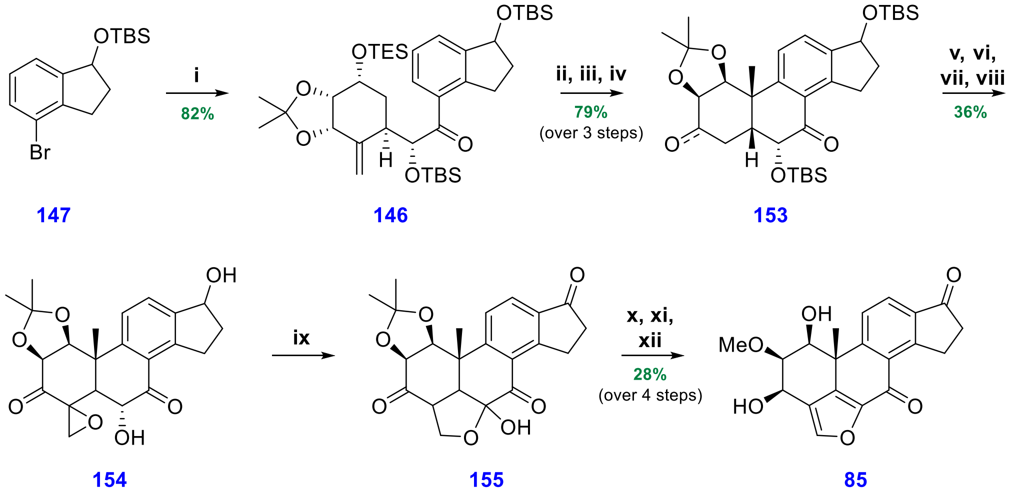
Scheme 0.38: *Gao's retrosynthetic analysis.*

The synthesis began with cross metathesis of ribose derivative **150**, prepared in three steps from L-ribose, to afford unsaturated ester **151** (***Scheme 0.39***). Treatment with hydroxylamine gave the oxime which was oxidised to the nitrile oxide which underwent intramolecular [3+2] cycloaddition with the alkene to give isoxazoline **149**. Protection, then reductive cleavage of this heterocycle yielded **152**, which was protected, then homologated, and converted to Weinreb amide **148**.



Scheme 0.39: *Synthesis of fragment* ***148****.* **Reagents and conditions: i)** ethyl acrylate, Grubbs II, CuI, Et_2O, 35 °C; **ii)** $NH_2OH \cdot HCl$, $NaHCO_3$, EtOH, then chloramine-T, 0 °C; **iii)** TESOTf, TEA, THF, –78 °C; **iv)** Raney Ni, $B(OMe)_3$, MeOH, H_2O, –78 °C; **v)** TBSOTf, 2,6-lut, THF, –78 °C; **vi)** $Ph_3P{=}CH_2$, tol, rt; **vii)** DMHH, *i*-PrMgCl, THF, –20 °C.

Bromide **147**, likewise prepared according to literature precedent, was lithiated and added into **148** to give cyclisation precursor **146** (***Scheme 0.40***). Upon treatment with Co(Salen$^{t\text{-}\mathrm{Bu},t\text{-}\mathrm{Bu}}$), **146** underwent the desired MHAT reaction, proceeding with precise steric control to give **153**, following deprotection and oxidation. Homologation at C-4, followed by TBS deprotection and epoxidation afforded **154**. Treatment of **154** with DMP oxidised both the C-3 and C-17 alcohols, with the enol of C-6 promoting epoxide opening to form pentacycle **155**. Deprotection and methylation gave viridin **84**, which could be selectively reduced at C-3 to give viridiol **85**.



Scheme 0.40: *Total synthesis of viridiol* ***85***. **Reagents and conditions: i) 148**, *t*-BuLi, Et_2O, –78 °C; **ii)** Co(Salen$^{t\text{-}Bu\textit{t}\text{-}Bu}$)Cl, $PhSiH_3$, acetone, 35 °C; **iii)** Dowex 50W, MeOH, 0 °C; **iv)** DMP, DCM, rt; **v)** L-proline, CH_2O, THF, 60 °C; **vi)** HF·Pyr, THF, 35 °C; **vii)** TMSOTf, TEA, THF, –78 °C; **viii)** *t*-BuO_2Li, THF, –10 °C; **ix)** DMP, DCM, 0 °C; **x)** H_2SO_4, MeCN, 35 °C; **xi)** Me_3OBF_4, DCM, rt, then H_2O, $TMSCHN_2$, 0°C; **xii)** $NaBH_4$, EtOH/DCM, –10 °C.

Project Aims

Viridiol's demonstrated phytotoxicity is of interest to Syngenta, although concerns regarding both generalised mammalian toxicity and product instability have limited its industrial applications thus far. Likewise, the documented therapeutic value of the ABE scaffold in wortmannin and hibiscone C makes further biological studies on viridiol very appealing. As such, access to viridiol and related analogues is highly desirable.

Engineered mutation of wild-type enzymes has already demonstrated a phenomenal capacity for enhancing both the selectivity and reactivity of cytochrome P450s. The potential to accept non-natural organic substrates, coupled with the mild reaction conditions and ability to effect kinetic resolution, has made enzymatic functionalisation a powerful synthetic tool. However, screening of more complex natural product-like substrates is necessary to build the comprehensive reactivity profile required to develop predictive tools which could streamline total synthesis further.

The aims of this project are therefore two-fold:

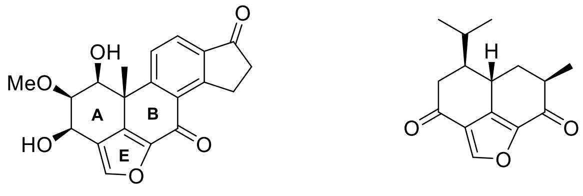
1. To synthesise carbon-rich cores relating to viridiol;
2. To effect enzymatic oxidation of these viridiol-like substrates to produce varied analogues and reactivity data.

To this end, two complementary synthetic approaches to the carbon skeleton of viridiol were envisaged, aiming to maximise the diversity of substrates available for subsequent enzymatic screening.

Chapter 1

Approaches to the ABE System of Viridiol

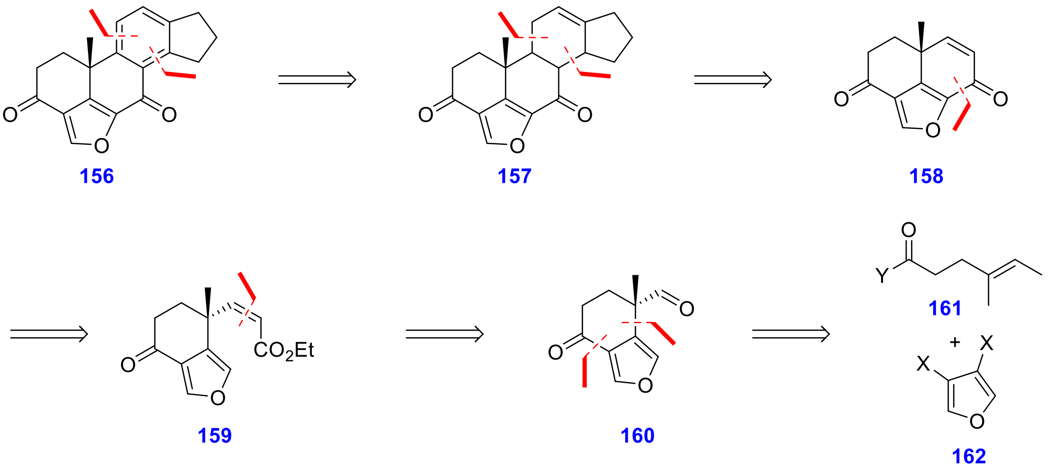
The first route aimed to construct the pentacyclic core of viridiol **85** around the furan unit, proceeding *via* tricycle **158**, similar in structure to the known bioactive hibiscone C **93** (***Figure 1.01***). It was anticipated that diketone **158** and subsequent intermediates would be suitable substrates for enzymatic screening.



viridiol **85** hibiscone C **93**

Figure 1.01: *Viridiol and hibiscone C.*

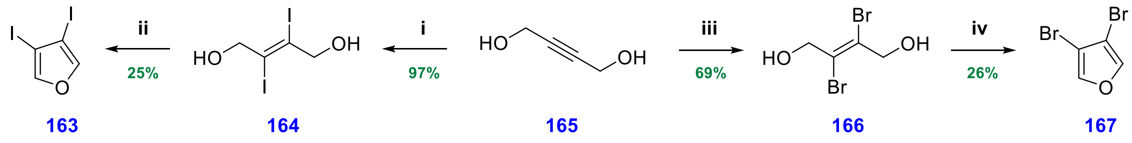
Pentacycle **156**, envisaged as the endpoint of the synthetic route, would be obtained by dehydrogenation of **157** (***Scheme 1.01***). Completion of the carbon skeleton was to be achieved by Diels–Alder reaction of vinyl cyclopentene with tricycle **158**, which was planned to arise from intramolecular Friedel–Crafts acylation of ester **159**. This, in turn, would be obtained from Still–Gennari reaction or an equivalent process from known intermediate **160**, accessible from **161** and **162**.[164]



Scheme 1.01: *Planned retrosynthetic route towards pentacyclic core* ***156****.*

Preliminary work on this route had already been undertaken within the group, reproducing the initial reported steps to aldehyde **160**.[*] In the course of this investigation, problems of reaction reproducibility and poor yields were quickly identified so subsequent work aimed to improve upon this synthesis.

The sequence commenced with the synthesis of diiodofuran **163**, in two steps from 1,4-butyne diol **165** (***Scheme 1.02***).[165] Iodination of the triple bond was straightforward, proceeding in almost quantitative yield; however, the reported oxidative cyclisation to **163** was plagued with difficulties.



Scheme 1.02: *Synthesis of halogenated furans* ***163*** *and* ***167****.* **Reagents and conditions: i)** I_2, H_2O, 70 °C, 2.5 h; **ii)** $K_2Cr_2O_7$, H_2SO_4, H_2O, NMP, heptane, 85 °C, 1 h; **iii)** Br_2, H_2O, 0 °C, 2 h; **iv)** $K_2Cr_2O_7$, H_2SO_4, H_2O, 120 °C, 2h.

The initial *E*-configuration of the double bond prevents trapping by the distal alcohol of the aldehyde generated *in situ* and an equilibrium amount of the *Z*-alkene must be produced before the reaction can proceed to cyclic intermediates. As a result, the reaction creates an unavoidable cocktail of over-oxidation products, leading to consistently low

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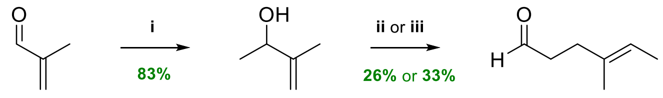
[*] Work undertaken by visiting student Anna Nikitjuka 2016.

yields. Furthermore, this process was found to be scale dependent, with small-scale iterations suffering far inferior yields. Although numerous practical alterations did improve the yield slightly, the complex experimental set-up, coupled with the instability of the product made a more robust alternative desirable and dibromofuran **167** was instead prepared. Whilst the yield of dibromide **167** was not significantly different from diiodide **163**, the greater experimental ease, especially as regarded the isolation of the product, made this the preferred substrate.

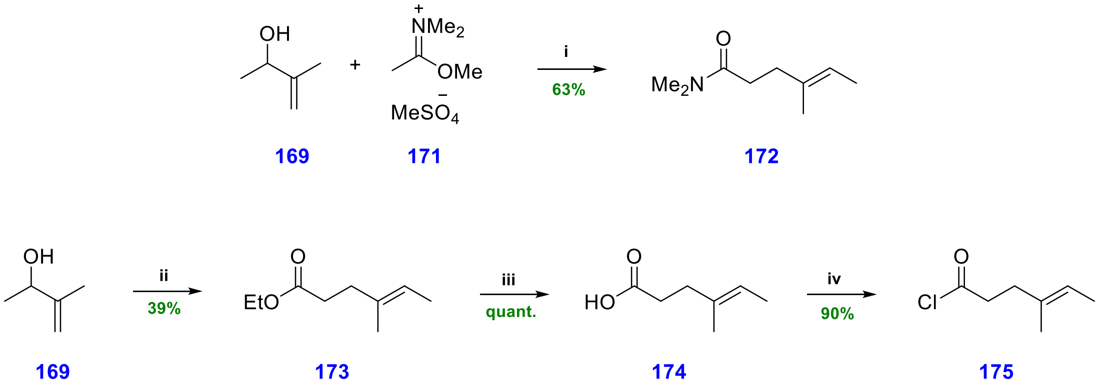
The synthesis of aldehyde **170** is summarised in ***Scheme 1.03***. Addition of methylmagnesium bromide to methacrolein afforded alcohol **169** in 83% yield, and subsequent Claisen rearrangement in the presence of butyl vinyl ether and $Hg(OAc)_2$ as catalyst gave aldehyde **170**.[166] Although clearly visible in the ^{1}H NMR spectrum of the crude product, this volatile compound could not be efficiently freed from the excess of butyl vinyl ether, and a low yield (26%) resulted. An alternative procedure was tested, repeating the Claisen rearrangement with a palladium phenanthroline complex and the higher-boiling triethylene glycol divinyl ether to facilitate isolation of the product aldehyde by direct distillation.[167] Whilst the reaction appeared efficient by ^{1}H NMR analysis, cleanly converting alcohol **169** into the desired aldehyde, isolation of this product remained a problem. Despite use of a cold trap during distillation, significant product losses were still incurred and **170** was obtained in 33% yield.



168 **169** **170**

Scheme 1.03: *Synthesis of aldehyde* ***170****.* **Reagents and conditions: i)** MeMgBr, Et_2O, 0 °C, 6 h; **ii)** butyl vinyl ether, $Hg(OAc)_2$, 110 °C, 20 h; **iii)** 5 mol% $Pd(phen)(tfa)_2$, DIPEA, DVE3, 120 °C, 4 days.

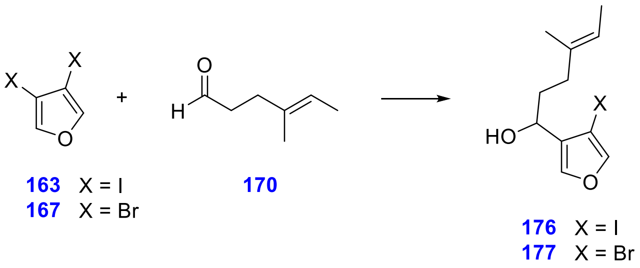
As a result of these disappointing yields, the less volatile acceptors amide **172** and acid chloride **175** were prepared, which were intended to also confer the advantage of producing the more stable furyl ketone directly in the addition step (***Scheme 1.04***). Dimethyl acetamide and dimethyl sulfate were heated together to give iminium salt **171** which was employed in an Eschenmoser–Claisen rearrangement with alcohol **169** to afford amide **172** in 63% yield.[168] Separately, alcohol **169** was subjected to Johnson–Claisen rearrangement conditions to give ester **173** which was hydrolysed to acid **174**, then treated with thionyl chloride and pyridine to give acid chloride **175** in 29% overall yield.[169]



Scheme 1.04: *Synthesis of amide* ***172*** *and chloride* ***175***. **Reagents and conditions: i)** MeLi, THF, 70 °C, 18 h; **ii)** triethyl orthoacetate, AcOH, 150 °C, 3 days; **iii)** NaOH, EtOH, Δ, 18 h; **iv)** thionyl chloride, pyridine, Et_2O, 0 °C, 3.5 h.

The union of fragments **163** and **167** with **170** proved far more troublesome than anticipated (***Table 1.01***). Lithiation of 3,4-dihalogenated furans is reported,[170] as is their addition into both aldehydes[171] and amides,[172] and precedent for the addition of lithiated furan **163** into aldehyde **170** reports a 62% yield of furyl alcohol **176**.[164] Exact reproduction of these conditions with both dibromo- **167** and diiodofuran **163** returned only starting furan (*entries **1** and **2***), indicating that halogen-metal exchange had not taken place. Alternative sources of butyllithium were trialled as variability in additives and salts between batches is reported to affect reaction outcomes[173] but neither *t*-butyllithium (*entry **3***), nor fresh *n*-butyllithium (*entry **4***) were successful. A different result was obtained when the solvent source was changed: use of ether stored over activated 3 Å molecular sieves for 24 h resulted in decomposition, which, whilst not useful, indicated that a change of solvent source was one factor affecting the course of the reaction (*entry **5***).

Table 1.01: *Addition of lithiated furans into aldehyde **170**.*



163 X = I
167 X = Br

170

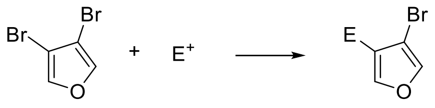
176 X = I
177 X = Br

Entry	Lithiating agent	Outcome
1[a]	*n*-BuLi	**163** only
2	*n*-BuLi	**167** only
3	*t*-BuLi	**167** only
4	*n*-BuLi (fresh)[c]	**167** only
5[b]	*n*-BuLi (fresh)[c]	Decomposition

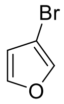
Reagents and conditions: ***167****, 1 eq 2.5 M BuLi, Et_2O, –78 °C, then 1 eq* ***170****.* [a] ***163****.* [b] *Dried over 3 Å molecular sieves for 24 h.* [c] *Titrated concentration 2.36 M.*

At this stage, use of aldehyde **170** was suspended until successful lithiation could be confirmed (***Table 1.02***) and inexpensive test electrophiles were used instead. Quenching of the reaction in ether with NH_4Cl resulted in decomposition but for the reaction in freshly-distilled THF, the first signs of product were evident by ^{1}H NMR analysis of the crude reaction mixture (*entries **1** and **2***). These conditions were repeated using a simple model aldehyde (isovaleraldehyde), then using aldehyde **170**, each giving low but isolable yields of expected products. These yields were improved by a slight modification to the work-up, including a $NaHCO_3$ wash to remove residual acidic impurities in order to prolong the life of the unstable furylic alcohol product. A model acid chloride **183** and amide **182** (see ***Table 1.02***) likewise were successful electrophiles, although yields were uniformly low (*entries **7** and **8***).

Table 1.02: *Addition of lithiated* ***167*** *into various acceptors.*



167



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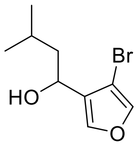

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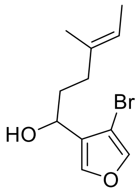

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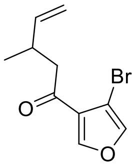

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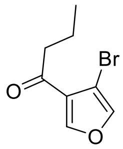


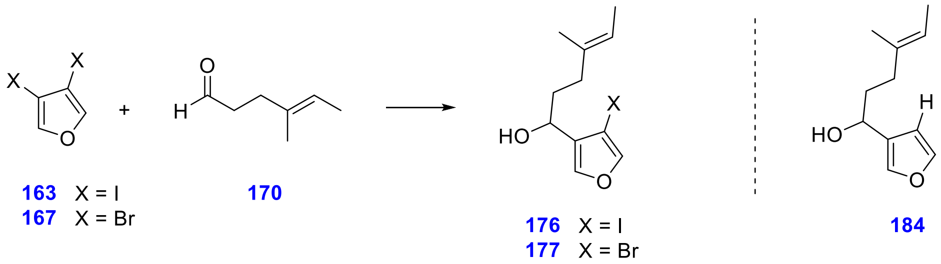
181

Entry	E+	Products
1[a]	NH_4Cl	Decomposition
2	NH_4Cl	**178** evident[c]
3	Isovaleraldehyde	37% **179**
4	**170**	11% **177**
5[b]	Isovaleraldehyde	46% **179**
6[b]	**170**	19% **177**
7[b]	*N*,*N*-Dimethyl 3-methylpent-4-enamide **182**	21% **180**
8[b]	Butyryl chloride **183**	10% **181**

Reagents and conditions: *1 eq 2.36 M n-BuLi, THF, –78 °C, then 1 eq acceptor.* [a] *Et_2O.* [b] *Modified work-up applied.* [c] *By ^1H NMR analysis of crude product.*

The ~20% yield of alcohol **177** obtained in *entry* ***6*** was encouraging. Iodofuran **163** is expected to more readily undergo halogen-metal exchange due to the weaker C-I bond so an improvement in yield was expected with this substrate. As butyllithium is known to behave differently in THF and diethyl ether, it was also desirable to repeat this reaction with a new source of ether.[174] Therefore, immediately prior to reaction, the requisite quantity of diethyl ether was distilled from CaH_2 to ensure dryness which it was hoped would result in a superior yield. In practice, neither modification improved the yield of the expected alcohol product (***Table 1.03***); the yields of furyl alcohol were higher for the bromo- substrate and, in both cases, the yields in ether were not significantly greater than those in THF.

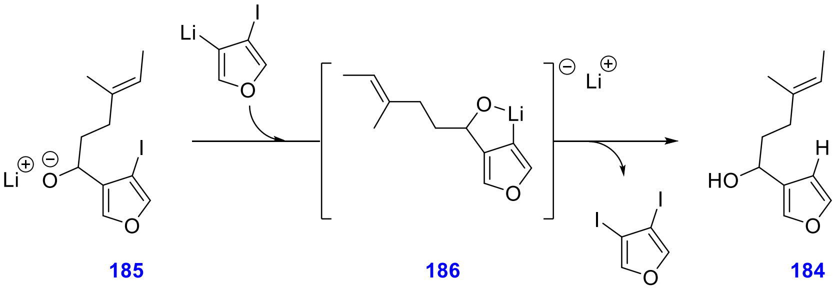
Table 1.03: *Screening of solvents for the lithiation and trapping of furans* ***163*** *and* ***167****.*



Entry	Furan	Solvent	176 or 177	184
1	**167**	Et_2O	16%	5%
2	**167**	THF	19%	—
3	**163**	Et_2O	12%	76%
4	**163**	THF	9%	—

Reagents and conditions: *1.0 eq 2.36 M BuLi, solvent, –78 °C, then 1.0 eq* ***170****.*

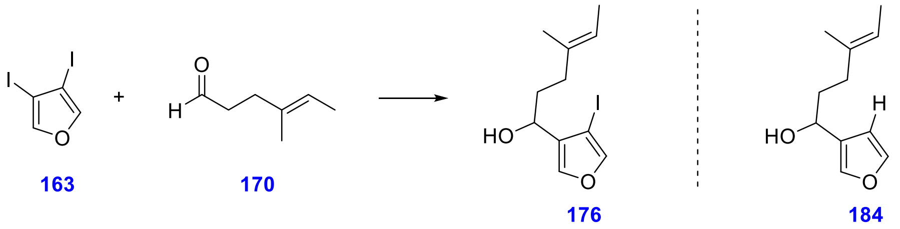
Isolation of side-product **184** suggested the operation of a competing reaction pathway, outlined in ***Scheme 1.05***. The furyl alkoxide intermediate is also able to undergo lithiation, likely facilitated by coordination with the alkoxide substituent, and the so-formed intermediate is quenched under the reaction conditions to give protonated product **184**. This proposed pathway suggests that the greater stability of **186** enables a further halogen-metal exchange with the simple lithiated furan, which is probably lost during the work-up as the volatile halogenated furan.



Scheme 1.05: *Proposed side reaction leading to* ***184****.*

Suppression of this side reaction requires a minimum concentration of furyllithium in the presence of the forming product. This was validated by reaction with 0.5 equivalents of butyllithium; formation of dehalogenated product **184** was virtually eliminated and the yield of the desired product **176** almost doubled (***Table 1.04***, *entry* ***2***). It was hoped that dilution of the aldehyde solution prior to addition to the furyllithium would constitute a sufficient reduction in the concentration but the reaction gave very poor mass returns, possibly owing to decomposition over the longer reaction time necessary for the greater volume of solution to be added (*entry* ***3***). Gratifyingly, inverse addition of the lithiated furan to the aldehyde solution did result in a noticeable and, more importantly, reproducible improvement (*entry* ***4***).

Table 1.04: *Attempts to suppress the formation of* ***184****.*



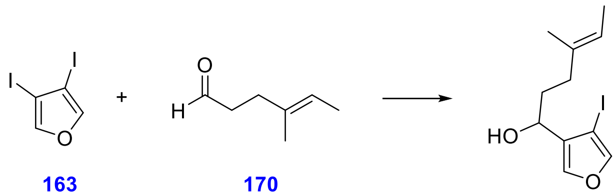
Entry	Method	176	184
1	—	12%	76%
2	0.5 eq BuLi	22%	Trace
3	Dilution of **170**	2%	6%
4	Inverse addition	29%	6%

Reagents and conditions: *1.0 eq 2.36 M n-BuLi, Et$_2$O, –78 °C, then 1.0 eq* ***170****.*

After extensive optimisation work, concerns remained around the disparity between literature and practical results for this reaction and the Trauner group, who had originally reported this sequence in 2003, were therefore contacted for additional insight. Whilst they had not observed formation of dehalogenated product **184**, the group experienced significant difficulty with diiodofuran **163**. Aside from the poor yield and challenge of handling, the group reported that even trace amounts of NMP remaining in the purified sample of diiodofuran **163** were sufficient to completely disrupt lithiation. As lithiation had now been established and no evidence of NMP could be detected by ^{1}H NMR spectroscopy, this issue could be dismissed. Additionally, Trauner *et al.* indicated that the reaction was extremely sensitive to substrate, and that all components – the furyl alcohol product, the diiodofuran, and the aldehyde – were unstable. Variations of both reaction time and temperature were attempted but all deviations from the optimised conditions resulted in decomposition or inferior yields.

The only remaining difference between this work and that of Trauner was the scale. In the work described so far, all iterations were carried out using 300 mg of starting dihalide whereas, in contrast, the Trauner procedure is described for reaction on a scale of 13.6 g of diiodide. Consequently, the reaction was trialled at a number of different scales in the hope that scale dependency was the dominant factor affecting yield (***Table 1.05***). Whilst this survey did not show the expected trend, it demonstrated that 500 mg was consistently the most efficient and all further reactions were run at this scale (*entry* ***2***).

Table 1.05: *Iterations at different scales.*



163

170

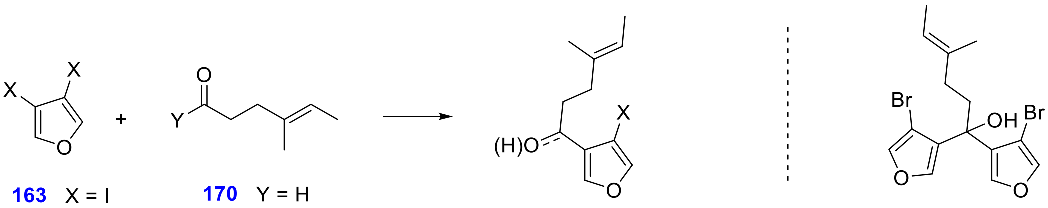
176

Entry	Scale (g)	176
1	0.3	29%
2	0.5	40%
3	1.5	27%
4	3.0	11%
5[a]	13.6	62%

Reagents and conditions: *1.0 eq 2.36 M n-BuLi, Et$_2$O, –78 °C, added to 1.0 eq* ***170****.* [a] *Result reported by Trauner et al.*[164]

Ultimately, it was disappointing that, when these modifications were applied to amide **172**, only amide **172** was returned (*entries* ***2*** *and* ***5***), despite prior evidence that amides were compatible with this process (***Table 1.06***). Chloride **175** was also unsuccessful, decomposing with **163** (*entry 3*) and over-reacting to give the double addition product **189** with **167** (*entry 6*). Therefore 40% and 27% had to be accepted as the highest achievable yields for this process in our hands (*entries **1** and **4***), and efforts turned to advancing the synthesis.

Table 1.06: *Screening of different acceptors.*



163 X = I
167 X = Br

170 Y = H
172 Y = NMe_2
175 Y = Cl

176 OH, X = I
177 OH, X = Br

187 =O, X = I
188 =O, X = Br

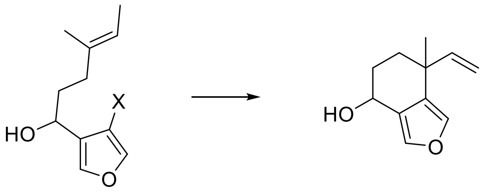
189

Entry	Furan	Acceptor	Outcome
1	**163**	**170**	40% **176**
2	**163**	**172**	**172** only
3	**163**	**175**	Decomposition
4	**167**	**170**	27% **177**
5	**167**	**172**	**172** only
6	**167**	**175**	27% **189**

Reagents and conditions: *1.0 eq 2.36 M BuLi, Et_2O, –78 °C, added to 1.0 eq acceptor.*

Both alkenes **176** and **177** were subjected to the conditions reported for the Heck cyclisation (***Table 1.07***).[164] The iodo-substrate **176** readily cyclised to give desired bicycle **190** in 77% yield as a 4:1 mixture of inseparable diastereomers. However, the bromo analogue **177** proved a more challenging substrate, requiring addition of a ligand to react at all.

***Table 1.07:** Heck cyclisation of furyl alcohols.*



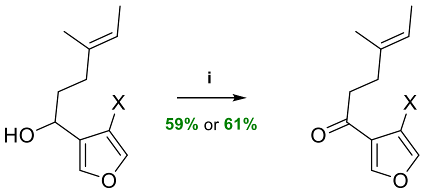
176 X = I
177 X = Br

190

Entry	Substrate	Deviation from reported conditions	Outcome
1	**176**	—	77% **190**
2	**177**	—	**SM**
3	**177**	5 mol% PPh_3	23% **190**
4	**177**	1.0 eq AgOAc	**SM**

***Reagents and conditions:** 5 mol% $Pd(OAc)_2$, 3.0 eq TBAB, 3.0 eq TEA, MeCN, H_2O, Δ.*

The analogous furyl ketones were expected to be more reactive substrates for the Pd oxidative insertion step so alcohols **176** and **177** were both oxidised to their respective ketones with Dess–Martin periodinane in 59% and 61% yields, respectively (***Scheme 1.06***).



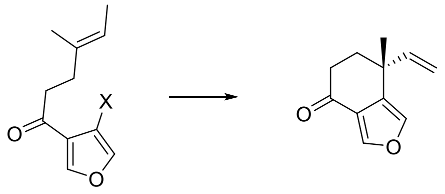
176 X = I
177 X = Br

187 X = I
188 X = Br

***Scheme 1.06:** Oxidation of alcohol products **176** and **177**.* **Reagents and conditions: i)** DMP, DCM, 0 °C, 2 h.

No improvement was observed for the bromo- substrate **188** but the iodo- variant **187** cyclised in quantitative yield to give furanocyclohexanone **191** (***Table 1.08**, entry **1***).

Table 1.08: *Heck cyclisation of furyl ketones.*



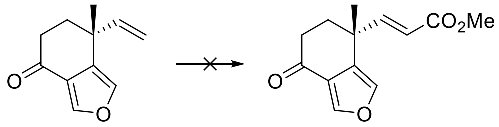
187 X = I
188 X = Br

191

Entry	Substrate	Deviation from reported conditions	Outcome
1	**187**	—	Quant. **191**
2	**188**	—	**SM**
3	**188**	5 mol% PPh_3	<5% **191**

Reagents and conditions: *5 mol% $Pd(OAc)_2$, 3.0 eq TBAB, 3.0 eq TEA, MeCN, H_2O, Δ.*

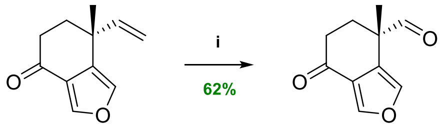
Previously, an attempted cross metathesis of olefin **191** with methyl acrylate had returned only starting material so an alternative strategy to elaborate this side chain was necessary (***Scheme 1.07***).*



191 **192**

Scheme 1.07: *Attempted cross metathesis with olefin* ***191****.*

Olefin **191** was oxidised and cleaved with OsO_4 and $NaIO_4$ to give aldehyde **160** in 62% yield (***Scheme 1.08***).



191 **160**

Scheme 1.08: *Formation of aldehyde* ***160****.* **Reagents and conditions: i)** OsO_4, $NaIO_4$, Py, H_2O, dioxane, rt, 18 h.

A Horner–Wadsworth–Emmons reaction was therefore proposed, successfully forming *E*-ester **192** in quantitative yield (***Scheme 1.09***). Hydrolysis of the ester revealed acid **193**

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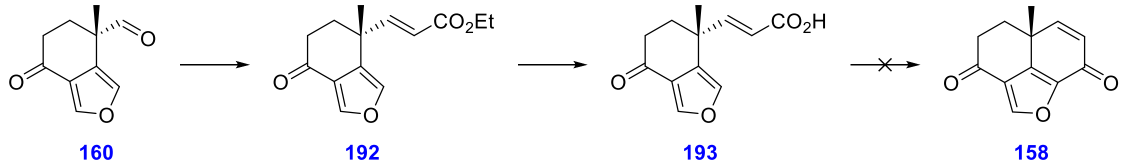
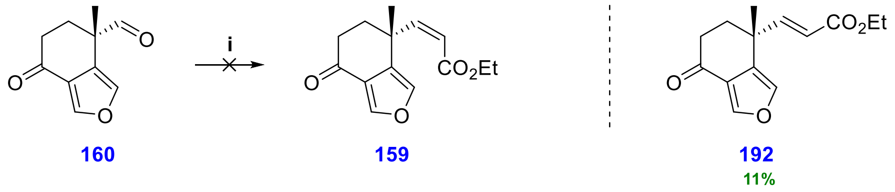
* Cross metathesis preliminary study performed by Anna Nikitjuka.

which was treated with trifluoroacetic anhydride to effect cyclisation. However, only acid **193** was returned and it was presumed that the *Z*- double bond geometry, necessary for cyclisation, was not accessible under the reaction conditions.*



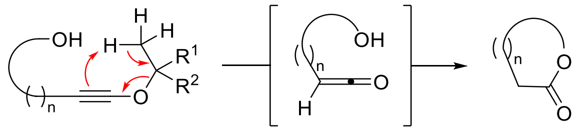
Scheme 1.09: *Attempted elaboration and cyclisation of aldehyde **160**.*

Aldehyde **160** was instead subjected to the conditions reported for Still–Gennari olefination (***Scheme 1.10***). It was disappointing to find that aldehyde **160** proved reluctant to react, likely due to steric hindrance, and only the *E*- isomer **192** was isolated in low yields. As only one such reaction on a tri-*α*-substituted aldehyde has been reported in the literature, an alternative strategy with a stronger precedent was sought.[175]



Scheme 1.10: *Attempted Still–Gennari olefination of aldehyde **160**.* **Reagents and conditions: i)** triethylphosphonoacetate, 18-Crown-6, KHMDS, THF, –78 °C, 30 min.

In 1993 MaGee *et al*. reported the thermolysis of ethoxyacetylenes to ketenes and their trapping *in situ* by an attached hydroxyl group to form lactones of varying sizes (***Scheme 1.11***).[176]



Scheme 1.11: *MaGee's lactonisation reaction via retro-ene reaction of ethoxyacetylenes.*

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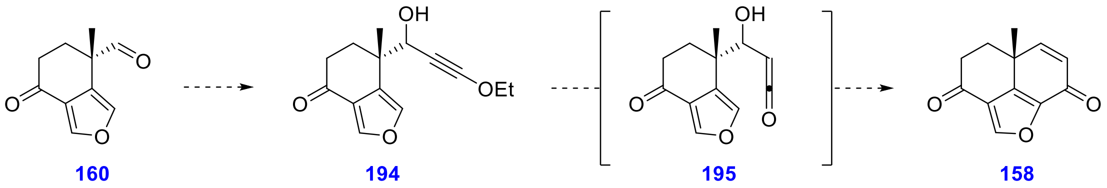
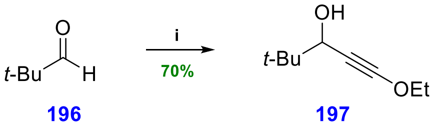
* Horner–Wadsworth–Emmons reaction performed by Anna Nikitjuka.

It was proposed that such a ketene, when tethered to a furanocyclohexane system, would be trapped by the furan to form tricycle **158** (***Scheme 1.12***) after β-elimination from the enol(ate) intermediate. The requisite ethoxyacetylene derivative **194** would, in turn, be accessed from aldehyde **160.**



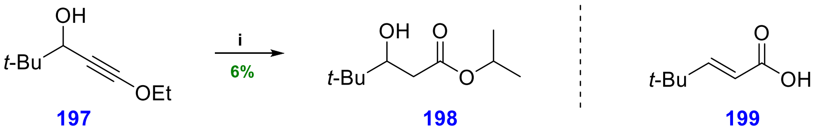
Scheme 1.12: *Proposed route to tricycle **158**.*

Owing to the difficulty of obtaining aldehyde **160**, this strategy was first tested on a model system: pivalaldehyde, a cheap, commercially available tri-α-substituted aldehyde. Ethoxy acetylene was deprotonated with butyllithium and pivalaldehyde added dropwise to give alkynyl alcohol **197** in 70% yield (***Scheme 1.13***).[177]



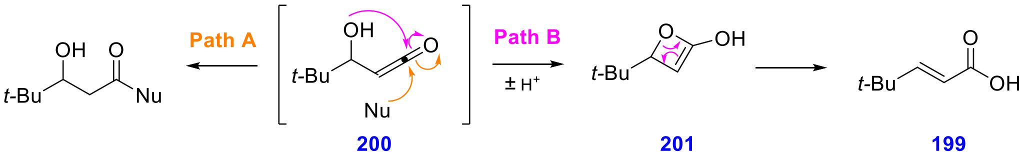
Scheme 1.13: *Preparation of alkynyl alcohol **197**.* **Reagents and conditions: i)** *n*-BuLi, ethoxyacetylene, THF, –78 °C, 7 h.

This alcohol was heated to 150 °C in xylene with the addition of two equivalents of isopropanol to act as an external nucleophile (***Scheme 1.14***). Traces of expected isopropylester **198** were observed by ^{1}H NMR analysis but the major product was unsaturated acid **199** (***Scheme 1.15***, ***Table 1.09***, *entry **1***).



Scheme 1.14: *Attempted trapping of the in-situ generated ketene intermediate with isopropanol.* **Reagents and conditions: i)** isopropanol, xylene, 150 °C, 2 h.

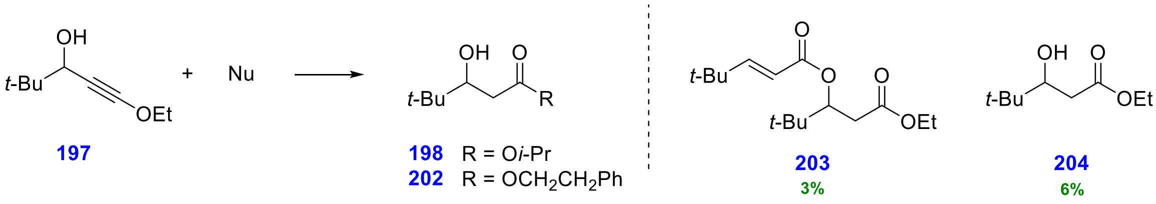
This acid is presumed to arise from trapping of the ketene by the proximal alcohol to form strained β-lactone enol **201** which undergoes thermal electrocyclic ring-opening under the reaction conditions (Path **B**, ***Scheme 1.15***). Although the use of two equivalents of isopropanol was expected to be a sufficient excess to favour Path **A**, it is likely that the relatively low boiling point of isopropanol led to significant loss of the external nucleophile, leading to Path **B** dominating.



Scheme 1.15: *Competing pathways to trap ketene* ***200****.*

On this basis, it was hoped that use of a non-volatile external nucleophile would maintain the required excess to favour Path **A** (***Table 1.09***). Gratifyingly, this theory was validated by the doubling of yield observed when phenethyl alcohol (bp 225 °C) was applied as the external nucleophile (*entry* ***2***). Whilst this represented a significant improvement over that obtained with (volatile) isopropanol (*entry* ***1***), the 11% yield remained too low to be synthetically useful and a comparable amount (14%) of the undesired acid side product **199** was still produced. As 76% of the phenethyl alcohol was returned unreacted, it was concluded that uptake of the nucleophile remained a problem. Application of benzofuran as both the solvent and the nucleophile was expected to overcome this limitation but no benzofuran incorporation was observed and the reaction returned predominantly acid side product **199** (*entry* ***3***). The two other products isolated from this reaction, esters **203** and **204**, indicated an acid-mediated process was competing with the desired reaction pathway. In response to this, the reaction with phenethyl alcohol was repeated, once with base-washed glassware (*entry* ***4***), and once with the addition of 20 mol% K_2CO_3 (*entry* ***5***), but in neither case did the yield of the desired ester improve.

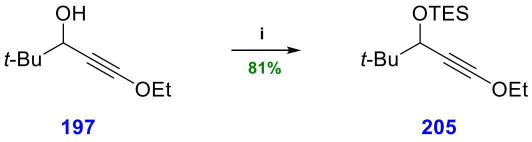
Table 1.09: *Attempted trapping of the in-situ generated ketene with external nucleophiles.*



Entry	Nu	Expected Product	Acid 199	Other Products
1	Isopropanol	6%	11%	—
2	Phenethyl alcohol	11%	14%	76% Nu
3[a]	Benzofuran	—	82%	**203** and **204**
4[b]	Phenethyl alcohol	8%	13%	67% Nu
5[c]	Phenethyl alcohol	8%	9%	64% Nu

Reagents and conditions: *xylene, 150 °C, 2 h.* [a]*Reaction neat in benzofuran.* [b]*Base-washed glassware.* [c]*20 mol% K_2CO_3.*

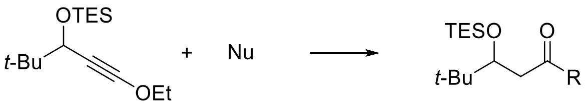
As the hydroxyl group of **197** was consistently problematic, its protection with chlorotriethylsilane and imidazole in DMF was conducted to give new substrate **205** in 81% yield (***Scheme 1.16***).



Scheme 1.16: *Protection of alkynyl alcohol **197**.* **Reagents and conditions: i)** chlorotriethylsilane, imidazole, DMF, 0 °C, 2 h.

As expected, subjecting this substrate to 150 °C in xylene containing phenethyl alcohol did not lead to formation of acid **199** (***Table 1.10***, *entry **1***). However, productive reaction was also inhibited, and product ester **206** was afforded in only 5% yield. Repetition with benzofuran yielded a complex mixture and it was concluded that the silyl group was interfering with the desired reactivity (*entry **2***). As multiple attempts at this thermolytic strategy had proven unpromising, this approach was discarded and efforts directed elsewhere.

***Table 1.10:** Attempted trapping of the in-situ generated ketene intermediate with protected substrate **205**.*



205

206 R = OCH_2CH_2Ph

Entry	Nu	Expected Product	Acid 199	Other Products
1	Phenethyl alcohol	5%	—	38% Nu
2[a]	Benzofuran	—	—	—

***Reagents and conditions:** xylene, 150 °C, 2 h. [a]Reaction neat in benzofuran.*

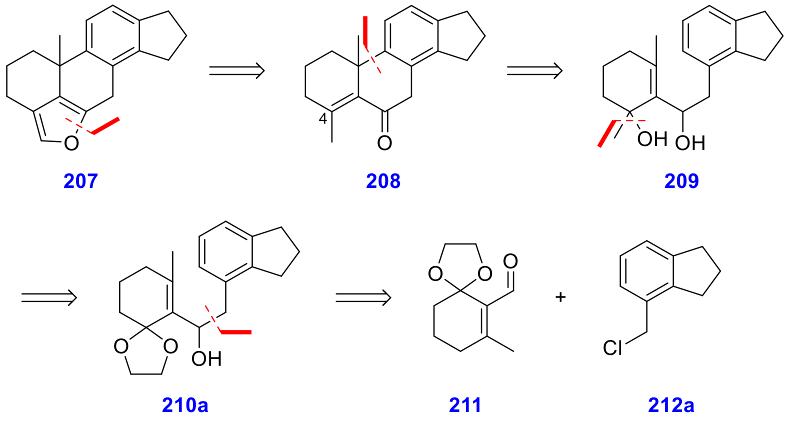
Progressing the synthesis from the furan unit had proven challenging owing to a combination of irreproducibility of literature transformations and the instability of almost all intermediates that frustrated attempts to obtain sufficient material to properly trial the later steps. As multiple strategies to elaborate the vinyl/aldehyde side chain had been unsuccessful, construction of the pentacyclic core around the furan unit was abandoned and a more productive synthetic strategy pursued.

Chapter 2

Approaches to the Steroidal Core of Viridiol

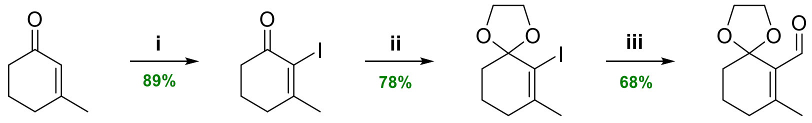
The second route aimed to complete the steroidal core prior to installation of the furan, thereby providing alternative intermediates for enzymatic screening and also avoiding the issues of product instability encountered previously.

Formation of the furan ring to complete the synthesis was envisaged to proceed *via* oxidation (either chemical or enzymatic) of the C-4 methyl group of tetracycle **208** (***Scheme 2.01***), followed by condensation *in situ*. An acid-mediated cyclisation and rearrangement were expected to form this tetracycle from the tethered precursor **209**, itself accessible *via* deprotection and reduction from **210a**, which, in turn, would be obtained by the union of known fragments **211** and **212a**, according to literature precedent.[178–180]



Scheme 2.01: *Planned retrosynthetic route towards pentacyclic core* ***207****.*

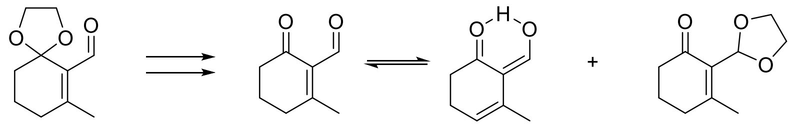
The synthesis commenced with the preparation of known aldehyde **211** from methylcyclohexenone **213** (***Scheme 2.02***).[178] Mild iodination with PIFA and I_2 in DCM gave iodide **214** in 89% yield, which was subsequently protected and formylated to provide aldehyde **211**.



213 **214** **215** **211**

Scheme 2.02: *Synthesis of aldehyde* ***211****.* **Reagents and Conditions: i)** I_2, PIFA, Py, DCM, rt, 18 h; **ii)** ethylene glycol, *p*-TsOH, benzene, 85 °C, 18 h; **iii)** *n*-BuLi, DMF, THF, –78 °C, 15 min.

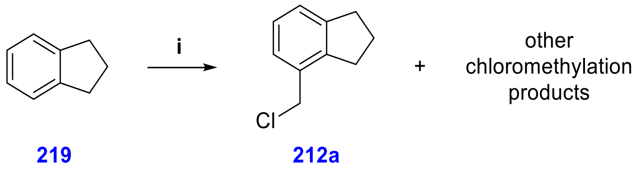
Aldehyde **211** was unstable towards long-term storage, with either loss or migration of the acetal protecting group occurring almost completely within a week (***Scheme 2.03***). The hydrolysis products **216** and **217** were isolated in only trace quantities and their formation was supposed to result from water contamination during storage. Acetal **218** was the major component of the decomposition mixture and, as aldehydes are generally more reactive than ketones, it was presumed that **218** was the more stable acetal and rearrangement of **211** to **218** was therefore inevitable in the presence of traces of acid catalysts. Consequently, only the amount of aldehyde **211** immediately required for subsequent reactions was prepared, and the remainder of the material stored as iodide **215**.



211 **216** **217** **218**

Scheme 2.03: *Decomposition of aldehyde* ***211****.*

The chloromethyl indane fragment was troublesome to synthesise in a pure form. Indane was heated to 65 °C with a mixture of conc. HCl, conc. H_2SO_4, and paraformaldehyde for 22 hours (***Scheme 2.04***).[179] The crude product under these conditions was a complex mixture, including decomposition and polymerisation products, but repeated Kugelrohr distillation enabled the isolation of a pungent colourless oil. ^{1}H NMR analysis identified this oil as a further mixture of products containing all of the possible mono- and bis-chloromethylated indanes. The non-polar character and structural similarity of these compounds made chromatographic separation challenging and three product mixtures were obtained which could not be purified further.



Scheme 2.04: *Chloromethylation of indane.* **Reagents and conditions: i)** $(CH_2O)_n$, conc. HCl, conc. H_2SO_4, 65 °C, 22 h.

The desired 4-chloromethyl indane **212a** was the minor component of a 3:1 mixture with its regioisomer 5-chloromethyl indane **212b**, presumably the favoured product owing to the higher steric accessibility of the 5-position (***Figure 2.01***). The two remaining mixtures contained the four possible bis-chloromethylated products: **220a** and **220b** in a 1:1.4 ratio, and **220c** and **220d** in a 1:1 ratio. Fortunately, mono-chloromethylation was favoured over bis-chloromethylation and **212a** and **212b** were the dominant products, obtained in 48% yield overall. Further attempts to separate these two components were made without success and, as separation could not be achieved, this mixture was therefore carried forward into the next reaction.

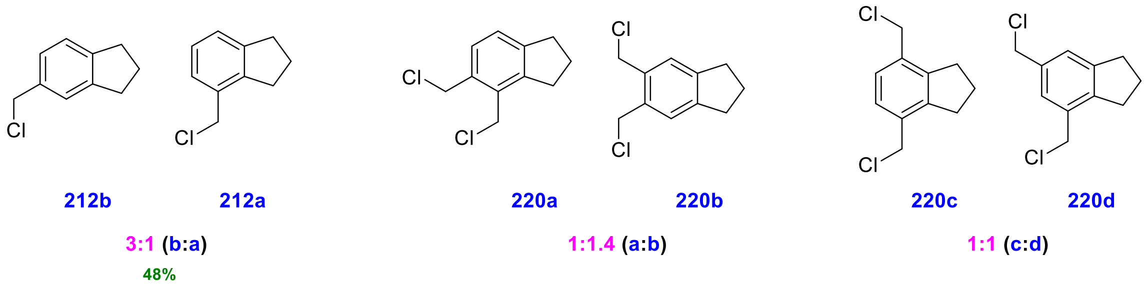
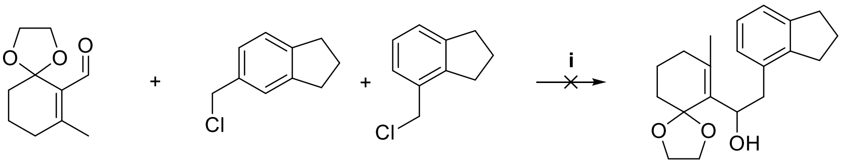


Figure 2.01: *Product mixtures from chloromethylation of indane.*

With both fragments in hand, attention turned to their union. Although perhaps the more obvious choice, we were discouraged from attempting a Grignard reaction initially because of the propensity of benzylic halides to homocouple, a problem observed within the group previously. A modification of this reaction, using lithium, is reported that cites higher yields and reduced homocoupling amongst its advantages.[180] Therefore, a neat mixture of aldehyde **211** and the chlorides **212a** and **212b** was slowly added to freshly-sliced lithium wire in THF at 0 °C (***Scheme 2.05***). Surprisingly, no reaction occurred, indicating that the lithium had failed to insert into the C–Cl bond, despite its activation by the aromatic ring. As the lithium wire had been stored for a significant time, it was proposed that oxidation over time may have rendered the metal unreactive, so an alternative source of lithium wire was found.

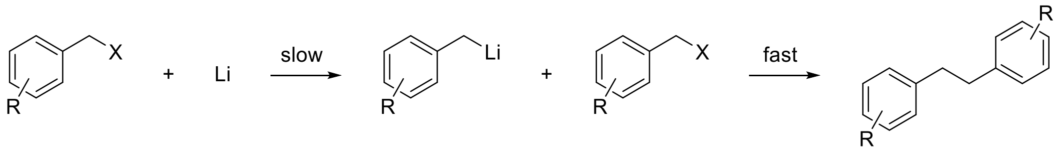


211 **212b** **212a** **210a**

3:1 (b:a)

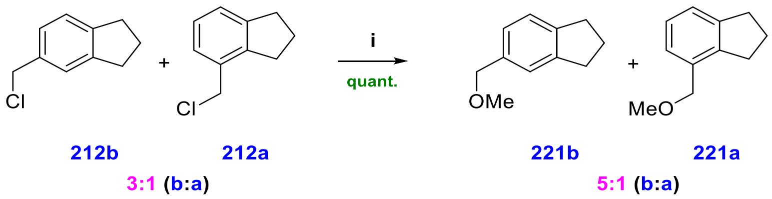
Scheme 2.05: *Attempted union of fragments **211** and **212a/212b** with lithium.* **Reagents and conditions: i)** lithium wire, THF, 0 °C, 5 min.

In addition to the change in lithium source, the authors recommended two modifications to this procedure in the case of benzylic halides, the reactivity of the benzyl lithium species increasing the probability of an undesirable Wurtz coupling, as observed in Grignard reactions of the same substrate. In this homocoupling process, the benzyl lithium species reacts immediately with any benzyl halide present to form the bibenzyl side product (***Scheme 2.06***). Conversion of the requisite chloride into the corresponding methyl ether prevents this side reaction as the methoxy group is less readily displaced than the chloride. Alternatively, a lithium electron acceptor complex may be used: these radical anions are extremely efficient single electron reductants. Rapid electron transfer facilitates immediate conversion to the benzyl lithium, minimising the standing concentration of benzyl halide, thereby suppressing the Wurtz coupling.



Scheme 2.06: *The Wurtz coupling.*

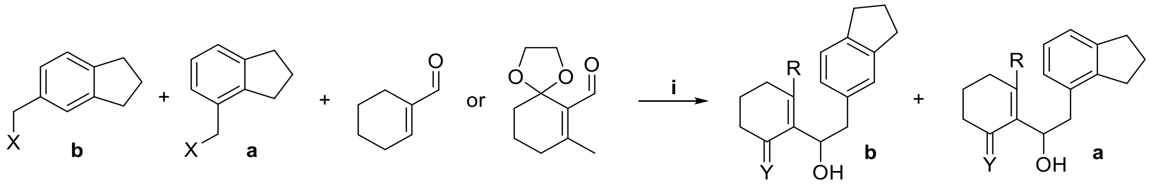
With this in mind, chloride mixture **212a**/**212b** was heated to reflux in methanol with sodium methoxide to afford the expected methyl ether mixture **221a**/**221b** in a 44% yield (***Scheme 2.07***). As with the chloromethyl compounds, these ethers could not be separated and the regioisomeric ratio suffered as a result of the transformation (5:1 **221b**:**221a** *cf.* 3:1 **212b**:**212a**). This likely arises from the same steric influences observed in the chloromethylation of indane: assuming the substitution proceeds *via* an S_N2 mechanism, the sterically more-accessible *5*-chloromethyl indane **212b** will be favoured for reaction, and, in the case of incomplete reaction, will result in a diminuition of the proportion of the desired *4*-substituted product **221a**.



Scheme 2.07: *Preparation of methyl ethers* ***221a*** *and* ***221b****.* **Reagents and Conditions: i)** NaOMe, MeOH, Δ, 8 h.

In the interest of preserving aldehyde **211**, commercially available aldehyde **222** was used for testing purposes and the addition reaction repeated for the methyl ether substrate. No reaction was observed and so the second modification was trialled for which a lithium acceptor complex was prepared. Fresh lithium wire was slowly added to a solution of naphthalene in THF under a flow of N_2 to form the characteristic dark green lithium naphthalenide complex. Slow addition of the neat mixture of aldehyde **222** and either chlorides **212a**/**212b** or methyl ethers **221a**/**221b** followed and, gratifyingly, the expected products were observed in both cases (*entries* ***1*** *and* ***2***, ***Table 2.01***). These conditions were repeated with aldehyde **211** but gave poor yields of 12% and 10% respectively (*entries* ***3*** *and* ***4***).

Table 2.01: *Union of indanyl and cyclohexyl fragments with lithium naphthalenide solution.*



212 X = Cl
221 X = OMe

222

211

223 Y = H,H and R = H
210 Y = $(OCH_2)_2$ and R = Me

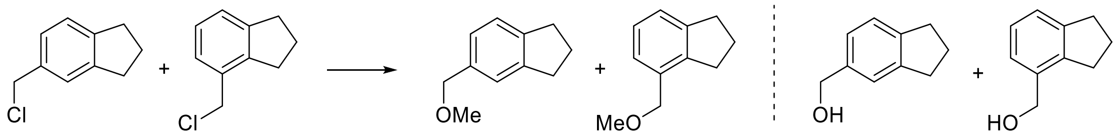
Entry	Indane Mixture	Aldehyde	Yield (%)	Regioisomeric ratio of product 5-:4-
1	**212**	**222**	49	5:1
2	**221**	**222**	21	3:1
3	**212**	**211**	12	4:1
4	**221**	**211**	10	5:1

Reagents and conditions: *lithium wire, naphthalene, THF, 0 °C, 5 min.*

The regioisomeric products of these reactions were also inseparable and the ratios were eroded still further as addition occurred preferentially with the 5-substrates. At this stage it was evident that the synthesis could not be advanced effectively with these mixtures and a strategy to isolate the 4-substituted compounds was required.

One such strategy arose from an observation made during the preparation of methyl ethers **221a** and **221b**. Initial conditions had resulted in incomplete reaction with the formation of some bismethoxy isomers so the reaction time and number of equivalents of NaOMe were varied (***Table 2.02***). These alterations resulted in both different combinations and ratios of products: reaction with four equivalents of NaOMe resulted in complete consumption of the starting chloride mixture **212a**/**212b** but the regioisomeric ratio of the product methyl ethers **221b**/**221a** was increasingly unfavourable at 6:1 (*entry **1***). Reduction of the number of equivalents of NaOMe slightly mitigated this effect, producing methyl ethers **221b** and **221a** in a 5:1 ratio (*entries **2** and **3***). Reaction under these conditions was incomplete, returning a significant proportion of starting chloride, but in a much more favourable regioisomeric ratio (1.7:1 (**212b**:**212a**) in *entry **3***).

Table 2.02: *Varying product ratios obtained during the methoxylation of chlorides **212a** and **212b**.*



212b **212a** **221b** **221a** **224b** **224a**

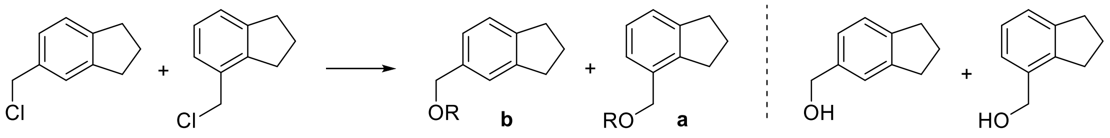
3:1 (**b**:**a**)

Entry	**Eq NaOMe**	**Time**	**Regioisomeric ratio of product 5-:4-**		
			221	**212**	**224**
1	4	8	6:1	—	3.5:1
2	1	5	5:1	2.3:1	3:1
3	1	8	5:1	1.7:1	—

Reagents and conditions: *NaOMe, MeOH, Δ.*

This enhancement of the ratio from 3:1 to 1.7:1 (*entry **3***) was significant and it was therefore postulated that a larger nucleophile might drive this enrichment further, reacting preferentially with the 5-chloromethyl indane to leave the 4-regioisomer untouched (***Table 2.03***). In practice, reaction with NaOEt in ethanol produced ethyl ethers **225b** and **225a** in the same 5:1 ratio as the methyl congener, without further enrichment of the starting chloride (*entry **2***). The *tert*-butyl ether was not obtained at all, and correspondingly the 3:1 ratio of **212b** and **212a** was not improved (*entry **3***). It was concluded that the steric influence of the nucleophile was insufficient to achieve the selectivity required and an alternative means of accessing 4-chloromethyl indane was sought.

Table 2.03: *Attempted alkoxylations of chlorides **212a** and **212b**.*



212b **212a**
3:1 (**b**:**a**)

221 X = OMe
225 X = OEt

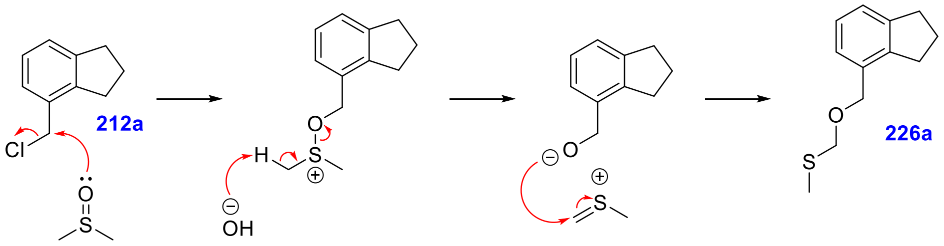
224b **224a**

Entry	**MOR**	**Solvent**	**Regioisomeric ratio of product 5-:4-**		
			221 or 225	**212**	**224**
1	NaOMe	MeOH	5:1	1.7:1	—
2	NaOEt	EtOH	5:1	1.7:1	3.7:1
3	KO*t*-Bu	*t*-BuOH	—	3.5:1	2.9:1

Reagents and conditions: *MOR, solvent, Δ, 8 h.*

During each of these attempted alkylations, a small amount of a mixture of alcohols **224a** and **224b** was produced. Although likewise chromatographically inseparable, the 5-regioisomer was reported to be purified by recrystallisation from pet. ether to give a white crystalline solid (**224b**) and a mother liquor enriched in **224a**.[179] The individual samples of mixed **224** were therefore combined to give 328 mg in a 5:1 ratio of **224b** to **224a**. Recrystallisation afforded 235 mg of pure **224b** and a mother liquor enriched to a 1:5 ratio of **224b** and **224a** respectively. Whilst this solution contained impurities and required further purification, such an improvement in ratio was worth pursuing and attention moved to directly converting chlorides **212a** and **212b** deliberately into alcohols **224a** and **224b** (***Table 2.04***).

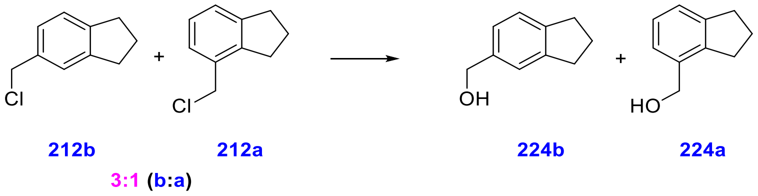
The chloride mixture **212a**/**212b** was heated to reflux in methanolic NaOH (3.0 M) but this merely improved the initial methoxylation reaction, producing methyl ethers **221a**/**221b** in quantitative yield (*entry **1***). To avoid alkoxylation, the solvent was changed: in acetone and THF, no reaction ensued (*entries **2** and **3***): in acetonitrile only very minor amounts of the desired alcohol were obtained in an unfavourable ratio of regioisomers (*entry **4***). As benzylic substrates support both S_N1 and S_N2 mechanisms, it was expected that moving to a more polar solvent would enhance the yield of alcohol product. Reaction in DMSO at 140 °C did indeed give an improved yield (of 29%) after 6 hours although the reaction had not reached completion (*entry* **5**). After 18 hours at 140 °C, all starting chloride had been consumed, yielding 29% of alcohols **224a** and **224b**, and 21% of methylthiomethyl ether products **226a** and **226b** (*entry* **6**). These products likely arise from *O*-activation of the DMSO by chloride **212**, followed by elimination with base and trapping of the resultant reactive methylmethylenesulfonium ion by the newly-formed alcohol (***Scheme 2.08***).



Scheme 2.08: *Formation of methylthiomethyl ether products* ***226a*** *and* ***226b*** *from chlorides* ***212a*** *and* ***212b****.* ***212a*** *only shown for clarity.*

As the elimination step requires high temperatures to occur, repetition of the reaction at room temperature with a higher concentration of base was attempted in order to suppress this side reaction but this failed to drive the reaction to completion and only a small amount of alcohol product was obtained (*entry* **7**). Complete omission of the organic co-solvent in a stronger hydroxide solution was likewise unsuccessful (*entry* **8**), as was addition of an equivalent of $AgNO_3$ to abstract the chloride (*entry* **9**). A phase transfer catalyst was also trialled to ensure effective mixing of the chloride and hydroxide solution but only traces of the alcohol were obtained (*entry* **10**). Heating in acid likewise failed to induce any reaction (*entry* **11**).

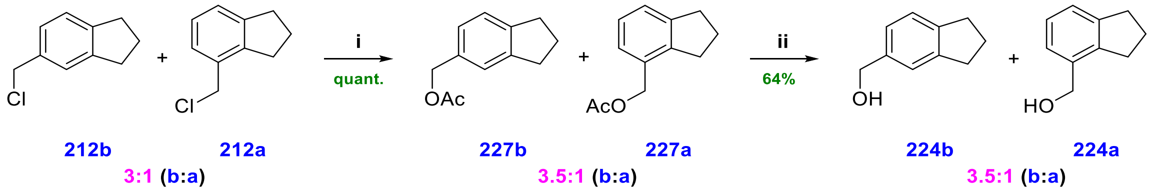
Table 2.04: *Conditions attempted to form alcohols **224a** and **224b** directly from chlorides **212a** and **212b**.*



Entry	**Solvent**	**Additive**	**Temperature (°C)**		**Products**	
				224	**212**	**Other**
1	MeOH	—	Δ	—	—	Quant / 4:1 **221**
2	Acetone	—	Δ	—	90% / 3.5:1	—
3	THF	—	Δ	—	76% / 3.5:1	—
4	MeCN	—	Δ	13% / 6:1	36% / 3.5:1	—
5	DMSO	—	140	29% / 5.5:1	36% / 5.5:1	—
6[a]	DMSO	—	140	29% / 4:1	—	21% / 3.5:1 **226**
7[b]	DMSO	—	rt	11% / 7.5:1	47% / 4.7:1	—
8[b]	—	—	100	Trace	73% / 3.4:1	—
9[b]	—	$AgNO_3$	100	Trace	90% / 3.4:1	—
10[b]	—	Bu_4NBr	125	Trace	69% / 4.6:1	—
11[c]	—	HCl	100	—	81% / 3.3:1	—

Reagents and conditions: *3 M NaOH, solvent, 6 h. [a] 18 h; [b] 5 M NaOH; [c] no base. Ratios given 5-:4-. Δ signifies temperature required for reflux.*

As an alternative, a stepwise method was explored, in which the chloride mixture was converted into the corresponding acetates, then ester hydrolysis effected to release the alcohols (***Scheme 2.09***).[181] Chloride mixture **212a**/**212b** was heated to reflux with NaOAc in acetic acid to form the inseparable esters **227a**/**227b**, which were solvolysed under basic conditions to give a 3.5:1 mixture of **224b** and **224a** respectively, in 64% yield.



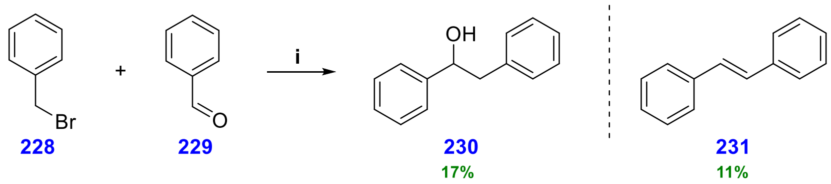
Scheme 2.09: *Synthesis of alcohols **224a** and **224b** via acetates **227a** and **227b**.* **Reagents and conditions: i)** NaOAc, AcOH, 120 °C, 5 h; **ii)** NaOH, MeOH, 70 °C, 1h.

Pure alcohol **224b** was readily obtained but repeat recrystallisations failed to isolate **224a** free of its regioisomer. Whilst enrichment was possible through this process, the product losses incurred were prohibitive and this strategy was therefore abandoned.

Rather than attempting to separate mixtures of regioisomers, it was determined that an alternative synthetic strategy was required, forming exclusively a 4-substituted indane, free from the 5- regioisomer. This endeavour was initially undertaken by other group members, enabling the focus to return to developing the subsequent steps.

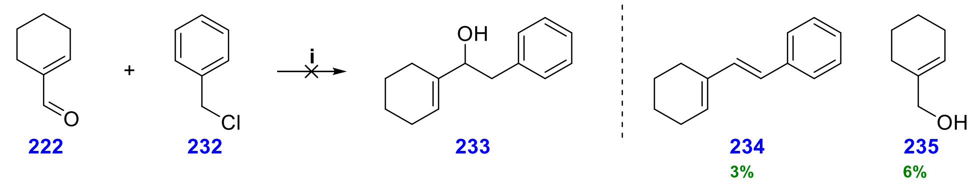
The lithium naphthalenide-mediated addition of chlorides **212a** and **212b** into aldehyde **211** had already shown some promise but the low yields still rendered this step synthetically unfeasible and formation of the active species was unreliable. It was therefore necessary to validate the effective formation of the lithium naphthalenide radical ion. An alternative preparation was sought and one equivalent each of lithium and naphthalene in THF was sonicated for 2 hours, then cooled to –78 °C.[182]

Two test reactions were performed with this solution: benzyl bromide **228** and benzaldehyde **229** afforded just 17% of the expected diphenylethanol product **230**, compared with 75% in the literature, and 11% of the elimination product stilbene **231** (***Scheme 2.10***).[183]



Scheme 2.10: *Test reaction with benzyl bromide and benzaldehyde to validate lithium naphthalenide.* **Reagents and conditions: i)** Li, naphthalene, THF, –78 °C, 5 min.

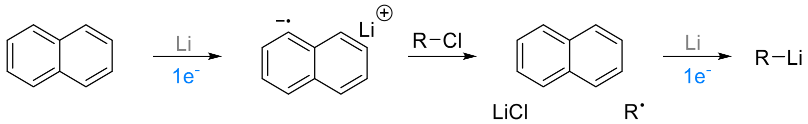
Reaction with benzyl chloride **232** and aldehyde **222**, chosen as being similar to the real system, gave an even worse outcome, giving only 3% of the elimination product **234** and 6% of the alcohol **233** (***Scheme 2.11***).



Scheme 2.11: *Test reaction with benzyl chloride and aldehyde* ***222*** *to validate lithium naphthalenide.* **Reagents and conditions: i)** Li, naphthalene, THF, –78 °C, 5 min.

Certainly, from the former of these two reactions, it was evident that the lithium naphthalenide solution was less active than expected. Ager reported a method for the quantitative estimation of lithium naphthalenide concentration so a fresh batch was prepared for titration.[184] As a radical anion, lithium naphthalenide is a poor nucleophile and its basic properties usually result from proton abstraction, meaning that it does not act as a base with all reagents typically used in the estimation of organolithiums. A double titration method may therefore be employed: quenching of an aliquot with a known quantity of water allows the determination of the total base concentration by titration with hydrochloric acid. Quenching of a second aliquot with methyl iodide permits determination of the residual base (*i.e.*, all basic components excepting the lithium naphthalenide) by the same method, and from this, the concentration of active lithium naphthalenide may be inferred. Aliquots taken at systematic intervals over a 24 hour period evidenced that not only was the active concentration much lower than expected, but it also varied greatly with time.

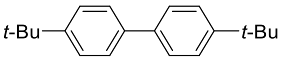
Consideration of a proposed mechanism explains these observations (***Scheme 2.12***).[185] A one-electron reduction of naphthalene by lithium metal gives the radical anion **237**, which rapidly reduces the alkyl halide to provide the alkyl radical **238**, naphthalene and LiCl. A second one-electron reduction by lithium metal forms the desired alkyllithium reagent **239**. However, both naphthalene and its radical anion **237** are readily intercepted by the intermediate alkyl radical **238** or the newly-formed alkyllithium **239**, resulting in numerous side-products and an unpredictable diminuition in the active concentration of alkyllithium.[185]



236 **237** **238** **239**

Scheme 2.12: *Mechanism of the formation of an alkyllithium via a lithium naphthalenide complex.*

In 1976, Freeman reported the exchange of naphthalene for di-*tert*-butylbiphenyl (DBB) **240** as a means of circumventing this undesirable activity (***Figure 2.02***).[186] The bulky *tert*-butyl groups block the participation of the DBB radical anion in these side reactions whilst also enhancing its electron donor ability. As such, formation of a Li-DBB complex was attempted.



240

Figure 2.02: *Di-tert-butylbiphenyl (DBB)*

Literature reports indicate that this is a somewhat condition-sensitive procedure.[187] Fresh lithium wire was cleaned, flattened into a ribbon, and cut into strips under a stream of argon. These lithium strips were stirred aggressively overnight in a Schlenk tube with DBB and anti-bumping granules until a fine grey powder formed. Distilled THF was added and the resulting blue/green solution cooled to –78 °C before a neat mixture of the substrates was added. In practice, an initial test reaction with benzyl chloride and aldehyde **241**[*] gave the expected product **242** in 41% yield (***Scheme 2.13***).

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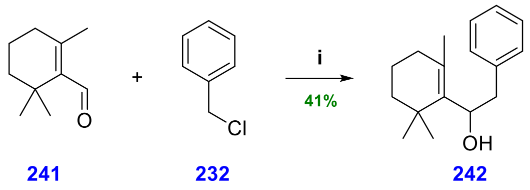
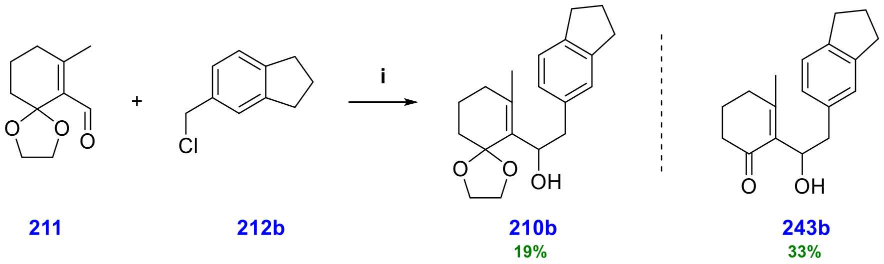
[*] Aldehyde **222** used previously no longer available.



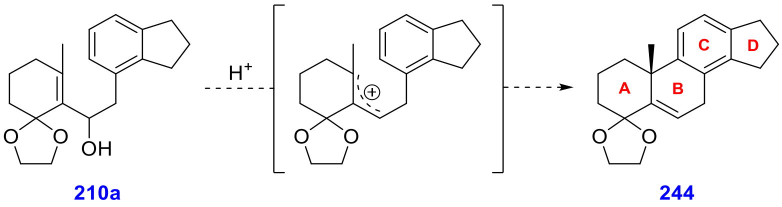
Scheme 2.13: *Test reaction with aldehyde* ***241*** *and benzyl chloride.* **Reagents and conditions: i)** Li wire, DBB, THF, –78 °C, 5 min.

With greater confidence, these conditions were repeated on aldehyde **211** and 5-chloromethyl indane **212b** (***Scheme 2.14***) and 19% of the expected product **210b** was isolated along with a further 33% of the deprotected adduct **243b**.



Scheme 2.14: *Test reaction with aldehyde* ***211*** *and 5-chloromethyl indane* ***212b****.* **Reagents and conditions: i)** Li wire, DBB, THF, –78 °C, 5 min.

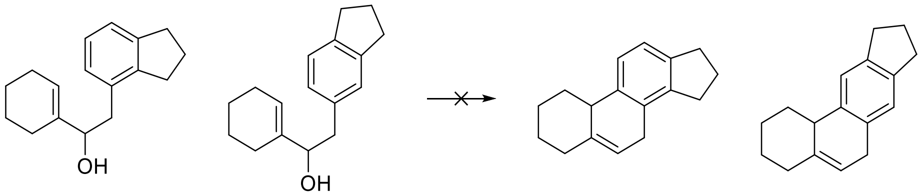
However, as access to the pure 4-chloromethyl indane had not at the time been achieved, this reaction could not be repeated on the real system. The expected product from this reaction, **210a**, was projected to undergo an acid-mediated cationic cyclisation in order to form the B-ring and complete the steroidal core (***Scheme 2.15***).



Scheme 2.15: *Envisaged cationic cyclisation to complete steroidal core* ***244****.*

Such cyclisations are well precedented with related substrates and so development of suitable conditions began with the mixed model system **223a**/**223b**, synthesised previously (***Table 2.05***).[188–192] Treatment with 90% H_2SO_4 proved too severe, resulting in complete decomposition of material at –5 °C, despite literature reports to the contrary (*entry **1***).[191] Milder reaction conditions, with *p*-toluenesulfonic acid, likewise failed to result in the isolation of any recognisable products, affording only a complex mixture and it was concluded that a substrate comprising a mixture of regioisomers was unsuitable for reaction development (*entry **2***).[192,190]

***Table 2.05:** Cyclisation attempts on model substrate **223a** and **223b**.*



223a **223b** **245a** **245b**

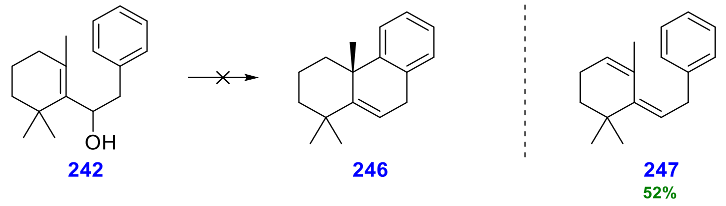
1:5 (a:b)

Entry	Acid	Temperature	Outcome
1	90% H_2SO_4	–5 °C	Decomposition
2[a]	10% *p*-TsOH	rt	Complex mixture

[a]in DCM.

Next, the simpler model substrate **242** was evaluated (***Table 2.06***). Treatment of this compound with H_2SO_4 again resulted in total decomposition (*entry **1***) and *p*-toluenesulfonic acid produced the elimination product **247** cleanly in 52% yield (*entry **2***). This result gave evidence that the allylic cation was able to form but that the aromatic ring was insufficiently electron rich to trap it; instead elimination predominated, suggesting that further cationic cyclisation attempts would be unproductive.

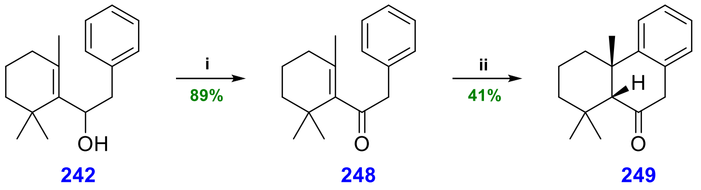
Table 2.06: *Cyclisation attempts on model substrate* ***242****.*



Entry	Acid	Temperature	Outcome
1	90% H_2SO_4	–5 °C	Decomposition
2[a]	10% *p*-TsOH	rt	52% **247**

[a]*in DCM.*

As an alternative, enone cyclisation was proposed, and alcohol **242** was oxidised to ketone **248** by Dess–Martin periodinane in 89% yield (***Scheme 2.16***). In 2004, Sames *et al.* reported $RuCl_3$/AgOTf as a mild catalyst for intramolecular electrophilic hydroarylation[188] and Crusco *et al.* subsequently applied this reagent system to ketone **248**.[193] These conditions were reproduced and expected tricycle **249** was obtained in 41% yield.*



Scheme 2.16: *Oxidation and cyclisation of model substrate* ***242****.* **Reagents and conditions: i)** DMP, DCM, 0 °C, 3 h; **ii)** 40 mol% AgOTf, 20 mol% $RuCl_3xH_2O$, DCE, 80 °C, 14 h.

With a model synthetic route now established, repetition with the indanyl system became desirable, although ongoing efforts within the group had still not facilitated access to a regioisomerically pure 4-indanyl fragment.

A previously rejected approach was then reconsidered: aldehydes **250a** and **250b** are reported to be chromatographically separable and are accessed *via* oxidation of alcohols **224a** and **224b** respectively.[179] However, in addition to the somewhat high step count

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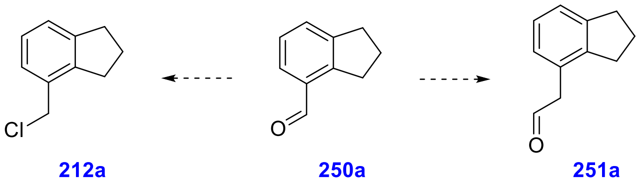
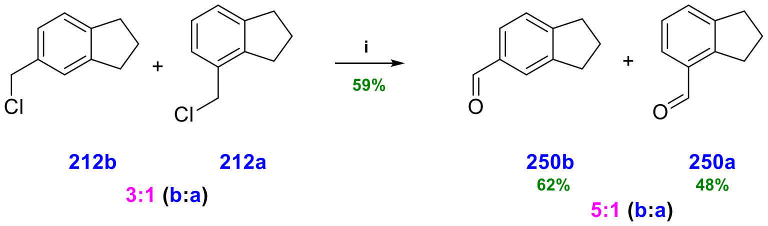
* The *cis*- ring fusion stereochemistry was confirmed by nOe analysis.

required to access these aldehydes (four steps from indane), further manipulations would also be required post-separation: either conversion back to chloride **212**, or homologation to aldehyde **251** and development of alternative conditions to facilitate the necessary addition reaction with iodide **214** or **215** (***Scheme 2.17***).



Scheme 2.17: *Post-separation manipulations of aldehyde* ***250a****.*

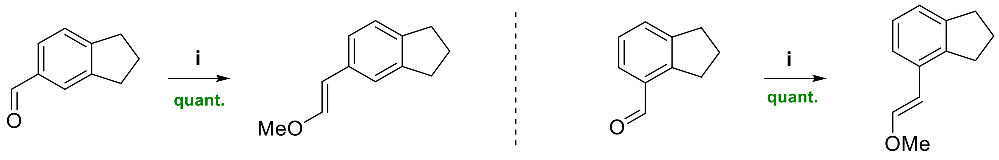
It was hoped that this sequence could be shortened by applying a Kornblum oxidation, thereby converting the inseparable chloride mixture **212a**/**212b** into aldehydes **250a**/**250b** directly, without intermediate steps. Thus, the 3:1 mixture of chlorides **212b** and **212a** was treated with DMSO and $NaHCO_3$ at 110 °C, affording a 5:1 mixture of aldehydes **250b** and **250a** in 59% yield (***Scheme 2.18***). These regioisomers were separated to give 5-formylindane **250b** in 62% yield and desired 4-formylindane **250a** in 48% yield, providing regioisomerically pure material for the first time.



Scheme 2.18: *Kornblum oxidation of mixed chlorides* ***212a*** *and* ***212b*** *to separable aldehydes* ***250a*** *and* ***250b****.* **Reagents and conditions: i)** $NaHCO_3$, DMSO, 110 °C, 1 h. Yields of individual regioisomers are given with respect to the 3:1 ratio of chloride regioisomers **212b** and **212a** present in the mixture.

As only small amounts of material were available, it was decided that subsequent reactions should first be validated on the 5-regioisomer. Because numerous procedures have been reported for the efficient homologation of aldehydes, this strategy was chosen in preference to a cumbersome conversion back to the chloride.

A one-pot Wittig homologation-hydrolysis sequence with NaHMDS, as the base for generating the ylid, and *p*-toluenesulfonic acid, as the catalyst for the hydrolysis step, reached only partial completion, resulting in a 17% yield of expected aldehyde **251b**.[194] Separation of these steps was more successful and Wittig homologation, with *t*-BuOK as the base, gave expected enol ether **252** in quantitative yield for both aldehydes **250a** and **250b** (***Scheme 2.19***).[195]

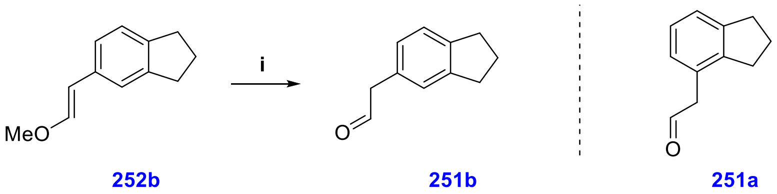


250b **252b** **250a** **252a**

Scheme 2.19: *Homologation of aldehydes **250a** and **250b**.* **Reagents and conditions: i)** (methoxymethyl) triphenylphosphonium chloride, *t*-BuOK, THF, –78 °C, 6 h.

Subsequent hydrolysis of this enol ether was more troublesome, likely owing to conjugation of the double bond with the aromatic ring. Treatment with 1.0 M aq. HCl resulted in partial reaction after 18 hours, giving 29% of expected aldehyde **251b** whilst returning 32% of the starting enol ether **252b** (***Table 2.07***, *entry **1***). A stronger acid was trialled to push the reaction but H_2SO_4 failed to improve upon this, with no observable reaction occurring at all (*entry **2***). Finally, TMSCl and NaI were found to induce full conversion, affording aldehydes **251b** and **251a** in 42% and 90% yields respectively (*entries **3** and **4***).[196]

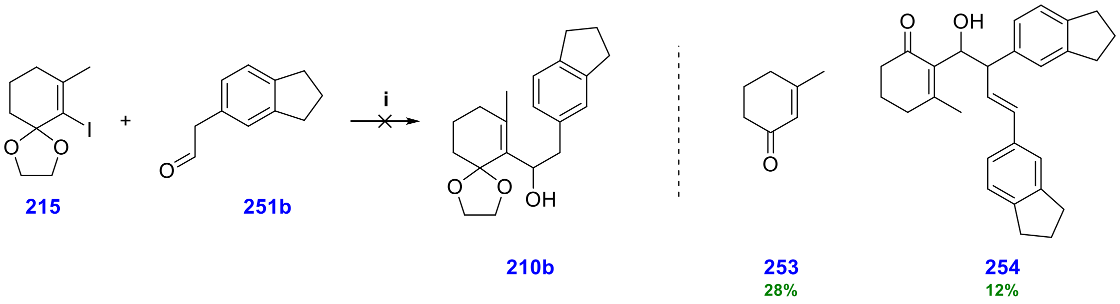
Table 2.07: *Enol ether hydrolysis.*



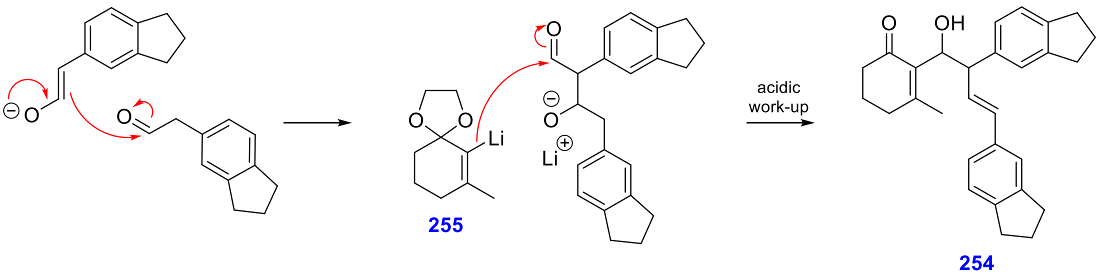
Entry	Conditions	Outcome
1	1.0 M HCl, THF, rt	29% **251b**, 32% **252b**
2	1.0 M H_2SO_4, THF, rt	No reaction
3	TMSCl, NaI, MeCN, rt	42% **251b**
4[a]	TMSCl, NaI, MeCN, rt	90% **251a**

*[a] from regioisomer **252a***

With both fragments in hand, a modified coupling strategy was evaluated. Iodide **215** was treated with *n*-butyllithium at –78 °C, then a solution of aldehyde **251b** was added (***Scheme 2.20***). From this reaction, no expected alcohol **210b** was isolated and, instead, the reduced iodide **253** was obtained, along with the di-indanyl adduct **254** (12% yield with respect to starting iodide **215**). Formation of this second product occurs because enolisation and aldol reaction of the aldehyde out-competes trapping with the lithiated cyclohexenyl unit **255** (***Scheme 2.21***). The resultant aldol adduct, that presumably accumulates in solution is instead trapped by **255** and acidic work-up releases the eliminated and deprotected adduct of this aldol product **254**.



Scheme 2.20: *Attempted addition of iodide **215** into aldehyde **251b**.* **Reagents and conditions: i)** *n*-BuLi, THF, –78 °C, 30 min.

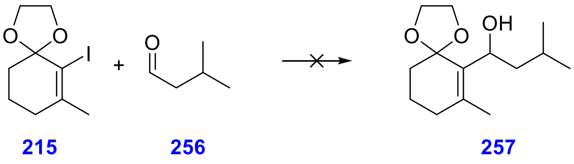


Scheme 2.21: *Formation of adduct* ***254****.*

Consideration was given to the use of a less basic Grignard reagent that should suppress this competing aldol pathway. Because formation of the organomagnesium derivative is not reported for iodide **215**, commercially available, enolisable isovaleraldehyde **256** was used in place of the more precious indanyl aldehydes for test reactions.

A variety of preparations of the organomagnesium were attempted (summarised in ***Table 2.08***): use of pre-activated Mg (Method **A**), activation with I_2 (Method **B**), and activation with heat (Method **C**) all failed to form the desired reagent and, in each case, only starting iodide **215** or a complex mixture was obtained.

Table 2.08: *Attempted formation of a Grignard reagent from iodide* ***215****.*

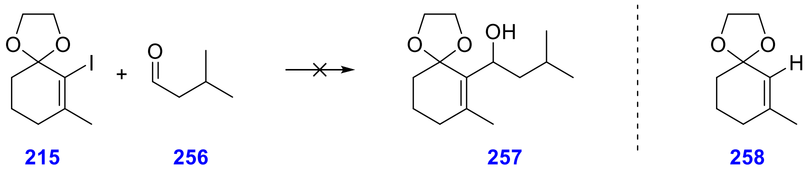


Entry	Mg (eq)	Mg activation	Solvent	Temperature (°C)	Time (h)	Outcome
1	1.1	**A**	THF	rt	2	Returned **215**
2	1.1	**A + B**	THF	rt	2	Returned **215**
3	1.1	**A + C**	THF	60	2	Returned **215**
4	6.6	**A**	Et_2O	0	2	Returned **215**
5	6.6	**A + B**	Et_2O	rt	1	Returned **215**
6	6.6	**B + C**	Et_2O	rt	2	Returned **215**
7	6.6	**B + C**	THF	Δ	2	Complex mixture

Mg activation conditions: Method **A** – stirred aggressively overnight under Ar.[197] Method B – I_2 crystal added to suspension of Mg turnings. Method **C** – gentle heating with a heat gun.

As lithiation of iodide **215** had already been achieved, transmetallation was next attempted. Addition of $MgBr_2{\cdot}OEt_2$ resulted only in formation of the proto-deiodinated product **258**, likely formed through quenching of the organolithium (possibly by the THF) before transmetallation could occur (***Table 2.09***, *entry **1***).[198] Use of a less basic organocerium reagent gave only a complex mixture (*entry **2***).[199]

Table 2.09: *Attempted transmetallation of iodide **215**.*



Entry	Transmetallation reagents	Time (h)	Outcome
1	*n*-BuLi, $MgBr_2{\cdot}OEt_2$	1.5	**258** only
2	*n*-BuLi, $CeCl_3$	6	Complex mixture

Reagents and conditions: *n*-BuLi, THF, –78°C.

As an alternative organometallic was not formed, it was necessary to reconsider the original lithiation strategy. Addition into the aldehyde had been shown to be possible but was just much less favourable than the aldol reaction. The initial procedure was therefore modified to suppress this undesired reaction as far as possible.

A more dilute solution of aldehyde **251b** was prepared (0.2 M in THF *cf.* 0.6 M) which was added *via* syringe pump at a rate of 1.5 cm^3/h in order to minimise the concentration of free aldehyde in solution. This strategy proved successful, and alcohol **210b** was isolated as the major product in 33% yield (***Scheme 2.22***).[*] Quantitative DMP oxidation gave ketone **259b** which was then cyclised to tetracycle **260b** using the AgOTf/$RuCl_3$ catalyst system to give an unoptimised yield of 18%.

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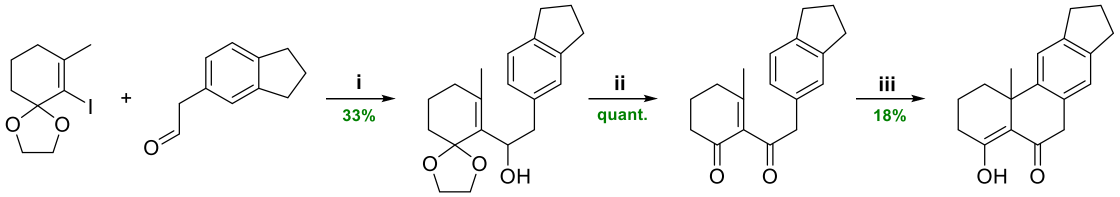
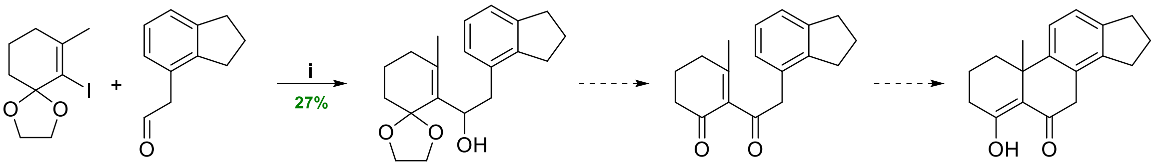
[*] An extensive mixture of other products account for the remainder of the mass and include reduced and/or deprotected iodide **215** and reduced **251b**.



215 **251b** **210b** **259b** **260b**

Scheme 2.22: *Synthesis of tetracycle* ***260b****.* **Reagents and conditions: i)** *n*-BuLi, THF, –78 °C, 2 h; **ii)** DMP, DCM, rt, 2 h; **iii)** 10% AgOTf, 5% $RuCl_3$, DCE, 80 °C, 14 h.

Repetition with aldehyde **251a** likewise gave alcohol **210a** in 27% yield but insufficient material was available to progress the synthesis further (***Scheme 2.23***). Whilst the aldehyde separation strategy had provided access to the necessary pure 4-substituted indane, the unfavourable regioisomeric ratio carried through the synthesis made this preparation too practically inefficient and, at the time, an alternative route to this fragment was showing more promise.



215 **251a** **210a** **259a** **260a**

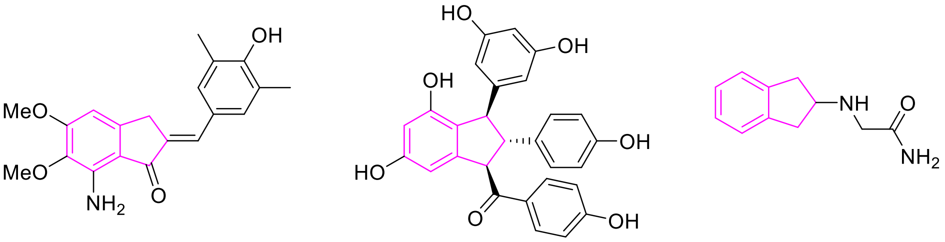
Scheme 2.23: *Repetition of sequence with aldehyde* ***251a****.* **Reagents and conditions: i)** *n*-BuLi, THF, –78 °C, 2 h.

Chapter 3

Synthesis of the 4-Indanyl Fragment

Synthetic Strategies Towards Functionalised Indanes

The indane nucleus is a bicyclic carbocycle featuring an aromatic ring fused to a cyclopentane unit. Derivatives of this motif are found in numerous compounds of biological interest such as indanocine **261** (anticancer),[200] caraphenol B **262** (antihypertension),[201] and indantadol **263** (relief of neuropathic pain),[202] and many strategies for the construction of this unit have been reported (***Figure 3.01***).[203–207]

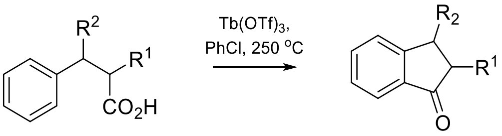


indanocine **261** caraphenol B **262** indantadol **263**

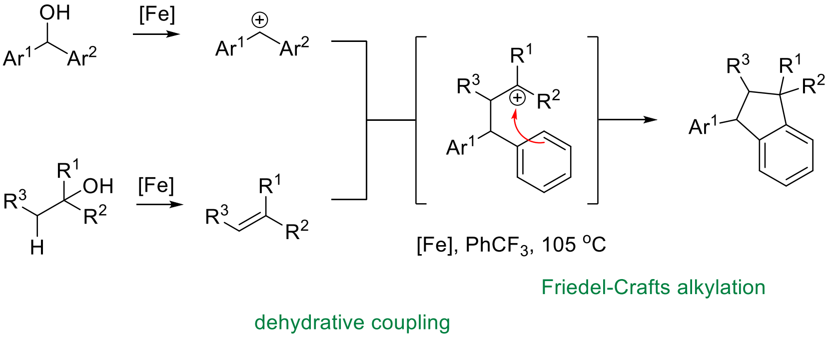
Figure 3.01: *Examples of compounds of therapeutic interest containing the indane nucleus (indicated in pink).*

- **Intramolecular Friedel–Crafts Reactions**

  Both acylation[208] and alkylation[209] reactions are used to construct the cyclopentane from either substituted[210] or tethered[211] aromatic substrates (***Scheme 3.01** and **Scheme 3.02***). Whilst acylation reactions produce indanones, other substituents are generally limited to alkyl groups and aromatics owing to the harsh conditions required to effect ring closure.



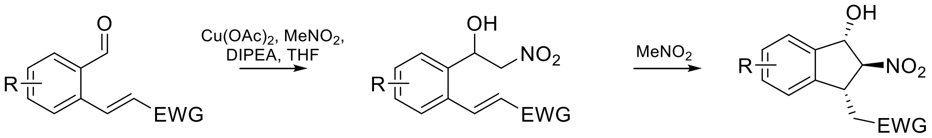
Scheme 3.01: *Synthesis of 1-indanones from 3-arylpropionic acids via Friedel–Crafts acylation (Cui et al.[208]).*



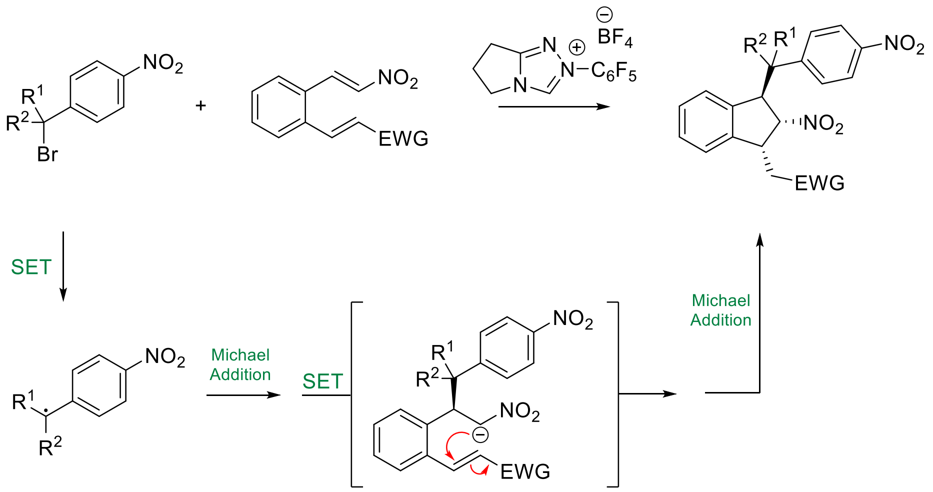
Scheme 3.02: *Synthesis of substituted indanes via dehydrative coupling/Friedel–Crafts alkylation of alcohols.[209]*

- **Intramolecular Michael Additions**

Michael additions into tethered electron-deficient alkenes have been extensively studied with a diverse range of cyclisation precursors enabling varied substitution patterns to be achieved with good diastereo- and enantio-selectivities.[212] Numerous cascade transformations have been reported, forming the Michael precursor through a variety of processes. Specific examples include the nitroaldol/Michael sequence reported by Gong *et al*. (***Scheme 3.03***),[213] Chi's NHC-mediated sequential Michael additions (***Scheme 3.04***),[202] and the Michael–Henry domino reaction reported by Enders *et al*.[214]



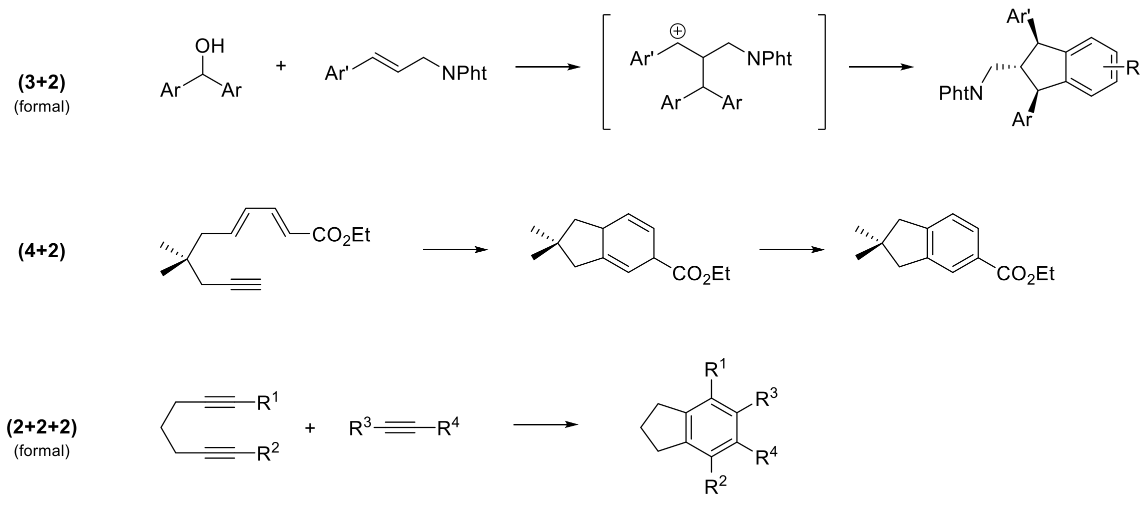
Scheme 3.03: *Nitroaldol/Michael sequence described by Gong et al.[213]*



Scheme 3.04: *NHC-mediated sequential Michael reactions.*[202]

- **Cycloaddition Reactions**

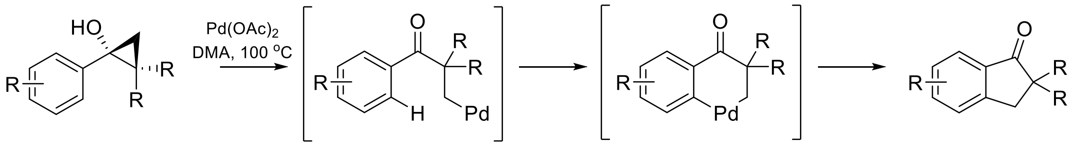
Cycloadditions are a versatile means of constructing the indane core with both inter- and intra-molecular variants reported. Early papers[215,216] describing (3+2) reactions between benzyl cations and styrenes illustrate routes to a range of diversely functionalised indanes from simple starting materials.[217,218] More recently, (4+2) reactions between 1,3-dienes and alkynes are perhaps the most common cycloaddition strategy applied, giving the dihydroindane adduct which is readily oxidised to the indane product.[219–221] Cyclotrimerisation of alkynes (a formal (2+2+2) process) is also described under various conditions (***Scheme 3.05***).[222]



Scheme 3.05: Examples of (formal) cycloadditions in the construction of the indane nucleus.

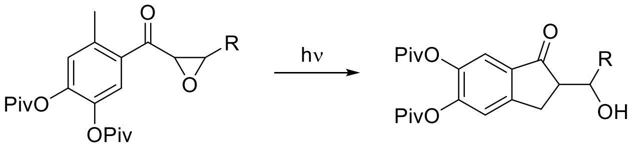
- **Ring Expansion and Contraction Processes**

Metal-induced ring opening of 3-[223] and 4-membered[224] tertiary alcohols has been reported, with subsequent ring closure being achieved by C-H activation of the connected aromatic ring (***Scheme 3.06***).



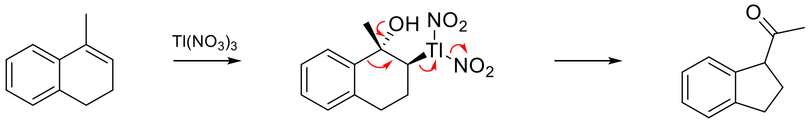
Scheme 3.06: *Ring expansion of cyclopropanols.*[223]

Photoenolisation of *o*-methylphenacyl epoxides to formal aldol adducts from indanone derivatives is also reported (***Scheme 3.07***).[225]



Scheme 3.07: *Photoenolisation of epoxides.*[225]

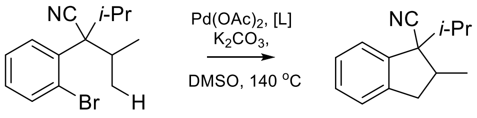
Oxidative ring contraction of 1,2-dihyronaphthalenes has been reported using a variety of oxidants, including: $Tl(NO_3)_3$ (***Scheme 3.08***),[226] hypervalent iodine reagents,[227,228] and $KMnO_4$/NaOH (Hooker oxidation conditions).[229]



Scheme 3.08: *Tl(NO$_3$)$_3$-mediated ring contraction of 1,2-dihydronaphthalene by Silva et al.*[226]

- **C-H Activation Processes**

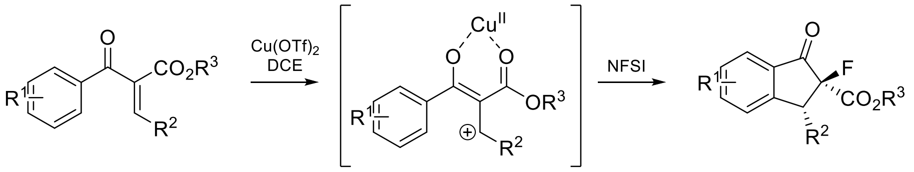
Inter- and intra-molecular C-H activation has been employed to form the cyclopentane ring (***Scheme 3.09***).[230–232]



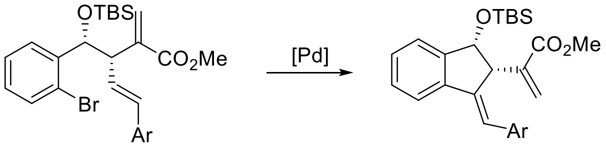
Scheme 3.09: *C-H arylation to form fused cyclopentane systems.*[231]

- **Miscellaneous Reactions**

Other synthetic approaches include: Nazarov cyclisations (***Scheme 3.10***),[233] radical-mediated processes,[234] cross-coupling processes (***Scheme 3.11***),[235,236] and cyclocarbonylation reactions.[237]



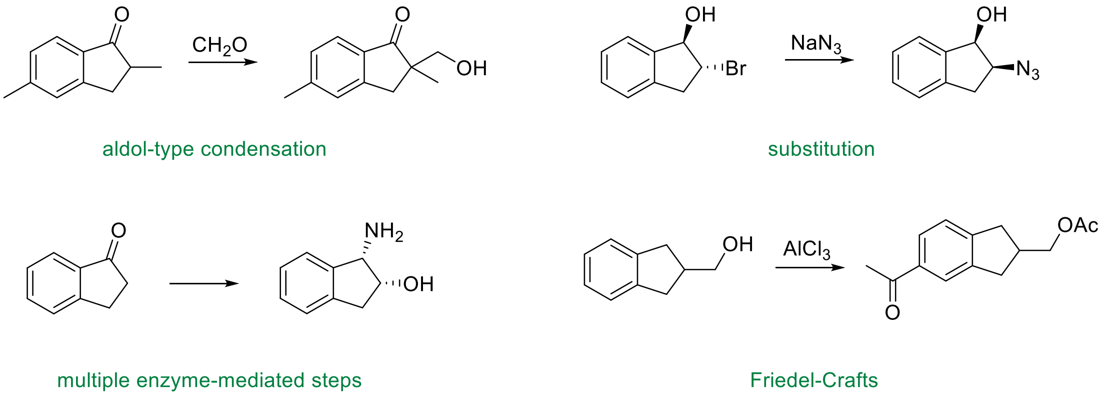
Scheme 3.10: *Synthesis of indanones by Nazarov cyclisation.*[233] *NFSI = N-Fluorobenzenesulfonimide.*



Scheme 3.11: *Heck-type cyclopalladation to form substituted indanes.*[236]

- **Functionalisation of Cyclic Precursors**

Derivatisation and desymmetrisation of existing carbocycles, both synthetically and enzymatically, is another method of obtaining synthetically useful indane fragments. Indanones and indanols are both common starting materials, enabling straightforward diversification of the cyclopentane ring under a range of conditions. Functionalisation of the aromatic ring is only reported under Friedel–Crafts conditions, thereby subject to steric constraints that favour reaction at the 5- position (***Scheme 3.12***).[238–241]



Scheme 3.12: *Functionalisation of existing indane nuclei.*

A limitation of all of the above approaches is the requirement for existing functionality when forming the indane unit, resulting in complex, poly-substituted products. Simpler, mono-substituted products are limited to oxidation of the cyclopentane unit, and Friedel–Crafts reaction at the 5-position of the aromatic, meaning few simple indanyl starting materials are readily accessible or commercially available.*

In the context of this project, the route to pentacyclic core **207** proposed retrosynthetically in Chapter 2 requires a 4-indanyl fragment **212a**, unfunctionalised in all other positions (***Scheme 3.13***). The previously attempted chloromethylation approach was unsuccessful, the reaction being dominated by steric factors and therefore affording mainly the 5-substituted product as part of an inseparable mixture. An alternative synthesis of this deceptively simple fragment was consequently necessary and investigations to that end are discussed in this chapter.

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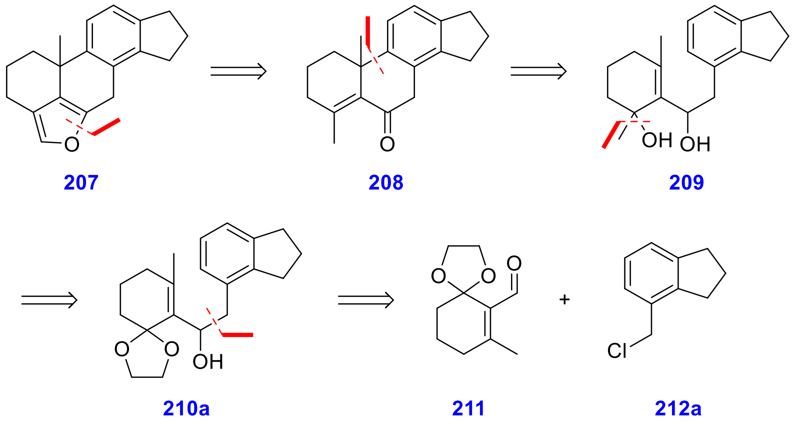
* 4-Nitroindane, 4-aminoindane, and 4-halogenated-1-indanones are commercially available but were deemed too expensive to be appropriate starting materials for large scale application.



Scheme 3.13: *Proposed retrosynthesis to pentacyclic core* ***207****.*

Attempted Syntheses in the Robertson Group

Efforts to obtain the 4- indanyl fragment were undertaken by several group members. Key results obtained in the work by Sutro (phthaloyl peroxide oxidation and C-H activation), Moloney (intramolecular Diels–Alder), and Keenlyside (Johnson–Claisen/Diels–Alder cascade) are summarised below.

Phthaloyl Peroxide Oxidation[*]

Phthaloyl peroxide is an arene-selective oxidant, proposed to operate *via* a radical rebound mechanism.[242,243] The previously unreported reaction with indane gave the separable indanols **265a** and **265b** in 87% yield and a 3:1 regioisomeric ratio (***Scheme 3.14***). However, owing to the explosive nature of this reagent, scale-up was deemed somewhat perilous and a safer alternative was sought.[244]

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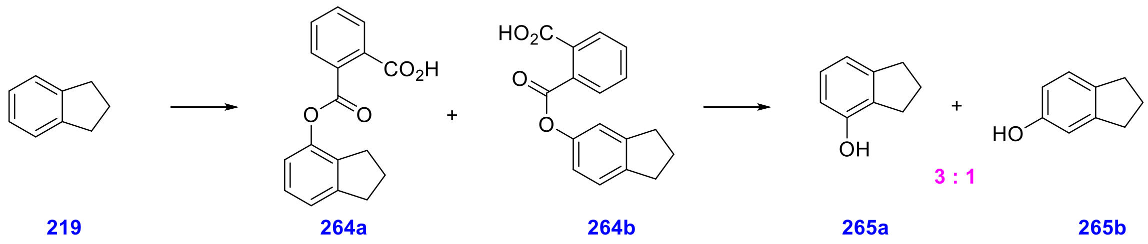
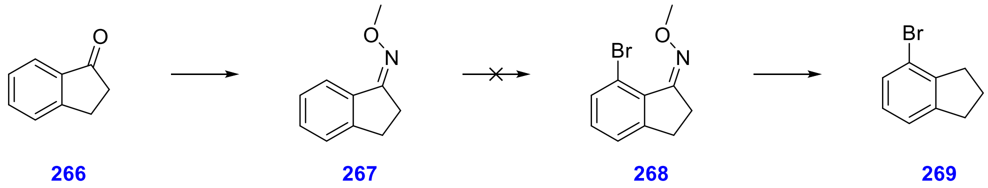
[*] Work carried out by Sutro.



Scheme 3.14: *Phthaloyl peroxide oxidation of indane.*

C-H Activation***

Sanford *et al*. reported a successful methyl oxime ether-directed C-H insertion for tetralone using Pd(OAc)$_2$ and NBS.[245] The same reaction conditions applied to indanone were unsuccessful and a complex mixture of products was generated (***Scheme 3.15***).[244]



Scheme 3.15: *Attempted C-H insertion for indanone.*

Intramolecular Diels–Alder Reaction*

Literature precedent exists for the intramolecular Diels–Alder reaction of a carbonyl dienophile across a furan diene to yield bridged indane-like systems.[246,247] The requisite unsaturated ester **270** was synthesised and the desired Diels–Alder reactivity was observed, with the formation of bridged intermediate **271** (***Scheme 3.16***). However, this intermediate was unavoidably intercepted by the Lewis acid catalyst for the cycloaddition (Me_2AlCl) to give the secondary alcohol side product **273**.[248]

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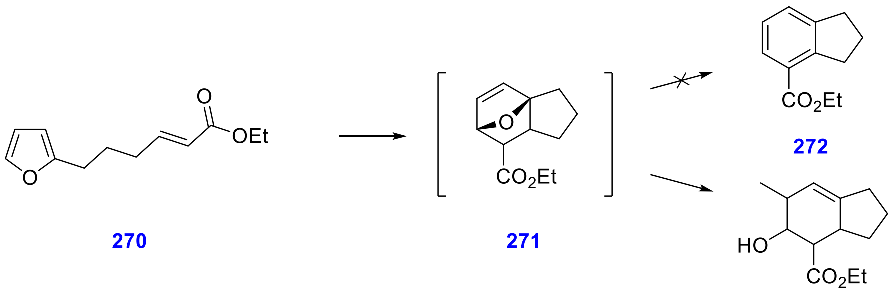
* Work carried out by Moloney.

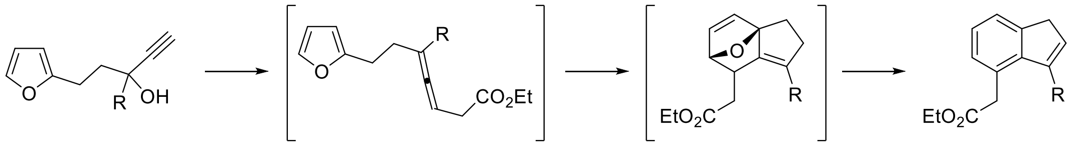


270 **271** **272** **273**

Scheme 3.16: *Intramolecular Diels–Alder reaction of unsaturated ester* ***270****.*

Johnson–Claisen/Diels–Alder Cascade*

A serendipitous discovery in a previous project within the group had demonstrated the possibility of obtaining indenes *via* a Johnson–Claisen/Diels–Alder cascade sequence.[249] Johnson–Claisen reaction of propargyl substrate **274** afforded the expected allene **276** but this immediately underwent rapid Diels–Alder cycloaddition and elimination to give indene **280** (***Scheme 3.17***). It was supposed that this reactivity could be reproduced on the nor-methyl substrate **275** and the resulting indene **281** reduced to the desired indane.



274 R = Me
275 R = H

276 R = Me
277 R = H

278 R = Me
279 R = H

280 R = Me
281 R = H

Scheme 3.17: *Johnson–Claisen/Diels–Alder cascade.*

In practice, this reactivity did not extend to substrate **275**, the reaction stalling at the allene intermediate **277**. As neither steric nor electronic influences were expected to be significant, it was proposed that the cyclisation occurs *via* a cationic intermediate arising from reversible protonation of the allene, rather than as a neutral [4+2] process (***Scheme 3.18***). Whilst the methyl substrate **276** is able to form a relatively stable tertiary/allylic

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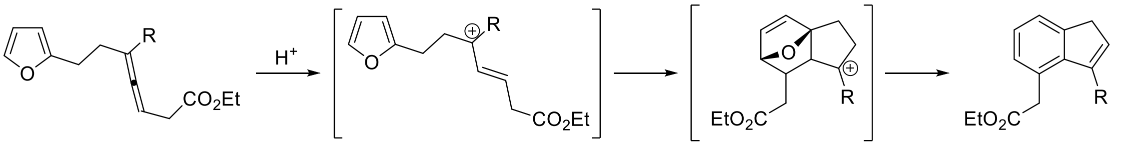
* Work carried out by Keenlyside.

cation, formation of the secondary/allylic cation on the nor-methyl substrate is presumably sufficiently less favourable to retard the cycloaddition step. This proposal was validated by attempted acid-catalysed-Diels–Alder reaction from both allenes, which yielded only methyl indene **280**, and so the route was abandoned.[250]

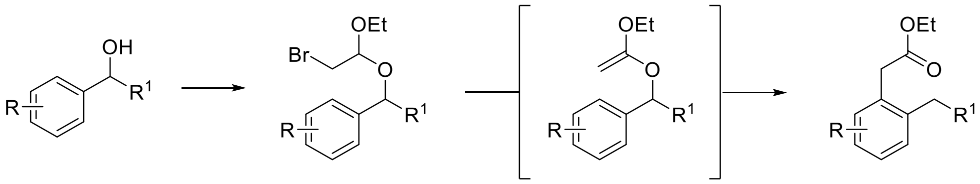


276 R = Me
277 R = H
282 R = Me
283 R = H
284 R = Me
285 R = H
280 R = Me
281 R = H

Scheme 3.18: *Cycloaddition via a proposed cationic intermediate.*

[3,3]-Sigmatropic Rearrangement

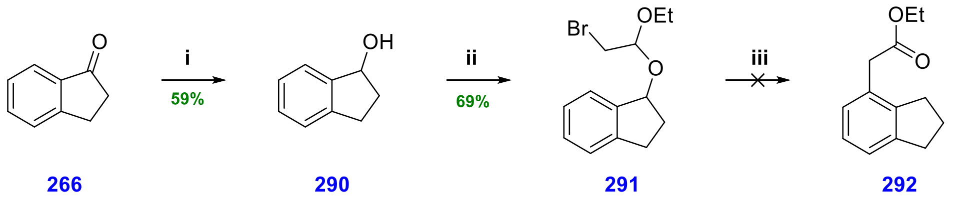
The Claisen rearrangement of benzyl vinyl ethers has historically been a missing substrate class, the loss of aromaticity during the reaction proving an insurmountable barrier. In 2017, McGeary *et al.* reported a one-pot procedure, overcoming this difficulty by use of an α-alkoxy substituent on the vinyl group to reduce the activation barrier.[251,252] Proceeding *via* a bromoacetal **287**, numerous benzylic alcohols **286** were converted into benzyl ketene acetals **288** which successfully underwent [3,3]-sigmatropic rearrangement to afford (*ortho*-tolyl)acetates **289** (***Scheme 3.19***). A brief investigation was instigated to see if this methodology would apply to an indane substrate.



286 **287** **288** **289**

Scheme 3.19: *McGeary's aromatic Claisen rearrangement.*

Indanone was reduced to 1-indanol **290** with sodium borohydride, and bromoacetalation gave intermediate **291** in 69% yield (***Scheme 3.20***). Following the reported protocol, bromoacetal **291** was stirred at room temperature with potassium *tert*-butoxide in DMF, to effect elimination to the ketene acetal, then the mixture was heated to 155 °C in order to effect the Claisen rearrangement.



Scheme 3.20: *Synthesis of* ***292*** *from indanone.* **Reagents and conditions: i)** $NaBH_4$, EtOH, rt, 3 h; **ii)** Br_2, ethyl vinyl ether, DIPEA, DCM, rt, 24 h; **iii)** KO*t*-Bu, DMF, 155 °C, 15 h.

These conditions failed to form any of the expected product ester **292**; instead, a mixture of products **290** and **293**–**296** (***Figure 3.02***) was generated. Alcohols **290** and **293** are both hydrolysis products of bromoacetal **291**, and aldehydes **294** and **295** are DMF-derivatives of these respective alcohols. The amine **296** likely arises from displacement of the bromine by decomposed DMF. Critically, this product distribution demonstrated that elimination from the bromoacetal was incomplete and the ketene acetal had not been formed under the reaction conditions prior to heating, therefore making rearrangement impossible.

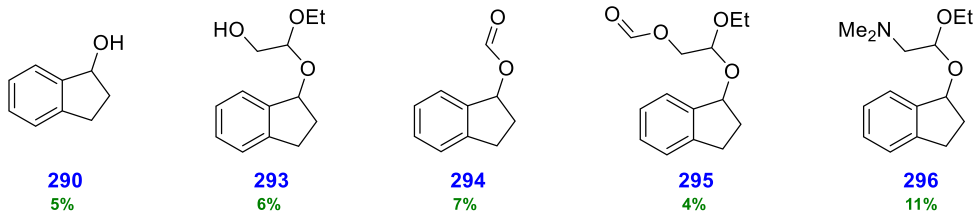
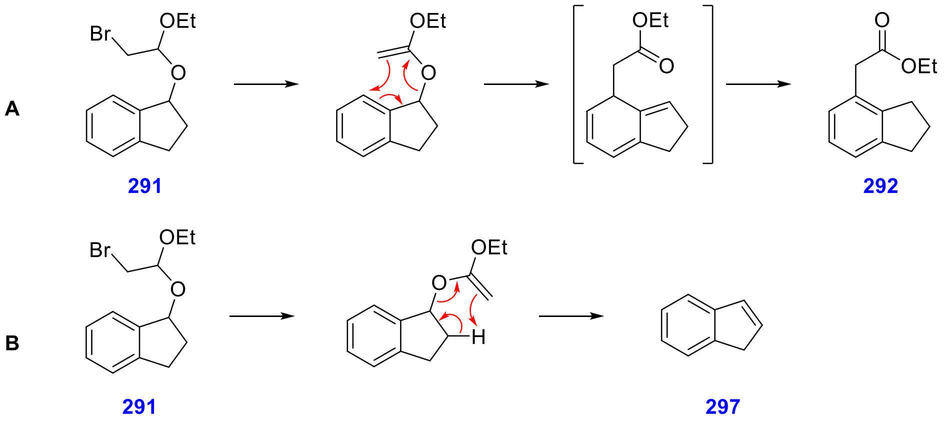


Figure 3.02: *Products arising from attempted rearrangement of* ***291****.*

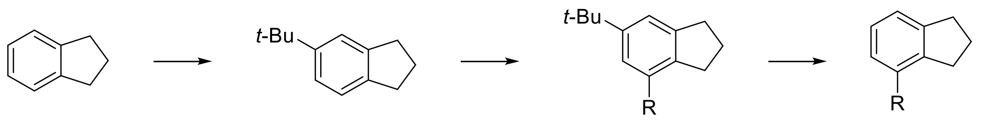
Work on this route was continued by Moloney and separation of the elimination and rearrangement steps was investigated.[248] Bromoacetal **291** was stirred at room temperature until full consumption of starting material was observed by TLC, then the temperature increased to induce rearrangement. Whilst small amounts of the product ester **292** were generated with this modification, repeat iterations were inconsistent and poor crude yields were obtained. In some cases, especially when the work-up solvent was incompletely evaporated, indene **297** was observed which suggested that a more-favourable retro-ene reaction competes with the desired Claisen rearrangement, which could also account for the poor mass return (***Scheme 3.21***). Crucially, the retro-ene reaction avoids the high-energy loss of aromaticity required in the Claisen rearrangement and, as this could not be overcome, this route also was abandoned.



***Scheme 3.21: A)** Desired Claisen rearrangement to form ester **292**. **B)** Proposed retro-ene reaction to form indene.*

Friedel–Crafts/Jocic Rearrangement Strategy

When Neudeck *et al.* encountered difficulties in accessing 4-chloromethyl indane **212a** free from the 5- regioisomer, they applied a blocking group strategy, first using Friedel–Crafts *tert*-butylation to block the more accessible 5-position, then introducing the desired functionality in the 4-position.[253] The *tert*-butyl group was subsequently removed to leave the 4-substituted indane (***Scheme 3.22***).



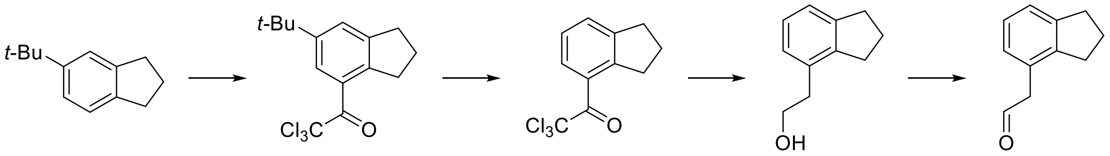
219 **298**

	R
299a	COMe
300	CO_2H
301	CO_2Me

	R
224a	CH_2OH
212a	CH_2Cl

Scheme 3.22: *Neudeck's route to 4-chloromethyl indane* ***212a****.*

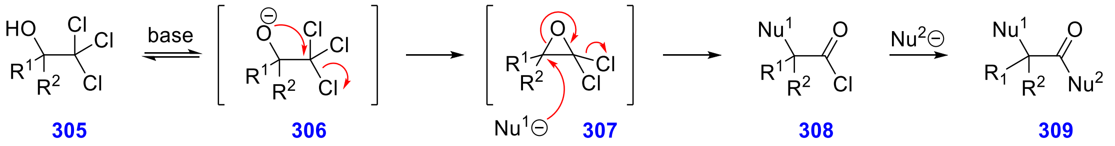
Significant functional group manipulation was required to obtain the chloromethyl product so a shorter route was proposed to indanyl aldehyde **251a**. It was hoped that by installing a trichloroacetyl group (**302**), in place of Neudeck's acetyl group, the resulting unblocked indane **303** would undergo a Jocic-type rearrangement under reductive conditions to generate alcohol **304**, which could then be oxidised to aldehyde **251a** (***Scheme 3.23***).



298 **302** **303** **304** **251a**

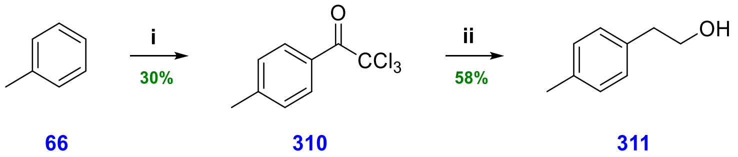
Scheme 3.23: *Proposed synthesis of aldehyde* ***251a****.*

The Jocic-type rearrangement proposed is actually a special case of the Bargellini reaction, famed for its adaptability and ease of modification.[254] Deprotonation of the carbinol **305** under basic conditions results in rapid formation of the *gem*-dichloro epoxide **307** (***Scheme 3.24***). This reactive intermediate is readily attacked by external nucleophiles that include phenol in the original Bargellini reaction,[255] azide in the Corey–Links reaction,[256] hydride in the Jocic reaction,[257] the solvent, or chloride (in a formal 1,2-chloride shift). The resultant α-substituted acyl chloride **308** then undergoes attack by a second nucleophile to give the product α-substituted carboxylic acid derivative **309**. Snowden *et al*. reported the use of this reaction for the homologation of primary alcohols and aldehydes *via* a (trichloromethyl)carbinol intermediate **305**.[257]



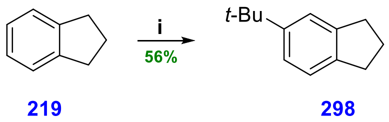
***Scheme 3.24:** Mechanism of the Bargellini rearrangement from (trichloromethyl)carbinol **305**.*

It was presumed that this reactivity would translate well to a starting keto substrate which would be reduced to the requisite carbinol under the reaction conditions. This was validated on a simple system: toluene was trichloroacetylated under standard conditions to afford **310** which was then subjected to a modified variant of Snowden's conditions. Hence, heating of **310** with five equivalents of $LiBH_4$ and three equivalents of NaOH in isopropanol gave expected product **311** in 58% yield (***Scheme 3.25***).



***Scheme 3.25:** Validation of strategy on toluene as a test substrate.* **Reagents and conditions: i)** trichloroacetyl chloride, $AlCl_3$, DCM, rt, 1 h; **ii)** $LiBH_4$, NaOH, IPA, 85 °C, 2 h.

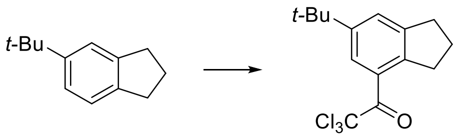
Synthesis of the requisite indanyl substrate thus commenced by stirring indane with $AlCl_3$ and *tert*-butylchloride at 0 °C, according to literature precedent, affording *tert*-butyl indane **298** in 56% yield after distillation (***Scheme 3.26***).



***Scheme 3.26:** tert-Butylation of indane.* **Reagents and conditions: i)** *t*-BuCl, $AlCl_3$, DCM, 0 °C, 2 h.

Subsequent trichloroacetylation proved problematic (***Table 3.01***). Standard acetylation conditions resulted in a complex mixture using either trichloroacetyl chloride or trichloroacetic anhydride (*entries **1** – **3***). The weaker Lewis acid $BF_3{\cdot}OEt_2$ failed to induce reaction at a range of temperatures, as did aluminium dodecatungstophosphate (*entries **4** – **6***).[258] Direct trichloroacetylation of **298** is not reported and this approach was ultimately abandoned as it was presumed that the electron donating *tert*-butyl group over-activated the indane, resulting in uncontrollable reaction.

Table 3.01: *Attempted trichloroacetylation of* ***298****.*



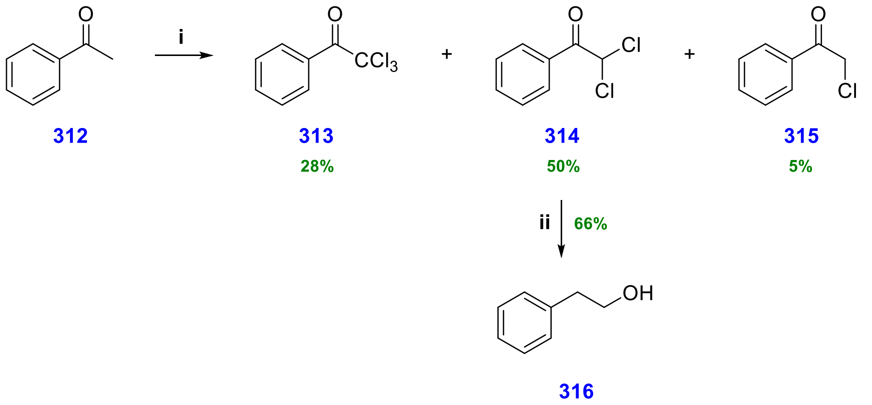
298 **302**

Entry	E+	Catalyst	Temperature/°C	Time/h	Outcome
1	A	$AlCl_3$	0	1	Decomposition
2	B	$AlCl_3$	0	1	Decomposition
3	C	$AlCl_3$	0	1	Decomposition
4	B	$BF_3 \cdot OEt_2$	0	3	**298** only
5	B	$BF_3 \cdot OEt_2$	0 to reflux	21	**298** only
6[a]	B	$AlPW_{12}O_{40}$	70	2.5	**298** only

Reagents and conditions: *1.1 eq of the electrophile, 0.5 M DCM.* [a]*No solvent. A = old trichloroacetyl chloride, B = new trichloroacetyl chloride, C = trichloroacetic anhydride.*

A stepwise strategy was therefore necessary. Because acetylation of **298** had been reported, its chlorination was investigated. Trichloroisocyanuric acid (TCCA) is a solid chlorine source, widely favoured commercially for its ease of use.[259] Each successive chlorination deactivates the system but control of time, temperature, and molar equivalents has been shown to allow selectivity for the extent of chlorination.

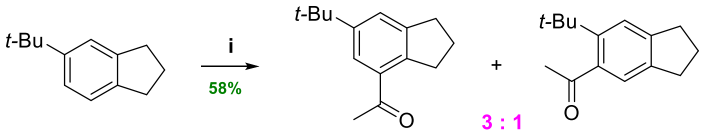
This process was first tested on acetophenone to establish the conditions necessary for the reaction to reach completion. After five hours of heating to reflux in acetic acid with two equivalents of TCCA, a mixture of the three chlorination products **313**, **314**, and **315** was obtained in 28%, 50%, and 5% yields, respectively (***Scheme 3.27***). The reaction was repeated over 8 hours but this did not increase the proportion of trichlorinated product **313**, instead resulting in a diminished mass return.



Scheme 3.27: Chlorination and rearrangement of acetophenone. **Reagents and conditions: i)** TCCA, acetic acid, Δ, 5 h; **ii)** $LiBH_4$, NaOH, IPA, 85 °C, 2 h.

However, given the reported flexibility of the Jocic reaction, we expected that rearrangement would still be possible from the dichlorinated product. Accordingly, dichloromethyl ketone **314** was subjected to the Jocic conditions and found to afford the expected benzyl carbinol product **316** in 66% yield.

Having established the feasibility of this stepwise strategy, attention returned to the indane system. *tert*-Butylindane **298** was acetylated according to literature precedent to afford a mixture of *tert*-butylacetyl indanes **299a** and **299b** in a 3:1 ratio (***Scheme 3.28***). Neudeck proposes that this arises through unavoidable partial cleavage of the *tert*-butyl group under the reaction conditions, leaving the more reactive 5- position vulnerable to reaction. However, it is more likely that the *tert*-butyl group reduces rather than prevents reaction in the 5- position. **299a** and **299b** decomposed upon attempted distillation and were only partially separable chromotographically meaning that limited quantities of pure **299a** could be obtained. As a result, the mixture was carried forward without further purification in the hope that later products would be easier to separate.



298 **299a** **299b**

Scheme 3.28: *Acetylation of tert-butyl indane* ***298****.* **Reagents and conditions: i)** $AlCl_3$, AcCl, DCM, –78 °C to rt, 1 h.

Attempted chlorination afforded a complex mixture so it was necessary to remove the *tert*-butyl group before further manipulation occurred. De-*tert*-butylation had been reported using two equivalents of $AlCl_3$ in toluene at 0 °C but application of these conditions to the **299a**/**299b** mixture failed to produce the desired result and 53% of the starting material was returned, heavily enriched in **299a**. A 19% yield of **317b** was also isolated, demonstrating that removal is possible under these conditions, but is more rapid for the 5- substituted indane (***Table 3.02****, entry* ***1***).

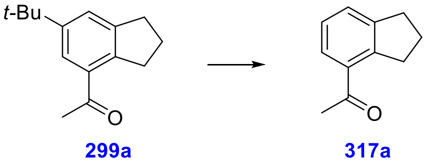
Purification of the *tert*-butylacetyl indane mixture was clearly essential: multiple chromatography columns afforded pure **299a** but significant loss of material was incurred. The reaction was repeated with this newly purified material at higher temperatures but this likewise returned only starting material (*entries* ***2*** *and* ***3***).

The quality of $AlCl_3$ is highly variable, depending on its exposure to air, so a portion was used that had been dried *in vacuo* prior to reaction but, again, only starting material was returned (*entry* ***4***). Mechanistically, removal of the *tert*-butyl group should require a proton, so $AlCl_3$ presumably catalyses the process *via* HCl present, or generated, *in situ*. With this in mind, a further two portions of $AlCl_3$ were dried *in vacuo* and the reaction repeated with the addition of 10% water and 10% concentrated HCl respectively.[260] However, no reaction was observed (*entries* ***5*** *and* ***6***). Repetition of the acylation conditions (minus the acetyl chloride) which Neudeck proposed had resulted in partial cleavage of the *tert*-butyl group were also trialled without success (*entry* ***7***).

There are limited reports of other reagents to achieve this transformation. Use of HBr in refluxing acetic acid, as reported by Wuest *et al.*,[261] returned only starting material, as did $Na_2S_2O_4$ in refluxing TFA (*entries **8** and **9***).[262] Several reports detail the use of clays and solid super acids which were not available to us but a similar reaction with activated Amberlyst also returned starting material (*entry **10***).[263,264]

A trace of product **317a** was observed when conc. H_2SO_4 in refluxing toluene was applied but predominantly the reaction returned starting material (*entry **11***). As no conditions could be found to remove the *tert*-butyl group, despite direct literature precedent, this promising approach towards fragment **212a** also had to be abandoned.

Table 3.02: *Attempted de-tert-butylation of **299a**.*



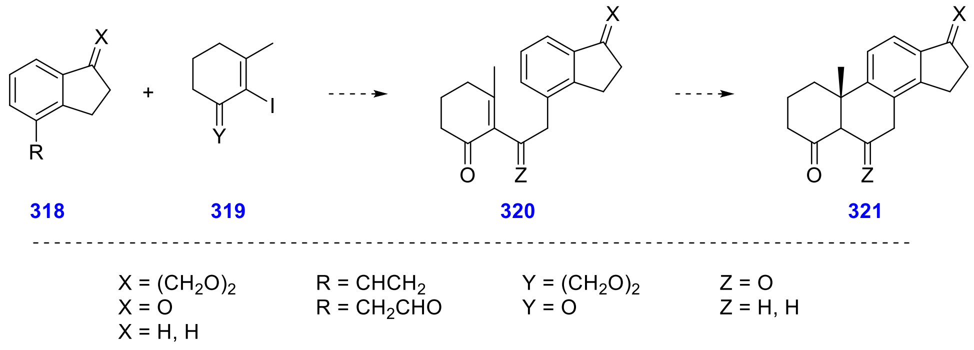
Entry	Catalyst	Catalyst Treatment	Solvent	Temperature / °C	Time / h	Outcome
1[a]	$AlCl_3$	-	Toluene	0	6	**299a** + 19% **317b**
2	$AlCl_3$	-	Toluene	65	24	**299a**
3	$AlCl_3$	-	Toluene	Δ	16	**299a**
4	$AlCl_3$	Dried o/n	Toluene	rt	7	**299a**
5	$AlCl_3$	Dried o/n + 10% water	Toluene	rt	6	**299a**
6	$AlCl_3$	Dried o/n + 10% HCl	Toluene	rt	6	**299a**
7	$AlCl_3$	-	DCM	rt	24	**299a**
8	HBr	-	AcOH	Δ	16	**299a**
9	$Na_2S_2O_4$	-	TFA	Δ	24	**299a**
10	Amberlyst	HCl activated	Toluene	Δ	5	**299a**
11	H_2SO_4	-	Toluene	Δ	3	**299a** + trace **317a**

[a] *Mixture of **299a** and **299b**. Δ signifies temperature required for reflux.*

Fries Rearrangement

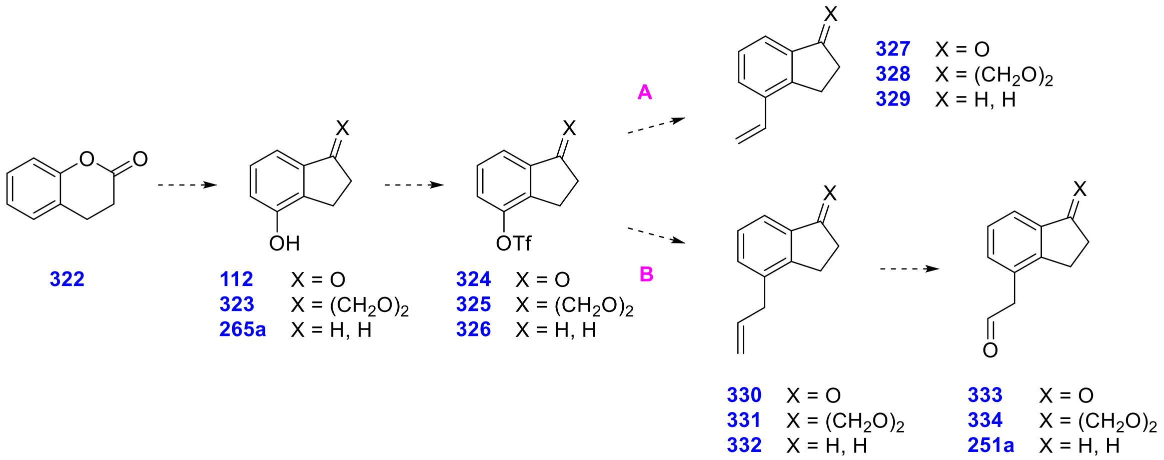
The Fries rearrangement of dihydrocoumarin to hydroxyindanone is an established reaction, proceeding *via* fusion of dihydrocoumarin with $AlCl_3$ at high temperatures in solvent-free conditions.[265] Previous attempts to repeat this reaction within the group had been unsuccessful and this,[244] coupled with the necessary reduction of the carbonyl group following the initial rearrangement, had discouraged pursuit of this strategy.

However, the difficulty of accessing suitable indanyl fragments by any other method led to re-evaluation of this approach. Given that the stated aim of this project is to screen as many different carbocyclic cores as possible, derivatisation of the indane fragment **318** prior to coupling with cyclohexyl fragment **319** would provide ready access to a number of subtly different cores **321** (***Scheme 3.29***).



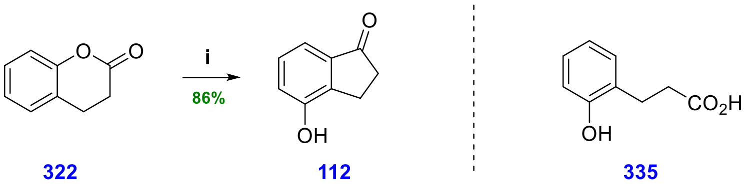
Scheme 3.29: *Proposed progression of viridiol synthesis from indanyl fragments.*

Therefore, a more divergent synthesis plan of the indane fragments was proposed, starting from dihydrocoumarin **322** (***Scheme 3.30***). Hydroxyindanone **112** could be protected as the acetal **323**, reduced to 4-indanol **265a**, or carried through directly. Triflation would therefore afford three different substrates for Stille (or similar) cross-coupling (**324**, **325**, and **326**), possible with either vinyl- (**A**) or allyl-tributylstannane (**B**). The vinyl products **327**–**329** could be used in a further coupling reactions with cyclohexenyl fragment **214** ultimately to give tetracycles **321** of the type Z = H, H. The allyl products could be cleaved oxidatively to give the respective aldehydes **333**, **334**, and **251a** for which coupling conditions are already established. Overall, this approach would give access to six different indane fragments, potentially resulting in six distinct carbon tetracycles.



Scheme 3.30: *Proposed divergent synthesis of indane fragments.*

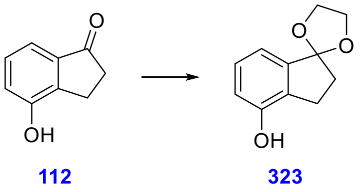
To begin with, dihydrocoumarin **322** was fused with $AlCl_3$ at 180 °C and stirred for three hours, following the conditions in the original report. As had been previously observed within the group, this did not yield the expected hydroxyindanone product. Instead, acid **335** was isolated, indicating that the lactone had been opened, but the oxonium ion intercepted by adventitious water rather than the aromatic. A more recent procedure was found and, with the addition of NaCl and a higher temperature, indanone derivative **112** was obtained as expected, in 86% yield (***Scheme 3.31***).[266]



Scheme 3.31: *Fries rearrangement of dihydrocoumarin **322**.* **Reagents and conditions:** $AlCl_3$, NaCl, 200 °C, 1 h.

A portion of this material was taken aside for the acetal protection. Under Dean–Stark conditions, reactions in either benzene or toluene were insufficiently vigorous to induce reaction, instead just returning starting material (***Table 3.03***). Protection of hydroxyindanone **112** in this way is not a reported transformation and conjugation of the carbonyl with the hydroxyl group, through the aromatic system, may account for this otherwise surprising lack of reactivity. In the interest of time, no further conditions were trialled.

Table 3.03: *Attempted protection of* ***3.03****.*



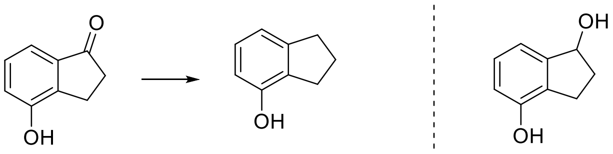
Entry	Solvent	Temperature/°C	Outcome
1	Benzene	100	**112**
2	Toluene	110	**112**

Reagents and conditions: *ethylene glycol, p-TsOH, 0.2 M solvent, 24 h.*

Attention moved to the removal of the ketone, which is reported under Clemmensen conditions.[265] Initial reaction gave a poor yield of 19% and repetition with a modified work-up gave an unavoidably contaminated product in a yield of 28% (***Table 3.04***, *entries* ***1*** and ***2***). Reduction with sodium cyanoborohydride and ZnI_2 resulted in a moderate improvement: 24% yield,[267] whereas hydrosilane reduction returned only starting material (*entries* ***3*** and ***4***).[268] Hydrogenation conditions with Pd/C gave the partial reduction product **336** in quantitative yield,[269] whilst hydrogenation with $Pd(OH)_2/C$ as catalyst failed to induce any reaction at all (*entries* **5** and **6**). A more aggressive approach was clearly necessary so the Wolff–Kishner reaction was investigated.[270] After a promising initial yield of 67%, subsequent reactions were found to be inconsistent and the harsh conditions resulted in damage to the glassware (*entry* ***7***). A milder variant of the reaction was attempted, using *p*-toluenesulfonyl hydrazide with a subsequent sodium cyanoborohydride reduction, giving **265a** in 39% yield (*entry* ***8***) and so the harsher

Wolff–Kishner conditions were ultimately chosen to advance the synthesis.[270] The aforementioned conjugation of the carbonyl, through the aromatic system, is likely to be enhanced under basic conditions, particularly when the hydroxyl group is deprotonated and is a potential reason for its resistance to reduction and the requirement for harsher conditions.

Table 3.04: *Reduction of hydroxyindanone* ***112****.*

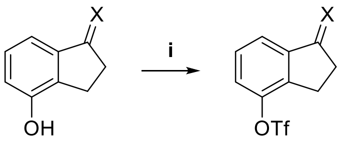


112 **265a** **336**

Entry	**Reagents**	**Solvent**	**Temperature/°C**	**Time/h**	**Outcome**
1	Activated Zn	AcOH	Δ	18	19% **265a**
2	Activated Zn	AcOH	Δ	18	28% **265a** (cont.)
3	ZnI_2, $NaBH_3CN$	DCE	Δ	2	24% **265a**
4	Triethyl silane, TFA	DCM	0 – rt	48	**112** only
5	H_2, Pd/C, TEA	EtOH	50	24	Quant. **336**
6	H_2, $Pd(OH)_2/C$	MeOH/THF	rt	24	**112** only
7	$N_2H_4 \bullet H_2O$	EtOH	130 – 180	22	67%, 21%, 47% **265a**
8	*p*-$TsNHNH_2$, $NaBH_3CN$	MeOH	Δ	22	39% **265a**

Δ signifies temperature required for reflux.

With **112** and **265a** in hand, triflation in pyridine was straightforward and gave cross-coupling substrates **324** and **326** in 67% and 65% yields, respectively (***Scheme 3.32***).

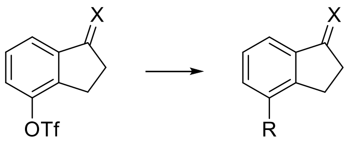


112 X=O **324** X=O 67%
265a X=H, H **326** X=H, H 65%

Scheme 3.32: *Triflation of* ***112*** *and* ***265a****.* **Reagents and conditions: i)** Tf_2O, pyridine, 0 °C, 5 min.

Standard Stille coupling conditions were found and, following a slight modification to the work-up, **327**, **329**, **330**, and **332** were all obtained in acceptable yields (***Table 3.05***).[270]

Table 3.05: *Stille coupling.*



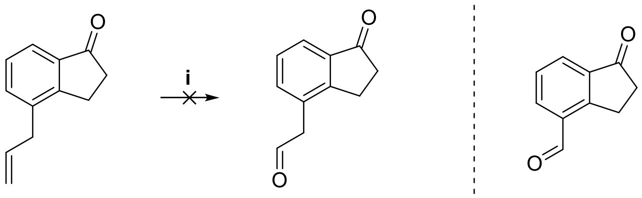
324 X=O
326 X=H, H

327 X=O and R = vinyl
329 X=H, H and R = vinyl
330 X=O and R = allyl
332 X=H, H and R = allyl

Entry	Triflate	Stannane	Yield
1	**324**	Vinyl	59%
2	**324**	Allyl	72%
3	**326**	Vinyl	Quant.
4	**326**	Allyl	87%

Reagents and conditions: *1.1 eq vinyl- or allyl-$SnBu_3$, 10 mol% $Pd(PPh_3)_4$, LiCl, toluene, Δ, 16 h.*

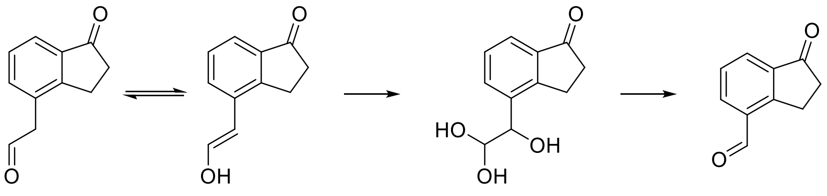
Allyl indanone **330** was then subjected to conditions for one-pot oxidative cleavage (OsO_4 and $NaIO_4$) but, surprisingly, this resulted in the quantitative formation of truncated aldehyde **337** (***Scheme 3.33***).



330 **333** **337**

Scheme 3.33: *Attempted one-pot oxidative cleavage of allyl indanone* ***330****.* **Reagents and conditions: i)** OsO_4, $NaIO_4$, pyridine, dioxane/water (3:1), dark, rt, 24 h.

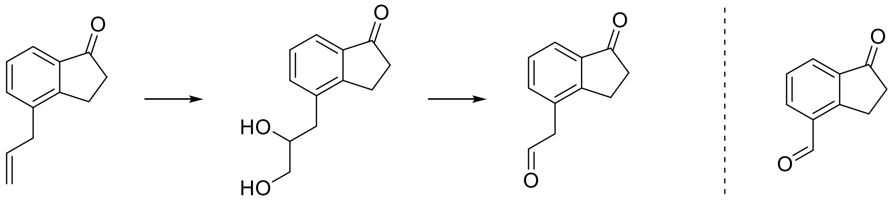
This product likely arises through oxidative cleavage of the expected product aldehyde **333** which might readily enolise under the reaction conditions (owing to conjugation with the aromatic), thereby providing another alkene for dihydroxylation and periodate cleavage (***Scheme 3.34***).



Scheme 3.34: *Proposed formation of truncated aldehyde* ***337****.*

To prevent this undesirable over-reaction, a stepwise approach was evaluated to ensure removal of all OsO_4 before cleavage could occur. Dihydroxylation proceeded in quantitative yield under both sets of conditions trailed but, despite thorough work-up (***Table 3.06***, *entry* ***2***), subsequent periodate cleavage still resulted in the formation of aldehyde **337**. This suggests that either trace OsO_4 still remained after work up, or that the chain shortening event occurs *via* a different mechanistic pathway.

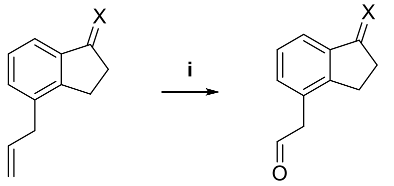
Table 3.06: *Oxidative cleavage following different dihydroxylation conditions.*



Entry	OsO_4	Dihydroxylation Solvent	Work-Up	Outcome
1	10 mol%	Acetone/*t*-BuOH/H_2O (20:3:1)	Concentration	Quant. **337**
2	2 mol%	THF/H_2O/acetone (1:1:1)	Full work-up	Mixture of **333** and **337**

Reagents and conditions: *NMO, rt, then $NaIO_4$, THF/H_2O (3:1), rt. Full work-up indicates quenching, extraction, and concentration prior to the periodate cleavage step.*

As this undesirable reactivity could not be suppressed, both allyl indane **332** and allyl indanone **330** were subjected to ozonolysis using a Wallace and Tiernan BA023012 ozoniser to give aldehydes **251a** and **333** in 41% and 30% yields, respectively (***Scheme 3.35***).

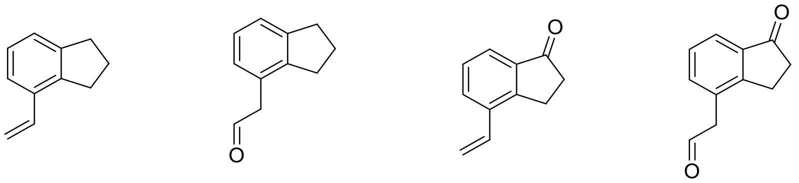


330 X = O
332 X = H, H

333 X = O 30%
251a X = H, H 41%

Scheme 3.35: *Ozonolysis of* ***330*** *and* ***332****.* **Reagents and conditions: i)** O_3, sudan red, DCM, –78 °C.

Through these routes, the four desired indane fragments **329**, **251a**, **327**, and **333** were obtained in good yields in between three and five steps from dihydrocoumarin (***Figure 3.03***).



329 Quant. **251a** 41% **327** 59% **333** 30%

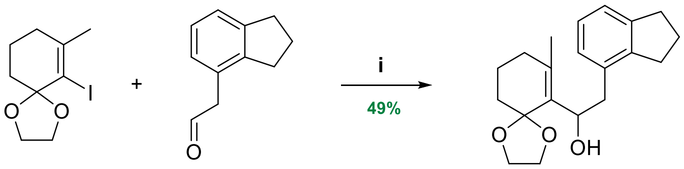
Figure 3.03: *Indane fragments successfully synthesised.*

Chapter 4

Completion of the Steroidal Core of Viridiol

Synthesis with Acetaldehyde-Indane Fragments 251a and 333

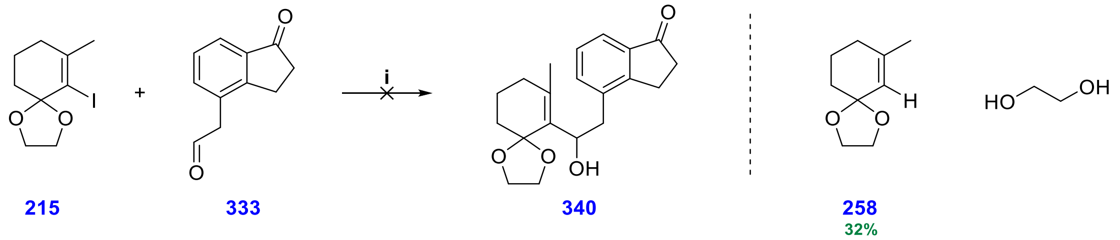
With aldehydes **251a** and **333** in hand, it was possible to return to the union of these fragments with iodide **215**. The conditions established in Chapter 2 were applied to aldehyde **251a** and pleasingly afforded expected alcohol **210a** in 49% yield (this substantial improvement upon the initial reaction discussed in Chapter 2 (27%) was attributed to the larger scale of this iteration) (***Scheme 4.01***).



215 **251a** **210a**

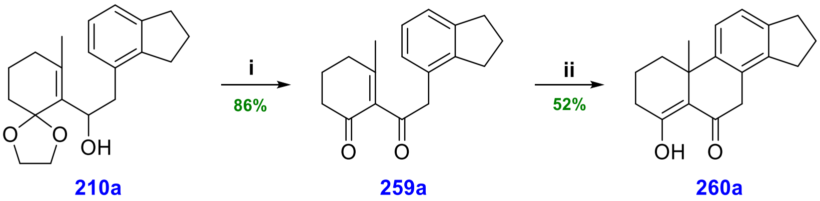
Scheme 4.01: *Union of iodide **215** with aldehyde **251a**.* **Reagents and conditions: i)** *n*-BuLi (1.6 M in hexanes), THF, –78 °C, 2 h.

However, repetition with aldehyde **333** was unsuccessful and a complex mixture of products was obtained (***Scheme 4.02***). Protodehalogenated compound **258** and ethylene glycol were identified by ^{1}H NMR analysis but the conspicuous lack of aromatic peaks indicated a problem with the availability of aldehyde fragment **333** during the reaction. The additional carbonyl present in **333** makes this compound vulnerable to polymerisation and it was supposed that various side reactions had sequestered the material before the intended addition reaction could take place. Consequently, no further reactions were attempted with this material.



Scheme 4.02: *Attempted union of iodide* ***215*** *with aldehyde* ***333***. **Reagents and conditions: i)** *n*-BuLi (1.6 M in hexanes), THF, –78 °C, 2 h.

Continuing the precedent set in Chapter 2, alcohol **210a** was oxidised (and deprotected) with DMP to afford diketone **259a** in 86% yield; this was subjected to the Sames catalyst system to effect cyclisation to tetracycle **260a** (52% yield) (***Scheme 4.03***).[188]



Scheme 4.03: *Synthesis of tetracycle* ***260a***. **Reagents and conditions: i)** DMP, DCM, rt, 2 h; **ii)** 10 mol% AgOTf, 5 mol% $RuCl_3$, DCE, 80 °C, 14 h.

Assignment of the enol form was confirmed by NMR analysis. The ^{13}C NMR spectrum revealed five quaternary carbon centres in the region 100 – 150 ppm, four belonging to the aromatic ring, and the last (112.5 ppm) assigned to the **C**=C-OH enol carbon at C-5 (***Figure 4.01***). This was supported by the absence of a resonance in the ^{1}H NMR spectrum for a bridgehead proton, and the HMBC correlation between the resonance at 112.5 ppm in the ^{13}C spectrum with the resonances belonging to both H-3 and H-7. The high-shift resonances at 195.6 and 184.8 ppm were assigned as the carbonyl and quaternary enol carbon respectively, enabling determination of their positions through HMBC analysis. Strong correlation of the pair of doublets at 3.6 ppm (H-7) with the ^{13}C resonance at 195.6 ppm but not with 184.8 ppm showed that the ketone was positioned at C-6 and the enol at C-4, an assignment further confirmed by the correlation of the ^{13}C resonance at 184.8 ppm with the H-3 resonances.

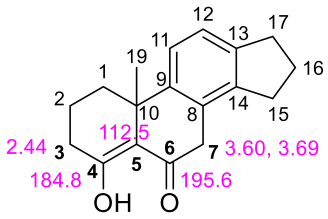
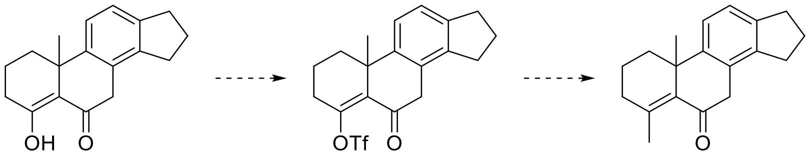


Figure 4.01: *Relevant ^{1}H and ^{13}C NMR resonances used in the identification of enol **260a**. All NMR chemical shifts are given in ppm.*

Continuation of this route is envisaged *via* enol triflation and a cross-coupling reaction (for example with Bu_3SnMe or similar) or cuprate addition to install the final carbon but time constraints and a lack of ready material prevented trial of these final steps (***Scheme 4.04***).



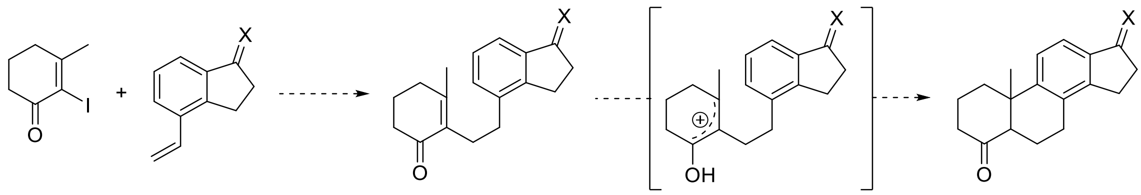
260a **341** **208**

Scheme 4.04: *Proposed completion of viridiol carbon skeleton.*

Completion of this route as proposed could be the subject of future work and would provide a viridiol-like carbon skeleton suitable for enzymatic study in a total of seven steps from commercially available starting materials.

Synthesis with Vinyl-Indane Fragments 327 and 329

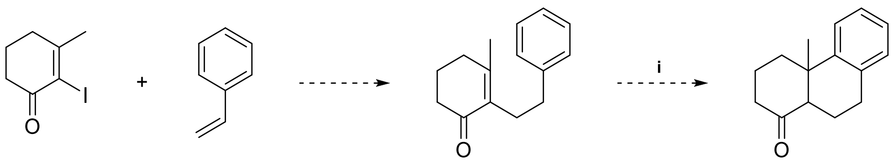
For vinyl fragments **327** and **329**, it was necessary to develop an alternative coupling strategy (***Scheme 4.05***). It was envisaged that a Suzuki cross-coupling would afford the tethered indane-cyclohexenyl unit **342** or **343**, which was expected to be a suitable substrate for an acid-mediated cyclisation to tetracycle **344** or **345**.



214
327 X = O
329 X = H, H
342 X = O
343 X = H, H
344 X = O
345 X = H, H

Scheme 4.05: *Proposed progression of viridiol synthesis with vinyl indane fragments.*

The cyclisation of the simpler phenyl substrate **347** is precedented and,[271] as both vinyl indanes **327** and **329** were precious materials, this strategy was first trialled using styrene as a model substrate (***Scheme 4.06***).

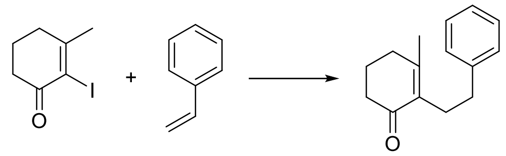


214 **346** **347** **348**

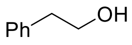
Scheme 4.06: *Proposed formation of model system **347** and its reported cyclisation.* **Reagents and conditions: i)** 85% H_3PO_4, 170 °C, 12 h, 70%.[271]

A report by Johnson *et al.* detailing Suzuki cross-couplings with α-iodoenones seemed a promising starting point for this system and the reported conditions ($PdCl_2$(dppf)•DCM with Ph_3As as a ligand) were duly tried (***Table 4.01**, entry **1***).[272] No cross-coupling was evidenced but alcohols **316** and **349** were isolated, likely arising from oxidation of any boranes present during work up. A variety of subtle modifications to the hydroboration and Suzuki steps were therefore tested and these, likewise, did not result in productive reaction, instead giving unexpected products **350** and **351** (*entries **2*** to ***5***).[273–276] Alkene **350** presumably forms through a competing Heck reaction (*entry **4***), whilst compound **351** seems to result from cross-coupling of the regioisomeric hydroboration product, likely present in equilibrium with the desired isomer at the higher reaction temperature (*entry **5***). Ultimately, low temperature hydroboration, followed by cross-coupling at 70 °C with NaOH as the base, gave the desired ketone in 9% yield (*entry **6***).

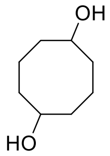
Table 4.01: *In-situ hydroboration and Suzuki cross-coupling conditions.*



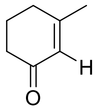
214 **346** **347**



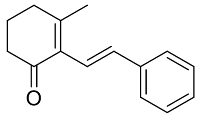
316



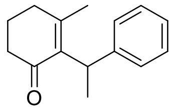
349



253



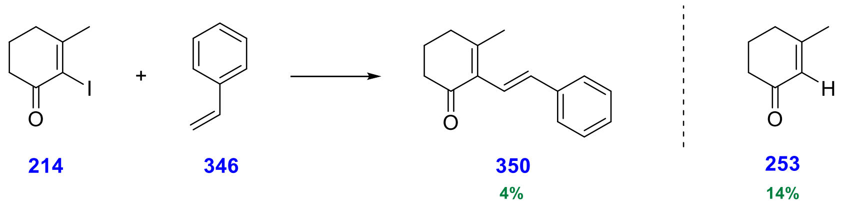
350



351

Entry	Hydroboration Conditions	Suzuki Conditions	Outcome
1	9-BBN (1 eq), THF, rt, 4 h	$PdCl_2$(dppf)•DCM (4 mol%), Ph_3As (15 mol%), Cs_2CO_3, DMF, rt, 18 h	12% **316**, 27% **349**
2	9-BBN (2 eq), THF, 50 °C, 2 h	$PdCl_2$(dppf)•DCM (4 mol%), Ph_3As (15 mol%), Cs_2CO_3, DMF, rt, 18 h	No products isolated
3	9-BBN (1 eq), THF, rt, 4 h	$Pd(PPh_3)_4$ (10 mol%), Cs_2CO_3, DMF, rt, 18 h	42% **214,** 8% **253**, 8% **349**
4	9-BBN (1 eq), THF, rt, 4 h	$PdCl_2$(dppf)•DCM (3 mol%), NaOH, THF, 70 °C, 18 h	19% **350**, 34% **253**
5	9-BBN (2 eq), THF, 50 °C, 2 h	$PdCl_2$(dppf)•DCM (3 mol%), NaOH, THF, 70 °C, 18 h	43% **351**, 48% **349**
6	9-BBN (1 eq), THF, 0 °C, 1 h, then rt 2 h	$PdCl_2$(dppf)•DCM (2 mol%), NaOH, THF, 70 °C, 18 h	9% **347**

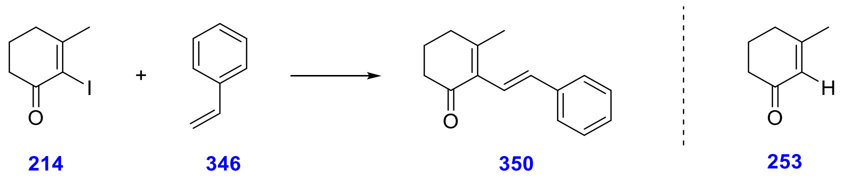
Despite this hint of success, the obtained 9% yield was too low to be practically useful and the Suzuki cross-coupling strategy was abandoned; however, the formation of unexpected alkene **350** under the conditions in *entry **4*** was interesting and a Heck reaction to form this product intentionally was therefore considered. In the first instance, the Suzuki conditions were repeated without prior formation of the trialkylboron, but surprisingly this reaction resulted in a very poor mass return and ultimately only 4% of the expected product **350** and 14% of the proto-dehalogenated iodide **253** were isolated (***Scheme 4.07***).



Scheme 4.07: *Repetition of the Suzuki cross-coupling conditions without formation of a trialkylboron.* **Reagents and conditions: i)** $PdCl_2$(dppf)•DCM (3 mol%), NaOH, THF, 70 °C, 18 h.

More conventional Heck conditions were therefore sought, and a standard procedure used within the group was trialled (***Table 4.02***). The initial outcome was modest with just 29% of the expected alkene product **350** isolated and it was considered that the large excess of triethylamine (five equivalents) may be deactivating the catalyst (*entry **1***). Reduction of this excess gave an encouraging increase in yield (*entry **2***) and switching to the more hindered base diisopropylethylamine, intended to supress nucleophilic attack of the Pd catalyst, afforded the expected alkene **350** in 62% yield (*entry **4***).

Table 4.02: *Heck conditions.*

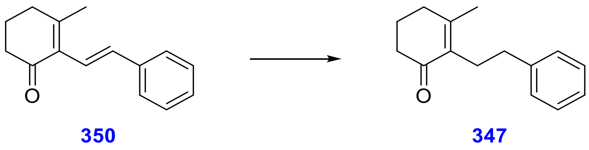


Entry	Base	Equivalents	350	253
1	TEA	5	29%	14%
2	TEA	2	40%	36%
3	TEA	1.2	SM only	
4	DIPEA	1.2	62%	—

Reagents and conditions: *$Pd(OAc)_2$ (2 mol%), $P(o\text{-}tol)_3$ (4 mol%), toluene, 110 °C, 18 h.*

Attempted hydrogenation of the linking double bond under standard conditions returned only starting material (***Table 4.03****, entry **1***) but reduction with diimide, generated *in situ* from 2,4,6-triisopropylbenzenesulphonyl hydrazide (TPSH), gave reduced product **347** cleanly in 75% yield (*entry **2***).

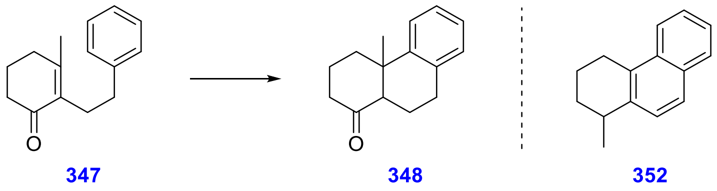
Table 4.03: *Reduction of the linking double bond.*



Entry	Conditions	Outcome
1	Pd/C, H_2, EtOAc, rt	SM
2	TPSH, TEA, THF, 70 °C	75% **347**

The cyclisation of ketone **347** to tricycle **348** had been reported by Stork to proceed in 70% yield upon heating with phosphoric acid.[271] In repeating this reaction, tricycle **348** was formed (as a mixture of *cis* and *trans* isomers) but the naphthyl product **352** was also produced through competitive cyclisation into the carbonyl, and the yields resulting from the need to separate reaction components chromatographically were lower than reported. Furthermore, repetitions of this procedure gave wildly varying results for no discernible reason, making this an unreliable reaction with which to progress the synthesis (***Table 4.04***).

Table 4.04: *Inconsistent results from Stork's cyclisation procedure.*



Entry	348	352
1	8%	26%
2	35%	20%
3	23%	15%
4	6%	14%

Reagents and conditions: *5 eq H_3PO_4, 170 °C, 12 h.*

Several alternatives to the Stork conditions were then considered (***Table 4.05***): In the first instance, the Sames catalyst system (AgOTf/$RuCl_3$), applied previously to both model system **248** and indanyl substrates **259a** and **259b**, was trialled but both returned only starting material (*entries **1** and **2***).[188*] A more recent amendment to Stork's conditions, intended to supress formation of the naphthyl side product **352** by forming the acid slowly *in situ* from methanesulphonic acid and P_2O_5 was next considered but reaction at both 35 °C and 100 °C likewise returned only starting material (*entries **3** and **4***).[277] Within Stork's original report was a second procedure for a related substrate, incorporating one equivalent of H_2SO_4 and a lower reaction temperature (120 °C). Under these conditions, the product tricycle **348** was formed, but the formation of a second (unidentified) product of similar mass, and the low isolated yield of tricycle **348** meant that this was still not a viable alternative (*entry **5***). It was then considered that dilution of the reaction would favour the intramolecular reaction under these conditions and limit the likely polymerisation of starting ketone **347**. A modification of Stork's initial conditions with 20 equivalents of H_3PO_4 (in place of five) gave an improved mass return and a higher yield of the product tricycle (*entry **6***); however, the loss of a comparable amount of material through the formation of naphthyl product **352** was problematic and dilution with a co-solvent was considered.

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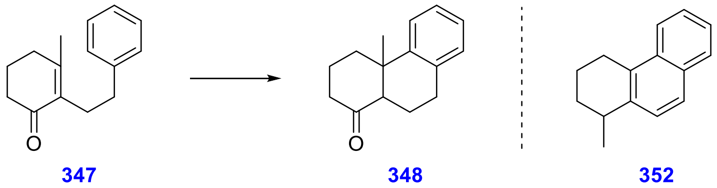
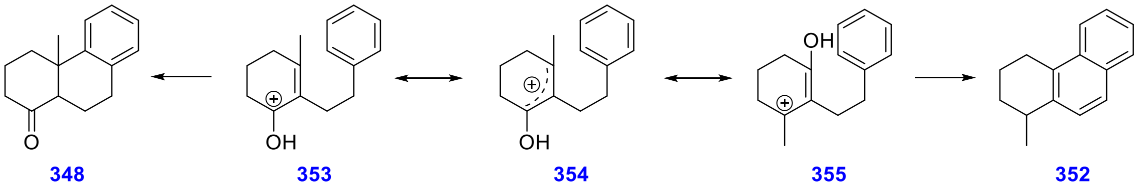
[*] The model substrate **248** and the indanyl substrates **259a** and **259b** required different ratios of the catalyst reagent system to effect cyclisation. Both conditions were trialled here.

Table 4.05: *Screen of cyclisation conditions.*



Entry	Conditions	Temperature (°C)	Outcome
1	0.1 eq AgOTf, 0.05 eq $RuCl_3$, DCE	85	SM
2	0.4 eq AgOTf, 0.2 eq $RuCl_3$, DCE	85	SM
3	2 eq MsOH, 0.2 eq P_2O_5	35	SM
4	2 eq MsOH, 0.2 eq P_2O_5	100	SM
5	5 eq H_3PO_4, 1 eq H_2SO_4	120	23% **348** + other products
6	20 eq H_3PO_4	170	37% **348**, 28% **352**

Under acidic conditions, the carbonyl of ketone **347** becomes protonated to form an unsymmetrical allylic cation **354** which forms either the naphthyl **352** or ketone product **348** depending on the end at which addition occurs (***Scheme 4.08***).



Scheme 4.08: *Formation of naphthyl and ketone products from common allylic cation.*

As the allylic system is flat, the benzyl group rotates out of the plane of the rest of the molecule, meaning that either the methyl or hydroxyl is slightly tucked under the aromatic, depending on the conformation (***Figure 4.02***). It was postulated that it may be possible to stabilise productive conformations of the cation by employing a polar solvent that might favour conformations in which the hydroxyl group is located on the accessible outside of the molecule (**A**).

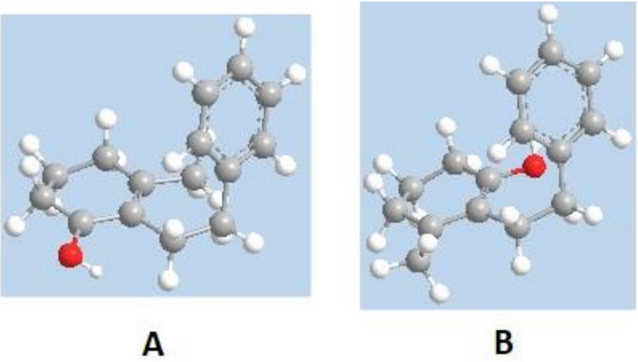
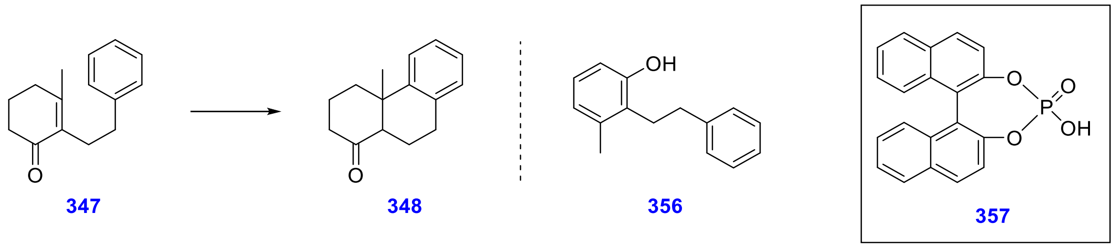


Figure 4.02: *3D representations of **A)** a productive conformation to give ketone product **348** with the hydroxyl group on the accessible outside face; **B)** an 'unproductive' conformation, giving naphthyl product **352** with the hydroxyl group slightly under the aromatic ring. Structures generated using Chem3D 18.2.*

Accordingly, a variety of polar protic and aprotic solvents were selected to test this hypothesis ($MeNO_2$, HFIP, and DCM) and ketone **347** was treated with two equivalents of H_3PO_4 and heated to reflux in each.[*] In the event, all reactions returned only starting material (table of individual results in **Appendix A**). Analogous reactions were trialled with proline and 1,1′-binaphthyl-2,2′-diyl hydrogenphosphate **357** which both had the potential advantage of providing a relatively bulky counterion able to coordinate with the protonated oxygen, possibly hindering 1,2-cyclisation and thus minimising formation of naphthyl product **352**. The majority of these reactions returned only starting material but the reactions in toluene and nitromethane also afforded small amounts of aromatised product **356** (***Scheme 4.09***).



Scheme 4.09: *Attempted cyclisation of ketone **347** under acidic conditions.* **Reagents and conditions:** 2 eq acid, 0.5 M in solvent, heated to reflux, 18 h.

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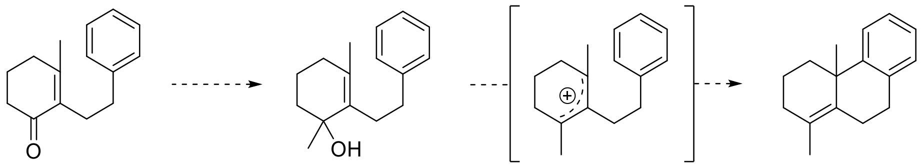
[*] Toluene was also used as an apolar comparison.

It was proposed that a more strongly activating effect was required to achieve cyclisation and ‘super Brønsted acid’ catalysis was therefore considered. A discussion by Yamamoto on the activation of unsaturated carbonyls by acids such as triflimide, in particular as its *N*-trimethylsilyl derivative, was interesting.[278] Triflimide was therefore stirred at room temperature with allyl trimethylsilane to form trimethylsilyltriflimide, obtained by vacuum distillation and used directly (***Scheme 4.10***).[279,280]

Scheme 4.10: *Formation of TMS-triflimide.* **Reagents and conditions: i)** rt, 1 h.

Screening of ketone **347** with both triflimide and TMS-triflimide resulted in either the complete loss or complete recovery of starting material (table of individual results in **Appendix A**). With this, and having trialled a variety of acidic conditions, none of which had achieved reliable cyclisation, it was concluded that the steric influence of the methyl group may contribute to the competing 1,2-cyclisation and an alternative approach was necessary.

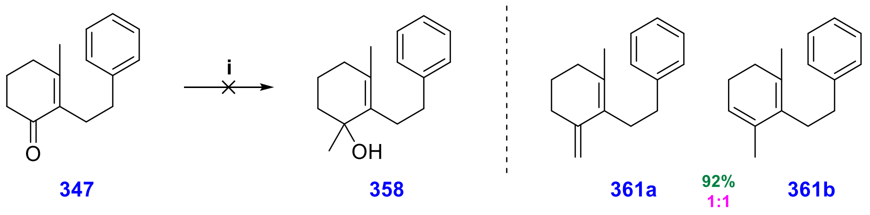
A slight modification was therefore envisaged (***Scheme 4.11***): formation of alcohol **358** by Grignard reaction with ketone **347**, followed by loss of water under acidic conditions would produce symmetrical allyl cation **359** which could be trapped *in situ* by the aromatic ring to form cyclised product **360**.



347 **358** **359** **360**

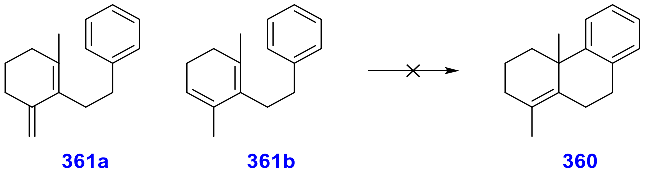
Scheme 4.11: *Proposed amendment of the cyclisation strategy.*

When ketone **347** was treated with methylmagnesium bromide, the expected alcohol **358** eliminated directly (most likely during the acidic work-up) and alkenes **361a** and **361b** were produced in a 1:1 ratio in 92% yield (***Scheme 4.12***).



Scheme 4.12: *Grignard addition into ketone **347**.* **Reagents and conditions: i)** MeMgBr (3.0 M in Et_2O), Et_2O, 0 °C, 16 h.

As cation **359** could still, mechanistically, be formed from these alkenes by protonation, this mixture was briefly investigated but no progression to cyclised products was observed under a variety of conditions (***Scheme 4.13***) (table of individual results in **Appendix B**).



Scheme 4.13: *Attempted cyclisation of alkenes **361a** and **361b** under acidic conditions.*

As no productive cyclisations could be achieved from ketone **347**, attention turned to preceding alkene **350**. The *E*-geometry of alkene **350** made cyclisation directly from this substrate problematic; however, it was envisaged that either thermal or photochemical conditions could promote equilibration with the *Z*-isomer to facilitate formal electrocyclisation (***Figure 4.03***).

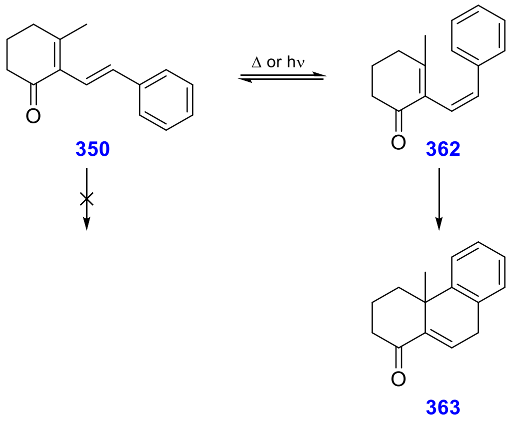
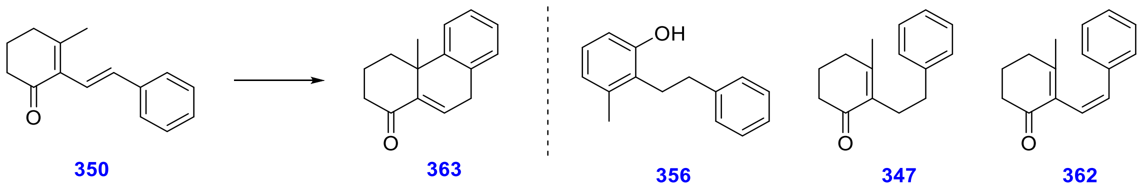


Figure 4.03: *Isomerisation of the double bond under thermal or photochemical conditions to facilitate formal electrocyclisation.*

Heating *E*-alkene **350** to reflux in both toluene and xylene resulted in no reaction (***Table 4.06**, entries **1** and **2***) but at 200 °C (in a pressure tube), apparent disproportionation to ketone **347** and alcohol **356** was observed (*entry **3***).

UV-vis spectrometry revealed λ_{max} = 285 nm for alkene **350** so it was considered that irradiation with a PenRay lamp would prove productive if there was a sufficient tail in the lamp's output, nominally rated at 254 nm. Although no cyclised product was formed, prolonged irradiation resulted in a 20% isolated yield of the *Z*-isomer (with 14% of *E*-**350** being recovered) (*entry **4***) which could, in principle, then be cyclised thermally.

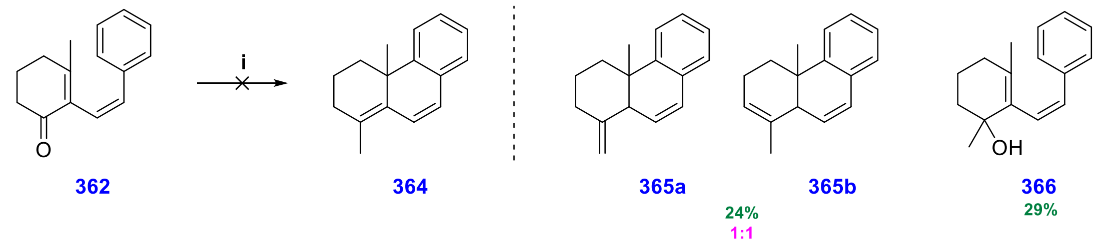
Table 4.06: *Attempted cyclisation of alkene **350** under thermal or photochemical conditions.*



Entry	**Solvent**	**Temperature (°C)**	**Outcome**
1	*o*-xylene	140	SM
2	Toluene	110	SM
3[a]	Toluene	200	9% **356**, 5% **347**
4[b]	MeCN	0	20% **362**, 14% SM

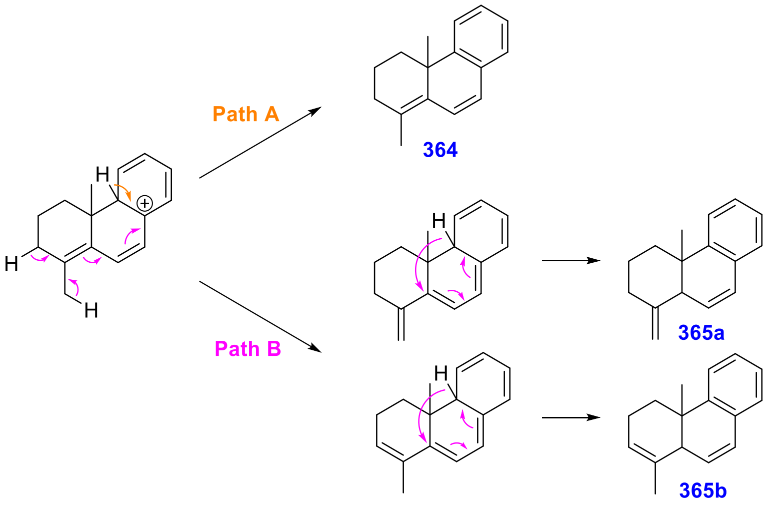
Reagents and conditions: *1.0 M in solvent.* [a] *pressure tube;* [b] *irradiation at 254 nm, 0.05 M in solvent, 20 h.*

Again, this idea proved unsuccessful and heating *Z*-alkene **362** to 200 °C in toluene (in a pressure tube) resulted in no reaction and starting material was returned. Following the approach taken with ketone **347**, Grignard addition into the ketone and elimination was next considered; gratifyingly, this resulted in the direct formation of cyclised products, with a further 29% of alcohol **366** isolated (due to incomplete elimination) (***Scheme 4.14***). Inseparable tricycles **365a** and **365b** (in a 1:1 ratio) were isolated in 24% yield and, whilst these were not the expected cyclic product, they represented a useful end-point for this model system.



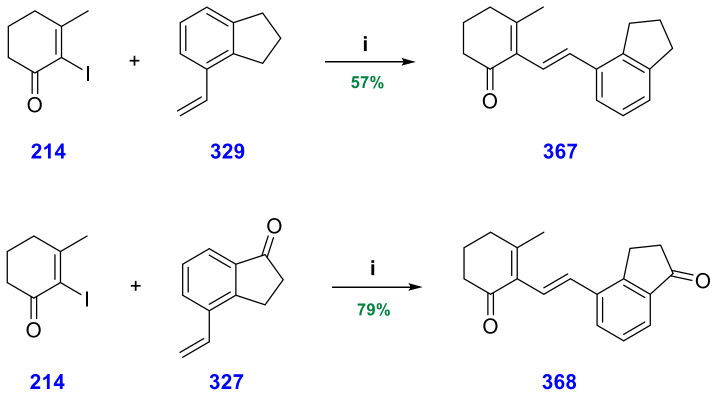
Scheme 4.14: *Grignard addition into ketone **362**.* **Reagents and conditions: i)** MeMgBr (3.0 M in Et_2O), Et_2O, 0 °C, 3 h.

Mechanistically, the formation of this pair of deconjugated alkenes is surprising. It was presumed that trapping of the aromatic cation would occur *via* the loss of the phenyl proton to give conjugated diene **364** (***Scheme 4.15***, Path **A**). However, the isolation of alkenes **365a** and **365b** suggests that loss of an aliphatic proton, either from the A-ring or methyl group, is a faster process and rearomatisation occurs following a [1,5]-shift of the phenyl proton to the bridgehead position (Path **B**).



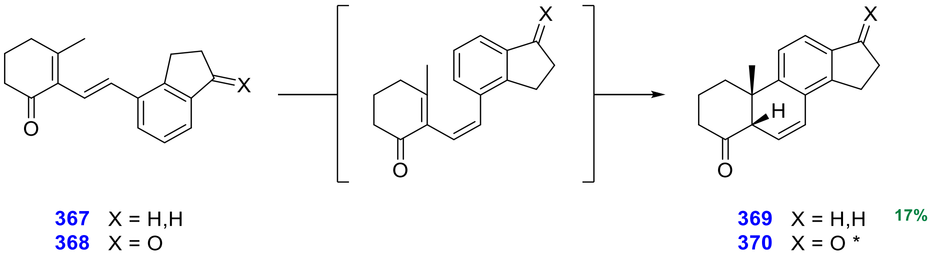
Scheme 4.15: *Proposed mechanism for the formation of deconjugated alkenes **365a** and **365b**.*

With an unoptimised route through to a cyclised product finally established, repetition with vinyl indane substrates **327** and **329** was then appropriate and treatment with $Pd(OAc)_2$ under Heck conditions furnished *E*-alkenes **367** and **368** in 57% and 79% yields respectively (***Scheme 4.16***).



Scheme 4.16: *Coupling with the vinyl substrates **327** and **329**.* **Reagents and conditions: i)** Pd(OAc)$_2$ (2 mol%), P(*o*-tol)$_3$ (4 mol%), DIPEA, toluene, 110 °C, 18 h.

Irradiation of both alkenes at 254 nm resulted in a complex mixture of products with none of the expected *Z*-product being isolated in either case. Nevertheless, small amounts of the steroidal cores **369** and **370** were obtained, presumably proceeding *via* the *Z*-isomer generated *in situ* (***Scheme 4.17***).



Scheme 4.17: *Photochemical cyclisation of alkenes **367** and **368**.* **Reagents and conditions:** MeCN, 0 °C, 254 nm light, 24 h. * The indanone product **370** could not be thoroughly separated and cleaned of impurities so a percentage yield is not given.

The stereochemistry of core **369** was determined by nOe analysis which revealed an interaction between the methyl group (C-19) and bridgehead proton (H-5), consistent with a *cis*-fused system (***Figure 4.04***).

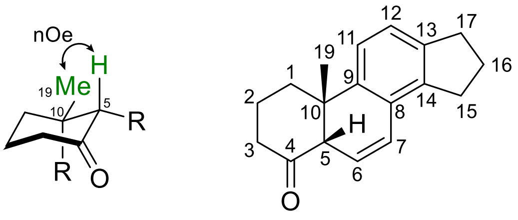
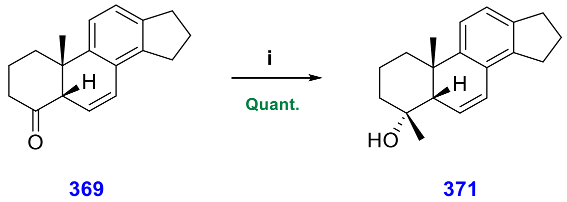


Figure 4.04: *Determination of stereochemistry for steroidal core **369**. Stereochemistry could not be determined for core **370** due to the presence of minor impurities.*

Finally, Grignard addition into the cyclohexanone was effected from core **369** to give alcohol **371** in quantitative yield, completing the carbon framework of viridiol in four steps from commercially available materials (***Scheme 4.18***).



Scheme 4.18: *Completion of the steroidal carbon core **371**.* **Reagents and conditions: i)** MeMgBr (3.0 M in Et_2O), Et_2O, 0 °C, 2.5 h.

The nOe detected between the methyl groups at C-19 and C-28, and the bridgehead proton H-5, confirmed the expected *cis*-geometry of these groups (***Figure 4.05***).

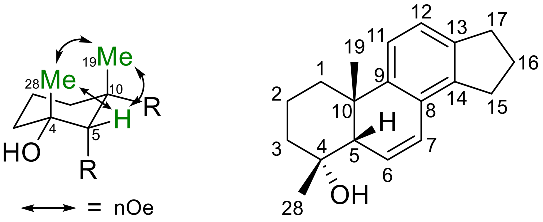


Figure 4.05: *Determination of stereochemistry for completed viridiol core **371**.*

The *cis*-decalin-type structure of ketone **369** probably exists in a rapidly equilibrating pair of conformers **369a** and **369b** (***Figure 4.06***). Nucleophilic attack occurs from the sterically less hindered *exo*-face to give alcohol **371** which likewise flips between two conformers **371a** and **371b**.

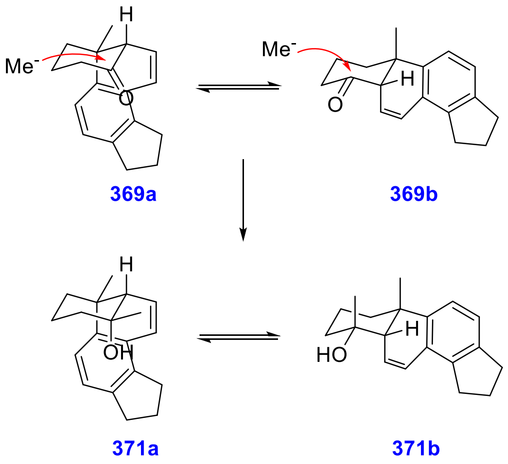
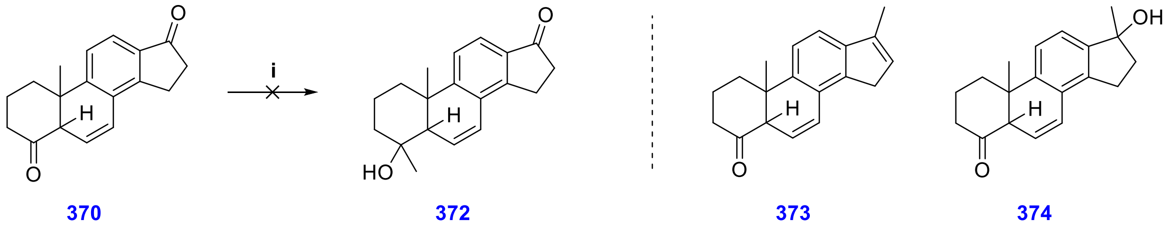


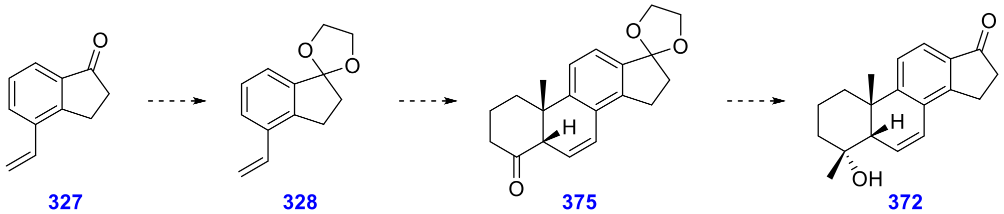
Figure 4.06: *Nucleophilic attack on the exo-face of ketone conformers **369a** and **369b**.*

Addition of the final carbon to core **370** was expected to be complicated by the presence of the strained and therefore potentially activated indanone carbonyl; indeed, treatment with methyl Grignard reagent afforded indene compound **373** and alcohol **374** as the major products (***Scheme 4.19***).



Scheme 4.19: *Attempted addition of final carbon to steroidal core **370**.* **Reagents and conditions: i)** MeMgBr (3.0 M in Et_2O), Et_2O, 0 °C, 2.5 h. Percentage yields not given due to minor impurities in starting ketone **370**. Alcohol **374** tentatively identified. Stereochemistry could not be determined.

Despite these promising developments, a lack of sufficient material, and constraints on time in producing more, prevented further elaboration of the indanone tetracycle. As the innate reactivity of the cyclopentanone could not be overcome, a protecting group strategy will be necessary in order to install the final carbon at the cyclohexanone. Although protection of the indanone carbonyl was not achieved from hydroxyindanone **112** (Chapter 3), probably owing to the deactivating effect of the *meta*-disposed hydroxyl group, it is likely that subsequent functionalisation of the phenol will have reduced the deactivation of the carbonyl group, suggesting that protection should be possible at a later stage. Future work could explore this possibility and potentially provide access to a further viridiol-like carbon skeleton for enzymatic study (***Scheme 4.20***).



327 **328** **375** **372**

Scheme 4.20: *Proposed protection strategy to complete viridiol-like carbon skeleton* ***372****.*

In summary, two synthetic routes towards differently oxidised viridiol skeletons have been developed and core **371**, prepared in four steps from commercially available materials, was obtained in sufficient quantity for enzymatic studies. Completion of tetracycles **260a** and **370** in a few steps can be envisaged, and two further viridiol skeletons should be accessible to provide invaluable contrasting substrates for P450-mediated oxidations.

Chapter 5

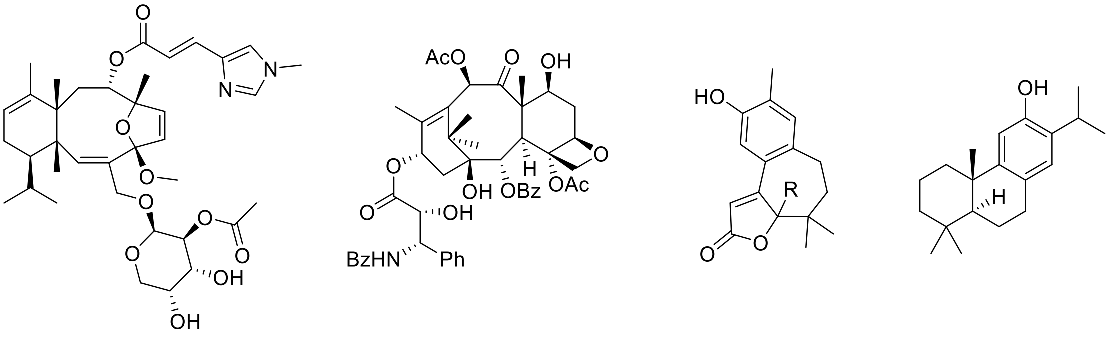
Late-Stage Oxidation with P450 Mutants

Research in the Wong Group

Research in the Wong group focusses on the development and characterisation of novel P450$_{BM3}$ mutants for selective oxidations in synthesis. The group have created a library of over 400 P450 variants, each with subtle differences in the size, shape, and polarity of the active site, to effect new or challenging C-H activations in a diverse range of compounds.

One area of particular interest is the application of P450 mutants to natural product-like scaffolds. The biosyntheses of many natural products include P450-mediated oxidations, and yet this is an under-utilised tool in chemical synthesis. In addition, modification and tuning of the active site enables C-H activation to occur at unactivated positions which would otherwise be inaccessible chemically. A collaboration between the Robertson and Wong groups is exploring this methodology.

Carbon-rich natural product frameworks are prepared chemically and screened against plates of 24 P450 mutants at analytical scale (µmol). Results are then analysed by GC to determine an approximate conversion of substrate into different products, and promising mutants are selected for preparative scale reactions (mmol), enabling isolation and identification of the oxidised products. Past targets have included eleutherobin **376**, taxol **47**, trigoxyphins K and L **377** and **378**, and ferruginol **379** (***Figure 5.01***).



eleutherobin **376**

taxol **47**

R = H trigoxyphin K **377**
R = OH trigoxyphin L **378**

ferruginol **379**

Figure 5.01: *Natural products studied in the Robertson and Wong group collaboration.*

Enzymatic Oxidation of Tricycle 249

Of particular relevance to this thesis is ferruginol, a diterpene belonging to the abietane family of compounds. Extracted first from conifers and later from a variety of other plant species, this compound (and others similar to it) has demonstrated both anti-inflammatory and anti-tumour activity against human cell lines.[281] In our group's work, the tricyclic unit **380** was prepared in three steps from geraniol and screened against two general enzyme plates (Y1 and Y5) and two steroid plates (SP1 and SP2).[282,283] Ten enzymes were selected for scale-up and ten oxidation products were isolated (***Figure 5.02***).

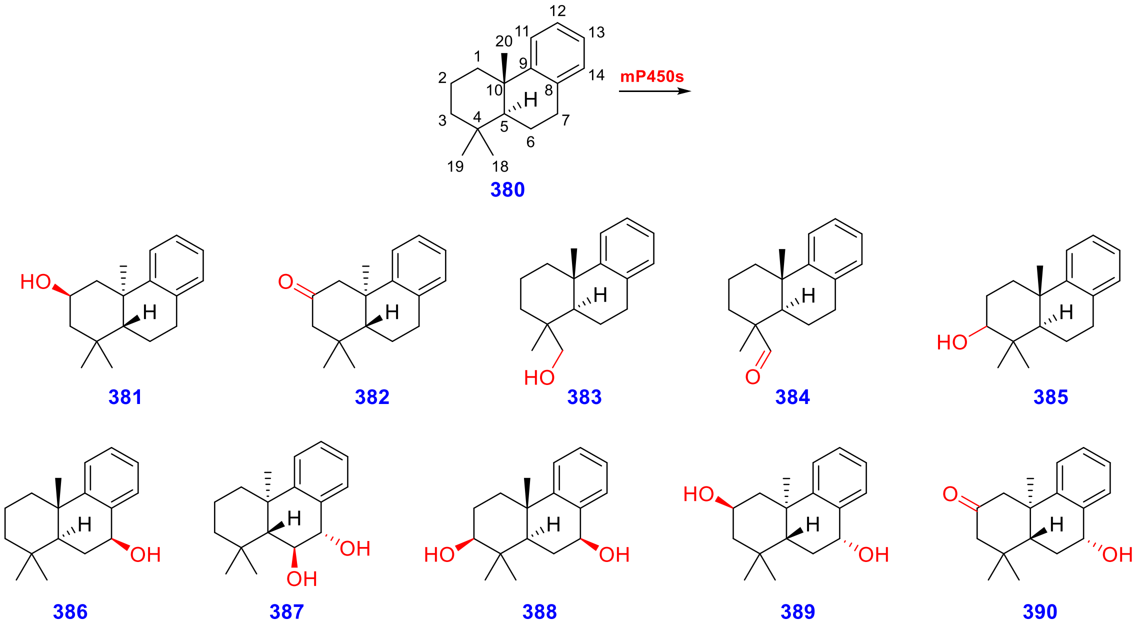
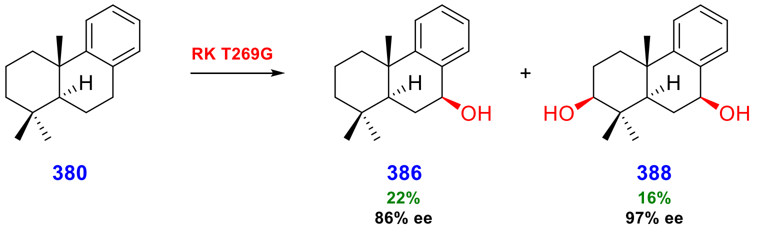


Figure 5.02: *Oxidation of racemic tricycle* ***380*** *with mutant P450 enzymes (mP450). Stereochemistry was not determined for all products.*

Mono-hydroxylation was observed at positions C-2, C-3, C-7 and C-18/19 (**381**, **383**, **385**, and **386**), with secondary oxidation also occurring at positions C-2 and C-18/19 (**382** and **384**). Three dihydroxylation products were isolated, each including oxidation at the benzylic position (**387**, **388**, and **389**), and a product resulting from three oxidation events was also obtained (**390**).

Previous work within the group had demonstrated kinetic resolution arising from P450 oxidations.[284] Two metabolites were therefore investigated (**386** and **388**) and, following preparation of racemic reference samples, the enantioenrichment was determined by chiral SFC (***Scheme 5.01***).



Scheme 5.01: *Kinetic resolution of tricycle* ***380****.*

The high % ee values obtained indicated a strong kinetic resolution effect for oxidation of substrate **380** with P450 mutant RK T269G and current work continues to investigate the behaviour of the racemate compared to the separate enantiomers to give insight into the kinetic resolution process.

The tricyclic model system **249** has many similarities to the ferruginol substrate, but the influence of the carbonyl and the *cis*-fused ring geometry was expected to result in a different product profile upon screening. The substrate was screened against general plates WY5 and WY6, the results of which are summarised in ***Chart 5.01*** and ***Chart 5.02***.

Chart 5.01: *Screening of tricycle **249** with plate WY5.*

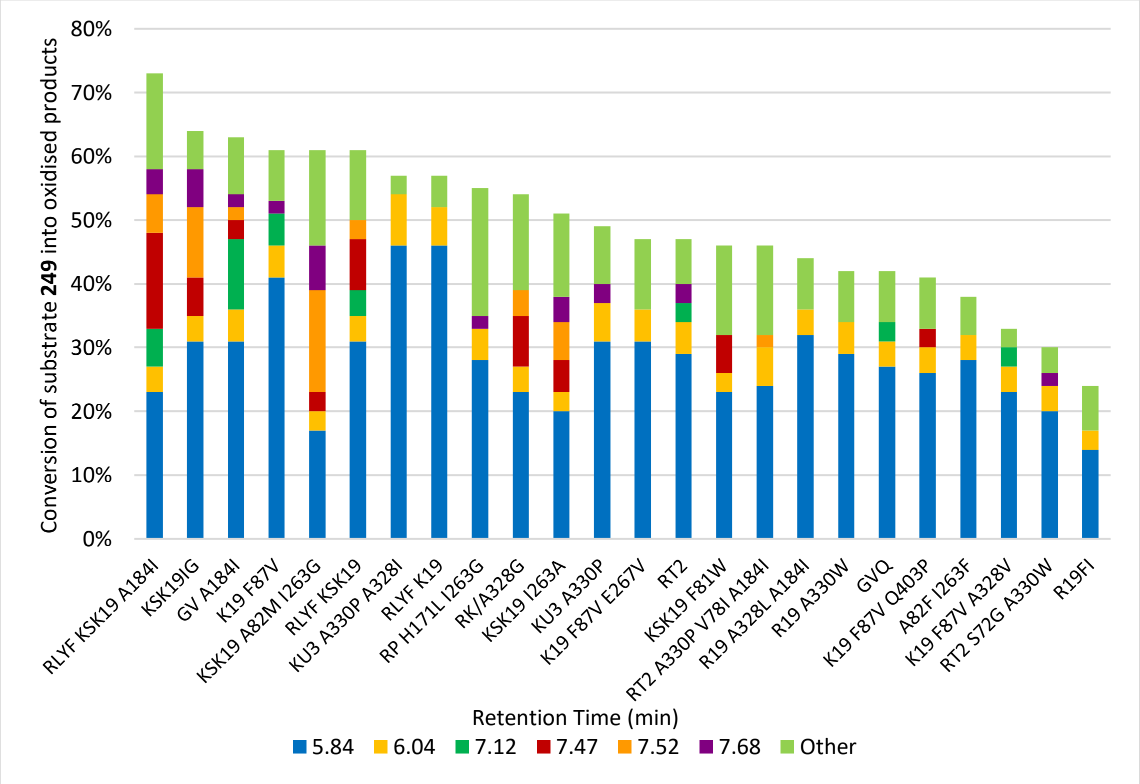
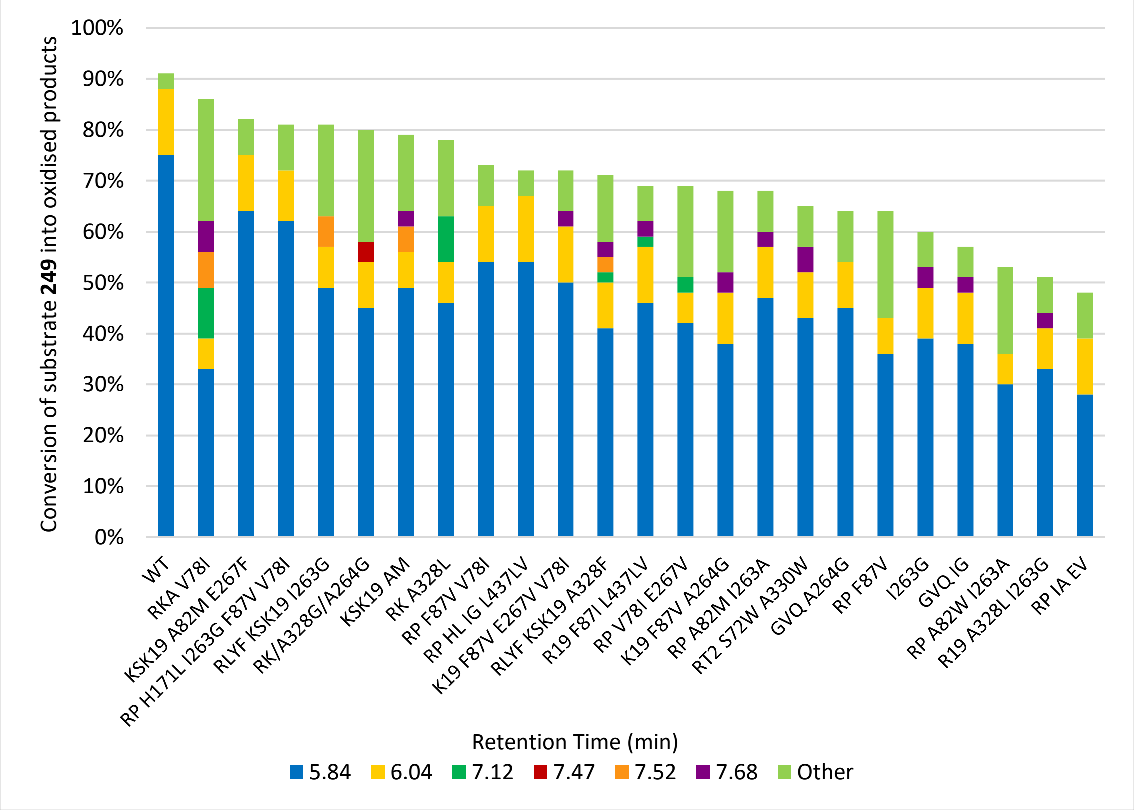


Chart 5.02: *Screening of tricycle **249** with plate WY6.*



Pleasingly, all mutants were active, with 34 out of 48 giving a conversion of >50%. Whilst one product dominated and was formed by every mutant, five other oxidised products were also detected by GC analysis. Three enzymes were then selected for scale-up: RLYF KSK19 AI, K19 F87V, and KSK19 AM IG (***Table 5.01***).

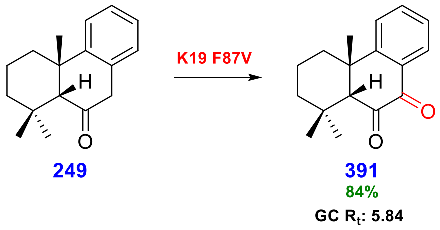
Table 5.01: *Enzymes selected for scale-up for the oxidation of tricycle **249**.*

Entry	**Mutant**	**Retention Time (min)**						
		4.38	**5.84**	**6.04**	**7.12**	**7.47**	**7.52**	**7.68**
1	RLYF KSK19 AI	27%	23%	4%	6%	15%	6%	4%
2	K19 F87V	39%	41%	5%	5%	—	—	2%
3	KSK19 AM IG	39%	17%	3%	—	3%	16%	7%

*GC R_t: substrate **249** (4.38 min), oxidised products (5.84 min, 6.04 min, 7.12 min, 7.47 min, 7.52 min, and 7.68 min). Percentages refer to relative area (%) of total GC peak integration. Complete screening data tables are available in **Appendix C**.*

In the first instance, it was desirable to identify the major product (retention time 5.84 minutes) and mutant K19 F87V was chosen for its good (61%) conversion, high selectivity for this product (67% of converted material), and excellent expression. Mutants KSK19 AM IG and RLYF KSK19 AI were both chosen for the diversity of products detected during screening, with the intention that multiple products could be isolated upon scale-up.

The three mutants were prepared according to a general procedure and reaction on a 24 mg scale with K19 F87V afforded 84% of diketone **391**, the major product of all screening reactions (***Scheme 5.02***).



Scheme 5.02: *Enzymatic oxidation of tricycle* ***249*** *with K19 F87V.* **Reagents and conditions:** K19 F87V (2000:1 substrate:enzyme), phosphate buffer, GDH, $NADP^+$, glucose, rt, 48 h.

This product was identified by the loss of the benzylic AB system in the ^{1}H NMR spectrum and the downfield shifting of the aromatic proton resonances (consistent with conjugation of the aromatic with a carbonyl). An additional ^{13}C peak in the 150 – 200 ppm region and comparison with literature data confirmed this assignment.[193]

Oxidation of tricycle **249** to diketone **391** in air is a reported process, making a series of control experiments necessary to determine the actual effect of the enzyme (***Table 5.02***). Three parallel reactions were therefore proposed: repetition of the enzyme-mediated oxidation (*entry* **2**); reaction without the P450 mutant (but still containing the cofactor regeneration system[*]) (*entry* **3**); and reaction in buffer only (*entry* **4**). After 24 hours the GC traces of each reaction were compared against that of the substrate at 0 hours (*entry* **1**). Although the conversion of substrate was similar for each reaction, the iteration with

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[*] Cofactor regeneration system is GDH, $NADP^+$ and glucose.

the P450 mutant showed a distinct preference to form diketone **391** (80% of converted material) compared with the moderate selectivity of the reactions without.

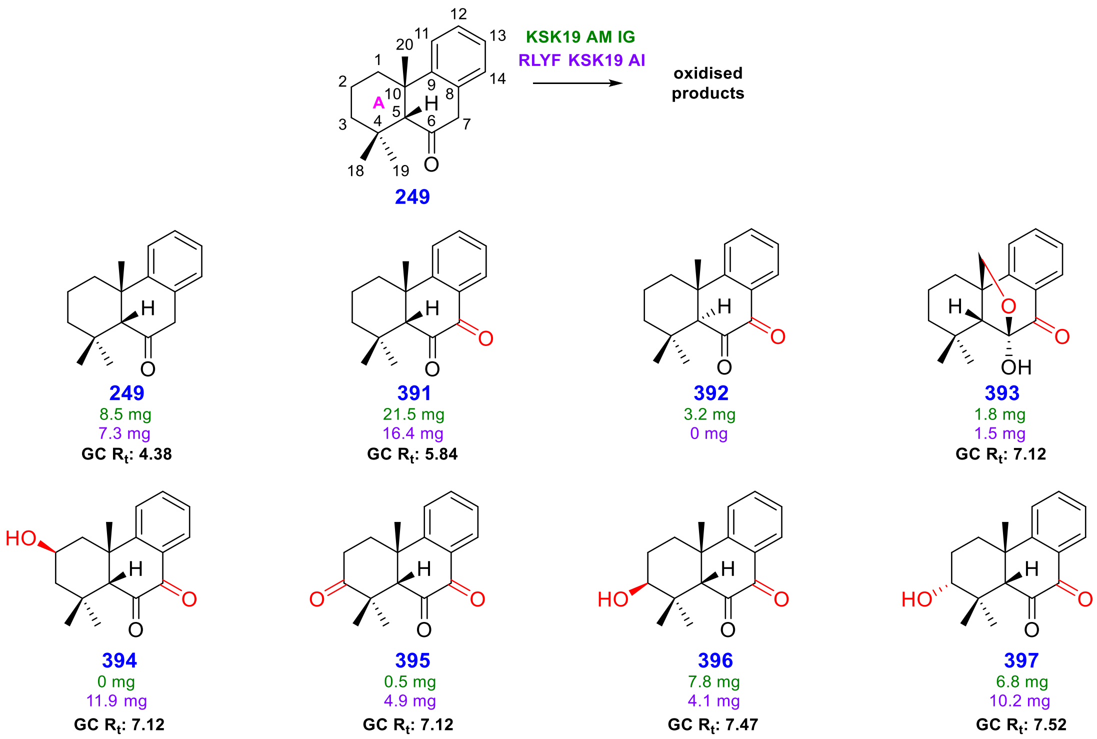
Table 5.02: *Control reactions for the oxidation of tricycle* ***249*** *with K19F87V.*

Entry	Reaction	Conversion	Of which is diketone 391
1	Substrate at 0 hrs	6%	42%
2	P450-oxidation	55%	80%
3	Without mP450	48%	56%
4	Buffer only	48%	50%

Reagents and conditions: *K19 F87V (2000:1 substrate:enzyme), phosphate buffer, GDH, NADP$^+$, glucose, rt, 24 h. *Reactions were run at screening scale.*

Therefore, whilst air oxidation was clearly a major process occurring during the enzyme-mediated reaction, the presence of the mutant P450 did affect the reaction outcome, either increasing the selectivity for the diketone product **391**, or increasing the rate of its formation from an intermediate oxidation product.

The reactions with KSK19 AM IG and RLYF KSK19 AI were both carried out at double scale (48 mg) as more products were expected. Oxidation of tricycle **249** with both mutants separately afforded six oxidised products each (in addition to returned starting material) (***Scheme 5.03***).



Scheme 5.03: *Enzymatic oxidation of tricycle* ***249*** *with KSK19 AM IG and RLYF KSK19 AI.* **Reagents and conditions:** P450 mutant (2000:1 substrate:enzyme), phosphate buffer, GDH, NADP$^+$, glucose, rt, 24 h (KSK19 AM IG) or 72 h (RLYF KSK19 AI). Masses are given in place of percentage yields as the metabolites were, in some cases, not able to be thoroughly separated or cleaned of minor impurities. Reactions were run using 48 mg of tricyclic substrate **249**.

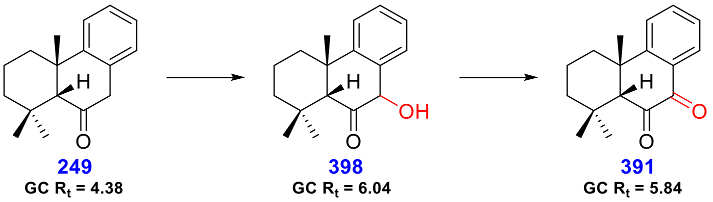
In all cases, double oxidation at the highly activated benzylic position was observed, again, identified by the downfield shifting of the aromatic proton resonances. The greater prevalence of benzylic oxidation compared with ferruginol substrate **380** is unsurprising, the position being additionally activated by the carbonyl. Epimer **392** was also isolated, likely arising from non-enzymatic reversible enolisation of the C-6 carbonyl in the reaction buffer or during product isolation. This process is likely to be slow, owing to the unfavourable bridgehead position of the C-H bond, and accounting for the small amounts of this product obtained.

Unusual hemiacetal **393** parallels some of the reactivity trends seen with the ferruginol substrate. Methyl oxidation was quickly identified by the presence of a pair of doublets at 3.4 and 4.0 ppm, and the absence of a third three-proton singlet in the ^{1}H NMR spectrum. The correlation of this pair of doublets to a quaternary carbon at 147.0 ppm (presumed to be C-9), and to a second peak at 102.9 ppm (assigned as C-6) revealed that not only had C-20 been hydroxylated, but that it had cyclised into the C-6 carbonyl.

The remaining A-ring oxidations were identified similarly. Oxidation in the C-2 position (**394**) was established from the triplet of triplets multiplicity of the C**H**OH proton resonance and equatorial hydroxylation was confirmed by the large coupling constant (11.5 Hz) and lack of nOe with either the bridgehead proton H-5, or C-20 methyl group. The C-3 position was highly active with axial, equatorial, and double oxidation products **396**, **397**, and **395** all being isolated. HMBC correlation with the *gem*-dimethyl groups indicated the C-3 position of the hydroxyl group and coupling constants and nOe were used to determine stereochemistry.

Following the identification of oxidised products, analysis of the screening results with respect to the mutations present leads to several general observations:[*]

- The products detected at 5.84 min and 6.04 min (shown in blue and yellow respectively on ***Chart 5.01*** and ***Chart 5.02***) are formed with every mutant. The major product diketone **391** (R_t = 5.84 min) is known to form readily in an air atmosphere, accounting for its ubiquity. The minor unidentified product is likely related to diketone **391**, perhaps as the precursor alcohol **398** (***Scheme 5.04***).



Scheme 5.04: *Proposed identity of product R_t = 6.04 min as intermediate in the formation of diketone* ***391****.*

- The two C-3 hydroxylation diastereomers **396** and **397** (R_t = 7.47 min (red) and R_t = 7.52 min (orange)) almost always occur together and of the 15 mutants which

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[*] A full list of mutations for each P450 mutant is available in **Appendix E**.

produced one or both of these products, all include a mutation at residue 87 (either F87V or F87A), known to influence the size of the active site. Most active mutants for these products also included H171L, Q307H, and N319Y mutations.

- Three very different products (**393**, **394** and **395**) share a retention time of 7.12 min (indicated in green) making relation of specific mutations with oxidised position challenging. Twelve mutants produced at least one of these products, amongst which two mutations were common: R47L and Y51F are residues near the entrance to the active site which are able to influence accessibility and binding of substrates.

Additional screening data for this substrate, particularly using plates based around specific combinations of mutations would be invaluable in conclusively determining a relationship between oxidised position and mutation. As alternative screening plates were not available at the time of work, it was proposed that diketone **391** would also be an interesting screening substrate, potentially providing insight into the oxidation of the A-ring positions without the dominating effect of benzylic oxidation. A racemic sample was readily obtained by separation of air-oxidised ketone **391** from unused substrate **249** and screened against plate WY5, but not a single mutant was active (>10% conversion of starting material).[193] Given the prevalence of multiple oxidation products in the initial screen, this result was surprising. However, it is likely that diketone **391** is produced from tricycle **249** within the active site of the enzyme (even if the process is partially air-mediated) and can therefore undergo further oxidation within the active site once formed. This may mean that, as the reaction substrate, diketone **391** cannot access the active site and enzymatic oxidation is not possible.

Kinetic Resolution of Metabolites

As racemic ketone **249** had been used for all reactions, kinetic resolution was possible and it was therefore desirable to determine whether any of the metabolites or returned substrate had become enantioenriched. The air oxidation of substrate **249** provided a racemic reference sample, so diketone **391** was selected for this analysis.

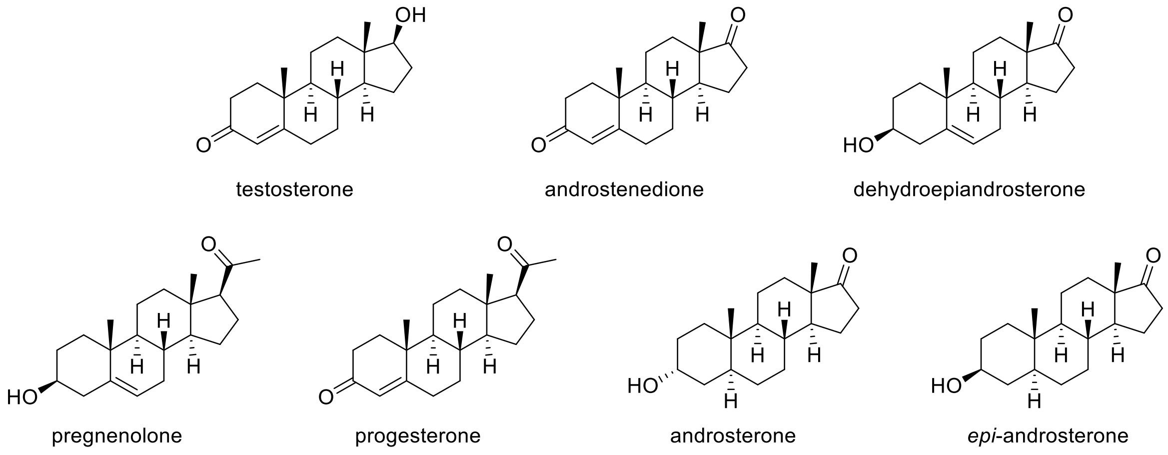
In the event, the enantiomers of the racemic reference sample could not be separated by chiral GC and, therefore, analysis of the enzymatically oxidised products was not possible. However, GC conditions were found that achieved substantial separation of the substrate enantiomers, and analysis of starting material returned from the reactions with both KSK19 AM IG and RLYF KSK19 AI was therefore possible. Substrate returned from the reaction with KSK19 AM IG was racemic; that from the RLYF KSK19 AI reaction had a 13% ee, indicating little or no overall kinetic resolution of the substrate.

Further investigation at this time was not possible but study into the enantioselective behaviour of mutant P450 enzymes with this substrate could be the subject of future work. The non-zero ee obtained for starting material returned by RLYF KSK19 AI is encouraging, but studies with returned starting material represent the average enantiodepletion of all reaction processes and therefore limited conclusions can be drawn from these results. It is likely that an alternative technique (such as chiral SFC) may be required to achieve separation of the enantiomers of the oxidised products, providing a clearer picture of any kinetic resolution effects.

Enzymatic Oxidation of Tetracycle 371

Whilst perhaps best known for their use in birth control, and in the media as substances of misuse and abuse, steroidal drugs are widely used to treat a variety of conditions ranging from asthma to cancer. Total synthesis remains challenging and most commercial drugs are produced through semi-synthesis from existing steroidal frameworks.[285,286] The biological activity of these compounds is subtly controlled by the steroidal cores' oxidation state and the nature of any side chains but the prevalence of inactivated sp^3 C-H bonds makes diversification of these compounds challenging.

As part of the Wong group's research into the oxidative diversification of existing pharmaceutical compounds, P450$_{\mathrm{BM3}}$ mutants were developed for the oxidation of several steroidal compounds (***Figure 5.03***). Existing enzymatic oxidations of steroidal frameworks are highly selective but extremely limited and most bacterial P450 enzymes have not shown any oxidative activity towards these substrates.



testosterone androstenedione dehydroepiandrosterone

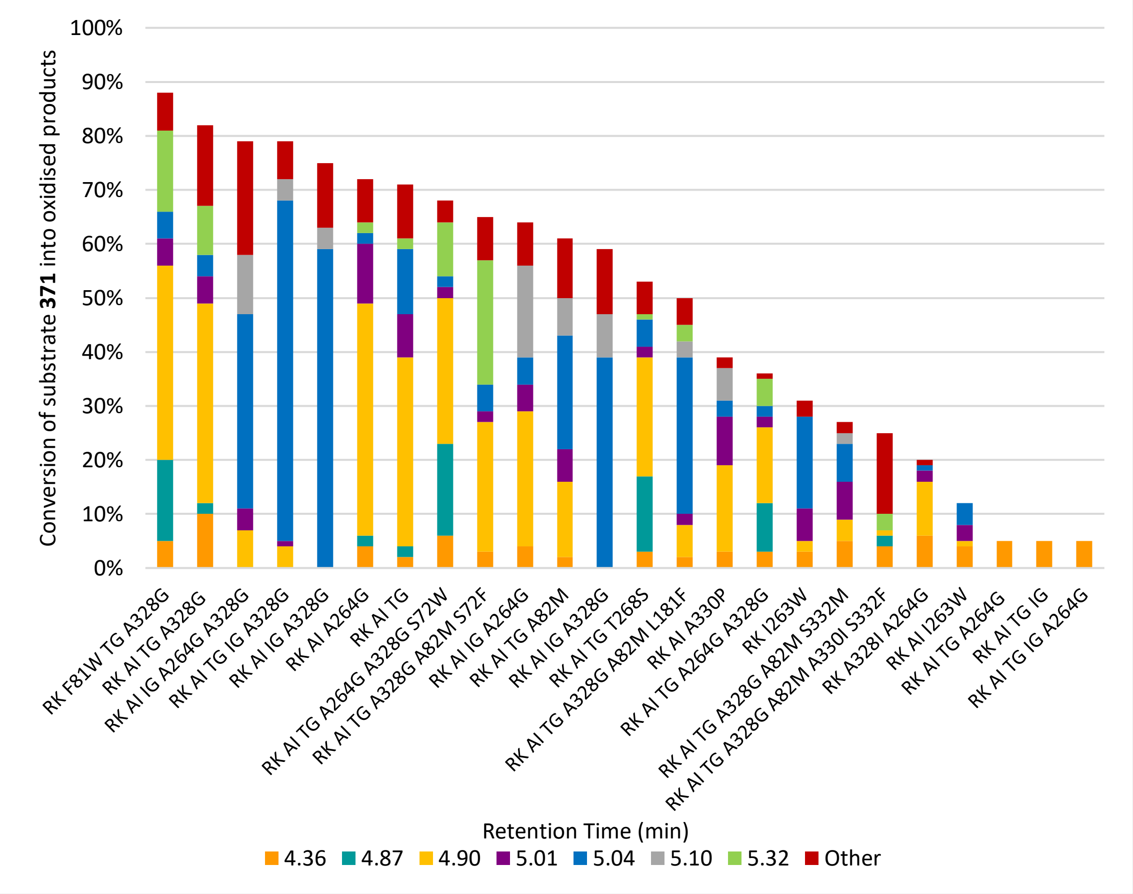
pregnenolone progesterone androsterone *epi*-androsterone

Figure 5.03: *Steroidal compounds under study by the Wong group.*

Comparison of crystal structures indicated an overly restrictive active site and a new library of evolved mutants was developed to facilitate the binding of the steroid substrate and favour the formation of new products.[287,288]

A sub-set of this new library was used to screen viridiol-like core **371** and, of the 24 mutants on steroid plate RK/AI, 16 gave a conversion of >50%, producing seven oxidised products (***Chart 5.03***).

Chart 5.03: *Screening of viridiol-like substrate* ***371*** *with plate RK/AI.*



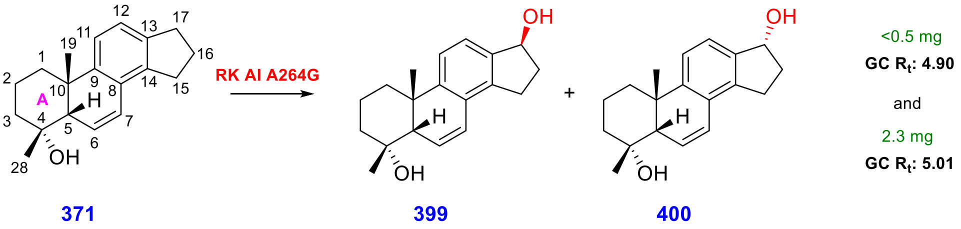
As such a limited amount of material was available, only two mutants (RK AI A264G and RK AI TG IG A328G) were selected for scale-up, both chosen on the basis of their good conversion (75% and 83%, respectively) to a different major product in each case (***Table 5.03***).

Table 5.03: *Enzymes selected for scale-up for the oxidation of tetracycle **371**.*

Entry	Mutant	Retention Time (min)							
		4.24	**4.36**	**4.87**	**4.90**	**5.01**	**5.04**	**5.10**	**5.32**
1	RK AI A264G	25%	4%	2%	43%	11%	2%	—	2%
2	RK AI TG IG A328G	17%	—	—	4%	1%	63%	4%	—

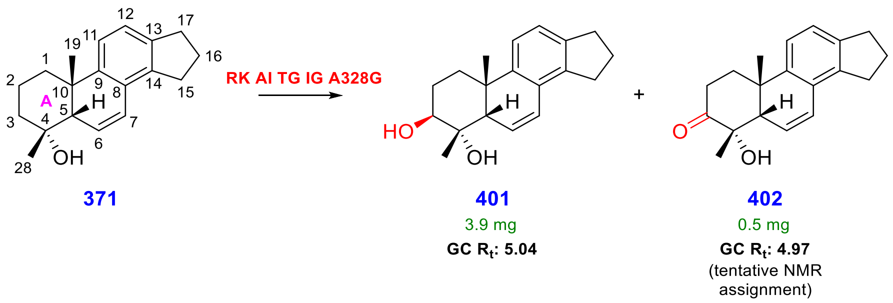
*GC R_t: substrate **371** (4.24 min), oxidised products (4.36 min, 4.87 min, 4.90 min, 5.01 min, 5.04 min, 5.10, and 5.32 min). Percentages refer to relative area (%) of total GC peak integration. Complete screening data tables are available in **Appendix D**.*

Both reactions were carried out at an 8.7 mg scale and two oxidised products were isolated from each (***Scheme 5.05*** and ***Scheme 5.06***).



Scheme 5.05: *Enzymatic oxidation of viridiol-like substrate **371** with RK AI A264G.* **Reagents and conditions:** RK AI A264G (500:1 substrate:enzyme), phosphate buffer, GDH, NADP$^+$, glucose, rt, 24 h. Stereochemistry of products could not be determined. Masses are given in place of percentage yields as the metabolites were, in some cases, not able to be thoroughly separated or cleaned of minor impurities.

Benzylic oxidation was expected and immediately identified from the deshielded C**H**OH resonances (around 5.3 ppm), and the downfield shifting of the aromatic protons relative to the substrate. HMBC correlation with these proton resonances further identified C-17 as the hydroxylated position and, as two such similar sets of spectra had been obtained, it was presumed that both diastereomers of the C-17 hydroxylation product (**399** and **400**) had formed. NOESY analysis was not helpful in determining the stereochemistry of the separated products owing to the lack of any suitable proximal groups.



Scheme 5.06: *Enzymatic oxidation of viridiol-like substrate* ***371*** *with RK AI TG IG A328G.* **Reagents and conditions:** RK AI TG IG A328G (500:1 substrate:enzyme), phosphate buffer, GDH, NADP$^+$, glucose, rt, 48 h. Masses are given in place of percentage yields as the metabolites were, in some cases, not able to be thoroughly separated or cleaned of minor impurities.

Pleasingly, oxidation was also observed on the A-ring. A C**H**OH triplet resonance at 3.6 ppm indicated aliphatic hydroxylation at either C-1 or C-3 and HMBC correlation with the bridgehead proton (H-5), and the C-28 methyl resonances placed the hydroxyl group at C-3 (**401**). The absence of an nOe correlation with the bridgehead proton or either methyl group then confirmed the *trans* stereochemistry of the diol. The small (3.0 Hz) coupling constant of the C**H**OH triplet suggests that conformer **401a** dominates in solution, probably owing to the sterically favourable equatorial orientation of both methyl groups (***Figure 5.04***).

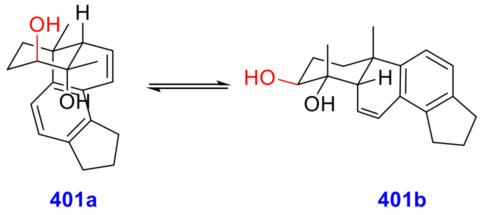


Figure 5.04: *Equilibrating conformers of diol product* ***401****.*

A second oxidation in the same position was also tentatively proposed. A pair of doublet – doublet of doublet pairs and an HMBC correlation between a ^{13}C peak at 208.9 ppm with the C-28 methyl protons, but not the C-19 protons suggested the presence of a carbonyl at C-3 (compound **402**) but full spectroscopic data could not be obtained from the small sample available.

A few general observations could now be made:*

- The pair of diastereomers **399** and **400** (R_t = 4.90 min (yellow) and R_t = 5.01 min (purple) (***Chart 5.03***)) were almost always formed together with the product at 4.90 min being favoured in almost all cases. 19 mutants produced one or both of these products and the T260G mutation was present in four of the top five mutants producing the alcohol detected at 4.90 min.
- Alcohol **401** (R_t = 5.04 min (indicated in blue on ***Chart 5.03***)) was formed by 20 of the 24 mutants, the top three of which all included the A328G mutation.

Additional material is required in order to complete the identification of all oxidised products and access to this material could be the subject of future work. Further screening of this substrate with related steroid plates would also be desirable to help identify the key mutations for oxidations in different positions and guide future enzyme evolution. Investigation into the kinetic resolution of oxidised products would likewise be invaluable and could lead to the preparation of enantiopure steroidal intermediates and their incorporation into the total synthesis of viridiol.

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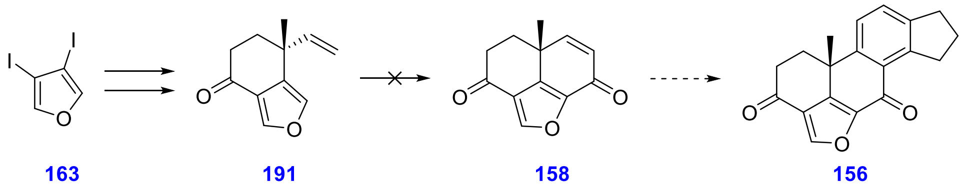
[*] A full list of mutations for each P450 mutant is available in **Appendix F**.

Summary and Future Work

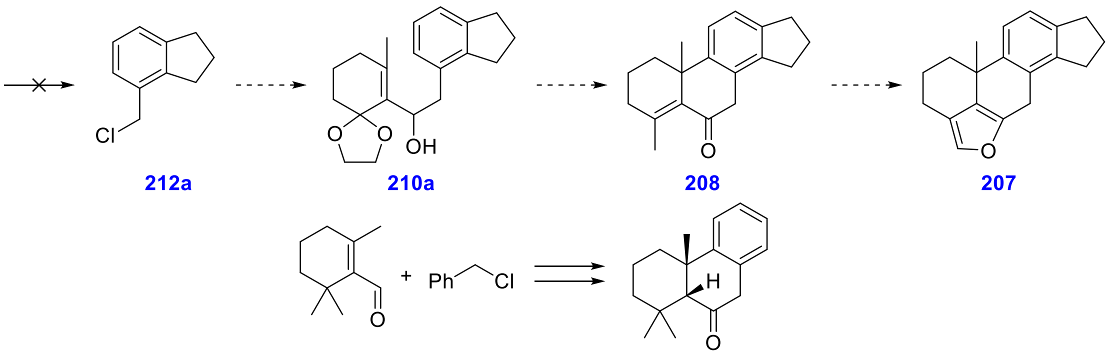
This project set out with the aim to prepare carbon-rich viridiol-like scaffolds which could be subjected to enzymatic oxidations by P450 mutants to produce varied analogues and to gain more information on the enzyme panel's capabilities, including any kinetic resolution of racemic substrates. Two synthetic strategies to viridiol-like carbon cores were envisaged:

The first of these focussed on constructing the pentacyclic system around the furan unit but efforts in this direction were hindered by the irreproducibility of literature procedures and the instability of almost all intermediates so that obtaining sufficient material for later steps became impractical. Multiple approaches to elaborate the side chain of the bicyclic system were unsuccessful, preventing an evaluation of the intended third ring closure and the route was ultimately abandoned (***Scheme 6.01***).



Scheme 6.01: *Summary of the first synthetic strategy.*

The second strategy aimed to build up the steroidal core initially, adding the furan ring as the final step. Attempts beginning with indane were frustrated by the formation of intractable mixtures of indanyl regioisomers so the synthetic strategy was developed on a simpler phenyl model system. A model tricycle was obtained efficiently and the applicability of this method to an analogous indanyl system was validated on a 5-substituted indanyl fragment (***Scheme 6.02***).

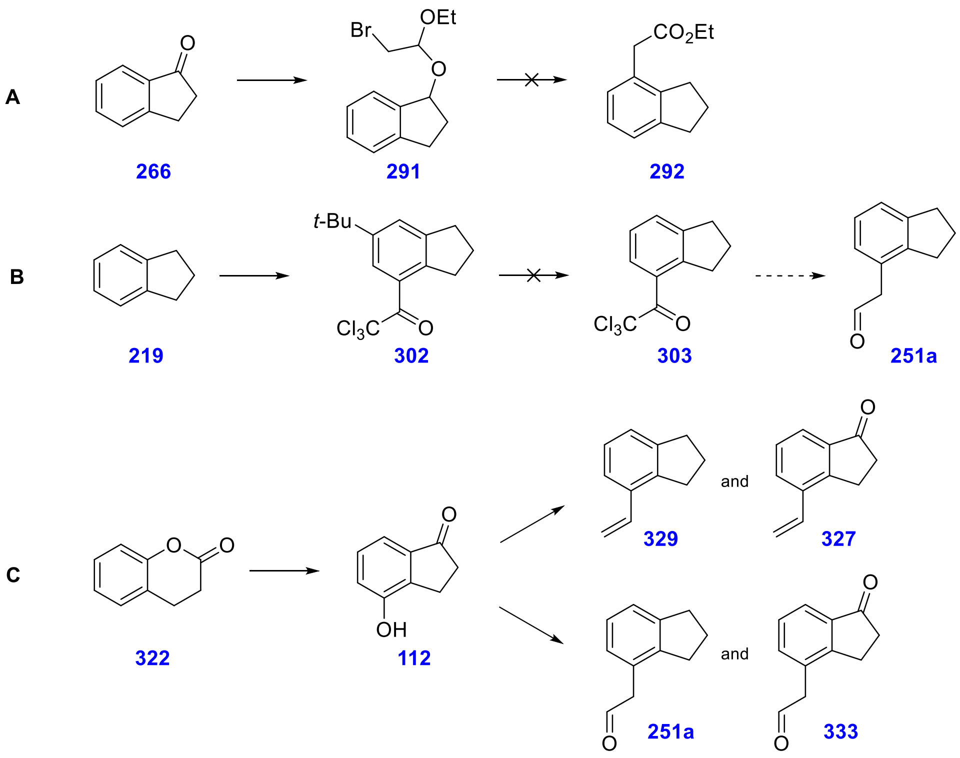


212a **210a** **208** **207**

241 **232** **249**

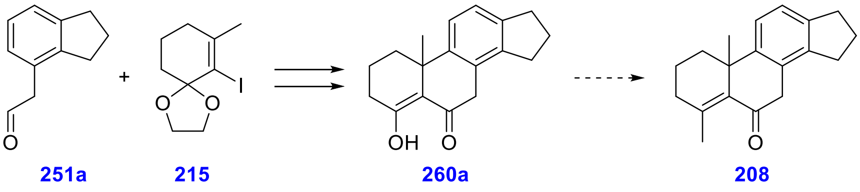
Scheme 6.02: *Summary of second synthetic route and development of the strategy on a model system.*

Multiple routes to 4-substituted indanes were then explored (***Scheme 6.03***). A [3,3]-sigmatropic rearrangement strategy was investigated but competing reaction pathways prevented reliable formation of the desired product (**A**). A promising Friedel–Crafts/Jocic rearrangement approach was also unsuccessful as no conditions could be found to remove the *tert*-butyl blocking group, despite literature precedent (**B**). Ultimately, in a modification of reported conditions, the Fries rearrangement of dihydrocoumarin to 4-hydroxyindanone, then a divergent synthetic approach from this intermediate, enabled the preparation of four suitable 4-substituted indanes with which to advance the synthesis of viridiol scaffolds (**C**).



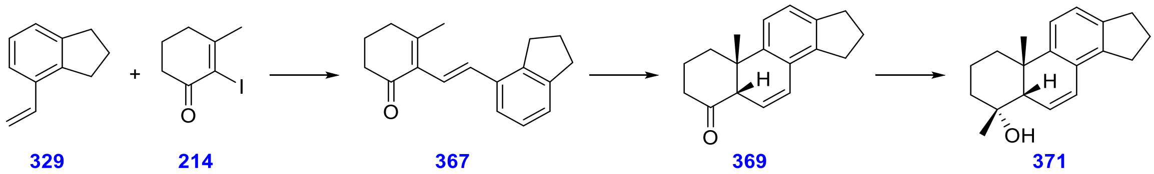
Scheme 6.03: *Summary of approaches towards 4-indanyl fragments.* ***A)*** *[3,3]-sigmatropic rearrangement;* ***B)*** *Friedel–Crafts/Jocic rearrangement and* ***C)*** *Fries rearrangement.*

With acetaldehyde-indane fragments in hand, the conditions previously established for the tricyclic model system were applied and a first steroidal tetracycle was accessed accordingly (***Scheme 6.04***). Future work could build on this synthetic route, installing the final carbon *via* triflation and cross-coupling to provide access to the complete carbon framework of viridiol which would be an ideal substrate for enzymatic study.



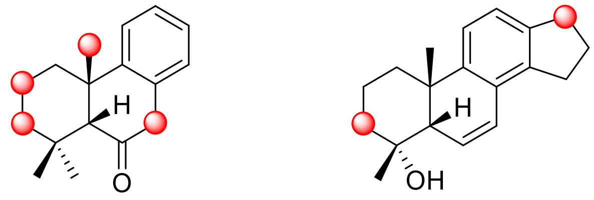
Scheme 6.04: *Summary of the synthesis from acetaldehyde-indane intermediate* ***251a*** *and proposed completion of the viridiol skeleton.*

A Heck reaction united the vinyl-indane fragments with the cyclohexenyl unit and, although reported acid-mediated cyclisation was ineffective, a second steroidal tetracycle was obtained *via* photochemical alkene isomerisation and cyclisation. Optimisation of this step would be a worthy focus for future efforts, facilitating the greater throughput of material for the final stages of the synthesis and potentially requiring the use of more specialised equipment. The subsequent methylation of the mono-ketone substrate successfully completed the viridiol skeleton and provided a suitable substrate for P450-mediated oxidation (***Scheme 6.05***). Incorrect chemoselectivity between two ketones prevented the completion of a second viridiol scaffold from vinyl indanone but incorporation of an indanone carbonyl protecting group could enable access to a complementary substrate for enzymatic study.



Scheme 6.05: *Summary of the completed synthesis of viridiol skeleton **371**.*

Finally, two substrates were subjected to enzymatic oxidation (***Figure 6.01***). The tricyclic model system, similar to other targets within the group, was screened against two plates of mutants and seven oxidised products were identified. Initial investigation into kinetic resolution effects was inconclusive but future work could further probe the enantioselective activity of P450 mutants with this substrate. The viridiol-like tetracycle was screened against a specialised plate of mutants engineered for steroids and four oxidised products were identified. With access to more material, additional scale-up reactions could be performed to identify the remaining oxidised products and further screening data would support the association of specific mutations with oxidised position. A study of kinetic resolution for viridiol-like substrates would also be worthwhile and, in the long term, could result in the preparation of enantiopure steroidal intermediates and their incorporation into the total synthesis of viridiol.



249 **371**

Figure 6.01: *Substrates subjected to P450-mediated oxidation. Oxidised positions indicated in red.*

Experimental Section

General Experimental

All solvents were commercially supplied or provided by the communal stills of the Chemistry Research Laboratory, Oxford. Petroleum ether refers to the fraction collected between 30-40 °C. Unless otherwise stated, reagents were obtained from commercial suppliers and used without further purification.

Flash column chromatography (FCC) was carried out using Merck Silicagel 60, particle size 40-63 μm. All reactions were followed by thin-layer chromatography (TLC) when practical, using Merck aluminium-backed Silicagel 60 F254 fluorescent treated silica which was visualised under UV light (λ_{max} = 254 or 365 nm) or by staining with aqueous basic potassium permanganate, anisaldehyde or vanillin solutions. Enzymatic reactions were followed by gas chromatography (GC) using 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 °C and the FID was at 250 °C. Chiral GC measurements were conducted on an HP6890 (H_2 as vector gas) or HP6850 (H_2 as vector gas) with a Lipodex® E column.

^{1}H, ^{13}C and ^{19}F NMR spectra were recorded using Bruker DPX-200, Bruker AVF-400, Bruker AVG-400, Bruker AVH-400, Bruker AVC-500 and Bruker NEO-600 spectrometers using $CDCl_3$ (or other deuterated solvent as specified). Chemical shifts are quoted in ppm relative to tetramethylsilane ($\delta = 0$) and referenced to the solvent residual. Coupling constants (*J*) are quoted in Hz, rounded to the nearest 0.5 Hz. The ^{1}H NMR spectra are reported as follows: ppm (multiplicity, coupling constants, number of protons, assignment). All NMR assignments use arbitrary numbering independent from IUPAC naming due to the difficulty of assigning spin systems in some molecules and assisted using two-dimensional (COSY, HSQC, HMBC, NOESY) NMR spectroscopy. Where an assignment could not be made unambiguously, possible assignments are listed.

Low resolution mass spectrometry (LRMS) (EI) was recorded on a Waters LCT Premier mass spectrometer. High resolution mass spectra (HRMS) were recorded on a Bruker µTOF mass spectrometer. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer as a thin film on a diamond ATR module. Only selected absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). UV-vis spectra were acquired on a Varian CARY50 spectrometer at 30 °C using 1 cm pathlength quartz cuvettes. Specific rotations were determined using a Perkin-Elmer 241 polarimeter at a wavelength 589 nm and at 25 °C, and are quoted in units of 10^{-1} deg cm^2 g^{-1} ; concentrations (*c*) are expressed in g/100 mL. Melting points were recorded in degrees Celsius (°C), using Griffin melting point apparatus. Boiling points for compounds purified by vacuum distillation were calculated using the Sigma Aldrich Pressure-Temperature Nomograph.

Ozonolysis was performed using a Wallace & Tiernan® ozoniser, type BA 023012, operating at 220 – 240 V and 50 Hz. Photochemical reactions were performed using a Pen-Ray® Light Source, model 11SC-1, emitting the mercury spectrum with the primary energy at 254 nm. Sonication was carried out using a Misonix 3000 sonicator and Fisher Scientific FB-505 ultrasonic processor. Deionised water ($\rho \sim 15$ $\text{M}\Omega\cdot\text{cm}^{-1}$) for enzymatic reactions was obtained from a Millipore ELIX 5 system and was used to prepare all the growth media and buffers.

Compound names are as generated by CambridgeSoft ChemBioDraw Ultra 18.2 and procedures are organised in numerical order as they appear in each chapter.

General Procedures

Enzyme Preparation (General Procedure A)

Deionised water (ρ ~ 15 MΩ·cm^{-1}) from a Millipore ELIX 5 system was used to prepare all the growth media and buffers (***Table GP1***). Media were autoclaved at 121 ºC for 30 min. All components added for bacterial culture growths were first sterilised *via* a syringe filter (0.22 μm).

Table GP1: *Media components.*

Media	Components (per litre deionised water)
SOC	Tryptone (20 g), yeast extract (5 g), NaCl (0.5 g). Then after autoclaving, $MgCl_2$ (10 mL, 1.0 M), $MgSO_4$ (10 mL, 1.0 M), glucose (2 mL, 20% w/v).
Phosphate buffer (pH 7.9)	KH_2PO_4 (5.38 g), K_2HPO_4 (27.8 g).
LB$_{ksn}$	Tryptone (10 g), yeast extract (5 g), NaCl (10 g), kanamycin (30 mg).
LB$_{agar}$	Tryptone (10 g), yeast extract (5 g), NaCl (10 g), kanamycin (30 mg), bacto-agar (15 g).
FB$_{glycerol}$	K_2HPO_4 (147 g), NaH_2PO_4 (31 g), $(NH_4)_2SO_4$ (25 g), Na_2SO_4 (20 g), Na_3citrate•$2H_2O$ (12 g), NH_4Cl (5 g), glycerol (60 g).
Inoculation solution/22.5 mL	Kanamycin (225 mg), $MgSO_4$ (1.83 g), thiamine•HCl (750 mg), trace elements solution (22.5 mL).
Trace elements solution	$CaCl_2·H_2O$ (740 mg), $CoCl_2·6H_2O$ (250 mg), $CuSO_4·5H_2O$ (100 mg), $FeCl_3·6H_2O$ (16.7 g), $MnSO_4·4H_2O$ (132 mg), Na_2EDTA (20.1 g), $ZnSO_4·7H_2O$ (180 mg).
Induction solution/40 mL	IPTG (380 mg), δ-ALA•HCl (672 mg), $FeSO_4$•$7H_2O$ (112 mg), kanamycin (600 mg), made up to 40 mL with deionised water.

Transformation

Maintaining the temperature at 0 °C, 1μL of the requisite DNA was added to an autoclaved Eppendorf® tube containing 40 μL of *E. coli* Bl21.DE3, followed by 0.8 mL of SOC solution. The mixture was warmed to 42 °C for 1 min, then cooled to 0 °C for 2 min to induce transformation. The transformed cells were left to shake at 37 °C and 220 rpm for 2 h, then the mixture centrifuged for 3 min at 13300 *g*. The supernatant was discarded, and cells resuspended in 100 μL phosphate buffer, then spread across LB$_{kan}$

agar plates and left in the oven at 37 °C for 18 h. After this time, an isolated colony from the plate was put into 6 mL LB_{kan} solution and shaken for 6 h at 220 rpm and 37 °C.

Inoculation

A 2 L conical flask containing 450 mL distilled water and 50 mL $FB_{glycerol}$ solution was autoclaved. Once the flask had cooled, 1.5 mL inoculation solution was added, followed by 100 µL of the enzyme pre-culture and the flask shaken for 24 h at 120 rpm and 37 °C.

Induction

The incubator temperature was lowered to 20 °C and 1 mL induction solution and 0.5 g tryptone added every 24 h until the solution turned red.

Purification

The cells were harvested by centrifugation at 4500 *g* for 15 min, after which the supernatant was discarded, and the pellet resuspended in 5 mL phosphate buffer. 10 mg lysozyme was added, and the solution shaken for 1 h at 20 °C and 120 rpm. The cell suspension was then transferred to a 100 mL beaker and the cells lysed by sonication on ice. The sonication procedure consisted of 20 cycles of 15-second pulses, followed by a 30-second cooling period at 55% amplitude. The lysed suspension was then centrifuged for 20 min at 9500 *g* at 4 °C to remove the cell debris.

The supernatant was subjected to $(NH_4)_2SO_4$ fractionation and a sufficient amount of solid $(NH_4)_2SO_4$ was added to achieve 40% saturation. Once fully dissolved, the solution was centrifuged for 20 min at 9500 *g* at 4 °C. The supernatant was retained and a further amount of solid $(NH_4)_2SO_4$ was added to achieve 60% saturation. Once fully dissolved, the solution was centrifuged for 20 min at 9500 *g* at 4 °C and the supernatant discarded. The resultant pellet (containing the enzyme) was resuspended in 5 mL of phosphate buffer.

Quantification

The concentration of the enzyme solution was determined by CO-difference assay. A 100 μL aliquot of the enzyme stock solution was diluted with 900 μL of phosphate buffer and the heme iron reduced by the addition of 5 mg $Na_2S_2O_4$. A UV-vis spectrum (200–700 mm) of this solution was recorded as the baseline, then CO bubbled slowly through the solution to give the CO-bound enzyme and a spectrum of this solution taken, showing the characteristic absorption at 450 nm. The concentration of the protein was then determined using the following equation:

$$[P450]\mu M = \frac{10\ \times (A_{450} - A_{490})}{\varepsilon} \times 10^6$$

Where ε is the extinction coefficient (91000 $M^{-1}cm^{-1}$).

Screening with General Plates (General Procedure B)

Tricyclic substrates **249** and **391** were screened against general plates WY5 and WY6 (kindly prepared by the Wong group) in a ratio of 2000:1 substrate to enzyme. Each well contained 100 µL of selected enzyme mutants (list available in **Appendix E**). A bulk solution of all other reaction components was prepared according to ***Table GP2*** and 900 µL aliquots added to each well to make the total volume 1 mL. Substrates were prepared as 0.2 M solutions in ethanol.

Table GP2: *Reaction components.*

Reagent	Bulk Solution		Per Well	
	Concentration	Volume	Volume	Concentration
Substrate	0.2 M	300 µL	10 µL	2 mM
Enzyme	10 µM	—	100 µL	1 µM
Glucose	1 M	3 mL	100 µL	100 mM
GDH	200 U mL^{-1}	300 µL	10 µL	2 U mL^{-1}
NADP$^+$	40 mM	300 µL	10 µL	400 µM
Phosphate Buffer	200 mM	23.1 mL	770 µL	154 mM

Plates were left to mechanically shake for 24 h at 120 rpm at rt. After this time, 500 µL from each reaction well was transferred to a separate 1.5 mL microcentrifuge tube and 300 µL EtOAc added. Each tube was agitated on the vortex for 10 s, then centrifuged for 1 min at 14800 *g*. 140 µL of the organic layer was transferred to a GC vial for analysis. The initial oven temperature was 200 °C, which was held for 1 min before ramping at a rate of 10 °C min^{-1} to a final temperature of 280 °C, which was held for 30 s.

Screening with Steroid Plates (General Procedure C)

Tetracyclic substrate **371** was screened against steroid plate RK AI (kindly prepared by the Wong group) in a ratio of 500:1 substrate to enzyme. Each well contained 100 µL of selected enzyme mutants (list available in **Appendix F**). A bulk solution of all other reaction components was prepared according to ***Table GP3*** and 400 µL aliquots added to each well to make the total volume 0.5 mL. The substrate was prepared as a 0.2 M solution in acetone.

Table GP3: *Reaction components.*

Reagent	Bulk Solution		Per Well	
	Concentration	Volume	Volume	Concentration
Substrate	0.2 M	65 µL	2.5 µL	1 mM
M-CD	0.2 M	65 µL	2.5 µL	1 mM
Enzyme	10 µM	—	100 µL	2 µM
Glucose	1 M	1.3 mL	50 µL	100 mM
GDH	200 U mL^{-1}	130 µL	5 µL	2 U mL^{-1}
NADP$^+$	40 mM	130 µL	5 µL	400 µM
Phosphate Buffer	200 mM	8.71 mL	335 µL	134 mM

Plates were left to mechanically shake for 24 h at 120 rpm at rt. After this time, each reaction well was transferred to a separate 1.5 mL microcentrifuge tube and 300 µL EtOAc added. Each tube was agitated on the vortex for 10 s, then centrifuged for 1 min at 14800 *g*. 140 µL of the organic layer was transferred to a GC vial for analysis. The initial oven temperature was 220 °C, which was held for 1 min before ramping at a rate of 20 °C min^{-1} to a final temperature of 300 °C, which was held for 3.5 min.

Preparative Scale Reactions (General Procedure D)

Preparative scale reactions were conducted in conical flasks at the same concentrations as the initial screenings and mechanically stirred in an incubator at 120 rpm at 20 °C. Reaction progress was monitored by GC analysis until no further product formation was detected (typically 24 – 78 h).* The completed reaction was quenched by the addition of EtOAc, and the mixture extracted (×3) by centrifugation at 4500 *g* with EtOAc. The combined organic layers were washed with water, then brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*.

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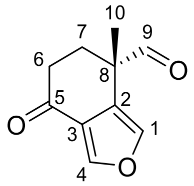
* In some cases, additional aliquots of enzyme and glucose, GDH, and NADP$^+$ were added where reaction seemed to have stalled.

Compounds from Chapter 1

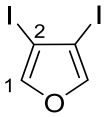
4-Methyl-7-oxo-4,5,6,7-tetrahydroisobenzofuran-4-carbaldehyde – 160



Synthesised according to a reported procedure.[301] To a solution of **191** (50.8 mg, 0.31 mmol, 1.0 eq) in a 3:1 mixture of dioxane:water (3.1 mL) were added pyridine (50 µL, 0.62 mmol, 2.0 eq), $NaIO_4$ (265 mg, 1.24 mmol, 4.0 eq), and OsO_4 (1.5 mg, 0.006 mmol, 0.02 eq). The reaction flask was coated in foil and the reaction left to stir at rt in the dark for 40 h. The mixture was diluted with water (2 mL) and DCM (3 mL), the layers separated, and the aqueous layer extracted with DCM (3 × 5 mL). The combined organic phases were washed with brine (10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **160** (39.4 mg, 23%) as an off-white solid.

Rf 0.15 (pentane:EtOAc, 6:1, stain: $KMnO_4$); **IR** ν_{max} 2923 (C-H), 1727 (C=O) (C-9), 1689 (C=O) (C-5), 1584 (C=C), 1538 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 9.55 (s, 1H, H-9), 8.04 (d, *J* = 1.5 Hz, 1H, H-1), 7.41 (d, *J* = 1.5 Hz, 1H, H-4), 2.63 – 2.57 (m, 2H, H-6), 2.47 – 2.39 (m, 1H, H-7), 2.04 – 1.95 (m, 1H, H-7), 1.46 (s, 3H, H-10); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 199.4 (C-9), 192.8 (C-5), 145.3 (C-1), 140.8 (C-4), 126.4 (C-2), 121.6 (C-3), 45.9 (C-8), 35.0 (C-6), 30.7 (C-7), 21.9 (C-10); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{10}H_{11}O_3$) requires *m/z* 179.0703, found *m/z* 179.0704; **mp** 67 – 68 °C.

3,4-Diiodofuran – 163



Recommended Set-up

Synthesised according to a modified literature procedure.[165] To a solution of **164** (45.1 g, 133 mmol, 1.0 eq) in 7.5 w/w% aq. H_2SO_4 (80 mL) was added hexane (130 mL) and NMP (130 mL) and the mixture heated to 85 °C. A solution of $K_2Cr_2O_7$ (39.0 g, 133 mmol, 1.0 eq) in H_2SO_4 (48 mL) and water (130 mL) was prepared and added without delay[*] to the solution of **164** *via* pressure-equalising dropping funnel at an initial rate of about 1 drop every 2 sec.[†] Once all of the $K_2Cr_2O_7$ solution had been added, the dropping funnel was removed and the reaction heated for a further 2 h, then cooled to rt. The phases were separated, and the aqueous layer was washed with hexane (3 × 400 mL).[‡] The combined organic layers were washed with sat. aq. Na_2CO_3 solution (1 L) and brine (1 L), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **163** (10.40 g, 25%) as a volatile, light-sensitive orange oil.[§] In some cases, dry column chromatography was required to obtain the pure product.[289][**]

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[*] Solution will become very hot, but if allowed to cool before addition, precipitation of the $K_2Cr_2O_7$ will occur.

[†] As the $K_2Cr_2O_7$ solution cools in the dropping funnel, precipitation of $K_2Cr_2O_7$ crystals occurs, inhibiting the dropwise addition. Small amounts of solid may be poked through the tap with a needle or something similar. Larger amounts can be re-dissolved by addition of small amounts of conc. H_2SO_4.

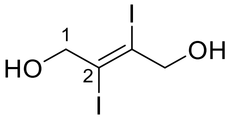
[‡] Because of the known light sensitivity of **163**, all flasks were coated in foil and charged with small amounts of BHT to act as a radical inhibitor.

[§] Protect from light and do not expose to pressures below 300 mbar due to product volatility.

[**] Pre-chilled pentane was used and all tubes were coated with foil and charged with BHT.

Rf 0.70 (pentane, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.45 (s, 2H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 146.4 (C-1), 77.7 (C-2). Data consistent with literature.[165]

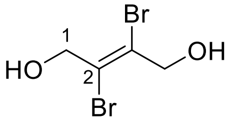
(*E*)-2,3-Diiodobut-2-ene-1,4-diol – 164



Synthesised according to a reported procedure.[290] To a solution of but-2-yne-1,4-diol (6.0 g, 69.7 mmol, 1.0 eq) in water (210 mL) was added KI (23.1 g, 139 mmol, 2.0 eq) and I_2 (18.6 g, 73.2 mmol, 1.05 eq) and the reaction stirred at 70 °C for 2.5 h. The mixture was cooled to 0 °C and left stirring for a further hour. The resulting precipitate was collected by filtration and dried *in vacuo* at 80 °C to afford title compound **164** (27.9 g, 83%) as a powdery yellow solid.

Rf 0.20 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **^{1}H NMR** (DMSO, 400 MHz) δ_H 5.54 (t, J = 6.0 Hz, 2H, OH), 4.21 (d, J = 6.0 Hz, 4H, H-1); **^{13}C NMR** (DMSO, 101 MHz) δ_C 105.1 (C-2), 73.9 (C-1); **mp** 165 – 166 °C, lit. 176 – 178 °C. Data consistent with literature.[290]

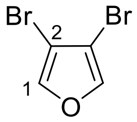
(*E*)-2,3-Dibromobut-2-ene-1,4-diol – 166



Synthesised according to a reported procedure.[291] To a 0 °C solution of but-2-yne-1,4-diol (7.56 g, 87.9 mmol, 1.0 eq) in water (175 mL) was added Br_2 (5.9 mL, 114 mmol, 1.3 eq). The reaction mixture was stirred at 0 °C for 30 min and rt for 90 min, after which time it was quenched with sat. aq. $Na_2S_2O_3$ solution (10 mL) and neutralised with 1.0 M aq. NaOH (15 mL). The organic product was extracted with EtOAc (3 × 30 mL) and the combined organic phases were washed with brine (60 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **166** (14.72 g, 69%) as a white solid.

Rf 0.15 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **^{1}H NMR** (DMSO, 400 MHz) δ_H 5.55 (t, *J* = 6.0 Hz, 2H, OH), 4.32 (d, *J* = 6.0 Hz, 4H, H-1); **^{13}C NMR** (DMSO, 101 MHz) δ_C 122.9 (C-2), 65.5 (C-1); **mp** 112 – 113 °C, lit. 114 – 115 °C. Data consistent with literature.[291]

3,4-Dibromofuran – 167



Synthesised according to a reported procedure.[292] To a two-necked flask equipped with distillation apparatus on the central neck were added **166** (32.4 g, 133 mmol, 1.0 eq) and 7% aq. H_2SO_4 (84 mL). A pressure-equalising dropping funnel was fitted to the other neck and charged with a solution of $K_2Cr_2O_7$ (23.5 g, 80 mmol, 0.6 eq) in H_2SO_4 (27 mL) and water (265 mL). The reaction mixture was stirred rapidly and heated to 120 °C until distillation began. The dichromate solution was then added dropwise over 1 h whilst distillation continued.[*] After addition was complete, the reaction was distilled for a further 1 h. The product was extracted from the distillate with PE (3 × 40 mL) and washed with sat. aq. Na_2CO_3 solution (60 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **167** (7.82 g, 26%) as a volatile pale-yellow oil.

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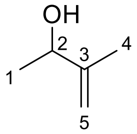
[*] Dissolution of the dichromate is very exothermic. As the solution cools, some precipitation in the dropping funnel may occur, inhibiting dropwise addition. Addition of small amounts of conc. H_2SO_4 will redissolve precipitate.

R

R_f 0.55 (pentane, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.45 (s, 2H); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 141.4 (C-1), 103.8 (C-2); **bp** 93 °C.[*] Data consistent with literature.[292]

3-Methylbut-3-en-2-ol – 169



Synthesised according to a reported procedure.[293] To a 0 °C solution of methylmagnesium bromide (66.7 mL, 3.0 M in Et_2O, 198 mmol, 1.1 eq) in Et_2O (36 mL) under N_2 was added dropwise, a solution of methacrolein (15.0 mL, 182 mmol, 1.0 eq) in Et_2O (36 mL). The reaction mixture was stirred at 0 °C and monitored by ^{1}H NMR until completion (6 h), then poured into a mixture of 1.0 M aq. HCl (100 mL) and Et_2O (100 mL). The phases were separated, and the aqueous layer was extracted with Et_2O (3 × 100 mL). The combined organic phases were mixed with solid $NaHCO_3$ (100 g), dried over anhydrous Na_2SO_4, and filtered. The solvent was removed by distillation at 1 atm with a fractionating column to afford title compound **169** (12.9 g, 83%) as a pale-yellow oil.[†]

R_f 0.15 (pentane:Et_2O, 15:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 4.92 (s, 1H, H-5), 4.75 (s, 1H, H-5), 4.20 (qd, J = 6.5 Hz, 3.5 Hz, 1H, H-2), 2.02 (br s, 1H, OH), 1.71 (s, 3H, H-4), 1.24 (d, J = 6.5 Hz, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 149.1 (C-3), 109.7 (C-5), 71.7 (C-2), 21.9 (C-1), 18.0 (C-4). Data consistent with literature.[293]

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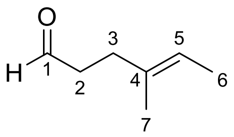
[*] Literature value not given.

[†] Characterised as a 2:1 mixture of **169** in Et_2O.

(*E*)-4-Methylhex-4-enal – 170



Method 1:[294] To a solution of **169** (6.00 g, 69.7 mmol, 1.0 eq) in butyl vinyl ether (70 mL) was added $Hg(OAc)_2$ (1.78 g, 5.6 mmol, 0.08 eq). The reaction mixture was stirred for 20 h in a pressure tube at 110 °C, then stirred for 2.5 h at 140 °C. The cooled crude product was purified by FCC (PE to PE:Et_2O, 20:1) and the solvent removed by distillation at 1 atm to afford title compound **170** (1.98 g, 29%) as a colourless oil.

Method 2:[167] To a solution of **169** (6.00 g, 69.7 mmol, 1.0 eq) in tri(ethylene glycol) divinyl ether (21.4 mL, 105 mmol, 1.5 eq) was added DIPEA (0.6 mL, 3.5 mmol, 0.05 eq) and **403** (178 mg, 0.35 mmol, 0.005 eq).[*] The reaction mixture was heated to 75 °C for 72 h,[†] after which time the temperature was increased to 120 °C until full consumption of the starting material was observed by ^{1}H NMR analysis. The reaction mixture was distilled immediately upon completion at *ca.* 120 °C and *ca.* 400 mbar to afford title compound **170** (2.22 g, 33%) as a volatile colourless oil.[‡]

***R**$_f$* 0.20 (PE, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 9.73 (t, *J* = 2.0 Hz, 1H, H-1), 5.22 (qq, *J* = 6.5 Hz, 1.5 Hz, 1H, H-5), 2.50 (td, *J* = 8.0 Hz, 2.0 Hz, 2H, H-2), 2.30 (t,

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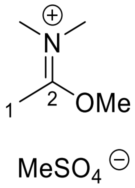
[*] **1,10-Phenanthroline-palladium trifluoroacetate – 403**
Synthesised from a reported procedure.[167] $Pd(TFA)_2$ (130 mg, 0.39 mmol, 1 eq) and 1,10-phenanthroline (70.0 mg, 0.39 mmol, 1 eq) were stirred together in DME (2 mL) at rt until a bright yellow precipitate formed (*ca.* 10 min). The precipitate was isolated by filtration, washed with Et_2O (3 mL) and dried under reduced pressure at 50 °C to afford **403** (179 mg, 89%) as a yellow powder.
***R**$_f$* 0.10 (EtOAc, stain: $KMnO_4$); **^{1}H NMR** (DMSO, 400 MHz) δ_H 9.04 (dd, *J* = 8.5 Hz, 2.5 Hz, 2H, H-Ar), 8.63 – 8.35 (m, 2H, H-Ar), 8.31 (d, *J* = 5.0 Hz, 2H, H-Ar), 8.14 (td, *J* = 5.0 Hz, 2.5 Hz, 2H, H-Ar); **^{13}C NMR** (DMSO, 101 MHz) δ_C 141.5 (C-1), 128.2 (C-4), 127.0 (C-2); **mp** 133 °C (dec.). Data consistent with literature.[323]

[†] Use of an oil bath and initially lower reaction temperature required to prevent decomposition of catalyst. Reaction carried out under air.

[‡] **Caution:** Further reduction of pressure can result in evaporation of product from the receiving flask. Use of a cold trap between distillation apparatus and pump is recommended. In some cases, a second distillation under the same conditions was required for optimal product purity.

J = 8.0 Hz, 2H, H-3), 1.59 (br s, 3H, H-7), 1.55 (dq, *J* = 6.5 Hz, 1.0 Hz, 3H, H-6); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 202.5 (C-1), 133.4 (C-4), 119.3 (C-5), 41.9 (C-2), 31.6 (C-3), 15.5 (C-7), 13.1 (C-6); **bp** 66 °C at 60 mbar, corrected to 145 °C at 1 atm.* Data consistent with literature.[295]

***N, N*-Dimethylacetamide-dimethyl sulfate complex – 171**



Synthesised according to a reported procedure.[296] Dimethylacetamide (46.5 mL, 500 mmol, 1 eq) and dimethyl sulfate (47.3 mL, 500 mmol, 1 eq) were heated at 75 °C under N_2 for 3 h. The reaction mixture was cooled to 0 °C and washed with Et_2O (5 × 100 mL). Most of the solvent was removed by syringe and the remainder removed *in vacuo* to afford **171** (88.8 g, 83%) as a viscous colourless oil.

Rf 0.10 (EtOAc, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 4.20 (s, 3H, SO_4Me), 3.56 (s, 3H, NMe), 3.38 (s, 3H, NMe), 3.21 (s, 3H, OMe), 2.56 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 176.8 (C-2), 60.6 (C-anion), 54.3 (NMe), 41.9 (NMe), 39.2 (OMe), 15.2 (C-1).† Data consistent with literature.[296]

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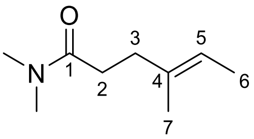
* Literature value not given.

† NMR spectra contain traces of dimethylacetamide which could not be removed.

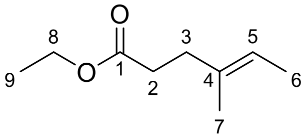
(*E*)-*N*,*N*,4-Trimethylhex-4-enamide – 172



To a solution of **169** (1.00 g, 11.6 mmol, 1 eq) in THF (35 mL) under N_2 was added methyllithium (7.3 mL, 1.6 M in Et_2O, 11.6 mmol, 1 eq) dropwise. The mixture was stirred for 5 min, then **171** (2.47 g, 11.6 mmol, 1 eq) was added. The reaction mixture was stirred at 70 °C for 18 h, then diluted with DCM (50 mL), and washed with sat. aq. $NaHCO_3$ solution (3 × 50 mL) and brine (100 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 5:1 to 1:2) to afford title compound **172** (1.13 g, 63%) as a colourless oil.

Rf 0.65 (pentane:Et_2O, 1:2, stain: vanillin); **IR** ν_{max} 2919 (C-H), 1641 (C=O), 1141 (C-N); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 5.28 – 5.20 (m, 1H, H-5), 3.01 (s, 3H, NMe), 2.94 (s, 3H, NMe), 2.43 – 2.36 (m, 2H, H-2), 2.34 – 2.26 (m, 2H, H-3), 1.63 (s, 3H, H-7), 1.57 (dq, *J* = 6.5 Hz, 1.0 Hz, 3H, H-6); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 173.0 (C-1), 135.0 (C-4), 119.0 (C-5), 37.4 (NMe), 35.5 (NMe), 35.0 (C-3), 32.4 (C-2), 15.9 (C-7), 13.5 (C-6); **LRMS** (ES+) mass found *m/z* 156.1 $[M+H]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_9H_{18}NO$) requires *m/z* 156.1383, found *m/z* 156.1382.

Ethyl (*E*)-4-methylhex-4-enoate – 173



Synthesised according to a reported procedure.[293] A mixture of **169** (1.0 g, 11.6 mmol, 1 eq), triethyl orthoacetate (10.6 mL, 58.0 mmol, 5 eq) and glacial acetic acid (69 µL, 1.2 mmol, 0.1 eq) was heated to 150 °C in a sealed tube for 72 h. The reaction was poured into 1.0 M aq. HCl (10 mL), the layers separated, and the aqueous layer extracted with EtOAc (3 × 15 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was distilled under reduced pressure (*ca*. 35 mbar) to afford title compound **173** (702 mg, 39%) as a colourless oil.

***R**f* 0.40 (pentane:Et_2O, 10:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 5.28 – 5.19 (m, 1H, H-5), 4.12 (q, *J* = 7.0 Hz, 2H, H-8), 2.42 – 2.35 (m, 2H, H-2), 2.33 – 2.26 (m, 2H, H-3), 1.61 (s, 3H, H-7), 1.56 (dq, *J* = 6.5 Hz, 1.0 Hz, 3H, H-6), 1.24 (t, *J* = 7.0 Hz, 3H, H-9); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 173.7 (C-1), 134.2 (C-4), 119.4 (C-5), 60.3 (C-8), 34.9 (C-3), 33.4 (C-2), 15.7 (C-7), 14.4 (C-9), 13.5 (C-6); **bp** 45 °C at 35 mbar, corrected to 141 °C at 1 atm.* Data consistent with literature.[293]

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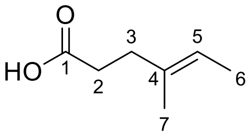
* Literature value not given.

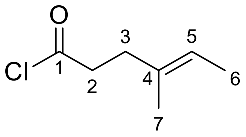
(*E*)-4-Methylhex-4-enoic acid – 174



A solution of **173** (657 mg, 4.2 mmol, 1 eq) and NaOH (1.05 mL, 5.0 M aq., 5.25 mmol, 1.25 eq) in EtOH (1.7 mL) was heated to reflux for 18 h. The cooled reaction mixture was acidified with 5.0 M aq. HCl (2 mL), the phases separated, and the aqueous layer extracted with Et_2O (3 × 5 mL). The combined organic phases were washed with water (15 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford title compound **174** (541 mg, quant.) as a colourless oil.

R R_f 0.20 (pentane:Et_2O, 5:1, stain: vanillin); **IR** ν_{max} 2919 (O-H), 1710 (C=O), 1413 (C-H), 1300 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 5.31 – 5.22 (m, 1H, H-5), 2.48 – 2.42 (m, 2H, H-2), 2.34 – 2.28 (m, 2H, H-3), 1.62 (s, 3H, H-7), 1.57 (dq, *J* = 6.5 Hz, 1.0 Hz, 3H, H-6); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 179.9 (C-1), 133.8 (C-5), 119.6 (C-4), 34.4 (C-3), 33.1 (C-2), 15.7 (C-7), 13.5 (C-6); **LRMS** (ES+) mass not found; **HRMS** (ESI) exact mass calculated for $[M-H]^-$ ($C_7H_{11}O_2$) requires *m/z* 127.0765, found *m/z* 127.0765.

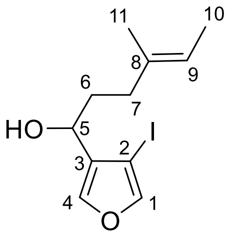
(*E*)-4-Methylhex-4-enoyl chloride – 175



A solution of **174** (400 mg, 3.12 mmol, 1.0 eq) in freshly distilled Et_2O (7.8 mL) was prepared at 0 °C. A separate solution of $SOCl_2$ (250 µL, 3.43 mmol, 1.1 eq) and pyridine (277 µL, 3.43 mmol, 1.1 eq) in Et_2O (4.4 mL) was prepared and added dropwise to the former solution. The reaction was stirred for 3.5 h, during which the reaction temperature was allowed to increase to rt. The resulting suspension was filtered, and the filtrate concentrated *in vacuo* to afford title compound **175** (410 mg, 90%) as a colourless oil.

R_f 0.20 (pentane:Et_2O, 2:1, stain: vanillin); **IR** ν_{max} 2921 (C-H), 1711 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 5.33 – 5.23 (m, 1H, H-5), 2.97 (ap. dd, *J* = 8.0 Hz, 7.0 Hz, 2H, H-2), 2.49 – 2.27 (m, 2H, H-3), 1.61 (s, 3H, H-7), 1.58 (dq, *J* = 7.0 Hz, 1.0 Hz, 3H, H-6); **LRMS** mass not found; **HRMS** mass not found.

(*E*)-1-(4-Iodofuran-3-yl)-4-methylhex-4-en-1-ol – 176



To a solution of **163** (500 mg, 1.56 mmol, 1.0 eq) in freshly distilled Et_2O (8.6 mL) under Ar at –78 °C was added *n*-butyllithium (0.62 mL, 2.5 M in hexanes, 1.56 mmol 1.0 eq) dropwise and the solution was stirred for 15 min. Meanwhile, a solution of **170** (152 mg, 1.36 mmol, 0.87 eq) in freshly distilled Et_2O (3.3 mL) was prepared under Ar and cooled to –78 °C. The previously prepared organolithium solution was added dropwise *via* cannula to the aldehyde solution (both maintained at –78 °C) and stirred for 20 min after addition was completed.[*] The reaction was quenched with sat. aq. NH_4Cl solution, the phases separated, and the aqueous phase extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 6:1) to afford title compound **176** (164 mg, 40%) as a yellow oil.

R_f 0.25 (pentane:Et_2O, 5:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.41 (d, *J* = 1.5 Hz, 1H, H-1), 7.35 (d, *J* = 1.5 Hz, H-4), 5.32 – 5.25 (m, 1H, H-9), 4.60 – 4.53 (m, 1H, H-5), 2.22 – 2.04 (m, 4H, H-6 and H-7), 1.64 (s, 3H, H-11), 1.58 (s, 3H, H-10); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 146.3 (C-1), 140.2 (C-4), 135.3 (C-8), 130.4 (C-3), 119.4 (C-9), 67.9 (C-5), 66.2 (C-2), 36.0 (C-6 or C-7), 35.0 (C-6 or C-7), 15.8 (C-11), 13.9 (C-10). Data consistent with literature.[297]

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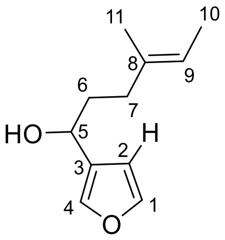
[*] A slower rate of addition was found to increase the yield (i.e., thinner cannula) although protracted addition times resulted in some degradation of the organolithium solution.

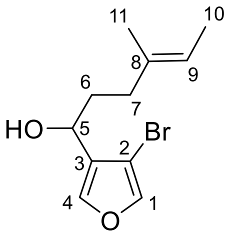
(*E*)-1-(Furan-3-yl)-4-methylhex-4-en-1-ol – 184



Compound **184** was isolated as a yellow oil and an unexpected side product in the formation of **176**.

R_f 0.15 (pentane:Et_2O, 5:1, stain: vanillin); **IR** ν_{max} 3373 (O-H), 2920 (C-H), 1503 (C=C), 1023 (C-O), 875 (=C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.41 – 7.37 (m, 2H, H-1 and H-4), 6.43 – 6.38 (m, 1H, H-2), 5.30 – 5.20 (m, 1H, H-9), 4.64 (t, *J* = 7.0 Hz, 1H, H-5), 2.14 – 2.00 (m, 2H, H-7), 1.89 – 1.77 (m, 3H, H-6 and OH), 1.61 (q, *J* = 1.0 Hz, 3H, H-11), 1.58 (dq, *J* = 6.5 Hz, 1.0 Hz, 3H, H-10); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 143.5 (C-4), 139.2 (C-1), 135.4 (C-8), 129.2 (C-3), 119.2 (C-9), 108.6 (C-2), 67.0 (C-5), 36.0 (C-6 or C-7), 35.9 (C-6 or C-7), 15.8 (C-11), 13.5 (C-10); **LRMS** mass not found; **HRMS** mass not found;

(*E*)-1-(4-Bromofuran-3-yl)-4-methylhex-4-en-1-ol – 177



To a solution of **167** (500 mg, 2.23 mmol, 1.0 eq) in freshly distilled Et_2O (11.1 mL) under Ar at –78 °C was added *n*-butyllithium (0.89 mL, 2.5 M in hexanes, 2.23 mmol 1.0 eq) dropwise and the solution stirred 15 min. Meanwhile, a solution of **170** (218 mg, 1.94 mmol, 0.87 eq) in freshly distilled Et_2O (4.7 mL) was prepared under Ar and cooled to –78 °C. The previously prepared organolithium solution was added dropwise *via* cannula to the aldehyde solution (both maintained at –78 °C) and stirred for 20 min after addition was completed.* The reaction was quenched with sat. aq. NH_4Cl solution, the phases separated, and the aqueous layer extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ solution (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 10:1) to afford title compound **177** (133.7 mg, 27%) as a yellow oil.

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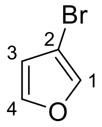
* A slower rate of addition was found to increase the yield (i.e., thinner cannula) although protracted addition times resulted in some degradation of the organolithium solution.

100.0 (C-2), 66.7 (C-5), 35.9 (C-7), 34.9 (C-6), 15.8 (C-11), 13.5 (C-10); **LRMS** mass not found; **HRMS** mass not found.

3-Bromofuran – 178



To a solution of **167** (500 mg, 2.23 mmol, 1 eq) in Et_2O (11.2 mL) under Ar at –78 °C was added *n*-butyllithium (0.95 mL, 2.36 M in hexanes, 2.23 mmol, 1 eq) dropwise. The mixture was stirred for 15 min, then quenched with sat. aq. NH_4Cl solution (10 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 10 mL). The combined organic phases were washed successively with water (20 mL), sat. aq. $NaHCO_3$ solution (20 mL), and brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*.[*] The crude product was purified by FCC (pentane:Et_2O, 20:1) to afford title compound **178** (26.2 mg, 12%) as a volatile yellow oil.

R$_f$ 0.25 (pentane:Et_2O, 20:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.44 (dd, *J* = 2.0 Hz, 1.0 Hz, 1H, H-1), 7.35 (ap. t, *J* = 2.0 Hz, 1H, H-4), 6.45 (dd, *J* = 2.0 Hz, 1.0 Hz, 1H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 143.7 (C-4), 141.1 (C-1), 113.5 (C-3), 99.8 (C-2). Data consistent with literature.[299]

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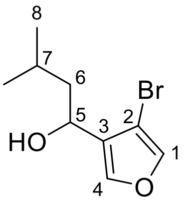
[*] Caution: product volatile.

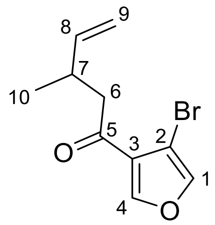
1-(4-Bromofuran-3-yl)-3-methylbutan-1-ol – 179



To a solution of **167** (300 mg, 1.34 mmol, 1 eq) in THF (2.7 mL) under Ar at –78 °C was added *n*-butyllithium (573 µL, 2.36 M in hexanes, 1.34 mmol, 1 eq) dropwise. The mixture was stirred for 3 h, then isovaleraldehyde (144 µL, 1.34 mmol, 1 eq) was added dropwise and the solution stirred for a further 2 h. The reaction was quenched with sat. aq. NH_4Cl solution (1 mL), the aqueous layer extracted with Et_2O (3 × 2 mL), and the combined organic phases washed with brine (5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 20:1) to afford title compound **179** (115 mg, 37%) as an off-white solid.

Rf 0.10 (pentane:Et_2O, 20:1, stain: vanillin); **IR** ν_{max} 3374 (O-H), 2957 (C-H), 1468 (C=C), 1048 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.41 (d, *J* = 2.0 Hz, 1H, H-1), 7.36 (d, *J* = 2.0 Hz, 1H, H-4), 4.77 – 4.70 (m, 1H, H-5), 1.87 – 1.77 (m, 1H, H-7), 1.76 – 1.70 (m, 1H, H-6), 1.69 – 1.58 (m, 1H, H-6), 0.97 (ap. t, *J* = 6.5 Hz, 6H, H-8); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 141.7 (C-1), 140.1 (C-4), 129.1 (C-3), 100.1 (C-2), 65.0 (C-5), 46.0 (C-6), 24.9 (C-7), 23.4 (C-8), 22.1 (C-8); **LRMS** mass not found; **HRMS** (GCMS CH_4 CI) exact mass calculated for $[M+H]^+$ ($C_9H_{14}O_2{}^{79}Br$) requires *m/z* 233.0172, found *m/z* 233.0177; **mp** 30 – 31 °C.

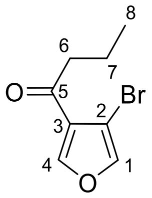
1-(4-Bromofuran-3-yl)-3-methylpent-4-en-1-one – 180



To a solution of **167** (200 mg, 0.89 mmol, 1 eq) in THF (1.8 mL) under Ar at –78 °C was added *n*-butyllithium (377 μL, 2.36 M in hexanes, 0.89 mmol, 1 eq) dropwise. The mixture was stirred for 3 h, then **182** (126 mg, 0.89 mmol, 1 eq) was added dropwise and the solution stirred for a further 2 h. The reaction was quenched with sat. aq. NH_4Cl solution (1 mL), the aqueous phase extracted with Et_2O (3 × 2 mL) and the combined organic phases washed with brine (5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by FCC (pentane:Et_2O, 20:1) to afford title compound **180** (46.2 mg, 21%) as an off-white solid.

***R**$_f$* 0.55 (pentane:Et_2O, 10:1, stain: $KMnO_4$); **IR** ν_{max} 2961 (C-H), 2925 (C-H), 1681 (C=O), 1640 (C=C), 1474 (C-C), 1089 (C-O), 957 (=C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.47 (d, *J* = 2.0 Hz, 1H, H-4), 6.62 (d, *J* = 2.0 Hz, 1H, H-1), 5.83 (ddd, *J* = 17.0 Hz, 10.5 Hz, 6.5 Hz, 1H, H-8), 5.03 (dt, *J* = 17.0 Hz, 1.5 Hz, 1H, H-9), 4.95 (dt, *J* = 10.5 Hz, 1.5 Hz, 1H, H-9), 3.00 – 2.93 (m, 1H, H-6), 2.92 – 2.86 (m, 1H, H-7), 2.85 – 2.79 (m, 1H, H-6), 1.09 (d, *J* = 6.5 Hz, 3H, H-10); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 188.5 (C-5), 148.4 (C-1), 145.3 (C-4), 142.9 (C-8), 117.6 (C-3), 113.3 (C-9), 107.0 (C-2), 46.0 (C-6), 33.4 (C-7), 19.9 (C-10); **LRMS** mass not found; **HRMS** (ES+) exact mass calculated for $[M+H]^+$ ($C_{10}H_{12}O_2{}^{79}Br$) requires *m/z* 243.0015, found *m/z* 243.0017.

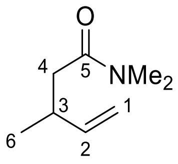
1-(4-Bromofuran-3-yl)butan-1-one – 181



To a solution of **167** (300 mg, 1.34 mmol, 1 eq) in THF (2.7 mL) under Ar at –78 °C was added *n*-butyllithium (573 µL, 2.36 M in hexanes, 1.34 mmol, 1 eq) dropwise. The mixture was stirred for 3 h, then butyryl chloride (138 µL, 1.34 mmol, 1 eq) was added dropwise and the solution stirred for a further 2 h. The reaction was quenched with sat. aq. NH_4Cl solution (1 mL), the aqueous layer extracted with Et_2O (3 × 2 mL) and the combined organic phases washed with brine (5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by FCC (pentane:Et_2O, 20:1) to afford title compound **181** (28.6 mg, 10%) as a pale-yellow oil.

R_f 0.40 (pentane:Et_2O 20:1, stain: vanillin); **IR** ν_{max} 2963 (C-H), 1684 (C=O), 1150 (C=C), 1145 (C-O), 871 (C-Br); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.99 (d, *J* = 1.5 Hz, 1H, H-4), 7.47 (d, *J* = 1.5 Hz, 1H, H-1), 2.76 (t, *J* = 7.5 Hz, 2H, H-6), 1.74 (hex, *J* = 7.5 Hz, 2H, H-7), 0.98 (t, *J* = 7.5 Hz, 3H, H-8); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 194.3 (C-5), 148.4 (C-1), 143.5 (C-4), 125.6 (C-3), 98.9 (C-2), 43.3 (C-6), 17.6 (C-7), 13.9 (C-8); **LRMS** mass not found; **HRMS** (APCI) exact mass calculated for $[M+H]^+$ ($C_8H_{10}O_2{}^{79}Br$) requires *m/z* 216.9859, found *m/z* 216.9856;

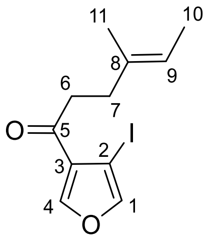
***N*,*N*,3-Trimethylpent-4-enamide – 182**



Synthesised according to a reported procedure.[298] To a solution of crotyl alcohol (0.8 mL, 9.4 mmol, 1 eq) in THF (15 mL) under N_2 was slowly added methyllithium (6 mL, 1.6 M in Et_2O, 9.4 mmol, 1 eq) dropwise. The mixture was stirred for 5 min, then **171** (2.0 g, 9.4 mmol, 1 eq) in THF (13 mL) was added. The reaction mixture was stirred at 70 °C for 18 h, then diluted with DCM (20 mL), and washed with sat. aq. $NaHCO_3$ solution (3 × 40 mL) and brine (50 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (PE:Et_2O, 3:1) to afford title compound **182** (375 mg, 28%) as an orange oil.

R_f 0.35 (pentane:Et_2O, 1:1, stain: vanillin); **IR** ν_{max} 2960 (C-H), 2930 (C-H), 1643 (C=O), 1337 (C-N), 1266 (C-N), 997 (C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 5.78 (ddd, *J* = 17.0 Hz, 10.5 Hz, 7.0 Hz, 1H, H-2), 4.98 (dt, *J* = 17.0 Hz, 1.5 Hz, 1H, H-1), 4.90 (dt, *J* = 10.5 Hz, 1.5 Hz, 1H, H-1), 2.96 (s, 3H, H-NMe), 2.90 (s, 3H, H-NMe), 2.71 (ap. dddd, *J* = 13.5 Hz, 6.5 Hz, 5.5 Hz, 1.5 Hz, 1H, H-3), 2.33 (dd, *J* = 15.0 Hz, 6.5 Hz, 1H, H-4), 2.21 (dd, *J* = 15.0 Hz, 7.5 Hz, 1H, H-4), 1.02 (d, *J* = 6.5 Hz, 3H, H-6); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 171.9 (C-5), 143.4 (C-2), 112.9 (C-1), 40.0 (C-4), 37.5 (C-NMe), 35.4 (C-NMe), 34.3 (C-3), 19.8 (C-6); **LRMS** mass not found; **HRMS** (ES+) exact mass calculated for $[M+H]^+$ ($C_8H_{16}NO$) requires *m/z* 142.1226, found *m/z* 142.1226.

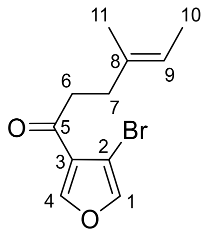
(*E*)-1-(4-Iodofuran-3-yl)-4-methylhex-4-en-1-one – 187



To a solution of alcohol **176** (58.9 mg, 0.19 mmol, 1.0 eq) in DCM (0.95 mL) under N_2 at 0 °C was added Dess-Martin periodinane (102 mg, 0.24 mmol, 1.25 eq). The reaction was allowed to warm to rt and its progress followed by TLC. The completed reaction (2 h) was quenched with IPA (1 mL) and the mixture evaporated to dryness. The crude residue was re-dissolved in Et_2O (5 mL) and washed successively with sat. aq. solutions of 1:1 $NaHCO_3/Na_2S_2O_3$ (5 mL), sat. aq. $NaHCO_3$ (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*, and the crude product purified by FCC (pentane:Et_2O, 20:1 to 10:1) to afford title compound **187** (35.0 mg, 61%) as a pale-yellow oil.

R_f 0.50 (pentane:Et_2O, 10:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.99 (d, *J* = 1.5 Hz, 1H, H-4), 7.47 (d, *J* = 1.5 Hz, 1H, H-1), 5.28 – 5.21 (m, 1H, H-9), 2.89 – 2.81 (m, 2H, H-6), 2.41 – 2.34 (m, 2H, H-7), 1.63 (s, 3H, H-11), 1.57 (d, *J* = 7.0 Hz, 3H, H-10); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 193.9 (C-5), 148.4 (C-4), 148.2 (C-1), 134.3 (C-8), 126.1 (C-3), 119.5 (C-9), 62.9 (C-2), 39.9 (C-6), 33.7 (C-7), 16.0 (C-11), 13.6 (C-10). Data consistent with literature.[164]

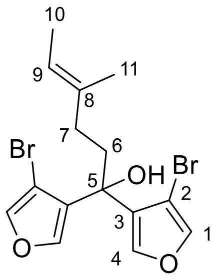
(*E*)-1-(4-Bromofuran-3-yl)-4-methylhex-4-en-1-one – 188



To a solution of alcohol **177** (72.4 mg, 0.28 mmol, 1.0 eq) in DCM (1.4 mL) under N_2 at 0 °C was added Dess-Martin periodinane (148 mg, 0.35 mmol, 1.25 eq). The reaction was allowed to warm to rt and its progress followed by TLC (pentane:Et_2O, 10:1). The completed reaction (2 h) was quenched with IPA (5 mL) and the mixture evaporated to dryness. The crude residue was re-dissolved in Et_2O (10 mL) and washed successively with sat. aq. solutions of 1:1 $NaHCO_3$/$Na_2S_2O_3$ (10 mL), sat. aq. $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*, and the crude product purified by FCC (pentane:Et_2O, 20:1) to afford title compound **188** (42.5 mg, 59%) as a pale-yellow oil.

Rf 0.70 (pentane:Et_2O, 10:1, stain: vanillin); **IR** ν_{max} 2918 (C-H), 1683 (C=O), 1227 (C-O), 912 (C-Br); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.99 (d, *J* = 1.5 Hz, 1H, H-4), 7.47 (d, *J* = 1.5 Hz, 1H, H-1), 5.28 – 5.21 (m, 1H, H-9), 2.90 – 2.84 (m, 2H, H-6), 2.37 (t, *J* = 7.5 Hz, 2H, H-7), 1.63 (s, 3H, H-11), 1.57 (dq, *J* = 6.5 Hz, 1.0 Hz, 3H, H-10); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 194.0 (C-5), 148.4 (C-1), 143.5 (C-4), 134.3 (C-8), 125.5 (C-3), 119.4 (C-9), 98.9 (C-2), 40.2 (C-6), 33.7 (C-7), 16.0 (C-11), 13.5 (C-10); **LRMS** mass not found; **HRMS** mass not found.

(*E*)-1,1-Bis(4-bromofuran-3-yl)-4-methylhex-4-en-1-ol – 189



Compound **189** was isolated as the unexpected major product in the attempted synthesis of **188** with acid chloride **175**.

To a solution of di-halogenated furan **167** (436 mg, 1.93 mmol, 1.0 eq) in freshly distilled Et_2O (9.7 mL) under Ar at –78 °C was added *n*-butyllithium (0.77 mL, 2.5 M in hexanes, 1.93 mmol, 1.0 eq) dropwise and the solution stirred 15 min. Meanwhile, a solution of acid chloride **175** (252 mg, 1.72 mmol, 0.87 eq) in freshly distilled Et_2O (4.6 mL) was prepared under Ar and cooled to –78 °C. The previously prepared organolithium solution was added dropwise *via* cannula to the acid chloride solution (both maintained at –78 °C) and stirred for 20 min after addition was completed.[*] The reaction was quenched with sat. aq. NH_4Cl solution (10 mL), the phases separated, and the aqueous extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ solution (30 mL) and brine (30 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 30:1) to afford title compound **189** (210 mg, 27%) as an off-white solid.

Rf 0.20 (pentane:Et_2O, 30:1, stain: vanillin); **IR** ν_{max} 3569 (O-H), 2919 (C-H), 1050 (C-O), 843 (=C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.42 (d, *J* = 2.0 Hz, 2H, H-4), 7.41 (d, *J* = 2.0 Hz, 2H, H-1), 5.29 – 5.21 (m, 1H, H-9), 2.69 (s, 1H, OH), 2.45 – 2.37 (m, 2H, H-

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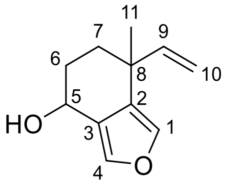
[*] A slower rate of addition was found to increase the yield (i.e., thinner cannula) although protracted addition times resulted in some degradation of the organolithium solution.

6), 2.06 – 1.98 (m, 2H, H-7), 1.64 (s, 3H, H-11), 1.57 (dq, *J* = 6.5 Hz, 1.0 Hz, 3H, H-10); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 142.8 (C-4), 142.0 (C-1), 135.6 (C-8), 127.9 (C-3), 119.4 (C-9), 98.9 (C-2), 71.7 (C-5), 36.8 (C-6), 33.6 (C-7), 16.0 (C-11), 13.6 (C-10); **LRMS** (ES+) mass not found; **HRMS** (ESI) exact mass calculated for $[M-OH]^+$ ($C_{15}H_{15}{}^{79}Br_2O$) requires *m/z* 384.9439, found *m/z* 384.9437; **mp** 43 – 45 °C.

7-Methyl-7-vinyl-4,5,6,7-tetrahydroisobenzofuran-4-ol – 190

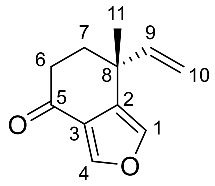


Synthesised according to a reported procedure.[164] To a solution of **176** (362 mg, 1.18 mmol, 1.00 eq) in acetonitrile (11.8 mL) was added H_2O (483 µL), TEA (487 µL, 3.49 mmol, 2.96 eq) and TBAB (1.14 g, 3.54 mmol, 3.00 eq). The solution was purged with N_2 for 15 minutes, then $Pd(OAc)_2$ (13.2 mg, 0.059 mmol, 0.05 eq) was quickly added. The reaction was stirred in a pre-heated oil bath at 75 °C for 17 h. Once cooled, the reaction was filtered through Celite® with EtOAc (25 mL) and the solution concentrated *in vacuo*. The resulting oil was triturated with Et_2O (25 mL) and the solution concentrated *in vacuo*. The crude product was purified by FCC (pentane:EtOAc, 6:1) to afford title compound **190** (161 mg, 77%) as a pale-yellow oil and a 4:1 mixture of inseparable diastereomers.

Rf 0.15 (pentane:EtOAc, 6:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.47 (t, *J* = 1.5 Hz, 0.2H, H-1_{min}), 7.45 (t, *J* = 1.5 Hz, 0.8H, H-1_{maj}), 7.19 (d, *J* = 1.5 Hz, 0.2H, H-4_{min}), 7.17 (d, *J* = 1.5 Hz, 0.8H, H-4_{maj}), 5.89 (dd, *J* = 17.0 Hz, 10.5 Hz, 0.8H, H-9_{maj}), 5.82 (dd, *J* = 17.0 Hz, 10.5 Hz, 0.2H, H-9_{min}), 5.01 (dd, *J* = 10.5 Hz, 1.5 Hz, 0.8H, H-

10_{maj}), 4.96 (dd, *J* = 10.5 Hz, 1.5 Hz, 0.2H, H-10_{min}), 4.85 (dd, *J* = 17.0 Hz, 1.5 Hz, 0.8H, H-10_{maj}), 4.79 – 4.69 (m, 1.2H, H-10_{min} and H-5), 2.04 – 1.94 (m, 1H, H-6), 1.85 – 1.76 (m, 1H, H-7), 1.73 – 1.62 (m, 1H, H-6), 1.59 – 1.50 (m, 2H, H-7 and OH), 1.35 (s, 0.6H, H-11_{min}), 1.29 (s, 2.4H, H-11_{maj}); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 147.0 (C-9_{maj}), 146.8 (C-9_{min}), 139.8 (C-1_{min}), 139.3 (C-1_{maj}), 138.9 (C-4_{maj}), 138.9 (C-4_{min}), 127.7 (C-2_{maj}), 127.2 (C-2_{min}), 125.7 (C-3_{maj}), 125.1 (C-3_{min}), 112.7 (C-10_{maj}), 112.7 (C-10_{min}), 64.2 (C-5_{maj}), 61.7 (C-5_{min}), 36.9 (C-8_{min}), 36.8 (C-8_{maj}), 34.9 (C-7_{maj}), 32.5 (C-7_{min}), 30.4 (C-6_{maj}), 29.5 (C-6_{min}), 29.0 (C-11_{min}), 28.9 (C-11_{maj}). Data consistent with literature.[164]

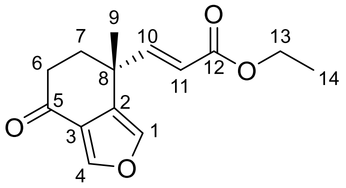
7-Methyl-7-vinyl-6,7-dihydroisobenzofuran-4(5*H*)-one – 191



Synthesised according to a reported procedure.[164] To a solution of **187** (81.7 mg, 0.27 mmol, 1.00 eq) in acetonitrile (2.7 mL) was added H_2O (111 µL), TEA (112 µL, 0.80 mmol, 2.96 eq) and TBAB (261.1 mg, 0.81 mmol, 3.00 eq). The solution was purged with N_2 for 15 minutes, then $Pd(OAc)_2$ (3.1 mg, 0.014 mmol, 0.05 eq) was quickly added. The reaction mixture was stirred in a pre-heated oil bath at 75 °C for 17 h. Once cooled, the reaction was filtered through Celite® with EtOAc (7 mL) and the solution concentrated *in vacuo*. The resulting oil was triturated with Et_2O (10 mL) and the solution concentrated *in vacuo* to afford title compound **191** (50.8 mg, quant.) as a brown oil which was used directly, without further purification.

R$_f$ 0.55 (pentane:EtOAc, 6:1, stain: vanillin); **IR** ν_{max} 2963 (C-H), 1688 (C=O), 1636 (C=C), 921 (=C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.99 (d, *J* = 1.5 Hz, 1H, H-1), 7.27 (d, *J* = 1.5 Hz, 1H, H-4), 5.89 (dd, *J* = 17.0 Hz, 10.5 Hz, 1H, H-9), 5.09 (dd, *J* = 10.5 Hz, 1.0 Hz, 1H, H-10), 4.79 (dd, *J* = 17.0 Hz, 1.0 Hz, 1H, H-10), 2.62 – 2.42 (m, 2H, H-6), 2.02 – 1.92 (m, 2H, H-7), 1.41 (s, 3H, H-11); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 195.2 (C-5), 144.6 (C-9), 144.5 (C-1), 139.4 (C-4), 114.6 (C-10), 37.2 (C-7), 36.5 (C-6), 27.4 (C-11); **LRMS** (ES+) mass found *m/z* 177.1 $[M+H]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{11}H_{13}O_2$) requires *m/z* 177.0910, found *m/z* 177.0912.

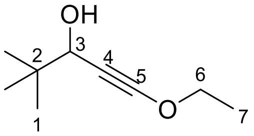
Ethyl (*E*)-3-(4-methyl-7-oxo-4,5,6,7-tetrahydroisobenzofuran-4-yl)acrylate – 192



Synthesised according to a reported procedure.[300] A solution of 18-crown-6 (238 mg, 0.90 mmol, 5 eq) and triethyl phosphonoacetate (29 μL, 0.18 mmol, 1 eq) in THF (1.8 mL) was prepared under N_2 and cooled to –78 °C, then KHMDS (360 μL, 0.5 M in toluene, 0.18 mmol, 1 eq) slowly added. A solution of **160** (32.7 mg, 0.18 mmol, 1 eq) in THF (1.8 mL) was added and the reaction stirred for 30 min. The reaction mixture was quenched with sat. aq. NH_4Cl solution (3 mL), the layers separated, and the aqueous layer extracted with Et_2O (3 × 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:EtOAc, 8:1) to afford title compound **192** (4.9 mg, 11%) as a yellow oil.

R$_f$ 0.15 (pentane:EtOAc, 8:1, stain: vanillin); **IR** ν_{max} 2963 (C-H), 2927 (C-H), 1717 (C=O), 1691 (C=O), 1267 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 8.01 (d, *J* = 1.5 Hz, 1H, H-4), 7.31 (d, *J* = 1.5 Hz, 1H, H-1), 7.00 (d, *J* = 15.5 Hz, 1H, H-10), 5.56 (d, *J* = 15.5 Hz, 1H, H-11), 4.17 (q, *J* = 7.0 Hz, 2H, H-13), 2.50 – 2.45 (m, 2H, H-6), 2.09 – 2.05 (m, 2H, H-7), 1.48 (s, 3H, H-9), 1.27 (t, *J* = 7.0 Hz, 3H, H-14); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 194.3 (C-5), 166.5 (C-12), 153.7 (C-10), 145.1 (C-4), 139.5 (C-1), 129.5 (C-2), 123.0 (C-3), 121.7 (C-11), 60.7 (C-13), 36.9 (C-7), 36.7 (C-8), 36.5 (C-6), 26.9 (C-9), 14.3 (C-14); **LRMS** (ES+) mass found *m/z* 271.1 $[M+Na]^+$; **HRMS** mass not found.

1-Ethoxy-4,4-dimethylpent-1-yn-3-ol – 197



Synthesised according to a reported procedure.[177] To a solution of ethoxy acetylene (1.2 mL, 40% in hexanes, 5.0 mmol, 2 eq) in THF (3 mL) at –78 °C was added *n*-butyllithium (3.1 mL, 1.6 M in hexanes, 5.0 mmol, 2 eq) dropwise. The reaction mixture was stirred for 2 h, then warmed to 0 °C and stirred a further 2 h. The reaction was cooled again to –78 °C and a solution of pivalaldehyde (272 μL, 2.5 mmol, 1 eq) in THF (3.5 mL) was added dropwise. The reaction mixture was stirred for 1 h, then warmed to rt and stirred for a further 2 h, then cooled to 0 °C and diluted with water (5 mL). The layers were separated and the aqueous layer extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **197** (433 mg, quant.) as a brown oil.

R_f 0.55 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **IR** ν_{max} 3419 (O-H), 2956 (C-H), 2263 (C≡C), 1000 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 4.09 (q, *J* = 7.0 Hz, 2H, H-6), 4.03 (d, *J* = 6.0 Hz, 1H, H-3), 1.38 (t, *J* = 7.0 Hz, 3H, H-7), 0.97 (s, 9H, H-1); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 94.4, 74.7, 71.5, 38.2, 36.2, 25.5, 14.5;[*] **LRMS** (ES+) mass found *m/z* 111.1 $[M-C_2H_5O]^+$; **HRMS** (ESI) exact mass calculated for $[M+Na]^+$ ($C_9H_{16}NaO_2$) requires *m/z* 179.1043, found *m/z* 179.1043.

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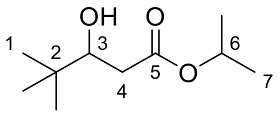
[*] No 2-D spectra available for this compound.

Isopropyl 3-hydroxy-4,4-dimethylpentanoate – 198

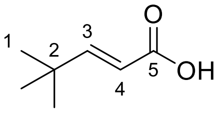


A solution of **197** (100 mg, 0.64, 1 eq) and isopropanol (98 µL, 1.28 mmol, 2 eq) in *m*-xylene (6 mL) was heated to 150 °C for 2 h. The reaction was then cooled to rt, the solvent removed *in vacuo*, and the crude product purified by FCC (pentane:Et_2O, 4:1) to afford title compound **198** (22.3 mg, 19%) as a colourless oil.

R

R_f 0.20 (pentane:Et_2O, 10:1, stain: $KMnO_4$); **IR** ν_{max} 3400 (O-H), 2966 (C-H), 1719 (C=O), 1163 (C-O), 1110 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 5.06 (p, *J* = 6.5 Hz, 1H, H-6), 3.69 (ddd, *J* = 10.5 Hz, 3.5 Hz, 2.0 Hz, 1H, H-3), 2.49 (dd, *J* = 16.0 Hz, 2.0 Hz, 1H, H-4)), 2.32 (dd, *J* = 16.0 Hz, 10.5 Hz, 1H, H-4), 1.25 (d, *J* = 6.5 Hz, 6H, H-7), 0.92 (s, 9H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 173.3 (C-5), 75.6 (C-3), 69.1 (C-6), 37.0 (C-4), 34.5 (C-2), 25.8 (C-1), 22.0 (C-7); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+Na]^+$ ($C_{10}H_{20}NaO_3$) requires *m/z* 211.1305, found *m/z* 211.1307.*

(*E*)-4,4-Dimethylpent-2-enoic acid – 199



Compound **199** was isolated as the unexpected major product in the synthesis of **198** and **202** as a pale-yellow oil in varying yields.

R_f 0.30 (pentane:Et_2O, 2:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.08 (d, *J* = 16.0 Hz, 1H, H-3), 5.75 (d, *J* = 16.0 Hz, 1H, H-4), 1.09 (s, 9H, H-1); **^{13}C NMR** ($CDCl_3$,

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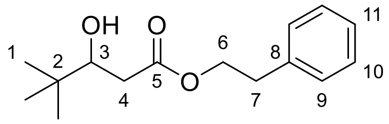
* Sample contained trace impurities.

101 MHz) δ_C 172.5 (C-5), 162.0 (C-3), 116.2 (C-4), 34.1 (C-2), 28.7 (C-1). Data consistent with literature.[302]

Phenethyl 3-hydroxy-4,4-dimethylpentanoate – 202



A solution of **197** (100 mg, 0.64, 1 eq) and phenethyl alcohol (76 µL, 0.64 mmol, 1 eq) in *m*-xylene (6 mL) was heated to 150 °C for 2 h. The reaction was then cooled to rt, the solvent removed *in vacuo*, and the crude product purified by FCC (pentane:Et_2O, 5:1) to afford title compound **202** (16.9 mg, 11%) as a colourless oil.

R_f 0.25 (pentane:Et_2O, 5:1, stain: $KMnO_4$); **IR** ν_{max} 3520 (O-H), 2958 (C-H), 1713 (C=O), 1161 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.34 – 7.28 (m, 2H, H-10), 7.25 – 7.20 (m, 3H, H-9 and H-11), 4.34 (t, *J* = 7.0 Hz, 2H, H-6), 3.66 (ddd, *J* = 10.5 Hz, 3.5 Hz, 2.0 Hz, 1H, H-3), 2.96 (t, *J* = 7.0 Hz, 2H, H-7), 2.51 (dd, *J* = 16.0 Hz, 2.0 Hz, 1H, H-4), 2.33 (dd, *J* = 16.0 Hz, 10.5 Hz, 1H, H-4), 0.91 (s, 9H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 174.8 (C-5), 139.3 (C-8), 129.0 (C-9), 128.7 (C-10), 127.4 (C-11), 75.6 (C-3), 65.3 (C-6), 36.8 (C-4), 35.2 (C-7), 34.5 (C-2), 25.7 (C-1); **LRMS** (ES+) mass found *m/z* 273.1 $[M+Na]^+$; **HRMS** (ESI) exact mass calculated for $[M+Na]^+$ ($C_{15}H_{22}NaO_3$) requires *m/z* 273.1461, found *m/z* 273.1462.[*]

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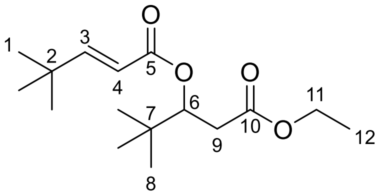
[*] Sample contained trace impurities.

1-Ethoxy-4,4-dimethyl-1-oxopentan-3-yl (*E*)-4,4-dimethylpent-2-enoate – 203

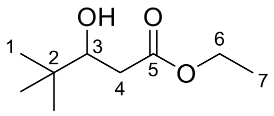


Compound **203** was isolated as an unexpected side product from the attempted *retro*-ene reaction of **197** in the presence of benzofuran.

A solution of **197** (150 mg, 0.96 mmol, 1.0 eq) in benzofuran (132 μL, 1.2 mmol, 1.25 eq) was heated to 150 °C for 2 h. The reaction was cooled, and the crude product purified by FCC (pentane:Et_2O, 10:1) to afford title compound **203** (8.5 mg, 3%) as a yellow oil.

Rf 0.15 (pentane:Et_2O, 10:1, stain: $KMnO_4$); **IR** ν_{max} 2963 (C-H), 1741 (C=O), 1650 (C=C), 1298 (C-O), 1159 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 6.96 (d, *J* = 16.0 Hz, 1H, H-3), 5.71 (d, *J* = 16.0 Hz, 1H, H-4), 5.22 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-6), 4.09 (qd, *J* = 7.0 Hz, 2.0 Hz, 2H, H-11), 2.59 (dd, *J* = 15.0 Hz, 3.0 Hz, 1H, H-9), 2.49 (dd, *J* = 15.0 Hz, 10.0 Hz, 1H, H-9), 1.21 (t, *J* = 7.0 Hz, 3H, H-12), 1.07 (s, 9H, H-1), 0.94 (s, 9H, H-8); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 171.4 (C-10), 166.7 (C-5), 159.5 (C-3), 116.7 (C-4), 76.7 (C-6), 60.8 (C-11), 36.2 (C-9), 34.9 (C-7), 33.9 (C-2), 28.8 (C-1), 25.9 (C-8), 14.2 (C-12); **LRMS** (ES+) mass found *m/z* 307.2 $[M+Na]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{16}H_{29}O_4$) requires *m/z* 285.2060, found *m/z* 285.2062.

Ethyl 3-hydroxy-4,4-dimethylpentanoate – 204

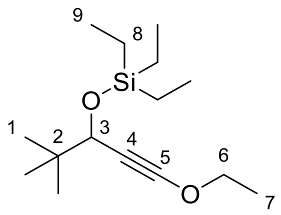


Compound **204** was isolated as an unexpected side product from the attempted *retro*-ene reaction of **197** in the presence of benzofuran.

A solution of **197** (150 mg, 0.96 mmol, 1.0 eq) in benzofuran (132 μL, 1.2 mmol, 1.25 eq) was heated to 150 °C for 2 h. The reaction was cooled, and the crude product purified by FCC (pentane:Et_2O, 10:1) to afford title compound **204** (10.6 mg, 6%) as a yellow oil.

R_f 0.15 (pentane:Et_2O, 5:1, stain: $KMnO_4$); **IR** ν_{max} 3489 (O-H), 2959 (C-H), 1733 (C=O), 1260 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 4.17 (q, *J* = 7.0 Hz, 2H, H-6), 3.70 (ddd, *J* = 10.5 Hz, 3.5 Hz, 2.0 Hz, 1H, H-3), 2.87 (d, *J* = 3.5 Hz, 1H, OH), 2.52 (dd, *J* = 16.0 Hz, 2.0 Hz, 1H, H-4), 2.35 (dd, *J* = 16.0 Hz, 10.5 Hz, 1H, H-4), 1.28 (t, *J* = 7.0 Hz, 3H, H-7), 0.92 (s, 9H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 174.1 (C-5), 75.6 (C-3), 60.9 (C-6), 36.7 (C-4), 34.5 (C-2), 25.7 (C-1), 14.3 (C-7); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+Na]^+$ ($C_9H_{18}NaO_3$) requires *m/z* 197.1148, found *m/z* 197.1151.

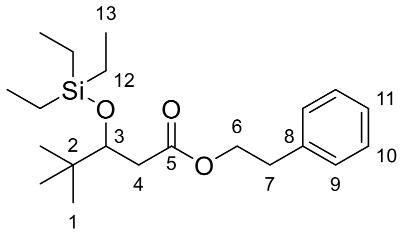
((1-Ethoxy-4,4-dimethylpent-1-yn-3-yl)oxy)triethylsilane – 205



To a solution of **197** (100 mg, 0.64 mmol, 1 eq) in DMF (1.3 mL) at 0 °C was added imidazole (131 mg, 1.92 mmol, 3 eq), then chlorotriethylsilane (215 µL, 1.28 mmol, 2 eq) dropwise. The reaction was stirred for 2 h at 0 °C, then quenched with water (5 mL) and diluted with Et_2O (20 mL). The organic layer was washed with water (3 × 20 mL), then brine (20 mL) and dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 5:1) to afford title compound **205** (139.4 mg, 81%) as a colourless oil.

Rf 0.45 (pentane, stain: $KMnO_4$); **IR** ν_{max} 2955 (C-H), 2265 (C=C), 1237 (C-O), 1072 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 4.06 (q, *J* = 7.0 Hz, 2H, H-6), 4.01 (s, 1H, H-3), 1.37 (t, *J* = 7.0 Hz, 3H, H-7), 0.98 (t, *J* = 8.0 Hz, 9H, H-9), 0.92 (s, 9H, H-1), 0.71 – 0.58 (m, 6H, H-8); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 95.3 (C-5), 74.2 (C-6), 71.5 (C-3), 38.8 (C-4), 36.6 (C-2), 25.7 (C-1), 14.6 (C-7), 7.1 (C-9), 5.1 (C-8); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{15}H_{31}O_2Si$) requires *m/z* 271.2088, found *m/z* 271.2090.

Phenethyl 4,4-dimethyl-3-((triethylsilyl)oxy)pentanoate – 206



A solution of **205** (150 mg, 0.55, 1 eq) and benzyl carbinol (66 µL, 0.55 mmol, 1 eq) in *m*-xylene (5.5 mL) was heated to 150 °C for 2 h. The reaction was then cooled to rt, the solvent removed *in vacuo*, and the crude product purified by FCC (pentane:Et_2O, 20:1) to afford title compound **206** (10.7 mg, 5%) as a pale-yellow oil.

R$_f$ 0.50 (pentane:Et_2O, 50:1, stain: $KMnO_4$); **IR** ν_{max} 2955 (C-H), 2876 (C-H), 1736 (C=O), 1163 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.34 – 7.28 (m, 2H, H-10), 7.25 – 7.21 (m, 3H, H-9 and H-11), 4.37 – 4.22 (m, 2H, H-6), 3.89 (dd, *J* = 7.5 Hz, 4.0 Hz, 3H, H-3), 2.96 (dt, *J* = 10.5 Hz, 7.0 Hz, 2H, H-7), 2.52 (dd, *J* = 16.0 Hz, 4.0 Hz, 1H, H-4), 2.32 (dd, *J* = 16.0 Hz, 7.5 Hz, 1H, H-4), 0.94 (t, *J* = 8.0 Hz, 9H, H-13), 0.85 (s, 9H, H-1), 0.58 (q, *J* = 8.0 Hz, 6H, H-12); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 173.0 (C-5), 138.0 (C-8), 129.0 (C-9 or C-11), 128.6 (C-10), 126.7 (C-9 or C-11), 77.0 (C-3), 65.1 (C-6), 39.3 (C-4), 35.7 (C-2), 35.2 (C-7), 26.0 (C-1), 7.2 (C-13), 5.4 (C-12); **LRMS** (ES+) mass found *m/z* 387.2 $[M+Na]^+$; **HRMS** (ESI) exact mass calculated for $[M+Na]^+$ ($C_{21}H_{36}NaO_3Si$) requires *m/z* 387.2326, found *m/z* 387.2328.[*]

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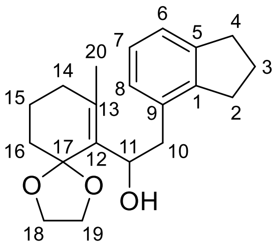
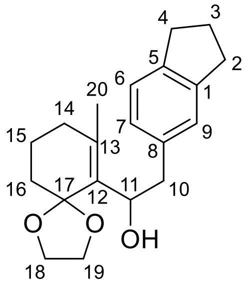
[*] Sample contained trace impurities.

Compounds from Chapter 2

2-(2,3-Dihydro-1*H*-inden-4-yl)-1-(7-methyl-1,4-dioxaspiro[4.5]dec-6-en-6-yl)ethan-1-ol – 210a & 2-(2,3-dihydro-1*H*-inden-5-yl)-1-(7-methyl-1,4-dioxaspiro[4.5]dec-6-en-6-yl)ethan-1-ol – 210b



a



b

Small, fresh slices of Li wire (23.6 mg, 3.4 mmol, 2.5 eq) were cut and added to a flask of naphthalene (474 mg, 3.7 mmol, 2.75 eq) under a stream of N_2. The flask was purged and sealed under N_2, then THF (14 mL) added and the mixture stirred at rt until a dark green solution formed (*ca*. 10 min). A mixture of **211** (245 mg, 1.34 mmol, 1.0 eq) and **212a** and **212b** (270 mg, 1:3 ratio, 1.62 mmol, 1.2 eq) in THF (0.2 mL) was added dropwise, accompanied by instantaneous colour change. The solid lithium was removed from the reaction and the remaining solution evaporated to dryness. The crude residue was hydrolysed with 1.0 M aq. HCl (5 mL), extracted with Et_2O (3 × 5 mL), and washed with brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated *in vacuo*, and the crude product purified by FCC (PE:Et_2O, 10:1) to afford title compounds **210a** and **210b** (56.0 mg, 12%) in a 1:4 ratio **a**:**b** of inseparable regioisomers, as a pale-yellow oil.*

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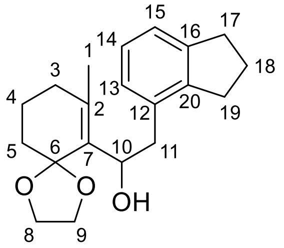
* Ratio calculated from H-11 peak integrations.

R_f 0.25 (pentane:Et_2O, 3:2, stain: vanillin); **IR** ν_{max} 3472 (O-H), 2934 (C-H), 1040 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.18 – 7.00 (m, 3H, H-Ar), 4.72 – 4.67 (m, 0.2H, H-11$_a$), 4.68 – 4.62 (m, 0.8H, H-11$_b$), 4.17 – 4.10 (m, 2H, H-18 or H-19), 4.08 – 4.02 (m, 2H, H-18 or H-19), 3.11 – 2.97 (m, 2H, H-10), 2.97 – 2.84 (m, 4H, H-2 and H-4), 2.11 – 2.01 (m, 4H, H-3 and H-14), 1.84 – 1.79 (m, 1H, H-16), 1.76 (s, 3H, H-20), 1.73 – 1.63 (m, 3H, H-15 and H-16); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 144.4 (C-5$_b$), 144.2 (C-5$_a$), 143.4 (C-1$_a$), 142.0 (C-1$_b$), 140.5 (C-12$_a$), 140.4 (C-12$_b$), 138.1 (C-8$_b$), 135.9 (C-9$_a$), 131.1 (C-13$_b$), 131.1 (C-13$_a$), 127.6 (C-8$_a$), 127.3 (C-6$_b$ or C-7$_b$ or C-9$_b$), 126.3 (C-6$_a$ or C-7$_a$), 125.5 (C-6$_b$ or C-7$_b$ or C-9$_b$), 124.2 (C-6$_b$ or C-7$_b$ or C-9$_b$), 122.4 (C-6$_a$ or C-7$_a$), 110.0 (C-17$_b$), 109.9 (C-17$_a$), 72.4 (C-11$_b$), 71.1 (C-11$_a$), 64.3 (C-18$_b$ or C-19$_b$), 64.2 (C-18$_a$ or C-19$_a$), 64.1 (C-18$_a$ or C-19$_a$), 64.1 (C-18$_b$ or C-19$_b$), 43.4 (C-10$_b$), 41.0 (C-10$_a$), 33.3 (C-4$_a$), 33.3 (C-4$_b$), 33.2 (C-16$_a$), 33.0 (C-16$_b$), 32.9 (C-14$_a$), 32.6 (C-14$_b$), 31.7 (C-2$_a$), 31.7 (C-2$_b$), 25.7 (C-3$_b$), 25.2 (C-3$_a$), 20.4 (C-20$_a$), 20.3 (C-20$_b$), 20.1 (C-15$_a$), 20.1 (C-15$_b$); **LRMS** (ES+) mass found *m/z* 337.2 [M+Na]$^+$; **HRMS** (ES+) exact mass calculated for [M+Na]$^+$ ($C_{20}H_{26}NaO_3$) requires *m/z* 337.1774, found *m/z* 337.1774.

2-(2,3-Dihydro-1*H*-inden-4-yl)-1-(7-methyl-1,4-dioxaspiro[4.5]dec-6-en-6-yl)ethan-1-ol – 210a

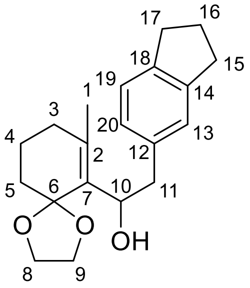


Compound **210a** was prepared separately as a single regioisomer from aldehyde **251a**.

To a solution of **215** (47.6 mg, 0.17 mmol, 1.0 eq) in THF (340 µL) under N_2 at –78 °C was added *n*-butyllithium (119 µL, 1.6 M in hexanes, 0.19 mmol, 1.1 eq) dropwise and the solution stirred for 5 min. A solution of **251a** (30.0 mg, 0.19 mmol, 1.1 eq) in THF (850 µL) was added to this, *via* syringe pump, at a rate of 1.78 mL/h. Once addition was complete, the mixture was allowed to stir for 15 minutes, then quenched at –78 °C with sat. aq. NH_4Cl solution (1 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 2 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 1:1) to afford title compound **210a** (14.4 mg, 27%) as a pale-yellow oil.

Rf 0.25 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **IR** ν_{max} 3464 (O-H), 2935 (C-H), 1039 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.12 – 7.04 (m, 3H, H-Ar), 4.73 – 4.63 (m, 1H, H-10), 4.16 – 4.09 (m, 2H, H-8 or H-9), 4.09 – 4.02 (m, 2H, H-8 or H-9), 3.12 – 2.97 (m, 2H, H-11), 2.97 – 2.88 (m, 4H, H-17 and H-19), 2.10 – 2.00 (m, 4H, H-3 and H-18), 1.84 – 1.78 (m, 1H, H-5), 1.76 (s, 3H, H-1), 1.74 – 1.67 (m, 3H, H-4 and H-5); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 144.2 (C-16), 143.4 (C-20), 140.5 (C-7), 135.9 (C-12), 131.1 (C-2), 127.6 (C-13), 126.3 (C-14 or C-15), 122.4, (C-14 or C-15), 109.9 (C-6), 71.1 (C-10), 64.2 (C-8 or C-9), 64.1 (C-8 or C-9), 41.0 (C-11), 33.3 (C-17), 33.2 (C-5), 33.0 (C-3), 31.7 (C-19), 25.2 (C-18), 20.4 (C-1), 20.1 (C-4); **LRMS** (ES+) mass found *m/z* 337.2 $[M+Na]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{20}H_{26}NaO_3$) requires *m/z* 337.1770, found *m/z* 337.1774.

2-(2,3-Dihydro-1*H*-inden-5-yl)-1-(7-methyl-1,4-dioxaspiro[4.5]dec-6-en-6-yl)ethan-1-ol – 210b



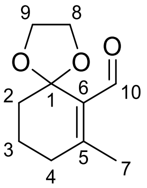
Compound **210b** was prepared separately as a single regioisomer from aldehyde **251b**.

To a solution of **215** (140 mg, 0.5 mmol, 1.0 eq) in THF (1 mL) under N_2 at –78 °C was added *n*-butyllithium (344 μL, 1.6 M in hexanes, 0.55 mmol, 1.1 eq) dropwise and the solution stirred for 5 min. A solution of **251b** (88.7 mg, 0.55 mmol, 1.1 eq) in THF (2.5 mL) was added to this, *via* syringe pump, at a rate of 1.78 mL/h. Once addition was complete, the mixture was allowed to stir for 15 minutes, then quenched at –78 °C with sat. aq. NH_4Cl solution (2 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 3 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 20:1) to afford title compound **210b** (46.8 mg, 33%) as a white solid.

R_f 0.20 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **IR** ν_{max} 3469 (O-H), 2935 (C-H), 1439 (C=C), 1041 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.16 – 7.13 (m, 2H, H-Ar), 7.06 – 7.01 (m, 1H, H-Ar), 4.64 (dt, *J* = 9.5 Hz, 5.0 Hz, 1H, H-10), 4.17 – 4.10 (m, 2H, H-8 or H-9), 4.09 – 4.02 (m, 2H, H-8 or H-9), 3.03 – 2.95 (m, 2H, H-11), 2.92 – 2.83 (m, 4H, H-15 and H-17), 2.10 – 1.99 (m, 4H, H-3 and H-16), 1.83 –1.77 (m, 1H, H-5), 1.76 (s, 3H, H-1), 1.73 – 1.65 (m, 3H, H-4 and H-5); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 144.4 (C-18), 142.0 (C-14), 140.4 (C-7), 138.1 (C-12), 131.1 (C-2), 127.3 (HC-Ar), 125.5 (HC-Ar), 124.2 (HC-Ar), 110.0 (C-6), 72.4 (C-10), 64.3 (C-8 or C-9), 64.1 (C-8 or C-9), 43.4 (C-11), 33.3 (C-

17), 33.0 (C-5), 32.6 (C-3), 31.7 (C-15), 25.7 (C-16), 20.3 (C-1), 20.1 (C-4); **LRMS** (ES+) mass found *m/z* 353.2 [M+K]$^+$; **HRMS** (ESI) exact mass calculated for [M+Na]$^+$ ($C_{20}H_{26}NaO_3$) requires *m/z* 337.1174, found *m/z* 337.1775; **mp** 79 – 81 °C.

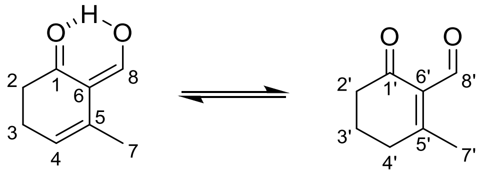
7-Methyl-1,4-dioxaspiro[4.5]dec-6-ene-6-carbaldehyde – 211



Synthesised according to a reported procedure.[178] To a solution of **215** (3.5 g, 12.4 mmol, 1 eq) in THF (52.5 mL) under N_2 at –78 °C was added dropwise *n*-butyllithium (8.5 mL, 1.6 M in hexanes, 13.6 mmol, 1.1 eq) and the reaction stirred for 5 min. DMF (1.05 mL, 13.6 mmol, 1.1 eq) was added dropwise and the mixture stirred a further 10 min. The reaction was quenched at –78 °C with 20% aq. $NaHPO_4$ solution (10 mL), warmed to rt, the phases separated and the aqueous layer extracted with Et_2O (3 × 20 mL). The combined organic phases were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (PE:EtOAc, 6:1) to afford title compound **211** (1.54 g, 68%) as a white solid.

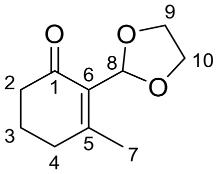
Rf 0.15 (pentane:EtOAc, 6:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 9.86 (s, 1H, H-10), 4.18 – 4.10 (m, 2H, H-8 or H-9), 4.08 – 3.99 (m, 2H, H-8 or H-9), 2.27 – 2.18 (m, 2H, H-4), 2.14 (s, 3H, H-7), 1.79 – 1.72 (m, 4H, H-2 and H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 192.1 (C-10), 158.3 (C-5), 131.9 (C-6), 107.2 (C-1), 65.4 (C-8), 65.4 (C-9), 34.5 (C-4), 33.6 (C-2), 20.6 (C-7), 19.9 (C-3); **mp** 50 – 51 °C, lit. 51 – 52 °C. Data consistent with literature.[178]

2-Methyl-6-oxocyclohex-1-ene-1-carbaldehyde – 216 (in equilibrium with 217)



Compound **216** (and **217**) was isolated as a yellow oil as an unexpected decomposition product of aldehyde **211**. It was identified tentatively by ^{1}H NMR but had decomposed further before full characterisation data could be obtained.

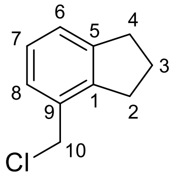
2-(1,3-Dioxolan-2-yl)-3-methylcyclohex-2-en-1-one – 218



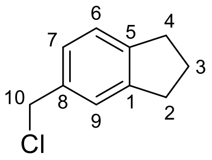
Compound **218** was isolated as a pale-yellow oil as an unexpected decomposition product of aldehyde **211**.

Rf 0.25 (pentane:EtOAc, 2:1, stain: $KMnO_4$); **IR** *νmax* 2951 (C-H), 2889 (C-H), 1662 (C=O), 1629 (C=C), 1077 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 5.94 (s, 1H, H-8), 4.13 – 4.07 (m, 2H, H-9 or H-10), 3.95 – 3.89 (m, 2H, H-9 or H-10), 2.44 – 2.37 (m, 4H, H-2 and H-4), 2.12 (s, 3H, H-7), 2.00 – 1.91 (m, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 197.6 (C-1), 165.3 (C-5), 129.9 (C-6), 98.3 (C-8), 65.0 (C-9), 65.0 (C-10), 37.8 (C-2), 34.5 (C-4), 21.9 (C-3), 21.3 (C-7); **LRMS** mass not found; **HRMS** (ES+) exact mass calculated for $[M+H]^+$ ($C_{10}H_{15}O_3$) requires *m/z* 183.1016, found *m/z* 183.1017; **mp** 45 – 47 °C.

4-(Chloromethyl)-2,3-dihydro-1*H*-indene – 212a & 5-(chloromethyl)-2,3-dihydro-1*H*-indene – 212b



a



b

Synthesised according to a reported procedure.[303] To a well-stirred mixture of indane (10.4 mL, 84.6 mmol, 1 eq), 37% (w/w) aq. formaldehyde (10.6 mL) and conc. HCl (18 mL) at 65 °C was added conc. H_2SO_4 (11.8 mL) *via* syringe pump and the reaction stirred for 22 h. The cooled mixture was poured into cold water (100 mL), the phases separated, and the aqueous layer extracted with Et_2O (3 × 100 mL). The combined organic phases were washed with water (200 mL), then brine (200 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford a thick brown sludge as the crude product. The crude was cleaned in batches *via* Kügelrohr distillation (<1 mbar, 200 °C) to produce a colourless liquid. Repeat distillations were required to extract all product from the crude mixture. The resulting colourless liquid was purified by FCC (PE)[*] to afford title compounds **212a** and **212b** (6.81 g, 48%) in a 1:3 ratio **a**:**b** of inseparable regioisomers, as a colourless liquid.[†]

R

R_f 0.75 (PE, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.27 – 7.13 (m, 3H, H-Ar), 4.59 (s, 0.5H, H-10$_a$), 4.58 (s, 1.5H, H-10$_b$), 3.04 – 2.87 (m, 4H, H-2 and H-4), 2.28 – 1.89 (m, 2H, H-5); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 145.2 (C-Ar$_a$), 145.1 (C-Ar$_b$), 144.9 (C-Ar$_b$), 143.6 (C-Ar$_a$), 135.5 (C-Ar$_b$), 133.1 (C-Ar$_a$), 126.8 (HC-Ar$_a$), 126.8 (HC-Ar$_b$),

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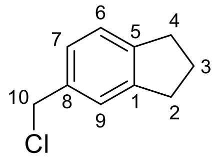
[*] Due to the extreme apolarity of this compound, and the impurities present, multiple consecutive columns may be necessary to fully purify large scale iterations of this reaction.

[†] Ratio calculated from H-10 peak integrations. Regiochemistry assigned by analogy with literature and later confirmed by isolation of **212b**.

124.9 (HC-Ar$_a$), 124.9 (HC-Ar$_a$), 124.9 (HC-Ar$_b$), 124.7 (HC-Ar$_b$), 46.9 (C-10$_b$), 44.9 (C-10$_a$), 33.0 (C-2$_a$ or C-4$_a$), 32.9 (C-2$_b$ or C-4$_b$), 32.8 (C-2$_b$ or C-4$_b$), 30.9 (C-2$_a$ or C-4$_a$), 25.6 (C-3$_b$), 25.0 (C-3$_a$); **bp** obs.: 225 °C, calc.: 424 °C. Data consistent with literature.[179]

5-(Chloromethyl)-2,3-dihydro-1*H*-indene – 212b

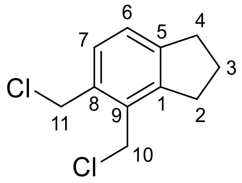


Chloride **212b** was prepared separately as a single regioisomer from pure **224b**.

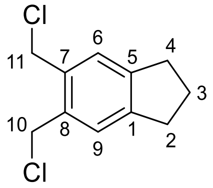
To a solution of **224b** (680 mg, 4.6 mmol, 1.0 eq) in DCM (23 mL) under N_2 were added TEA (836 µL, 6.0 mmol, 1.3 eq) and DMAP (56.2 mg, 0.46 mmol, 0.1 eq). Tosyl chloride (1.14 g, 6.0 mmol, 1.3 eq) was added slowly under a stream of N_2 and the reaction mixture stirred at rt for 24 h. The reaction was quenched with 1.0 M aq. HCl (15 mL) and the aqueous layer extracted with DCM (3 × 15 mL). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane), to afford title compound **212b** (422 mg, 55%) as a colourless oil.

R$_f$ 0.75 (PE, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.26 (s, 1H, H-9), 7.21 (d, *J* = 8.0 Hz, 1H, H-6 or H-7), 7.15 (d, *J* = 8.0 Hz, 1H, H-6 or H-7), 4.58 (s, 2H, H-10), 2.91 (ap. td, *J* = 7.5 Hz, 3.5 Hz, 4H, H-2 and H-4), 2.08 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 145.1 (C-Ar), 144.9 (C-Ar), 135.5 (C-Ar), 126.8 (HC-Ar), 124.9 (HC-Ar), 124.7 (HC-Ar), 46.9 (C-10), 32.9 (C-2 or C-4), 32.8 (C-2 or C-4), 25.6 (C-3). Data consistent with literature.[179]

4,5-Bis(chloromethyl)-2,3-dihydro-1*H*-indene – 220a & 5,6-bis(chloromethyl)-2,3-dihydro-1*H*-indene – 220b



a



b

Compounds **220a** and **220b** were isolated as an inseparable mixture of isomers in a 1:1.4 ratio **a**:**b**[*] as an unexpected side product (white solid) in the formation of chlorides **212a** and **212b**.

R_f 0.30 (pentane, stain: $KMnO_4$); **IR** ν_{max} 2953 (C-H), 1447 (C=C), 690 (C-Cl); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ_H 7.26 (s, 2H, H-6_b and H-9_b), 7.21 (d, *J* = 7.5 Hz, 0.7H, H-6_a or H-7_a), 7.18 (d, *J* = 7.5 Hz, 0.7H, H-6_a or H-7_a), 4.77 (s, 1.4H, H-10_a), 4.75 (s, 4H, H-10_b and H-11_b), 4.74 (s, 1.4H, H-11_a), 3.01 (t, *J* = 7.5 Hz, 1.4H, H-2_a or H-4_a), 2.95 (t, *J* = 7.5 Hz, 1.4H, H-2_a or H-4_a), 2.91 (t, *J* = 7.5 Hz, 4H, H-2_b and H-4_b), 2.17 – 2.04 (m, 3.4H, H-3_a and H-3_b); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ_C 146.3 (C-Ar_b), 146.0 (C-Ar_a), 145.5 (C-Ar_a), 134.2 (C-7_b and C-8_b), 134.0 (C-Ar_a), 132.1 (C-Ar_a), 129.2 (C-6_a and C-7_a), 126.9 (C-6_b and C-9_b), 125.3 (C-Ar_a), 43.9 (C-10_b and C-11_b), 43.8 (C-11_a), 40.5 (C-10_a), 33.2 (C-2_a or C-4_a), 32.7 (C-2_b and C-4_b), 31.1 (C-2_a or C-4_a), 25.5 (C-3_b), 25.1 (C-3_a); **LRMS** mass not found; **HRMS** (GCMS EI) exact mass calculated for $[M+H]^+$ ($C_{11}H_{12}Cl_2$) requires *m/z* 214.0316, found *m/z* 214.0308.

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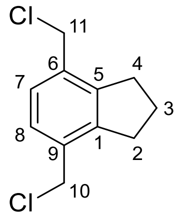
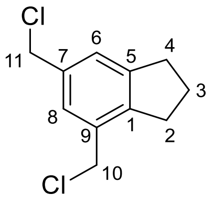
[*] Ratio calculated from H-10 and H-11 peak integrations. Regiochemistry determined from symmetry evident in NMR spectra of major product.

4,7-Bis(chloromethyl)-2,3-dihydro-1*H*-indene – 220c & 4,6-bis(chloromethyl)-2,3-dihydro-1*H*-indene – 220d



c



d

Compounds **220c** and **220d** were isolated as an inseparable mixture of isomers in a 1:1 ratio **c**:**d**[*] as an unexpected side product (white solid) in the formation of chlorides **212a** and **212b**.

R$_f$ 0.20 (pentane, stain: $KMnO_4$); **IR** ν_{max} 2958 (C-H), 1444 (C=C), 705 (C-Cl); **^{1}H NMR** (CD_2Cl, 500 MHz) δ_H 7.25 (s, 1H, H-6$_d$ or H-8$_d$), 7.18 (s, 1H, H-6$_d$ or H-8$_d$), 7.17 (s, 2H, H-7$_c$ and H-8$_c$), 4.59 (s, 8H, H-10$_c$, H-11$_c$, H-10$_d$, and H-11$_d$), 3.01 (t, *J* = 7.5 Hz, 4H, H-2$_c$ and H-4$_c$), 2.96 (ap. dt, *J* = 15.0 Hz, 7.5 Hz, 4H, H-2$_d$ and H-4$_d$), 2.21 – 2.08 (m, 4H, H-3$_c$ and H-3$_c$); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ_C 146.0 (C-Ar$_c$), 144.3 (C-Ar$_d$), 144.0 (C-Ar$_d$), 136.2 (C-Ar$_d$), 133.5 (C-Ar$_c$), 129.1 (C-Ar$_d$), 127.4 (C-7$_c$ and C-8$_c$), 127.1 (C-6$_d$ or C-8$_d$), 125.1 (C-6$_d$ or C-8$_d$), 46.3 (C-10$_d$ or C-11$_d$), 45.8 (C-10$_d$ or C-11$_d$), 44.4 (C-10$_c$ and C-11$_c$), 32.8 (C-2$_d$ or C-4$_d$), 30.9 (C-2$_c$ and C-4$_c$), 30.6 (C-2$_d$ or C-4$_d$), 25.0 (C-3$_c$), 24.5 (C-3$_d$); **LRMS** mass not found; **HRMS** (GCMS EI) exact mass calculated for $[M+H]^+$ ($C_{11}H_{12}Cl_2$) requires *m/z* 214.0316, found *m/z* 214.0313.

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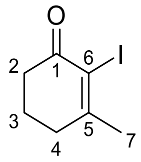
[*] Ratio calculated from aromatic peak integrations.

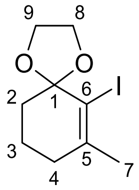
2-Iodo-3-methylcyclohex-2-en-1-one – 214



Synthesised according to a reported procedure.[178] To a solution of I_2 (2.74 g, 10.8 mmol, 0.6 eq) and pyridine (1.75 mL, 21.6 mmol, 1.2 eq) in DCM (20 mL) was added [bis(trifluoroacetoxy)iodo]benzene (4.64 g, 10.8 mmol, 0.6 eq) and the reaction mixture stirred at rt until the iodine colouration faded (*ca*. 1 h). 3-Methyl-2-cyclohexenone (2.06 mL, 18.0 mmol, 1.0 eq) was added and the reaction mixture stirred for a further 18 h, then quenched with sat. aq. $Na_2S_2O_3$ solution (15 mL). The phases were separated, the aqueous layer extracted with DCM (3 × 20 mL) and the combined organic phases dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by FCC (PE:EtOAc, 10:1 to 1:1) to afford title compound **214** (3.78 g, 89%) as an orange oil.

R$_f$ 0.30 (PE:EtOAc, 8:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 2.60 (t, *J* = 6.5 Hz, 2H, H-2), 2.55 (t, *J* = 6.5 Hz, 2H, H-4), 2.26 (s, 3H, H-7), 1.99 (p, *J* = 6.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 192.1 (C-1), 166.7 (C-5), 106.9 (C-6), 36.4 (C-2), 34.3 (C-4), 32.0 (C-7), 22.2 (C-3). Data consistent with literature.[304]

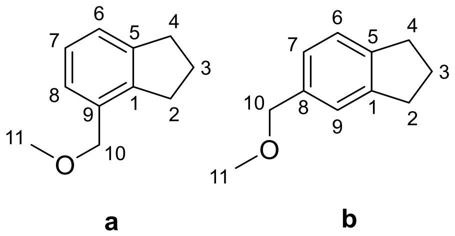
6-Iodo-7-methyl-1,4-dioxaspiro[4.5]dec-6-ene – 215



Synthesised according to a reported procedure.[178] To a solution of **214** (5.5 g, 23.3 mmol, 1 eq) and ethylene glycol (23.1 mL, 372.8 mmol, 16 eq) in benzene (245 mL) was added *p*-toluenesulfonic acid monohydrate (88.4 mg, 0.047 mmol, 0.002 eq) and the reaction heated to reflux under Dean-Stark conditions for 18 h. The reaction was cooled to rt, quenched with sat. aq. $NaHCO_3$ solution (100 mL), the phases separated, and the aqueous layer extracted with Et_2O (3 × 100 mL). The combined organic phases were washed with brine (200 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (PE:Et_2O, 9:1) to afford title compound **215** (5.12 g, 78%) as a white solid.

Rf 0.20 (PE:Et_2O, 9:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 4.29 – 4.17 (m, 2H, H-8 and H-9), 4.03 – 3.92 (m, 2H, H-8 and H-9), 2.20 (t, *J* = 6.0 Hz, 2H, H-4), 1.95 (s, 3H, H-7), 1.92 – 1.86 (m, 2H, H-2), 1.81 – 1.70 (m, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 147.4 (C-5), 107.3 (C-1), 104.9 (C-6), 65.6 (C-8), 65.6 (C-9), 34.1 (C-2), 33.4 (C-4), 30.1 (C-7), 20.5 (C-3); **mp** 74 – 75 °C, lit. 76 – 77 °C. Data consistent with literature.[178]

4-(Methoxymethyl)-2,3-dihydro-1*H*-indene – 221a & 5-(methoxymethyl)-2,3-dihydro-1*H*-indene – 221b



A solution of **212a** and **212b** (500 mg, 1:3 ratio, 3.0 mmol, 1.0 eq) and NaOH (144 mg, 3.0 M aq. solution, 3.6 mmol, 1.2 eq) in methanol (6 mL) was heated to reflux for 20 h. Once cooled, the reaction mixture was evaporated to dryness and re-dissolved in EtOAc (3 mL). The solution was washed with water (3 mL), then brine (3 mL), and the organic layer dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compounds **221a** and **221b** (456 mg, quant.) in a 1:4 ratio **a**:**b** of inseparable regioisomers, as a yellow oil.[*]

***R**f* 0.55 (pentane:Et_2O, 10:1, stain: vanillin); **IR** *νmax* 2930 (C-H), 2845 (C-H), 2818 (C-H), 1491 (C=C), 1439 (C=C), 1192 (C-O), 1098 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.24 – 7.08 (m, 3H, H-Ar), 4.45 (s, 0.4H, H-10_a), 4.43 (s, 1.6H, H-10_b), 3.40 (s, 0.6H, H-11_a), 3.39 (s, 2.4H, H-11_b), 2.97 – 2.86 (m, 4H, H-2 and H-4), 2.16 – 2.01 (m, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 144.7 (C-Ar_b), 143.9 (C-Ar_b), 142.9 (C-Ar_a), 136.1 (C-Ar_b), 133.8 (C-Ar_a), 130.6 (C-Ar_a), 126.4 (HC-Ar_a), 126.0 (HC-Ar_b), 125.8 (HC-Ar_a), 124.3 (HC-Ar_b), 124.1 (HC-Ar_b), 123.9 (HC-Ar_a), 75.1 (C-10_b), 73.2 (C-10_a), 58.3 (C-11_a), 58.1 (C-11_b), 33.0 (C-2_a or C-4_a), 32.9 (C-2_b or C-4_b), 32.7 (C-2_b or C-4_b), 31.0 (C-2_a or C-4_a), 25.7 (C-3_b), 25.1 (C-3_a); **LRMS** mass not found; **HRMS** (GCMS NH_3 Cl)

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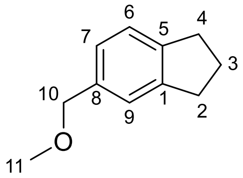
[*] Ratio calculated from H-10 peak integrations.

exact mass calculated for $[M+NH_4]^+$ ($C_{11}H_{18}NO$) requires *m/z* 180.1383, found *m/z* 180.1384.

5-(Methoxymethyl)-2,3-dihydro-1*H*-indene – 221b

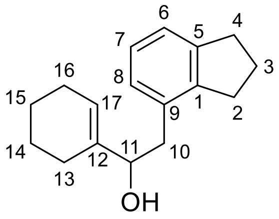


Methyl ether **221b** was prepared separately as a single regioisomer from pure **224b**.

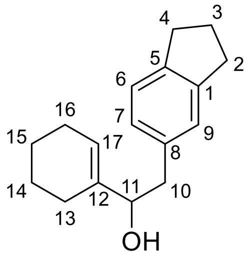
To a suspension of 60% NaH (54.3 mg, 1.36 mmol, 2 eq) in THF (6.8 mL) under N_2 at 0 °C was added a solution of **224b** (100 mg, 0.68 mmol, 1 eq) in THF (6.8 mL) dropwise. Methyl iodide (86.5 µL, 1.36 mmol, 2 eq) was added dropwise. The reaction was heated to reflux for 5 h, cooled to rt, and quenched with sat. aq. NH_4Cl solution (5 mL). The aqueous layer was extracted with EtOAc (3 × 10 mL) and the combined organic layers washed with brine (25 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **221b** (109 mg, quant.) as a brown oil which was used without further purification.

R_f 0.55 (pentane:Et_2O, 10:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.22 – 7.18 (m, 2H, H-Ar), 7.10 (d, *J* = 7.5 Hz, 1H, H-Ar), 4.43 (s, 2H, H-10), 3.39 (s, 3H, H-11), 2.90 (ap. td, *J* = 7.5 Hz, 3.0 Hz, 4H, H-2 and H-4), 2.07 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 144.7 (C-Ar), 143.9 (C-Ar), 136.1 (C-Ar), 126.0 (HC-Ar), 124.4 (HC-Ar), 124.2 (HC-Ar), 75.1 (C-10), 58.1 (C-11), 32.9 (C-2 or C-4), 32.8 (C-2 or C-4), 25.7 (C-3).

1-(Cyclohex-1-en-1-yl)-2-(2,3-dihydro-1*H*-inden-4-yl)ethan-1-ol – 223a & 1-(cyclohex-1-en-1-yl)-2-(2,3-dihydro-1*H*-inden-5-yl)ethan-1-ol – 223b



a



b

Small, fresh slices of Li wire (26.8 mg, 3.8 mmol, 2.5 eq) were cut and added to a flask of naphthalene (526 mg, 4.1 mmol, 2.75 eq) under a stream of N$_2$. The flask was purged and sealed under N$_2$, then THF (15 mL) added and the mixture stirred at rt until a dark green solution formed (*ca*. 10 min). A neat mixture of cyclohex-1-ene-1-carbaldehyde (300 mg, 1.8 mmol, 1.2 eq) and **212a** and **212b** (165 mg, 1:3 ratio, 1.5 mmol, 1.0 eq) was added dropwise, accompanied by instantaneous colour change. The solid lithium was removed from the reaction and the remaining solution evaporated to dryness. The crude residue was hydrolysed with 1.0 M aq. HCl (5 mL), extracted with Et$_2$O (3 × 5 mL), and washed with brine (10 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, concentrated *in vacuo*, and the crude product purified by FCC (PE:Et$_2$O, 10:1) to afford title compounds **223a** and **223b** (179 mg, 49%), in a 1:5 ratio **a**:**b** of inseparable regioisomers, as an off-white solid.[*]

R$_f$ 0.15 (PE:Et$_2$O, 10:1, stain: vanillin); **IR** ν_{max} 3427 (O-H), 2926 (=C-H), 2855 (C-H), 1491 (C=C), 1136 (C-O); **^{1}H NMR** (CDCl$_3$, 400 MHz) δ_H 7.16 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.13 – 7.09 (m, 1H, H-Ar), 7.03 – 6.97 (m, 1H, H-Ar), 5.72 – 5.66 (m, 1H, H-17), 4.19 (dd, *J* = 9.0 Hz, 4.0 Hz, 0.17H, H-11$_a$), 4.15 (dd, *J* = 9.0 Hz, 4.0 Hz, 0.83H, H-11$_b$),

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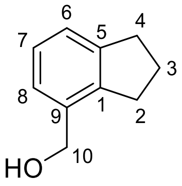
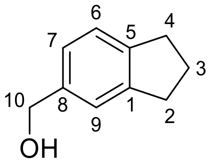
[*] Ratio calculated from H-11 peak integrations.

2.98 – 2.80 (m, 5H, H-2, H-4 and H-10), 2.81 – 2.65 (m, 1H, H-10), 2.21 – 2.09 (m, 1H, H-3), 2.10 – 1.98 (m, 5H, H-3, H-13 and H-16), 1.76 – 1.55 (m, 4H, H-14 and H-15); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 144.8 (C-Ar$_b$ or C-12$_b$), 143.5 (C-Ar$_a$ or C-12$_a$), 143.4 (C-Ar$_a$ or C-12$_a$), 142.5 (C-Ar$_b$ or C-12$_b$), 140.0 (C-Ar$_a$ or C-12$_a$), 139.6 (C-Ar$_b$ or C-12$_b$), 138.2 (C-Ar$_a$ or C-12$_a$), 136.5 (C-Ar$_b$ or C-12$_b$), 127.3 (HC-Ar$_a$), 127.3 (HC-Ar$_b$), 126.5 (HC-Ar$_a$), 126.5 (HC-Ar$_b$), 124.5 (HC-Ar$_b$), 123.1 (C-17$_b$), 122.9 (C-17$_a$), 122.8 (HC-Ar$_a$), 77.3 (C-11$_b$), 76.2 (C-11$_a$), 42.3 (C-10$_b$), 40.2 (C-11$_a$), 33.3 (C-2$_a$ or C-4$_a$), 32.9 (C-2$_b$ or C-4$_b$), 32.7 (C-2$_b$ or C-4$_b$), 31.6 (C-2$_a$ or C-4$_a$), 25.6 (C-13$_a$ or C-16$_a$), 25.6 (C-13$_b$ or C-16$_b$), 25.1 (C-13$_b$ or C-16$_b$), 25.1 (C-13$_a$ or C-16$_a$), 24.3 (C-3$_b$), 24.3 (C-3$_a$), 22.8 (C-14$_b$ or C-15$_b$), 22.8 (C-14$_a$ or C-15$_a$), 22.8 (C-14$_b$ or C-15$_b$), 22.7 (C-14$_a$ or C-15$_a$); **LRMS** mass not found; **HRMS** (ES+) exact mass calculated for $[M+Na]^+$ ($C_{17}H_{22}ONa$) requires *m/z* 265.1563, found *m/z* 265.1565.

(2,3-Dihydro-1*H*-inden-4-yl)methanol – 224a & (2,3-dihydro-1*H*-inden-5-yl)methanol – 224b



a



b

Synthesised according to a reported procedure.[181] A solution of **227a** and **227b** (344 mg, 1:3.5 ratio, 1.80 mmol, 1 eq) and NaOH (0.45 mL, 4.0 M aq., 1.8 mmol, 1 eq) in methanol (0.72 mL) was heated to reflux for 1 h. The cooled reaction was acidified with conc. HCl (0.25 mL), diluted with DCM (5 mL) and washed with water (5 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford title compounds **224a** and **224b** (172 mg, 64%) in a 1:3.5 ratio **a**:**b** as an off-white solid.[*]

R_f 0.10 (pentane:Et_2O, 5:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.26 – 7.11 (m, 3H, H-Ar), 4.68 (s, 0.44H, H-10_a), 4.65 (s, 1.56H, H-10_b), 2.97 – 2.86 (m, 4H, H-2 and H-4), 2.16 – 2.03 (m, 2H, H-3), 1.60 (s, 1H, OH); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 144.8 (C-Ar_b), 143.9 (C-Ar_b), 141.6 (C-Ar_a), 138.8 (C-Ar_b), 136.3 (C-Ar_a), 132.9 (C-Ar_a), 126.6 (HC-Ar_a), 125.2 (HC-Ar_b), 124.7 (HC-Ar_a), 124.4 (HC-Ar_b), 123.8 (HC-Ar_a), 123.3 (HC-Ar_b), 65.7 (C-10_b), 63.7 (C-10_a), 32.8 (C-2_a or C-4_a), 32.8 (C-2_b or C-4_b), 32.6 (C-2_b or C-4_b), 30.7 (C-2_a or C-4_a), 25.6 (C-3_b), 25.0 (C-3_a). Data consistent with literature.[181]

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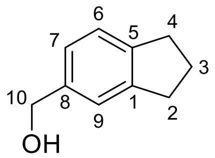
[*] Ratio calculated from H-10 peak integrations.

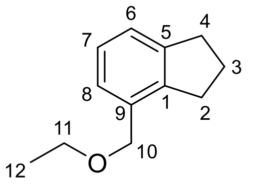
(2,3-Dihydro-1*H*-inden-5-yl)methanol – 224b



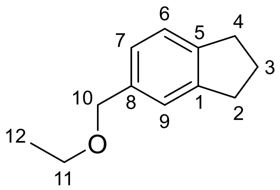
Pure *5*-regioisomer **224b** was isolated as a white solid *via* repeat recrystallisation of the mixture of **224a** and **224b** (1:3.5) in PE.

Rf 0.10 (pentane:Et_2O, 5:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.25 (s, 1H, H-9), 7.22 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.14 (d, *J* = 7.5 Hz, 1H, H-Ar), 4.65 (d, *J* = 6.0 Hz, 1H, H-10), 2.90 (ap. td, *J* = 7.5 Hz, 3.5 Hz, 4H, H-2 and H-4), 2.08 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 144.8 (C-Ar), 143.9 (C-Ar), 138.8 (C-Ar), 125.2 (HC-Ar), 124.4 (HC-Ar), 123.3 (HC-Ar), 65.7 (C-10), 32.8 (C-2 or C-4), 32.6 (C-2 or C-4), 25.6 (C-3); **mp** 65 – 67 °C, lit. 70 – 72 °C. Data consistent with literature.[181]

4-(Ethoxymethyl)-2,3-dihydro-1*H*-indene – 225a & 5-(ethoxymethyl)-2,3-dihydro-1*H*-indene – 225b



a



b

A solution of **212a** and **212b** (300 mg, 1:3 ratio, 1.8 mmol, 1 eq) and NaOEt (123 mg, 1.8 mmol, 1 eq) in ethanol (3.6 mL) was heated to reflux under N_2 for 18 h. Once cooled to rt, the reaction mixture was evaporated to dryness and re-dissolved in EtOAc (2 mL). The crude product was washed with water (2 mL), then brine (2 mL), and the organic layer dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (PE:Et_2O, 40:1) to afford title compounds **225a** and **225b** (85.3 mg, 24 %) in a 1:5 ratio **a**:**b** of inseparable regioisomers, as an off-white solid.*

R_f 0.30 (PE:Et_2O, 50:1, stain: vanillin); **IR** ν_{max} 2933 (C-H), 1095 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.25 – 7.10 (m, 3H, H-Ar), 4.51 (s, 0.33H, H-10$_a$), 4,49 (s, 1.66H, H-10$_b$), 3.55 (q, J = 7.0 Hz, 2H, H-11), 2.98 – 2.87 (m, 4H, H-2 and H-4), 2.09 (p, J = 7.5 Hz, 2H, H-3), 1.26 (t, J = 7.0 Hz, 3H, H-12); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 147.2 (C-Ar$_a$), 144.6 (C-Ar$_b$), 143.7 (C-Ar$_b$), 142.9 (C-Ar$_a$), 136.5 (C-Ar$_b$), 134.2 (C-Ar$_a$), 126.4 (HC-Ar$_a$), 126.0 (HC-Ar$_b$), 125.7 (HC-Ar$_a$), 124.3 (HC-Ar$_b$), 124.1 (HC-Ar$_b$), 123.8 (HC-Ar$_a$), 73.1 (C-10$_b$), 71.2 (C-10$_a$), 65.9 (C-11$_a$), 65.7 (C-11$_b$), 32.9 (C-2$_a$ or C-4$_a$), 32.9 (C-2$_b$ or C-4$_b$), 32.7 (C-2$_b$ or C-4$_b$), 31.0 (C-2$_a$ or C-4$_a$), 25.7 (C-3$_b$), 25.1 (C-3$_a$), 15.4 (C-12); **LRMS** mass not found; **HRMS** (ES+) exact mass calculated for $[M+Na]^+$ ($C_{12}H_{16}ONa$) requires *m/z* 199.1093, found *m/z* 199.1096.

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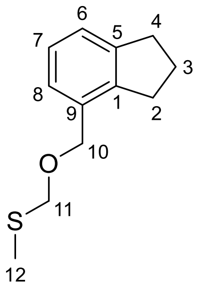
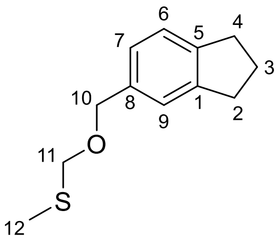
[*] Ratio calculated from H-10 peak integrations.

(((2,3-Dihydro-1*H*-inden-4-yl)methoxy)methyl)(methyl)sulfane – 226a & (((2,3-dihydro-1*H*-inden-5-yl)methoxy)methyl)(methyl)sulfane – 226b



a



b

A solution of **212a** and **212b** (1.0 g, 1:3 ratio, 6.0 mmol, 1.0 eq) and NaOH (2.4 mL, 3.0 M aq., 7.2 mmol, 1.2 eq) in DMSO (12 mL) was heated to 140 °C for 18 h. The cooled reaction was diluted with water (25 mL), the aqueous layer extracted with Et_2O (5 × 30 mL), and the combined organic layers washed with brine (120 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FFC (PE:Et_2O, 50:1 to 4:1) to afford title compounds **226a** and **226b** (262 mg, 21%) as a 1:3.5 ratio **a**:**b** of inseparable regioisomers, as a pale-yellow oil.[*][†]

R$_f$ 0.45 (pentane:Et_2O, 40:1, stain: $KMnO_4$); **IR** *ν$_{max}$* 2951 (C-H), 1438 (C=C), 1225 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.25 – 7.12 (m, 3H, H-Ar), 5.10 (s, 0.44H, H-10$_a$), 5.08 (s, 1.56H, H-10$_b$), 3.02 – 2.87 (m, 6H, H-2, H-4 and H-11), 2.19 – 2.04 (m, 5H, H-3 and H-12); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 145.4 (C-Ar$_a$), 144.9 (C-Ar$_a$), 144.9 (C-Ar$_b$), 144.7 (C-Ar$_b$), 135.4 (C-Ar$_a$), 133.8 (C-Ar$_b$), 129.0 (HC-Ar$_a$), 126.6 (HC-Ar$_b$), 125.3 (HC-Ar$_a$), 124.9 (HC-Ar$_a$), 124.8 (HC-Ar$_b$), 124.5 (HC-Ar$_b$), 66.8 (C-10$_b$), 64.9 (C-10$_a$), 33.3 (C-2$_b$ or C-4$_b$ or C-11$_b$), 33.0 (C-2$_a$ or C-4$_a$ or C-11$_a$), 32.9 (C-2$_b$ or C-4$_b$ or C-11$_b$), 32.8 (C-2$_b$ or C-4$_b$ or C-11$_b$), 32.5 (C-2$_a$ or C-4$_a$ or C-11$_a$), 31.0 (C-2$_a$ or C-4$_a$ or C-11$_a$),

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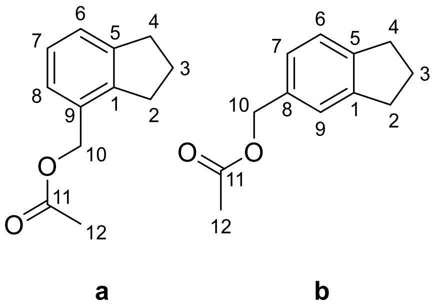
[*] Ratio calculated from H-10 peak integrations.

[†] Also contains aldehyde contaminants which could not be removed.

25.6 (C-3$_b$), 25.0 (C-3$_a$), 21.2 (C-12$_b$), 21.1 (C-12$_a$); **LRMS** mass not found; **HRMS** mass not found.

(2,3-Dihydro-1*H*-inden-4-yl)methyl acetate – 227a & (2,3-dihydro-1*H*-inden-5-yl)methyl acetate – 227b



Synthesised according to a reported procedure.[181] A solution of **212a** and **212b** (300 mg, 1:3 ratio, 1.8 mmol, 1.0 eq), NaOAc·3H_2O (308 mg, 2.3 mmol, 1.25 eq) and acetic acid (0.6 mL) was heated to reflux for 5 h. Once cooled to rt, the reaction mixture was evaporated to dryness and the residue diluted with water (3 mL). The aqueous layer was extracted with toluene (3 × 5 mL) and the combined organic phases were washed with water (15 mL), then sat. aq. $NaHCO_3$ solution (15 mL), then brine (15 mL). The solution was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford title compounds **227a** and **227b** (344 mg, quant.) in a 1:3.5 ratio **a**:**b** of inseparable regioisomers, as a colourless oil.*

R$_f$ 0.60 (pentane:Et_2O, 5:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.35 – 7.09 (m, 3H, H-Ar), 5.11 (s, 0.44H, H-10$_a$), 5.08 (s, 1.56H, H-10$_b$), 3.04 – 2.83 (m, 4H, H-2 and H-4), 2.22 – 1.95 (m, 5H, H-3 and H-12); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 171.1 (C-11$_b$), 171.1 (C-11$_a$), 144.9 (C-Ar$_a$), 144.9 (C-Ar$_b$), 144.7 (C-Ar$_b$), 143.3 (C-Ar$_a$), 133.8

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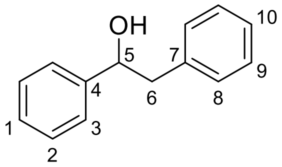
* Ratio calculated from H-10 peak integrations.

(C-Ar$_b$), 131.5 (C-Ar$_a$), 126.7 (HC-Ar$_b$), 126.3 (HC-Ar$_a$), 125.3 (HC-Ar$_a$), 124.8 (HC-Ar$_b$), 124.6 (HC-Ar$_b$), 123.4 (HC-Ar$_a$), 66.8 (C-10$_b$), 64.9 (C-10$_a$), 33.0 (C-2$_a$ or C-4$_a$), 32.9 (C-2$_b$ or C-4$_b$), 32.8 (C-2$_b$ or C-4$_b$), 31.0 (C-2$_a$ or C-4$_a$), 25.6 (C-3$_b$), 25.0 (C-3$_a$), 21.2 (C-12$_b$), 21.1 (C-12$_a$). Data consistent with literature.[181]

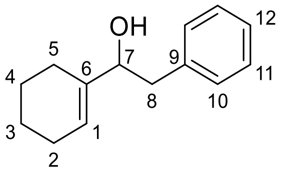
1,2-Diphenylethan-1-ol – 230



A solution of Li (69.4 mg, 10.0 mmol, 1.0 eq) and naphthalene (1.28 g, 10.0 mmol, 1.0 eq) in THF (100 mL) under N_2 was sonicated for 2 h. The solution was cooled to –78 °C. In a separate flask, a portion of this solution (35 mL, 3.50 mmol, 2.5 eq) was warmed to 0 °C. Benzyl bromide (200 µL, 1.68 mmol, 1.2 eq) and benzaldehyde (142 µL, 1.40 mmol, 1.0 eq) were added dropwise as a neat mixture. Immediate colour changes were observed, and the reaction was stirred for 5 min. The excess Li was removed and the THF removed *in vacuo*. The resultant residue was hydrolysed with 1.0 M aq. HCl (5 mL) and extracted with Et_2O (3 × 5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 5:1) to afford title compound **230** (47.2 mg, 17%) as a white solid.

R_f 0.40 (pentane:Et_2O, 5:1, no stain); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.39 – 7.18 (m, 10H, H-Ar), 4.91 (ddd, *J* = 8.0 Hz, 5.0 Hz, 3.0 Hz, 1H, H-5), 3.09 – 2.96 (m, 2H, H-6), 1.92 (d, *J* = 3.0 Hz, 1H, OH); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 144.0 (C-Ar), 138.2 (C-Ar), 129.7 (HC-Ar), 128.7 (HC-Ar), 128.6 (HC-Ar), 127.8 (HC-Ar), 126.8 (HC-Ar), 126.0 (HC-Ar), 75.5 (C-5), 46.3 (C-6); **mp** 53 – 55 °C, lit. 60 – 61 °C. Data consistent with literature.[305]

1-(Cyclohex-1-en-1-yl)-2-phenylethan-1-ol – 233

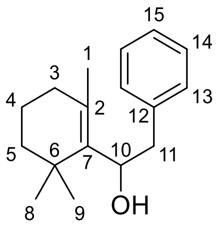


Naphthalene (846 mg, 6.6 mmol, 1.1 eq), in THF (6.9 mL) was stirred at rt under N_2 for 30 min. Chunks of Li were added under a stream of N_2, resulting in the immediate formation of a deep green solution. This was stirred under N_2 for 16 h.

The lithium napthalenide solution (4.2 mL, 3.63 mmol, 2.5 eq) was cooled to 0 °C. Benzyl chloride (200 μL, 1.74 mmol, 1.2 eq) and 1-cyclohexene-1-carboxyaldehyde (165 μL, 1.45 mmol, 1.0 eq) were combined and added to the cooled lithium naphthalenide solution as a neat mixture. The reaction was stirred at 0 °C for 5 min, then the THF removed *in vacuo*. The resulting residue was hydrolysed with 1.0 H aq. HCl (2 mL) and extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 5:1) to afford title compound **233** (73.1 mg, 21%) as a yellow oil.

R_f 0.20 (pentane:Et_2O, 5:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.34 – 7.28 (m, 2H, H-Ar), 7.25 – 7.20 (m, 3H, H-Ar), 5.70 – 5.63 (m, 1H, H-1), 4.18 (dt, *J* = 8.5 Hz, 3.5 Hz, 1H, H-7), 2.88 (dd, *J* = 13.5 Hz, 4.5 Hz, 1H, H-8), 2.78 (dd, *J* = 13.5 Hz, 8.5 Hz, 1H, H-8), 2.19 – 2.08 (m, 3H, H-2, H-3 and OH), 1.74 – 1.50 (m, 5H, H-3, H-4 and H-5); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 139.4 (C-6 or C-Ar), 138.8 (C-6 or C-Ar), 129.5 (HC-Ar), 128.6 (HC-Ar), 126.5 (HC-Ar), 123.4 (C-1), 77.4 (C-7), 42.3 (C-8), 25.1, 24.3, 22.8, 22.7 (C-2, C-3, C-4 and C-5). Data consistent with literature.[306]

2-Phenyl-1-(2,6,6-trimethylcyclohex-1-en-1-yl)ethan-1-ol – 242



Li/DBB solution was prepared following Donohoe's protocol.[307] Lithium wire[*] was placed between two sheets of foil and flattened into a thin ribbon (by rolling a jar over it) which was cut into small strips with a scalpel. To an oven-dried Schlenck tube were added lithium strips (34.7 mg, 5.0 mmol, 2.50 eq), DBB (1.33 g, 5.0 mmol, 2.48 eq), and anti-bumping granules (100 mg). The Schlenck tube was evacuated and charged with argon on the high-vac manifold three times and the dry mixture left to stir at maximum speed for 16 h until a dark grey powder had formed. The stirring was reduced and freshly distilled THF (10 mL) added, immediately forming a deep green solution.[†] The solution was cooled to –78 °C and the temperature of the bath monitored. A neat mixture of benzyl chloride (276 μL, 2.4 mmol, 1.20 eq) and β-cyclocitral (323 μL, 2.0 mmol, 1.00 eq) was added dropwise. The reaction was allowed to stir for 5 min at –78 °C, then quenched with sat. aq. NH_4Cl solution whilst still cold. The quenched mixture was allowed to warm to rt and hydrolysed with 1.0 M aq. HCl (10 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 10:1) to afford title compound **242** (198 mg, 41%) as a colourless oil.

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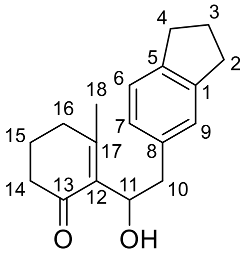
[*] Stored under oil but washed with pentane prior to use.

[†] Where this colour change did not occur, the Li/DBB complex was not correctly formed and the reaction was abandoned. Specific troubleshooting may be found in Donohoe's protocol.

R_f 0.20 (pentane:Et_2O, 10:1, stain: vanillin); **IR** ν_{max} 3462 (O-H), 3027 (C-H), 2927 (C-H), 1495 (C=C), 1077 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.37 – 7.21 (m, 5H, H-Ar), 4.46 (dd, *J* = 10.5 Hz, 3.0 Hz, 1H, H-10), 3.14 (dd, *J* = 14.0 Hz, 10.5 Hz, 1H, H-11), 2.86 (dd, *J* = 14.0 Hz, 3.0 Hz, 1H, H-11), 2.05 – 1.98 (m, 2H, H-3), 1.97 (s, 3H, H-1), 1.64 – 1.54 (m, 2H, H-4), 1.49 – 1.42 (m, 2H, H-5), 1.11 (s, 3H, H-8 or H-9), 1.01 (s, 3H, H-8 or H-9); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 140.1 (C-12), 139.3 (C-7), 132.0 (C-2), 129.5 (CH-Ar), 128.7 (CH-Ar), 126.6 (CH-Ar), 75.5 (C-10), 43.4 (C-11), 40.1 (C-5), 35.0 (C-6), 34.3 (C-3), 28.8 (C-8 or C-9), 28.2 (C-8 or C-9), 21.4 (C-1), 19.5 (C-4); **LRMS** (ES+) mass found *m/z* 267.2 $[M+Na]^+$; **HRMS** (ESI) exact mass calculated for $[M+Na]^+$ ($C_{17}H_{24}NaO$) requires *m/z* 267.1719, found *m/z* 267.1720.

2-(2-(2,3-Dihydro-1*H*-inden-5-yl)-1-hydroxyethyl)-3-methylcyclohex-2-en-1-one – **243b**



Li/DBB solution was prepared following Donohoe's protocol.[307] Lithium wire[*] was placed between two sheets of foil and flattened into a thin ribbon (by rolling a jar over it) which was cut into small strips with a scalpel. To an oven-dried Schlenck tube were added lithium strips (17.4 mg, 2.5 mmol, 2.50 eq), DBB (666 mg, 2.5 mmol, 2.48 eq), and anti-bumping granules (50 mg). The Schlenck tube was evacuated and charged with argon on the high-vac manifold three times and the dry mixture left to stir at maximum speed for 16 h until a dark grey powder had formed. The stirring was reduced and freshly distilled THF (5 mL) added, immediately forming a deep green solution.[†] The solution was cooled to –78 °C and the temperature of the bath monitored. A mixture of **212b** (199 mg, 1.2 mmol, 1.20 eq) and aldehyde **211** (182 mg, 1.0 mmol, 1.00 eq) in THF (0.2 mL) was added dropwise. The reaction was allowed to stir for 5 min, then quenched with sat. aq. NH_4Cl solution whilst still cold. The quenched mixture was allowed to warm to rt and hydrolysed with 1.0 M aq. HCl (5 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 10:1) to afford title compound **243b** (119 mg, 33%) as a yellow solid.

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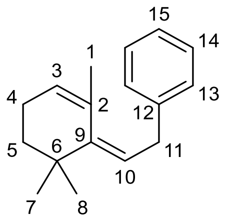
[*] Stored under oil but washed with pentane prior to use.

[†] Where this colour change did not occur, the Li/DBB complex was not correctly formed and the reaction was abandoned. Specific troubleshooting may be found in Donohoe's protocol.

R_f 0.20 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **IR** ν_{max} 3468 (O-H), 2946 (C-H), 1642 (C=O), 1490 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.10 (d, *J* = 7.5 Hz, 1H, H-6), 7.00 (s, 1H, H-9), 6.89 (d, *J* = 7.5 Hz, 1H, H-7), 4.66 (dt, *J* = 11.0 Hz, 7.5 Hz, 1H, H-11), 4.45 (d, *J* = 11.0 Hz, 1H, OH), 3.11 (dd, *J* = 13.0 Hz, 7.5 Hz, 1H, H-10), 2.91 – 2.82 (m, 5H, H-10, H-4 and H-2), 2.42 (t, *J* = 7.0 Hz, 2H, H-14), 2.35 – 2.17 (m, 2H, H-16), 2.05 (p, *J* = 7.5 Hz, 2H, H-3), 1.99 – 1.85 (m, 2H, H-15), 1.56 (s, 3H, H-18); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 202.0 (C-13), 158.8 (C-12), 144.4 (C-5), 142.2 (C-8), 136.5 (C-1), 134.5 (C-17), 127.4 (C-7), 125.6 (C-9), 124.2 (C-6), 72.4 (C-11), 43.7 (C-10), 38.8 (C-14), 33.3 (C-16), 32.9 (C-2), 32.6 (C-4), 25.7 (C-3), 22.1 (C-15), 20.8 (C-18); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+Na]^+$ ($C_{18}H_{22}NaO_2$) requires *m/z* 293.1512, found *m/z* 293.1511; **mp** 72 – 74 °C.

(*E*)-(2-(2,6,6-Trimethylcyclohex-2-en-1-ylidene)ethyl)benzene – 247



To a solution of **242** (40.0 mg, 0.16 mmol, 1.0 eq) in DCM (160 µL) was added *p*-toluenesulfonic acid monohydrate (2.7 mg, 0.016 mmol, 0.1 eq) and the reaction stirred for 3 h at rt. The reaction was diluted with Et_2O (2 mL) and washed with sat. aq. $NaHCO_3$ solution (2 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane) to afford title compound **247** (16.5 mg, 52%)* as a colourless oil.

R_f 0.55 (pentane, stain: vanillin); **IR** ν_{max} 3026 (=C-H), 2959 (C-H), 2919 (C-H), 699 (=C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.33 – 7.27 (m, 2H, H-Ar), 7.25 – 7.18 (m, 3H, H-Ar), 5.68 (t, *J* = 4.5 Hz, 1H, H-3), 5.54 (t, *J* = 7.5 Hz, 1H, H-10), 3.73 (d, *J* = 7.5 Hz, 2H, H-11), 2.13 – 2.07 (m, 2H, H-4), 1.79 (q, *J* = 1.5 Hz, 3H, H-1), 1.52 (t, *J* = 6.0 Hz, 2H, H-5), 1.29 (s, 6H, H-7 and H-8); **^{13}C NMR** ($CDCl_3$, 125 MHz) δ_C 144.1 (C-9), 142.4 (C-12), 133.7 (C-2), 128.6 (CH-Ar), 128.6 (CH-Ar), 126.3 (C-3), 126.0 (CH-Ar), 124.6 (C-10), 40.6 (C-5), 36.3 (C-11), 34.9 (C-6), 28.5 (C-7), 28.5 (C-8), 23.0 (C-4), 22.1 (C-1); **LRMS** mass not found; **HRMS** mass not found.

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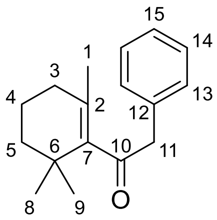
[*] Contains <5% inseparable impurity.

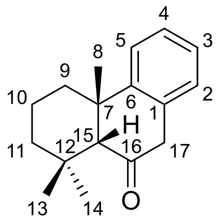
2-Phenyl-1-(2,6,6-trimethylcyclohex-1-en-1-yl)ethan-1-one – 248



To a solution of **242** (880 mg, 3.6 mmol, 1.0 eq) in DCM (18 mL) under N_2 at 0 °C was added Dess-Martin periodinane (2.29 g, 5.4 mmol, 1.25 eq). The reaction was allowed to warm to rt and its progress followed by TLC (pentane:Et_2O, 10:1). The reaction was quenched with isopropanol (20 mL) after 2 h and the mixture evaporated to dryness. The crude residue was re-dissolved in Et_2O (25 mL) and washed successively with sat. aq. solutions of 1:1 $NaHCO_3$/$Na_2S_2O_3$ (25 mL), $NaHCO_3$ (25 mL), water (25 mL), and brine (25 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford **248** (778 mg, 89%) as a pale-yellow oil which was used without further purification.

Rf 0.25 (pentane:Et_2O, 20:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.36 – 7.27 (m, 3H, H-Ar), 7.24 –7 .20 (m, 2H, H-Ar), 3.86 (s, 2H, H-11), 2.02 – 1.95 (m, 2H, H-3), 1.73 – 1.64 (m, 2H, H-4), 1.60 (s, 3H, H-1), 1.49 – 1.44 (m, 2H, H-5), 1.10 (s, 6H, H-8 and H-9); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 208.4 (C-10), 143.4 (C-7), 134.1 (C-12), 130.1 (HC-Ar), 129.7 (C-2), 128.5 (HC-Ar), 127.0 (HC-Ar), 52.2 (C-11), 39.1 (C-5), 33.5 (C-6), 31.3 (C-3), 28.9 (C-8), 28.9 (C-9), 21.3 (C-1), 19.0 (C-4). Data consistent with literature.[193]

4b,8,8-Trimethyl-4b,6,7,8,8a,10-hexahydrophenanthren-9(5*H*)-one – 249



Synthesised according to a reported procedure.[193] A mixture of AgOTf (5.9 mg, 0.023 mmol, 0.4 eq) and $RuCl_3•3H_2O$ (2.5 mg, 0.012 mmol, 0.2 eq) was stirred in DCE (87 μL) at rt for 1 h. A solution of **248** (14.1 mg, 0.058 mmol, 1.0 eq) in DCE (203 μL) was added dropwise and the reaction heated to 80 °C for 14 h. The cooled reaction was purified by FCC (pentane:Et_2O, 20:1) to afford title compound **249** (5.5 mg, 40%) as a yellow solid.*

Rf 0.30 (pentane:Et_2O, 20:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.35 (d, *J* = 7.5 Hz, 1H, H-5), 7.26 (t, *J* = 7.5 Hz, 1H, H-3), 7.19 (t, *J* = 7.5 Hz, 1H, H-4), 7.07 (d, *J* = 7.5 Hz, 1H, H-2), 3.72 (d, *J* = 23.5 Hz, 1H, H-17), 3.54 (d, *J* = 23.5 Hz, 1H, H-17), 2.55 (d, *J* = 14.5 Hz, 1H, H-9), 2.13 (s, 1H, H-15), 1.62 – 1.47 (m, 2H, H-10), 1.40 – 1.25 (m, 3H, H-9 and H-11), 1.08 (s, 3H, H-8), 0.96 (s, 3H, H-14), 0.31 (s, 3H, H-14); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 212.4 (C-16), 141.7 (C-6), 134.2 (C-1), 128.7 (C-2), 127.3 (C-3), 126.5 (C-4), 124.1 (C-5), 66.6 (C-15), 44.2 (C-17), 42.3 (C-11), 38.9 (C-7), 36.1 (C-9), 34.4 (C-12), 33.4 (C-8), 32.3 (C-13), 22.6 (C-14), 19.0 (C-10); **mp** 55 – 57 °C.† Data consistent with literature.[193]

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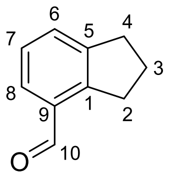
[*] Stereochemistry confirmed by NOESY analysis.

[†] Literature value not given.

2,3-Dihydro-1*H*-indene-4-carbaldehyde – 250a



To a solution of **212a** and **212b** (635 mg, 1:3 ratio, 3.8 mmol, 1 eq) in DCM (7.6 mL) was added $NaHCO_3$ (1.28 g, 15.2 mmol, 4 eq) and Ar bubbled through. The reaction flask was placed in a pre-heated oil bath at 110 °C for 1 h, cooled to rt, and diluted with water (5 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 40:1) to afford title compound **250a** (67.1 mg, 48%) as a colourless oil, and its regioisomer **250b** (259.3 mg, 62%) as a pale-yellow oil.*

R_f 0.50 (pentane:Et_2O, 20:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 10.15 (s, 1H, H-10), 7.63 (d, *J* = 7.5 Hz, 1H, H-8), 7.47 (d, *J* = 7.5 Hz, 1H, H-6), 7.32 (t, *J* = 7.5 Hz, 1H, H-7), 3.29 (t, *J* = 7.5 Hz, 2H, H-2), 2.94 (t, *J* = 7.5 Hz, 2H, H-4), 2.15 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 192.9 (C-10), 146.5 (C-Ar), 146.4 (C-Ar), 132.5 (C-Ar), 130.1 (C-6), 129.3 (C-8), 126.8 (C-7), 32.2 (C-4), 31.9 (C-2), 25.4 (C-3); Data consistent with literature.[179]

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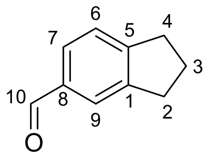
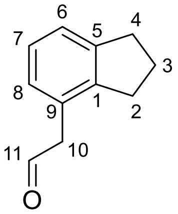
* Percentage yields calculated relative to the amount of regioisomer present in the starting mixture of **212a** and **212b**.

2,3-Dihydro-1*H*-indene-5-carbaldehyde – 250b



R_f 0.35 (pentane:Et_2O, 20:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 9.96 (s, 1H, H-10), 7.73 (s, 1H, H-9), 7.65 (d, J = 8.0 Hz, 1H, H-7), 7.36 (d, J = 8.0 Hz, 1H, H-6), 2.97 (t, J = 7.5 Hz, 4H, H-2 and H-4), 2.13 (p, J = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 192.4 (C-10), 152.2 (C-Ar), 145.4 (C-Ar), 135.5 (C-Ar), 129.1 (C-7), 125.3 (C-6), 125.0 (C-9), 33.3 (C-2 or C-4), 32.5 (C-2 or C-4), 25.5 (C-3). Data consistent with literature.[179]

2-(2,3-Dihydro-1*H*-inden-4-yl)acetaldehyde – 251a

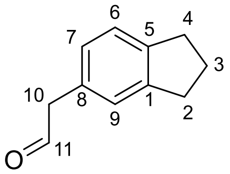


Method 1:[196] Synthesised according to a reported procedure. To a solution of **252a** (36.9 mg, 0.21 mmol, 1 eq) and NaI (31.5 mg, 0.21 mmol, 1 eq) in acetonitrile (4 mL) under Ar was added TMSCl (26 µL, 0.21 mmol, 1 eq) dropwise. The reaction was stirred at rt for 1.5 h, then quenched with aq. sat. NaS_2O_3 solution (3 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 5 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 40:1) to afford title compound **251a** (30.7 mg, 90%) as a colourless oil.

Method 2: In a two-necked flask, a solution of **332** (160 mg, 1.01 mmol, 1 eq) in DCM (20 mL) was prepared. A spatula tip of sudan red was added and the mixture gently swirled until the solution was a uniform pink colour. On one neck was placed a thermometer adapter fitted with a glass pipette which reached below the level of the solution (to allow bubbling of gas through the solution). To the second neck was attached an outlet tube passing through a bubbler containing a 1.0 M aq. solution of KI. The solution was cooled to –78 °C and the end of the pipette fitted to the ozoniser. O_2 was bubbled through the solution at a flow rate of 80 mL/min for 5 min, after which time the ozoniser was switched on and O_3 was bubbled through the solution (also at 80 mL/min) until a colour change to pale yellow was observed (20 min). The ozoniser was switched off and O_2 bubbled through the solution for a further 5 min to remove all traces of O_3. The reaction was quenched with dimethyl sulfide (371 µL, 5.05 mmol, 5 eq) at –78 °C and then the reaction mixture allowed to warm to rt. This mixture was stirred for 24 h at rt, then the solvent was removed *in vacuo*. The crude product was purified by FCC (pentane:EtOAc, 2:1) to afford title compound **251a** (66.7 mg, 41%) as a yellow oil.

Rf 0.10 (pentane:Et_2O, 40:1, stain: $KMnO_4$); **IR** ν_{max} 2950 (C-H), 1724 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 9.71 (t, *J* = 2.5 Hz, 1H, H-11), 7.20 (dd, *J* = 7.5 Hz, 1.5 Hz, 1H, H-Ar), 7.16 (t, *J* = 7.5 Hz, 1H, H-7), 7.00 (dd, *J* = 7.5 Hz, 1.5 Hz, 1H, H-Ar), 3.67 (d, *J* = 2.5 Hz, 2H, H-10), 2.96 (t, *J* = 7.5 Hz, 2H, H-2 or H-4), 2.84 (t, *J* = 7.5 Hz, 2H, H-2 or H-4), 2.09 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 199.2 (C-11), 145.1 (C-Ar), 144.0 (C-Ar), 127.9 (C-9), 127.7 (HC-Ar), 127.0 (C-7), 123.9 (HC-Ar), 48.9 (C-10), 33.2 (C-2 or C-4), 31.6 (C-2 or C-4), 24.9 (C-3); **LRMS** mass not found; **HRMS** (APCI) exact mass calculated for $[M+H]^+$ ($C_{11}H_{13}O$) requires *m/z* 161.0961, found *m/z* 161.0962.

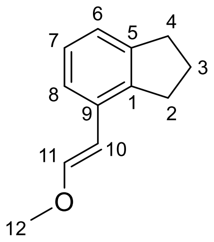
2-(2,3-Dihydro-1*H*-inden-5-yl)acetaldehyde – 251b



Synthesised according to a reported procedure.[196] To a solution of **252b** (228 mg, 1.31 mmol, 1 eq) and NaI (196 mg, 1.31 mmol, 1 eq) in acetonitrile (26 mL) under Ar was added TMSCl (166 μL, 1.31 mmol, 1 eq) dropwise. The reaction was stirred at rt for 1.5 h, then quenched with aq. sat. NaS_2O_3 solution (20 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in* *vacuo*. The crude product was purified by FCC (pentane:Et_2O, 40:1) to afford title compound **251b** (88.7 mg, 42%) as a pale-yellow oil.

R_f 0.30 (pentane:Et_2O, 40:1, stain: vanillin); **IR** ν_{max} 2946 (C-H), 1722 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 9.73 (t, *J* = 2.5 Hz, 1H, H-11), 7.22 (d, *J* = 7.5 Hz, 1H, H-6), 7.08 (s, 1H, H-9), 6.98 (d, *J* = 7.5 Hz, 1H, H-7), 3.65 (d, *J* = 2.5 Hz, 2H, H-10), 2.95 – 2.86 (m, 4H, H-2 and H-4), 2.08 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 200.0 (C-11), 145.4 (C-Ar), 143.7 (C-Ar), 129.6 (C-8), 127.6 (C-6), 125.8 (C-9), 125.0 (C-7), 50.7 (C-10), 32.9 (C-2 or C-4), 32.7 (C-2 or C-4), 25.6 (C-3); **LRMS** mass not found; **HRMS** (APCI) exact mass calculated for $[M+H]^+$ ($C_{11}H_{13}O$) requires *m/z* 161.0961, found *m/z* 161.0960.

(*E*)-4-(2-Methoxyvinyl)-2,3-dihydro-1*H*-indene – 252a

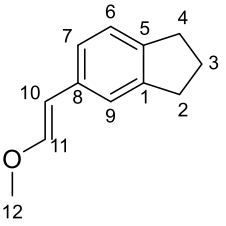


To a solution of (methoxymethyl)triphenylphosphonium chloride (219 mg, 0.64 mmol, 2.2 eq) in THF (2.9 mL) under N$_2$ at –78 °C was added ^{t}BuOK (580 µL, 1.0 M in THF, 0.58 mmol, 2.0 eq) dropwise and the mixture stirred for 30 min. A solution of **250a** (42.1 mg, 0.29 mmol, 1.0 eq) in THF (290 µL) was added dropwise. The reaction mixture was warmed to rt, stirred for 6 h, then diluted with brine (3 mL). The layers were separated and the aqueous layer extracted with Et$_2$O (3 × 5 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous MgSO$_4$, filtered, and concentrated *in vacuo* to afford title compound **252a** (36.9 mg, quant.) as a 2:1 mixture of *trans*:*cis* isomers, as a colourless oil. This product was used in the next step without further purification.

R_f 0.55 (pentane:Et$_2$O, 40:1, stain: KMnO$_4$); **IR** ν_{max} 2934 (C-H), 1641 (C=C), 1100 (C-O); **^{1}H NMR** (CDCl$_3$, 400 MHz) δ_H 7.77 (d, *J* = 7.5 Hz, 0.33H, H-Ar$_C$), 7.33 – 7.30 (m, 0.66H, H-Ar$_T$), 7.16 – 7.03 (m, 2H, H-Ar), 6.99 (d, *J* = 13.0 Hz, 0.66H, H-11$_T$), 6.18 (d, *J* = 7.0 Hz, 0.33H, H-11$_C$), 5.85 (d, *J* = 13.0 Hz, 0.66H, H-10$_T$), 5.27 (d, *J* = 7.0 Hz, 0.33H, H-10$_C$), 3.78 (s, 1H, H-12$_C$), 3.70 (s, 2H, H-12$_T$), 2.91 (ap. dt, *J* = 14.0 Hz, 7.5 Hz, 4H, H-2 and H-4), 2.08 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** (CDCl$_3$, 100 MHz) δ_C 149.5 (C-11$_T$), 148.1 (C-11$_C$), 144.7 (C-Ar$_T$), 143.9 (C-Ar$_C$), 141.6 (C-Ar$_C$), 141.0 (C-Ar$_T$), 137.4 (C-Ar$_C$), 137.3 (C-Ar$_T$), 128.8 (HC-Ar$_T$), 126.6 (HC-Ar$_T$), 126.3 (HC-Ar$_C$), 126.1 (HC-Ar$_C$), 122.1 (HC-Ar$_C$), 121.9 (HC-Ar$_T$), 103.6 (C-10$_T$), 103.2 (C-10$_C$), 60.7 (C-12$_C$), 56.7 (C-12$_T$), 33.4 (C-2$_C$ or C-4$_C$), 33.3 (C-2$_T$ or C-4$_T$), 32.0 (C-2$_T$ or C-4$_T$), 31.8 (C-2$_C$

or C-4$_C$), 24.9 (C-3$_T$), 24.8 (C-3$_C$); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for [M+H]$^+$ ($C_{12}H_{15}O$) requires *m/z* 175.1117, found *m/z* 175.1118.

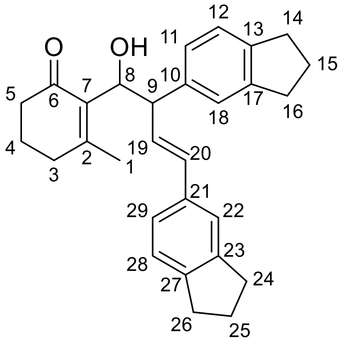
(*E*)-5-(2-Methoxyvinyl)-2,3-dihydro-1*H*-indene – 252b



To a solution of (methoxymethyl)triphenylphosphonium chloride (994 mg, 2.9 mmol, 2.2 eq) in THF (13 mL) under N_2 at –78 °C was added ^{t}BuOK (2.6 mL, 1.0 M in THF, 2.6 mmol, 2.0 eq) dropwise and the mixture stirred for 30 min. A solution of **250b** (190 mg, 1.3 mmol, 1.0 eq) in THF (1.3 mL) was added dropwise. The reaction was warmed to rt, stirred for 6 h, then diluted with brine (10 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 15 mL). The combined organic layers were washed with brine (40 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **252b** (226 mg, quant.) as a yellow oil. This product was used in the next step without further purification.

R$_f$ 0.60 (pentane:Et_2O, 40:1, stain: $KMnO_4$); **IR** ν_{max} 2935 (C-H), 1650 (C=C), 1435 (C=C), 1099 (C-O), 697 (=C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.15 – 7.11 (m, 2H, H-Ar), 7.03 – 6.97 (m, 2H, H-Ar and H-11), 5.82 (d, *J* = 13.0 Hz, 1H, H-10), 3.67 (s, 3H, H-12), 2.87 (ap. ddd, *J* = 9.0 Hz, 5.0 Hz, 2.0 Hz, 4H, H-2 and H-4), 2.06 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 148.3 (C-Ar), 137.4 (C-Ar), 137.3 (C-Ar), 128.9 (C-11), 124.6 (HC-Ar), 123.5 (HC-Ar), 121.1 (HC-Ar), 105.6 (C-10), 56.6 (C-12), 33.0 (C-2 or C-4), 32.7 (C-2 or C-4), 25.6 (C-3); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for [M+H]$^+$ ($C_{12}H_{15}O$) requires *m/z* 175.1117, found *m/z* 175.1119.

(*E*)-2-(2,4-Bis(2,3-dihydro-1*H*-inden-5-yl)-1-hydroxybut-3-en-1-yl)-3-methylcyclohex-2-en-1-one – 254



To a solution of **215** (109 mg, 0.39 mmol, 1.0 eq) in THF (0.8 mL) under N_2 at –78 °C was added *n*-butyllithium (269 µL, 1.6 M in hexanes, 0.43 mmol, 1.1 eq) dropwise and the mixture stirred for 5 min. A solution of **251b** (68.8 mg, 0.43, 1.1 eq) in THF (0.8 mL) at –78 °C was added dropwise and the reaction stirred for 30 min. The reaction was quenched with 20% aq. NaH_2PO_4 solution (2 mL) and 1.0 M aq. HCl (2 mL) whilst cold. The mixture was warmed to rt, the layers separated, and the aqueous layer extracted with Et_2O (3 × 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 3:1) to afford unexpected title compound **254** (19.1 mg, 12%) as a pale-yellow oil.

R_f 0.20 (pentane:Et_2O, 1:1, stain: vanillin); **IR** ν_{max} 3453 (O-H), 2943 (C-H), 1648 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.14 (d, *J* = 7.5 Hz, 1H, H-11 or H-12 or H-28 or H-29), 7.11 (d, *J* = 7.5 Hz, 1H, H-11 or H-12 or H-28 or H-29), 6.98 (s, 1H, H-18 or H-22), 6.96 (s, 1H, H-18 or H-22), 6.88 (d, *J* = 7.5 Hz, 1H, H-11 or H-12 or H-28 or H-29), 6.84 (d, *J* = 7.5 Hz, 1H, H-11 or H-12 or H-28 or H-29), 5.87 (td, *J* = 7.5 Hz, 2.0 Hz, 1H, H-19), 5.29 (d, *J* = 10.5 Hz, 1H, H-8), 4.84 (d, *J* = 10.5 Hz, 1H, OH), 3.21 (d, *J* = 7.5 Hz, 2H, H-9), 2.93 – 2.81 (m, 8H, H-14, H-16, H-24 and H-26), 2.38 – 2.30 (m, 2H, H-5), 2.29 –

2.20 (m, 2H, H-3), 2.05 (p, *J* = 7.5 Hz, 4H, H-15 and H-25), 1.97 – 1.76 (m, 2H, H-4), 1.67 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 201.6 (C-6), 158.9 (C-7), 144.5-144.0-143.1-142.8-141.7-139.2 (C-10, C-13, C-17, C-21, C-23 and C-27), 133.5 (C-2), 127.1 (C-11 or C-12 or C-28 or C-29), 126.2 (C-11 or C-12 or C-28 or C-29), 125.2 (C-18 or C-22), 125.1 (C-19), 124.5 (C-11 or C-12 or C-28 or C-29), 124.3 (C-18 or C-22), 124.0 (C-11 or C-12 or C-28 or C-29), 73.7 (C-8), 38.7 (C-5), 34.8 (C-9), 33.3 (C-3), 33.0-33.0-32.8-30.5 (C-14, C-16, C-24 and C-26), 25.7-25.5 (C-15 and C-25), 22.0 (C-4), 21.1 (C-1);[*] **LRMS** (ES+) mass found *m/z* 413.2 $[M+H]^+$; **HRMS** (ESI) exact mass calculated for $[M+Na]^+$ ($C_{29}H_{32}NaO_2$) requires *m/z* 435.2295, found *m/z* 435.2295.

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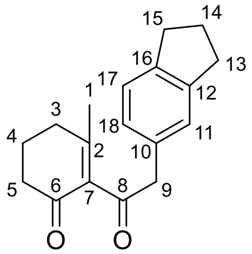
[*] HMBC not available for this compound.

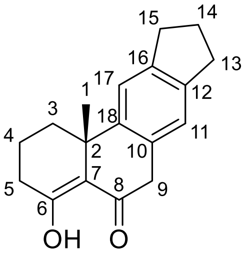
2-(2-(2,3-Dihydro-1*H*-inden-5-yl)acetyl)-3-methylcyclohex-2-en-1-one – 259b



To a solution of **210b** (45.0 mg, 0.14 mmol, 1.0 eq) in DCM (0.7 mL) under N_2 at 0 °C was added Dess-Martin periodinane (76.3 mg, 0.18 mmol, 1.25 eq). The reaction was allowed to warm to rt and its progress followed by TLC (pentane:Et_2O, 1:1). The reaction was quenched with isopropanol (1 mL) after 2 h and the mixture evaporated to dryness. The crude residue was re-dissolved in Et_2O (5 mL) and washed successively with sat. aq. solutions of 1:1 $NaHCO_3$/$Na_2S_2O_3$ (5 mL), $NaHCO_3$ (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford title compound **259b** (36.6 mg, quant.) as a pale-yellow oil which was used without further purification.

R_f 0.55 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **IR** ν_{max} 2943 (C-H), 1692 (C=O), 1438 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.15 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.09 (s, 1H, H-11), 6.97 (d, *J* = 7.5 Hz, 1H, H-Ar), 3.88 (s, 2H, H-9), 2.88 (ap. q, *J* = 7.0 Hz, 4H, H-13 and H-15), 2.10 – 2.00 (m, 4H, H-3 and H-14), 1.85 – 1.71 (m, 4H, H-4 and H-5), 1.56 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 208.1 (C-6 or C-8), 205.8 (C-6 or C-8), 144.5 (C-Ar or C-2 or C-7), 142.8 (C-Ar or C-2 or C-7), 140.6 (C-Ar or C-2 or C-7), 135.7 (C-Ar or C-2 or C-7), 132.0 (C-Ar or C-2 or C-7), 127.9 (HC-Ar), 126.1 (C-11), 124.3 (HC-Ar), 51.3 (C-9), 33.0 (C-13 or C-15), 32.9 (C-13 or C-15), 32.7 (C-5), 31.1 (C-3), 25.6 (C-14), 20.7 (C-1), 19.8 (C-4); **LRMS** (ES+) mass found *m/z* 269.2 $[M+H]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{18}H_{21}O_2$) requires *m/z* 269.1536, found *m/z* 269.1536.

4-Hydroxy-11b-methyl-1,2,3,6,8,9,10,11b-octahydro-5*H*-cyclopenta[b]phenanthren-5-one – 260b

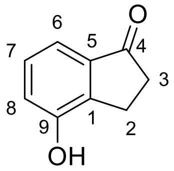


Synthesised according to a reported procedure.[193] A mixture of AgOTf (2.8 mg, 0.011 mmol, 0.1 eq) and $RuCl_3$•$3H_2O$ (1.2 mg, 0.11 mmol, 0.05 eq) was stirred in DCE (165 µL) under N_2 at rt for 1 h. A solution of **259b** (30.0 mg, 0.11 mmol, 1.0 eq) in DCE (885 µL) was added dropwise and the reaction heated to 80 °C for 14 h. The cooled reaction was purified by FCC (pentane:Et_2O, 10:1) to afford title compound **260b** (5.3 mg, 18%) as a pale-yellow oil.

***R**f* 0.40 (pentane:Et_2O, 10:1, stain: $KMnO_4$); **IR** ν_{max} 2941 (C-H), 1604 (C=O), 1412 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.24 (s, 1H, H-17), 7.01 (s, 1H, H-11), 3.86 (d, *J* = 19.0 Hz, 1H, H-9), 3.52 (d, *J* = 19.0 Hz, 1H, H-9), 2.95 – 2.83 (m, 4H, H-13 and H-15), 2.48 – 2.34 (m, 3H, H-3 and H-5), 2.13 – 2.03 (m, 2H, H-14), 2.04 – 1.96 (m, 2H, H-4), 1.93 – 1.81 (m, 1H, H-3), 1.30 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 197.0 (C-8), 183.7 (C-6), 143.6 (C-18), 143.5 (C-12 or C-16), 142.7 (C-12 or C-16), 130.0 (C-10), 124.2 (C-11), 119.4 (C-17), 112.4 (C-7), 41.4 (C-9), 37.8 (C-2), 34.9 (C-3), 33.0 (C-13), 32.6 (C-15), 31.4 (C-5), 29.9 (C-7), 29.1 (C-1), 25.7 (C-14), 17.8 (C-4); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{18}H_{21}O_2$) requires *m/z* 269.1536, found *m/z* 269.1536.

Compounds from Chapter 3

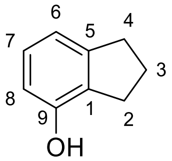
4-Hydroxy-2,3-dihydro-1*H*-inden-1-one – 112



Synthesised according to a reported procedure.[266] $AlCl_3$ (17.4 g, 130.4 mmol, 5.5 eq) and NaCl (4.5 g, 75.8 mmol, 3.2 eq) were mixed and heated to 150 °C under an atmosphere of N_2. Dihydrocoumarin (3.0 mL, 23.7 mmol, 1.0 eq) was added dropwise whilst the reaction was maintained at this temperature. When addition was complete, the temperature was increased to 200 °C and the reaction stirred for 1 h. The mixture was cooled to 0 °C and quenched with crushed ice (30 g) and water (15 mL), then warmed to rt. To this suspension was then added 3.0 M aq. HCl (100 mL) and the mixture stirred at rt for a further 30 min. The resultant solid was collected by filtration and re-dissolved in Et_2O (250 mL). This solution was washed with 3.0 M aq. HCl until all traces of solid aluminium residue were removed. The organic layer was then dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **112** (3.0 g, 86%) as a grey powder.

R_f 0.25 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **IR** ν_{max} 3167 (O-H), 2980 (C-H), 1677 (C=O), 1596 (C=C); **^{1}H NMR** (DMSO, 400 MHz) δ_H 9.98 (s, 1H, OH), 7.23 (t, *J* = 7.5 Hz, 1H, H-7), 7.11 – 7.04 (m, 2H, H-6 and H-8), 2.93 (t, *J* = 6.0 Hz, 2H, H-2), 2.62 – 2.57 (m, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 206.6 (C-4), 155.1 (C-9), 141.8 (C-Ar), 138.4 (C-Ar), 128.6 (HC-Ar), 119.8 (HC-Ar), 113.3 (HC-Ar), 35.8 (C-3), 22.3 (C-2); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_9H_9O_2$) requires *m/z* 149.0597, found *m/z* 149.0597; **mp** (dec) 221 – 223 °C, lit. 231 – 232 °C;

2,3-Dihydro-1*H*-inden-4-ol – **265a**



Method 1: To a solution of **112** (100 mg, 0.67 mmol, 1 eq) in AcOH (22 mL) was slowly added activated Zn dust (1.1 g, 16.8 mmol, 25 eq).* The reaction was heated to 100 °C and stirred for 18 h, after which time it was cooled to rt and diluted with water (50 mL). The unreacted Zn was filtered off and the mixture extracted with EtOAc (3 × 200 mL). The combined organic layers were washed sequentially with water (2 × 500 mL), sat. aq. $NaHCO_3$ solution (500 mL), and brine (500 mL), then dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 10:1) to afford title compound **265a** (38 mg, 28%) as a pale-yellow oil.

Method 2: To a solution of **112** (100 mg, 0.67 mmol, 1 eq) in DCE (1.9 mL) under N_2 were added ZnI_2 (638 mg, 2.0 mmol, 3 eq) and $NaBH_3CN$ (126 mg, 2.0 mmol, 3 eq). The reaction mixture was heated to reflux for 2 h, cooled, and filtered through silica with EtOAc. The resulting solution was concentrated *in vacuo* and the crude product purified by FCC (pentane:Et_2O, 5:1) to afford title compound **265a** (21 mg, 24%) as a pale-yellow oil.

Method 3: To a solution of **112** (100 mg, 0.67 mmol, 1.0 eq) in ethylene glycol (1 mL) were added hydrazine monohydrate (302 µL, 6.23 mmol, 9.3 eq), then ground KOH pellets (112 mg, 2.0 mmol, 3.0 eq). The reaction mixture was heated to 130 °C for 2 h, then the temperature increased to 180 °C and the mixture stirred a further 20 h. After this

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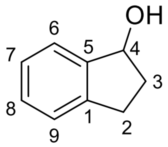
* The zinc dust was activated and dried immediately prior to use according to a reported procedure.[324]
A suspension of Zn dust (5 g) in 1.0 M aq. HCl (50 mL) was stirred at rt for 15 min. The zinc was removed by filtration and washed sequentially with water (2 × 50 mL) and Et_2O (2 × 50 mL), then dried under high vacuum at 50 °C.

time, the reaction mixture was cooled to rt and poured into water (10 mL). The organic component was extracted with Et_2O (3 × 15 mL) and the combined organic layers washed with brine (45 mL). The organic layer was then dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **265a** (60 mg, 67%) as a pale-yellow oil which was used without further purification.

Method 4: A solution of **404** (212 mg, 0.67 mmol, 1 eq) and $NaBH_3CN$ (84 mg, 1.34 mmol, 2 eq) was prepared in methanol (3.2 mL) under Ar, then cooled to –10 °C. Acetyl chloride (48 µL, 0.67 mmol, 1 eq) was added slowly, at a rate to maintain the temperature below 0 °C. Once addition was complete, the reaction mixture was stirred for 1 h at 0 °C, after which time a solution of $NaOAc•3H_2O$ (912 mg, 6.7 mmol, 10 eq) in methanol (3.2 mL) was added dropwise. The reaction mixture was heated to reflux for 3 h, then cooled, and the solvent removed *in vacuo*. The residue was diluted with water (10 mL) and the aqueous phase extracted with Et_2O (3 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 5:1) to afford title compound **265a** (35 mg, 39%) as a pale-yellow oil.

R_f 0.25 (pentane:Et_2O, 5:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.05 (t, *J* = 7.5 Hz, 1H, H-7), 6.83 (d, *J* = 8.0 Hz, 1H, H-6), 6.62 (d, *J* = 7.0 Hz, 1H, H-8), 4.51 (s, 1H, OH), 2.93 (t, *J* = 7.5 Hz, 2H, H-4), 2.86 (t, *J* = 7.5 Hz, 2H, H-2), 2.11 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 152.0 (C-9), 145.9 (C-5), 129.9 (C-1), 127.9 (C-7), 116.7 (C-6), 113.2 (C-8), 33.4 (C-4), 28.8 (C-2), 23.6 (C-3). Data consistent with literature.[317]

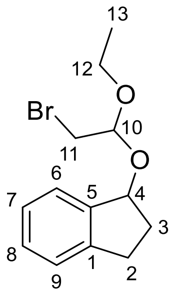
2,3-Dihydro-1*H*-inden-1-ol – 290



Indanone (1.0 g, 7.6 mmol, 1.0 eq) and $NaBH_4$ (318 mg, 8.4 mmol, 1.1 eq) were stirred together at rt in EtOH (7.6 mL) until completion by TLC (*ca*. 3 h). The mixture was concentrated and the crude residue redissolved in DCM (4 mL) and washed with water (4 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **290** (662 mg, 59%) as an off-white slurry.

Rf 0.30 (pentane:EtOAc, 4:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.45 – 7.40 (m, 1H, H-Ar), 7.28 – 7.23 (m, 3H, H-Ar), 5.25 (td, *J* = 7.0 Hz, 5.0 Hz, 1H, H-4), 3.12 – 3.02 (m, 1H, H-2), 2.88 – 2.77 (m, 1H, H-2), 2.55 – 2.45 (m, 1H, H-3), 2.00 – 1.91 (m, 1H, H-3), 1.76 (d, *J* = 7.0 Hz, 1H, OH); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 145.1 (C-Ar), 143.5 (C-Ar), 128.5 (HC-Ar), 126.9 (HC-Ar), 125.0 (HC-Ar), 124.3 (HC-Ar), 76.6 (C-4), 36.1 (C-3), 29.9 (C-2). Data consistent with literature.[308]

1-(2-Bromo-1-ethoxyethoxy)-2,3-dihydro-1*H*-indene – 291



Synthesised according to a reported procedure.[251] Br_2 (164 µL, 3.2 mmol, 0.7 eq) was dissolved in DCM (40 mL) under Ar at 0 °C. Ethyl vinyl ether (690 µL, 7.2 mmol, 1.6 eq) was added dropwise and the solution stirred 5 min. DIPEA (1.25 mL, 7.2 mmol, 1.6 eq) was added, followed by **290** (600 mg, 4.5 mmol, 1.0 eq) and the reaction mixture stirred at rt for 22 h. This was then washed with sat. aq. $NaHCO_3$ solution (30 mL), then brine (30 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 50:1) to afford title compound **291** (919 mg, 69%) as a 1:1 mixture of diastereomers as a colourless oil.

R R_f 0.20 (pentane:Et_2O, 50:1, stain: $KMnO_4$); **IR** ν_{max} 1057 (C-O), 1460 (C-C), 2975 (C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.45 – 7.40 (m, 1H, H-Ar), 7.28 – 7.21 (m, 3H, H-Ar), 5.25 (dd, *J* = 6.5 Hz, 5.0 Hz, 0.5H, H-4_A), 5.17 (dd, *J* = 6.5 Hz, 4.5 Hz, 0.5H, H-4_B), 4.93 (td, *J* = 5.5 Hz, 3.5 Hz, 1H, H-10), 3.82 – 3.58 (m, 2H, H-12), 3.50 – 3.36 (m, 2H, H-11), 3.15 – 3.03 (m, 1H, H-2), 2.88 – 2.76 (m, 1H, H-2), 2.49 – 2.33 (m, 1H, H-3), 2.21 – 2.07 (m, 1H, H-3), 1.28 (ap. q, *J* = 7.0 Hz, 3H, H-13); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 143.9 (C-Ar), 142.6 (C-Ar), 128.7 (HC-Ar), 126.6 (HC-Ar), 125.1 (HC-Ar), 125.0 (HC-Ar), 101.5 (C-10), 100.6 (C-10), 81.6 (C-4_B), 80.7 (C-4_A), 61.6 (C-12), 61.5 (C-12), 34.2 (C-3), 33.0 (C-3), 32.4 (C-11), 32.3 (C-11), 30.3 (C-2), 30.2 (C-2), 15.5 (C-13), 15.4 (C-

13);[*] **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+Na]^+$ ($C_{13}H_{17}O_2{}^{79}BrNa$) requires *m/z* 307.0304, found *m/z* 307.0306.

293 to 296

Compounds **293** to **296** were isolated as unexpected products from the attempted rearrangement of **291**.

To a solution of **291** (908 mg, 3.0 mmol, 1.0 eq) in DMF (6 mL) under N_2 was added KO^tBu (370 mg, 3.3 mmol, 1.1 eq) and the reaction mixture stirred at rt for 30 min. The mixture was then heated to 155 °C for 10 h, cooled to rt, and poured into distilled water (6 mL). The aqueous phase was extracted with hexane (5 × 15 mL) and the combined organic layers washed with water (50 mL) then brine (50 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O 5:1) to afford unexpected compounds **293**, **294**, **295**, and **296**.

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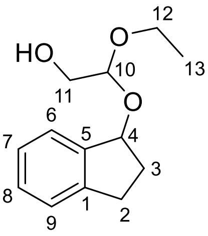
[*] Duplicate signals due to diastereomers.

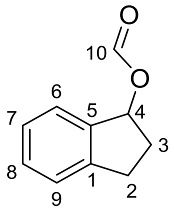
2-((2,3-Dihydro-1*H*-inden-1-yl)oxy)-2-ethoxyethan-1-ol – 293



293 (38.9 mg, 6%) was obtained as a 1:2 mixture of diastereomers A:B, as a yellow oil from the failed rearrangement of **291**.

R$_f$ 0.15 (pentane:Et$_2$O, 2:1, stain: KMnO$_4$); **IR** *ν*$_{max}$ 3452 (O-H), 2973 (C-H), 1054 (C-O); **^{1}H NMR** (CDCl$_3$, 400 MHz) δ$_H$ 7.47 – 7.35 (m, 1H, H-Ar), 7.29 – 7.18 (m, 3H, H-Ar), 5.31 – 5.14 (m, 1H, H-4), 4.89 – 4.78 (m, 1H, H-10), 3.92 – 3.76 (m, 1H, H-12), 3.72 – 3.55 (m, 3H, H-11 and H-12), 3.16 – 3.01 (m, 1H, H-2), 2.90 – 2.76 (m, 1H, H-2), 2.50 – 2.36 (m, 1H, H-3), 2.22 – 2.00 (m, 1H, H-2), 1.60 (br s, OH), 1.32 – 1.23 (m, 3H, H-13); **^{13}C NMR** (CDCl$_3$, 100 MHz) δ$_C$ 143.9 (C-Ar), 142.8 (C-Ar), 128.7 (HC-Ar$_B$), 128.6 (HC-Ar$_A$), 126.7 (HC-Ar$_A$), 126.6 (HC-Ar$_B$), 125.2 (HC-Ar$_B$), 125.0 (HC-Ar$_A$), 125.0 (HC-Ar$_A$), 124.8 (HC-Ar$_B$), 102.3 (C-10$_B$), 101.2 (C-10$_A$), 81.6 (C-4$_B$), 80.8 (C-4$_A$), 63.6 (C-11), 62.6 (C-12$_B$), 62.5 (C-12$_A$), 34.2 (C-2$_B$), 33.3 (C-2$_A$), 30.3 (C-3$_B$), 30.1 (C-3$_A$), 15.7 (C-13$_A$), 15.6 (C-13$_B$); **LRMS** (ES+) mass found *m/z* 261.1 [M+K]$^+$; **HRMS** (ESI) exact mass calculated for [M+Na]$^+$ (C$_{13}$H$_{18}$O$_3$Na) requires *m/z* 245.1148, found *m/z* 245.1147.

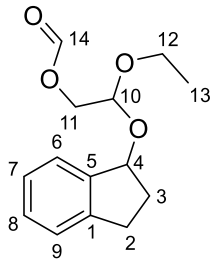
2,3-Dihydro-1*H*-inden-1-yl formate – 294



294 (36.2 mg, 7%) was obtained as a yellow oil from the failed rearrangement of **291**.

R_f 0.70 (pentane:Et_2O, 2:1, stain: $KMnO_4$); **IR** *ν*max 2924 (C-H), 1719 (C=O), 1172 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 8.12 (s, 1H, H-10), 7.46 – 7.41 (m, 1H, H-Ar), 7.35 – 7.21 (m, 3H, H-Ar), 6.34 (dd, *J* = 7.0 Hz, 3.5 Hz, 1H, H-4), 3.20 – 3.09 (m, 1H, H-2), 2.97 – 2.86 (m, 1H, H-2), 2.60 – 2.47 (m, 1H, H-3), 2.21 – 2.11 (m, 1H, H-3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 161.1 (C-10), 144.6 (C-Ar), 140.6 (C-Ar), 129.3 (HC-Ar), 127.0 (HC-Ar), 125.8 (HC-Ar), 125.0 (HC-Ar), 78.3 (C-4), 32.4 (C-3), 30.4 (C-2); **LRMS** mass not found; **HRMS** mass not found.

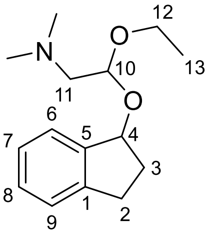
2-((2,3-Dihydro-1*H*-inden-1-yl)oxy)-2-ethoxyethyl formate – 295



295 (33.1 mg, 4%) was obtained as a 1:1 mixture of diastereomers as a brown oil from the failed rearrangement of **291**.

R_f 0.55 (pentane:Et_2O, 2:1, stain: $KMnO_4$); **IR** ν_{max} 2975 (C-H), 1728 (C=O), 1127 (C-O), 1066 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 8.10 (s, 0.5H, H-14), 8.06 (s, 0.5H, H-14), 7.45 – 7.34 (m, 1H, H-Ar), 7.28 – 7.18 (m, 3H, H-Ar), 5.26 (dd, J = 6.5 Hz, 5.0 Hz, 0.5H, H-4_A), 5.18 (dd, J = 6.5 Hz, 4.5 Hz, 0.5H, H-4_B), 4.98 (dt, *J* = 6.5 Hz, 5.5 Hz, 1H, H-10), 4.31 – 4.18 (m, 2H, H-11), 3.86 – 3.59 (m, 2H, H-12), 3.15 – 3.01 (m, 1H, H-2), 2.89 – 2.75 (m, 1H, H-2), 2.47 – 2.34 (m, 1H, H-3), 2.21 – 2.00 (m, 1H, H-3), 1.27 (ap q, *J* = 7.0 Hz, 3H, H-13); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 160.8 (C-14), 160.7 (C-14), 143.9 (C-Ar), 142.6 (C-Ar), 128.7 (HC-Ar), 126.6 (HC-Ar), 125.0 (HC-Ar), 124.9 (HC-Ar), 99.1 (C-10), 98.3 (C-10), 81.4 (C-4_B), 80.8 (C-4_A), 63.7 (C-11), 63.7 (C-11), 62.0 (C-12), 61.8 (C-12), 34.2 (C-3), 33.2 (C-3), 30.3 (C-2), 30.1 (C-2), 15.5 (C-13), 15.4 (C-13); **LRMS** (ES+) mass found *m/z* 273.1 $[M+Na]^+$; **HRMS** (ESI) exact mass calculated for $[M+Na]^+$ ($C_{14}H_{18}O_4Na$) requires *m/z* 273.1097, found *m/z* 273.1097.

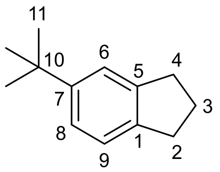
2-((2,3-Dihydro-1*H*-inden-1-yl)oxy)-2-ethoxy-*N*,*N*-dimethylethan-1-amine – 296



296 (87.2 mg, 11%) was obtained as a 1:2 mixture of diastereomers A:B, as a grey-green oil from the failed rearrangement of **291**.

Rf 0.10 (EtOAc, stain: $KMnO_4$); **IR** ν_{max} 2972 (C-H), 1459 (C=C), 1131 (C-N), 1039 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.46 – 7.37 (m, 1H, H-Ar), 7.26 – 7.18 (m, 3H, H-Ar), 5.24 (t, *J* = 6.0 Hz, 0.33H, H-4_A), 5.16 (dd, *J* = 6.0 Hz, 5.0 Hz, 0.66H, H-4_B), 4.91 – 4.83 (m, 1H, H-10), 3.84 – 3.57 (m, 2H, H-12), 3.12 – 3.00 (m, 1H, H-2), 2.86 – 2.75 (m, 1H, H-2), 2.64 – 2.55 (m, 1H, H-11), 2.52 – 2.35 (m, 2H, H-3 and H-11), 2.31 (s, 2H, NMe_A), 2.28 (s, 4H, NMe_B), 2.19 – 2.01 (m, 1H, H-3), 1.30 – 1.23 (m, 3H, H-13); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 143.8 (C-Ar_B), 143.6 (C-Ar_A), 143.3 (C-Ar_A), 143.2 (C-Ar_B), 128.3 (HC-Ar_B), 128.3 (HC-Ar_A), 126.6 (HC-Ar_A), 126.4 (HC-Ar_B), 125.0 (HC-Ar_A), 125.0 (HC-Ar_B), 125.0 (HC-Ar_B), 124.8 (HC-Ar_A), 101.4 (C-10_B), 100.3 (C-10_A), 80.6 (C-4_B), 79.8 (C-4_A), 62.4 (C-11_B), 62.4 (C-11_A), 61.0 (C-12_B), 60.8 (C-12_A), 46.5 (NMe_A), 46.5 (NMe_B), 34.3 (C-3_B), 33.3 (C-3_A), 30.3 (C-2_B), 30.1 (C-2_A), 15.5 (C-13_A), 15.5 (C-13_B); **LRMS** (ES+) mass found *m/z* 272.2 $[M+Na]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{15}H_{24}NO_2$) requires *m/z* 250.1802, found *m/z* 250.1801.

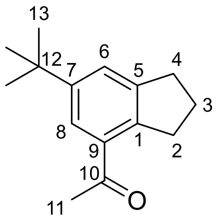
5-(*tert*-Butyl)-2,3-dihydro-1*H*-indene – 298



Synthesised according to a reported procedure.[253] Indane (3.0 mL, 24.5 mmol, 1.0 eq) and $AlCl_3$ (240 mg, 1.8 mmol, 0.075 eq) were stirred in DCM (18.4 mL) under N_2 at 0 °C for 2 h. A solution of *tert*-butyl chloride (2.7 mL, 24.5 mmol, 1.0 eq) in DCM (6.1 mL) was added dropwise and stirred for 2 h. The completed reaction was poured slowly into ice cold 1.0 M aq. HCl (25 mL), the layers separated, and the aqueous layer extracted with DCM (3 × 25 mL). The combined organic phases were washed with water (75 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by vacuum distillation at *ca.* 100 °C and 13 mbar to afford title compound **298** (2.4 g, 56%) as a colourless oil.

R_f 0.70 (pentane, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.29 (s, 1H, H-6), 7.19 – 7.17 (m, 2H, H-8 and H-9), 2.90 (dt, *J* = 11.0 Hz, 7.5 Hz, 4H, H-2 and H-4), 2.08 (p, *J* = 7.5 Hz, 2H, H-3), 1.33 (s, 9H, H-11); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 149.3 (C-Ar), 144.2 (C-Ar), 141.4 (C-Ar), 124.0 (C-8 or C-9), 123.3 (C-8 or C-9), 121.4 (C-6), 34.7 (C-10), 33.2 (C-2 or C-4), 32.6 (C-2 or C-4), 31.8 (C-11), 25.7 (C-3); **bp** 103 °C at 13 mbar, lit. 108 °C at 13 mbar. Data consistent with literature.[253]

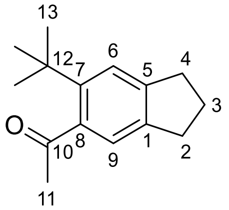
1-(6-(*tert*-Butyl)-2,3-dihydro-1*H*-inden-4-yl)ethan-1-one – 299a



Synthesised according to a modified literature procedure.[309] To a suspension of $AlCl_3$ (228 mg, 1.71 mmol, 3.0 eq) in DCM (11.4 mL) at –78 °C under N_2 was added slowly a solution of **298** (100 mg, 0.57 mmol, 1.0 eq) in DCM (11.4 mL). Acetyl chloride (102 μL, 1.43 mmol, 2.5 eq) was added dropwise and the reaction warmed to rt and stirred for 1 h. The reaction was cooled to 0 °C, quenched carefully with water (10 mL), and diluted with DCM (15 mL). The layers were separated, and the organic layer washed with 2.0 M aq. HCl (2 × 15 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 50:1) to afford title compound **299a** as a 3:1 mixture with its regioisomer **299b**. This mixture was re-purified by FCC (toluene) to afford **299a** (54.0 mg, 44%) as a white solid.

R_f 0.25 (pentane:Et_2O, 40:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.68 (s, 1H, H-8), 7.46 (s, 1H, H-6), 3.21 (t, *J* = 7.5 Hz, 2H, H-2), 2.91 (t, *J* = 7.5 Hz, 2H, H-4), 2.60 (s, 3H, H-11), 2.08 (p, *J* = 7.5 Hz, 2H, H-3), 1.35 (s, 9H, H-13); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 200.3 (C-10), 149.7 (C-Ar), 146.3 (C-Ar), 142.5 (C-Ar), 133.9 (C-Ar), 125.9 (C-6), 124.4 (C-8), 33.9 (C-2), 32.6 (C-4), 31.6 (C-13), 28.7 (C-11), 25.4 (C-3); **mp** 30 – 31 °C, lit. 39 °C; Data consistent with literature.[253]

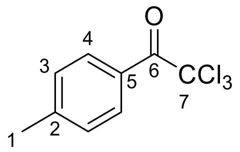
1-(6-(*tert*-Butyl)-2,3-dihydro-1*H*-inden-5-yl)ethan-1-one – 299b



The second regioisomer **299b** (17.3 mg, 15%) was also obtained as a colourless oil.

***R**$_f$* 0.50 (toluene, stain: $KMnO_4$); **IR** *ν*$_{max}$ 2935 (C-H), 1699 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.34 (s, 1H, H-Ar), 7.01 (s, 1H, H-Ar), 2.90 (ap. dt, *J* = 11.5 Hz, 7.5 Hz, 4H, H-2 and H-4), 2.59 (s, 3H, H-11), 2.09 (p, *J* = 7.5 Hz, 2H, H-3), 1.37 (s, 9H, H-13); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 202.2 (C-10), 145.6 (C-Ar), 145.2 (C-Ar), 142.0 (C-Ar), 140.5 (C-Ar), 123.7 (HC-Ar), 121.9 (HC-Ar), 35.9 (C-12), 33.1 (C-2 or C-4), 32.7 (C-11), 32.5 (C-2 or C-4), 32.2 (C-13), 25.5 (C-3); **LRMS** (ES+) mass found *m/z* 239.3 $[M+Na]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{15}H_{21}O$) requires *m/z* 217.1587, found *m/z* 217.1588.

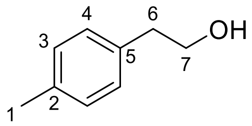
2,2,2-Trichloro-1-(*p*-tolyl)ethan-1-one – 310



Synthesised according to a reported procedure.[310] A suspension of $AlCl_3$ (693 mg, 5.2 mmol, 1.1 eq) in DCM (10 mL) was stirred under N_2 at 0 °C. Toluene (0.5 mL, 4.7 mmol, 1.0 eq) was added dropwise, followed by dropwise addition of trichloroacetyl chloride (580 µL, 5.2 mmol, 1.1 eq) and the reaction stirred for 1 h. The completed reaction was quenched with sat. aq. $NaCO_3$ solution (5 mL) and washed with brine (10 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by vacuum distillation at *ca.* 115 °C and 10 mbar to afford title compound **310** (336 mg, 30%) as a yellow oil.

R_f 0.70 (pentane:Et_2O, 20:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 8.17 (d, *J* = 8.0 Hz, 2H, H-4), 7.30 (d, *J* = 8.0 Hz, 2H, H-3), 2.45 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ_C 181.1 (C-6), 145.7 (C-5), 131.9 (C-4), 129.3 (C-3), 126.5 (C-2), 93.0 (C-7), 21.9 (C-1); **bp** 115 °C at 10 mbar, lit. 115 °C at 1 mbar. Data consistent with literature.[310]

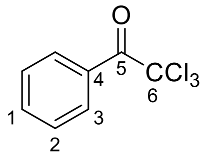
2-(*p*-Tolyl)ethan-1-ol – 311



Synthesised according to a modified literature procedure.[257] In a two-necked flask fitted with a condenser under N_2 was added **310** (200 mg, 0.85 mmol, 1 eq) in IPA (3.5 mL). $LiBH_4$ (2.1 mL, 2.0 M in THF, 4.25 mmol, 5 eq) and powdered NaOH (102 mg, 2.55 mmol, 3 eq) were added and the reaction heated to reflux for 2 h until completion by TLC. The cooled reaction was quenched with sat. aq. NH_4Cl solution (5 mL) and the aqueous layer saturated by addition of solid NaCl. The layers were separated and the aqueous phase extracted with EtOAc (5 × 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 2:1) to afford title compound **311** (67.2 mg, 58%) as a pale-yellow oil.

R

2,2,2-Trichloro-1-phenylethan-1-one – 313



Synthesised according to a reported procedure.[312] A solution of acetophenone (200 µL, 1.7 mmol, 1 eq) and trichloroisocyanuric acid (790 mg, 3.4 mmol, 2 eq) in acetic acid (3.4 mL) was heated to reflux for 5 h. The reaction was cooled, the precipitate washed with acetic acid (2 mL), and the filtrate diluted with water (5 mL). The aqueous layer was extracted with pentane (4 ×15 mL) and the combined organic layers washed with brine (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane to pentane:Et_2O, 50:1) to afford title compound **313** (105.5 mg, 28%) as a pale-yellow oil.

R_f 0.45 (pentane, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 8.26 (d, *J* = 7.5 Hz, 2H, H-3), 7.64 (t, *J* = 7.5 Hz, 1H, H-1), 7.50 (t, *J* = 7.5 Hz, 2H, H-2); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 181.4 (C-5), 134.4 (C-1), 131.7 (C-3), 129.3 (C-4), 128.6 (C-2).* Data consistent with literature.[313]

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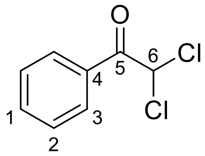
* C-6 not visible in ^{13}C NMR spectrum.

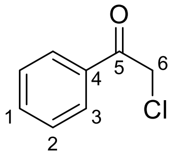
2,2-Dichloro-1-phenylethan-1-one – 314



Dichlorinated product **314** (160.7 mg, 50%) was also isolated as a yellow oil during the formation of trichlorinated **313**.

R$_f$ 0.15 (pentane, stain: KMnO$_4$); **^{1}H NMR** (CDCl$_3$, 400 MHz) δ_H 8.09 (d, *J* = 8.0 Hz, 2H, H-3), 7.66 (t, *J* = 8.0 Hz, 1H, H-1), 7.52 (t, *J* = 8.0 Hz, 2H, H-2), 6.68 (s, 1H, H-6); **^{13}C NMR** (CDCl$_3$, 101 MHz) δ_C 186.1 (C-5), 134.7 (C-1), 131.5 (C-4), 129.9 (C-3), 129.1 (C-2), 67.9 (C-6). Data consistent with literature.[314]

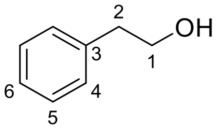
2-Chloro-1-phenylethan-1-one – 315



Monochlorinated product **315** (14.3 mg, 5%) was also isolated as a yellow solid during the formation of trichlorinated **313**.

R$_f$ 0.20 (pentane:Et$_2$O, 40:1, stain: KMnO$_4$); **^{1}H NMR** (CDCl$_3$, 400 MHz) δ_H 7.96 (d, *J* = 7.5 Hz, 2H, H-3), 7.62 (t, *J* = 7.5 Hz, 1H, H-1), 7.50 (t, *J* = 7.5 Hz, 2H, H-2), 4.72 (s, 2H, H-6); **^{13}C NMR** (CDCl$_3$, 101 MHz) δ_C 191.2 (C-5), 134.4 (C-4), 134.2 (C-1), 129.1 (C-2), 128.7 (C-3), 46.1 (C-6); **mp** 43 – 45 °C, lit. 50 – 51 °C. Data consistent with literature.[315]

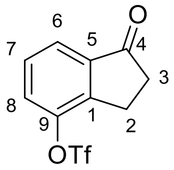
2-Phenylethan-1-ol – 316



To a solution of **314** (136.3 mg, 0.73 mmol, 1 eq) in IPA (2.9 mL) under N_2 were added $LiBH_4$ (79.5 mg, 3.65 mmol, 5 eq) and powdered NaOH (87.6 mg, 2.19 mmol, 3 eq). The reaction was heated to 85 °C and monitored by TLC (pentane:Et_2O, 5:1) until completion (*ca*. 2 h). The cooled reaction was quenched with sat. aq. NH_4Cl solution (4 mL) and saturated with solid NaCl. The phases were separated and the aqueous layer extracted with EtOAc (5 × 5 mL), then the combined organic phases were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 2:1) to afford title compound **316** (59.1 mg, 66%) as a colourless oil.

R$_f$ 0.25 (pentane:Et_2O, 2:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.35 – 7.29 (m, 2H, H-Ar), 7.26 – 7.22 (m, 3H, H-Ar), 3.87 (t, *J* = 6.5 Hz, 2H, H-1), 2.88 (t, *J* = 6.5 Hz, 2H, H-2); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 138.6 (C-3), 129.2 (HC-Ar), 128.8 (HC-Ar), 126.7 (C-6) , 63.9 (C-1), 39.4 (C-2). Data consistent with literature.[316]

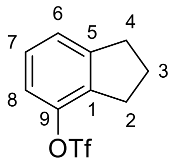
1-Oxo-2,3-dihydro-1*H*-inden-4-yl trifluoromethanesulfonate – 324



To a solution of **112** (500 mg, 3.37 mmol, 1 eq) in pyridine (1.3 mL) under N_2 at 0 °C was added trifluoromethanesulfonic anhydride (567 µL, 3.37 mmol, 1 eq). The reaction was stirred for 5 min at 0 °C, then warmed to rt and diluted with EtOAc (15 mL). The mixture was washed sequentially with water (10 mL), 1.0 M aq. HCl (10 mL), water (10 mL), and brine (10 mL), then the organic layer dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 5:1) to afford title compound **324** (624 mg, 66%) as a pale yellow oil.

R_f 0.30 (pentane:Et_2O, 8:1, stain: $KMnO_4$); **IR** ν_{max} 1726 (C=O), 1423 (C-H), 1212 (C-F), 1139 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.80 (dd, *J* = 6.0 Hz, 2.5 Hz, 1H, H-Ar), 7.52 – 7.49 (m, 2H, H-Ar), 3.25 (t, *J* = 6.5 Hz, 2H, H-2), 2.81 – 2.76 (m, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 204.5 (C-4), 147.3 (C-5), 146.5 (C-1), 140.8 (C-9), 129.8 (HC-Ar), 127.0 (HC-Ar), 124.0 (HC-Ar), 118.8 (q, *J* = 321 Hz, CF_3), 36.0 (C-3), 22.8 (C-2); **^{19}F NMR** ($CDCl_3$, 377 MHz,) δ_F -73.37; **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{10}H_8F_3O_4S$) requires *m/z* 281.0090, found *m/z* 281.0093.

2,3-Dihydro-1*H*-inden-4-yl trifluoromethanesulfonate – 326



To a solution of **265a** (124.2 mg, 0.93 mmol, 1 eq) in pyridine (372 μL) under N_2 at 0 °C was added trifluoromethanesulfonic anhydride (156 μL, 0.93 mmol, 1 eq). The reaction was stirred for 5 min at 0 °C, then warmed to rt and diluted with EtOAc (10 mL). The mixture was washed sequentially with water (10 mL), 1.0 M aq. HCl (10 mL), water (10 mL), and brine (10 mL), then the organic layer dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane) to afford title compound **326** (159.4 mg, 65%) as a colourless oil.

***R*_f_** 0.50 (pentane, stain: $KMnO_4$); **IR** ν_{max} 2962 (C-H), 1421 (C=C), 1211 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.25 – 7.17 (m, 2H, H-6 and H-7), 7.04 (d, J = 7.5 Hz, 1H, H-8), 3.07 – 2.96 (m, 4H, H-2 and H-4), 2.14 (p, J = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 148.7 (C-Ar), 146.5 (C-Ar), 136.7 (C-Ar), 128.3 (C-6 or C-7), 124.5 (C-6 or C-7), 118.7 (C-8), 117.3 (CF_3)*, 33.5 (C-2 or C-4), 30.2 (C-2 or C-4), 25.3 (C-3); **^{19}F NMR** ($CDCl_3$, 377 MHz,) δ_F -73.76; **LRMS** mass not found; **HRMS** (EI) exact mass calculated for $[M]^{+\bullet}$ ($C_{10}H_9F_3O_3S$) requires *m/z* 266.0224, found *m/z* 266.0225.

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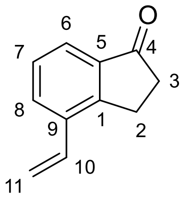
* Splitting with ^{19}F not visible in ^{13}C NMR spectrum.

4-Vinyl-2,3-dihydro-1*H*-inden-1-one – 327



A solution of **324** (300.0 mg, 1.07 mmol, 1.0 eq) was prepared in dry dioxane (4.3 mL) under N_2 at rt. Pre-dried anhydrous LiCl[*] (136.1 mg, 3.21 mmol, 3.0 eq) was quickly added, followed by freshly washed $Pd(PPh_3)_4$[†] (123.6 mg, 0.107 mmol, 0.1 eq), then vinyl tributylstannane (345 μL, 1.18 mmol, 1.1 eq). The reaction was heated to reflux for 16 h, cooled, and quenched with sat. aq. $NaHCO_3$ solution (4 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 4 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 8:1) to afford title compound **327** (99.7 mg, 59%) as a colourless oil.

R_f 0.25 (pentane:Et_2O, 5:1, stain: $KMnO_4$); **IR** ν_{max} 2923 (C-H), 1709 (C=O), 788 (=C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.73 (d, *J* = 7.5 Hz, 1H, H-8), 7.68 (d, *J* = 7.5 Hz, 1H, H-6), 7.38 (t, *J* = 7.5 Hz, 1H, H-7), 6.87 (dd, *J* = 17.5 Hz, 11.0 Hz, 1H, H-10), 5.82 (d, *J* = 17.5 Hz, 1H, H-11), 5.44 (d, *J* = 11.0 Hz, 1H, H-11), 3.20 – 3.13 (m, 2H, H-2), 2.74 – 2.69 (m, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 207.1 (C-4), 152.0 (C-1), 138.0 (C-5), 135.8 (C-9), 134.0 (C-10), 131.4 (C-8), 128.4 (C-7), 123.1 (C-6), 115.7 (C-11), 36.7 (C-3), 25.0 (C-2); **LRMS** mass not found; **HRMS** (APCI) exact mass calculated for $[M+H]^+$ ($C_{11}H_{11}O_2$) requires *m/z* 159.0804, found *m/z* 159.0804.

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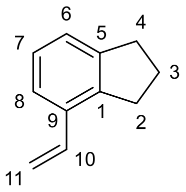
[*] The requisite amount of LiCl was dried under high vacuum at 100 °C for 6 h.

[†] A sample of $Pd(PPh_3)_4$ was washed with hot ethanol and dried under high vacuum at rt for 6 h. The requisite amount was then weighed under a stream of N_2.

4-Vinyl-2,3-dihydro-1*H*-indene – 329



A solution of **326** (103.6 mg, 0.39 mmol, 1.0 eq) was prepared in dry dioxane (1.6 mL) under N_2 at rt. Pre-dried anhydrous LiCl[*] (49.6 mg, 1.17 mmol, 3.0 eq) was quickly added, followed by freshly washed $Pd(PPh_3)_4$[†] (45.1 mg, 0.039 mmol, 0.1 eq), then vinyltributylstannane (126 µL, 0.43 mmol, 1.1 eq). The reaction was heated to reflux for 16 h, cooled, and quenched with sat. aq. $NaHCO_3$ solution (2 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 2 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane) to afford title compound **329** (100.0 mg, quant.) as a colourless oil.

Rf 0.65 (pentane, stain: $KMnO_4$); **IR** ν_{max} 2955 (C-H), 2918 (C-H), 2849 (C-H), 1463 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.34 – 7.29 (m, 1H, H-Ar), 7.17 – 7.12 (m, 2H, H-Ar), 6.82 (dd, *J* = 17.5 Hz, 11.0 Hz, 1H, H-10), 5.70 (dd, *J* = 17.5 Hz, 1.5 Hz, 1H, H-11_{trans}), 5.29 (dd, *J* = 11.0 Hz, 1.5 Hz, 1H, H-11_{cis}), 2.95 (ap. p, *J* = 7.5 Hz, 4H, H-2 and H-4), 2.09 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 144.8 (C-Ar), 142.3 (C-Ar), 135.3 (C-10), 133.9 (C-Ar), 126.6 (HC-Ar), 123.8 (HC-Ar), 122.9 (HC-Ar), 114.8 (C-11), 33.1 (C-2 or C4), 31.7 (C-2 or C-4), 25.1 (C-3); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{11}H_{13}$) requires *m/z* 145.1012, found *m/z* 145.1012.

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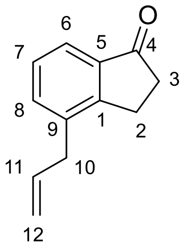
[*] The requisite amount of LiCl was dried under high vacuum at 100 °C for 6 h.

[†] A sample of $Pd(PPh_3)_4$ was washed with hot ethanol and dried under high vacuum at rt for 6 h. The requisite amount was then weighed under a stream of N_2.

4-Allyl-2,3-dihydro-1*H*-inden-1-one – 330



A solution of **324** (300.0 mg, 1.07 mmol, 1.0 eq) was prepared in dry dioxane (4.3 mL) under N_2 at rt. Pre-dried anhydrous LiCl[*] (136.1 mg, 3.21 mmol, 3.0 eq) was quickly added, followed by freshly washed $Pd(PPh_3)_4$[†] (123.6 mg, 0.107 mmol, 0.1 eq), then allyltributylstannane (366 μL, 1.18 mmol, 1.1 eq). The reaction was heated to reflux for 16 h, cooled, and quenched with sat. aq. $NaHCO_3$ solution (4 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 4 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 5:1) to afford title compound **330** (126.2 mg, 68%) as a yellow solid.

R_f 0.40 (pentane:Et_2O, 5:1, stain: $KMnO_4$); **IR** ν_{max} 1714 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.64 (d, *J* = 7.5 Hz, 1H, H-6), 7.42 (d, *J* = 7.5 Hz, 1H, H-8), 7.34 (t, *J* = 7.5 Hz, 1H, H-7), 5.96 (ddt, *J* = 17.0 Hz, 10.0 Hz, 6.5 Hz, 1H, H-11), 5.12 (dq, *J* = 10.0 Hz, 1.5 Hz, 1H, H-12), 5.06 (dq, *J* = 17.0 Hz, 1.5 Hz, 1H, H-12), 3.45 (d, *J* = 6.5 Hz, 2H, H-10), 3.09 – 3.04 (m, 2H, H-2), 2.72 – 2.67 (m, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 207.4 (C-4), 152.6 (C-Ar), 137.8 (C-Ar), 137.3 (C-Ar), 135.5 (C-11), 134.7 (C-8), 127.9 (C-7), 121.4 (C-6), 116.8 (C-12), 36.6 (C-10), 36.3 (C-3), 24.5 (C-2); **LRMS** mass not

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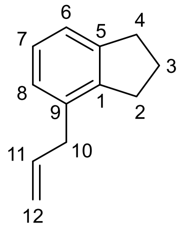
[*] The requisite amount of LiCl was dried under high vacuum at 100 °C for 6 h.

[†] A sample of $Pd(PPh_3)_4$ was washed with hot ethanol and dried under high vacuum at rt for 6 h. The requisite amount was then weighed under a stream of N_2.

found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{12}H_{13}O$) requires *m/z* 173.0961, found *m/z* 173.0963; **mp** 41 – 43 °C.

4-Allyl-2,3-dihydro-1*H*-indene – 332



A solution of **326** (100.0 mg, 0.38 mmol, 1.0 eq) was prepared in dry dioxane (1.5 mL) at rt. Pre-dried anhydrous LiCl[*] (48.3 mg, 1.14 mmol, 3.0 eq) was quickly added, followed by freshly washed $Pd(PPh_3)_4$[†] (43.9 mg, 0.038 mmol, 0.1 eq), then allyltributylstannane (130 µL, 0.42 mmol, 1.1 eq). The reaction was heated to reflux for 16 h, cooled, and quenched with sat. aq. $NaHCO_3$ solution (2 mL). The layers were separated and the aqueous layer extracted with Et_2O (3 × 2 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane) to afford title compound **332** (53.0 mg, 87%) as a yellow oil.

Rf 0.55 (pentane, stain: $KMnO_4$); **IR** ν_{max} 3078 (=C-H), 2956 (C-H), 2843 (C-H), 1638 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.11 (d, *J* = 4.5 Hz, 2H, H-6 and H-8), 6.97 (t, *J* = 4.5 Hz, 1H, H-7), 5.95 (ddt, *J* = 18.0 Hz, 9.0 Hz, 7.0 Hz, 1H, H-11), 5.06 (t, *J* = 1.5 Hz, 1H, H-12), 5.03 (dq, *J* = 7.0 Hz, 1.5 Hz, 1H, H-12), 3.36 (dt, *J* = 7.0 Hz, 1.5 Hz, 2H, H-10), 2.93 (t, *J* = 7.5 Hz, 2H, H-4), 2.87 (t, *J* = 7.5 Hz, 2H, H-2), 2.07 (p, *J* = 7.5 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 144.5 (C-Ar), 143.0 (C-Ar), 136.8 (C-11), 135.8 (C-9), 126.6 (C-6), 126.3 (C-7), 122.4 (C-8), 115.7 (C-12), 38.2 (C-10), 33.2 (C-4), 31.3 (C-2), 25.0 (C-3); **LRMS** mass not found; **HRMS** mass not found.

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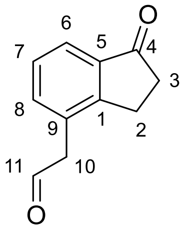
[*] The requisite amount of LiCl was dried under high vacuum at 100 °C for 6 h.

[†] A sample of $Pd(PPh_3)_4$ was washed with hot ethanol and dried under high vacuum at rt for 6 h. The requisite amount was then weighed under a stream of N_2.

2-(1-Oxo-2,3-dihydro-1*H*-inden-4-yl)acetaldehyde – 333



In a two-necked flask, a solution of **330** (250 mg, 1.45 mmol, 1 eq) in DCM (29 mL) was prepared. A spatula tip of sudan red was added and the mixture gently swirled until the solution was a uniform pink colour. On one neck was placed a thermometer adapter fitted with a glass pipette which reached below the level of the solution (to allow bubbling of gas through the solution). To the second neck was attached an outlet tube passing through a bubbler containing a 1.0 M aq. solution of KI. The solution was cooled to –78 °C and the end of the pipette fitted to the ozoniser.* O_2 was bubbled through the solution at a flow rate of 80 mL/min for 5 min, after which time the ozoniser was switched on and O_3 was bubbled through the solution (also at 80 mL/min) until a colour change to pale yellow was observed (3 min). The ozoniser was switched off and O_2 bubbled through the solution for a further 5 min to remove all traces of O_3. The reaction was quenched with dimethyl sulphide (532 µL, 7.25 mmol, 5 eq) at –78 °C and then the reaction mixture allowed to warm to rt. This mixture was stirred for 24 h at rt, then the solvent was removed *in vacuo*. The crude product was purified by FCC (pentane:EtOAc, 2:1) to afford title compound **333** (76 mg, 30%) as a yellow oil.

***R**$_f$* 0.10 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **IR** ν_{max} 1711 (C=O) (C-11), 1686 (C=O) (C-4), 1584 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 9.79 (t, *J* = 2.0 Hz, 1H, H-11), 7.72 (d, *J* = 7.5 Hz, 1H, H-6), 7.45 (d, *J* = 7.5 Hz, 1H, H-8), 7.40 (t, *J* = 7.5 Hz, 1H, H-7), 3.82 (d, *J* = 2.0 Hz, 2H, H-10), 3.05 – 2.99 (m, 2H, H-2), 2.73 – 2.68 (m, 2H, H-3); **^{13}C NMR**

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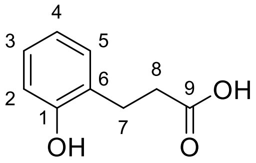
* Wallace and Tiernan, Type 023012 ozoniser. Set to 220 – 240 V and 50 Hz.

($CDCl_3$, 101 MHz) δ_C 206.7 (C-4), 197.7 (C-11), 154.6 (C-1), 137.8 (C-5), 135.9 (C-8), 130.3 (C-9), 128.2 (C-7), 123.2 (C-6), 47.3 (C-10), 36.2 (C-3), 24.7 (C-2); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{11}H_{11}O_2$) requires *m/z* 175.0754, found *m/z* 175.0755;

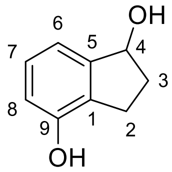
3-(2-Hydroxyphenyl)propanoic acid – 335



335 was isolated as the unexpected major product in an attempted Fries rearrangement of dihydrocoumarin. Dihydrocoumarin (1 mL, 7.9 mmol, 1 eq) was fused with $AlCl_3$ (2.1 g, 15.8 mmol, 2 eq) under N_2 by heating to 180 °C for 3h. The reaction mixture was cooled to rt, treated with cold conc. HCl (30 mL), and the organic component extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with $NaHCO_3$ (150 mL), then 1.0 M aq. NaOH (150 mL) and put aside. The combined aqueous layers were carefully acidified and re-extracted with Et_2O (3 × 300 mL). The organic layers from both extractions were combined and dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 2:1) to afford title compound **335** (515 mg, 39%) as an off-white powder.

R_f 0.25 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.25 (t, *J* = 8.0 Hz, 1H, H-Ar), 7.20 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.10 (td, *J* = 8.0 Hz, 1.0 Hz, 1H, H-Ar), 7.05 (dd, *J* = 8.0 Hz, 1.0 Hz, 1H, H-Ar), 3.01 (t, *J* = 7.5 Hz, 2H, H-7), 2.82 – 2.76 (m, 2H, H-8); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 167.7 (C-9), 153.0 (C-1), 128.4 (HC-Ar), 126.8 (HC-Ar), 125.1 (HC-Ar), 123.3 (C-6), 117.8 (HC-Ar), 29.4 (C-8), 23.9 (C-7); **mp** 74 – 75 °C, lit. 81 – 83 °C. Data consistent with literature.[318]

2,3-Dihydro-1*H*-indene-1,4-diol – 336



Compound **336** was the unexpected product obtained from the partial reduction of **112**. To a solution of **112** (100 mg, 0.67 mmol, 1.00 eq) and TEA (5 μL, 0.034 mmol, 0.05 eq) in ethanol (13.4 mL) under N_2 was quickly added Pd/C (67 mg, 0.63 mmol, 0.95 eq). The flask was purged with H_2 and stirred at 50 °C under H_2 for 24 h. The reaction was filtered through Celite® with ethanol and concentrated *in vacuo* to afford title compound **336** (100 mg, quant.) as a sticky pinkish solid.

R_f 0.40 (Et_2O, stain: $KMnO_4$); **IR** ν_{max} 3316 (O-H), 2961 (C-H), 1594 (C=C), 1469 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.14 (t, *J* = 7.5 Hz, 1H, H-7), 7.01 (d, *J* = 7.5 Hz, 1H, H-6), 6.73 (d, *J* = 7.5 Hz, 1H, H-8), 5.25 (dd, *J* = 7.0 Hz, 5.0 Hz, 1H, H-4’), 3.01 (ddd, *J* = 15.5 Hz , 8.5 Hz, 5.0 Hz, 1H, H-2’), 2.75 (ddd, *J* = 15.5 Hz, 8.5 Hz, 6.0 Hz, 1H, H-2), 2.51 (dddd, *J* = 13.5 Hz, 8.5 Hz, 7.0 Hz, 5.0 Hz, 1H, H-3’), 1.99 (dddd, *J* = 13.5 Hz, 8.5 Hz, 6.0 Hz, 5.0 Hz, 1H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 152.0 (C-9), 147.5 (C-5), 129.0 (C-1), 128.7 (C-7), 116.6 (C-6), 114.8 (C-8), 76.5 (C-4)[*] 33.5 (C-3), 27.0 (C-2); **LRMS** mass not found; **HRMS** mass not found. **mp** 58 – 60 °C.

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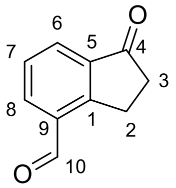
[*] Peak under $CDCl_3$ but visible by HSQC.

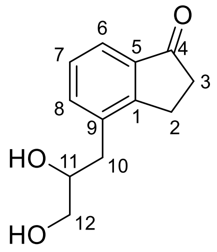
1-Oxo-2,3-dihydro-1*H*-indene-4-carbaldehyde – 337



To a solution of **338** (60 mg, 0.29 mmol, 1 eq) in 3:1 THF:water (9.7 mL) was added $NaIO_4$ (124 mg, 0.58 mmol, 2 eq) and the reaction mixture stirred at rt for 2 h. The mixture was concentrated *in vacuo* and the residue diluted with Et_2O (5 mL). The layers were separated and the aqueous phase extracted with Et_2O (3 × 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 4:1) to afford title compound **337** (46 mg, quant.) as a cloudy oil.

R_f 0.10 (pentane:Et_2O, 4:1, stain: $KMnO_4$); **IR** ν_{max} 2922 (C-H), 1709 (C=O) (C-10), 1686 (C=O) (C-4), 1583 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 10.25 (s, 1H, H-10), 8.07 (dd, J = 7.5 Hz, 1.0 Hz, 1H, H-8), 8.01 (dd, J = 7.5 Hz, 1.0 Hz, 1H, H-6), 7.61 (tt, J = 7.5 Hz, 1.0 Hz, 1H, H-7), 3.56 – 3.51 (m, 2H, H-2), 2.80 – 2.75 (m, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 206.1 (C-4), 191.8 (C10), 156.1 (C-1), 138.7 (C-5), 138.3 (C-8), 133.8 (C-9), 129.2 (C-6), 128.2 (C-7), 36.3 (C-3), 26.1 (C-2); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{10}H_9O_2$) requires *m/z* 161.0597, found *m/z* 161.0597.

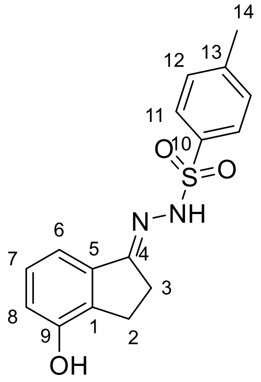
4-(2,3-Dihydroxypropyl)-2,3-dihydro-1*H*-inden-1-one – 338



A solution of **330** (50 mg, 0.29 mmol, 1.0 eq) in 1:1:1 THF:water:acetone (1.3 mL) was prepared at rt. 4-Methylmorpholine *N*-oxide (45 mg, 0.38 mmol, 1.3 eq) was added, then OsO_4 (120 µL, 2.5 % in *t*-BuOH, 0.010 mmol, 0.02 eq) was added and the reaction mixture stirred at rt for 5 h. The reaction mixture was diluted with brine (5 mL) and the organic component extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **338** (60 mg, quant.) as a pale-yellow oil which was used without further purification.

Rf 0.05 (Et_2O, stain: $KMnO_4$); **IR** ν_{max} 3410 (O-H), 1696 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.66 (dd, *J* = 7.5 Hz, 1.0 Hz, 1H, H-6), 7.49 (dd, *J* = 7.5 Hz, 1.0 Hz, 1H, H-8), 7.36 (t, *J* = 7.5 Hz, 1H, H-7), 4.08 – 4.00 (m, 1H, H-11), 3.76 (dd, *J* = 11.0 Hz, 3.0 Hz, 1H, H-12), 3.57 (dd, *J* = 11.0 Hz, 7.0 Hz, 1H, H-12), 3.19 – 3.06 (m, 2H, H-2), 2.90 – 2.85 (m, 2H, H-10), 2.70 (t, *J* = 6.0 Hz, 2H, H-3); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 207.3 (C-4), 154.5 (C-1), 137.5 (C-5), 136.2 (C-9), 135.5 (C-8), 127.9 (C-7), 122.3 (C-6), 72.2 (C-11), 66.3 (C-12), 36.3 (C-3), 35.8 (C-10), 24.8 (C-2); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{12}H_{15}O_3$) requires *m/z* 207.1016, found *m/z* 207.1019.

(*E*)-*N*'-(4-Hydroxy-2,3-dihydro-1*H*-inden-1-ylidene)-4-methylbenzenesulfonohydrazide – 404



A solution of **112** (100 mg, 0.67 mmol, 1.0 eq) and *p*-toluenesulfonyl hydrazide (138 mg, 0.74 mmol, 1.1 eq) was prepared in ethanol (0.67 mL) under N_2. The mixture was heated to reflux and stirred for 30 min, then left standing at rt for 18 h. The resulting crystalline precipitate was filtered, washed with cold ethanol (10 mL), and dried under high vacuum to afford title compound **404** (211 mg, quant.) as a brown sludge which was used without further purification.

R

R$_f$ 0.40 (Et_2O, stain: $KMnO_4$); **IR** ν_{max} 3480 (O-H), 3217 (N-H), 1596 (C=C), 1163 (C-O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.90 (d, *J* = 8.5 Hz, 2H, H-11) 7.36 (d, *J* = 8.0 Hz, 1H, H-6), 7.32 – 7.27 (m, 2H, H-12), 7.12 (t, *J* = 8.0 Hz, 1H, H-7), 6.77 (d, *J* = 8.0 Hz, 1H, H-8), 2.99 – 2.93 (m, 2H, H-2 or H-3), 2.68 – 2.62 (m, 2H, H-2 or H-3), 2.40 (s, 3H, H-14); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 152.7 (C-4 or C-9), 144.4 (C-4 or C-9), 135.4 (C-Ar), 130.1 (C-Ar), 129.7 (C-6), 128.9 (C-7), 128.4 (C-Ar), 128.3 (C-Ar), 128.2 (C-11), 117.1 (C-8), 114.8 (C-12), 26.9 (C-2 or C-3), 25.1 (C-2 or C-3), 21.7 (C-14);* **LRMS** (ES+) mass found *m/z* 317.1 $[M+H]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{16}H_{17}N_2O_3S$) requires *m/z* 317.0954, found *m/z* 317.0955.1;

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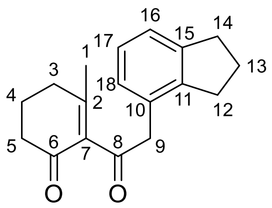
* Crude sample contains impurities.

Compounds from Chapter 4

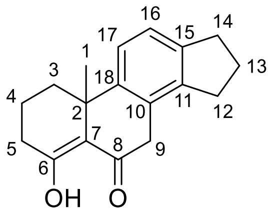
2-(2-(2,3-Dihydro-1*H*-inden-4-yl)acetyl)-3-methylcyclohex-2-en-1-one – 259a



To a solution of **210a** (45.2 mg, 0.14 mmol, 1.0 eq) in dry DCM (0.7 mL) under N_2 at 0 °C was added Dess-Martin periodinane (76.3 mg, 0.18 mmol, 1.25 eq). The reaction was allowed to warm to rt and its progress followed by TLC (pentane:Et_2O, 1:1). The reaction mixture was quenched with isopropanol (3 mL) after 2 h and the mixture evaporated to dryness. The crude residue was re-dissolved in Et_2O (5 mL) and washed successively with sat. aq. solutions of 1:1 $NaHCO_3$/$Na_2S_2O_3$ (5 mL), $NaHCO_3$ (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to afford title compound **259a** (32.3 mg, 86%) as a pale-yellow oil which was used without further purification.

***R**$_f$* 0.30 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **IR** ν_{max} 2923 (C-H), 1699 (C=O), 1664 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.12 (t, *J* = 7.0 Hz, 1H, H-17), 7.07 (d, *J* = 7.0 Hz, 1H, H-16), 6.93 (d, *J* = 7.0 Hz, 1H, H-18), 3.93 (s, 2H, H-9), 2.92 (t, *J* = 7.5 Hz, 2H, H-14), 2.85 (t, *J* = 7.5 Hz, 2H, H-12), 2.41 (t, *J* = 6.0 Hz, 2H, H-5), 2.31 (t, *J* = 6.0 Hz, 2H, H-3), 2.04 (p, *J* = 7.5 Hz, 2H, H-13), 1.96 (p, *J* = 6.0 Hz, 2H, H-4), 1.71 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 203.5 (C-8), 197.1 (C-6), 160.7 (C-2), 144.6 (C-15), 144.2 (C-11), 139.1 (C-7), 129.8 (C-10), 128.0 (C-18), 126.5 (C-16), 123.4 (C-17), 49.3 (C-9), 37.5 (C-5), 33.3 (C-14), 32.3 (C-3), 31.8 (C-12), 25.1 (C-13), 21.9 (C-4), 21.7 (C-1); **LRMS** (ES+) mass found *m/z* 291.1 $[M+Na]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{18}H_{21}O_2$) requires *m/z* 269.1536, found *m/z* 269.1537.

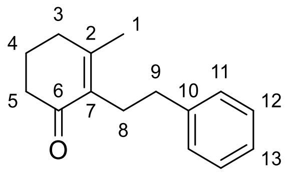
4-Hydroxy-10-methyl-1,2,3,7,10,15,16,17-octahydro-6*H*-cyclopenta[a]phenanthren-6-one – 260a



A mixture of AgOTf (2.6 mg, 0.01 mmol, 0.1 eq) and $RuCl_3$•$3H_2O$ (1.0 mg, 0.005 mmol, 0.05 eq) was stirred in DCE (150 µL) under N_2 at rt for 1 h. A solution of **259a** (27.6 mg, 0.10 mmol, 1.0 eq) in DCE (350 µL) was added dropwise and the reaction heated to 80 °C for 14 h. The cooled reaction was purified by FCC (pentane:Et_2O, 10:1) to afford title compound **260a** (13.9 mg, 52%) as a colourless oil.

***R**f* 0.50 (pentane:Et_2O, 10:1, stain: $KMnO_4$); **IR** ν_{max} 2952 (C-H), 1604 (C=O), 1411 (C=C); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 7.16 (ap. q, *J* = 8.0 Hz, 2H, H-16 and H-17), 3.69 (d, *J* = 20.0 Hz, 1H, H-9), 3.60 (d, *J* = 20.0 Hz, 1H, H-9), 2.99 – 2.90 (m, 2H, H-14), 2.90 – 2.81 (m, 2H, H-12), 2.51 – 2.36 (m, 3H, H-3 and H-5), 2.11 (p, *J* = 7.5 Hz, 2H, H-13), 2.05 – 1.97 (m, 2H, H-4), 1.88 (td, *J* = 13.0 Hz, 4.5 Hz, 1H, H-3), 1.30 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 195.6 (C-8), 184.8 (C-6), 143.3 (C-18), 142.5 (C-12 or C-14), 142.3 (C-12 or C-14), 128.0 (C-10), 123.0 (C-16), 121.5 (C-17), 112.5 (C-7), 38.1 (C-9), 37.7 (C-2), 34.9 (C-3), 32.9 (C-14), 31.6 (C-5), 31.4 (C-12), 29.8 (C-1), 25.2 (C-13), 17.9 (C-4); **LRMS** (ES+) mass found 269.2 $[M+H]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{18}H_{21}O_2$) requires *m/z* 269.1537, found *m/z* 269.1537.

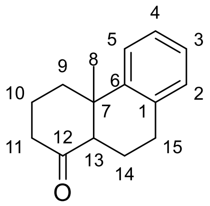
3-Methyl-2-phenethylcyclohex-2-en-1-one – 347



To a solution of **350** (111.5 mg, 0.53 mmol, 1.0 eq) and **405** (238.7 mg, 0.80 mmol, 1.5 eq) in THF (13.3 mL) under N_2 was added TEA (223 µL, 1.60 mmol, 3.0 eq) dropwise and the reaction heated to reflux for 2 h. After this time, the reaction was cooled, concentrated *in vacuo*, and the crude product purified by FCC (pentane:Et_2O, 8:1) to afford title compound **347** (49.1 mg, 43%) as a yellow oil.

Rf 0.15 (pentane:Et_2O, 8:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.29 – 7.23 (m, 2H, H-Ar), 7.20 – 7.16 (m, 3H, H-Ar), 2.66 – 2.54 (m, 4H, H-8 and H-9), 2.39 (t, *J* = 6.5 Hz, 2H, H-5), 2.29 (t, *J* = 6.5 Hz, 2H, H-3), 1.93 (p, *J* = 6.5 Hz, 2H, H-4), 1.74 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 198.8 (C-6), 156.2 (C-2), 142.4 (C-10), 134.8 (C-7), 128.8 (HC-Ar), 128.3 (HC-Ar), 125.9 (HC-Ar), 38.1 (C-5), 35.3 (C-9), 33.0 (C-3), 27.7 (C-8), 22.5 (C-4), 21.2 (C-1). Data consistent with literature.[319]

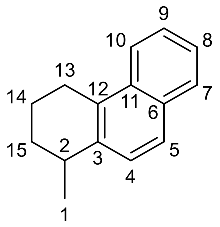
4a-Methyl-3,4,4a,9,10,10a-hexahydrophenanthren-1(2*H*)-one – 348



Compound **347** (36.8 mg, 0.17 mmol, 1 eq) and 85% H_3PO_4 (56 µL, 0.85 mmol, 5 eq) were heated together at 170 °C for 12 h. The cooled mixture was extracted with Et_2O (3 × 5 mL) and the combined organic phases washed with water (15 mL), then sat. aq. $NaHCO_3$ solution (15 mL). The organic phase was then dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* and the crude product purified by FCC (pentane to pentane:Et_2O, 20:1) to afford title compound **348** (9.5 mg, 26%) as a mixture of diastereomers as a pale-yellow oil.

Rf 0.15 (pentane:Et_2O, 40:1, stain: $KMnO_4$); **IR** ν_{max} 2931 (C-H), 1708 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.26 (d, *J* = 7.5 Hz, 1H, H-5), 7.16 (t, *J* = 7.5 Hz, 1H, H-3), 7.11 (t, *J* = 7.5 Hz, 1H, H-4), 7.06 (d, *J* = 7.5 Hz, 1H, H-2), 2.96 (t, *J* = 6.0 Hz, 0.4H, H-15), 2.93 (t, *J* = 6.0 Hz, 0.6H, H-15), 2.82 – 2.76 (m, 1H, H-15), 2.44 – 2.40 (m, 1H, H-13), 2.38 (dd, *J* = 9.0 Hz, 6.0 Hz, 1H, H-11), 2.33 – 2.29 (m, 1H, H-11), 2.29 – 2.23 (m, 1H, H-9), 2.23 – 2.16 (m, 1H, H-14), 1.93 – 1.85 (m, 2H, H-10 and H-14), 1.84 – 1.73 (m, 2H, H-9 and H-10), 1.36 (s, 3H, H-8); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 213.8 (C-12), 143.3 (C-6), 135.7 (C-1), 129.5 (C-2), 126.3 (C-3), 126.1 (C-4), 125.9 (C-5), 57.2 (C-13), 40.8 (C-7), 39.3 (C-11), 36.8 (C-9), 29.8 (C-8), 28.4 (C-15), 22.1 (C-14), 21.8 (C-10); **LRMS** mass not found; **HRMS** mass not found;

1-Methyl-1,2,3,4-tetrahydrophenanthrene – 352



Compound **352** (2.7 mg, 8%) was isolated as a pale-yellow oil as a major side product in the reported preparation of **348**.

R R_f 0.55 (hexane, stain: $KMnO_4$); **IR** ν_{max} 2926 (C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.98 (d, *J* = 8.5 Hz, 1H, H-Ar), 7.78 (d, *J* = 8.0 Hz, 1H, H-Ar), 7.63 (t, *J* = 8.5 Hz, 1H, H-Ar), 7.53 – 7.44 (m, 1H, H-Ar), 7.44 – 7.37 (m, 1H, H-Ar), 7.33 (d, *J* = 8.5 Hz, 1H, H-Ar), 3.34 – 3.23 (m, 2H), 2.96 – 2.88 (m, 1H, H-2), 1.96 – 1.89 (m, 2H), 1.76 – 1.65 (m, 2H), 1.36 (d, *J* = 7.0 Hz, 3H, H-1);[*] **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 128.5, 127.5, 125.9, 124.9, 124.5, 123.2, (6 × HC-Ar), 36.8, (C-2) 32.6, 27.8, 27.0, (3 × CH_2) 23.0 (C-1);[†] **LRMS** mass not found; **HRMS** mass not found.

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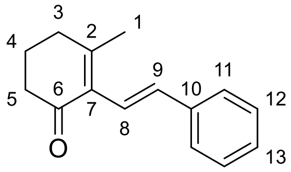
[*] Complete assignments of all peaks could not be confirmed from available data.
[†] Quaternary carbons not visible in obtained ^{13}C spectrum.

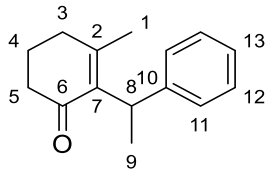
(*E*)-3-Methyl-2-styrylcyclohex-2-en-1-one – 350



To a solution of tri(*o*-tolyl)phosphine (19.5 mg, 0.064 mmol, 0.04 eq) in toluene (2 mL) under N_2 was added $Pd(OAc)_2$ (7.2 mg, 0.032 mmol, 0.02 eq). To this solution were sequentially added: a solution of **214** (373.5 mg, 1.60 mmol, 1.0 eq) in toluene (3 mL); DIPEA (331 μL, 1.90 mmol, 1.2 eq); then a solution of styrene (184 μL, 1.60 mmol, 1.0 eq) in toluene (3 mL). The reaction mixture was heated to reflux and stirred for 18 h. Once cooled, the reaction was diluted with EtOAc (40 mL), filtered through Celite®, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 8:1) to afford title compound **350** (246.2 mg, 73%) as a yellow oil.

R_f 0.10 (pentane:Et_2O, 10:1, stain: vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.45 (d, *J* = 7.5 Hz, 2H, H-11), 7.32 (t, *J* = 7.5 Hz, 2H, H-12), 7.25 – 7.20 (m, 1H, H-13), 6.97 (d, *J* = 16.5 Hz, 1H, H-9), 6.87 (d, *J* = 16.5 Hz, 1H, H-8), 2.48 (t, *J* = 6.5 Hz, 4H, H-3 and H-5), 2.13 (s, 3H, H-1), 1.99 (p, *J* = 6.5 Hz, 2H, H-4); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 199.0 (C-6), 157.2 (C-2), 138.0 (C-10), 134.2 (C-9), 132.9 (C-7), 128.7 (C-12), 127.6 (C-13), 126.6 (C-11), 122.0 (C-8), 38.7 (C-3 or 5), 34.9 (C-3 or 5), 24.5 (C-1), 22.0 (C-4). Data consistent with literature.[320]

3-Methyl-2-(1-phenylethyl)cyclohex-2-en-1-one – 351

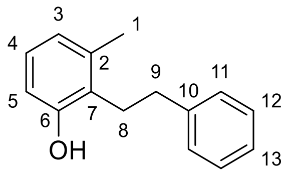


Compound **351** was obtained as the unexpected major product in the attempted Suzuki coupling of styrene and **214** following a reported procedure.[275]

To a solution of styrene (53 µL, 0.46 mmol, 1.1 eq) in THF (2.5 mL) under N_2 at rt was added 9-BBN (1.8 mL, 0.5 M in THF, 0.92 mmol, 2.2 eq) and the reaction mixture stirred at 50 °C for 2 h. A separate flask, fitted with a reflux condenser and under N_2, was charged with $PdCl_2(dppf)_2$•DCM (10.6 mg, 0.013 mmol, 0.03eq) and to this were added the former reaction solution, a solution of **214** (100.0 mg, 0.42 mmol, 1.0 eq) in THF (2.1 mL), and 3.0 M aq. NaOH (0.42 mL). The mixture was heated to reflux for 16 h, then cooled and diluted with Et_2O (10 mL). The phases were separated and the aqueous phase extracted with Et_2O (3 × 15 mL) and the combined organic phases washed with brine (40 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 8:1) to afford title compound **351** (42.0 mg, 43%) as a colourless oil.

R_f 0.25 (pentane:Et_2O, 8:1, stain: $KMnO_4$); **IR** ν_{max} 2934 (C-H), 1660 (C=O), 1620 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.27 – 7.20 (m, 4H, H-11 and H-12), 7.15 – 7.11 (m, 1H, H-13), 4.46 (q, J = 7.0 Hz, 1H, H-8), 2.43 – 2.38 (m, 2H, H-5), 2.36 – 2.32 (m, 2H, H-3), 1.94 (p, J = 6.5 Hz, 2H, H-4), 1.78 (s, 3H, H-1), 1.50 (d, J = 7.0 Hz, 3H, H-9); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 198.4 (C-6), 156.5 (C-2), 145.4 (C-10), 140.1 (C-7), 128.1 (C-12), 127.1 (C-11), 125.4 (C-13), 38.5 (C-5), 34.6 (C-8), 34.0 (C-3), 22.3 (C-4), 21.9 (C-1), 17.4 (C-9); **LRMS** (ES+) mass found *m/z* 215.1 $[M+H]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{15}H_{19}O_X$) requires *m/z* 215.1430, found *m/z* 215.1430.

3-Methyl-2-phenethylphenol – 356

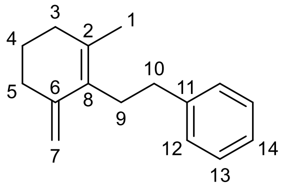


Compound **356** was obtained as an unexpected product from the attempted thermal cyclisation of **347**.

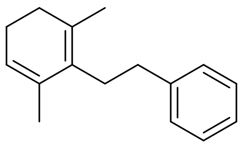
A solution of **347** (50.0 mg, 0.24 mmol, 1 eq) in dry toluene (240 µL) under Ar was heated to 200 °C in a pressure tube for 24 h. Once cooled, the solvent was removed *in vacuo* and the crude product purified by FCC (pentane:Et_2O 10:1) to afford title compound **356** (4.6 mg, 9%) as a pale-brown oil.

R_f 0.30 (pentane:Et_2O, 8:1, stain: $KMnO_4$); **IR** ν_{max} 3418 (O-H), 1468 (C=C); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.30 (t, *J* = 7.5 Hz, 3H, H-Ar), 7.22 (d, *J* = 7.5 Hz, 2H, H-Ar), 6.98 (t, *J* = 8.0 Hz, 1H, H-4), 6.76 (d, *J* = 8.0 Hz, 1H, H-3), 6.62 (d, *J* = 8.0 Hz, 1H, H-5), 4.53 (s, 1H, OH), 2.96 – 2.90 (m, 2H, H-8), 2.85 – 2.79 (m, 2H, H-9), 2.26 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 153.8 (C-6), 142.4 (C-10), 138.1 (C-2), 128.6 (HC-Ar), 126.7 (C-4), 126.7 (HC-Ar), 126.1 (HC-Ar), 123.0 (C-3), 113.2 (C-5), 35.4 (C-8), 29.0 (C-9), 19.5 (C-1); **LRMS** mass not found; **HRMS** mass not found.

(2-(2-Methyl-6-methylenecyclohex-1-en-1-yl)ethyl)benzene – 361a and (2-(2,6-dimethylcyclohexa-1,5-dien-1-yl)ethyl)benzene – 361b



a



b

To a solution of **347** (52.2 mg, 0.24 mmol, 1.0 eq) in Et_2O (0.96 mL) under N_2 at 0 °C was added MeMgBr (97 µL, 3.0 M in Et_2O, 0.29 mmol, 1.2 eq) and the reaction stirred at 0 °C for 20 h. The reaction was then poured into a 1:1 mixture of Et_2O:1.0 M aq. HCl (10 mL), the layers separated, and the aqueous layer extracted with Et_2O (3 × 10 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to give title compounds **361a** and **361b** (47.0 mg, 92%) in a 1:1 ratio as a colourless oil which was used without further purification.*

***R*$_f$** 0.55 (pentane, stain: $KMnO_4$); **IR** ν_{max} 3026 (C-H), 2929 (C-H), 1453 (C=C), 699 (=C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.31 – 7.26 (m, 2H), 7.22 – 7.18 (m, 3H), 5.52 (s, 0.5 H), 4.95 (s, 0.5 H), 4.75 (s, 0.5 H), 2.71 – 2.66 (m, 1H), 2.64 – 2.59 (m, 1H), 2.58 – 2.53 (m, 1H), 2.50 – 2.44 (m, 1H), 2.38 – 2.32 (m, 1H), 2.11 (t, *J* = 6.5 Hz, 1H), 2.01 (d, *J* = 2.0 Hz, 2.2H), 1.85 (d, *J* = 1.5 Hz, 1.7H), 1.69 (s, 2.2H), 1.66 (s, 1.4H); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 144.3, 142.9, 142.5, 134.9, 133.8, 130.5, 130.3, 128.6, 128.6, 128.4, 125.9, 125.8, 120.9, 105.9, 36.3, 35.3, 33.5, 33.4, 30.5, 30.5, 30.4, 23.5, 22.9, 20.4, 20.2, 19.6; **LRMS** mas not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{16}H_{21}$) requires *m/z* 213.1638, found *m/z* 213.1639.

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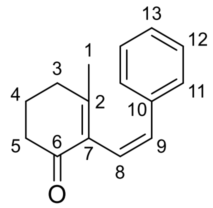
[*] Ratio determined from ^{1}H NMR resonances at 5.52 ppm (H-5_b) and resonances at 4.95 and 4.75 ppm (H-7_a).

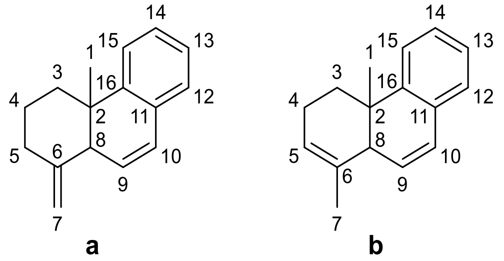
(*Z*)-3-Methyl-2-styrylcyclohex-2-en-1-one – 362



A solution of **350** (100 mg, 0.47 mmol, 1 eq) was prepared in dry acetonitrile (9.4 mL) in a PenRay photochemical vessel fitted to an Ar line with an exit bubbler attached. Ar was bubbled through the solution (*via* needle) for 10 minutes. The photochemical vessel was immersed in a foil-lined ice bath and irradiated at 254 nm at 0 °C under a constant stream of Ar for 20 h. After this time, the light source was switched off and the solution concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 10:1 to 8:1) to afford title compound **362** (22.4 mg, 22%) as a yellow oil.

Rf 0.15 (pentane:Et_2O, 8:1, stain: $KMnO_4$); **IR** ν_{max} 2922 (C-H), 1664 (C=O), 697 (=C-H); **^{1}H NMR** ($CDCl_3$, 500 MHz) δ_H 7.26 – 7.21 (m, 2H, H-11 or H-12), 7.20 – 7.14 (m, 3H, H-11 or H-12 and H-13), 6.66 (d, *J* = 12.0 Hz, 1H, H-9), 6.20 (d, *J* = 12.0 Hz, 1H, H-8), 2.47 (t, *J* = 6.5 Hz, 2H, H-5), 2.31 (t, *J* = 6.5 Hz, 2H, H-3), 1.99 (p, *J* = 6.5 Hz, 2H, H-4), 1.64 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 198.7 (C-6), 157.4 (C-2), 138.0 (C-10), 133.6 (C-7), 133.1 (C-9), 128.3 (C-11 or C-12), 128.3 (C-11 or C-12), 127.2 (C-13), 125.0 (C-8), 38.1 (C-5), 32.6 (C-3), 22.5 (C-1), 22.1 (C-4); **LRMS** (ES+) mass found *m/z* 235.1 $[M+Na]^+$; **HRMS** (APCI) exact mass calculated for $[M+H]^+$ ($C_{15}H_{17}O$) requires *m/z* 213.1274, found *m/z* 213.1275;

4a-Methyl-1-methylene-1,2,3,4,4a,10a-hexahydrophenanthrene – 365a and 1,4a-Dimethyl-3,4,4a,10a-tetrahydrophenanthrene – 365b



A solution of **362** (18.2 mg, 0.086 mmol, 1.0 eq) was prepared in dry Et_2O (172 µL) under N_2 and cooled to 0 °C. Methylmagnesium bromide (32 µL, 3.0 M in Et_2O, 0.095 mmol, 1.1 eq) was added dropwise and the mixture stirred at 0 °C for 2 h. After this time, the solution was warmed to rt, poured into a mixture of Et_2O (5 mL) and 3.0 M aq. HCl (5 mL), and allowed to stir at rt for a further 30 min. The layers were separated and the aqueous phase extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane to pentane:Et_2O, 5:1) to afford title compounds **365a** and **365b** (15.2 mg, 84%) as a colourless oil as a 1:1.25 mixture of alkenes **a**:**b**.*

R$_f$ 0.80 (pentane, stain: $KMnO_4$); **IR** ν_{max} 3024 (C-H), 2927 (C-H), 1447 (C=C), 693 (=C-H); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 7.35 (d, J = 7.0 Hz, 1.8H, H-Ar), 7.32 (d, J = 7.0 Hz, 1.4H, H-Ar), 7.26 – 7.21 (m, 2.6H, H-Ar), 7.18 (t, J = 7.0 Hz, 1.4H, H-Ar), 6.51 (d, J = 12.0 Hz, 0.8H, H-10_a), 6.45 (d, J = 12.0 Hz, 1H, H-10_b), 6.18 (d, J = 12.0 Hz, 1H, H-9_b), 6.11 (d, J = 12.0 Hz, 0.8H, H-9_a), 5.57 (s, 1H, 5_b), 4.93 (s, 0.8H, H-7_a), 4.70 (s, 0.8H, H-7_a), 2.40 (s, 1.6H, H-5_a), 2.14 – 2.08 (m, 3.6H, 1 × H-4_a and H-8_a and 2 × H-4_b), 2.08 – 2.03 (m, 1.8H, 1 × H-4_a and H-8_b), 1.75 (t, J = 6.5 Hz, 3.6H, H-3), 1.68 (d, J = 2.0 Hz,

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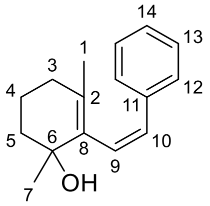
[*] Ratio determined from H-1 methyl peaks at 1.63 ppm (H-1_b) and 1.55 ppm (H-1_a).

3H, H-7$_b$), 1.63 (s, 3H, H-1$_b$), 1.55 (s, 2.4H, H-1$_a$); **^{13}C NMR** (CDCl$_3$, 151 MHz) δ_C 131.3 (C-10$_a$), 130.8 (C-10$_b$), 128.9 (C-9$_a$), 128.6 (C-9$_b$), 128.4 (HC-Ar), 128.2 (HC-Ar), 128.2 (HC-Ar), 126.9 (HC-Ar), 120.0 (C-5$_b$), 108.4 (C-7$_a$), 32.5, 32.3 (C-5$_a$), 30.0, 23.2, 22.7, 21.3 (C-1$_a$), 20.7 (C-7$_b$), 20.7 (C-1$_b$);* **LRMS** mass not found; **HRMS** mass not found.

(*Z*)-1,3-Dimethyl-2-styrylcyclohex-2-en-1-ol – 366



Alcohol **366** (4.7 mg, 24%) was isolated as a colourless oil as a major side product during the preparation of compounds **365a** and **365b**.

R$_f$ 0.25 (pentane:Et$_2$O, 5:1, stain: KMnO$_4$); **IR** ν_{max} 2923 (C-H); **^{1}H NMR** (CDCl$_3$, 400 MHz) δ_H 7.46 – 7.37 (m, 2H, H-Ar), 7.24 – 7.15 (m, 3H, H-Ar), 6.55 (d, *J* = 12.5 Hz, 1H, H-9 or H-10), 6.21 (d, *J* = 12.5 Hz, 1H, H-9 or H-10), 2.03 – 1.94 (m, 2H), 1.91 – 1.75 (m, 2H), 1.71 – 1.60 (m, 2H), 1.43 (s, 3H, Me), 1.39 (s, 3H, Me); **LRMS** (ES+) mass found *m/z* 229.2 [M+H]$^+$; **HRMS** mass not found.†

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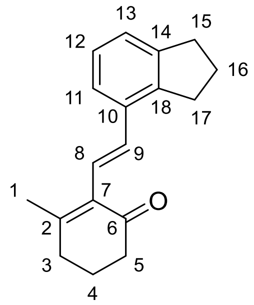
[*] Quaternary carbons not visible in obtained ^{13}C NMR spectrum.

[†] No ^{13}C data available.

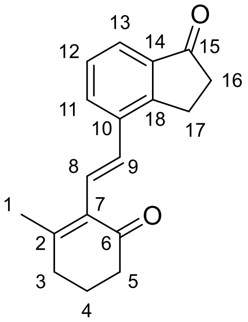
(*E*)-2-(2-(2,3-Dihydro-1*H*-inden-4-yl)vinyl)-3-methylcyclohex-2-en-1-one – 367



To a solution of tri(*o*-tolyl)phosphine (3.4 mg, 0.011 mmol, 0.04 eq) in toluene (0.4 mL) under N_2 was added $Pd(OAc)_2$ (1.4 mg, 0.0056 mmol, 0.02 eq). To this solution were added sequentially: a solution of **214** (66.1 mg, 0.28 mmol, 1.0 eq) in toluene (0.5 mL); DIPEA (59 μL, 0.34 mmol, 1.2 eq); then a solution of **329** (40 μL, 1.60 mmol, 1.0 eq) in toluene (0.5 mL). The reaction mixture was heated to reflux and stirred for 18 h. Once cooled, the reaction was diluted with EtOAc (10 mL), filtered through Celite®, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 8:1) to afford title compound **367** (34.5 mg, 50%) as a bright yellow solid.

R_f 0.15 (pentane:Et_2O, 8:1, stain: $KMnO_4$); **IR** ν_{max} 2944 (C-H), 1667 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.38 – 7.34 (m, 1H, H-11), 7.15 – 7.10 (m, 2H, H-12 and H-13), 7.04 (d, *J* = 16.5 Hz, 1H, H-9), 6.79 (d, *J* = 16.5 Hz, 1H, H-8), 2.98 (t, *J* = 7.5 Hz, 2H, H-17), 2.92 (t, *J* = 7.5 Hz, 2H, H-15), 2.48 (t, *J* = 7.0 Hz, 4H, H-3 and H-5), 2.13 (s, 3H, H-1), 2.07 (p, *J* = 7.5 Hz, 2H, H-16), 2.03 – 1.95 (m, 2H, H-4); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 198.6 (C-6), 157.0 (C-2), 144.7 (C-14), 142.5 (C-18), 134.2 (C-10), 133.3 (C-7), 132.6 (C-9), 126.6 (C-12), 123.5 (C-13), 122.8 (C-11), 122.8 (C-8), 38.7 (C-5), 33.9 (C-3), 33.1 (C-15), 31.9 (C-17), 25.1 (C-16), 22.6 (C-1), 22.0 (C-4); **LRMS** (ES+) mass found *m/z* 275.1 $[M+Na]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{18}H_{21}O$) requires *m/z* 253.1587, found *m/z* 253.1588; **mp** 61 – 63 °C.

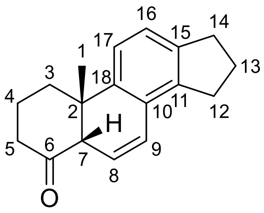
(*E*)-4-(2-(2-Methyl-6-oxocyclohex-1-en-1-yl)vinyl)-2,3-dihydro-1*H*-inden-1-one – 368



To a solution of tri(*o*-tolyl)phosphine (3.9 mg, 0.013 mmol, 0.04 eq) in toluene (0.6 mL) under N_2 was added $Pd(OAc)_2$ (1.4 mg, 0.0064 mmol, 0.02 eq). To this solution were sequentially added: a solution of **214** (75.5 mg, 0.32 mmol, 1.0 eq) in toluene (0.5 mL); DIPEA (66 μL, 0.38 mmol, 1.2 eq); then a solution of **327** (50 μL, 0.32 mmol, 1.0 eq) in toluene (0.5 mL). The reaction mixture was heated to reflux and stirred for 18 h. Once cooled, the reaction was diluted with EtOAc (10 mL), filtered through Celite®, and concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 5:1 to 1:1) to afford title compound **368** (43.5 mg, 50%) as a white solid.

R$_f$ 0.25 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **IR** ν_{max} 2921 (C-H), 1705 (C=O), 1667 (C=O); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.78 (d, *J* = 7.5 Hz, 1H, H-11), 7.66 (d, *J* = 7.5 Hz, 1H, H-13), 7.38 (t, *J* = 7.5 Hz, 1H, H-12), 7.18 (d, *J* = 16.5 Hz, 1H, H-9), 6.91 (d, *J* = 16.5 Hz, 1H, H-8), 3.18 (t, J = 6.0 Hz, 2H, H-17), 2.71 (t, *J* = 6.0 Hz, 2H, H-16), 2.51 (q, *J* = 6.5 Hz, 4H, H-3 and H-5), 2.16 (s, 3H, H-1), 2.01 (p, *J* = 6.5 Hz, 2H, H-4);**^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 207.4 (C-15), 198.5 (C-6), 158.3 (C-2), 153.2 (C-18), 137.5 (C-14), 136.3 (C-10), 132.6 (C-7), 130.6 (C-11), 129.6 (C-9), 127.9 (C-12), 124.5 (C-8), 122.7 (C-13), 38.9 (C-5), 36.3 (C-16), 33.9 (C-3), 25.1 (C-17), 22.6 (C-1), 21.9 (C-4); **LRMS** mass not found; **HRMS** mass not found; **mp** 94 – 96 °C.

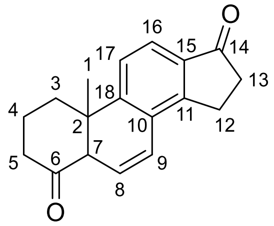
10-Methyl-1,2,3,5,10,15,16,17-octahydro-4*H*-cyclopenta[a]phenanthren-4-one – 369



A solution of **367** (50 mg, 0.2 mmol, 1 eq) was prepared in dry acetonitrile (4 mL) in a PenRay photochemical vessel fitted to an Ar line with an exit bubbler attached. Ar was bubbled through the solution (*via* needle) for 10 minutes. The photochemical vessel was immersed in a foil-lined ice bath and irradiated at 254 nm at 0 °C under a constant stream of Ar for 20 h. After this time, the light source was switched off and the solution concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 10:1 to 2:1) to afford title compound **369** (8.4 mg, 17%) as a colourless oil.

Rf 0.30 (pentane:Et_2O, 10:1, stain: $KMnO_4$); **IR** ν_{max} 2931 (C-H), 1714 (C=O); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 7.10 (s, 2H, H-16 and H-17), 6.62 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-9), 6.43 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-8), 3.42 (t, *J* = 3.0 Hz, 1H, H-7), 2.99 (dt, *J* = 15.5 Hz, 7.5 Hz, 1H, H-12), 2.93 – 2.87 (m, 2H, H-14), 2.85 (dd, *J* = 15.5 Hz, 7.5 Hz, 1H, H-12), 2.48 – 2.39 (m, 3H, H-3 and H-5), 2.23 – 2.16 (m, 1H, H-4), 2.13 – 2.06 (m, 4H, H-3, H-4 and H-13), 0.99 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 210.0 (C-6), 142.9 (C-15), 142.1 (C-18), 141.4 (C-11), 128.5 (C-10), 125.3 (C-9), 124.1 (C-8), 123.6 (C-16 or C-17), 120.9 (C-16 or C-17), 55.5 (C-7), 42.8 (C-2), 40.9 (C-5), 34.9 (C-3), 32.8 (C-14), 31.0 (C-12), 25.2 (C-13), 22.9 (C-4), 20.5 (C-1); **LRMS** (ES+) mass found 253.2 $[M+H]^+$; **HRMS** mass not found.

10-Methyl-2,3,5,10,15,16-hexahydro-1*H*-cyclopenta[a]phenanthrene-4,17-dione – 370



A solution of **368** (50 mg, 0.19 mmol, 1 eq) was prepared in dry acetonitrile (3.8 mL) in a PenRay photochemical vessel fitted to an Ar line with an exit bubbler attached. Ar was bubbled through the solution (*via* needle) for 10 minutes. The photochemical vessel was immersed in a foil-lined ice bath and irradiated at 254 nm at 0 °C under a constant stream of Ar for 20 h. After this time, the light source was switched off and the solution concentrated *in vacuo*. The crude product was purified by FCC (pentane:Et_2O, 2:1 to 1:1) to afford title compound **370** (4.7 mg) as a colourless oil.*

Rf 0.10 (pentane:Et_2O, 2:1, stain: $KMnO_4$); **IR** ν_{max} 2917 (C-H), 2849 (C-H), 1708 (C=O); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 7.65 (d, *J* = 8.0 Hz, 1H, H-17), 7.36 (d, *J* = 8.0 Hz, 1H, H-16), 6.71 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-8), 6.58 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-9), 3.48 (t, *J* = 3.0 Hz, 1H, H-7), 3.24 – 3.14 (m, 1H, H-12), 3.11 – 3.00 (m, 1H, H-12), 2.76 – 2.69 (m, 2H, H-13), 2.57 – 2.48 (m, 1H, H-5), 2.48 – 2.43 (m, 2H, H-3 and H-5), 2.19 – 2.07 (m, 2H, H-3 and H-4), 2.04 – 1.99 (m, 1H, H-4),1.28 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 208.9 (C-6), 206.8 (C-14), 150.5 (C-18), 137.6 (C-15), 135.9 (C-11), 130.3 (C-10), 126.0 (C-9), 123.2 (C-8), 123.0 (C-17), 122.6 (C-16), 55.0 (C-7), 43.3 (C-2), 40.7 (C-5), 36.5 (C-13), 34.7 (C-3), 24.3 (C-12), 22.6 (C-4), 20.0 (C-1); **LRMS** mass not found; **HRMS** mass not found.†

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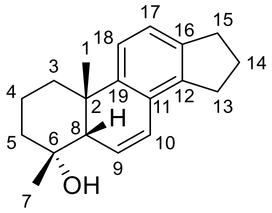
[*] Compound **368** could not be thoroughly separated and cleaned of impurities so percentage yield not given.
[†] Relative stereochemistry could not be assigned.

4,10-Dimethyl-2,3,4,5,10,15,16,17-octahydro-1*H*-cyclopenta[a]phenanthren-4-ol – 371

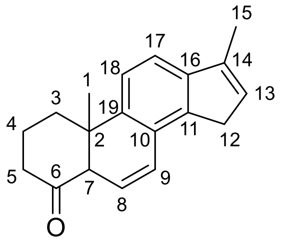


A solution of **369** (12.0 mg, 0.048 mmol, 1.0 eq) was prepared in dry Et_2O (96 µL) under N_2 and cooled to 0 °C. Methylmagnesium bromide (18 µL, 3.0 M in Et_2O, 0.053 mmol, 1.1 eq) was added dropwise and the mixture stirred at 0 °C for 2.5 h. After this time, the solution was warmed to rt, poured into a mixture of Et_2O (2 mL) and 3.0 M aq. HCl (2 mL), and allowed to stir at rt for a further 20 min. The layers were separated and the aqueous phase extracted with Et_2O (3 × 5 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **371** (6.5 mg, 50%) as an off-white solid.

Rf 0.10 (pentane:Et_2O, 10:1, stain: $KMnO_4$); **IR** ν_{max} 3440 (O-H), 2929 (C-H), 1459 (C=C), 786 (=C-H); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 7.08 (d, *J* = 7.5 Hz, 1H, H-17), 7.00 (d, *J* = 7.5 Hz, 1H, H-18), 6.69 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-10), 6.20 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-9), 2.99 (dt, *J* = 15.0 Hz, 7.5 Hz, 1H, H-13), 2.94 – 2.80 (m, 3H, H-13 and H-15), 2.24 (t, *J* = 3.0 Hz, 1H, H-8), 2.23 – 2,21 (m, 1H, H-3), 2.12 – 2.05 (m, 2H, H-14), 1.99 (qt, *J* = 14.0 Hz, 3.5 Hz, 1H, H-4), 1.80 – 1.75 (m, 1H, H-5), 1.69 (dt, *J* = 14.0 Hz, 3.5 Hz, 1H, H-4), 1.63 (td, *J* = 1.0 Hz, 3.5 Hz, 1H, H-3), 1.50 – 1.43 (m, 1H, H-5), 1.31 (s, 3H, H-7), 1.20 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 145.6 (C-19), 142.0 (C-16), 140.8 (C-12), 128.6 (C-11), 128.1 (C-9), 126.4 (C-10), 123.6 (C-17), 120.2 (C-18), 71.9 (C-6), 50.1 (C-8), 40.3 (C-5), 37.8 (C-2), 36.0 (C-3), 32.8 (C-15), 31.0 (C-

13), 30.2 (C-7), 25.3 (C-14), 20.6 (C-1), 18.4 (C-4); **LRMS** mass not found; **HRMS** mass not found.

10,17-Dimethyl-1,2,3,5,10,15-hexahydro-4*H*-cyclopenta[a]phenanthren-4-one – 373



A solution of **370** (15.0 mg, 0.056 mmol, 1.0 eq) was prepared in dry Et_2O (112 µL) under N_2 and cooled to 0 °C. Methylmagnesium bromide (21 µL, 3.0 M in Et_2O, 0.062 mmol, 1.1 eq) was added dropwise and the mixture stirred at 0 °C for 2.5 h. After this time, the solution was warmed to rt, poured into a mixture of Et_2O (2 mL) and 3.0 M aq. HCl (2 mL), and allowed to stir at rt for a further 20 min. The layers were separated and the aqueous phase extracted with Et_2O (3 × 5 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford title compound **373** (<0.5 mg)* as a pale-yellow oil.

R$_f$ 0.20 (pentane:Et_2O, 4:1, stain: $KMnO_4$); **IR** ν_{max} 3629 (O-H), 2919 (C-H); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 7.22 – 7.16 (m, 2H, H-17 and H-18), 6.79 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-9), 6.27 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-8), 6.16 (ap. q, *J* = 2.0 Hz, 1H, H-13), 3.35 (t, *J* = 2.0 Hz, 1H, H-12), 3.24 (t, *J* = 2.0 Hz, 1H, H-12), 2.34 – 2.24 (m, 2H, H-3 and H-7), 2.15 (d, *J* = 2.0 Hz, 3H, H-15), 2.08 – 1.96 (m, 1H, H-4), 1.82 – 1.76 (m, 1H, H-5), 1.75 – 1.63 (m, 2H, H-3 and H-4), 1.50 – 1.45 (m, 1H, H-5), 1.23 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 197.0 (C-6), 144.3 (C-14 or C-19), 144.2 (C-14 or C-19),

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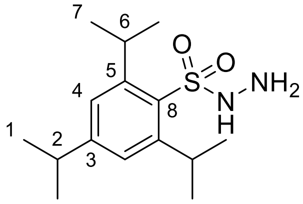
* Mass could not be accurately determined so no yield given.

140.7 (C-11 or C-16), 140.1 (C-11 or C-16), 128.6 (C-8), 128.1 (C-10), 128.0 (C-13), 125.9 (C-9), 120.3 (C17 or C-18), 118.1 (C-17 or C-18), 50.5 (C-7), 40.4 (C-5), 38.0 (C-2), 36.1 (C-3), 36.1 (C-12), 21.0 (C-1), 18.4 (C-4), 13.2 (C-15); **LRMS** mass not found; **HRMS** mass not found.*

2,4,6-Triisopropylbenzenesulfonohydrazide – 405



A solution of 2,4,6-triisopropylbenzenesulfonyl chloride (3.0 g, 9.9 mmol, 1.0 eq) in THF (12 mL) was prepared at –10 °C under N_2. To this solution was added hydrazine monohydrate (1.1 mL, 21.8 mmol, 2.2 eq) dropwise, maintaining the temperature below 0 °C. The mixture was allowed to warm to 0 °C and was stirred for 3 h, then the solvent was removed *in vacuo*, still maintaining the temperature below 0 °C. The crude product was dried under high vacuum to afford title compound **405** (2.95 g, quant.) as a white solid.

R_f 0.25 (pentane:Et_2O, 2:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.20 (s, 2H, H-4), 5.43 (s, 1H, NH), 4.16 (p, *J* = 6.5 Hz, 2H, H-6), 3.65 (s, 2H, NH_2), 2.92 (p, *J* = 7.0 Hz, 1H, H-2), 1.27 (d, *J* = 6.5 Hz, 12H, H-7), 1.26 (d, *J* = 7.0 Hz, 6H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 154.0 (C-Ar), 152.0 (C-Ar), 124.2 (C-4), 34.4 (C-2), 30.0 (C-6), 25.1 (C-7), 23.7 (C-1); **mp** 120 °C (dec). Data consistent with literature.[321]

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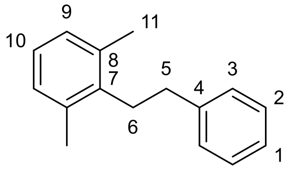
* Relative stereochemistry could not be assigned.

1,3-Dimethyl-2-phenethylbenzene – 406



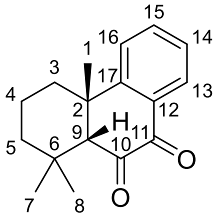
Compound **406** was obtained as the unexpected major product during the attempted acid-mediated cyclisation of inseparable alkenes **361a** and **361b** under the following conditions.

A solution of inseparable alkenes **361a** and **361b** (1:1) (18.7 mg, 0.088 mmol, 1 eq) in glacial acetic acid (88 µL) was heated to reflux for 18 h. The cooled reaction mixture was diluted with water (5 mL) and extracted with Et_2O (3 × 5 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (15 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by FCC (pentane) to afford title compound **406** (7.0 mg, 38%) as a pale-yellow oil.

R_f 0.35 (pentane, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.35 – 7.30 (m, 2H, H-Ar), 7.27 – 7.21 (m, 3H, H-Ar), 7.04 (s, 3H, H-Ar), 2.97 – 2.89 (m, 2H, H-6), 2.80 – 2.73 (m, 2H, H-5), 2.36 (s, 6H, H-11); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 142.4 (C-Ar), 138.7 (C-Ar), 136.3 (C-Ar), 128.6 (HC-Ar), 128.4 (HC-Ar), 128.3 (HC-Ar), 126.1 (HC-Ar), 126.0 (HC-Ar), 35.5 (C-5), 32.2 (C-6), 19.9 (C-11). Data consistent with literature.[322]

Compounds from Chapter 5

1,1,4a-Trimethyl-1,2,3,4,4a,10a-hexahydrophenanthrene-9,10-dione – 391



Method 1: Diketone **391** was synthesised according to **General Procedure D** with **249** (24 mg, 0.1 mmol) and P450 mutant K19F87V (36 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1) to afford title compound **391** (21.2 mg, 82%) as a yellow solid. **$[\alpha]_D^{25}$** = +10.5 (c = 1.00, $CHCl_3$).

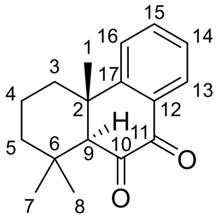
Method 2: Diketone **391** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant KSK19 AM IG (48 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **391** (21.5 mg, 42%) as a yellow solid. **$[\alpha]_D^{25}$** = –22.5 (c = 0.40, $CHCl_3$).

Method 3: Diketone **391** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant RLYF KSK19 AI (72 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **391** (16.4 mg, 32%) as a yellow solid. **$[\alpha]_D^{25}$** = –3.7 (c = 0.65, $CHCl_3$).

R_f 0.15 (pentane:Et_2O, 10:1, stain: $KMnO_4$); **IR** ν_{max} 2953 (C-H), 1723 (C=O), 1685 (C=O); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 8.14 (dd, *J* = 8.0 Hz, 1.5 Hz, 1H, H-13), 7.67 (dd, *J* = 8.0 Hz, 1.5 Hz, 1H, H-15), 7.48 (dd, *J* = 8.0 Hz, 1.5 Hz, 1H, H-16), 7.41 (td, *J* = 8.0 Hz, 1.5 Hz, 1H, H-14), 2.69 (s, 1H, H-9), 2.61 (dt, *J* = 14.5 Hz, 3.0 Hz, 1H, H-3), 1.65 – 1.52 (m, 2H, H-4), 1.48 – 1.31 (m, 3H, H-3 and H-5), 1.23 (s, 3H, H-1), 0.98 (s, 3H, H-7), 0.38 (s, 3H, H-8); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 199.0 (C-10), 181.5 (C-11), 150.0 (C-17), 135.7 (C-15), 133.9 (C-12), 130.4 (C-13), 127.6 (C-14), 124.8 (C-16), 69.0 (C-

9), 42.1 (C-5), 39.8 (C-2), 38.8 (C-1), 36.2 (C-3), 35.6 (C-6), 31.5 (C-7), 24.2 (C-8), 18.9 (C-4); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{17}H_{21}O_2$) requires *m/z* 257.1536, found *m/z* 257.1536; **mp** 117 – 119 °C. Data consistent with literature.[193]

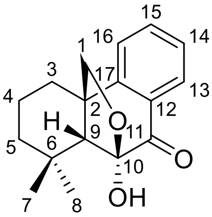
1,1,4a-Trimethyl-1,2,3,4,4a,10a-hexahydrophenanthrene-9,10-dione – 392



Diketone **392** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant KSK19 AM IG (48 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **392** (3.2 mg) as a colourless oil.

R_f 0.20 (pentane:Et_2O, 10:1, stain: $KMnO_4$); **IR** ν_{max} 2981 (C-H), 2917 (C-H), 1749 (C=O); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 7.95 – 7.91 (m, 1H, H-13), 7.56 – 7.54 (m, 2H, H-15 and H-16), 7.40 – 7.36 (m, 1H, H-14), 2.79 (s, 1H, H-9), 2.65 – 2.58 (m, 1H, H-3), 1.82 – 1.75(m, 1H, H-5), 1.75 – 1.67 (m, 1H, H-4), 1.66 – 1.59 (m, 1H, H-4), 1.57 (s, 3H, H-1), 1.55 – 1.53 (m, 1H, H-3), 1.48 – 1.44 (m, 1H, H-5), 1.25 (s, 3H, H-7 or H-8), 0.95 (s, 3H, H-7 or H-8); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 168.4 (C-10), 164.1 (C-11), 147.0 (C-17), 133.7 (C-15), 133.4 (C-13), 129.4 (C-12), 127.7 (C-14), 126.9 (C-16), 65.1 (C-9), 39.4 (C-2), 37.8 (C-3), 37.5 (C-5), 33.2 (C-6), 31.6 (C-7 or C-8), 30.5 (C-1), 29.5 (C-7 or C-8), 18.9 (C-4); **LRMS** (ES+) mass found 295.1 $[M+K]^+$; **HRMS** mass not found.

9-Hydroxy-8,8-dimethyl-5,6,7,8,8a,9-hexahydro-10*H*-9,4b-(epoxymethano)phenanthren-10-one – 393

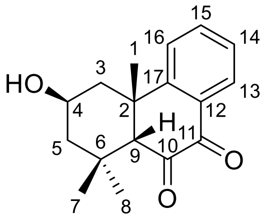


Method 1: Hemiacetal **393** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant KSK19 AM IG (48 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **393** (1.8 mg) as a pale-yellow oil.

Method 2: Hemiactetal **393** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant RLYF KSK19 AI (72 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **393** (1.5 mg) as a pale-yellow oil.

***R**f* 0.05 (pentane:Et_2O, 5:1, stain: $KMnO_4$); **IR** ν_{max} 3671 (O-H), 2961 (C-H), 2918 (C-H), 1716 (C=O); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 8.17 (d, *J* = 7.5 Hz, 1H, H-Ar), 7.66 (t, *J* = 7.5 Hz, 1H, H-Ar), 7.46 – 7.42 (m, 2H, H-Ar), 4.01 (d, *J* = 7.0 Hz, 1H, H-1), 3.39 (d, *J* = 7.0 Hz, 1H, H-1), 2.87 – 2.74 (m, 2H, CH_2), 2.63 – 2.52 (m, 1H, CH_2), 2.53 – 2.47 (m, 1H, CH_2), 2.34 (s, 1H, H-9), 2.16 – 2.07 (m, 2H, CH_2), 1.07 (s, 3H, C-7), 0.18 (s, 3H, C-8); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 191.7 (C-11), 147.0 (C-17), 135.1 (HC-Ar), 127.9 (HC-Ar), 127.4 (HC-Ar), 124.1 (HC-Ar), 102.9 (C-10), 77.3 (C-1), 61.5 (C-9), 32.0 (C-7), 23.8 (C-8); **LRMS** (ES+) mass found 273.1 $[M+H]^+$; **HRMS** mass not found.

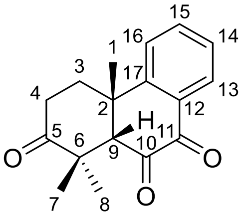
3-Hydroxy-1,1,4a-trimethyl-1,2,3,4,4a,10a-hexahydrophenanthrene-9,10-dione – 394



Diketone **394** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant RLYF KSK19 AI (72 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **394** (10.2 mg) as a pale-yellow oil.

Rf 0.30 (Et_2O, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 8.14 (dd, *J* = 7.5 Hz, 1.5 Hz, 1H, H-13), 7.69 (td, *J* = 7.5 Hz, 1.5 Hz, 1H, H-15), 7.58 (d, *J* = 7.5 Hz, 1H, H-16), 7.44 (td, *J* = 7.5 Hz, 1.5 Hz, 1H, H-14), 3.90 (tt, *J* = 11.5 Hz, 4.0 Hz, 1H, H-4), 2.90 (ddd, *J* = 14.0 Hz, 4.0 Hz, 2.0 Hz Hz, 1H, H-3), 2.68 (s, 1H, H-9), 1.77 (ddd, *J* = 12.5 Hz, 4.0 Hz, 2.0 Hz, 1H, H-5), 1.38 – 1.33 (m, 2H, H-3 and H-5), 1.31 (s, 3H, H-1), 1.04 (s, 3H, H-7), 0.43 (s, 3H, H-8); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 198.3 (C-10), 181.1 (C-11), 149.3 (C-17), 136.0 (C-15), 133.3 (C-12), 130.5 (C-13), 128.0 (C-14), 124.5 (C-16), 68.1 (C-9), 64.0 (C-4), 51.2 (C-5), 45.0 (C-3), 41.2 (C-2), 38.7 (C-1), 37.5 (C-6), 31.5 (C-7), 25.0 (C-8); **LRMS** (ES+) mass found 273.1 $[M+H]^+$; **HRMS** mass not found.

1,1,4a-Trimethyl-3,4,4a,10a-tetrahydrophenanthrene-2,9,10(1*H*)-trione – 395

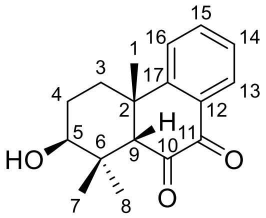


Method 1: Triketone **395** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant KSK19 AM IG (48 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **395** (0.5 mg) as a pale-yellow oil.

Method 2: Triketone **395** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant RLYF KSK19 AI (72 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **395** (4.9 mg) as a pale-yellow oil.

R_f 0.60 (Et_2O, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 8.24 (dd, *J* = 8.0 Hz, 1.5 Hz, 1H, H-13), 7.78 (td, *J* = 8.0 Hz, 1.5 Hz, 1H, H-15), 7.58 (d, *J* = 8.0 Hz, 1H, H-16), 7.52 (t, *J* = 8.0 Hz, 1H, H-14), 3.04 (s, 1H, H-9), 2.83 (dt, *J* = 15.0 Hz, 4.5 Hz, 1H, H-3), 2.76 (td, *J* = 15.0 Hz, 4.5 Hz, 1H, H-4), 2.43 (dt, *J* = 15.0 Hz, 4.0 Hz, 1H, H-4), 1.95 (td, *J* = 15.0 Hz, 4.0 Hz, 1H, H-3), 1.34 (s, 3H, H-1), 1.16 (s, 3H, H-7), 0.64 (s, 3H, H-8); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 211.2 (C-5), 195.9 (C-10), 180.8 (C-11), 148.0 (C-17), 136.4 (C-15), 133.8 (C-12), 130.9 (C-13), 128.5 (C-14), 124.9 (C-16), 68.2 (C-9), 47.7 (C-6), 40.0 (C-2), 37.4 (C-1), 35.3 (C-3), 34.9 (C-4), 23.9 (C-7), 23.8 (C-8); **LRMS** (ES+) mass found 271.1 $[M+H]^+$; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{17}H_{19}O_3$) requires *m/z* 271.1329, found *m/z* 271.1329.

2-Hydroxy-1,1,4a-trimethyl-1,2,3,4,4a,10a-hexahydrophenanthrene-9,10-dione – 396

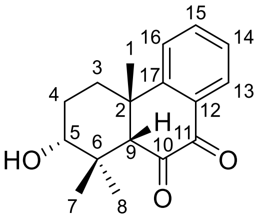


Method 1: Diketone **396** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant KSK19 AM IG (48 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **396** (7.8 mg) as a pale-yellow oil.

Method 2: Diketone **396** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant RLYF KSK19 AI (72 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **396** (11.9 mg) as a pale-yellow oil.

R_f 0.30 (Et_2O, stain: $KMnO_4$); **IR** ν_{max} 3519 (O-H), 2957 (C-H), 2917 (C-H), 1720 (C=O), 1686 (C=O); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 8.15 (dd, *J* = 8.0 Hz, 1.5 Hz, 1H, H-13), 7.68 (td, *J* = 8.0 Hz, 1.5 Hz, 1H, H-15), 7.51 (d, *J* = 8.0 Hz, 1H, H-16), 7.42 (t, *J* = 8.0 Hz, 1H, H-14), 3.40 (t, *J* = 3.0 Hz, 1H, H-5), 3.14 (s, 1H, H-9), 2.31 (dt, *J* = 14.0 Hz, 3.0 Hz, 1H, H-3), 1.97 (td, *J* = 14.0 Hz, 3.0 Hz, 1H, H-3), 1.89 (tt, *J* = 14.0 Hz, 3.0 Hz, 1H, H-4), 1.75 (dq, *J* = 14.0 Hz, 3.0 Hz, 1H, H-4), 1.25 (s, 3H, H-1), 1.01 (s, 3H, H-7), 0.37 (s, 3H, H-8); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 199.3 (C-10), 181.2 (C-11), 150.2 (C-17), 135.9 (C-15), 133.8 (C-12), 130.6 (C-13), 127.7 (C-14), 124.5 (C-16), 75.1 (C-5), 63.1 (C-9), 39.5 (C-2), 39.4 (C-6), 38.6 (C-1), 28.0 (C-3), 26.2 (C-7), 25.7 (C-4), 24.2 (C-8); **LRMS** (ES+) mass found 273.1 $[M+H]^+$; **HRMS** mass not found.

2-Hydroxy-1,1,4a-trimethyl-1,2,3,4,4a,10a-hexahydrophenanthrene-9,10-dione – 397

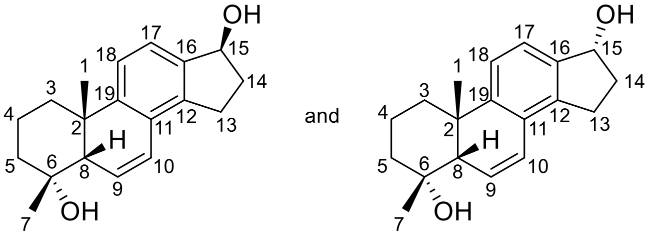


Method 1: Diketone **397** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant KSK19 AM IG (48 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **397** (6.8 mg) as a pale-yellow oil.

Method 2: Diketone **397** was synthesised according to **General Procedure D** with **249** (48 mg, 0.2 mmol) and P450 mutant RLYF KSK19 AI (72 h reaction). The crude product was purified by FCC (pentane:Et_2O, 20:1 to Et_2O) to afford title compound **397** (4.1 mg) as a pale-yellow oil.

***R**_f_* 0.20 (Et_2O, stain: $KMnO_4$); **IR** ν_{max} 3505 (O-H), 2964 (C-H), 2917 (C-H), 1722 (C=O), 1685 (C=O); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 8.16 (d, *J* = 7.5 Hz, 1H, H-13), 7.70 (t, *J* = 7.5 Hz, 1H, H-15), 7.48 (d, *J* = 7.5 Hz, 1H, H-16), 7.44 (t, *J* = 7.5 Hz, 1H, H-14), 3.45 (dd, *J* = 11.5 Hz, 4.0 Hz, 1H, H-5), 2.70 (s, 1H, H-9), 2.63 (dt, *J* = 14.5 Hz, 3.5 Hz, 1H, H-3), 1.80 (dq, *J* = 13.0 Hz, 3.5 Hz, 1H, H-4), 1.68 – 1.60 (m, 1H, H-4), 1.59 – 1.51 (m, 1H, H-3), 1.23 (s, 3H, H-1), 1.06 (s, 3H, H-7), 0.34 (s, 3H, H-8); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 197.5 (C-10), 181.2 (C-11), 149.3 (C-17), 136.0 (C-15), 133.8 (C-12), 130.6 (C-13), 127.8 (C-14), 124.5 (C-16), 78.0 (C-5), 68.1 (C-9), 40.0 (C-6), 39.9 (C-2), 38.3 (C-1), 34.3 (C-3), 27.6 (C-4), 26.7 (C-7), 17.3 (C-8); **LRMS** (ES+) mass found 295.1 $[M+Na]^+$; **HRMS** mass not found.

4,10-Dimethyl-2,3,4,5,10,15,16,17-octahydro-1*H*-cyclopenta[a]phenanthrene-4,17-diol – 399 and 4,10-dimethyl-2,3,4,5,10,15,16,17-octahydro-1*H*-cyclopenta[a]phenanthrene-4,17-diol – 400



Alcohols **399** and **400** were synthesised according to **General Procedure D** with **371** (8.7 mg, 0.032 mmol) and P450 mutant RK AI A264G (24 h reaction). The crude product was purified by FCC (pentane:Et_2O, 1:1) to afford title compounds **399** and **400** as pale-yellow oils.[*]

Product 1 (<0.5 mg): ***R_f*** 0.25 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **IR** ν_{max} 3440 (O-H), 2926 (C-H); **^{1}H NMR** ($CDCl_3$, 400 MHz) δ_H 7.27 (d, *J* = 8.0 Hz, 1H, H-17), 7.12 (d, *J* = 8.0 Hz, 1H, H-18), 6.69 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-9), 6.24 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-10), 5.21 (t, *J* = 6.0 Hz, 1H, H-15), 3.13 (ddd, *J* = 16.0 Hz, 8.5 Hz, 5.0 Hz, 1H, H-13), 2.76 – 2.69 (m, 1H, H-13), 2.50 (dddd, *J* = 13.0 Hz, 8.5 Hz, 7.0 Hz, 5.0 Hz, 1H, H-14), 2.28 – 2.21 (m, 3H, H-3 and H-4 and H-8), 2.03 – 1.95 (m, 1H, H-14), 1.78 (d, *J* = 14.0 Hz, 1H, H-5), 1.70 (dq, *J* = 14.0 Hz, 3.5 Hz, 1H, H-4), 1.63 (td, *J* = 13.0 Hz, 3.5 Hz, 1H, H-3), 1.51 – 1.44 (m, 1H, H-5), 1.31 (s, 3H, H-7), 1.20 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 101 MHz) δ_C 147.9 (C-19), 142.9 (C-16), 139.8 (C-12), 129.1 (C-11), 128.7 (C-10), 125.7 (C-9), 123.4 (C-17), 121.1 (C-18), 76.6 (C-15), 71.9 (C-6), 49.9 (C-8), 40.3 (C-5), 38.0 (C-2), 36.0 (C-14), 36.0 (C-3), 30.2 (C-7), 28.0 (C-13), 20.5 (C-1), 18.4 (C-4); **LRMS** mass not found; **HRMS** mass not found.

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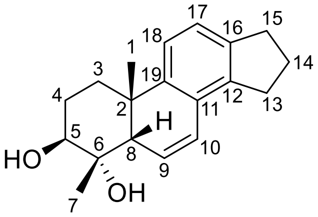
[*] Stereochemistry could not be definitively assigned.

Product 2 (2.3 mg): ***R**$_f$* 0.15 (pentane:Et$_2$O, 1:1, stain: KMnO$_4$); **IR** $\nu_{\max}$ 3385 (O-H), 2917 (C-H); **^{1}H NMR** (CDCl$_3$, 600 MHz) δ_H 7.13 (d, *J* = 8.0 Hz, 1H, H-18), 7.10 (d, *J* = 8.0 Hz, 1H, H-17), 7.08 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-10), 6.26 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-9), 5.34 (d, *J* = 5.0 Hz, 1H, H-15), 3.12 (dt, *J* = 16.0 Hz, 8.0 Hz, 1H, H-13), 2.76 (ddd, *J* = 16.0 Hz, 9.0 Hz, 4.0 Hz, 1H, H-13), 2.39 (ddt, *J* = 14.0 Hz, 9.0 Hz, 7.0 Hz, 1H, H-14), 2.25 – 2.20 (m, 2H, H-3 and H-8), 2.07 (dddd, *J* = 14.0 Hz, 8.0 Hz, 4.0 Hz, 2.5 Hz, 1H, H-14), 2.00 (qt, *J* = 14.0 Hz, 4.0 Hz, 1H, H-4), 1.81 – 1.75 (m, 1H, H-5), 1.73 – 1.67 (m, 1H, H-4), 1.62 (td, *J* = 13.0 Hz, 4.0 Hz, 1H, H-3), 1.50 – 1.44 (m, 1H, H-5), 1.32 (s, 3H, H-7), 1.23 (s, 3H, H-1); **^{13}C NMR** (CDCl$_3$, 151 MHz) δ_C 146.6 (C-19), 146.1 (C-Ar), 141.8 (C-Ar), 140.1 (C-Ar), 129.4 (C-9), 125.5 (C-10), 123.9 (C-17), 122.8 (C-18), 75.6 (C-15), 71.9 (C-6), 50.3 (C-8), 40.3 (C-5), 38.1 (C-2), 36.2 (C-3), 35.8 (C-14), 30.1 (C-7), 29.7 (C-13), 20.6 (C-1), 18.5 (C-4); **LRMS** mass not found; **HRMS** mass not found.

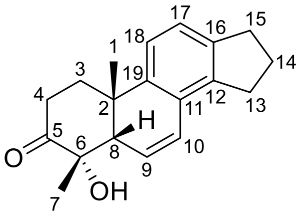
4,10-Dimethyl-2,3,4,5,10,15,16,17-octahydro-1*H*-cyclopenta[a]phenanthrene-3,4-diol – 401



Diol **401** was synthesised according to **General Procedure D** with **371** (8.7 mg, 0.032 mmol) and P450 mutant RK AI TG IG A328G (48 h reaction). The crude product was purified by FCC (pentane:Et$_2$O, 1:1) to afford title compound **401** (3.9 mg) as a pale-yellow oil.

R$_f$ 0.15 (pentane:Et$_2$O, 1:1, stain: KMnO$_4$); **^{1}H NMR** (CDCl$_3$, 600 MHz) δ_H 7.09 (d, *J* = 8.0 Hz, 1H, H-18), 7.01 (d, *J* = 8.0 Hz, 1H, H-17), 6.72 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-10), 6.15 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-9), 3.64 (t, *J* = 3.0 Hz, 1H, H-5), 2.99 (dt, *J* = 15.0 Hz, 7.5 Hz, 1H, H-15), 2.94 – 2.86 (m, 2H, H-13), 2.86 – 2.80 (m, 1H, H-15), 2.60 (t, *J* = 3.0 Hz, 1H, H-8), 2.35 (tdd, *J* = 14.5 Hz, 4.0 Hz, 2.5 Hz, 1H, H-4), 2.12 – 2.03 (m, 3H, H-3 and H-14), 1.98 (dt, *J* = 13.0 Hz, 3.5 Hz, 1H, H-3), 1.79 (dq, *J* = 14.5 Hz, 3.5 Hz, 1H, H-4), 1.38 (s, 3H, H-7), 1.19 (s, 3H, H-1); **^{13}C NMR** (CDCl$_3$, 151 MHz) δ_C 145.3 (C-19), 142.1 (C-16), 140.7 (C-12), 128.5 (C-11), 127.6 (C-9), 127.0 (C-10), 123.6 (C-18), 120.2 (C-17), 74.5 (C-5), 74.1 (C-6), 44.6 (C-8), 37.6 (C-2), 32.8 (C-13), 31.0 (C-15), 29.1 (C-3), 26.5 (C-7), 25.6 (C-4), 25.3 (C-14), 20.5 (C-1); **LRMS** mass not found; **HRMS** mass not found.

4-Hydroxy-4,10-dimethyl-1,2,4,5,10,15,16,17-octahydro-3*H*-cyclopenta[a]phenanthren-3-one – 402



Ketone **402** was synthesised according to **General Procedure D** with **371** (8.7 mg, 0.032 mmol) and P450 mutant RK AI TG IG A328G (48 h reaction). The crude product was purified by FCC (pentane:Et_2O, 1:1) to afford title compound **402** (0.5 mg) as a pale-yellow oil.

R_f 0.25 (pentane:Et_2O, 1:1, stain: $KMnO_4$); **^{1}H NMR** ($CDCl_3$, 600 MHz) δ_H 7.11 (d, *J* = 8.0 Hz, 1H, H-18), 6.89 (d, *J* = 8.0 Hz, 1H, H-17), 6.77 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-10), 6.22 (dd, *J* = 10.0 Hz, 3.0 Hz, 1H, H-9), 3.05 – 2.96 (m, 2H, H-13), 2.95 – 2.83 (m, 3H, H-3 and H-15), 2.79 (t, *J* = 3.0 Hz, 1H, H-8), 2.75 (d, *J* = 12.5 Hz, 1H, H-3), 2.65 – 2.54 (m, 2H, H-4), 2.13 – 2.08 (m, 2H, H-14), 1.48 (s, 3H, H-7), 1.18 (s, 3H, H-1); **^{13}C NMR** ($CDCl_3$, 151 MHz) δ_C 208.9 (C-5), 143.0 (C-19), 142.9 (C-16), 141.3 (C-12), 128.3 (C-11), 127.3 (C-10), 126.5 (C-9), 124.0 (C-18), 120.0 (C-17), 75.5 (C-6), 55.4 (C-4), 52.2 (C-3), 49.8 (C-8), 42.8 (C-2), 32.8 (C-15), 31.1 (C-13), 30.4 (C-7), 25.3 (C-14), 21.9 (C-1); **LRMS** mass not found; **HRMS** (ESI) exact mass calculated for $[M+H]^+$ ($C_{19}H_{23}O_2$) requires *m/z* 283.1693, found *m/z* 283.1693.

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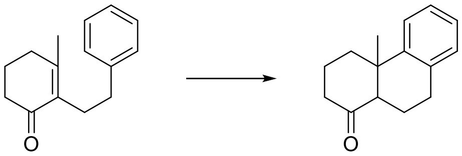
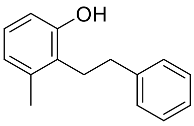
Appendix A – Attempted Cyclisation of Ketone 347

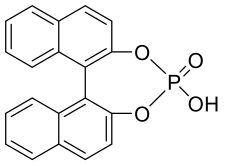
Table A1: *Attempted acid-mediated cyclisation of ketone **347**.*



347 **348**



356



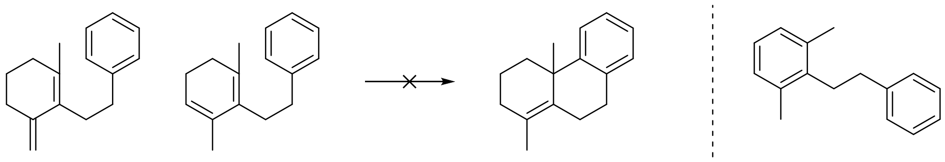
357

Entry	Acid	Solvent	Outcome
1	H_3PO_4	Toluene	SM
2	H_3PO_4	HFIP	SM
3	H_3PO_4	DCM	SM
4	H_3PO_4	Nitromethane	SM
5	Proline	Toluene	SM
6	Proline	HFIP	SM
7	Proline	DCM	SM
8	Proline	Nitromethane	SM
9	**357**	Toluene	SM + trace **356**
10	**357**	HFIP	SM
11	**357**	DCM	SM
12	**357**	Nitromethane	SM + trace **356**
13	Tf_2NH	Toluene	Complex mixture
14	Tf_2NH	HFIP	SM + trace other
15	Tf_2NH	DCM	SM + trace other
16	Tf_2NH	Nitromethane	Nothing recovered
17	$TMSNTf_2$	Toluene	Nothing recovered
18	$TMSNTf_2$	HFIP	SM + trace other
19	$TMSNTf_2$	DCM	SM
20	$TMSNTf_2$	Nitromethane	Complex mixture

Reagents and conditions: *2 eq acid, 0.5 M in solvent, heated to reflux, 18 h.*

Appendix B – Attempted Cyclisation of Alkenes 361a and 361b

***Table B1:** Attempted cyclisation of alkenes **361a** and **361b** under acidic conditions.*



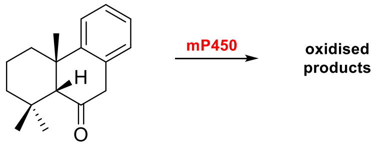
361a **361b** **360** **406**

Entry	Acid	Solvent	Temperature	Outcome
1[a]	AcOH	—	120 °C	38% **406**
2	10% H_2SO_4	THF	rt	SM + trace other
3	10% TFA	THF	70 °C	SM
4	10% TFA	DCM	rt	SM + trace other
5	10% TfOH	DCM	rt	SM + complex mixture
6	2% AgOTf	DCM	rt	SM
7[b]	5% $Au(PPh_3)_3Cl$, 7% $AgSbF_6$	DCM	rt	SM

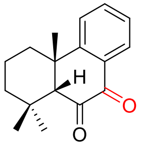
***Reagents and conditions:** 0.5 M in solvent. [a] 1.0 M in glacial acetic acid; [b] 0.1 M in DCM.*

Appendix C – Screening Data for Tricycle 249 with General Plates WY5 and WY6

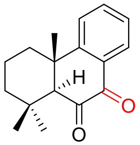
Table C1: *Screening data for tricycle **249** with general plates WY5 and WY6.*



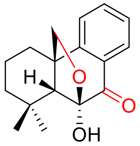
249
GC R_t: 4.38



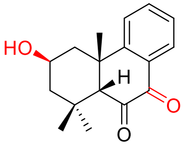
391
GC R_t: 5.84



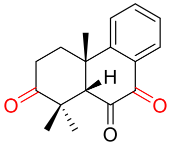
392



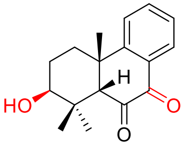
393
GC R_t: 7.12



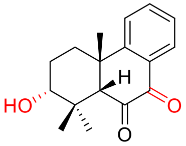
394
GC R_t: 7.12



395
GC R_t: 7.12



396
GC R_t: 7.47



397
GC R_t: 7.52

		Retention Time (min)							
Entry	**Enzyme**	**4.38**	**5.84**	**6.04**	**7.12**	**7.47**	**7.52**	**7.68**	**Other**
		249	**291**		**393 – 395**	**396**	**397**		**Inc. 392**
1	RLYF KSK19 A184I	27%	23%	4%	6%	15%	6%	4%	15%
2	KSK19IG	36%	31%	4%	—	6%	11%	6%	6%
3	GV A184I	37%	31%	5%	11%	3%	2%	2%	9%
4	K19 F87V	39%	41%	5%	5%	—	—	2%	8%
5	KSK19 A82M I263G	39%	17%	3%	—	3%	16%	7%	15%

6	RLYF KSK19	39%	31%	4%	4%	8%	3%	—	11%
7	KU3 A330P A328I	43%	46%	8%	—	—	—	—	3%
8	RLYF K19	43%	46%	6%	—	—	—	—	5%
9	RP H171L I263G	45%	28%	5%	—	—	—	2%	20%
10	RK/A328G	46%	23%	4%	—	8%	4%	—	15%
11	KSK19 I263A	49%	20%	3%	—	5%	6%	4%	13%
12	KU3 A330P	51%	31%	6%	—	—	—	3%	9%
13	K19 F87V E267V	53%	31%	5%	—	—	—	—	11%
14	RT2	53%	29%	5%	3%	—	—	3%	7%
15	KSK19 F81W	54%	23%	3%	—	6%	—	—	14%
16	RT2 A330P V78I A184I	54%	24%	6%	—	—	2%	—	14%
17	R19 A328L A184I	56%	32%	4%	—	—	—	—	8%
18	R19 A330W	58%	29%	5%	—	—	—	—	8%
19	GVQ	58%	27%	4%	3%	—	—	—	8%
20	K19 F87V Q403P	59%	26%	4%	—	3%	—	—	8%
21	A82F I263F	62%	28%	4%	—	—	—	—	6%
22	K19 F87V A328V	58%	23%	4%	3%	—	—	—	3%
23	RT2 S72G A330W	70%	20%	4%	—	—	—	2%	4%

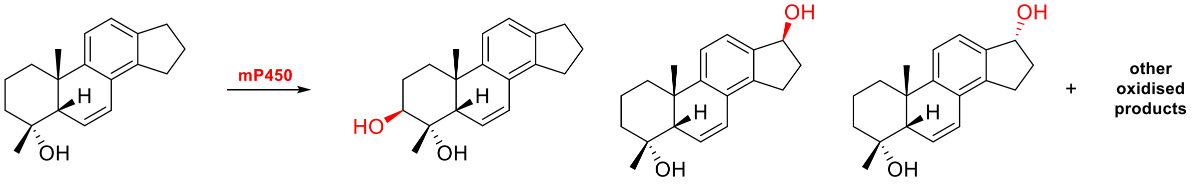
24	R19FI	76%	14%	3%	—	—	—	—	7%
25	WT	9%	75%	13%	—	—	—	—	3%
26	RKA V78I	22%	33%	6%	10%	—	7%	6%	24%
27	KSK19 A82M E267F	18%	64%	11%	—	—	—	—	7%
28	RP H171L I263G F87V V78I	19%	62%	10%	—	—	—	—	9%
29	RLYF KSK19 I263G	19%	49%	8%	—	—	6%	—	18%
30	RK/A328G/A264G	20%	45%	9%	—	4%	—	—	22%
31	KSK19 AM	19%	49%	7%	—	—	5%	3%	15%
32	RK A328L	22%	46%	8%	9%	—	—	—	15%
33	RP F87V V78I	27%	54%	11%	—	—	—	—	8%
34	RP HL IG L437LV	28%	54%	13%	—	—	—	—	5%
35	K19 F87V E267V V78I	28%	50%	11%	—	—	—	3%	8%
36	RLYF KSK19 A328F	29%	41%	9%	2%	—	3%	3%	13%
37	R19 F87I L437LV	31%	46%	11%	2%	—	—	3%	7%
38	RP V78I E267V	31%	42%	6%	3%	—	—	—	18%
39	K19 F87V A264G	32%	38%	10%	—	—	—	4%	16%
40	RP A82M I263A	32%	47%	10%	—	—	—	3%	8%
41	RT2 S72W A330W	35%	43%	9%	—	—	—	5%	8%

42	GVQ A264G	36%	45%	9%	—	—	—	—	10%
43	RP F87V	36%	36%	7%	—	—	—	—	21%
44	I263G	40%	39%	10%	—	—	—	4%	7%
45	GVQ IG	43%	38%	10%	—	—	—	3%	6%
46	RP A82W I263A	47%	30%	6%	—	—	—	—	17%
47	R19 A328L I263G	49%	33%	8%	—	—	—	3%	7%
48	RP IA EV	52%	28%	11%	—	—	—	—	9%

Screening of tricycle **249** performed according to General Procedure **B**. The oxidised products were detected by GC analysis and the product distributions determined from the integration of peak areas. Oxidised products were subsequently identified by NMR analysis following scale-up of selected mutants.

Appendix D – Screening Data for Tetracycle 371 with Steroid Plate RK AI

Table D1: *Screening data for tetracycle **371** with steroid plate RK AI.*



371 GC R_t: 4.24

401 GC R_t: 5.04

399 GC R_t: 4.90 and **400** GC R_t: 5.01

Entry	Enzyme	Retention Time (min)								
		4.24	**4.36**	**4.87**	**4.90**	**5.01**	**5.04**	**5.10**	**5.32**	**Other**
		371			**399 and 400**		**401**			
1	RK F81W TG A328G	7%	5%	15%	36%	5%	5%	—	15%	7%
2	RK AI TG A328G	8%	10%	2%	37%	5%	4%	—	9%	15%
3	RK AI IG A264G A328G	16%	—	—	7%	4%	36%	11%	—	21%
4	RK AI TG IG A328G	17%	—	—	4%	1%	63%	4%	—	7%
5	RK AI IG A328G	21%	—	—	—	—	59%	4%	—	12%
6	RK AI A264G	25%	4%	2%	43%	11%	2%	—	2%	8%
7	RK AI TG	26%	2%	2%	35%	8%	12%	—	2%	10%
8	RK AI TG A264G A328G S72W	30%	6%	17%	27%	2%	2%	—	10%	4%
9	RK AI TG A328G A82M S72F	31%	3%	—	24%	2%	5%	—	23%	8%

10	RK AI IG A264G	33%	4%	—	25%	5%	5%	17%	—	8%
11	RK AI TG A82M	33%	2%	—	14%	6%	21%	7%	—	11%
12	RK AI IG A328G	39%	—	—	—	—	39%	8%	—	12%
13	RK AI TG T268S	46%	3%	14%	22%	2%	5%	—	1%	6%
14	RK AI TG A328G A82M L181F	45%	2%	—	6%	2%	29%	3%	3%	5%
15	RK AI A330P	55%	3%	—	16%	9%	3%	6%	—	2%
16	RK AI TG A264G A328G	61%	3%	9%	14%	2%	2%	—	5%	1%
17	RK I263W	66%	3%	—	2%	6%	17%	—	—	3%
18	RK AI TG A328G A82M S332M	69%	5%	—	4%	7%	7%	2%	—	2%
19	RK AI TG A328G A82M A330I S332F	70%	4%	2%	1%	—	—	—	3%	15%
20	RK A328I A264G	77%	6%	—	10%	2%	1%	—	—	1%
21	RK AI I263W	85%	4%	—	1%	3%	4%	—	—	—
22	RK AI TG A264G	93%	5%	—	—	—	—	—	—	—
23	RK AI TG IG	93%	5%	—	—	—	—	—	—	—
24	RK AI TG IG A264G	93%	5%	—	—	—	—	—	—	—

Screening of tetracycle **371** performed according to General Procedure **C**. The oxidised products were detected by GC analysis and the product distributions determined from the integration of peak areas. Oxidised products were subsequently identified by NMR analysis following scale-up of selected mutants.

Appendix E – Mutations List for General Plates WY5 and WY6

Enzyme	Mutations from WT at numbered residues																									
	47	51	72	74	78	81	82	87	171	184	188	191	239	259	263	264	267	276	307	319	328	330	353	401	403	437
AF/IF							AF								IF											
GV/AI				AG				FV		AI																
GVQ				AG				FV			LQ															
GVQ/AG				AG				FV			LQ					AG										
GVQ/IG				AG				FV			LQ				IG											
IG															IG											
K19/FV								FV	HL										QH	NY						
K19/FV/AG								FV	HL							AG			QH	NY						
K19/FV/AV								FV	HL										QH	NY	AV					
K19/FV/EV								FV	HL								EV		QH	NY						
K19/FV/EV/VI					VI			FV	HL								EV		QH	NY						
K19/FV/QP								FV	HL										QH	NY					QP	
KSK19/AM							AM	FA	HL										QH	NY						
KSK19/AM/EF							AM	FA	HL								EF		QH	NY						
KSK19/AM/IG							AM	FA	HL						IG				QH	NY						
KSK19/FW						FW		FA	HL										QH	NY						
KSK19/IA								FA	HL						IA				QH	NY						
KSK19/IG								FA	HL						IG				QH	NY						
KU3/AP													NH	IV				AT				AP				
KU3/AP/AI													NH	IV				AT			AI	AP				
	47	51	72	74	78	81	82	87	171	184	188	191	239	259	263	264	267	276	307	319	328	330	353	401	403	437

	47	51	72	74	78	81	82	87	171	184	188	191	239	259	263	264	267	276	307	319	328	330	353	401	403	437
R19/AL/AI	RL	YF							HL	AI									QH	NY	AI					
R19/AL/IG	RL	YF							HL						IG				QH	NY	AI					
R19/AW	RL	YF							HL										QH	NY		AW				
R19/FI	RL	YF						FI	HL										QH	NY						
R19/FI/LLV	RL	YF						FI	HL										QH	NY						LLV
RK/AG	RL	YF						FV	HL										QH	NY	AG					
RK/AG/AG	RL	YF						FV	HL							AG			QH	NY	AG					
RK/AL	RL	YF						FA	HL										QH	NY	AL					
RKA/VI	RL	YF			VI			FA	HL										QH	NY	AI					
RL/YF/K19	RL	YF							HL										QH	NY						
RL/YF/KSK19	RL	YF						FA	HL										QH	NY						
RL/YF/KSK19/AF	RL	YF						FA	HL										QH	NY	AF					
RL/YF/KSK19/AI	RL	YF						FA	HL	AI									QH	NY						
RL/YF/KSK19/IG	RL	YF						FA	HL						IG				QH	NY						
RP/AM/IA	RL	YF					AM								IA									IP		
RP/AW/IA	RL	YF					AW								IA									IP		
RP/FV	RL	YF						FV																IP		
RP/FV/VI	RL	YF			VI			FV																IP		
RP/HL/IG	RL	YF							HL						IG									IP		
RP/HL/IG/FV/VI	RL	YF			VI			FV	HL						IG									IP		
RP/HL/IG/LLV	RL	YF							HL						IG									IP		LLV
RP/IA/EV	RL	YF													IA		EV							IP		
RP/VI/EV	RL	YF			VI												EV							IP		
RT/SW/AW	RL	YF	SW									AT	NH	IV				AT				AW	LI			
	47	51	72	74	78	81	82	87	171	184	188	191	239	259	263	264	267	276	307	319	328	330	353	401	403	437

	47	51	72	74	78	81	82	87	171	184	188	191	239	259	263	264	267	276	307	319	328	330	353	401	403	437
RT2	RL	YF										AT	NH	IV				AT					LI			
RT2/AP/VI/AI	RL	YF			VI					AI		AT	NH	IV				AT				AP	LI			
RT2/SG/AW	RL	YF	SG									AT	NH	IV				AT				AW	LI			
WT																										
	47	51	72	74	78	81	82	87	171	184	188	191	239	259	263	264	267	276	307	319	328	330	353	401	403	437

Appendix F – Mutations List for Steroid Plate RK AI

Enzyme	Mutations from WT at numbered residues																
	47	51	72	81	82	87	171	181	184	260	263	264	268	307	319	328	330	332
RK/AI/TG	RL	YF				FA	HL		AI	TG				QH	NY			
RK/AI/TG/AM	RL	YF			AM	FA	HL		AI	TG				QH	NY			
RK/AI/TG/IG	RL	YF				FA	HL		AI	TG	IG			QH	NY			
RK/AI/TG/IG/AG	RL	YF				FA	HL		AI	TG	IG	AG		QH	NY			
RK/AI/TG/IG/AG	RL	YF				FA	HL		AI	TG	IG			QH	NY	AG		
RK/AI/TG/AG	RL	YF				FA	HL		AI	TG		AG		QH	NY			
RK/AI/TG/AG/AG	RL	YF				FA	HL		AI	TG		AG		QH	NY	AG		
RK/AI/TG/AG/AG/SW	RL	YF	SW			FA	HL		AI	TG		AG		QH	NY	AG		
RK/AI/TG/TS	RL	YF				FA	HL		AI	TG			TS	QH	NY			
RK/AI/TG/AG	RL	YF				FA	HL		AI	TG				QH	NY	AG		
RK/AI/TG/AG/AM/SF	RL	YF	SF		AM	FA	HL		AI	TG				QH	NY	AG		
RK/AI/TG/AG/AM/LF	RL	YF			AM	FA	HL	LF	AI	TG				QH	NY	AG		
RK/AI/TG/AG/AM/AI/SF	RL	YF			AM	FA	HL		AI	TG				QH	NY	AG	AI	SF
RK/AI/TG/AG/AM/SM	RL	YF			AM	FA	HL		AI	TG				QH	NY	AG		SF
RK/AI/IG/AG	RL	YF				FA	HL		AI		IG	AG		QH	NY			
RK/AI/IG/AG/AG	RL	YF				FA	HL		AI		IG	AG		QH	NY	AG		
RK/AI/IG/AG	RL	YF				FA	HL		AI		IG			QH	NY	AG		
RK/AI/IW	RL	YF				FA	HL		AI		IW			QH	NY			
RK/AI/AG	RL	YF				FA	HL		AI			AG		QH	NY			
RK/AI/AP	RL	YF				FA	HL		AI					QH	NY		AP	
RK/FW/TG/AG	RL	YF		FW		FA	HL			TG				QH	NY	AG		
RK/IW	RL	YF				FA	HL				IW			QH	NY			
RK/AI/AG	RL	YF				FA	HL					AG		QH	NY	AI		
	47	51	72	81	82	87	171	181	184	260	263	264	268	307	319	328	330	332